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***The serotonergic and noradrenergic systems:
Their involvement in the antidepressant effect.***

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the requirement of the doctoral degree

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This thesis is comprised of six co-authored papers. I have written all papers with the helpful guidance and thoughtful criticism of all the co-authors. All of the experimental work presented in these papers have been carried out by myself with the exception of the radioligand binding experiments presented in chapter I. These experiments were skilfully carried out by Normand Lavoie.

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ABBREVIATIONS

ACh: Acetylcholine

AHP: Afterhyperpolarization

cAMP: adenosine 3',5'-cyclic monophosphate

4-AP: 4-Aminopyridine

CA₁₋₄: *Cornu ammonis* (Ammon's horns of the hippocampus)

Ca²⁺: Calcium ion

Cl⁻: Chloride ion

cDNA: Copied deoxyribonucleic acid

CNS: Central nervous system

CRF: Corticotropin-releasing factor

DA: Dopamine

5,7-DHT: 5,7-Dihydroxytryptamine

DSP-4: *N*-(2-chloroethyl)-*N*-ethyl-2-bromobenzylamine

EAA: Excitatory amino acid

EEDQ: *N*-Ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline

GABA: γ -aminobutyric acid

GDP: Guanosine diphosphate

GDP β S: Guanosine 5'-O-(2-thiodiphosphate)

GTP: Guanosine triphosphate

GTP γ S: Guanosine 5'-O-(3-thiotriphosphate)

HRP: Horseradish peroxidase

5-HT: 5-Hydroxytryptamine, serotonin

i.p.: Intra peritoneal

IP₃: 1,4,5 Inositol triphosphate

i.v.: Intravenous

K⁺: Potassium ion

LSD: *Lyserg Säure Diäthylamid* (Lysergic diethylamide acid)

MAO: Monoamine oxidase

MAOI: Monoamine oxidase inhibitor

mRNA: Messenger ribonucleic acid

Na⁺: Sodium ion

NE: Norepinephrine

6-OHDA: 6-Hydroxydopamine

8-OH-DPAT: 8-Hydroxy-2(di-N-propylamino)tetralin

PCA: Parachloroamphetamine

PCPA: Parachlorophenylalanine

Pgi: (Nucleus) Paragigantocellularis

PI: Phosphatidyl inositol

PKA: Protein kinase A

PKC: Protein kinase C

PLC: Phospholipase C

POP: Pseudo-one-pulse

PrH: (Nucleus) Prepositus hypoglossi

s.c.: Sub cutaneous

SSRI: Selective Serotonin Reuptake Inhibitors

TCA: Tricyclic Antidepressant

TTX: Tetrodotoxin

ABSTRACT

There is an historical debate in which the role and importance of the serotonergic (5-HT) and noradrenergic (NE) systems as being the key components in the antidepressant response is disputed. Alternatively, this contentious debate has directly led to the hypothesis which posits that a combined pharmacological action on both these systems might result in an enhanced antidepressant efficacy. The development of the dual 5-HT/NE reuptake inhibitor venlafaxine has allowed to put to the test this hypothesis. Hence, clinical insight into this problem was provided by studies that documented a greater efficacy of venlafaxine in major depression when used at high doses. The present research endeavour, undertaken in parallel at the Neurobiological Psychiatry Unit with clinical studies, sought to determine the effects of acute and prolonged treatments with venlafaxine on the rat 5-HT and NE systems such as to suggest a biological substratum for its clinical features. Using in vivo electrophysiological paradigms in the rat dorsal hippocampus, dorsal raphe and locus coeruleus, we have shown that: 1) venlafaxine blocks the reuptake of 5-HT more potently than that of NE; 2) venlafaxine and the SSRI paroxetine are equipotent in blocking the reuptake of 5-HT; 3) the NE reuptake inhibitor desipramine is more potent than venlafaxine in blocking the reuptake of NE. We have documented an important discrepancy between the affinity of venlafaxine for both the 5-HT and NE transporters binding sites and its in vivo reuptake inhibition potencies: the in vivo 5-HT and NE reuptake inhibition potencies of venlafaxine are much greater than could have been predicted from its affinities for the 5-HT and NE transporters. The disclosure of this peculiarity sheds doubt on the exact nature of the mechanism by which venlafaxine blocks both reuptake processes and, ultimately, exerts its antidepressant effect. The complementary use of in vivo electrophysiological and in vitro superfusion paradigms was used to ascertain the sensitivity and/or function of somatodendritic and postsynaptic 5-HT_{1A} receptors, postsynaptic α_2 -adrenergic receptors, presynaptic 5-HT_{1B} autoreceptors and α_2 -adrenergic auto- and heteroreceptors following long-term administration of a high and a low dose of venlafaxine. The temporal pattern of firing activity of both dorsal raphe 5-HT and locus coeruleus NE neurons during sustained administration of both dose regimens of venlafaxine was also determined. Finally, in a corollary set of experiments, we showed that the mixed β -adrenergic/5-HT_{1A} receptors antagonist (-)pindolol potentiated the activation of postsynaptic 5-HT_{1A} receptors induced by venlafaxine. Moreover, we have confirmed the discriminative antagonism of (-)pindolol between somatodendritic and postsynaptic 5-HT_{1A} receptors since

it blocked the former and not the latter. Therefore, we propose that (-)pindolol potentiates the activation of postsynaptic 5-HT_{1A} receptors by circumventing the homeostatic activity of the inhibitory somatodendritic 5-HT_{1A} autoreceptor.

RÉSUMÉ

Il existe un débat historique dans lequel le rôle et l'importance respectifs des systèmes sérotoninergiques (5-HT) et noradrénergiques (NE) dans la médiation de l'effet antidépresseur sont débattus. Parallèlement, ce contentieux a mené à l'élaboration de l'hypothèse soutenant qu'une action combinée sur ces deux systèmes pourrait résulter en une efficacité thérapeutique accrue. Le développement de la venlafaxine, un bloqueur combiné de la recapture de la 5-HT et de la NE, a permis d'apporter une nouvelle lumière sur cette problématique. Aussi, des études cliniques ont démontrées une efficacité accrue de la venlafaxine dans le traitement de la dépression majeure lorsqu'utilisée à hautes doses. L'effort de recherche présenté ici, entrepris à l'Unité de Neurobiologie Psychiatrique en parallèle à des études cliniques, vise essentiellement à déterminer les effets d'administrations aiguës et prolongées de venlafaxine sur certains éléments impliqués dans les neurotransmissions sérotoninergique et noradrénergique du rat et ce, de façon à identifier le substratum biologique sous-tendant son efficacité thérapeutique. En utilisant des paradigmes électrophysiologiques dans l'hippocampe dorsal, le raphé dorsal ainsi que dans le locus coeruleus, nous avons démontré que: 1) la venlafaxine bloque la recapture de la 5-HT plus puissamment que celle de la NE; 2) la venlafaxine et la paroxétine (un bloqueur sélectif de la recapture de la 5-HT) bloquent, avec une puissance égale, la recapture de la 5-HT, et 3) la désipramine (un bloqueur sélectif de la recapture de la NE) bloque plus puissamment la recapture de la NE que la venlafaxine. De plus, nous avons documenté la présence d'une importante discordance entre la puissance in vivo de la venlafaxine pour bloquer les 2 mécanismes de recapture et ses affinités pour les transporteurs de la 5-HT et de la NE. En fait, il appert que la venlafaxine bloque, in vivo, les recaptures de façon beaucoup plus puissante que celle prédite sur la base de ses affinités pour les deux transporteurs. La démonstration de cette équivoque soulève conséquemment un doute sur les mécanismes précis par lesquels la venlafaxine exerce son blocage de la recapture et, conséquemment, sur les mécanismes régissant son efficacité thérapeutique. Nous avons utilisé une combinaison d'approches expérimentale électrophysiologiques in vivo et de superfusion in vitro afin de déterminer la sensibilité et/ou la fonction des récepteurs 5-HT_{1A} somatodendritiques et postsynaptiques, α_2 -adrénergiques postsynaptiques, des autorécepteurs 5-HT_{1B} présynaptiques ainsi que des auto- et hétérorécepteurs α_2 -adrénergiques présynaptique. Le décours temporel du taux de décharge des neurones 5-HT et NE du raphé dorsal et du locus coeruleus, respectivement, a été

déterminé pendant des traitements avec la venlafaxine. Finalement, dans une série expérimentale corollaire, nous avons démontré qu'une administration de (-)pindolol, un antagoniste mixte des récepteurs $5\text{-HT}_{1A}/\beta$ adrénergique, potentialise l'activation des récepteurs 5-HT_{1A} postsynaptiques induite par la venlafaxine. Nous avons confirmé l'antagonisme différentiel exercé par le (-)pindolol entre les récepteurs 5-HT_{1A} somatodendritiques et postsynaptiques. Aussi, nous proposons que cet effet potentialisateur du (-)pindolol résulte d'un antagonisme sélectif des autorécepteurs somatodendritiques 5-HT_{1A} .

INTRODUCTION

The discovery in the 1950's that tricyclic antidepressants (TCAs) and monoamine oxidase inhibitors (MAOIs) were clinically effective in the treatment of major depression has given strength to the tenet of a biological basis for the illness. As these drugs were not ideal and plagued by numerous side-effects, it became mandatory to elucidate the mechanisms of action by which these drugs elicit their therapeutic efficacy in order to develop more selective drugs directly targeted at purported relevant site of action. In this regard, it was demonstrated that imipramine and amitriptyline, two effective TCAs, inhibit the reuptake of the catecholamine norepinephrine (NE) in the rat brain (Glowinski and Axelrod 1964; Ross and Renyi 1967). It was also later shown that other TCA's effective in major depression inhibits the reuptake of not only a catecholamine, but also of the indolamine serotonin (5-hydroxytryptamine; 5-HT) (Ross and Renyi 1969; Carlsson 1970). Combined with the fact that the MAOIs inhibits the catabolism of these monoamines, it soon became apparent that the 5-HT and NE systems were playing a critical role in the therapeutic efficacy of these drugs.

Despite the fact that the discovery of these first effective antidepressant treatments was derived from serendipity, the study of their mechanism of action has allowed for the rational development of more selective drugs aimed at specific targets. In this regard, the introduction of the Selective Serotonin Reuptake Inhibitors (SSRIs) which have proven to bear a similar therapeutic efficacy with the older agents while displaying fewer side-effects, represents a successful, if not spectacular, direct result of such a rational approach. Notwithstanding the progression achieved with this new class of drugs, these agents are still characterized by the relative high number of non-responders and by their delayed onset of therapeutic action. Indeed, these two major drawbacks represent the main challenge to which new antidepressant strategies are aimed at.

As for the etiological involvement of the monoamines 5-HT and NE in major depression, the role of 5-HT in the pathophysiology of affective disorders has been somewhat more robustly demonstrated compared to that for NE. For one, the widespread serotonergic innervation of the brain subserves its important modulation of many brain functions that underlie somatic functions (sleep, appetite, sexual function, etc.; Barchas and Usdin, 1973) that are altered in patients suffering from depression (see Maes and Meltzer 1995 for review). Using a more direct approach, Shopsin *et al.* (1975 and 1976) showed that the administration of the tryptophan hydroxylase inhibitor PCPA to patients who had recovered from depression

with imipramine or tranylcypromine, most patients showed a rapid and pronounced return of depressive symptoms. Similarly, the tryptophan depletion paradigm was also able to delineate a role for 5-HT in the depressive disorders (Delgado *et al.* 1990).

These first research endeavours have directly lead to the elaboration of the monoamine theory of depression which states, in its simplest form, that the depressive disorder results from an alteration in monoamine neurotransmission (Coppen *et al.* 1972; Maes and Meltzer 1995; Siever 1987). Although this hypothesis has received ever since tremendous attention and has hence been refined, it is yet to be definitive or conclusive.

In any event, as both the 5-HT and NE systems appear to be involved in the therapeutic action of currently available drugs, a research commitment was consequently undertaken to study the physiology and pharmacology of both these system's neurotransmissions, as well as, but to a lesser degree, to the study of their mutual interactions. Amongst these tasks, a particular attention, by our and other laboratories, was given to the effects on these two systems of chronic administration of antidepressant treatments. The rationale of this paradigm being first to mimic the therapeutic regimen that is given to depressed patients and second to identify the potential homeostatic mechanisms that may be responsible for the therapeutic action of these drugs that occur after 3 to 4 weeks of treatments. The general hypothesis supported by the Neurobiological Psychiatry Unit is that all effective antidepressant treatments exert their therapeutic action through increasing 5-HT neurotransmission and this, via different mechanisms (Blier and de Montigny 1994). The purpose of the present work is to present, in an orderly fashion, relevant and representative findings on the physiology and pharmacology of both the 5-HT and NE system. A particular effort has been also denoted to describe some of the functional interconnectivity that exists between these systems. Furthermore, these two systems are endowed with homeostatic mechanisms that display a remarkable plasticity in response to prolonged pharmacological treatments. The nature and relevance of some of these adaptative changes following prolonged antidepressant treatments will be covered in such way as to attempt to present a tenable, yet critical, hypothesis for the mechanism of action of antidepressant treatments and how a combined action on both these systems may display therapeutic benefits. The availability of the dual 5-HT/NE reuptake inhibitor venlafaxine, which is an effective antidepressant, has permitted to gain privileged window into this problematic. The goal of the experimental section of the present thesis (presented in a more elaborate manner in section 12) is to fully characterize the neurobiological actions of venlafaxine. As

the studies presented in the present thesis mainly involved the use, on the one hand, of the raphe-hippocampus pathway and on the other hand the locus coeruleus-hippocampus pathway, our discussion will be mainly focused on issue directly relevant to these structures.

1. ON THE ANATOMY OF THE 5-HT SYSTEM

The most extensive monoaminergic system in the brain stem is the serotonergic system, whose neurons outnumber both the noradrenergic and dopaminergic cells of the brain stem. The anatomy of this system will be briefly covered herein with a particular attention to structures of relevance to the present work (see chapter III, IV, V and VI).

The first detailed anatomical map of the 5-HT system in a mammalian brain was provided by Dahlström and Fuxe 1964. This was rendered possible by the development of specific methods that allowed, first, the retention of 5-HT in tissue by freeze-drying, and second, by a method that allowed for conversion of 5-HT into a fluorophore detectable by fluorescence microscopy. This is known as the Falk-Hillarp method. Over the years, progress in immunohistochemistry has allowed the development of more precise mapping of the system. Thus, specific antibodies developed for 5-HT, 5-hydroxytryptophan, tryptophan hydroxylase and the 5-HT transporters have been used to this end. The use of these various techniques has allowed to determine that the 5-HT system consists of clusters of neurons containing 5-HT that are found in the central gray, in the surrounding reticular formation although most of the serotonergic cell bodies originate from a cell cluster located in the centre, hence *raphe* from Greek meaning midline.

1.1. The raphe nuclei

The subdivision of the raphe nuclei by (Dahlström and Fuxe 1964) involved the subdivision of 5-HT containing cells in 9 groups, referred to as B₁ to B₉, classified in a caudal to rostral axis (see table 1).

TABLE 1: Nomenclature Assigned to 5-HT Neurons**B1 Raphe pallidus nucleus**

Caudal ventrolateral medulla

B2 Raphe obscurus nucleus**B3 Raphe magnus nucleus**

Rostral ventrolateral medulla

Lateral paragigantocellular reticular nucleus

B4 Central gray of the medulla oblongata**B5 Pontine median raphe nucleus****B6 Pontine dorsal raphe nucleus****B7 Midbrain dorsal raphe nucleus****B8 Midbrain median raphe nucleus**

Caudal linear nucleus

B9 Medial lemniscus

The group formed of the B1 to B4 cell group of the classification of Dahlström and Fuxe (1964) are sometimes collectively referred to as the Caudal Linear Nucleus (Törk 1990). The 5-HT neurons of this group are located rostral to the caudal pole of the oculomotor nuclei and extend dorsally from the anterior edge of the interpeduncular nucleus to blend with the rostral part of the dorsal raphe nucleus. The projections from these regions extend to the thalamus and cortex.

Dorsal Raphe Nucleus. The dorsal raphe nucleus corresponds essentially to the B7 cell group of Dahlström and Fuxe (1964). The B6 group is the caudal extension of the dorsal raphe nucleus in the pons whereas the B7 group is located within the midbrain central gray. The dorsal raphe nucleus is the largest raphe nucleus (that displays a characteristic fountain shape in transverse sections) and contains approximately 50% of the 5-HT neurons of the rat brain (Descarries *et al.* 1990). The main projection system from this cell group is from 5-HT neurons that compose approximately two third of the entire neuronal population of the nucleus. It is believed that the ratio of 5-HT/non-5HT cells is higher in the dorsal raphe nucleus than in the other raphe cell group (Steinbusch and Nieuwenhuys 1999). The 5-HT neurons of the dorsal raphe are considered being leptodendritic cells, *i.e.*, they show few, although relatively long, dendrites arising from a fusiform perikaryon. More detailed and recent mappings have furthered the cytoarchitectonic classification of the dorsal raphe nucleus by subdividing the dorsal raphe nucleus into a principal part (B7 of Dahlström and Fuxe 1964)) which is itself subdivided into a medial, a lateral (the wings) and a caudal component. The medial component is further divided into a medial (dorso- and ventral medial part) and an interfascicular component. The superior component is in the central gray just below the cerebral aqueduct. The interfascicular component surrounds the medial longitudinal fibers and is especially prominent between the fasciculi. These neurons blend with the caudal median raphe nucleus. The lateral component (the wings) forms the largest division of the dorsal raphe nucleus and extends as far rostrally as the oculomotor nuclei. In the human, the lateral wings can be divided into a dorsal and ventrolateral subdivision. The wings are much more prominent in primates than in rodents and appear to be associated with more complex and advance level of CNS organization. Finally, the caudal component: this is the caudal most portion of the dorsal raphe nucleus (which corresponds to the B6 region of Dahlström and Fuxe 1964) that extends in the pons. It flanks the median sulcus of the aqueduct, above the interfascicular subnucleus.

The median raphe nucleus consists of a paramedian and median cluster of cells, lying below and caudal to the superior cerebellar decussating. Dahlström and Fuxe (1964) divide the median raphe in a caudal (B5) and a rostral (B8) component. Scattered 5-HT cells of the median raphe nucleus are seen ventrolaterally. These laterally situated cells lie in the nucleus pontis centralis oralis and form a ring around the central tegumental tract, one of the most primitive ascending pathways carrying reticulothalamic axons. The median raphe nucleus

consists of the second largest cluster of 5-HT neurons in the mammalian brain (Baker *et al.* 1990).

1.2. Efferent system

The 5-HT system gives rise to an extremely widespread efferent system. Of all the neurotransmitter systems within the vertebrate CNS, the 5-HT system is the most expansive. However, and importantly the 5-HT efferent system is neither ubiquitous nor nonspecific. For instance, within a particular region some portions may receive a very dense 5-HT innervation, whereas neighboring regions may receive only sparse 5-HT fibers.

The 5-HT system sends sparse projections to various brainstem structures, cerebellar cortex and spinal cord although its main efference is to the forebrain (See figure 1). The serotonergic fibers projecting to the forebrain originate mainly in the rostral group (B₅ to B₉) of raphe nuclei, whereas those projecting to the spinal cord originate from the caudal groups (B₁ to B₄). Several pathways have been described in rats and primates.

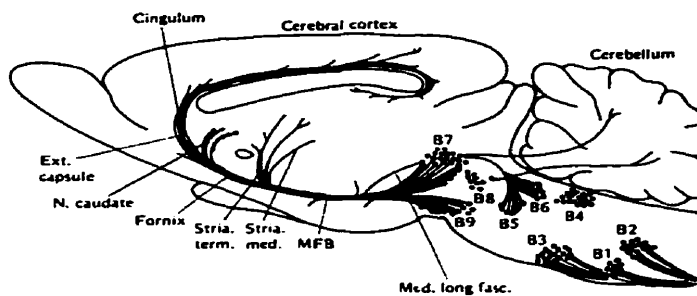


Figure 1: Schematic diagram illustrating the distribution of the main serotonin-containing pathways in the rat central nervous system. In the rat the largest pathway is the medial forebrain bundle which carries fibers from the median raphe nucleus and the dorsal raphe nucleus to a wide range of target areas in the forebrain(

Figure taken from Cooper *et al.* 1996.

In primates, a significant number of these fibers (~25%) are heavily myelinated whereas in rats this percentage falls to less than 1 % (Azmitia and Gannon, 1983). Virtually every cell in the brain is in close proximity to a 5-HT fibre. In the rat adult forebrain, a dense 5-HT innervation is seen in the suprachiasmatic nucleus of the hypothalamus (rhythm center), the substantia nigra, limbic centers, and around and in the ventricles. Serotonergic fibers in the cortex are abundant in limbic areas, and the primary sensory and association areas.

The different raphe nuclei innervate specific terminal areas in the neuraxis. Moreover, each terminal area has its topographic representation within the respective nuclei. Whereas the dorsal raphe nucleus preferentially innervates the striatum, the substantia nigra, the

amygdala, and the nucleus accumbens, the median raphe nucleus preferentially innervates the cingulate cortex, septal nuclei, and the hippocampus. However, these innervation patterns are not absolute: for instance, whereas the hippocampus receives 5-HT fibres originating from the median raphe nucleus, some fibres also originate from the dorsal raphe nucleus and B9 regions. In the hippocampus, the 5-HT innervation is highly organized. The 5-HT innervation from the median raphe nucleus enters through the fimbria and other dorsal pathways and terminates in the strata oriens and radiatum of the CA₃ region. The hippocampal innervation from the dorsal raphe nucleus, however, enters the hippocampus from the entorhinal cortex and into the dentate gyrus. Interestingly, the 5-HT projections from a single neuron or group of neurons can innervate several target regions. This sort of architecture allows for a relatively small number of cells to influence a great number of brain regions and hence brain functions.

In addition to its forebrain projections, there is a substantial 5-HT innervation to motor areas such as on motor neurons in the ventral horn, the motor nucleus of the trigeminal and the facial motor nucleus. For instance, microiontophoretic application of 5-HT onto motor neurons results in an increased excitability. Moreover, secondary motor structures such as the substantia nigra, globus pallidus and habenula receive serotonergic innervation. This pattern of innervation provides an anatomical correlate to the well described and typical motor “serotonin syndrome” which can be induced pharmacologically in laboratory animals, as well in humans, following administration of various serotonergic compounds such as precursors, releasers or agonists. This syndrome is characterized in the rat by hyperactivity, head shakes or “the wet dog” shakes, tremor, rigidity and reciprocal forepaw treading.

1.3. Afferent to the raphe

There are many regions of the brain that send projections to the raphe nuclei for which multiple neurotransmitters (excitatory amino acid, GABA, substance P, NE, DA and neuropeptides) have been implicated. For instance, one of the most relevant of these projections to the present work arises from the locus coeruleus and uses NE as its neurotransmitter (Baraban and Aghajanian 1981). The functional role of this afferent pathway is discussed in greater details in section 3.1.2. In addition to this, some of the most important pathways to the dorsal raphe nucleus include a GABAergic innervation originating from the lateral habenula (Hjorth *et al.* 1982), EAA innervation from specific hypothalamic nuclei and from the habenula nuclei (Hillion *et al.* 1994; Kalen *et al.* 1986; Marcinkiewicz *et*

al. 1989). A detailed description of afferent systems to the raphe of less importance has been reviewed by Pyneyro and Blier (1999).

1.4. Integrative aspect

Cytoarchitectonic and immunohistochemical studies have revealed that the 5-HT neurons of the raphe cell group are heterogenous in shape as well as in size. Some authors classify these cells as being small, medium, large and very large (Steinbusch and Nieuwenhuys 1999). The relative distribution of the cells within different raphe cell groups is also heterogenous. For instance, studies by Steinbush reveal that the small 5-HT containing cells can occur as being either spherical, ellipsoidal or piriform. Thus, whereas the small spherical 5-HT cells are observed in the dorsal raphe nucleus, the small ellipsoidal 5-HT cells are found in the nucleus centralis superior. Large 5-HT cells can be found as being either fusiform or multipolar. In the dorsal raphe nucleus, very large 5-HT cells are found to be only of multipolar appearance. There are of course other examples. These ones were only presented for illustrative purposes.

Altogether, there is a variability in cytoarchitecture, dendritic pattern, afferent and efferent systems that have been described between the different raphe nuclei. These observations thus render unlikely the fact that these nuclei constitute a single functional entity and this should obviously be taken into consideration in the context of functional studies.

2. ON THE STRUCTURE, FUNCTION, LOCALIZATION AND PHARMACOLOGY OF EFFECTOR ELEMENTS OF THE 5-HT SYSTEM

2.1 The 5-HT transporter

In order to keep an adequate temporal resolution, all neurotransmitters released in the synaptic cleft must have their action rapidly terminated by a biological process. These include degradative metabolism of the neurotransmitters by specific enzymes (for example, acetylcholinesterase or monoamine oxidase) or active transport by specific transport proteins located in the plasma membrane of presynaptic terminals. Such carriers exist for GABA, glycine, glutamate as well as for the catechol monoamines (DA, epinephrine and NE) and for the indolamine 5-HT. These proteins have three major features. First, as mentioned above, these carriers clear neurotransmitters from the synaptic cleft much faster than passive diffusion thus allowing for a better temporal resolution of consecutive release events. Second, an

improved spatial resolution is also achieved as the neurotransmitters are confined to a smaller area. This, in turn, will favour the development and maintenance of postsynaptic membrane specializations. Third, biological economy is attained as neurotransmitters once carried into the presynaptic terminals can be re-packaged into secretory vesicles and are thus made available for another secretory event. In this section, specific issues related to the reuptake process for 5-HT, *i.e.*, the transporter protein will be discussed.

The cDNA for the brain 5-HT transporter has been cloned from rat (Blakely *et al.* 1991; Hoffman *et al.* 1991), mouse (Chang *et al.* 1996) and human (Lesch *et al.* 1993a; Lesch *et al.* 1993b; Ramamoorthy *et al.* 1993). The transporter has 12 putative transmembrane domains, cytoplasmic N- and C- termini and N-glycosylation sites within the large extracellular loop between transmembrane segments 3 and 4. It has a calculated molecular mass of around 70 000 Da. Rat and human transporters differ in their amino acid sequence by 8.1 % (*i.e.*, by 51 amino acids). One of the direct applications of the identification of various cDNA coding for the 5-HT transporters has been the demonstration by Lesch *et al.* (1993b) that the human platelet 5-HT transporter was identical to that cloned from brain tissues. These results have thus given further credence to the long-entertained assumption that human platelet 5-HT uptake sites could be used as a valid model of brain 5-HT transporters function in health and disease and holds therefore profound implications in the interpretation of existing data generated in the context of trying to elucidate altered reuptake function in many psychiatric disorders.

The availability of various tritiated ligands that bind to the 5-HT transporters (*i.e.*, 5-HT reuptake inhibitors) has allowed to determine their localization. Whereas [³H]imipramine has been extensively used for this purpose, it is now well acknowledged that this compound is far from being ideal for this purpose (Hrdina *et al.* 1990; D'Amato *et al.* 1987). Studies with more suitable compounds such as [³H]paroxetine, [³H]citalopram and [³H]cyanoimipramine have revealed that in rodent and human brain, high levels of 5-HT transporters are consistently found in substantia nigra, amygdaloid nuclei and raphe nuclei (Plenge and Mellerup 1985; Biegon and Mathis 1993; Gobbi *et al.* 1990a; Kovachich *et al.* 1988; Laruelle *et al.* 1988). The hippocampus usually show a moderate level of binding whereas the cerebellum displays low to undetectable level of labelling. Moreover, lesions of 5-HT neurons with PCA or with 5, 7-DHT lead to a drastic decrease of binding to the 5-HT transporter throughout the brain (Hrdina *et al.* 1990; Kovachich *et al.* 1988) thus suggesting that the 5-HT transporters are

located mostly, if not exclusively, on 5-HT terminals. In keeping with this, the group of Descarries has developed a sampling technique that allows for the autoradiographic counting of both 5-HT axonal varicosities and of the 5-HT transporter (labelled with [^3H]cyanoimipramine) and have provided strong evidence supporting the notion that the 5-HT transporters are located on 5-HT terminals. Consistent with this interpretation, *in situ* hybridization studies have revealed the presence of 5-HT transporter mRNA in the raphe nuclei (Fujita *et al.* 1993; Blakely *et al.* 1991). More recently, the development of specific antibodies for the 5-HT transporters has further allowed for a detailed analysis of their localisation (Sur *et al.* 1996; Lawrence *et al.* 1995b; Lawrence *et al.* 1995a; Qian *et al.* 1995). Consistent with the data obtained by autoradiographical methods, the highest level of 5-HT transporter immunoreactivity was found in the dorsal raphe itself (Qian *et al.* 1995; Sur *et al.* 1996) with moderate labelling in the neighbouring central gray (Sur *et al.* 1996). In the hippocampus, the distribution of the 5-HT transporter displays a differential regional and laminar distribution. The stratum oriens layer of CA₂-CA₃ region displayed a higher immunocytochemical labelling than that of the CA₁ region (Qian *et al.* 1995; Sur *et al.* 1996). Within the CA₃ and CA₁ regions, higher labelling occurs in the stratum oriens layer than in the stratum lacunosum molecular layer (Sur *et al.* 1996). The stratum pyramidale exhibits low density of 5-HT transporters (Sur *et al.* 1996). These results are consistent with the study of Oleskevich and Descarries (1990) that have quantified the 5-HT innervation in the different fields of the Ammon's horn and its layers. They have observed a high density of varicosities in the stratum molecular of CA₁ and of the stratum oriens of CA₃ whereas the stratum pyramidale of the entire CA₃-CA₁ region as well as the granule cell layer of dentate gyrus displayed lower densities of varicosities. Neurons of the locus coeruleus and of substantia nigra do not stain with 5-HT transporters antibody, although 5-HT transporters-immunoreactive fibres can be detected in these nuclei (Qian *et al.* 1995; Zhou *et al.* 1996).

One of the prominent features of the 5-HT carrier-mediated function is the absolute requirement for extracellular Na⁺ ions. Indeed, the reuptake of 5-HT is coupled to the energetically favourable Na⁺ ions influx which occurs down its concentration gradient. A requirement for Cl⁻ ions, although not as stringent than that for Na⁺ ions, has also been documented for 5-HT transport. Moreover, intracellular K⁺ ion accelerates the influx of 5-HT. Based on this experimental evidence, the following mechanistic model for 5-HT transporter function has been proposed: Na⁺, Cl⁻, and a protonated 5-HT molecule binds to the carrier

protein which then undergoes a conformational change allowing for the transport of the molecule and of the ions into the cytoplasm. At this stage the transporter must be “recycled” in order for it to conduct another transport process. This occurs via intracellular K⁺ ions that reorients the transporters for another round.

The 5-HT transporter is the target of a variety of drugs that induce a wide variety of distinct behaviour. These drugs range from various tricyclic and heterocyclic compounds that are antidepressant agents to the psychoactive agent cocaine and various amphetamine and phenylethylamine derivatives that act as 5-HT releasers [for example MDMA (“ecstasy”), and fenfluramine]. The pharmacological profile of the rodent 5-HT transporters has been extensively studied with various tritiated compounds such as [³H]imipramine (Sette *et al.* 1983; Wood *et al.* 1986; Cortes *et al.* 1988; Plenge *et al.* 1990; Hrdina *et al.* 1990; Plenge *et al.* 1991), [³H]cyanoimipramine (Dumbrille-Ross and Tang 1983; Kovachich *et al.* 1988; Marcusson and Ross 1990; Soucy *et al.* 1994; Gurevich and Joyce 1996; Descarries *et al.* 1995), [³H]paroxetine (Plenge *et al.* 1991; Cortes *et al.* 1988; Marcusson *et al.* 1988; Dewar *et al.* 1992; Marcusson *et al.* 1992; Cheetham *et al.* 1993; Gurevich and Joyce 1996; Plenge *et al.* 1990; Hrdina *et al.* 1990), [³H]citalopram (D'Amato *et al.* 1987; Plenge *et al.* 1990; Plenge *et al.* 1990; Plenge *et al.* 1991; Descarries *et al.* 1995; Strazielle *et al.* 1996) and the cocaine analog [³H]RTI55 (Bengel *et al.* 1997; Rothman *et al.* 1995). These studies have first permitted to determine the ionic requirement for the binding of various compounds to the 5-HT transporters. Based on displacement studies of cold compounds and 5-HT by various tritiated compounds, the current model of 5-HT reuptake inhibition indicates that the binding of the various 5-HT reuptake inhibitors (imipramine, paroxetine and citalopram) to the transporter is partly overlapping (as they share at least one common site, i.e., that for 5-HT itself) (Graham *et al.* 1989; Marcusson and Ross 1990; Marcusson *et al.* 1989). Nonetheless, the cocaine analog β-CIT has been shown to bind to the 5-HT transporter in a unique way with regard to the ionic and pH requirements for binding. Indeed, while binding of the antidepressants (those inhibiting 5-HT reuptake) have a Cl⁻ ion dependency, β-CIT does not. Moreover, unlike imipramine, β-CIT binding is markedly pH sensitive (Wall *et al.* 1993; Humphreys *et al.* 1994). These studies indicate that there is perhaps more than one binding site on the 5-HT transporter.

Various compounds bind to the 5-HT transporter with more or less affinity and hence lead to the inhibition of the reuptake of 5-HT (Hyttel *et al.* 1982, Bolden-Watson and Richelson

1993; Tatsumi et al, 1997). The affinities with which the 5-HT reuptake inhibitors bind to the 5-HT transporter are well correlated with their potencies to inhibit the reuptake of [³H]5-HT from synaptosomes (Cheetham *et al.* 1993). In the case of antidepressants, tertiary amines such as imipramine and chloroimipramine are more potent at the 5-HT transporter than at the NE transporter, whereas secondary amine tricyclics such as desipramine and nortryptiline are more potent at the NE transporters.

2.2 The 5-HT_{1A} receptor

Shortly after the report by Peroutka and Snyder (Peroutka and Snyder 1981), identifying two 5-HT receptor subtypes, Pedigo *et al.* (1981) first identified the 5-HT_{1A} receptor. The description of the first selective 5-HT_{1A} agonist 8-OH-DPAT was done as early as 1982 by Hjorth *et al.* (1982). The availability of this compound as well as its tritiated form has paved the way to the study of the function and localization of the 5-HT_{1A} receptor in the brain. Further studies (Hamon *et al.* 1984; Hall *et al.* 1985; Hoyer *et al.* 1985b) have permitted to establish this compound as the prototypical 5-HT_{1A} agonist (despite the fact that it does have some affinity for the 5-HT₇ receptor (see Hoyer *et al.* 1994) and despite the development of more recent agonists). These include drugs such as alnespirone, ALK-3, 5-Carbocamidotryptamine, S 14506, flesinoxan, ipsapirone, buspirone and lesopitron (Hjorth *et al.* 1989; Casanovas *et al.* 1997; Gozlan *et al.* 1983; Hoyer *et al.* 1994). Although some of these drugs have been used clinically, most do not show the combination of selectivity and potency compared to 8-OH-DPAT for the 5-HT_{1A} receptor. As an example, the anxiolytic buspirone possesses affinity for DA receptors whereas it, along with ipsapirone, gepirone, and tandospirone share a common metabolite, 1-pyrimidinyl-piperazine (1-PP), which is a potent α_2 -adrenergic antagonist.

The synthesis and/or characterization of effective 5-HT_{1A} antagonists have lagged that for the agonist. Indeed, the first 5-HT_{1A} antagonists were discovered in the process of characterizing their effects on other receptor subtypes. For instance, the DA antagonist spiperone was one of the first 5-HT_{1A} antagonists used (Pedigo *et al.* 1981; Alexander and Wood 1988; Blier *et al.* 1989). The β -adrenergic antagonists (-)propranolol, (-)penbutolol, (-)alprenolol, (-)tertatolol and (-)pindolol display relatively high affinity for the 5-HT_{1A} receptor (Alexander and Wood 1988; Sanchez *et al.* 1996) and show in various electrophysiological and biochemical assay antagonistic activities (Blier *et al.* 1989; Lum and Piercey 1988;

Göthert and Kollmeier 1986; Hjorth 1992), although in some conditions agonistic (partial) actions are also observed with some of these compounds (Haddjeri *et al.* 1998a; Newman-Tancredi *et al.* 1998; Clifford *et al.* 1998). Moreover, these compounds do not show adequate selectivity as some bind, in addition to β -adrenergic receptors, to other receptor subtypes such as the 5-HT_{1B} receptor (Hoyer *et al.* 1985a). Other antagonists for the 5-HT_{1A} receptor include BMY 7378, WAY 100135, NAN-190 and others, for which partial agonistic activities have been observed. WAY100635 has been shown to be a powerful antagonist of 5-HT_{1A} receptor-mediated functions (Fletcher *et al.* 1996; Corradetti *et al.* 1996; Hajós *et al.* 1995; Sanchez *et al.* 1996). This compound possesses high affinity for the 5-HT_{1A} receptor (Fletcher *et al.* 1996; Khawaja *et al.* 1995b; Sanchez *et al.* 1996), shows very good selectivity (displays only weak affinity for the α_1 -adrenergic receptors (Forster *et al.* 1995) and does not show agonistic activity using various experimental approaches (Newman-Tancredi *et al.* 1997; Alper and Nelson 1998; Corradetti *et al.* 1996), hence the term “silent” antagonist (Fletcher *et al.* 1996). These properties have rendered the use of the tritiated form of the compound of great use. One of the main features proper to the use of [³H]WAY 100635 instead of [³H]8-OH-DPAT, is the consistently higher labelling of sites (Gozlan *et al.* 1995; Khawaja 1995a). This results from the fact that antagonists, in contrast to agonists, label with equal affinity both coupled and uncoupled form of the receptor to G proteins (Gozlan *et al.* 1995). NAD-299 also shows very good selectivity and potency for the 5-HT_{1A} receptor (Johansson *et al.* 1997; Stenfors *et al.* 1998).

The first 5-HT_{1A} receptor was cloned in 1987 from a human genomic library (Kobilka *et al.* 1987) (identified as so only later by Raymond *et al.* (1989)), which was followed by the cloning of its rat homologue (Albert *et al.* 1990). In both cases, a β_2 -receptor sequence was used to screen the genomic library. Indeed, these studies have permitted to show that the 5-HT_{1A} and β_2 -receptor share a high sequence homology (*ca.* 45%). The rat and human 5-HT_{1A} receptors consist of a protein of 422 amino acids and contain a classical G-protein associated receptor structure of 7 transmembrane hydrophobic domains.

The regional distribution of the 5-HT_{1A} receptor was made possible by the availability of a tritiated form of what is still the prototypical 5-HT_{1A} receptor agonist, *i.e.*, [³H]8-OH-DPAT. Noteworthy, other tritiated 5-HT_{1A} ligands are available, such as ipsapirone, alnespirone, S 14506, and WAY 100635. The use of these ligands has permitted to show that the 5-HT_{1A} receptor binding is notably high in limbic regions such as the dentate gyrus, CA₁, CA₂, CA₃

fields (somewhat higher in CA₁) of the hippocampus, lateral septum, frontal and entorhinal cortex, whereas lower levels are expressed in the spinal cord, the amygdala and hypothalamic nuclei. Little or no labelling is observed in globus pallidus, substantia nigra and cerebellum. The highest labelling is found in the dorsal raphe with somewhat lower densities in other raphe nuclei (Verge *et al.* 1986; Marcinkiewicz *et al.* 1984; Pazos and Palacios 1985; Hensler *et al.* 1991; Verge *et al.* 1985; Li *et al.* 1997; Pompeiano *et al.* 1992). Relatively early studies by (Verge *et al.* 1986; Verge *et al.* 1985; Hensler *et al.* 1991; Weissmann-Nanopoulos *et al.* 1985) were able to further characterize the location of these receptors by showing that selective degeneration of serotonergic neurons produced by an intracerebral injection of 5, 7-DHT is associated with a significant loss of 5-HT_{1A} binding (as assessed with [³H]8-OH-DPAT) only in the raphe nuclei, thereby providing evidence for the somatodendritic location of these receptors on 5-HT neurons of the raphe by a 5, 7-DHT lesion. This assertion is further supported by the high correlation observed with mRNA coding the 5-HT_{1A} receptor and [³H]8-OH-DPAT labelling found in the raphe nuclei and also by the loss of both [³H]8-OH-DPAT labelling and 5-HT_{1A} hybridization signal in the dorsal raphe following a 5, 7-DHT lesion (Pompeiano *et al.* 1992). In terminal areas, essentially, the labelling is unaltered. Labelling of 5-HT_{1A} receptors is well correlated with the presence of mRNA for the receptor (determined by *in situ* hybridization) in all brain regions where the signal is detected (with the exception of the septum and dorsal horn of the spinal cord; Pompeiano *et al.* 1992; Albert *et al.* 1990), thus suggesting that these receptors are located somatodendritically in most regions. Interestingly, there is an apparent discrepancy in the hippocampus. Indeed, in the CA₁ field, whereas high labelling is found in the strata oriens, radiatum and lacunosum moleculare, no mRNA is found in those subregions. The mRNA coding for the 5-HT_{1A} receptor is primarily found in the pyramidal cell layer which shows lower receptor labelling. This finding is fully consistent with the dendritic arborisation found in the hippocampus and thus indicates that the 5-HT_{1A} receptors are mainly found in the dendrites of pyramidal neurons (Pompeiano *et al.* 1992).

The group of Hamon and coworkers have developed a specific antibody raised against a highly selective portion of the 5-HT_{1A} receptor protein (the third cytoplasmic loop) which allowed for detailed studies of the ultrastructural localisation of the 5-HT_{1A} receptor (Kia *et al.* 1996b; el Mestikawy *et al.* 1996; Riad *et al.* 1991; Kia *et al.* 1996a). Thus, immunohistochemistry at the electron microscope level show that the 5-HT_{1A} receptor is

located in the plasma membrane around the soma and dendrites of various neuronal types (Kia *et al.* 1996a; Kia *et al.* 1996b) including those found in dorsal and median raphe nuclei, septum and cerebral cortex. The dorsal raphe is the most heavily stained of the raphe groups. Interestingly, cells showing labelling in the median raphe are less abundant than in the dorsal raphe although the intensity in a given positive cell is stronger in the vicinity of the plasma membrane. In the dorsal raphe 5-HT_{1A}-like immunoreactivity is found both around cell bodies and on dendritic processes. In the hippocampus, 5-HT_{1A} receptors are only detected in the dendritic area while the cell bodies are immunonegative (Kia *et al.* 1996b). This is consistent with the observation that 5-HT_{1A} binding is especially high in the stratum oriens, stratum radiatum and stratum lacunosum molecular where the dendrites of pyramidal and granules cells are located (Verge *et al.* 1986; Pompeiano *et al.* 1992). It is also always interesting to acknowledge the presence of findings from functional studies that are not only congruent, but also supportive, of data obtained in anatomical studies. Indeed, in *in vitro* electrophysiological study in hippocampal slices, it was observed that the 5-HT-induced hyperpolarization (a 5-HT_{1A}-mediated event) elicited by microiontophoretic applications of 5-HT is more effective (in terms of hyperpolarization, in mv) as the microiontophoretic application is moved, in a somatodendritic axis, from the stratum pyramidale to the stratum radiatum (see figure 2).

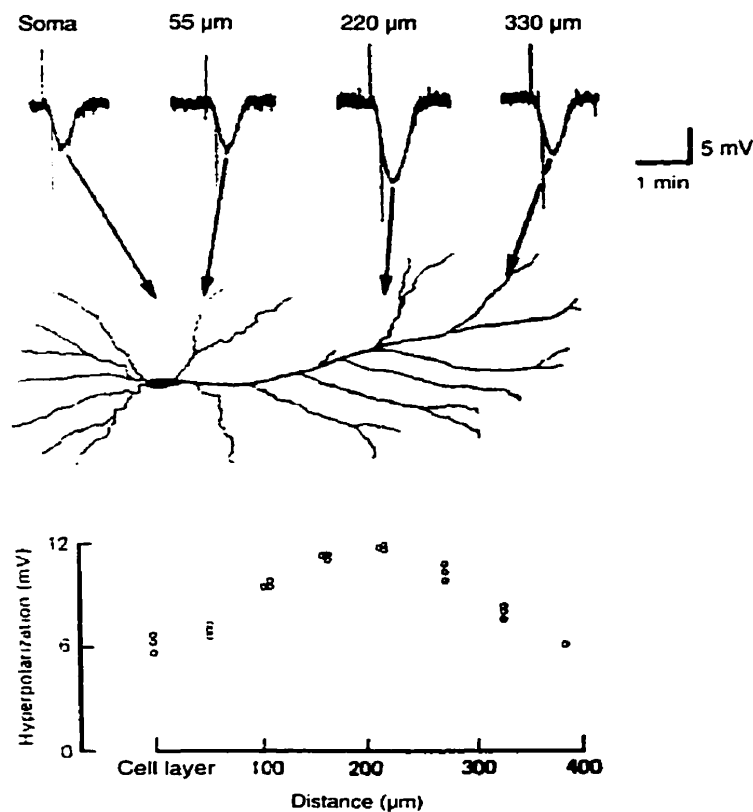


Figure 2: Distribution of hyperpolarizing responses. A, sample chart records of responses obtained from various sites on a pyramidal cell. An ionophoretic pipette was positioned immediately above the slice and 12s current pulses were applied to the pipette. B, graph of the responses partially illustrated for the cell in part A. Resting potential = -62 mV. Figure taken from Andrade and Nicoll, 1987b)

This general pattern of localization of 5-HT_{1A} immunoreactivity was however not replicated in the primate brain using another antibody (Azmitia *et al.* 1995). For instance, in the hippocampus, pyramidal cells showed a discrete localization of 5-HT_{1A} receptors of the initial axon segment, the axon hillock, with little staining of dendrites (Azmitia *et al.* 1995). In the raphe, however, labelling is consistent with that observed by (Kia *et al.* 1996b), *i.e.*, labelling of both soma and dendrites. The reasons for this discrepancy could involve the fact that different species were studied, but also by the use of different antibodies. The latter contention seems the more likely since, whereas the antibody in the study of Kia *et al.* (1996b) was raised against a portion of the third cytoplasmic loop, that of the study of Azmitia *et al.* (1995) was raised against the second extracellular loop. Moreover, the presence of a fundamental difference between the two antibodies is further shown by the fact that, whereas the former does not label glial cells (with small exceptions) the latter labels 5-HT_{1A} receptor on glial cells throughout the CNS of rats and monkeys (Whitaker-Azmitia *et al.* 1993; Azmitia

et al. 1995). Another unresolved issue regards the locus coeruleus. Indeed, Azmitia *et al.* (1995) report that many large neurons in the monkey's locus coeruleus stain for 5-HT_{1A} receptor immunoreactivity. However, it has been reported that the rat locus coeruleus is not noticeably enriched with mRNA coding for this receptor nor with 8-OH-DPAT labelling (Wright *et al.* 1995; Pompeiano *et al.* 1992). Future studies involving *in situ* hybridization in primate brain might perhaps help clarify this issue, although the selectivity of the antibody used in the reports of Whitaker-Azmitia *et al.* (1993) and Azmitia *et al.* (1995) appears to be questionable.

Numerous experiments have permitted to show that 5-HT_{1A} receptor activation leads to an inhibition of adenylate cyclase activity, when activity is first stimulated with forskolin (Pauwels *et al.* 1993; Zgombick *et al.* 1989; Devivo and Maayani 1985; De Vivo and Maayani 1986; De Vivo and Maayani 1990; Schoeffter and Hoyer 1988). The attenuation of this effect, at least in the hippocampus, by pertussis toxin demonstrates the involvement of a G_i protein (Harrington *et al.* 1988; Clarke *et al.* 1987). Moreover and as covered elsewhere, electrophysiological studies have shown that the electrophysiological effects mediated by the 5-HT_{1A} receptor is linked to a Gi protein in the hippocampus (Andrade *et al.* 1986), in the raphe (Penington *et al.* 1993b), as well as in the ventromedial hypothalamus (Newberry and Priestley 1988; Newberry 1992) although cAMP production does not seem to be involved in these electrophysiological effects (see section 3.1 and 4.1). It needs also to be added that 5-HT_{1A} receptor activation is also known to directly stimulate the activity of adenylate cyclase (Markstein *et al.* 1986; Markstein *et al.* 1999).

The cloning of the 5-HT_{1A} receptor has allowed careful analysis its coupling mechanism in various expression system such as COS-7, HeLa, CHO, GH₄C₁ cells as well as in neuronal cell lines. Some of these experiments confirmed the negative effect on adenylate cyclase by 5-HT_{1A} receptors (Albert *et al.* 1990; Fargin *et al.* 1989). The details of the results obtained with these different assay systems is beyond the scope of the present review. Indeed, different conditions obtained using different assay introduces variations in the operational and pharmacological properties of the receptor. Nonetheless, it can be mentioned that in addition to the well-described inhibition of adenylate cyclase, stimulation of the latter enzymatic system has also been observed. Moreover, stimulation of PI hydrolysis and elevation of Ca²⁺ accumulation has been observed in these systems (Hoyer and Martin 1996).

2.3. The 5-HT_{1B/1D} receptor

Over recent years, there have been a profound reorganization and rethinking of the framework used for the classification of 5-HT receptors. This was rendered necessary because of the approaching day where all human receptors will have been cloned. Therefore, there is a need for an alignment of nomenclature with the human genome product in the process of the ultimate attempt to provide a unitary scheme for receptor classification. As radioligand binding techniques were invaluable in providing clear evidence for the existence and characterization of various receptor classes (e.g., 5-HT₁, 5-HT₂, 5-HT₃) and sub-types (5-HT_{1A}, 5-HT_{1B/D}, 5-HT_{1E}, 5-HT_{1F}), a lack of reliability soon emerged when traditional pharmacological approaches were applied to receptor sub-type characterization (all the more so in the content of species variants). This outcome is perhaps best illustrated for the classification of the 5-HT_{1B/D} receptor subtypes which has been the subject of debate in terms of classification for which various reorganisations have been proposed. Only the current status of the most recent nomenclature proposed by Hoyer *et al.* (1994) and Hartig *et al.* (1996) with regard to the 5-HT_{1B/D} receptors will be presented herein. For a review on the principles used for the newer classification scheme, as well as for the different contentions from which have stemmed the confusion around the classification of these receptors see Hoyer and Martin (1997) and Hartig *et al.* (1996). It needs however to be emphasized that the determining principle governing the elaboration of the classification scheme lies in the fact that preeminence has been given to the human gene product so that the classification lies *a priori* in the identification of the human receptor.

In order to have an adequate appraisal of the classification used, some general contentions must first be clearly stated. First, both 5-HT_{1B} and 5-HT_{1D} are found in rodents and humans. The former 5-HT_{1D α} gene product refers now to the h 5-HT_{1D} receptor whereas the 5-HT_{1D β} gene product refers now to the h5-HT_{1B} receptor. In rats, both r5-HT_{1B} and r5-HT_{1D} are found. As was first suggested by (Massingham *et al.* 1979), it is now well recognized that the r5-HT_{1B} and h5-HT_{1B} are species homologue. In light of the working scheme adopted, it must be clear in mind that even though two receptors share the same name, they do not necessarily share the same pharmacology.

2.3.1 The 5-HT_{1B} receptor

The r5-HT_{1B} receptor (a single protein chain of 386 amino acids) shares *ca.* 92%

homology (96% in the transmembrane domain) with the human, guinea pig, bovine and pig 5-HT_{1B} receptors (former 5-HT_{1DB} receptors), which is 390 amino acids long (Voigt *et al.* 1991; Hamblin *et al.* 1992; Weinshank *et al.* 1992). Despite this high homology, these receptors present fundamental and important pharmacological differences (Hamblin *et al.* 1992; Weinshank *et al.* 1992; Voigt *et al.* 1991; Adham *et al.* 1992). First, the discriminatory affinities of some β -adrenergic receptor antagonist for these receptor subtypes will be considered. Indeed, whereas drugs such as (-)propranolol, (-)pindolol and cyanopindolol display high affinities for the r5-HT_{1B} receptor, those for the non rodent and guinea pig 5-HT_{1B} receptor are clearly less (Waeber *et al.* 1988; Waeber *et al.* 1989). Moreover, drugs such as CP 93, 129 and (-)SDZ 21009 also show greater affinities for the r5-HT_{1B} receptors than for non-rodent and guinea pig 5-HT_{1B} receptors. In addition, the 5-HT_{1A} agonist 8-OH-DPAT, although exhibiting very low affinity for the r5-HT_{1B} receptor, displays moderate affinity for the non-rodent and guinea pig 5-HT_{1B} receptor. Accordingly, in functional models, 8-OH-DPAT acts as a weak agonist on the terminal 5-HT_{1D} receptor of the guinea-pig without showing significant activity at the terminal receptor in the rat (Starke *et al.* 1989). It is also worth mentioning that other compounds such as rauwolscine, yohimbine and sumatriptan have also been used to discriminate the two receptor subtypes, these compounds exhibiting higher affinities for the non-rodent and guinea pig 5-HT_{1B} receptors (Göthert *et al.* 1995). It is striking to realize that a remarkably subtle difference in amino acid sequence (to a single amino acid difference in this case (Parker *et al.* 1993; Oksenberg *et al.* 1992)) accounts for these pharmacological differences. Altogether, these contentions reveal that, since the guinea-pig 5-HT_{1B} receptor's pharmacology resembles more the h5-HT_{1B} than does the r5-HT_{1B}, the guinea pig is considered best to study the function of this receptor in a transposition context from animal data to humans.

Under the appropriate masking conditions, tritiated iodocyanopindolol, as well as iodo-GTI has been used to label 5-HT_{1B} sites in the rat. Because of the complex pharmacology of the 5-HT_{1B/D} receptors, the specific labelling of 5-HT_{1B} in higher species has been more difficult to achieve. Thus, in the rat, 5-HT_{1B} binding sites are found in high concentration in the striatum, the substantia nigra and in the dorsal subiculum. Interestingly, the dorsal raphe nucleus displays low 5-HT_{1B} labelling, despite showing a high level of mRNA coding for this receptor (Doucet *et al.* 1995; Bruinvels *et al.* 1994; Bruinvels *et al.* 1993; Hamblin *et al.* 1992). This is consistent with functional studies that have failed to demonstrate any effect for

the 5-HT_{1B} receptor in the dorsal raphe of rat (Piñeyro *et al.* 1995b; Sprouse and Aghajanian 1987). The combination of *in situ* hybridization and autoradiography have shown similar mismatches in other brain regions as well, such as the hippocampus (CA₁) and substantia nigra and deep nuclei of the cerebellum (Bruinvels *et al.* 1994; Doucet *et al.* 1995). This has been interpreted as being indicative that 5-HT_{1B} are predominantly found on axon terminals in various brain regions and thus serve as heteroreceptors, in addition to autoreceptors. More recently, the development of an antibody has permitted to further characterize the cellular location of the 5-HT_{1B} receptor. Preliminary studies report that the receptor is indeed located on axons, although in some regions it is found preferentially in pre-terminal axons than in terminal axons (Sari *et al.* 1997; Riad *et al.* 1996). The 5-HT_{1B} receptors have been shown in various models to inhibit the accumulation of forskolin stimulated cAMP (Hamblin *et al.* 1992; Zgombick *et al.* 1993; Hoyer *et al.* 1990; Weinshank *et al.* 1992; Schoeffter and Hoyer 1989). In addition to this, it has also been shown that the 5-HT_{1B} receptor activation can elevate intracellular Ca²⁺ and stimulate phospholipase C (Zgombick *et al.* 1993).

2.3.2 The 5-HT_{1D} receptor.

The 5-HT_{1D} receptor (former 5-HT_{1D α} receptor) shares a modest transmembrane homology of 74% (overall homology of *ca.* 62 %) with the 5-HT_{1B} (former 5-HT_{1D β} receptor; Hamblin *et al.* 1992; Weinshank *et al.* 1992). Although the 5-HT_{1D} receptor was initially believed not to be found in the rat, its presence in this specie has been convincingly demonstrated (Hamblin *et al.* 1992; Bruinvels *et al.* 1993). The rat 5-HT_{1D} receptor is a 374 amino acid long polypeptide which is 90% (95% of transmembrane homology) identical to its human analogue (Weinshank *et al.* 1992; Hamblin *et al.* 1992). Despite the overt difference in their sequence, the 5-HT_{1B} and 5-HT_{1D} receptors share a pharmacology that is remarkably similar (Hamblin *et al.* 1992). Indeed, these receptors were initially thought to be pharmacologically indistinguishable. However, it is now known that drugs such as ritanserin and ketanserin can be used to differentiate the 5-HT_{1B} from the 5-HT_{1D} receptors. For instance, ketanserin is used in functional models for this purposes as it displays a 75-fold greater affinity for the latter compared to the former receptor (Zgombick *et al.* 1995).

In the rat, the distribution of the 5-HT_{1D} receptor is roughly comparable to that of the 5-HT_{1B} receptor although its expression is consistently lower: in many regions it is only slightly above detection limits (Bruinvels *et al.* 1993). Moreover, no regions are found to express

solely 5-HT_{1D} receptors. Similar results are also observed in human brain (Castro *et al.* 1997). The levels of 5-HT_{1D} mRNA in rats are also low and detected in only a few structures of the brain. The highest levels of this mRNA species are found in the dorsal raphe, locus coeruleus, striatum, red nucleus and periaqueductal grey (Bruinvels *et al.* 1994). In the dorsal raphe, there is no mismatch between 5-HT_{1D} receptor binding and mRNA levels (Hamblin *et al.* 1992; Bruinvels *et al.* 1993; in contrast to what is observed for the 5-HT_{1B} receptor), thus supporting its role as a somatodendritic autoreceptor in the rat. This is consistent with what was demonstrated functionally by (Piñeyro *et al.* 1995b). Finally, the 5-HT_{1D} receptors were found in various models to inhibit the accumulation of forskolin stimulated cAMP via a pertussis toxin sensitive G protein (Hamblin *et al.* 1992; Hamblin and Metcalf 1991; Hoyer *et al.* 1990; Weinshank *et al.* 1992; Schoeffter and Hoyer 1989). In addition to this, it has also been shown that the 5-HT_{1D} receptor activation can elevate intracellular Ca²⁺ and stimulate phospholipase C (Zgombick *et al.* 1993).

3. ON FUNCTIONAL ASPECTS OF THE 5-HT NEURONS

3.1. Electrophysiological properties of raphe 5-HT neurons

3.1.1 Basic characteristics

In the anaesthetized rats, neurons of the dorsal raphe exhibit a spontaneous, slow (1 to 5 Hz), regular discharge pattern displaying biphasic or triphasic action potentials of 1.5-3.0 ms duration (Aghajanian 1982b; Aghajanian and VanderMaelen 1982; VanderMaelen and Aghajanian 1983). Similar patterns of firing are also observed for 5-HT neurons of the median raphe, although the mean firing activity is significantly lower in these neurons (Hajós *et al.* 1995). Recorded intracellularly, these neurons display action potentials of about 1.8 ms duration, which are followed by large (10-20 mV) after (or postspike) hyperpolarizations which normally last 200-800 ms. Those hyperpolarizations are followed by interspikes depolarisation that leads to the following spike. The spontaneous activity of these cells stems from the succession of this cycle of interspikes hyperpolarizations and depolarization which has been termed “pacemaker potentials” (Aghajanian and VanderMaelen 1982). Experiments in acutely dissociated dorsal raphe nucleus neurons (Penington and Kelly 1990; Penington *et al.* 1991) have permitted to show that the fast component of the action potential is TTX sensitive. In addition, these experiments have permitted to unravel the presence of a low and a high threshold Ca²⁺ component of the action potential. The T-type Ca²⁺ channel seems to be

involved in initiating the low threshold Ca^{2+} current whereas N- and L-type (and perhaps a third one) would be responsible for the high-threshold current. The afterhyperpolarization is mediated by a Ca^{2+} -dependent K^{+} current (Aghajanian 1985). As Ca^{2+} is sequestered and extruded, the K^{+} current diminishes and the membrane depolarizes until it reaches the threshold for the low-threshold Ca^{2+} channel and another action potential is triggered.

3.1.2 Noradrenergic input to the raphe

One of the prominent features of dorsal raphe 5-HT neurons firing activity is its dependence on a noradrenergic input. As such, a tonic facilitatory role for NE on 5-HT neurons firing activity has been suggested based on the observation that microiontophoretic and systemic administration of α_1 -adrenergic antagonists suppress the firing activity of dorsal raphe 5-HT neurons (Hertel *et al.* 1997; Baraban and Aghajanian 1900; Baraban and Aghajanian 1980). The requirement of an intact noradrenergic system is further outlined by a marked suppression of firing activity of 5-HT neurons following a pretreatment with the noradrenergic selective toxin 6-OHDA. Interestingly, several days after the lesioning, there is a recovery of firing activity by the 5-HT neurons which results from an “unknown mechanism . . .” as stated by (Baraban and Aghajanian 1980). Indirect evidence supporting the presence of this tonic noradrenergic-mediated facilitatory activity is given by the use of raphe brain slices. Indeed, in the slice preparation, otherwise mostly silent cells are excited by NE or by the α_1 -adrenergic agonist phenylephrine, which effect is reduced or blocked by α_1 -adrenergic receptor antagonists such as prazosin (VanderMaelen and Aghajanian 1983). Moreover, the addition of amphetamine depolarizes 5-HT cells which effect is blocked by prazosin, thus further indicating that the release of endogenous NE increases the excitability of 5-HT neurons (Pan and Williams 1989a). Thus, the predominant presence of silent neurons in the raphe slice preparation is most probably due to a disfacilitation of firing activity of 5-HT neurons as a consequence of the removal of the noradrenergic input.

The actions of NE on the excitability of 5-HT neurons are complex. It is now well established that the excitation of 5-HT neurons involves the suppression of at least two types of K^{+} currents. First, Aghajanian (Aghajanian 1985) has demonstrated the expression of the I_A current in 5-HT neurons of the dorsal raphe. The I_A current is an early transient K^{+} current (which is activated by depolarizing voltage steps from potentials more negative than rest and blocked by 4-aminopyridine). In dorsal raphe 5-HT neurons, this early transient outward

current is suppressed by NE and the α_1 -adrenergic agonist phenylephrine (Aghajanian 1985). This suppression of I_A may account, in part, for the acceleration of pacemaker activity of 5-HT neurons induced by α_1 -adrenergic agonists (Aghajanian 1985). Moreover, it was shown that the α_1 -adrenergic-mediated depolarization is accompanied by a decrease in a resting K^+ conductance (Aghajanian 1985; Pan *et al.* 1994), which effect, most probably, also contributes to the acceleration of firing activity of 5-HT neurons. Paradoxically, it was also shown that activation the α_1 -adrenergic receptors induce an increase of the late afterhyperpolarization (AHP) (Freedman and Aghajanian 1987; Pan *et al.* 1994) which effect would offset the general excitatory effect of NE on the excitability of 5-HT neurons. These studies thus unravel the presence of a complex pleiotropic action of α_1 -adrenergic activation of 5-HT neurons. The signal transduction mechanisms underlying the expression of these currents are still ill-defined. It appears that intracellular Ca^{2+} is not required for the NE-induced depolarization although there is a requirement for external Ca^{2+} as the depolarizing effect of NE are blocked by various Ca^{2+} channels blockers (Pan *et al.* 1994). As for the effect of NE on the late-AHP, it is believed that it is mediated by the activation of the phospholipase C pathway that, in turn, leads to the release of internal stores of Ca^{2+} from an IP_3 -sensitive pool (Pan *et al.* 1994).

3.2. Release of 5-HT in the raphe

The use of the *in vitro* slice preparation, *in vivo* push-pull cannula and finally of *in vivo* microdialysis has been extensively employed to assess 5-HT release in various regions of the brain, including in the raphe. The two latter techniques offer the advantage of being carried out in the whole brain of either awake or anaesthetized animals in such ways that the complete circuitry and interconnectivity of the different structures are preserved to a much greater extent than that is achieved in the *in vitro* slice preparation.

Electrical stimulation, perfusion with a high concentration of K^+ in *in vitro* mesencephalic slices containing the raphe and high K^+ perfusion *in vivo* in the raphe, all enhance the release of 5-HT (Hery *et al.* 1982; Piñeyro and Blier 1996; El Mansari and Blier 1996; Starkey and Skingle 1994; Adell *et al.* 1993; Bosker *et al.* 1994; Bagdy and Harsing, Jr. 1995). The electrically-evoked release of [^3H]5-HT from mesencephalic slices as well as that induced by high K^+ stimulation, is Ca^{2+} -dependent (Bagdy and Harsing, Jr. 1995; Piñeyro *et al.* 1995b; Hery *et al.* 1986; Starkey and Skingle 1994; O'Connor and Kruk 1991), indicative the 5-HT release in the raphe follows a classical vesicular fusion-mediated release. Moreover, blockade

of the N-, and not the L-type of Ca^{2+} channels reduces the electrically evoked release of 5-HT from raphe slices (Bagdy and Harsing, Jr. 1995). Consistent with the notion of vesicular-mediated neurotransmitter release, 5-HT release in raphe was shown to be diminished by reserpine (Adell and Artigas 1998) although this was shown not to be the case by a previous study by the same group (Adell *et al.* 1993). In addition, the electrically-evoked release of [^3H]5-HT (Starkey and Skingle 1994; O'Connor and Kruk 1991), but not that induced by high K^+ stimulation (Piñeyro and Blier 1996; El Mansari and Blier 1996) has consistently been shown to be reduced by perfusion with TTX. But why does TTX prevent the electrically-induced 5-HT release and not high K^+ induced 5-HT release? The most likely reason for this relies in the intrinsic difference in transmitter release induced by the two methods (high K^+ vs electrical stimulation). There is evidence that the electrically-induced release of 5-HT stems from a synaptically-driven mechanism. Indeed, it was shown that the electrical stimulation of mesencephalic slices evokes multiple synaptic potentials, one of which being an EAA-mediated depolarization (Pan and Williams 1989b). Further studies by the same group have further identified a 5-HT mediated IPSP evoked by electrical stimulation of those slices (Pan *et al.* 1989). Most interestingly to the present discussion, is the fact that this 5-HT-mediated IPSP was, at least partly, dependent of the excitatory amino acid-mediated depolarization (Pan *et al.* 1989). These results were interpreted as indicating that the IPSP recorded in dorsal raphe neurons was caused by 5-HT released at least in part from indirect (synaptically induced) excitation of 5-HT-containing cells within the slice. Hence, these results provide a likely mechanism for the fact that the electrically-evoked release of 5-HT in raphe slices is TTX sensitive. For the case of high K^+ -induced release of 5-HT from mesencephalic slices, it is well possible that the resultant depolarization directly activates voltage-dependent Ca^{2+} channels (presumably the N-type) and hence triggers 5-HT release and this, in a TTX-independent manner. There is a fundamental and imperative need, however, to assess the 5-HT release in the raphe in physiological conditions. To this end, the use of *in vivo* microdialysis or push-pull cannula has proven to be most valuable. Most of the studies concurred in showing that the basal release of 5-HT in the raphe nuclei is TTX sensitive (Hery *et al.* 1982; Bosker *et al.* 1994; Adell and Artigas 1998; Matos *et al.* 1996) although this has not universally been shown (Adell *et al.* 1993). Three considerations must be put forward when considering these data. First, electrophysiological work by (Penington *et al.* 1991) has shown, in current clamp mode, that there is an important TTX sensitive component of the

action potential recorded from 5-HT neurons, whereas the other components is mediated by Ca^{2+} channels. This contention by itself, can explain the presence of TTX-sensitive 5-HT release. Secondly, there is a need to consider the various excitatory inputs to the raphe and especially the tonic noradrenergic input to 5-HT neurons. Indeed, TTX would functionally isolate 5-HT neurons from those excitatory inputs and hence reduce 5-HT release. Finally, it is possible that part of the 5-HT release in the raphe is of axonal origin hence explaining the reduction of 5-HT release by TTX.

There is evidence that there could exist a basal release of 5-HT that would not involve a classical vesicular release of 5-HT in the raphe. Indeed, in mesencephalic slices, there is a spontaneous release of 5-HT that is TTX insensitive as well as Ca^{2+} independent (Piñeyro and Blier 1996; Piñeyro *et al.* 1995b). These studies were however carried out in mesencephalic slices and thus questions the occurrence of this basal release in physiological conditions. Taken together, these results indicate that, in the raphe, there exist a classical vesicular induced Ca^{2+} dependent release of 5-HT and that, in physiological conditions, is likely to be the most conspicuous one.

3.3. Function of somatodendritic 5-HT autoreceptors

3.3.1. Electrophysiological aspects

i) Basic characteristics and pharmacology

Serotonergic cell bodies in the raphe nuclei contain a high density of 5-HT_{1A} receptors (Verge *et al.* 1985; Weissmann-Nanopoulos *et al.* 1985; Hensler *et al.* 1991; Pazos and Palacios 1985). Even before the advent of autoradiography, the presence of the so-called somatodendritic autoreceptor had been postulated by Aghajanian on the basis of electrophysiological studies (Aghajanian *et al.* 1972; Aghajanian *et al.* 1968; Aghajanian 1978). Indeed, systemic administration of the hallucinogen LSD¹ was found to have a potent suppressant effect on spontaneously active 5-HT neurons (Aghajanian *et al.* 1968). The location of this inhibitory element on the somatodendritic region was later unravelled by the observation that the microiontophoretic applications of LSD as well as of 5-HT onto 5-HT

¹LSD: Lysergic diethylamide acid. LSD actually refers to “*Lyserg Säure Diäthylamid*”, which compound was the 25th compound (hence LSD₂₅) out of a series of 27 alkaloid synthesized in 1938 in Switzerland by Sandoz. Its psychotropic properties were discovered accidentally in 1943 by one of the researcher.

neurons of the dorsal raphe suppress their firing activity (Aghajanian *et al.* 1972). The term somatodendritic 5-HT autoreceptor was then proposed. Interestingly, although some indolamine hallucinogens share with LSD the capacity to suppress the firing activity of 5-HT neurons, mescaline and other substituted phenethylamine hallucinogens when intravenously administered or directly applied by microiontophoresis on dorsal raphe 5-HT neurons do not (Haigler and Aghajanian 1973; Penington and Reiffenstein 1986). On the one hand, these results provided strong evidence that suppressing firing activity of 5-HT neurons is not a common mechanism for hallucinogens (this is all the more so important that LSD and mescaline displays similar clinical effects and do show cross-tolerance in humans). On the other hand, these findings prompted the search for a mutually exclusive target that would mediate the inhibitory effect of LSD. This aspect was cleared years later, as it was shown that LSD, and not mescaline, displays high affinity for the 5-HT_{1A} receptor (Hoyer *et al.* 1994). Finally, the fact that other 5-HT_{1A} agonists that share the ability to suppress firing activity of these neurons and that also have also anxiolytic and antidepressive (and not hallucinogenic) properties, precluded the involvement of the latter receptor in the behavioral expression of hallucinogens.

There is now little doubt that the somatodendritic 5-HT autoreceptor controlling firing activity of 5-HT neurons is of the 5-HT_{1A} subtype. This certainty arises from an impressive corpus of both *in vitro* and *in vivo* electrophysiological studies that show that the systemic and local administration of a variety of compounds that share an affinity for 5-HT_{1A} receptors (LSD, 8-OH-DPAT, buspirone, flesinoxan, ipsapirone, tandospirone) suppress the spontaneous firing activity of 5-HT neurons of the dorsal raphe nucleus which effect is reversed or prevented by 5-HT_{1A} antagonists such as BMY 7378, spiperone or WAY 100635 (for example, (VanderMaelen *et al.* 1986; Morot-Gaudry *et al.* 1981; Hajós *et al.* 1995; Sinton and Fallon 1988; Hodgkiss *et al.* 1992). Moreover, the physiological importance of these receptors is further demonstrated by the observations that the administration of various drugs that possess either 5-HT releasing or 5-HT reuptake inhibiting properties such a fenfluramine, cocaine, non-selective 5-HT reuptake inhibitors, selective reuptake inhibitors such as indalpine, citalopram, zimelidine and paroxetine, as well as MAOI's suppresses the firing activity of 5-HT neurons which effect was shown in some studies to be reversed or prevented by various 5-HT_{1A} antagonists (see for example de Montigny *et al.* 1990; Blier *et al.* 1984; Scuvée-Moreau and Dresse 1979; Chaput *et al.* 1986b; de Montigny *et al.* 1981; Kasamo *et al.* 1996;

Sheard *et al.* 1972; Hajós *et al.* 1995; Aghajanian *et al.* 1970; Sharp *et al.* 1997; Quinaux *et al.* 1982; Scuvée-Moreau and Dresse 1990; Cunningham and Lakoski 1990; Cunningham and Lakoski 1988; Pitts and Marwah 1986.

ii) Ionic basis

The use of conventional sharp microelectrode recording on mesencephalic slices preparation (containing the dorsal raphe) has offered the means to investigate the ionic basis for the inhibitory effect of 5-HT_{1A} receptors on the firing activity of 5-HT neurons. Initial studies have shown that when the neuronal firing of a 5-HT neuron is inhibited by LSD, the pacemaker potentials (see above) no longer occur and the cell remains in a hyperpolarized state (Aghajanian and VanderMaelen 1982). It has also been demonstrated that it is most likely the opening of a K⁺ channel, by inducing a hyperpolarization (accompanied by a decrease in input resistance), that subserves the inhibitory role of 5-HT_{1A} receptors on the firing activity of dorsal raphe 5-HT neurons (Aghajanian and Lakoski 1984; Hodgkiss *et al.* 1992; Williams *et al.* 1988). Studies by (Williams *et al.* 1988) have also shown that the 5-HT-induced current displays inwardly rectifying properties. Moreover work by (Penington *et al.* 1993b), confirmed and extended these findings in acutely isolated dorsal raphe 5-HT neurons: 5-HT evokes a small outward current with a reversal potential close that predicted for a K⁺ current. Moreover, this current showed inward rectification in accordance with the finding of Williams *et al.* (1988). The 5-HT_{1A} nature of the response is suggested by the fact that bath application of 5-HT_{1A} agonists such as ipsapirone or 8-OH-DPAT mimics the action of 5-HT (Hodgkiss *et al.* 1992; Davidson and Stamford 1995). In addition to the opening of a K⁺ channel, whole cell recordings from acutely dissociated raphe neurons show that 5-HT decreases the high threshold Ca²⁺ current (described in section 3.1), probably via 5-HT_{1A} receptor activation since this effect is also mimicked by 8-OH-DPAT (Penington and Kelly 1990) and by LSD (Penington and Fox 1994). This Ca²⁺ current is virtually insensitive to L-type Ca²⁺ channel blocker but is partially sensitive to ω -conotoxin, a N-type channel blocker (Penington *et al.* 1991).

It is noteworthy that high concentrations of 5-HT (up to 100 μ M) are routinely used in raphe slices. Many considerations may be put forward in an attempt to explain this observation. For instance, the slow perfusion rate in the chambers in combination with the very high density of 5-HT transporters in the raphe most probably greatly hampers the

bioavailability of 5-HT, hence the need for concentration much greater than one might expect for an action on the 5-HT_{1A} receptor. In support of this assertion is the observation made by the group of Kelly in Edimburg that under whole-cell patch-clamp configuration made on acutely isolated 5-HT neurons from the dorsal raphe, much lower concentrations of 5-HT are needed (10 vs 100 μ M) than that used by the same group in 400 μ m thick raphe slices (Hodgkiss *et al.* 1992; Hodgkiss *et al.* 1992; Penington *et al.* 1993a).

iii) Signal transduction pathway

There is ample evidence demonstrating that the 5-HT_{1A}-mediated inhibitory response in the dorsal raphe is mediated through a G protein. Indeed, pretreatment with pertussis toxin (intracerebroventricularly or *in situ* 48 hours prior or *in vitro* for 13 hours) causes an almost total prevention of the inhibitory and hyperpolarizing effects of 5-HT (Innis *et al.* 1988; Williams *et al.* 1988; Penington *et al.* 1993b). Consistent with this, the suppressant effect of the 5-HT_{1A} receptor agonists 8-OH-DPAT, ipsapirone and LSD on the firing activity of dorsal raphe 5-HT neurons is prevented by pertussis toxin (Innis *et al.* 1988). Moreover, irreversible activation of G protein by intracellular injection of the non-hydrolyzable GTP analog GTP γ S mimics, and is non-additive with, the hyperpolarizing action of 5-HT (Innis *et al.* 1988). It has also been observed that the addition of cAMP in the patch pipette or of an analog in the perfusion medium does not alter the response to 5-HT (Penington *et al.* 1993b) suggesting that the effect of 5-HT on K⁺ channels does not involve cAMP (nor any diffusible second messenger) but rather a direct coupling of the G-protein with the channel. This is consistent with the lack of 5-HT_{1A} inhibition of adenylate cyclase activity in the dorsal raphe (Clarke *et al.* 1996). Note also that similar observations for 5-HT_{1A} receptor-mediated effect on cellular excitability have been made in other regions such as the dorsal root ganglia (Crain *et al.* 1987), ventromedial hypothalamus (Newberry and Priestley 1988) and also in the hippocampus (Andrade *et al.* 1986; see section 4.1.2).

3.3.2. 5-HT autoreceptors and somatodendritic release

i) Somatodendritic 5-HT_{1A} autoreceptors.

In *In vivo* microdialysis experiments in the raphe, local infusion of low concentrations (1 and 10 μ M) of 8-OH-DPAT in the raphe does not change basal output of 5-HT (Adell *et al.* 1993), whereas higher concentrations (100 μ M to 10 mM) increase the extracellular

concentration of 5-HT in the raphe, perhaps by to a reported reuptake blockade of 5-HT by the compound (Assié and Koek 1996). Given that 8-OH-DPAT is known to inhibit firing activity of 5-HT neurons both *in vivo* and *in vitro*, these results support the idea that basal output of 5-HT in the raphe is impulse flow independent. Nonetheless, in mesencephalic brain slices, 8-OH-DPAT concentration-dependently inhibits the electrically-evoked release of tritiated 5-HT without however affecting the spontaneous release (Bagdy and Harsing, Jr. 1995; Piñeyro *et al.* 1995b). This effect was further shown to be 5-HT_{1A}-mediated since it is antagonized by the 5-HT_{1A} antagonist WAY 100135 (Bagdy and Harsing, Jr. 1995). Moreover, 8-OH-DPAT was shown not to alter the K⁺-induced release of [³H]5-HT (Middlemiss 1987). The inhibitory effect of 8-OH-DPAT on the electrically-evoked release of [³H]5-HT from raphe slices is blocked by the voltage-sensitive K⁺ channel blocker 4-AP (Bagdy and Harsing, Jr. 1995) as was that on the firing activity of raphe 5-HT neurons *in vitro* (Haj-Dahmane *et al.* 1991).

ii) Somatodendritic 5-HT_{1D/B} autoreceptors

The presence of 5-HT_{1D} receptors has been demonstrated in the dorsal raphe of the rat by an autoradiographic study (Bruinvels *et al.* 1993). Pharmacological studies undertaken in the rat *in vivo* as well as *in vitro* in the rat (Piñeyro *et al.* 1995b), mouse (Piñeyro *et al.* 1995a) and guinea-pig (Starkey and Skingle 1994), have demonstrated that this receptor subtype inhibits the electrically-evoked release of [³H]5-HT from mesencephalic slices. Whereas (Piñeyro *et al.* 1995b) have shown that the 5-HT_{1B} receptor agonist CP 93, 129 does not influence the electrically-evoked release of 5-HT in raphe slices, Bagdy and Harsing, Jr. (1995) observed that the 5-HT_{1B} receptor agonist CGS-12066B does. Nonetheless, it must be underscored that the latter compound was reported to poorly discriminate between 5-HT_{1B} and 5-HT_{1A} receptors.

The unraveling functional 5-HT_{1A} as well as 5-HT_{1D} receptors modulating raphe release of 5-HT prompted the study of their mutual interactions. In brief, the hypothesis states that, in the presence of a tonic activation of the somatodendritic 5-HT_{1D} autoreceptor, the presence of an antagonist would elevate the extracellular concentration of 5-HT in the raphe and thereby activate the somatodendritic 5-HT_{1A} receptor leading to a decrease in firing activity and hence of terminal 5-HT release. This hypothesis has been verified experimentally. The systemic administration of GR 127935 (0.3 mg/kg i.p.), produces a significant decrease in extracellular 5-HT in frontal cortex and striatum of the guinea pig (Roberts *et al.* 1997; Roberts *et al.* 1998). Interestingly, it was reported in the dentate gyrus (which is almost exclusively a median raphe

innervated area), GR 127935 significantly increases extracellular 5-HT (Roberts *et al.* 1998). The fact that the 5-HT_{1B} antagonist SB-224289 (which displays about a 60-fold selectivity for the 5-HT_{1B} over the 5-HT_{1D} receptor) does not alter the extracellular concentration of 5-HT in frontal cortex and in the striatum (Roberts *et al.* 1998) indicates the effect observed with the GR compound was indeed mediated by the 5-HT_{1D} receptor. However, in the dentate gyrus, SB-224289, like GR 12793, increases extracellular level of 5-HT. Thus, it was hypothesized that the 5-HT_{1D} receptor would contribute to regulation of 5-HT neurotransmission only in neurons originating in the dorsal, and not in median raphe (Roberts *et al.* 1998). Within this context, the enhancement of extracellular 5-HT in the dentate gyrus by GR 127935, would be consistent with the presence of a local tonic activation of the terminal 5-HT_{1D} autoreceptor of the guinea pig.

3.4. Release of 5-HT in terminal regions

3.4.1. Basic characteristics

There are several pieces of evidence from the *in vitro* slice or synaptosomes preparation suggesting that the release of 5-HT in terminal area involves an exocytotic mechanism (Starke *et al.* 1989). This contention was verified more recently with the use of *in vivo* microdialysis studies that have shown that the release of 5-HT is reduced by *ca.* 70% by omitting Ca⁺⁺ from the perfusion fluid in various terminal regions such as the hypothalamus (Auerbach *et al.* 1989), striatum (Kalen *et al.* 1988), hippocampus (Adell *et al.* 1993) and frontal cortex (Carboni and Di Chiara 1989). However, increasing the extracellular concentration of Ca⁺ actually increases the release of 5-HT. That the release is of neuronal origin and involves the propagation of action potentials is further shown by the reduced 5-HT output in regions such as the striatum (Kalen *et al.* 1988) and hypothalamus (Auerbach *et al.* 1989) following perfusion with TTX. It is very important, however, to note that TTX does not completely inhibit the release of 5-HT (as well as of NE), indicating that there is small amount (*ca.* 20%) of action potential-insensitive release of 5-HT (Westerink, *et al.*). Although one might argue that this residual release of 5-HT might be the result of incomplete blockade of Na⁺ channels by TTX consequent to an insufficient diffusion of the drug, TTX was shown to totally abolish the release of ACh and DA. There are several mechanisms on the 5-HT neurons that subserves an homeostatic role on 5-HT function. In this section, 3 of these mechanisms will be

discussed: 1) the somatodendritic 5-HT_{1A} receptors, 2) the terminal 5-HT_{1BD} receptors and 3) the terminal α_2 -adrenergic heteroreceptors.

3.4.2. Somatodendritic 5-HT autoreceptor and terminal release

i) 5-HT_{1A} agonists

The presence and electrophysiological properties of somatodendritic 5-HT_{1A} autoreceptors in the raphe nuclei has been covered elsewhere. In brief, the acute administration of various 5-HT_{1A} receptor agonist induces the suppression of firing activity of 5-HT neurons, which, presumably, would reduce the net outflow of 5-HT in terminal areas. Indeed, this has been assumed, like dogma, for many years. *In vivo* microdialysis studies have experimentally assessed this outcome by showing that systemic, as well as intra-raphé, administration of various 5-HT_{1A} agonists such as 8-OH-DPAT, ipsapirone, gepirone and buspirone reduce the extracellular concentrations of 5-HT in terminal regions of the brain such as in the dorsal hippocampus, hypothalamus and frontal cortex. This effect was sometimes prevented by 5-HT_{1A} antagonists (Auerbach *et al.* 1989; Sharp *et al.* 1989a; Muth *et al.* 1986; Sharp *et al.* 1989b). Nonetheless, it must be borne in mind that the effects mediated by systemic administration of 5-HT_{1A} agonists, as is the case for the firing activity of 5-HT neurons, may not necessarily be mediated entirely by the somatodendritic 5-HT_{1A} autoreceptors. There is indeed evidence suggesting that a postsynaptic 5-HT_{1A} receptor may be involved in the effect of systemic administration of 8-OH-DPAT (see section 4 and 5). In keeping with *in vivo* electrophysiological data that shows that WAY 100635 has little effect on the firing activity of 5-HT neurons, systemic administration of this compound by itself has very weak effect on the extracellular concentrations of 5-HT hypothalamus, striatum, frontal cortex and hippocampus (Stenfors *et al.* 1999). Taken together, the results suggest that in the anaesthetised rodent, the somatodendritic 5-HT_{1A} autoreceptor is not the subject of a tonic activation by endogenous 5-HT in the raphe nuclei.

In vivo microdialysis studies have shown that somatodendritic autoreceptor activation by 5-HT_{1A} agonist unevenly reduces 5-HT release in the rat brain (Adell and Artigas 1991; Adell *et al.* 1993; Casanovas and Artigas 1996; Casanovas *et al.* 1997; Kreiss and Lucki 1994; Romero and Artigas 1997). On the basis of the differential innervation of various terminal areas by neurons originating from neurons of the dorsal vs the median raphe nuclei, it has been suggested that neurons from the dorsal raphe are more sensitive to autoreceptor stimulation

than those of the median raphe. This interpretation is consistent with results obtained from *in vivo* voltammetry showing that whereas a small dose of the selective 5-HT_{1A} agonist 8-OH-DPAT produces a decrease of 5-HT levels in the dorsal raphe and in the frontal cortex, it was ineffective in the median raphe and in the dentate gyrus (which receives its 5-HT innervation exclusively from the median raphe). Moreover, the microiontophoretic application of 8-OH-DPAT directly onto 5-HT neurons is three times more potent in suppressing the firing activity of dorsal raphe 5-HT neurons than that of their median raphe congeners (Blier *et al.* 1990). However, studies aimed at determining potencies of acute administration of the 5-HT_{1A} agonists 8-OHDPAT to suppress the firing activity of 5-HT neurons of the median raphe vs the dorsal raphe have generated conflicting results. Indeed, whereas an early electrophysiological study has shown that the systemic administration of 5-HT_{1A} agonists are more potent (30 fold) to suppress the firing activity of 5-HT neurons of the dorsal raphe than that of the median raphe (Sinton and Fallon 1988), this finding was not replicated by Hajós *et al.* (1995). It is important here to underscore the need for caution in interpreting, in this context, results obtained with the acute systemic administration of 8-OHDPAT, as there is now ample evidence that a postsynaptic 5-HT_{1A} receptor located outside the raphe, participates in its effects on the firing activity of at least, 5-HT neurons of the dorsal raphe (see section 4 and 5).

It is interesting to note that these differences in sensitivity are most probably not accounted for by different levels of expression of 5-HT_{1A} receptors by neurons of the subgroup of raphe nuclei. Despite the presence of great variability between studies in terms of absolute numerical data, autoradiographical counts of 5-HT_{1A} receptors in the raphe area reports that, irrespective of the ligand used (*i.e.*, 8-OH-DPAT or WAY 100635), there is more 5-HT_{1A} receptor labelling in the dorsal raphe (*ca.* 3.4 times) than in the median raphe (Li *et al.* 1997; Weissmann-Nanopoulos *et al.* 1985; Khawaja 1995a). However, this outcome is most probably not involved in the greater sensitivity of 5-HT neurons of the dorsal raphe considering the fact that there is about 3 times more 5-HT neurons in the dorsal raphe than in the median raphe (Wiklund *et al.* 1981). Therefore, it seems reasonable to affirm that individual 5-HT neurons of the dorsal raphe express about the same number of 5-HT_{1A} receptors as their congeners of the median raphe. Therefore, the difference in sensitivity of neurons to autoreceptor stimulation cannot be explained in terms of difference in receptor expression.

The finding of Hajós *et al.* (1995) that the 5-HT reuptake inhibitor paroxetine is equipotent in suppressing the firing activity of median and dorsal raphe neuron seems incongruent with this contention. However, one must underscore that the suppression of firing activity induced by a reuptake inhibitor is an indirect measure of the sensitivity of the somatodendritic autoreceptor. Indeed, the determination of the ratio of 5-HT_{1A} receptor/5-HT transporter is the predominant feature to assess. Thus, autoradiography studies with markers of the 5-HT transporter report that the density of the 5-HT transporter is invariably greater in the dorsal raphe than in the median raphe (Biegon and Mathis 1993; De Souza and Kuyatt 1987; Hrdina *et al.* 1990; Cortes *et al.* 1988; Gobbi *et al.* 1994) with a mean ratio of 1.7 (calculated from the values provided by these studies). It is thus possible, although speculative, that assuming the law of mass-action, the greater functional sensitivity of the 5-HT_{1A} autoreceptor of the dorsal raphe nucleus would be cancelled out by the greater absolute number of 5-HT transporter to be blocked by paroxetine in the dorsal raphe hence explaining the equipotency of paroxetine to suppress firing activity of 5-HT neurons in dorsal and median raphe neurons.

ii) 5-HT reuptake inhibitors

There is a myriad of studies that have assessed the effects of 5-HT reuptake inhibitors on the release of 5-HT in terminal areas. As was covered in section 3.3.1., it is now well established that the acute intravenous administration of various 5-HT reuptake inhibitors suppresses the firing activity of the 5-HT neurons of the dorsal and median raphe nuclei through a somatodendritic 5-HT_{1A} autoreceptor-mediated mechanism (Gartside *et al.* 1995; de Montigny *et al.* 1981; Blier *et al.* 1984; Chaput *et al.* 1986b; Hajós *et al.* 1995). The functional outcome and relative importance of the suppression of firing activity of 5-HT neurons by the acute administration of 5-HT reuptake inhibitors were later put into perspective by *in vivo* microdialysis studies which showed that the acute systemic administration of 5-HT reuptake inhibitors results in an enhanced output of 5-HT in terminal regions and this, despite the presence of presumably silent 5-HT neurons (Invernizzi *et al.* 1995; Kreiss and Lucki 1995; Malagie *et al.* 1995; Rutter *et al.* 1995; Jordan *et al.* 1994; Romero and Artigas 1997; Adell and Artigas 1991; Rollema *et al.* 1996, see figure). A similar outcome is also observed with the MAO inhibitor tranylcypromine (Sharp *et al.* 1997). Given that such treatment induces a 100% suppression of firing activity of raphe neurons, these results suggest that there is a basal, firing-independent release of 5-HT. This is fully consistent with the data covered

in section 3.4.1. indicating that TTX does not fully inhibit the release of 5-HT in terminal areas. Earlier studies assessing the effect of systemic administration of 5-HT reuptake inhibitors on the extracellular concentration of 5-HT in terminal areas were suggesting that the increase in 5-HT was of greater magnitude in areas such as the hippocampus than in the frontal cortex (see Fuller 1994) for review). Nonetheless, this issue has been the focus of a more recent and rigorous appraisal that reveals that, in fact, the systemic administration of 5-HT reuptake inhibitors increases evenly the extracellular levels of 5-HT in terminal areas (Romero and Artigas 1997) although this is not replicated by all authors (Roberts *et al.* 1998).

The increase of extracellular levels of 5-HT in terminal areas induced by systemic administration of 5-HT reuptake inhibitors is greatly hampered by the concurrent activation of somatodendritic 5-HT_{1A} autoreceptors and hence suppression of firing activity. In keeping with this, several groups have reported that various selective and non-selective 5-HT_{1A} antagonist (such as (-)-pindolol) potentiates the 5-HT reuptake inhibitor-mediated increase of extracellular concentration of 5-HT (Hjorth 1993; Hjorth and Auerbach 1994a; Malagié *et al.* 1996; Hjorth *et al.* 1997; Romero and Artigas 1997; Romero *et al.* 1996; Dreshfield *et al.* 1996; Hjorth *et al.* 1996; Moret and Briley 1986; Gartside *et al.* 1995; Invernizzi *et al.* 1997; Arborelius *et al.* 1996). Also, see figure 3. That this effect is the result of an antagonism of somatodendritic 5-HT_{1A} autoreceptor is shown by several observations: First, it is well known that administration of 5-HT_{1A} antagonists reverses the suppressant effect of SSRI's on the firing activity of raphe 5-HT neurons (Hajós *et al.* 1995; Gartside *et al.* 1997; Gartside *et al.* 1995; Kasamo *et al.* 1996). In addition, the local administration in the raphe by reverse dialysis of 5-HT_{1A} antagonists also potentiates the SSRI increase of extracellular levels of 5-HT in terminal regions (Romero and Artigas 1997; Matos *et al.* 1996). Inasmuch as an increase of 5-HT neurotransmission is the substratum for the efficacy of antidepressants, these findings have constituted the rationale for the elaboration of one the most important hypotheses for attaining a better antidepressant response (see chapter VI for further discussion on this topic).

When 5-HT transporters in terminal areas are blocked by local infusion of a 5-HT reuptake inhibitor, the systemic administration of a 5-HT reuptake inhibitor leads to a decrease of extracellular levels of 5-HT in that same terminal area (Romero and Artigas 1997; Rutter *et al.* 1995; Hervas and Artigas 1990). This effect is due to the activation of somatodendritic 5-HT_{1A} autoreceptors as it is reversed or prevented by 5-HT_{1A} antagonists and that it is mimicked

by intraraphe administration of SSRI's (Hervas and Artigas 1980; Romero *et al.* 1996; Rutter *et al.* 1995; Romero and Artigas 1997). Accordingly, the magnitude of this effect varies with terminal areas and shows a regional pattern that is similar to that observed with 5-HT_{1A} agonist, *i.e.*, greater in regions preferentially innervated by the dorsal raphe (Romero and Artigas 1997; Hervas and Artigas 1980). These observations thus further corroborate the idea that 5-HT neurons of the dorsal raphe are more sensitive than their congener of the median raphe to autoreceptor stimulation (see above). This contention is also reflected on the magnitude of the potentiation of SSRI's-induced rise of extracellular levels of 5-HT in terminal areas by 5-HT_{1A} antagonists. Indeed, the effect was shown to occur with greater magnitude in forebrain areas that exhibit a preferential innervation from the dorsal raphe (cortex and striatum) rather than those mainly innervated by the median raphe nucleus (hippocampus; Malagié *et al.* 1996; Romero and Artigas 1997; see figure3).

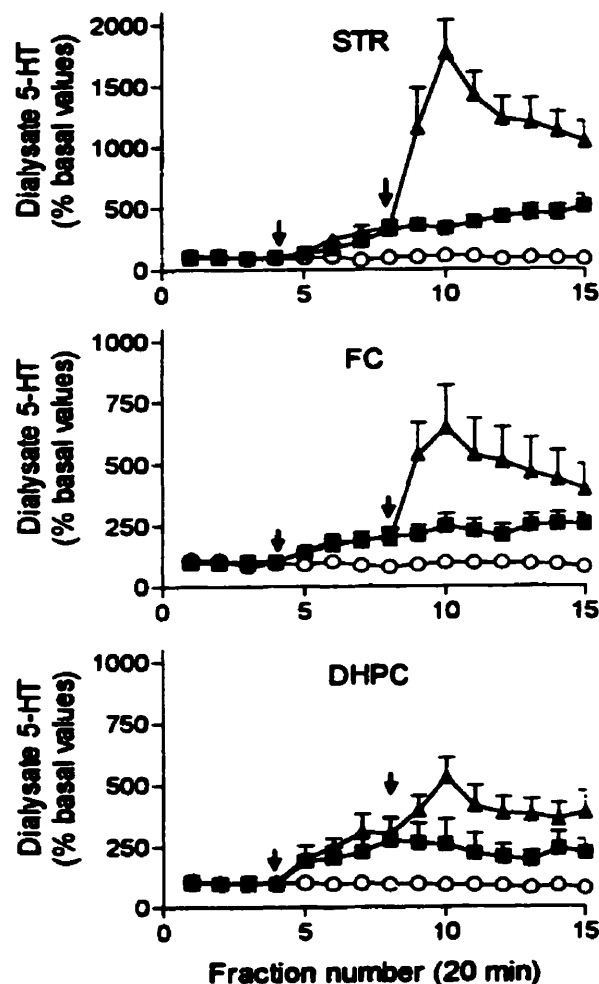


Figure 3: Effect of administration of WAY-100635 (1 mg/kg, s.c.) or saline 80 min after administration of paroxetine (3 mg/kg, i.p.) on dialysate 5-HT in dorsal striatum (STR), frontal cortex (FC), and dorsal hippocampus (DHPC). First and second arrows denote injections of paroxetine (or saline) and WAY-100635 (or saline), respectively. Data are mean $\pm$ SEM (bars) values. Experimental groups were as follows: saline + WAY-100635 ($\circ$), paroxetine + saline ($\blacksquare$), and paroxetine + WAY-100635 ($\blacktriangle$). Note the different scales used along the y-axes. Figure taken from Romero and Artigas (1997).

Considering that 5-HT neurons of the dorsal raphe are more sensitive to autoreceptor stimulation than those of the median raphe, it is striking that the systemic administration of SSRI's enhance evenly the extracellular levels of 5-HT in terminal areas. In search of possible explanation for this, Romero and Artigas (1997) have hypothesised that terminal 5-HT release might be differentially controlled in dorsal raphe and median-raphe innervated terminal regions. In order to put to the test their hypothesis, they have determined the effects of local application of the 5-HT_{1B} antagonist methiothepin in the frontal cortex, striatum and hippocampus. However, the magnitude of the increase was the same in all regions, thus indicating that the control of release is similar and thus cannot account for the comparable effect of SSRI's in these terminal regions.

3.4.3. Terminal 5-HT_{1B/D} receptors

Although the presence of an autoregulatory element modulating the release of transmitters has been hypothesised on the basis of indirect evidence as early as 1961 (Koelle 1961), several years past before experimental evidence clearly allowed the suggestions that cholinergic (Polak 1971), noradrenergic (Farnebo and Hamberger 1971), GABAergic (Johnston and Mitchell 1971), DA (Farnebo *et al.* 1970) and 5-HT (Farnebo *et al.* 1970) axon terminals had such "presynaptic autoreceptors" (see Kirpekar 1984; Langer 1997; Starke *et al.* 1989, for review).

The first evidence toward the demonstration that a 5-HT receptor inhibits the release of 5-HT was achieved by Farnebo *et al.* (1970) who showed that LSD inhibits the electrically-evoked release of [³H]5-HT from rat brain cortex slices. Subsequently, it was shown that 5-HT itself had the same effect, which was reversed by the 5-HT antagonist methiothepin (Göthert and Weinheimer 1979). Because there was a crucial lack of 5-HT₁ subtype selective drugs at the time of these early studies, the identification of the receptor that was modulating the terminal release of 5-HT relied on the use of a large number of agonists and antagonists and subsequent correlative data analysis. The details of the pharmacological analysis have been extensively reviewed elsewhere (Göthert 1990; Starke *et al.* 1989). Essentially, these studies showed that the terminal receptor modulating the release of 5-HT in the rat is of the 5-HT_{1B} receptor subtype. Interestingly, there is a series of β -adrenergic antagonists possessing affinity for the 5-HT_{1B} receptor such as propranolol, cyanopindolol and pindolol that

antagonize the inhibitory effect of 5-HT agonists. Noteworthy is the fact that more recent functional studies have been able to confirm the 5-HT_{1B} nature of this receptor by using newer compounds that showed better selectivity (Bagdy and Harsing, Jr. 1995; Piñeyro *et al.* 1995b; Sharp *et al.* 1989a). This pharmacological work was nonetheless rapidly confronted with the realm of species-differences. Indeed, the pharmacological identification of the 5-HT_{1B} receptor did not hold true when assessed in either rabbit, guinea pigs or in human brains. Indeed, the pharmacological profile of this release-modulating autoreceptor suited more that for the 5-HT_{1D} receptor, then recently identified (Bühlen *et al.* 1996; DeVane 1994; Hoyer and Middlemiss 1989; Limberger *et al.* 1991; Schlicker *et al.* 1989).

The pharmacological identification of a receptor modulating release does not prove its “autoreceptor” nature. Indeed, there is the possibility that the receptor identified is actually on another neuronal element (for example a GABAergic interneurons) thereby participating in a polysynaptic pathway. For the case of presynaptic receptor modulating release, essentially two approaches have been used to experimentally address this issue. First, the use of brain synaptosomes, as opposed to brain slices. Synaptosomes (sometimes referred to as “pinched off” nerve endings) consist mainly of isolated varicosities which do not contain functional interneurons that could participate in the modulation of release. Therefore, the disclosure of a receptor modulating release in such a preparation does not necessarily imply that it is an autoreceptor. The second approach consists of functionally isolating the nerve terminal in brain slices by perfusing TTX. This will block propagation of action potential in any neuronal element that might be present in the slices. In these conditions, K⁺-evoked release of the neurotransmitter is thus not influenced by any other neuronal element. The basic concept and application of this approach are also widely, and perhaps even more routinely, used *in vitro* electrophysiological studies. Thus, the presynaptic location of the 5-HT autoreceptor has been demonstrated in the cerebral cortex of the rat (Göthert and Weinheimer 1979), the guinea pig (Bühlen *et al.* 1996; Limberger *et al.* 1991). and man (Fink *et al.* 1995; Schlicker *et al.* 1996) and in the hippocampus (Maura *et al.* 1982b), nucleus accumbens (Drescher and Hetey 1988), hypothalamus (Cox and Ennis 1982) and spinal cord (Brown *et al.* 1988) of the rat. It is also noteworthy that the presynaptic nature of a given receptor can theoretically be assessed using autoradiography following a specific lesion, in this case, of the 5-HT system. Nonetheless, this approach has proven to be largely unsuccessful in the case of the 5-HT_{1B} receptor. Indeed, studies assessing this issue have largely found no significant decrease of 5-

HT_{1B} receptor levels following a 5, 7-DHT lesions. This observation has led to the conclusion that these release-modulating receptors would not be located on 5-HT terminals, which is at variance with the conclusion drawn from functional studies. This is most probably due to the presence of a high number of postsynaptic 5-HT_{1B} receptors such as the decrease of presynaptic receptor following lesioning goes undetected with the traditional autoradiography method (see Starke *et al.* 1989, for discussion of this issue).

Early studies carried out in rat brain slices showed that the perfusion of 5-HT₁ antagonists such as methiothepin increased the release of 5-HT in various regions of the brain, although this finding is generally not replicated in synaptosomal preparation (Starke *et al.* 1989). Indeed, in this experimental approach, 5-HT is washed out much more efficiently and thus removed from the biophase much more rapidly. The presence of a tonic activation is also not observed when the pseudo-one-pulse (POP) stimulation paradigm is applied, since the amount of 5-HT release is too low to activate the autoreceptor (Singer, 1988). The use of *in vivo* microdialysis presents potentially the best method to assess the presence of a tonic activation on terminal 5-HT_{1B/1D} by endogenous 5-HT in a physiologically relevant condition. Unfortunately, these studies have not been able to present a clear and consistent picture on that matter due to many factors that render necessary a discussion. The presence of a tonic activation was assessed with the use of the 5-HT_{1B/1D} antagonist GR 127935 and assessing the release of 5-HT in terminal areas. Thus, it has been found that GR 127935 increases the extracellular level of 5-HT in the frontal cortex (Stenfors *et al.* 1999) and hypothalamus (Rollema *et al.* 1996) but not the striatum (Stenfors *et al.* 1999) of the guinea pig. However, others have found that this compound did not significantly increase cortical 5-HT efflux when given either systemically or by infusion via the probe directly into the cortex (Hutson *et al.* 1995) of guinea pigs. Studies in the rat have also shown that this compound administered locally in dorsal and ventral hippocampus, striatum and the dorsal and median raphe nucleus was devoid of any significant effect (it even tends to reduce 5-HT levels; Hervas *et al.* 1998). Nonetheless, these authors (Hervas *et al.* 1998) have observed that methiothepin, perfused locally, increased the level of 5-HT in the hippocampus, frontal cortex, striatum and dorsal and median raphe nucleus. Thus, the authors conclude, that in the rat, the control of 5-HT release by 5-HT_{1B} receptors is of the same magnitude in these terminal areas.

i) Mechanistic aspects

From a mechanistic point of view, one can imagine several ways by which a receptor can reduce the release of a neurotransmitter. One of central concept evolves around the notion if the inhibition of release involves inhibition of the propagation of an action potential or from directly inhibiting the mechanism by which synaptic vesicles fuse with the membrane during the secretory event. One argument that argues against a modulation of the propagation of action potentials, is that K⁺-induced depolarization, even in the presence of TTX, is as sensitive than electrically-evoked release of [³H]5-HT to terminal autoreceptor stimulation (Starke *et al.* 1989). It is now well established that the 5-HT_{1B} receptors belong to the G protein coupled superfamily (see section 2.3). Thus, it seems plausible that a G protein might be involved on the inhibitory effect of terminal autoreceptor stimulation on 5-HT release. Although (Passarelli *et al.* 1988) found the inhibitory effect of two 5-HT receptor agonists (LSD and 5-methoxytryptamine) on the evoked release of 5-HT in the hippocampus to be pertussis toxin sensitive, (Bluer 1991) failed to converge to the same conclusion as the inhibitory effect of terminal 5-HT_{1B} receptor stimulation was not altered by a pretreatment with either pertussis toxin or NEM. Some methodological factors belittling the results obtained by (Passarelli *et al.* 1988) have been suggested by (Bluer 1991). Studies in rat hypothalamic slices (Ramdine *et al.* 1989) have shown that inhibition of PKC attenuates the inhibitory effect of 5-methoxytryptamine of 5-HT release whereas activation enhance the electrically-evoked overflow of [³H]5-HT in rat hypothalamic slices (Ramdine *et al.* 1989) and in rabbit hippocampus slices (Feuerstein *et al.* 1987). These results suggest the involvement of the PLC-PKC pathway in the terminal 5-HT autoreceptor control of 5-HT release.

3.4.4. Adrenergic receptors modulating the release of 5-HT

The presence of an adrenergic receptor modulating the release of 5-HT from brain slices (Frankhuyzen and Mulder 1982) has first been described by (Starke and Montel 1973) who showed that clonidine inhibits the release of [³H]5-HT from preloaded cerebral cortex slices. The function this α_2 -adrenergic receptor has been extensively studied using both *in vivo* and *in vitro* paradigms (Frankhuyzen and Mulder 1982; Göthert and Huth 1980; Göthert *et al.* 1981; Maura *et al.* 1982a; Mongeau *et al.* 1993; Numazawa *et al.* 1995). Whereas Starke *et al.* (1995) has shown that in the rat, both the α_2 -adrenergic auto- and heteroreceptors are of the α_{2D} subtype whereas, in the rabbit, both are of the α_{2A} subtype (Trendelenburg *et al.* 1994), there are several studies that have provided evidence that the adrenergic receptor modulating

the release of 5-HT is different than that of NE (Frankhuyzen and Mulder 1982; Gobbi *et al.* 1990b; Maura *et al.* 1992; Mongeau *et al.* 1993; Preziosi *et al.* 1989).

The use of an *in vivo* electrophysiological approach has permitted the study of these receptors in more relevant physiological context. One of the most salient results from these studies has been the demonstration by Mongeau *et al.*, (1993) tonic activation of NE on the α_2 -adrenergic heteroreceptor controlling release of 5-HT in the hippocampus. This conclusion was reached in light of 3 sets observation 1) the effectiveness of stimulation of the 5-HT pathway is diminished by desipramine, which effect was shown to be due to α_2 -adrenergic heteroreceptor activation by endogenous NE, 2) selectively reducing NE output (by a low dose of clonidine) enhanced the efficacy of 5-HT pathway stimulation and 3) the efficacy of this stimulation was enhanced following blockade of the α_2 -adrenergic heteroreceptor. As an addition to this, one could add that following a 6-OHDA lesion, the sensitivity of the α_2 -adrenergic heteroreceptor was enhanced, indicative that the system reacted to a decrease of tonic activation by endogenous NE.

This conclusion is however at variance with that reached by, *in vitro* experiments as they fail to detect the presence of a tonic activation of α_2 -adrenergic heteroreceptors by endogenous NE (Schlicker *et al.* 1983; Göthert *et al.* 1981; Galzin *et al.* 1984). It is possible that in the *in vitro* slice preparation endogenous NE does not reach the 5-HT terminals in sufficient concentrations due to the continuous washing of the slices in those experiments.

Experiments have been carried out in order to determine the signal transduction pathway employed by these receptors. The involvement of a G protein was delineated by the observation that the inhibitory effect of clonidine on the release of [³H]5-HT was reduced by a NEM pretreatment (Maura *et al.* 1992).

4. ON THE EFFECTS OF 5-HT ON POSTSYNAPTIC NEURONS

The use of various electrophysiological approaches has proven invaluable in the process of elucidating the effects mediated by 5-HT on postsynaptic neurons. Valuable information was generated by such techniques on the role of 5-HT in controlling firing activity, and on the underlying cellular mechanisms and signal transducing pathways involved, as well in the generation and maintenance of various neural networks. In the following section, the electrophysiological effects of 5-HT mediated by the 5-HT_{1A} receptor will be presented.

4.1. Electrophysiological properties of postsynaptic 5-HT_{1A} receptors

4.1.1. Basic characteristics, ionic basis and pharmacology

Most of the representative electrophysiological work on the function of 5-HT receptors of limbic areas was carried out in the dorsal hippocampus. A predominant inhibitory role for 5-HT was soon delineated as it was observed that the microiontophoretic application of 5-HT directly onto dorsal hippocampus pyramidal neurons suppresses the firing activity of these neurons (Bloom *et al.* 1972). The involvement of the 5-HT_{1A} receptor mediates this effect as it is mimicked by the microiontophoretic application of various 5-HT_{1A} agonists such as gepirone, flesinoxan, flibanserin, Bay x 3702 and the prototypical agonist 8-OH-DPAT and blocked by 5-HT_{1A} antagonists (Blier and de Montigny 1990) (Dong *et al.* 1998; Hadrava *et al.* 1994; Hadrava *et al.* 1996; Hadrava *et al.* 1995; Rueter *et al.* 1998a). Moreover, the acute systemic administration of all the 5-HT_{1A} agonists tested thus far, *i.e.*, 8-OHDPAT, PAPP, gepirone, buspirone, tandospirone, flesinoxan and flibanserin also suppresses the firing activity of pyramidal neurons of the CA₃ field of the hippocampus (Blier and de Montigny 1987; Godbout *et al.* 1991; Hadrava *et al.* 1995; Rueter *et al.* 1998a). The amplitude of population spikes recorded in the hippocampus elicited by stimulation of the schaffers collateral is also suppressed by 5-HT_{1A} receptor activation as it is mimicked by various 5-HT_{1A} agonist and blocked by 5-HT_{1A} antagonist (Beck and Goldfarb 1985; Bijak and Tokarski 1994; Bijak *et al.* 1996; Dijcks *et al.* 1994; Rowan and Anwyl 1986).

Although these studies unravelled a prominent inhibitory effect of 5-HT on the firing activity of pyramidal neurons, the cellular and molecular mechanisms underlying the effects of 5-HT on membrane excitability were unknown. This issue has been widely studied with the use of *in vitro* electrophysiological recording techniques carried out in hippocampal slices. The effects of 5-HT on membrane excitability are complex as bath application of 5-HT evokes a biphasic response from CA₁ pyramidal neurons of the hippocampus (Andrade and Nicoll 1987b). Whereas the most conspicuous response consists of a hyperpolarization, it is followed by a longer lasting depolarization. This depolarization was later shown to be mediated by the 5-HT₄ receptor (Andrade and Chaput 1991; Chaput *et al.* 1990; Torres *et al.* 1996). We will however herein focus on the 5-HT_{1A} receptor-mediated response. Thus, application of electrophysiological protocols in the CA₃, as well as in CA₁ region of the hippocampus demonstrated that the electrophysiological response elicited by 5-HT_{1A} receptors

involves an increase in K^+ permeability (Andrade and Nicoll 1987b; Beck and Choi 1991; Beck *et al.* 1992; Jahnsen 1980; Okuhara and Beck 1994; Segal 1980). Indeed, the 5-HT-induced hyperpolarization is associated with a decrease in input resistance, is blocked by extracellular barium and intracellular caesium (two K^+ channel blockers), is unaffected by the Cl^- gradient and has a reversal potential near that for K^+ . The pharmacology of this hyperpolarizing response of 5-HT was shown to be 5-HT_{1A}-mediated since, first, it is mimicked by the 5-HT_{1A} agonist 8-OHDPAT and not by the 5-HT_{1B} agonist TFMPP and, second, blocked by spiperone and methiothepin and not by ketanserin, a 5-HT₂ antagonist (Andrade *et al.* 1986; Andrade and Nicoll 1987b). Interestingly, 8-OHDPAT in those conditions behaves as a partial agonist (Andrade and Nicoll 1987b) which finding has been subsequently replicated using various experimental approaches (Colino and Halliwell 1986; Hadrava *et al.* 1996; Markstein *et al.* 1986). Taken together, these results strongly suggest that most, if not all, 5-HT_{1A}-induced inhibitory effects on neuronal firing observed in the nervous system can be attributable to the opening of a K^+ channel.

4.1.2. Signal transduction pathway

There has been a research endeavour seeking to elucidate the molecular identity of the effector system that mediates the effects of 5-HT_{1A} receptor activation on cellular excitability. In this regard, (Andrade *et al.* 1986) have shown that the 5-HT_{1A} receptor mediated hyperpolarization is prevented by a pretreatment with pertussis toxin, an inactivator of $G_{i/o}$ protein, thereby demonstrating the involvement of the latter protein in this cellular response. Moreover, the fact that the intracellular injection of the hydrolysis-resistant GDP analog GDP β S and GTP analog GTP γ S reduced and mimicked, respectively, the hyperpolarizing effect of 5-HT further corroborates the involvement of a G protein in mediating the latter effect. However and most interestingly, the fact that the electrophysiological response could not be mimicked nor altered by manipulating second messenger system (*i.e.*, cAMP, intracellular Ca^{2+} and inositol phospholipid turnover) prompted the author to hypothesize that the hyperpolarizing effect mediated by 5-HT_{1A} receptor involves a direct coupling of the G protein with the K^+ channel (Andrade *et al.* 1986). Even though some yet unidentified cellular mediators could theoretically mediate the response, their conclusion has not yet been challenged. In addition, the inhibition of the population spike amplitude elicited by 5-HT_{1A} receptor activation as well as the effect of microiontophoretically-applied 5-HT (known to be

5-HT_{1A} mediated) is also pertussis toxin-sensitive (Blier *et al.* 1993; Clarke *et al.* 1987).

4.2 Intra vs extrasynaptic 5-HT_{1A} receptors.

Very few studies have addressed the issue of postsynaptic 5-HT_{1A} receptor activation by endogenous 5-HT. Indeed, whereas, for example, the selective release of endogenous glutamate in the CA₁ region by the electrical stimulation of the Schaffer's collaterals (CA₃ - CA₁ pathway) is routinely used in the *in vitro* slice preparation, that for 5-HT by electrical activation of the afferent 5-HT pathway cannot be achieved in an *in vitro* preparation. However the effect of the electrical stimulation of the main ascending 5-HT pathway in the ventral tegmental area on the firing activity of dorsal hippocampus pyramidal neurons can be assessed *in vivo* (Chaput *et al.* 1986a). Such stimulation produces a short lasting (30-50 ms) suppression of firing activity of these neurons. That this phenomenon is indeed mediated by the release of endogenous 5-HT is shown by its abolition by the destruction of the 5-HT neurons by the 5-HT neurotoxin 5, 7-DHT as well by its blockade by the 5-HT_{1A} antagonist BMY 7378.

This paradigm has been used, amongst others, to study the signal transduction pathway that underlies the effect of stimulating the ascending 5-HT pathway. Thus, it was demonstrated that the response of pyramidal neurons to the stimulation of the 5-HT pathway is left unaltered by a pretreatment with pertussis toxin whereas the suppressant effect of microiontophoretic application of 5-HT is markedly reduced by the pertussis pretreatment (Blier *et al.* 1993). Taken together, these observations prompted the authors to suggest that "extrasynaptic" 5-HT_{1A} receptor (*i.e.*, those mediating the effect of microiontophoretic application of 5-HT) might be distinct from the "intrasympaptic" 5-HT_{1A} receptors (*i.e.*, those mediating the response to the stimulation of the ascending 5-HT pathway) inasmuch as the former but not the latter receptor, are coupled to a G_{i/o} protein (Blier *et al.* 1993). The authors further confirmed their conclusion by demonstrating that a much higher dose of the 5-HT_{1A} receptor antagonist BMY 7378 is needed to block the effect of endogenous 5-HT than for microiontophoretically applied 5-HT.

The fact that a 5, 7-DHT lesions prevented the effect of the stimulation show that the effect to a 5-HT receptor or to any chemical messenger that would be co-released with 5-HT upon electrical stimulation of the pathway that might have an electrophysiological effect that would be blocked by only a high dose of BMY 7378. Another interpretation might rely on the fact

that in control condition, endogenous 5-HT would tonically activate the “intrasynaptic” population of “5-HT_{1A}” receptors and thereby prevent their inactivation by pertussis toxin. There are several problems with this interpretation. For instance, it has been shown that the intravenous administration of WAY 100635 does not prevent the suppressant effect induced by stimulation of the ascending 5-HT pathway thus implying that intravenous administration of WAY 100635 does not antagonize the action of endogenous 5-HT on the intrasynaptic population of postsynaptic 5-HT_{1A} receptors of the dorsal hippocampus (Haddjeri *et al.* 1998b).

4.3. Distinct properties of somatodendritic and postsynaptic 5-HT_{1A} receptors

The use of radioligand binding techniques and of molecular biological approaches have been of considerable use in the subclassification of receptors within the scope of a stringent nomenclature. In the case of the 5-HT_{1A} receptor, based on experimental evidence acquired with the use of these techniques, it has been assumed that the somatodendritic and postsynaptic population of 5-HT_{1A} receptors were identical. This assumption is further supported by various physiological evidence, for instance, those showing that the 5-HT_{1A} response both in the hippocampus and in dorsal raphe involves the opening of a K⁺ channel occurring *via* a cAMP-independent, G-protein mediated process (Blier *et al.* 1993; Innis and Aghajanian 1987; Innis *et al.* 1988). Nonetheless, there is a rather abundant number of experimental evidence supporting the presence of a heterogeneity within this receptor subtype. Given the central role of this receptor in the homeostasis of the serotonergic system, as well as in mediating the therapeutic response of antidepressant drugs, such a heterogeneity deserves attention herein. Hence, pharmacological, biochemical and functional evidence suggests that there are fundamental differences between the 5-HT_{1A} receptor found somatodendritically in the raphe and those found postsynaptically in the hippocampus. These contentions will be considered here separately.

4.3.1. Pharmacological evidence

One of the first hunch suggestive that the raphe somatodendritic and postsynaptic 5-HT_{1A} receptors might have different properties was provided by observations using microiontophoretic applications of 5-HT as well as of agonists onto dorsal raphe 5-HT neurons and dorsal hippocampus neurons of the CA₃ region. Whereas 5-HT is more effective to

suppress firing activity of CA₃ pyramidal neurons than 5-HT neurons, the inverse is true for all 5-HT_{1A} agonists tested thus far, *i.e.*, they are more potent in the raphe than in the hippocampus (Blier and de Montigny 1987). Another observation, obtained using both *in vivo* and *in vitro* electrophysiological paradigms, is that most 5-HT_{1A} receptor agonists exhibit partial agonism in the dorsal hippocampus and full agonist activity in the raphe. This was first suggested by the group of Aghajanian who has observed that the direct microiontophoretic application of 5-HT_{1A} agonists (8-OH-DPAT, ipsapirone and LY 165163) is much more effective to suppress the firing activity of dorsal raphe 5-HT neurons than hippocampus pyramidal neurons (Sprouse and Aghajanian 1988). Full agonistic activity of various 5-HT_{1A} agonists was observed by others in the dorsal raphe (Cox *et al.* 1993) This hypothesis was further substantiated by both *in vivo* and *in vitro* electrophysiological studies that concurred in showing that in the hippocampus 5-HT_{1A} agonist can block the effect of 5-HT on pyramidal neurons (Andrade and Nicoll 1987b; Blier and de Montigny 1990; Godbout *et al.* 1991; Hadrava *et al.* 1995), consistent with partial agonism. This effect is not observed in the dorsal raphe (Blier and de Montigny 1990). The third pharmacological evidence involves the differential response of these two populations of receptors to antagonists, *i.e.*, spiperone, and BMY-7378. When studied *in vivo* in the anaesthetized animal, it is striking to observe that spiperone readily antagonizes the effects of microiontophoretic application of 5-HT and 8-OH-DPAT on the firing activity of 5-HT neurons, whereas it is devoid of antagonistic activity in the dorsal hippocampus in the same conditions. Spiperone will also antagonize endogenous 5-HT on 5-HT neurons as it will reverse the suppressant effect of 5-HT reuptake inhibitors on the firing activity of 5-HT neurons (Kasamo *et al.* 1996). It is noteworthy that spiperone, in addition to its affinity for the 5-HT_{1A} receptors, also displays 5-HT₂ receptor affinity. That this latter outcome is not involved in these results is shown by the lack of antagonism of the electrophysiological effect of 5-HT by the 5-HT₂ antagonist ketanserin *in vivo* (Lakoski and Aghajanian 1985) as well as *in vitro* (Andrade and Nicoll 1987b; Williams *et al.* 1988). Taken together, these results therefore show that the butyrophenone spiperone displays a preferential antagonism on somatodendritic and postsynaptic 5-HT_{1A} receptors. Finally, the exact opposite situation is obtained with the buspirone analog BMY-7378 that displays a preferential postsynaptic antagonism. Indeed, when microiontophORIZED onto dorsal hippocampus pyramidal neurons, it readily antagonizes the effect of 5-HT and 8-OH-DPAT whereas it lacks such an effect using a similar paradigm on 5-HT neurons of the dorsal raphe. In addition, a

differential effect of the mix B₂/5-HT_{1A} receptor ligand (-)pindolol has been observed in the hippocampus and in the dorsal raphe. This issue is discussed in chapter VI.

4.3.2. Functional evidence

Another line of evidence supporting the heterogeneity of the 5-HT_{1A} receptors involves the different regulatory properties of the two populations of 5-HT_{1A} receptors, *i.e.*, that of the hippocampus and that of the dorsal raphe following long-term pharmacologic treatment. Although this issue is extensively covered elsewhere (see section 5), one must underscore the fact that the long-term administration of various drugs induces adaptative mechanisms that are different for these two populations of receptors. Three examples will be given for illustrative purposes. First, following long-term administration of various TCA's, whereas the postsynaptic population of 5-HT_{1A} receptors will sensitize, that of the dorsal raphe is unchanged (Blier and de Montigny 1980; de Montigny and Aghajanian 1978). Secondly, following long-term administration with various 5-HT reuptake inhibitors, somatodendritic autoreceptors desensitize whereas the postsynaptic 5-HT_{1A} receptors are unchanged. Thirdly, and perhaps most importantly in the context of the present discussion, is the fact that following sustained administration of 5-HT_{1A} agonists (that presumably exert a similar activation of both populations of receptors) leads to a desensitization of only somatodendritic 5-HT_{1A} autoreceptors. (See section 5 for a more detailed discussion and for respective references).

4.3.3. Biochemical evidence (signal transduction)

It is well demonstrated that the suppressant effect of 5-HT_{1A} receptor activation on firing activity is mediated by a pertussis toxin-sensitive mechanism that does not require a second messenger and this both in the raphe (Innis and Aghajanian 1987; Innis *et al.* 1988) and in postsynaptic regions such as the hippocampus (Andrade *et al.* 1986). This outcome was confirmed by an *in vivo* electrophysiological study that the suppressant effect of microiontophoretic applications of both 5-HT and 8-OH-DPAT were pertussis-toxin in both the raphe and in the hippocampus (Blier *et al.* 1993). Nonetheless, the effects of microiontophoretic applications of 5-HT and 8-OH-DPAT are unaltered in the raphe by cholera toxin, whereas those in the hippocampus were greatly diminished by this toxin (Blier *et al.* 1993). Another line of evidence has been provided by biochemical approaches. It has been covered elsewhere that 5-HT_{1A} receptor activation leads to an inhibition of forskolin-

stimulated cAMP production. Clarke *et al.* (1996) have replicated this in hippocampal homogenates with a wide variety of 5-HT_{1A} agonists but they have shown that forskolin-stimulated production of cAMP is unaltered by 5-HT_{1A} receptor activation in raphe homogenate. In addition, the basal stimulation of cAMP production by 5-HT_{1A} agonist observed in the hippocampus was also not observed in the raphe. These experiments, on the one hand, provide indirect support to the hypothesis that, in the raphe, 5-HT_{1A} receptor interaction with the K⁺ channel is independent of cAMP accumulation (or inhibition of) and on the other hand, provides strong evidence (although not definite) that there is a profound and significant difference in the signal transduction pathway used by the 5-HT_{1A} receptor in the raphe and in the hippocampus.

4.3.4. Molecular biology

There are yet no definite indications provided by molecular biological approach that are supportive of the view of a heterogeneous population of 5-HT_{1A} receptors. Northern blot analysis by (Albert *et al.* 1990) showed three different weights of mRNA coding for the 5-HT_{1A} receptor (3.9, 3.6 and 3.3 kb) suggesting that there could be either transcriptional start or perhaps different polyadenylation sites. It is still unclear whether the presence of three mRNA's has functional significance and also whether it could provide a likely explanation for the different properties that are observed between pre- and postsynaptic 5-HT_{1A} receptors.

4.3.5. Integrative aspects

The presence of a heterogeneity of 5-HT_{1A} receptors has been received with skepticism probably because of the lack of a clear-cut evidence from molecular biology supporting it. Various explanations have been forwarded to account for the results covered above. For instance, the presence of a different receptor reserve between the raphe and hippocampus has been often proposed to underlie the functional differences observed between pre- and postsynaptic 5-HT_{1A} receptors.

The "receptor reserve" theory postulates that the relationship between drug effect and receptor occupancy is a nonlinear function (Furchgott 1966; Stephenson 1956). Thus, an effector system that would be endowed with a receptor reserve would require only a fraction of its receptors to be activated in order to display a full response. Experimentally, this can be assessed by eliciting a given response with agonists in the presence of a receptor inactivator.

In the case of the monoamine systems, the irreversible receptor blocker EEDQ has been mostly used as it inactivates 5-HT, DA and NE receptors. Thus, for the population of 5-HT_{1A} receptors in the dorsal raphe, it was shown that for a series of 5-HT_{1A} agonists (8-OH-DPAT, ipsapirone and BMY 7378 administered intravenously), treatment with EEDQ shifts the agonists dose-response curves to the right but does not reduce the maximal suppressant effect (of 100%) on the firing activity of dorsal raphe 5-HT neurons. These results thus suggest the presence of a considerable (>50%) 5-HT_{1A} receptor reserve in the raphe for inhibiting firing activity (Cox *et al.* 1993). In contrast, in the dorsal hippocampus, a pharmacological analysis revealed that the inhibition of forskolin-stimulated cAMP accumulation by 5-HT_{1A} receptor stimulation does not benefit from any significant receptor reserve (Yocca *et al.* 1992). These results, at first sight, could explain some of the functional differences observed between pre- and postsynaptic 5-HT_{1A} receptors. Indeed, it has often been demonstrated that in various expression systems, the manipulation of the number of receptors expressed can alter the pharmacology of various compounds. In the case of 5-HT_{1A} agonists, it has been demonstrated that the agonist efficacy of various compounds (ipsapirone, buspirone or eltoprazine) increases (from weak partial agonism or even antagonism to full agonism) with the increase of receptor expression (Boddeke *et al.* 1992; Newman-Tancredi *et al.* 1997; Varrault and Bockaert 1992a; Varrault *et al.* 1992b). With this in mind, the findings that the 5-HT_{1A} agonists are more potent in the raphe than in the hippocampus and that they act as full agonists in the former region and as partial agonists in the latter, can find a likely explanation in the fact that both regions benefit from different receptor reserve (see above). Nonetheless, the presence of such a differential receptor reserve cannot reconcile the other evidence indicative of a heterogeneous population of 5-HT_{1A} receptors, including the fact that 5-HT itself is more potent in the hippocampus than in the dorsal raphe (see above). The peculiar differential antagonism observed with various compounds is also explained with difficulty on the grounds of a differential receptor reserve. In addition, it needs to be emphasized that the disclosure of different receptor reserve in these two regions was documented for different functions carried out by the 5-HT_{1A} receptors. Indeed, whereas that for the raphe was shown for suppressing firing activity, that in the hippocampus was for suppressing forskolin-activated cAMP production. Thus, there is not a perfect symmetry in the experimental approaches, therefore presenting *per se* a limitation in the interpretation of the results. This all the more important considering, first, that the electrophysiological effect of 5-HT_{1A} receptor activation is

independent of cAMP in the hippocampus: it is thus not known whether the system involved in the direct coupling of the receptor to the K⁺ channel possesses a receptor reserve. It is indeed possible that one 5-HT_{1A} mediated function (for example inhibition of adenylate cyclase) might benefit from a receptor reserve and not the other (for example opening of a K⁺ channel). As a corollary illustration of the latter aspect, is the observation that whereas the 5-HT_{1A} mediated suppression of firing activity of dorsal raphe 5-HT neurons benefits from a large receptor reserve, the inhibition of 5-HT synthesis by low efficacy 5-HT_{1A} agonists in these same neuron benefits from a much smaller reserve (Meller *et al.* 1990). This outcome, on the one hand, provides an example of two different functions carried out by the same receptor that benefit from a different receptor reserve and, on the other hand, suggests the presence of a functional heterogeneous population of 5-HT_{1A} receptors within the same population of neurons, *i.e.*, those on the 5-HT neurons.

Altogether, these observations, within the context of our current understanding, prompt for caution in the applicability and interpretation of the incomplete assessments of receptor reserve for 5-HT_{1A} receptor-mediated functions. In closing, one might add that if the receptor reserve theory, or any other, were to account for all of the differences that are observed between different population of receptors, and this in the absence of different amino acid sequence, this by no mean would diminish or even refute the presence of a functional heterogeneity. The rationality supportive of attempts to take advantage of the latter heterogeneity, in the context of pharmacological interventions, is therefore preserved.

4.4. Integrative aspects

For sake of brevity and conciseness, only the actions of 5-HT on membrane excitability in the hippocampus (essentially those generated by 5-HT_{1A} receptors) have been covered herein, as they are the ones that best relates to the interpretation of the work presented in chapter II, III, IV and VI. It needs nonetheless to be emphasized that the actions of 5-HT on membrane excitability are complex as, for one, they vary with brain regions (as a result of differential expression of the many 5-HT receptors) and also, as one given neuron can functionally express 5-HT receptors of different and opposing effects. Indeed, whereas a general inhibitory role on cellular excitability is recognized for 5-HT_{1A} receptors, 5-HT₂, 5-HT₃, 5-HT₄ and 5-HT₇ have been associated with excitatory responses (Aghajanian *et al.* 1990; Andrade and Chaput 1991; Araneda and Andrade 1991; Marek and Aghajanian 1999;

McCormick and Wang 1991). Moreover, in certain cases, opposing responses elicited by different 5-HT receptor subtypes were shown to prevail in the same cell (Andrade and Nicoll 1987a; Andrade and Nicoll 1987b; Araneda and Andrade 1991). Another example of a mixed response to 5-HT involves the excitatory effect of 5-HT₂ receptors in pyramidal cells of the piriform cortex with a concurrent activation (also through 5-HT₂ receptors) of local inhibitory GABAergic interneurons impinging on the pyramidal neurons (Marek and Aghajanian 1996). These contentions have rendered very difficult the process of providing a single, unifying and holistic role of 5-HT based on its electrophysiological responses. One can appreciate the extent of this assertion by considering the general inhibitory and excitatory role, respectively, attributed to GABA and glutamate, for example. Attempts to provide such a holistic role has nonetheless been attempted by several authors. Perhaps the single most decisive clues supportive of the existence of a holistic role is provided mainly by the work of B. Jacobs by showing that 5-HT neurons (at least those of the dorsal raphe) displays a concerted activity across different behavioural states (*i.e.*, the sleep-wake cycle). In trying to integrate the actions of 5-HT at the cellular level with those attributed to various systems at a behavioral level, G. Aghajanian (*Serotonin notes*, Vol.4 No 2, September 1997) has forwarded a very interesting proposition that deserves mention here: “...(5-HT) is responsible for the simultaneous fine tuning of neuronal excitability throughout the central nervous system to coordinate the activity of individual neurons and networks in the service of a given behavioral state”. As was alluded by himself, only time will tell if this proposition will survive with our increasing knowledge on the actions of 5-HT.

5. ON THE EFFECTS OF ANTIDEPRESSANT TREATMENTS ON THE 5-HT SYSTEM

The discovery by Ross and Renyi (1969) that tricyclic compounds effective in the treatment of major depression inhibited the uptake of radioactive 5-HT from brain slices has fostered a tremendous attention to the 5-HT system as being a key mediator in the antidepressive response elicited by pharmacological treatments. Whereas the biologic effects of antidepressants supervene within minutes after their administration, the clinical onset of therapeutic activity generally takes several weeks to fully developed. This constitutes the fundamental rationale for studying the effects of prolonged and sustained treatments of antidepressants to laboratory animals on specific targets involved in regulating the overall 5-

HT neurotransmission. Thus, in the following section, relevant data obtained on the effects of various antidepressant treatments on the function of somatodendritic 5-HT_{1A} autoreceptors, presynaptic 5-HT_{1B/D} receptors, α_2 -adrenergic heteroreceptors and postsynaptic 5-HT_{1A} receptors will be covered.

5.1. Somatodendritic 5-HT_{1A} autoreceptors

5.1.1. Serotonin reuptake inhibitors (SSRI's)

The sustained administration of the SSRI's zimelidine, indalpine and citalopram induces a near total suppression of the firing activity of the 5-HT dorsal raphe neurons when administered for two days. However, this initial decrease of firing activity is followed by a gradual recovery, such that 5-HT neurons display a mean firing activity that is up to control value by 21 days of treatment (Blier and de Montigny 1983; Blier *et al.* 1984; Chaput *et al.* 1986b). In the search to identify the neurobiological substratum responsible for this plasticity, the sensitivity of the somatodendritic 5-HT_{1A} receptors following long-term administration of SSRI's was sought for. The best experimental approach to assess this issue is to use the most specific 5-HT_{1A} agonist and to apply it locally onto raphe 5-HT neurons in order to activate the somatodendritic 5-HT_{1A} receptor population. Over the years, three methods have been mostly used to this end. First, the direct microiontophoretic application of 5-HT and 5-HT_{1A} agonists directly onto 5-HT neurons, second, bath application of 5-HT and of 5-HT_{1A} agonists in mesencephalic slices containing the raphe and third, intraraphe application using reverse dialysis. In addition to those experimental approaches, in *in vivo* studies (electrophysiology and microdialysis), agonists have also been administered via a systemic route. This method, although of easier technical use, is more indirect and present limitations (discussed below). Table 2 presents a summary of different studies that have directly assessed the sensitivity of somatodendritic autoreceptors following long-term administration of SSRI's. The approaches that have yielded the most consistent results are those that involve either intraraphe microiontophoretic application or bath application in the raphe slice preparation, since these studies have all detected a desensitization of the somatodendritic 5-HT_{1A} receptor modulating firing activity following long-term and sustained SSRI's treatments. However, those studies whereby the systemic administration of 8-OH-DPAT was used to test this issue have generated conflicting results, as some have detected a desensitization whereas others have failed (see

table 2). Of particular importance is the fact that the intravenous administration of LSD has shown to be a more reliable way to assess the sensitivity of the somatodendritic 5-HT_{1A} receptors. This issue is discussed below more extensively. Taken together, the long-term administration of SSRI's induces a desensitization of the somatodendritic 5-HT_{1A} autoreceptors.

Table 2.

Drug and regimen	Agonist used	Result and comments	References
Citalopram, 5 mg/kg, bid x 14 days	8-OH-DPAT, s.c. <i>in vivo</i> microdialysis	Not desensitized; probe implanted in hippocampus or frontal cortex	(Hjorth and Auerbach 1994b)
Alnespirone, 5 mg/kg/day x 21 days i.p.	8-OH-DPAT bath application; <i>in vitro</i> electrophysiology	Desensitized	(Le Poul <i>et al.</i> 1999)
Flibanserin 5 mg/kg/day x 21 days	LSD, i.v. <i>In vivo</i> electrophysiology	Desensitized	(Rueter and Blier 1999)
Gepirone, 15 mg/kg/day x 21 days	LSD, i.v.; <i>in vivo</i> electrophysiology	Desensitized	(Blier and de Montigny 1987)
Gepirone, 15 mg/kg/day x 21 days	8-OH-DPAT, i.v. <i>in vivo</i> electrophysiology	Not desensitized	(Blier and de Montigny 1987)
Gepirone, 20 mg/kg/day x 14 days	8-OH-DPAT, <i>in vitro</i> assay: 5-HTP accumulation in cortex and hippocampus	Desensitized	(Bohmker <i>et al.</i> 1993)
Gepirone, 15 mg/kg/day x 21 days	8-OH-DPAT, LSD, gepirone or 5-HT by microiontophoresis: <i>in vivo</i> electrophysiology	Desensitized	(Blier and de Montigny 1987)
Ipsapirone, 5 mg/kg ip, bid x 14 days	Ipsapirone and 8-OH-DPAT, bath application, <i>in vitro</i> electrophysiology	Desensitized	(Schechter <i>et al.</i> 1990)

Ipsapirone, 15 mg/kg/day, s.c. (O.m.p. ² .) Or 7.5 mg/kg bid, s.c.	5-HT and 8-OH-DPAT, by microiontophoresis; <i>in vivo</i> electrophysiology	Desensitized	(Dong <i>et al.</i> 1997)
Ipsapirone, 15 mg/kg/day, s.c. (O.m.p.) Or 7.5 mg/kg bid, s.c.	LSD, i.v.; <i>In vivo</i> electrophysiology	Desensitized	(Dong <i>et al.</i> 1997)
Ipsapirone, 15 mg/kg/day, s.c. (O.m.p.) Or 7.5 mg/kg bid, s.c.	8-OH-DPAT, i.v., <i>In vivo</i> electrophysiology	Not desensitized	(Dong <i>et al.</i> 1997)
Ipsapirone 20 mg/kg, bid x 14 days	8-OH-DPAT, <i>in vitro</i> assay: 5-HTP accumulation in cortex and hippocampus	Desensitized	(Bohmker <i>et al.</i> 1993)
8-OH-DPAT 0.2 or 2.0 mg/kg bid x 14 days or 0.4 or 4 mg/kg/day x 14 days	8-OH-DPAT, <i>in vitro</i> assay: 5-HTP accumulation in cortex and hippocampus	Not desensitized	(Bohmker <i>et al.</i> 1993)
8-OH-DPAT, 1 mg/kg/day x 7 or 14 days s.c.	8-OH-DPAT, s.c.; <i>in vivo</i> microdialysis	Probe in striatum: desensitized after 7 or 14 days. Probe in hippocampus: trend for desensitization after 14 days	(Kreiss and Lucki 1997)
8-OH-DPAT, 1 mg/kg x 7 days, s.c.	8-OH-DPAT, <i>in vivo</i> microdialysis	Desensitized; probe in the striatum	(Kreiss and Lucki 1992)
Cerclamine, 16 mg/kg bid, s.c.x 14 days	8-OH-DPAT, and ipsapirone bath application; <i>in vitro</i> electrophysiology	Desensitized	(Jolas <i>et al.</i> 1994)

²O.m.p.: Osmotic mini-pump.

Cericlamine, 16 mg/kg bid, s.c. x 14 days	8-OH-DPAT and ipsapirone, systemic administration: <i>in vivo</i> electrophysiology	Not desensitized	(Jolas <i>et al.</i> 1994)
Citalopram, 10 mg/kg bid x 14 days	8-OH-DPAT; s.c.; <i>in</i> <i>vivo</i> microdialysis	In hippocampus	(Invernizzi <i>et al.</i> 1995)
Citalopram, 20 mg/kg/day ip x 14 days	LSD, i.v. <i>In vivo</i> electrophysiology	Desensitized	(Chaput <i>et al.</i> 1986b)
Citalopram, ?? x 14 days	Citalopram, s.c. <i>in vivo</i> microdialysis	Not desensitized, probe in hippocampus or frontal cortex	(Auerbach and Hjorth 1995)
Fluoxetine, 10 mg/kg/day x 14 days, s.c.	8-OH-DPAT, <i>in vivo</i> microdialysis	Desensitized; probe in the diencephalon	(Rutter <i>et al.</i> 1994)
Fluoxetine 5 mg/kg ip x 3 or 7 days	Ipsapirone, bath application; <i>in vitro</i> electrophysiology	Desensitized	(Le Poul <i>et al.</i> 1997)
Fluoxetine, 5 mg/kg ip x 3 days	8-OH-DPAT, bath application; <i>in vitro</i> electrophysiology	Desensitized	(Le Poul <i>et al.</i> 1995)
Indalpine 5 mg/kg/day ip x 14 days	LSD, i.v. and 5-HT by microiontophoresis: <i>In</i> <i>vivo</i> electrophysiology	Desensitized	(Blier <i>et al.</i> 1984)
Paroxetine, 10 mg/kg x 21 days, p.o.	8-OH-DPAT, <i>in vitro</i> electrophysiology, bath application	Desensitized	(Davidson and Stamford 1998)
Paroxetine, 10 mg/kg x 21 days, p.o.	8-OH-DPAT, <i>in vitro</i> raphe slice	Not desensitized: 5- HT _{1A} receptor modulating release of 5- HT	(Davidson and Stamford 1998)
Paroxetine 5 mg/kg ip x3 days	8-OH-DPAT, bath application; <i>in vitro</i> electrophysiology	Desensitized	(Le Poul <i>et al.</i> 1995)

Paroxetine, 10 mg/kg/day x 21 days (w/o)	8-OH-DPAT, bath application; <i>In vitro</i> [³ H]5-HT release	Desensitized	(Piñeyro and Blier 1996)
Zimelidine, 5 mg/kg/day	LSD, i.v. <i>In vivo</i> electrophysiology	Desensitized	(Blier and de Montigny 1983)
Amiflamine, 2 mg/kg, i.p. bid x 21 days (w/o)	LSD, i.v. <i>In vivo</i> electrophysiology	Desensitized	(Blier <i>et al.</i> 1986a)
Befloxatone, 0.75 mg/kg/day x 21 days (w/o)	8-OH-DPAT, bath application; <i>In vitro</i> [³ H]5-HT release	Desensitized	(Piñeyro and Blier 1996)
Clorgyline, 2.5 mg/kg/day x 21 days	LSD, i.v. <i>In vivo</i> electrophysiology	Desensitized	(Blier and de Montigny 1985)
Phenelzine, 2.5 mg/kg/day x 21 days	LSD, i.v. <i>In vivo</i> electrophysiology	Desensitized	(Blier and de Montigny 1985)
Imipramine 5 and 10 mg/kg/day or iprindole, desipramine and femoxetine, 5 mg/kg/day x 14 days	LSD, i.v. or 5-HT and LSD by microiontophoresis; <i>in</i> <i>vivo</i> electrophysiology	Not desensitized	(Blier and de Montigny 1980)

5.1.2. Monoamine oxidase inhibitors (MAOI's)

The acute administration of MAO type A inhibitors leads to a suppression of the firing activity of 5-HT neurons (Aghajanian *et al.* 1970). In the course of a long-term administration of MAOI's such as phenelzine, clorgyline and amiflamine, 5-HT neurons of the dorsal raphe recover from the initial decrease of firing activity to normal after 21 days of treatment (Blier and de Montigny 1985; Blier *et al.* 1986a). This phenomenon is accompanied by a desensitization of the somatodendritic 5-HT_{1A} receptors, as indicated by a shift to the right of the dose-response curve of the suppressant effect of LSD on the firing activity of 5-HT neurons (Blier and de Montigny 1985; Blier *et al.* 1986a). Somatodendritic 5-HT_{1A} receptors that modulate the release of 5-HT in raphe slices were also shown to be desensitized by long-term administration of befloxadone (Piñeyro and Blier 1996).

5.1.3. Serotonin 5-HT_{1A} agonists

The effects of long-term administration of 5-HT_{1A} agonists on the 5-HT neurons essentially mimic those observed following long-term administration of agents that enhanced raphe concentration of extracellular 5-HT, *i.e.*, SSRI's and MAOI's. Indeed, in the midst of a long-term administration of 5-HT_{1A} agonists, the initial suppression of firing activity gradually fades so that the firing activity of 5-HT neurons is back to normal after 21 days of treatment (Blier and de Montigny 1987). This recovery is accompanied by a desensitization of the somatodendritic 5-HT_{1A} receptors as assessed by the reduced responsiveness of dorsal raphe 5-HT neurons to the direct microiontophoretic applications of 5-HT, LSD, gepirone and 8-OH-DPAT (Blier and de Montigny 1987). Most interestingly, the dose response curve of the suppressant effect of intravenous administration of LSD is shifted to the right in gepirone treated animals but not that for 8-OH-DPAT. These results have lead to the hypothesis that the suppressant effect of systemic administration of 8-OH-DPAT would not be only mediated by a direct activation of the somatodendritic 5-HT_{1A} receptors but also by the activation of a population of postsynaptic 5-HT_{1A} receptors acting on raphe 5-HT neurons through a long-loop (see below for further discussion). Interestingly, exactly the same scheme has been observed following long-term administration of ipsapirone. Indeed, the desensitization of the somatodendritic 5-HT_{1A} autoreceptors was unequivocally demonstrated by the reduced sensitivity of the 5-HT neurons to microiontophoretic application of 5-HT and 8-OH-DPAT (Dong *et al.* 1997) as well as to the bath application in raphe slices of 8-OH-DPAT and ipsapirone (Hamon *et al.* 1990). Whereas the dose- response curve generated with the intravenous administration of LSD was shifted to the right in those treated animals (indicative of a desensitization), that with intravenous administration of 8-OH-DPAT was not (Dong *et al.* 1997). The long-term administration of other 5-HT_{1A} agonists such as alnespirone and flibanserin also leads to a desensitization of the somatodendritic 5-HT_{1A} autoreceptor as assessed using electrophysiological paradigms (Le Poul *et al.* 1999; Rueter and Blier 1999).

It is well established that the acute systemic administration of 5-HT_{1A} agonist leads to a decrease of the extracellular concentration of 5-HT in different areas of the brain consequent of the decrease of firing activity of 5-HT neurons. The degree of the decrease has been often taken as an index of the sensitivity of the somatodendritic autoreceptors. For instance (Kreiss and Lucki 1997) has assessed the degree of decrease of the extracellular level of 5-HT in both the striatum and dorsal hippocampus of rats treated with 8-OH-DPAT for 1, 7 or 14 days. Interestingly, they have found that the decrease of 5-HT levels induced by a 5-HT_{1A} agonist

was blunted but was so to a greater extent in the striatum than in the hippocampus. These authors have suggested that, given the contentions that the striatum is preferentially innervated by the dorsal raphe whereas the hippocampus by the median raphe, the somatodendritic 5-HT_{1A} receptors of the dorsal raphe would be easier to desensitize than their median raphe congener.

5.1.4. Tricyclic antidepressants (TCA's)

As chronic TCAs treatment induced a supersensitivity of postsynaptic 5-HT neurons, Blier and de Montigny (1980) investigated whether a similar mechanism would occur at the level of the somatodendritic 5-HT_{1A} autoreceptors located on the dorsal raphe 5-HT neurons. They reported that the sensitivity of these somatodendritic receptors was unaltered following long-term treatment with the TCAs desipramine and imipramine. In light of this, it appears peculiar that TCA's that are endowed with a substantial 5-HT reuptake inhibition potencies do not induce such a desensitization. To the best of my knowledge, this is the only study that has directly assessed the sensitivity of somatodendritic 5-HT_{1A} autoreceptors following a prolonged treatment with tricyclic antidepressants (see table 2). Nonetheless, the level of receptor number as well as the level of mRNA coding for the 5-HT_{1A} receptors has been assessed following long-term administration of TCA's. Consistent with the above findings, long-term administration of TCA's does not alter either the level of receptors or of mRNA in the raphe (Welner *et al.* 1989; Burnet *et al.* 1994).

5.1.5. Electroconvulsive shocks (ECS)

The sensitivity of the 5-HT_{1A} autoreceptors to the microiontophoretic application of 5-HT and of 8-OH-DPAT is unaltered in rats treated with repeated electroconvulsive therapy (Blier and Bouchard 1992). Consistent with this, the level of mRNA coding the 5-HT_{1A} receptors is unaltered following repeated ECS to rats (Burnet *et al.* 1995).

5.1.6. Integrative aspect

When one considers the corpus of data that has been generated on the effect of antidepressant treatments on the function and sensitivity of the somatodendritic 5-HT_{1A} autoreceptor (table 2), there are several issues that need attention. In terms of general consensus, it seems first unmitigated that sustained administration with tricyclic antidepressants and repeated ECS does not alter the sensitivity of somatodendritic 5-HT_{1A}

autoreceptors. As for 5-HT_{1A} agonists and SSRI's treatments, the situation appears more complex due to the different results obtained following seemingly similar treatment regimens. However, a scrutiny of the different paradigms can easily demonstrate two consensuses. First, when the methods of applications of different agonists allow for their application directly onto raphe 5-HT neurons (*i.e.*, either microiontophoretic or bath application in mesencephalic slices), all studies concur in showing that long-term administrations of either SSRI's or 5-HT_{1A} agonists induces a desensitization of somatodendritic 5-HT_{1A} autoreceptors. Secondly, the systemic administration of the 5-HT_{1A} agonist 8-OH-DPAT proves to be a very poor probe to detect the desensitization of this receptor following prolonged treatments. Perhaps the two best studies to illustrate this issue are those of Jolas *et al.* (1994) and of Dong *et al.* (1997). However, and in sharp contrast to the situation that prevails for 8-OH-DPAT, the systemic administration of the 5-HT non selective agonist LSD has, paradoxically, proved to be a much more reliable probe to disclose the presence of an altered function of the somatodendritic 5-HT_{1A} receptors (see table 2). This is but one of the experimental evidence that suggests that the firing activity of 5-HT neurons of the dorsal raphe (perhaps also of the other raphe nuclei, see Bosker *et al.* 1997) are controlled not only by the activity of the somatodendritic 5-HT_{1A} autoreceptors, but also by postsynaptic 5-HT_{1A} receptors. This hypothesis, formulated as early as 1987, has been received with skepticism. However, and especially lately, it has received various confirmation from different laboratories such as it is now widely accepted. Amongst these confirmations, is the study of Blier *et al.* 1987b, that shows that following a long-term treatment with lithium, which is known to sensitize postsynaptic 5-HT_{1A} receptors of the hippocampus (see below), induces a sensitization of the effect of systemic administration of 8-OH-DPAT for reducing the firing activity without any change in the sensitivity of the somatodendritic 5-HT_{1A} receptors, as assessed by local microiontophoretic application of 5-HT_{1A} agonist. Moreover, in rats in which somatodendritic 5-HT_{1A} receptors are inactivated by intraraphe injection of pertussis toxin, the systemic administration of 8-OH-DPAT is still capable of reducing the level of striatal release (Romero *et al.* 1994) thus again suggesting the presence of a non-raphe 5-HT_{1A}-mediated control of firing activity of 5-HT neurons. The regional location of such a postsynaptic 5-HT_{1A} receptor has been suggested by Ceci *et al.* (1994) by showing that the suppressant effect of systemic administration of 8-OH-DPAT on the firing activity of 5-HT neurons of the dorsal raphe is diminished in rats subject to a fronto-cortical deafferentation. This attempt was implemented by that of Hajos *et al.* (1999) showing

that a transection of the medial prefrontal cortex, and not of the parietal cortex, attenuated the effect of systemic 8-OH-DPAT on the firing activity of dorsal raphe nucleus 5-HT neurons and this, without altering their sensitivity to microiontophoretic application 8-OH-DPAT in the raphe. These authors also report the presence of neurons of the prefrontal cortex that are excited by 8-OH-DPAT and that could putatively be involved in this long loop. The same group has also demonstrated both functionally and anatomically that neurons of the ventral prefrontal cortex have an inhibitory projection to 5-HT neurons of the raphe nuclei (Hajos *et al.* 1998). The neurotransmitter that mediates this effect has not been yet identified. Altogether, it is now perhaps universally accepted that the 5-HT_{1A} receptor-mediated control of firing activity of 5-HT neurons does not only imply a somatodendritic location, but also a postsynaptic 5-HT_{1A} receptor located on neuronal element distant of the raphe, in the prefrontal cortex. The unraveling of this pathway reconciles many previous apparent discrepancies and also, prompts for caution when interpreting studies involving effects mediated by 5-HT_{1A} agonists, all the more so when administered via a systemic route.

5.2. Presynaptic 5-HT_{1B/1D} receptors

There are three main experimental approaches that have been used to assess the function and/or sensitivity of the terminal 5-HT_{1B/D} receptor following prolonged antidepressant treatments. These include *in vitro* release experiments in brain slices or synaptosomes, *in vivo* microdialysis experiments and third, a paradigm that involves the *in vivo* stimulation of the ascending 5-HT pathway. The use of the *vivo* stimulation paradigm, essentially used only in our laboratory, has permitted to show that the function of the terminal 5-HT autoreceptor was decrease following long-term administration of various 5-HT reuptake inhibitors such as zimelidine, citalopram, paroxetine and fluoxetine (Chaput *et al.* 1991; Blier *et al.* 1988; Chaput *et al.* 1986b; de Montigny *et al.* 1990; Blier and de Montigny 1983). Similar results have also been obtained in release experiments carried out in brain slices from various regions including the hippocampus and hypothalamus (Moret and Briley 1990; Blier and Bouchard 1994; El Mansari *et al.* 1995). It is tempting to speculate that the desensitization of the terminal 5-HT_{1B} receptors supervenes as a result of an enhanced concentration of extracellular 5-HT acting upon these receptors in an excessive manner. If this were the case, one could expect that a similar pattern of desensitization would prevail following a prolonged treatment with other agents that enhance the synaptic availability of 5-HT. Prolonged administration of

MAOI's (that also enhance the extracellular concentration of 5-HT (Sharp *et al.* 1997) was however shown not to desensitize the terminal 5-HT_{1B} autoreceptors (Blier *et al.* 1988). These results are intriguing in light of the fact that the amount of 5-HT released by action potentials is enhanced in these treated animals (Blier *et al.* 1986b; Chaput *et al.* 1991; Blier *et al.* 1988). Taken together, these sets of observations raise two important issues: 1) what is the mechanism responsible for the enhanced output of 5-HT following electrical stimulation of the ascending 5-HT pathway of rats treated with MAOI's and 2) what is the mechanism responsible for the desensitization of the terminal 5-HT_{1B/D} receptor, since it does not seem to be related to an enhanced concentration of 5-HT. In response to the first question, it is still unclear why this output is enhanced after a prolonged treatment as it does not seem to be related to a desensitization of the 5-HT_{1B} receptor. It has however been suggested to the result of a desensitization of the α_2 -adrenergic heteroreceptor controlling 5-HT release (see below). Assuming that the desensitization of this receptor develops in response to a sustained enhanced activation of the receptor (by endogenous NE), it is unclear how such a desensitization would lead to a greater output of 5-HT. Indeed, the development of such a desensitization would be expected to normalize the inhibitory effect of NE on 5-HT output to that prevailing in control situations. It seems that inhibiting the catabolism of 5-HT leads to a greater release of 5-HT per action potentials. In response to the second question, Blier *et al.* (1988) proposed that a functional link between the 5-HT transporter and the terminal 5-HT_{1B/D} receptor could exist, which link would be activated by 5-HT reuptake inhibitors and hence lead to a desensitization of the terminal 5-HT_{1B/D} receptors. In contrast, MAOI's would not activate this link and would thus not induce such a desensitization. Although this hypothesis has not yet been refuted, the presence of such a "molecular link" between the two entities has not been convincingly demonstrated. There exist however several studies claiming to have identified such a link. The proponents of this view rely mainly on experiment showing that the inhibitory effect of terminal 5-HT autoreceptor agonists is diminished in the presence of a 5-HT reuptake inhibitor (Langer and Moret 1982; Galzin *et al.* 1985; Moret and Briley 1988). However, experiments using different experimental paradigms, *i.e.*, POP stimulation, or synaptosomes combined with high rates of perfusion show that the effects observed in slices in the presence of 5-HT reuptake inhibitor is mainly due to a competitive effect of endogenous 5-HT and not through a "molecular link" and thus refute such an interpretation on a methodological basis. It is possible that the reasons why sustained and prolonged

administration of MAOI's does not induce a desensitization relies on the extent of exposure of the terminal 5-HT_{1B} receptor to extracellular 5-HT induced by MAOI's and SSRI's treatments. Indeed, both drugs enhance extracellular concentration of 5-HT by fundamentally different ways. SSRI's by blocking the reuptake of released 5-HT may lead to an enhanced concentration of 5-HT in a much greater area, well beyond the synaptic cleft. In contrast, MAOI's, by enhancing the amount of released 5-HT per action potential, would enhance the concentration 5-HT in smaller area around the synaptic cleft. Indeed, upon each action potential, there would more 5-HT released, greater activation of postsynaptic 5-HT receptors, but the 5-HT would quickly be taken by the nerve terminals by active transport and thus precludes excessive diffusion to the 5-HT_{1B} receptors and hence prevents the development of a desensitization. This contention is further supported by the observation that the 5-HT_{1B} receptors are not confined to terminal axons but would be found preferentially in pre-terminal axons (Sari *et al.* 1997; Riad *et al.* 1996).

5.3. α_2 -adrenergic heteroreceptors located on the 5-HT terminals

The sensitivity of the α_2 -adrenergic heteroreceptors modulating the release of 5-HT has been assessed following treatments with various antidepressant treatments. One of the experimental approaches used involves determining *in vivo* the effects of the α_2 -adrenergic receptor agonist clonidine on the efficacy of the stimulation of the ascending 5-HT pathway on the firing activity of CA₃ pyramidal neurons (an index of 5-HT release). It has indeed been shown that a high dose of clonidine preferentially acts on the α_2 -adrenergic heteroreceptors, thereby decreasing the release of 5-HT (Mongeau *et al.* 1993). The use of this approach has shown that long-term treatments with drugs that enhance the synaptic availability of NE (befloxatone and nisoxetine) leads to diminished effect of the high dose of clonidine on the release of 5-HT, indicative of a desensitization of the α_2 -adrenergic heteroreceptors (Mongeau *et al.* 1994b). *In vivo* microdialysis and *in vitro* release studies have also demonstrated a functional desensitization of the latter receptor following the long-term administration of drugs that enhance the synaptic concentration of NE, including desipramine (Blier and Bouchard 1994; Yoshioka *et al.* 1995).

Long-term administration of type A MAOI clorgyline, phenylzine and amiflamine increases the efficacy of the electrical stimulation of the 5-HT pathway for suppressing firing

activity of CA₃ pyramidal neurons (Blair *et al.* 1986a; Blair *et al.* 1986b). This outcome was however not replicated with the reversible MAOI-A inhibitor befloxatone (Haddjeri *et al.* 1998b). As for the effects of the former three MAOI's is known (see above) not to result from a 5-HT_{1B/1D} desensitization, this increased 5-HT neurotransmission could be the result of a modification of a functionally important modulation of the 5-HT system by the noradrenergic system. *In vitro* slices superfusion (Blair and Bouchard 1994) and *in vivo* electrophysiological experiments have shown that a long-term treatment with befloxatone induces a desensitization of rodent terminal α_2 -adrenergic heteroreceptor controlling 5-HT release. The requirement of an intact noradrenergic system in mediating this effect is clearly demonstrated as this α_2 heteroreceptor desensitization is prevented by a 6-OHDA pretreatment (Mongeau *et al.*, 1994). It is however unclear how the desensitization of the terminal α_2 adrenergic heteroreceptor by itself can lead to an enhanced 5-HT output (induced by electrical stimulation of the 5-HT bundle). As an illustration of this, long-term administration of nisoxetine (a NE reuptake inhibitor) desensitizes the α_2 adrenergic heteroreceptor and will not result in an enhanced output of 5-HT. Presumably, the desensitization of the heteroreceptor leads to a normalization to control level of the inhibitory effect of this receptor on 5-HT release. Accordingly, the desensitization of the α_2 -adrenergic heteroreceptor by some MAOI's will not lead *per se* to an enhanced 5-HT output, but will hamper the inhibitory role of the heteroreceptor to control value. It thus appears that the enhanced output of 5-HT induced by some MAOI's would be dependent, but not the result, on a desensitization of the α_2 -adrenergic heteroreceptor.

5.4. Postsynaptic 5-HT_{1A} receptors

5.4.1. Tricyclic antidepressants (TCA's)

Postsynaptic 5-HT receptors in limbic areas such as the lateral geniculate nucleus (Menkes and Aghajanian 1981; de Montigny and Aghajanian 1978) the amygdala (Wang and Aghajanian 1980), the facial motor nucleus (Menkes *et al.* 1980) and hippocampus (Chaput *et al.* 1991; Gravel and de Montigny 1987; Gallagher and Bunney, Jr. 1979) (de Montigny and Aghajanian 1978) were shown to be sensitized following long-term, but not acute or subchronic, administration of TCAs such as desipramine, imipramine, iprindole, amitriptyline, chloroimipramine. Interestingly, this was not observed with other psychoactive

drugs such as chlorpromazine (de Montigny and Aghajanian 1978). In keeping with this sensitizing effect on postsynaptic 5-HT receptor function, the efficacy of the stimulation of the ascending 5-HT pathway (mediated by activation of postsynaptic 5-HT_{1A} receptors by endogenous 5-HT) is enhanced following a long-term administration of TCA's. It is here important to emphasize the sensitization of postsynaptic 5-HT_{1A} receptors induced by long-term administration of iprindole. Indeed, this tricyclic compound presents, unlike the other TCA's, the remarkable feature of whereas possessing antidepressant activity, it is devoid of any 5-HT or NE reuptake inhibition properties. It can thus be inferred from this contention that the sensitizing effect of long-term administration of TCA's is independent of reuptake inhibition but would be related somehow on the tricyclic moiety. In keeping with this, SSRI's do not sensitize postsynaptic 5-HT_{1A} receptors (see below). The availability of selective NE reuptake inhibitors that do not show a tricyclic moiety being only recent, it will be interesting to assess the sensitivity of postsynaptic 5-HT_{1A} receptors although, in light of the results obtained with iprindole, it seems reasonable to predict that such compound will not induce any sensitization of postsynaptic 5-HT_{1A} receptors.

The function of postsynaptic 5-HT_{1A} receptors following long-term administration of TCA's was also assessed using an *in vitro* electrophysiological approach that, however, generated conflicting results. Indeed, whereas some authors have replicated the general consensus brought by the *in vivo* electrophysiological studies (see above) (Dijcks *et al.* 1991; Bijak and Papp 1995; Bijak and Tokarski 1994; Bijak *et al.* 1996) others have not (Beck and Halloran 1989; Olpe *et al.* 1984; Rowan and Anwyl 1985). A scrutiny of the various approach reveals that most of the inconsistencies rely on methodological considerations. Indeed, all studies involving extracellular recordings of population spikes in the CA₁ region of the hippocampus (elicited by stimulation of the Schaffer's collateral) have shown that the suppressant effect of 5-HT (and also of 8-OH-DPAT) on the population spike amplitude is enhanced following prolonged treatment with TCA's such as desipramine, imipramine and oxaprotiline (Bijak and Papp 1995; Dijcks *et al.* 1991; Bijak *et al.* 1996; Bijak and Tokarski 1994). The study of Rowan and Anwyl (1985) reported however a decrease of the 5-HT induced decrease in population spike amplitude in imipramine treated animals. In this study, the authors have used a high resistance microelectrode to stimulate the stratum radiatum and as suggested by (Bijak *et al.* 1996)“ . . . a field excitatory postsynaptic potential seemed to dominate the population spike in the recording and, therefore, possible effect of 5-HT on

glutamate release or on the local circuitry were likely to prevail". It is thus possible that the response elicited in the study of Rowan and Anwyl (1985) was different from that of other studies. In support of this, is the fact that the response elicited by 5-HT on the effects of the stimulation of the stratum radiatum was blocked by cyproheptadine and ketanserin (Rowan and Anwyl 1985) and thus resembles more a 5-HT₂ response than one mediated by 5-HT_{1A} receptors. In addition, intracellular recordings of hippocampal pyramidal neurons have not been able to show any change following prolonged treatment with imipramine on 5-HT-induced membrane hyperpolarization or reduction of the amplitude of the slow afterhyperpolarization elicited by a train of action potential (Beck and Halloran 1989). Finally, TCA treatments, including iprindole (Newman *et al.* 1992) do not alter the 5-HT_{1A}-mediated inhibition of adenylate cyclase activity in hippocampal homogenate (Varrault *et al.* 1991). These divergent results suggest that the response involved in the coupling of the 5-HT_{1A} receptor to the K⁺ channel, and not that involved for coupling to the adenylate cyclase would be sensitized.

It is obviously tempting to assume that the functional sensitization of the 5-HT_{1A} mediated response following treatment with various TCA would be accompanied by a concurrent increase in 5-HT_{1A} receptor number. To test this hypothesis, autoradiography method has been used to quantify the level of expression of 5-HT_{1A} receptors in different regions of the brain following prolonged treatment with TCA's. As can be seen from table 3, these studies have not been able to generate a suitable consensus. Indeed, the result of our search for studies assessing the receptor densities following long-term treatment with TCA's shows that whereas three studies report an increase in 5-HT_{1A} receptor number, four studies report no change and two studies even report a decrease in 5-HT_{1A} receptor number. We have failed to provide a suitable explanation for this discrepancy within a methodological context although one can ponder on the suggestion of Burnet *et al.* (1994) that some of the differences might be due to strain differences. This difference is substantiated by *in situ* hybridization, as it was shown that following long-term administration of imipramine, the level of mRNA coding for the 5-HT_{1A} receptor is unchanged in the hippocampus of LEW/n rats, whereas it is decreased in that of F344/n rats. Taken together, however, it is striking to compare the strong consensus obtained from functional electrophysiological studies showing an increase in the sensitivity of postsynaptic 5-HT_{1A} receptor following long-term administration of TCA's to the absence of a consensus obtained from quantitative autoradiography. In light of this, it would seem

hazardous to conclude that the enhanced responsiveness of postsynaptic 5-HT_{1A} receptors would find its substrate in an increase receptor number and thus, we face a situation whereby the mechanism underlying a phenomenon discovered more than 20 years ago is yet to be elucidated.

Table 3.

Drug and Regimen	Ligand	Change in Bmax and region	References
Alnespirone 5 mg/kg ip x 21 days	[³ H]8-OH-DPAT	N.c. in all regions	(Le Poul <i>et al.</i> 1999)
Buspirone 10 mg/kg/day ip, bid x 21 days	[³ H]5-HT	Decrease in dorsal raphe	(Gobbi <i>et al.</i> 1991)
Gepirone, 10 mg/kg/day x 21 days	[³ H]8-OH-DPAT	Decrease in dorsal raphe, n.c. in hippocampus	(Welner <i>et al.</i> 1989)
Ipsapirone 10 mg/kg ip x 21 days	[³ H]8-OH-DPAT	Decrease in DRN N.c. in median raphe, and hippocampus (N.c. in raphe after 14 days)	(Fanelli and McMonagle-Strucko 1992)
Ipsapirone 5 mg/kg/day ip, bid x 14 days	[³ H]8-OH-DPAT	N.c. in all regions studied	(Schechter <i>et al.</i> 1990)
Fluoxetine and Gepirone, 10 mg/kg/day x 21 days	[³ H]8-OH-DPAT	Decrease in dorsal raphe, n.c. in hippocampus	(Welner <i>et al.</i> 1989)
Citalopram, 20 mg/kg, i.p. x 21 days	[³ H]8-OH-DPAT	n.c. ³ in all regions studied	(Hensler <i>et al.</i> 1991)
Sertraline 5 mg/kg bid x 21 days	[³ H]8-OH-DPAT	N.c. in all regions studied	(Hensler <i>et al.</i> 1991)

³N.c.: No change

Fluoxetine 10 mg/kg/day ip x 3, 7, 14 or 22 days	[³ H]8-OH-DPAT	N.c. in all regions tested (Hypothalamus, hippocampus, raphe, Cortex <i>inter alii</i>)	(Li <i>et al.</i> 1997)
Paroxetine 10 mg/kg/day, po bid (w/o)	[³ H]8-OH-DPAT	Decrease in hippocampus	(Maj <i>et al.</i> 1996)
Amitriptyline 10 mg/kg/day x 21 days	[³ H]8-OH-DPAT	Increase in dorsal hippocampus, n.c. in dorsal raphe	(Welner <i>et al.</i> 1989)
Desipramine 10 mg/kg/day ip x 28 or 35 days	[³ H]5-HT	N.c. where ?	(Newman <i>et al.</i> 1990)
Imipramine mg/kg/day x days	[³ H]8-OH-DPAT	N.c. in hippocampus	(Hayakawa <i>et al.</i> 1994)
Imipramine 10 mg/kg/day, po bid	[³ H]8-OH-DPAT	Increase in hippocampus	(Maj <i>et al.</i> 1996)
Imipramine, 20 mg/kg/day, ip x 21 days	[³ H]8-OH-DPAT	Decrease in hippocampus and frontal cortex	(Mizuta and Segawa 1989; Mizuta and Segawa 1988)
Imipramine, 10 mg/kg bid x 14 days	[³ H]8-OH-DPAT	N.c. in all regions of the hippocampus	(Bijak <i>et al.</i> 1996)
Imipramine 5 mg/kg/day x 56 days	[³ H]8-OH-DPAT	Increase in hippocampus of LEW/N rats N.c. in hippocampus of F344/N rats N.c. in raphe of both strains	(Burnet <i>et al.</i> 1994)
Imipramine mg/kg/day x 14 days	[³ H]8-OH-DPAT	N.c. in hippocampus	(Watanabe <i>et al.</i> 1993)
Imipramine 15 mg/kg/day ip x 14 days	[³ H]8-OH-DPAT	N.c. in frontal cortex and hippocampus	(Newman <i>et al.</i> 1991)
Clorgyline, 1 mg/kg i.p. x 21 days	[³ H]8-OH-DPAT	Essentially n.c. in all regions studied (small decrease in hypothalamus)	(Hensler <i>et al.</i> 1991)

Phenelzine, 5 mg/kg, i.p. x 21 days	[³ H]8-OH-DPAT	Essentially n.c. in all regions studied (small decrease in hypothalamus)	(Hensler <i>et al.</i> 1991)
Tranylcypromine, 5 mg/kg i.p. x 21 days	[³ H]8-OH-DPAT	Essentially n.c. in all regions studied (small increase in CA _{2/3} region of hippocampus)	(Hensler <i>et al.</i> 1991)
ECS, 5 times/day x 10 days	[³ H]8-OH-DPAT	Increase in DG, decrease in CA _{3c} , n.c. in other regions including dorsal raphe and CA ₁ and CA _{3b}	(Burnet <i>et al.</i> 1995)
ECS	[³ H]8-OH-DPAT	Increase in dentate gyrus	(Hayakawa <i>et al.</i> 1994)
ECS	[³ H]8-OH-DPAT	Increase in frontal cortex	(Nowak and Dulinski 1991)

5.4.2. Electroconvulsive shocks (ECS)

Repeated, but not single, ECS therapy to rats was shown to be accompanied by an increase in the sensitivity of hippocampus pyramidal neurons to 5-HT (*i.e.*, postsynaptic 5-HT_{1A} receptors) and to NE or GABA (de Montigny 1984; Mongeau *et al.* 1994a). Consistently, repeated ECS therapy to rats is also accompanied by an enhanced efficacy of the stimulation of the ascending 5-HT pathway for suppression of the firing activity of CA₃ pyramidal neurons of the dorsal hippocampus (Chaput *et al.* 1991). Paradoxically, repeated ECS induces a slight, but significant, reduction in 5-HT's ability to inhibit adenylate cyclase activity in hippocampal homogenate (Varrault *et al.* 1991; Newman and Lerer 1988). This may appear consistent with the reduction in the 5-HT_{1A} receptor number following repeated ECS in the CA₃ region of the hippocampus (Burnet *et al.* 1995). However, repeated ECS consistently leads to an increase of [³H]8-OH-DPAT level in the dentate gyrus which is also accompanied by an increase in mRNA coding for the 5-HT_{1A} receptor in this region (Hayakawa *et al.* 1994; Burnet *et al.* 1995). In the frontal cortex, repeated ECS also leads to an increase in 5-HT_{1A} receptor densities (Nowak and Dulinski 1991).

5.4.3. Monoamine oxidase inhibitors (MAOI's)

The sensitivity of postsynaptic 5-HT_{1A} receptors following long-term treatment with various MAOI's has been assessed using an *in vivo* electrophysiological approach. Thus, long-term administration of deprenyl and phenelzine does not alter the sensitivity of postsynaptic 5-HT_{1A} receptors (Blier *et al.* 1986b). However, the efficacy of the stimulation of the ascending pathway is enhanced following a long-term treatment with phenelzine and not with diphenyl. This indicates that there is most probably an enhanced synaptic release of 5-HT upon each electrical stimulation in phenelzine treated animals. Diphenyl, by blocking only MAO type A, does not enhance the releasable pool of 5-HT. These results are nonetheless at variance with those generated by the inhibition of adenylate cyclase activity by 5-HT in hippocampal homogenate. Indeed, long-term administration of tranylcypromine (Sleight *et al.* 1988) as well as of clorgyline (Sleight *et al.* 1988; Varrault *et al.* 1991) results in a reduction of the ability of 5-HT to inhibit the production of cAMP.

Interestingly, this desensitization following long-term administration of clorgyline was also observed using an *in vivo* electrophysiological paradigm (Blier *et al.* 1986b). This observation is also substantiated by a quantitative autoradiography study that shows a decreased number of [³H]8-OH-DPAT binding sites following a clorgyline treatment. Nonetheless, and in contrast to the results obtained by (Varrault *et al.* 1991; Sleight *et al.* 1988), this desensitization of postsynaptic 5-HT_{1A} receptors is observed only with clorgyline and not with tranylcypromine (Blier *et al.* 1986b).

5.4.4. Serotonin reuptake inhibitors (SSRI's)

Long-term administration of various 5-HT reuptake inhibitors such as fluoxetine (de Montigny and Aghajanian 1978) does not result in any change in the sensitivity of postsynaptic 5-HT_{1A} receptors when assessed using an *in vivo* electrophysiological paradigm. At variance to this, is the observation that prolonged treatment with zimelidine and fluoxetine were shown to decrease the ability of 5-HT to inhibit forskolin-stimulated accumulation of cAMP (Newman *et al.* 1992). This outcome was however not replicated by Varrault *et al.* (1991) using also fluoxetine. As for the 5-HT_{1A} receptor number in the hippocampus following long-term administration of SSRI's, autoradiography studies have also generated conflicting results (see table 3) although there is a stronger consensus that SSRI's administration does not alter the receptor number in the hippocampus.

5.4.5. Serotonin 5-HT_{1A} receptor agonists

Long-term administration of 5-HT_{1A} agonists does not alter the sensitivity of postsynaptic 5-HT_{1A} receptors in the hippocampus when assessed by *in vivo* electrophysiological paradigms (Blier and de Montigny 1987). Indeed, the effect of the microiontophoretic application of 5-HT and 5-HT_{1A} agonists was not altered in rats that were treated for 21 days with gepirone nor was the effect of the stimulation of the ascending 5-HT pathway (Blier and de Montigny 1987). In keeping with this, autoradiography studies have consistently failed to detect any change in the 5-HT_{1A} receptor number in the hippocampus following long-term administration of various 5-HT_{1A} agonists. Moreover, a treatment with ipsapirone does not alter the ability of 5-HT to inhibit forskolin stimulated accumulation of cAMP in the dorsal raphe (Varrault *et al.* 1991)

5.5. Integrative aspect

Determining the isolated effects of acute or long-term effects of antidepressant treatments on specific targets provides very little valuable information if not considered in a functional and global context. With this in mind, the corpus of data summarized above can be integrated in such way as to propose that effective antidepressant treatments increase the 5-HT neurotransmission and this, with a time scale that is congruent with the clinical onset of these therapies in treating major depression. This proposition can further be refined as it was lately shown that various effective antidepressant treatments all enhanced the tonic activation of postsynaptic 5-HT_{1A} receptors (Haddjeri *et al.* 1998). However, inasmuch these antidepressant treatments enhance 5-HT_{1A}-mediated neurotransmission, they do so *via* distinct mechanisms. For instance, the main effect of treatment with TCA's on 5-HT related synaptic event is the induction of an enhanced sensitivity of postsynaptic 5-HT_{1A} receptors. This effect is shared with repeated administration of ECS. Treatments with SSRI's, conversely, imply essentially a presynaptic effect that leads to an increase of 5-HT release. Whereas such an enhancement of 5-HT output could be expected to occur earlier in treatment as a result of acute 5-HT reuptake blockade, it is hampered by an overactivation of two major serotonergic homeostatic mechanisms, *i.e.*, the somatodendritic 5-HT_{1A} and terminal 5-HT_{1B} autoreceptors. In the midst of treatment, these two populations of receptors desensitize and thus allow for the development of a parallel enhancement of the net throughput of 5-HT, which occurs in

presence of continuous 5-HT reuptake blockade. This outcome is experimentally reflected by *in vivo* microdialysis studies showing that the extracellular concentration of 5-HT in terminal areas is greater after long-term treatments with agents that block 5-HT reuptake than what can be obtained in control conditions (Bel and Artigas 1993; Kreiss and Lucki 1995). The long-term administration of 5-HT_{1A} agonists, in turn, involves both a pre- and a postsynaptic mechanisms. Indeed, this leads to activation of presumably both the somatodendritic and postsynaptic 5-HT_{1A} receptors and hence reduces the firing activity of 5-HT neurons. In the midst of treatment with 5-HT_{1A} agonists, somatodendritic 5-HT_{1A} autoreceptors desensitize, hence normalizing the firing activity of 5-HT neurons. Given that their clinical effects also supervene after several weeks of treatments, it seems that the sole activation of postsynaptic 5-HT_{1A} receptors is insufficient to sustain an antidepressant effect, as a recovery of firing activity of 5-HT neurons parallel faithfully the time course observed clinically. Much like SSRI's, treatments with MAOI's enhance the activation of postsynaptic 5-HT_{1A} receptors by enhancing the output of 5-HT. Whereas the acute blockade of the catabolism of 5-HT enhances the extracellular concentrations of 5-HT in the terminal regions, it also does so in the raphe (Bel and Artigas 1995; Celada and Artigas 1993; Ferrer and Artigas 1994) and thus leads to a reduction of firing activity of 5-HT neurons. Therefore, the sustained blockade of MAO in combination with a normal firing activity of 5-HT neurons would underlie the capacity of treatments with MAOI's to enhance the tonic activation of postsynaptic 5-HT_{1A} receptors. Mirtazapine is an α_2 -adrenergic, 5-HT₂, 5-HT₃ and H₁ receptor antagonist that has been shown to possess antidepressant activity. Although it has not been covered hereabove, the neurobiological effects of this antidepressant deserve attention in the present discussion. Long-term administration with mirtazapine enhances the tonic activation of postsynaptic 5-HT_{1A} receptors in the hippocampus (Haddjeri *et al.* 1998). Experimental data suggest that this phenomenon develops mainly by enhancing the output of 5-HT by a presynaptic mechanism. It is proposed that the antidepressant effect of mirtazapine is due to its α_2 -adrenergic receptor antagonism. As such, an antagonism of mirtazapine has convincingly been demonstrated on the somatodendritic α_2 -adrenergic autoreceptors, on terminal α_2 -adrenergic auto- and heteroreceptors, as well as on postsynaptic α_2 -adrenergic receptors using *in vivo* electrophysiological paradigms (Aulakh *et al.* 1996). Moreover, the acute administration of mirtazapine enhances the extracellular concentrations of 5-HT (De Boer *et al.* 1994). This phenomenon has been proposed to result from the blockade of terminal α_2 -adrenergic

heteroreceptors located on 5-HT terminals as well as from blockade of α_2 -adrenergic autoreceptors that would enhance the firing activity of 5-HT neurons (by enhancing the α_1 -adrenergic mediated tonic excitatory input). Nonetheless, this acute enhancement of 5-HT is insufficient for antidepressant efficacy. Studies on the effects of long-term administration of mirtazapine have documented that, first, mirtazapine leads to marked increases in the firing activity of 5-HT neurons and second, leads to a desensitization of the α_2 -adrenergic heteroreceptors (Haddjeri *et al.* 1997). It is thus possible that these properties act in concert to enhance 5-HT output in a way to enhance the tonic activation of postsynaptic 5-HT_{1A} receptors.

Altogether, one is confronted with an impressive corpus of data that converge in demonstrating a commonality of actions of effective antidepressants on the 5-HT neurotransmission. By a simple transposition process, strategies aimed at increasing this neurotransmission should thus be useful in the treatment of major depression. This contention has received over the years tremendous attention and still constitutes the primary premise to most rational approach aimed at developing more efficacious and faster therapies for the treatment of major depression. It is interesting however to note that there exist newer antidepressants which primary pharmacological actions suggest that they are not acting on 5-HT neurotransmission. In this regard, the efficacy of the NE reuptake inhibitor reboxetine (Berzowski *et al.* 1997) and of the substance P antagonist (Kramer *et al.* 1998) in the treatment of major depression represent the most notable examples. It will be of utmost interest to assess the effects of these agents on parameters involved in mediating the 5-HT neurotransmission and thus to determine if they conform to the commonality of actions antidepressant treatments described herein.

6. ON THE ANATOMY OF THE NE SYSTEM

There are seven NE cell groups in the CNS, denoted A1 to A7. There are two principal groups of nuclei in the brain stem that contains NE: the locus coeruleus and the lateral tegmental neurons which are more scattered in the medullary lateral pontine tegmentum. The former noradrenergic cell group will be most described here.

6.2 The locus coeruleus

The neurons of the locus coeruleus give rise to the brain's major NE innervation. This system is uniquely divergent in such ways that the locus coeruleus is the most widely projecting CNS nucleus known (Foote, 1983). The nucleus locus coeruleus is named so because it shows a slightly blue colour when examined in fresh human tissue (from Latin *caeruleum* meaning sky, blue). There are two loci coeruleus located bilaterally. Under Nissl-stained sections, the locus coeruleus appears as a densely packed cluster of darkly stained cells. Each nucleus is shaped like a tapered cylinder that extends *ca.* 1 mm in the rostrocaudal axis and is roughly 300 μm in diameter at its widest dorsoventral extent (Bezin *et al.* 1994). The locus coeruleus runs along the ventrolateral edge of the fourth ventricle. Laterally and ventrally, the locus coeruleus is bordered by the mesencephalic V (trigeminal) nerve which is often used as an anatomical landmark for *in vivo* electrophysiological recordings.

It is interesting to note that, unlike what is observed in cats, rabbits and guinea pigs, the rat and primate locus coeruleus nuclei are composed almost exclusively of NE neurons. Various studies have estimated the number of NE neurons per locus coeruleus nucleus (see Aston-Jones *et al.* 1995) which range from 1400 to 1800 neurons. The dendritic arbor of any individual locus coeruleus neuron is preferentially oriented in the horizontal plane along the rostrocaudal axis and extends throughout and beyond the cell body region (Shipley *et al.* 1996; Ivanov and Aston-Jones 1995; Aston-Jones *et al.* 1991) into pericoerulear area (See figure 4). This region thus participates in the integration of afferent inputs to the locus coeruleus.



Figure 4: Horizontal section through the LC showing a single biocytin-filled NE neurons. The axon of this cell is indicated by the arrows. Note that the dendrites extend beyond the limits of the LC proper, indicated by the dashed lines. The figure is adapted from Shipley et al. 1996.

A careful scrutiny of the morphology of different cells was achieved in the study of Loughlin *et al.* (1986). Indeed, it was observed that cells within the locus coeruleus are morphologically diverse and heterogenous. First, large, multipolar cells,, with no obvious orientation of cell body along any dimension are preferentially found in the ventral locus coeruleus. They show at least three dendrites. These cells project mainly to the spinal cord. Second, fusiform cells that have a length to width ratio of at least 2:1, the width being at least $12\ \mu\text{m}$ have been identified. These cells show a strong antero-posterior orientation. The locus coeruleus has also medium-size cells of around 15 to $20\ \mu\text{m}$ in diameter, with at least three visible dendrites distributed throughout the body of the locus coeruleus. Last, small round cells of less than $10\ \mu\text{m}$ can be observed. The function and nature of these cells still remain debated. It appears however that they are probably not NE-containing cells (Loughlin *et al.* 1986).

6.2. Lateral tegmental Nucleus:

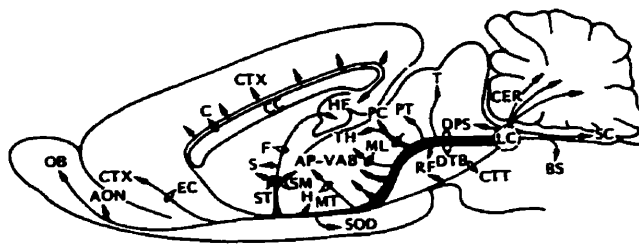
The locus coeruleus contains approximately half of the NE containing neurons in the brain stem (see below). The rest is found diffusely throughout the lateral tegmentum. These

neurons compose what has been referred to as the A5 and A7 cell groups. They project to the spinal cord, the brain stem, thalamus, cerebellar and cerebral cortices. It is noteworthy that in these cortices, the input from these neurons is of minor importance compared to that of the locus coeruleus. In general, the projections from A5 and A7 neurons do not overlap with those of the locus coeruleus. Essentially, it can be assumed that whereas the locus coeruleus provides the major noradrenergic input to the forebrain, A5 and A7 noradrenergic neurons project mainly to the brainstem and spinal cord. These neurons will not be dealt with any further details herein.

6.3 EFFERENT SYSTEM OF THE LOCUS COERULEUS

It is interesting to note that no one-to-one relationship exists between cell type and efferent target, *i.e.*, that no one cell type described above, provides the sole innervation to any one terminal field. Nonetheless, whereas there is a clear heterogeneity within the locus coeruleus with respect to morphology and preferred efferent, there is still a certain degree of efferent specialisation that is emerging. For instance, there is a regional specificity as only cells located

dorsally project to the hippocampus, whereas those located ventrally project to the spinal cord.



Only cells of the locus coeruleus send projections in the dorsal tegmental bundle which emerges in a rostroventral

direction (Lindvall and Bjorklund 1974; Luppi *et al.* 1995). This bundle then gives rise to many decussating fibers that innervate many regions of the brain (See figure 5).

Figure 5: Diagram of the projections of the locus ceruleus viewed in the sagittal plane. Abbreviations: AON, anterior olfactory nucleus; AP-VAB, ansa peduncularis-ventral amygdaloid bundle system; BS, brainstem nuclei; C, cingulum; CC, corpus callosum; CER, cerebellum; CTT, central tegmental tract; CTX, cerebral neocortex; DPS, dorsal periventricular system; DTB, dorsal catecholamine bundle; EC, external capsule; F, fornix; H, hypothalamus; HF, hippocampal formation; LC, locus ceruleus; ML, medial lemniscus; MT, mammillothalamic tract; OB, olfactory bulb; PC, posterior commissure; PT, pretectal area; RF, reticular formation; S, septal area; SC, spinal cord; SM, stria medullaris; SOD, supraoptic decussations; ST, stria terminalis; T, tectum; TH, thalamus. Figure taken from Cooper *et al.*, 1996.

The central tegmental tract is also composed of projection fibers from the locus coeruleus. Axons originating in the locus coeruleus run also in the medial forebrain bundle that also innervate the hypothalamus, thalamus and amygdala. This bundle, ventral to the corpus callosum, divides in a caudal and a rostral branch. The rostral thus run back along the corpus callosum in the fornix superior and eventually carry noradrenergic fibers to the hippocampus (Lindvall and Bjorklund 1974). The entire NE innervation of the hippocampus arises from the locus coeruleus. In addition, the locus coeruleus neurons projecting to the locus coeruleus are found in the upper one third of the locus coeruleus. Every layer of the hippocampus is innervated by noradrenergic axons (Oleskevitch, *et al.* 1989). Noradrenergic axons are found to make synaptic contacts on GABAergic interneurons of the hippocampus as well as on pyramidal neurons. Of note, only a small proportion of noradrenergic varicosities form synapses (ca. 15%; Umbriaco *et al.* 1995).

6.4. Afferent system to the locus coeruleus

There is a general consensus in the literature that the afferent network to the locus coeruleus is much more restricted than its efferent projection system. Nonetheless, there is debate on the exact nature and extent of the afferent system to the locus coeruleus due, amongst others perhaps, on different retrograde tracing techniques. Early retrograde tracer studies using iontophoretic application of horseradish peroxidase (HRP) have found that regions such as insular cortex, the central nucleus of the amygdala as well as the hypothalamus send projections to the locus coeruleus. Moreover, the areas of catecholamine cell groups A1, A2 and A5 appeared to contain many reactive cells and were thus believed to participate in the afferent system to the locus coeruleus (Cedarbaum and Aghajanian 1978; Clavier *et al.* 1976). The brainstem raphe nuclei was also shown to project to the locus coeruleus (Cedarbaum and Aghajanian 1978; Clavier *et al.* 1976; Morgane and Jacobs 1979). Finally, the locus coeruleus was shown to receive afferents from the marginal zones of the dorsal horns of the spinal cord. The existence of these sensory afferents from the spinal cord and the nucleus of the solitary tract is of course consistent with the responsiveness of locus coeruleus neurons to noxious stimuli (Cedarbaum and Aghajanian 1976; Graham and Aghajanian 1971; Nyback *et al.* 1975). In contrast to these reports, a more recent study suggests the presence of an even more restricted efferent projection system to the locus coeruleus (Aston-Jones *et al.* 1986). Indeed these authors suggest that direct afferent to the locus coeruleus arise almost exclusively from

the nucleus paragigantocellularis (PGi; located in the rostral ventrolateral medulla) and from the prepositus hypoglossi (PrH; located in the dorsomedial rostral medulla, slightly rostral to the PGi). This study used very small injections of wheat germ agglutinin conjugated to HRP (WGA-HRP) which injections, according to the authors, were restricted to the locus coeruleus proper. It was thus suggested that the most likely explanation for the presence of this discrepancy with regard to the extent of the afferent system to the locus coeruleus across different studies, is that, in the previous studies, there would have been a spread of the injected tracer to the surrounding area of the locus coeruleus. This outcome would have thus presumably confounded earlier studies that lacked good spatial discrimination so as to reveal a more extensive afferent system. According to this view, the nuclei previously found to innervate the locus coeruleus would be, in fact, strongly innervating areas proximal to the locus coeruleus proper. This view is however not universally accepted (Craig *et al.* 1989) The hypothesis that PGi and PrH are the predominant afferents to the locus coeruleus is also not supported by the demonstration that the destruction of these nuclei fails to block locus coeruleus responses to somatosensory stimuli. This issue has further been studied by (Luppi *et al.* 1995). These authors have observed that retrograde labelling to most, if not all, regions studied was more intense when the injection of the tracer was achieved in the pericoerulear region in addition to that in the locus coeruleus proper. This is fully consistent with the previous observations that locus coeruleus dendrites extend for considerable distance into peri-locus coeruleus regions, especially in the rostromedial and caudal juxtaependymal zones (Shipley *et al.* 1996). This is also important in the case of the serotonergic innervation to the locus coeruleus from the raphe. Indeed, it has been shown that whereas the dorsal raphe innervates only minimally the core of the locus coeruleus, the pericoerulear area are well innervated from the dorsal raphe (Luppi *et al.* 1995). Taken together, it seems that the most important afferent system to the locus coeruleus proper originates from the PGi and PrH, whereas peri-locus coeruleus area (which contains locus coeruleus NE neurons dendrites) would receive inputs from a more extensive network *i.e.*, from other regions such as the central nucleus of the amygdala, spinal cord, prefrontal cortex, dorsal raphe and others. Such a view can reconcile most of the discrepancies reported above.

Numerous transmitters have been postulated to be involved in the afferent systems to the locus coeruleus. Various pharmacological and anatomical studies suggest that the afferent system to the locus coeruleus from the PGi involves a glutamatergic excitatory input. There

is also an adrenergic inhibitory input to the locus coeruleus, of less importance, that originates from the PGI. As for the other major anatomical afferent to the locus coeruleus proper, that from the PrH, it has been shown to involve a GABAergic pathway. Moreover, GABAergic innervation to the locus coeruleus has been reported to arise from neurons located in the preoptic and posterior hypothalamic areas, the ventrolateral part of the periaqueductal gray and also from the dorsal PGI nucleus. In addition to these neurotransmitters systems, enkephalin input arising from both the PGI and PrH, corticotropin-releasing factor (CRF) arising from PGI (although a minor extent), pericoerulear area and from paraventricular hypothalamic nucleus have also been described.

7. ON THE STRUCTURE, FUNCTION, LOCALIZATION AND PHARMACOLOGY OF EFFECTOR ELEMENTS OF THE NE SYSTEM

7.1. The NE transporter

The NE transporter was the first of the monoamine transporter to be cloned from humans (Pacholczyk *et al.* 1991). It has also been cloned from bovine (Lingen *et al.* 1994) and rat brain (Brüss *et al.* 1997). Similar to the 5-HT transporter, it has 12 putative transmembrane domains. The transporter bears a large hydrophilic region (extracellular) between transmembrane regions 3 and 4. The rat transporter possesses a 93% and 91% identity of its amino acid sequence with its human and bovine, respectively (Brüss *et al.* 1997; Lingen *et al.* 1994; Pacholczyk *et al.* 1991)

Receptor autoradiography with various NE reuptake inhibitors has been used to determine the brain distribution of the NE transporter. Whereas ligands such as [³H]desipramine, [³H]tomoxetine or [³H]mazindol were shown to be of limited use because of various methodological issues (high non-specific binding and heterogenous binding for desipramine and binding to other transporter for mazindol and tomoxetine), [³H]nisoxetine has proven to be much more reliable ligand for this purpose (Tejani-Butt *et al.* 1990; Gehlert *et al.* 1995). Thus, very high expression of the NE transporter is found in the locus coeruleus and several thalamic nuclei. Moderate to high levels are found in regions such as the dentate gyrus, raphe nuclei (dorsal>median raphe) and CA₂ and CA₃ regions of the hippocampus. The CA₁ region shows somewhat a lower level of expression. The pattern of expression generally is in accord with that observed for NE. [³H]Nisoxetine has also been used to label the NE transporters in human brain, in which it was observed there is an uneven distribution of NE transporters in a rostro-caudal axis in the locus coeruleus. Such an uneven distribution is also observed in the raphe (Ordway *et al.* 1997). That the NE transporter is expressed mainly, if not solely, on NE terminals is shown by the drastic reduction of labelling in most regions following a NE denervation using either 6-OHDA or DSP-4 (Tejani-Butt *et al.* 1990; Tejani-Butt 1992; Cheetham *et al.* 1996). This is fully consistent with *in situ* hybridization studies that have shown that NE transporter mRNA is detected in the locus coeruleus of rats (Lorang *et al.* 1994) as well as in humans (Eymin *et al.* 1995). Interestingly, the central nucleus of the amygdala, the dorsal medial nucleus of the hypothalamus and the locus coeruleus show little

or no change in transporter levels following a DSP-4, whereas they show diminished levels following a 6-OHDA lesion. These results are likely explained considering the fact that DSP-4 lesioning seems to be mostly effective to target NE terminals that arise from the locus coeruleus compared to those from other NE cell groups. Indeed, the noradrenergic innervation to the amygdala and hypothalamus was shown to arise predominantly from non-locus coeruleus NE neurons. As for the locus coeruleus, it has been shown that the cell bodies are more resistant than terminals to the toxicity of 6-OHDA and DSP-4.

Like the 5-HT transporter, the NE transporter shows an absolute dependence on extracellular Na^+ (Brüss *et al.* 1997; Harder and Bonisch 1985; Friedrich and Bonisch 1986; Lingen *et al.* 1994), a feature now known to be characteristic of Na^+ /cotransport processes, where energy for inward solute transfer is coupled to the energetically favorable influx of Na^+ down its concentration gradient. NE influx also is sensitive to intracellular K^+ , but available data are more consistent with K^+ stimulation at a modulatory site rather than direct K^+ efflux (Harder and Bonisch 1985). Moreover, uptake of NE is Cl^- dependent (Lingen *et al.* 1994; Brüss *et al.* 1997; Harder and Bonisch 1985; Friedrich and Bonisch 1986) and thus, the electrogenic process for NE transport is both Na^+ and Cl^- dependent. However, a novel transport mechanism for NE transport has been identified by patch-clamp analysis of NE transporter functions. Indeed these studies have revealed that in addition to this electrogenic process (that shows a fixed-stoichiometry), the NE transporter shows properties of a channel-like pore in that it would transport NE showing an indefinite stoichiometry (Galli *et al.* 1995; Galli *et al.* 1996). These authors have further shown that both these transport mechanisms are blocked by cocaine and desipramine (Galli *et al.* 1996).

Like the binding of substrates, binding of reuptake inhibitors to the NE transporter is dependent on extracellular Na^+ . For example, the binding of [^3H]desipramine to the NE transporter is Na^+ - and Cl^- -dependent (Schömig and Bonisch 1986; Bonisch and Harder 1986). These results were interpreted as being indicative that reuptake inhibitors binds to the transporter on the substrate recognition site. Moreover, kinetics analysis on NE uptake mechanism carried out in phaeochromocytoma cells (PC12) have supported this view (Schömig *et al.* 1988a; Schömig and Bonisch 1986; Schömig *et al.* 1988b). The fact that drugs such as cocaine and desipramine inhibits [^3H]NE uptake in a competitive manner is further supportive of such a model (Hara *et al.* 1998). However, there are several observations that are difficult to reconcile with a model that states that NE and reuptake inhibitors bind to a

common site. For instance,, in contrast to 5-HT for [3H]cyanoimipramine binding (Kovachich *et al.* 1988), NE itself (NE > 1 μ M) was shown to be incapable of displacing [3H]nisoxetine from the transporter (Tejani-Butt 1992) thus suggesting that the binding site of nisoxetine does not overlap the binding site for NE. Another study, however, reports that whereas NE is able to inhibit the binding of [3H]desipramine, it does so less potently than the uptake of [3H]NE itself (Raisman *et al.* 1982). These results can be interpreted as indicative that, perhaps, NE can allosterically regulate the binding of desipramine. Moreover, although some NE reuptake inhibitors competitively inhibit the uptake of NE, there are some compounds that do so in a non-competitive manner, such as ketamine, PCP as well as some sigma receptor ligands(Barker and Blakely 1995; Hara *et al.* 1998), thus demonstrating the presence of an allosteric site on the transporter. It is still unsure on how exactly all the different NE reuptake inhibitors bind on the transporter and make use of such an allosteric site.

There are several clinically effective antidepressant drugs that have NE reuptake inhibition properties that have been shown to bind to the NE transporter, as assessed with [3H]nisoxetine [3H]desipramine, [3H]tomoxetine or [3H]mazindol displacement studies (Cheetham *et al.* 1996; Gehlert *et al.* 1995; Tejani-Butt 1992). In general, there is a very good correlation between the affinities of compounds for the NE transporters and their potencies to inhibit the uptake of [3H]NE from brain slices or synaptosomes (Cheetham *et al.* 1996).

7.2. The α -adrenergic receptors

The presence of two subtypes of adrenergic receptors has been demonstrated more than 50 years ago by (Ahlquist.R.P. 1948) and classified, on pharmacological basis, into α and β subtypes. Whereas, three subtypes of β adrenergic receptors are now acknowledged (Emorine *et al.* 1989), two subtypes of α -adrenergic receptors have been proposed (Albano and Amchin 1995). Traditional radioligand binding techniques were able to identify four subtypes of α -adrenergic receptors, *i.e.*, α_{1A} , α_{1B} , α_{2A} , α_{2B} . This was based on different order of affinities of receptor ligand. Molecular approaches were able to further subdivide these families of into more subgroups.

7.2.1. The α_1 -adrenergic receptors.

Three distinct α_1 -adrenergic receptors are found in the brain, α_{1A} -, α_{1B} - and α_{1D} -adrenergic receptors (Pieribone *et al.* 1994; Nicholas *et al.* 1996). Briefly, according to IUPHAR, lowercase subscript ($\alpha_{1a/1b/1d}$) refers to the cloned whereas upper-case subscript refers to their pharmacological homologues ($\alpha_{1A/1B/1D}$). It is of note that considerable confusion has existed regarding the cloned cDNA sequences and endogenous receptor subtypes as defined by pharmacological criteria. Indeed, there has been a misclassification between α_{1A} - and α_{1D} -adrenergic receptor as their pharmacological profile is very similar. Indeed, there is no ligand that allows for their pharmacological discrimination, the latter being only achieved by rank order of potencies of various ligands. One of the main pharmacological criteria is that NE is more potent than adrenaline at the α_{1D} -adrenergic receptor whereas the inverse is true at the α_{1A} -adrenergic receptor (Nicholas *et al.* 1996).

The mRNAs coding for the α_{1a} -, α_{1b} - and α_{1d} -adrenergic receptors are widely distributed in the rat CNS. Up to recently (Day *et al.* 1997; Domyancic and Morilak 1997), the mapping of the α_{1A} -adrenergic receptor was not available. The α_{1a} -adrenergic mRNA (Day *et al.* 1997; Domyancic and Morilak 1997) has a unique and diverse pattern of expression. It is found in brain and spinal cord. High levels are found in the olfactory system, some septal and thalamic nuclei, hypothalamus and moderate levels are found throughout the cortices. In the hippocampus, as well as in the dorsal raphe, generally, low levels are expressed (Day *et al.* 1997; Domyancic and Morilak 1997). Interestingly, in the hippocampus, expression was observed to be confined in non-pyramidal cells (Day *et al.* 1997) although this finding was not observed in the study of Domyancic and Morilak (1997). Very low levels of this mRNA species are expressed in basal ganglia and median raphe. The locus coeruleus expresses low to moderate levels. Conversely, the α_{1b} -adrenergic mRNAs are found in cerebral cortices, thalamus and in dorsal and in median raphe and spinal cord, whereas low (or no) signals are found in hypothalamus and cerebellar cortex and no (or very low) signal is observed in hippocampus, striatum and locus coeruleus (Day *et al.* 1997; Pieribone *et al.* 1994; McCune *et al.* 1993; Nicholas *et al.* 1996). Signal for the mRNAs coding for the α_{1d} -adrenergic receptors are found in the cerebral cortex, thalamus, spinal cord and at very high levels in all regions of the hippocampus while, and this consistently observed across different studies, no signal is found in locus coeruleus, striatum, dorsal raphe as well as hypothalamus (Day *et al.* 1997; Pieribone *et al.* 1994; McCune *et al.* 1993). Thus, it is this receptor subtype

mRNA that predominates in the hippocampus, which expression, in the CA fields, is found in pyramidal cells. It is interesting to note that whereas the locus coeruleus shows high densities of α_1 -adrenergic receptors as assessed with [^3H]prazosin (Chamba *et al.* 1991), no labeling for mRNA coding for either α_{1B} - and α_{1D} -adrenergic receptors is found. These results were interpreted as indicative that in the locus coeruleus, α_1 -adrenergic receptors are mainly found on non-noradrenergic nerve terminals and would act as heteroreceptors (Nicholas *et al.* 1996). However, at that time, the distribution of α_{1A} -adrenergic receptors' mRNA had not been mapped yet. It is thus possible that the α_1 -adrenergic receptors labelling seen in the locus coeruleus might represent α_{1A} -adrenergic receptors (Day *et al.* 1997). Although these receptors can theoretically be somatodendritic autoreceptors, double-labelling studies with catecholaminergic marker are mandatory to ascertain this issue.

The α_1 -adrenergic receptors produce their physiological actions primarily via the phospholipase C-protein kinase C (PLC-PKC) pathway (Summers and McMartin 1993). Activation of the PI pathway would lead, via the generation of diacylglycerol and IP_3 -mediated release of Ca^{2+} from internal stores, to the activation of protein kinase C and to the phosphorylation and regulation of many types of channels and pumps, depending on the cell type. Release of Ca^{2+} from internal stores would also lead to activation of Ca^{2+} /calmodulin-dependent protein kinases and the subsequent phosphorylation and regulation of other channels and pumps. In addition, release of Ca^{2+} from internal stores can influence directly Ca^{2+} -activated K^+ channels.

7.2.2. The α_2 -adrenergic receptors

The α_2 -adrenergic receptors are defined as having a high affinity for rauwolscine and its diastereomer yohimbine. Soon, however, heterogeneity was observed by several laboratories within this subset of α -adrenergic receptors (Bylund and U'Prichard 1983; Vizi *et al.* 1983; Waterfall *et al.* 1985). Molecular biology has allowed to identify three distinct α_2 -adrenergic receptors: α_{2A} , α_{2B} , and α_{2C} . There is also the α_{2D} -subtype but it will generally be discussed herein together with the α_{2A} adrenergic receptor as these two subtypes are species homologues. The α_2 -adrenergic receptors have seven clusters of hydrophobic residues 21-28 amino acid long that presumably represent membrane-spanning domains. These domains are highly conserved among the various subtypes while it is the sequence that codes for the intra-

and extracellular loop which shows different degree of divergence. Molecular probes have shown that the α_{2C} -adrenergic, in contrast to the α_{2A} - and the α_{2B} -adrenergic, can be found at intracellular sites (Rosin *et al.* 1996).

Various tritiated compounds have been used to localize the α_2 -adrenergic receptor expression throughout the brain. These include clonidine, rauwolscine, idazoxan, yohimbine, RX821002 and rimenidine. However, still today, the available ligands poorly discriminate the different subtypes of α_2 -adrenergic receptors. The development of antibodies in combination with that of *in situ* hybridization techniques with specific probes for these receptors subtypes has allowed a much more precise mapping of receptor expression than that achieved with traditional radioligand binding techniques. For these reasons, studies involving the latter techniques will be most dealt with herein. The mRNA's coding for the α_{2A} -adrenergic receptors are present in various regions such as the spinal cord and hypothalamus with especially high amounts in the locus coeruleus (Nicholas *et al.* 1993; Scheinin *et al.* 1994). This receptor subtype mRNA is absent in the dorsal raphe and striatum whereas there is a small signal, just above background, in the hippocampus and thalamus. The α_{2B} -adrenergic receptor mRNA is absent in most regions of interest of the brain with the exception of the thalamus. Finally, α_{2C} -adrenergic mRNA's receptors are found in many regions such as the cerebral as well as cerebellar cortices, hippocampus and striatum, although no signal is detected in the locus coeruleus and dorsal raphe (Nicholas *et al.* 1993; Scheinin *et al.* 1994). In the hippocampus, both of the latter studies concurred in showing that high levels of mRNA are found in the CA₁ region. However, in the CA₃ region, whereas (Nicholas *et al.* 1993) observed low levels, (Scheinin *et al.* 1994) did not detect any signal for this receptor mRNA. The α_{2C} -adrenergic receptor labelling with [³H]MK912 show high level in striatum (Uhlén *et al.* 1997). Interestingly, in this region, the noradrenergic innervation is negligible. A selective α_{2C} -adrenergic antibody has been developed (Rosin *et al.* 1996). Thus, labelling is found in hippocampus, with very high labelling in CA₁ region, consistent with mRNA localization (Nicholas *et al.* 1993; Scheinin *et al.* 1994). Interestingly, the receptors were observed in aggregates frequently distributed in a polarized fashion towards the apical dendrites. Moreover, the labelling of CA₃ cells was of a much lesser intensity (although in caudal regions of the hippocampus, some pyramidal cells of the CA₃ regions displayed high levels of the receptors). The α_{2C} -like immunoreactivity is found in nearly all raphe cell groups. The dorsal

raphe exhibits moderate labelling that increases as we move caudally. The median raphe contains more receptors whereas the most intense labelling is found in the raphe magnus (Rosin *et al.* 1996). Whereas no mRNA is found for this receptor in the raphe region, moderately high binding levels are found when using [³H]rauwolscine (Zilles *et al.* 1993). These results suggest that these receptors are not expressed on 5-HT neurons. Interestingly, nearly every cell in the locus coeruleus is labelled with this antibody (Rosin *et al.* 1996). Although (Rosin *et al.* 1996) report a purely somatic distribution of this receptor, (Lee *et al.* 1998) report that this receptor is located in dendrites of locus coeruleus neurons. That these dendrites were noradrenergic was confirmed by a double-labelling procedure with tyrosine hydroxylase (Lee *et al.* 1998). Recall however that mRNA coding for the α_{2C} -adrenergic receptor is absent in the locus coeruleus (Nicholas *et al.* 1993). The reason underlying this discrepancy is unclear. Finally, the α_2 -adrenergic receptors have been linked in many tissues, including brain, to inhibition of adenylate cyclase activity via activation of G_i/G_o proteins (Summers and McMartin 1993).

8. ON THE FUNCTIONAL ASPECT OF NE NEURONS OF THE LOCUS COERULEUS

8.1. Electrophysiological properties of locus coeruleus NE neurons

Studies in freely moving rats and cats have shown that locus coeruleus NE neurons display a spontaneous firing activity of *ca.* 2 Hz during wakefulness, decrease their activity during slow wave sleep (to a mean firing activity lower than 1 Hz), and are nearly quiescent during paradoxical sleep (Aston-Jones and Bloom 1981a; Gervasoni *et al.* 1998). These studies have also shown that locus coeruleus NE firing activity is markedly responsive to simple stimuli in every modalities tested (visual, auditory, olfactory, somatosensory) consisting in short-latency responses followed by a brief diminished activity (few hundred msec; Aston-Jones and Bloom 1981b; Foote *et al.* 1980; Aston-Jones *et al.* 1991). NE neurons of the locus coeruleus, when recorded *in vivo* in the anaesthetized rats displays typical characteristics including a spontaneous, pacemaker-like, slow (1 to 5 Hz) firing activity with the prominent feature of responding to noxious stimulation (e.g., of contralateral paw) by a burst of firing followed by a quiescent period (Cedarbaum and Aghajanian 1976; Graham and Aghajanian 1971; Nyback *et al.* 1975).

Such pacemaker-like activity is also observed in *in vitro* slice preparations (Andrade *et al.* 1983; Williams *et al.* 1985; Andrade and Aghajanian 1984b; Graham and Aghajanian 1971; Wang and Aghajanian 1987; Williams *et al.* 1984) as well as in acutely dissociated NE neurons (Arima *et al.* 1998; Masuko *et al.* 1986). The latter observations, in contrast to what is observed in raphe cells, strongly implies that the pacemaker activity of locus coeruleus neurons represents an intrinsic property of these neurons, as the neurons in such preparations are devoid of any afferent input that might modulate ionic conductances. The spontaneous firing activity of locus coeruleus NE neurons is believed to result essentially from a balance between two ionic conductances. First, the generation of the action potential results from a Na^+ current that activates near threshold for action potential generation. This action potential will favour the entry of Ca^{2+} in the cell which, in turn, will activate a Ca^{2+} -activated K^+ channel that decays over a period of 1-2 seconds (Aghajanian *et al.* 1983; Andrade and Aghajanian 1984b). This is thought to be a major determinant of the pacemaker cycle. The search for delineating the molecular mechanism responsible for this pacemaker activity has been able to demonstrate a role for the cAMP-dependent phosphorylation pathway. The first clue for the involvement of a diffusible intracellular molecule in mediating the pacemaker activity was provided by the observation that the firing activity of an important fraction of locus coeruleus NE neurons decreases rapidly upon establishing whole-cell patch-clamp recordings (Alreja and Aghajanian 1991a). Indeed, an important wash-out of intracellular components into the low resistance patch pipette is a phenomenon known to be intrinsic to this type of recording. As for the identification of the specific pathway involved, the observation that agents that elevate cAMP levels increase the firing activity of locus coeruleus neurons (Wang and Aghajanian 1987) whereas one (PKI_{5-24}) that inhibits cAMP-dependent protein kinase will hyperpolarize locus coeruleus NE neurons (Alreja and Aghajanian 1991b) suggested a role for the cAMP-PKA pathway. Further evidence was provided by the observation that the run-down in firing activity of locus coeruleus NE neurons under patch-clamp recording is prevented when the patch-electrodes are filled with 500 nM cAMP whereas the firing activity is increased with higher cAMP concentration (Alreja and Aghajanian 1991a). Similar observations were obtained with 8-Br-cAMP (Alreja and Aghajanian 1991a). It is noteworthy that indirect elevation of cAMP by stimulating G_s protein [with intracellular application of the active subunit of cholera toxin] also increased the firing activity of locus coeruleus NE neurons, which effect is blocked by PKI_{5-24} . These findings thus clearly indicate the involvement of the

cAMP-PKA pathway in generating the pacemaker cycle although it is yet to be determined which current exactly is activated by this cascade. The presence of a Ca^{2+} dependent K^{+} current in these neurons was covered above. Given that it is known that cAMP can induce a suppression of Ca^{2+} dependent K^{+} current in the hippocampus (see section 9.1), it represents a likely target. However, it was shown that this current is unlikely to be involved in this case as whereas Co^{2+} will block the latter current (Madison and Nicoll 1986b), it will not block the excitation of locus coeruleus NE firing activity induced by bath application of 8-Br-cAMP (Wang and Aghajanian 1987). Taken together, it appears that in locus coeruleus neurons, endogenous cAMP induce a persistent Ca^{2+} -independent/TTX-insensitive inward current that depolarize the cell membrane and hence represent a major determinant for the generation of the pacemaker cycle of locus coeruleus NE neurons.

8.2. Release of NE in the locus coeruleus

Very few studies have assessed the basic characteristic of NE release directly in the locus coeruleus, owing to its small size. The use of voltammetric techniques, push-pull canulae and more recently of *in vivo* microdialysis have proven more convenient than traditional *in vitro* slice preparations. Whereas the level of DA in the locus coeruleus is diminished by more than 70% by local administration of TTX, that of NE was reduced by only 40% (Singewald *et al.* 1994). These results suggest that whereas some of the NE in the locus coeruleus is dependent on impulse flow, there is an important fraction that is not. The presence of the somatodendritic release of NE in the locus coeruleus is substantiated by the observation that the systemic administration of desipramine enhances the extracellular concentration of NE in the locus coeruleus (Mateo *et al.* 1998).

8.3. Function of the somatodendritic α_2 -adrenergic autoreceptor

8.3.1. Basic characteristics and pharmacology

Microiontophoretic application, bath applications in locus coeruleus slices, as well as of intravenous administrations, of various α_2 -adrenergic receptor agonists such as clonidine suppress the firing activity of locus coeruleus NE neurons which, in some cases, was shown to be reversed by α_2 -adrenergic antagonists (Freedman and Aghajanian 1984; Myers *et al.* 1998; Svensson *et al.* 1975; Cedarbaum and Aghajanian 1977; Aghajanian 1982a; Marwaha and Aghajanian 1982; Lacroix *et al.* 1991b). These observations have provided the basis for

hypothesizing the presence of the so-called somatodendritic NE autoreceptors. Antidromic stimulation of the dorsal NE bundle invariably leads to a poststimulus inhibition of firing of locus coeruleus NE neurons (Aghajanian *et al.* 1977). The fact that this inhibition is dependent on an intact NE bundle and that systemic as well as microiontophoretic applications of piperoxane onto NE cells prevents the appearance of this inhibition provides strong indirect support to the hypothesis of collateral inhibition mediated by an α_2 -adrenergic somatodendritic autoreceptor (Andrade and Aghajanian 1984a). The observation that microiontophoretic application of desipramine in the locus coeruleus prolonged the poststimulus inhibition further supports this hypothesis (Aghajanian *et al.* 1977). Electrical field stimulation of locus coeruleus slices induces the appearance of an α_2 -adrenergic receptor mediated hyperpolarization. Finally, it has been shown by many authors that the acute intravenous administration of various NE reuptake inhibitors suppresses the firing activity of locus coeruleus NE neurons which effect is reversed by α_2 -adrenergic antagonists (Ceci and Borsini 1996; Haskins *et al.* 1985; Scuvée-Moreau and Dresse 1979; Nyback *et al.* 1975; Quinaux *et al.* 1982; Mateo *et al.* 1998). A similar outcome is also observed with other drugs that enhance the synaptic concentration of NE such as duloxetine (a dual 5-HT/NE reuptake inhibitor) and of various monoamine releasers (Kasamo *et al.* 1996; Scuvée-Moreau and Dresse 1990; Graham and Aghajanian 1971).

More recently, there has been an effort to identify which subtype of α_2 -adrenergic receptor is indeed mediating the suppression of firing activity. A pharmacological investigation in acutely dissociated neurons has suggested the involvement of the α_{2B}/α_{2C} adrenergic receptors. This was based on the observation that the NE-induced current was mimicked by the α_2 -adrenergic agonist UK 14, 304 but not by the α_{2A} -adrenergic agonist oxymethazoline. The NE-mediated current was also blocked by the α_{2B}/α_{2C} antagonists ARC 239 and by the α_1 - and α_{2B}/α_{2C} antagonist prazosin (Arima *et al.* 1998). However, another pharmacological analysis carried out in slices reached a different conclusion (Nörenberg *et al.* 1997). Indeed, these authors suggest that the adrenergic receptor controlling firing activity of locus coeruleus neurons is of the α_{2AD} -adrenergic subtype. In this study, in contrast to that of Arima *et al.* (1998), oxymetazoline was shown to suppress the firing activity of locus coeruleus NE neurons. Moreover, whereas prazosin was able to reverse the NE-induced current, in the study of Arima *et al.* 1998) it was devoid of any antagonistic activity in the

study (Nörenberg *et al.* 1997). The reasons underlying these fundamental differences are unclear all the more so that methodological considerations can hardly be hypothesised to be responsible for these differences.

The presence of a tonic activation on the somatodendritic α_2 -adrenergic autoreceptor controlling the firing activity of locus coeruleus NE neurons has been repeatedly demonstrated, since the systemic administration of various α_2 -adrenergic antagonists enhances the firing activity of locus coeruleus NE neurons (Marwaha and Aghajanian 1982; Cedarbaum and Aghajanian 1976; Engberg and Eriksson 1991; Cedarbaum and Aghajanian 1977; Rasmussen and Jacobs 1986c). The finding that the irreversible receptor inactivator EEDQ also increases the firing activity of locus coeruleus neurons is also consistent with this notion (Pineda *et al.* 1997). The presence of a tonic activation by endogenous NE on the somatodendritic is also reflected in studies assessing the release of NE in the locus coeruleus. Indeed, the systemic administration of piperoxane and yohimbine increase the level of NE in the locus coeruleus (Quintin *et al.* 1986). Finally, the observation that in locus coeruleus slices, this enhancing effect is observed with BRL 4408 and not with ARC 239, suggest that the α_2 -adrenergic receptor mediating the release of NE in the locus coeruleus is of the α_{2A} -adrenergic receptor subtype (Callado and Stamford 1999).

Both *in vivo* and *in vitro* approaches concurred in showing that the suppressant effect on the firing activity of locus coeruleus NE neurons induced by the α_2 -adrenergic autoreceptor benefits from a large receptor reserve (Pineda *et al.* 1997; Engberg and Eriksson 1991). These studies involved Furchgott's pharmacological analysis (Furchgott 1966) carried out with the irreversible receptor inactivator EEDQ. Of note, other α_2 -adrenergic receptors-mediated effects were shown to benefit from a receptor reserve. These includes mydriasis (Menargues *et al.* 1991), hypotension (Hamilton and Reid 1985) sedation and hypothermia (Durcan *et al.* 1994).

8.3.2. Ionic mechanism and second messenger pathway

Voltage-clamp analysis in locus coeruleus slices reveals that activation of α_2 -adrenergic autoreceptor leads to the opening of an inwardly rectifying K^+ channels. A similar conclusion was also reached by studies carried out in acutely dissociated locus coeruleus NE neurons (Arima *et al.* 1998). Although the most prominent effect of NE on locus coeruleus cells involves an action on K^+ channels, Williams and North (1985) have documented an

inhibition by NE of Ca^{2+} action potentials but that was not mimicked by clonidine. Such an effect most probably participates in a NE-mediated homoeostatic action on NE neurons although its exact physiological role is still unclear. In any event, the second messenger system underlying the suppressant effect of α_2 -adrenergic receptor stimulation has been sought for. The involvement of a $G_{i/o}$ protein was first shown by the elimination of the α_2 -adrenergic receptor-mediated hyperpolarization of locus coeruleus neurons by a pretreatment with pertussis-toxin (Aghajanian and Wang 1986). Studies in acutely dissociated neurons have confirmed this by showing that the I_{NE} was mimicked and prevented, respectively by GTP γ S and GTP β S. The requirement of a G-protein was further outlined by two other sets of experiments: 1) I_{NE} is reduced by perfusion with the sulfhydryl alkylating agent N-ethylmaleimide (NEM) which uncouples receptors from pertussis toxin-sensitive G-protein and 2) there is a greater incidence of opening of channels when GTP is perfused rather than GDP in the inside-out patch recording mode (Arima *et al.* 1998). Moreover, in the latter study, none of the compounds tested known to act on intracellular signalling pathways displayed any effect on I_{NE} . This outcome had indeed partly been previously observed by Andrade and Aghajanian (1985) who have shown that 8-br-cAMP by itself did not have any effect on the basal firing activity of locus coeruleus NE neurons. However, they have observed that this compound reversed the inhibition of firing induced by clonidine as well as morphine. Considering that both these drugs act on receptors that inhibit the accumulation of cAMP, these results suggest that these receptors suppress firing activity by decreasing the levels of cAMP. This interpretation intrinsically assumes that cAMP tonically activates or “drives” the firing activity of these neurons. Note that this has indeed been observed (see above)

8.4. Serotonergic input to the locus coeruleus

There is a dense innervation of 5-HT containing fibers in the locus coeruleus (Leger *et al.* 1980). This is also substantiated by the observation that intense labelling (comparable to those found in the median raphe but lower than those in the dorsal raphe) of the 5-HT transporter (which is a valid index of serotonergic innervation (Soucy *et al.* 1994)) is found in the locus coeruleus of rodents (Hrdina *et al.* 1990; De Souza and Kuyatt 1987; Biegon and Mathis 1993) and of humans (Cortes *et al.* 1988). An integrative approach to various anatomical reports (Cedarbaum and Aghajanian 1978; Luppi *et al.* 1995) allows to suggest that whereas the pericoerulear regions receive dense innervation from the dorsal raphe, the

serotoninergetic innervation from the dorsal raphe to the core of the locus coeruleus is minimal. The innervation arising from the median raphe is also greater to peri-locus coeruleus regions than to the core of the locus coeruleus. In keeping with these data, anterograde studies with proline show that both the median and dorsal raphe project only minimally to the core of the locus coeruleus. It has also been observed that whereas a lesion of the dorsal raphe does not induce any discernable decrease in the density of 5-HT immunoreactive fibers in the locus coeruleus (Pieribone and Aston-Jones 1990), one of the median raphe induces a partial decrease of 5-HT terminals in the locus coeruleus (Leger *et al.* 1980), suggesting that the median raphe innervation to the locus coeruleus is greater than that from the dorsal raphe. This finding is however at variance with the study of Luppi *et al.* (1995). The different methodologies used can be responsible. This issue was directly assessed and preliminary data from the group of Aston-Jones, using a combination of retrograde staining with 5-HT immunoreactivity, show that following an intense injection in the core of the locus coeruleus, only a small number of cells were double-stained in the dorsal raphe whereas a greater number is observed in the median raphe and B9 5-HT cell group. It is thus possible that the greater number of dorsal raphe cells compared to median raphe cells observed following injection in the core of the locus coeruleus in the study of Luppi *et al.* (1995) were not 5-HT cells. Still, it must be emphasized that an injection directly in the core of the locus coeruleus yields very little staining in the dorsal raphe compared to what is found following an injection into peri-locus coeruleus regions.

Taken together, it appears that the serotoninergetic innervation to the locus coeruleus proper arises mainly from the median raphe and B9 5-HT cell groups. Moreover, this innervation, regardless of whether it originates from the dorsal or median raphe, is more intense in pericoerulear regions. The importance of this latter contention, in a functional context, needs nonetheless to be minimized. Indeed, it is well established that locus coeruleus NE neurons extends dendrites beyond the core of the locus coeruleus thus potentially making synaptic contact to any nerve terminals found in the peri-locus coeruleus areas especially in the rostromedial zones. Hence, it can be assumed that the 5-HT innervation to these regions can be expected to participate in the synaptic input to locus coeruleus NE cells.

Extracellular concentrations of 5-HT can be monitored in the locus coeruleus. That it is of neuronal origin is shown by the fact that TTX and veratridine, suppress and enhance, respectively, the extracellular levels of 5-HT in the locus coeruleus of freely moving rats

(Singewald *et al.* 1997).

There is now ample evidence suggesting that the serotonergic system exert an inhibitory effect on the noradrenergic neurons of the locus coeruleus. However, there is still a lack of knowledge on the intricacies and details of the circuitry responsible for this effects. The presence of a tonic inhibitory action of the serotonergic system is shown by the observation that both a PCPA pretreatment (Reader *et al.* 1986; Ferron 1988) and a 5, 7-DHT lesions (Haddjeri *et al.* 1997) lead to an increased spontaneous firing activity of locus coeruleus NE neurons. This inhibitory input to the locus coeruleus is further evidenced by the decrease in spontaneous and evoked firing activity of locus coeruleus NE neurons by stimulation of the dorsal raphe nucleus, which effect is abolished in PCPA or 5, 7-DHT treated rats (Segal 1979). In addition, the microiontophoretic application of 5-HT was shown in some studies (Segal 1979; Aston-Jones *et al.* 1991), but not others (Haddjeri *et al.* 1997; Gorea *et al.* 1991), to suppress the firing activity of locus coeruleus NE neurons. This effect was, in one study, shown to be antagonized by concurrent microiontophoretic applications of the 5-HT antagonist methysergide (Segal 1979). Noteworthy is the fact that the studies that report a suppression of spontaneous locus coeruleus NE neuron firing activity by microiontophoretic 5-HT have used a higher concentration of 5-HT than that used in studies that report no change. This can be interpreted as being indicative that the receptors mediating the effect of microiontophoretically-applied 5-HT would be located away from the cell body, perhaps on the dendritic tree. This is fully consistent with the anatomical data showing that locus coeruleus NE neurons give rise to an extensive network of dendrites beyond the locus coeruleus proper into pericoerulear zones (Shipley *et al.* 1996). In this regard, whereas an *in vitro* study on locus coeruleus slices report no effects of bath application of 5-HT or 5-CT on membrane potential, firing rate and input resistance of NE neurons (Bobker and Williams 1989), it was shown that bath application of 5-HT elicits a decrease of spontaneous firing activity of NE neurons (accompanied by a decrease in membrane potential; Chiu *et al.* 1995). The latter effect was however observed when only remarkably high concentrations of 5-HT were used thus raising the issue of selectivity of action. Altogether, it seems likely that the important serotonergic input to the locus coeruleus does not exert the bulk of its effect directly on the locus coeruleus NE neurons.

Two main 5-HT receptors have been implicated in the serotonergic modulation of locus coeruleus NE neurons, *i.e.*, the 5-HT_{1A} and the 5-HT₂ receptors.

8.4.1. Mediation by the 5-HT_{1A} receptors

Whereas there is an abundant labeling of [³H]5-HT in the locus coeruleus, that for [³H]8-OH-DPAT is much more scarce (Pompeiano *et al.* 1992). The labelling of neither [³H]5-HT, nor that of [³H]8-OH-DPAT, is significantly altered in 5, 7-DHT treated animals indicating that the 5-HT receptors are mostly located postsynaptically in the locus coeruleus and not on 5-HT nerve terminals (Weissmann-Nanopoulos *et al.* 1985). Moreover, the fact that no hybridization signal for the presence of mRNA coding the 5-HT_{1A} receptors is found in the locus coeruleus (Pompeiano *et al.* 1992), suggest that the 5-HT_{1A} receptors are located on nerve terminals of other neuronal elements such as glutamatergic and GABAergic (see below).

The acute systemic administration of various 5-HT_{1A} agonists enhances the firing activity of locus coeruleus NE neurons (Sanghera *et al.* 1982; Sanghera *et al.* 1990; Piercey *et al.* 1994; Engberg 1992; Broderick and Piercey 1991). Although some of these compounds have 1-(2-pyrimidinyl)-piperazine (which is an α_2 -adrenergic antagonist) as a common metabolite (hence possibly explaining the enhancement of firing activity), the fact that 8-OH-DPAT (that does not generate this metabolite) also induces an increase in firing activity suggests that it is a 5-HT_{1A}-mediated effect (Engberg 1992; Piercey *et al.* 1994). This enhancement of firing activity is reflected in *in vivo* voltammetric experiments which show that the systemic administration of 8-OH-DPAT enhances the DOPAC levels in the locus coeruleus (Clement *et al.* 1992). These results, however, do not provide indications on the location of the 5-HT_{1A} receptor mediating these effects. Three possibilities will be considered herein: 1) the somatodendritic 5-HT_{1A} receptor located on 5-HT neurons; 2) a postsynaptic 5-HT_{1A} receptor located on a non-NE neuronal element (thus participating in a polysynaptic circuit) and 3) a postsynaptic 5-HT_{1A} receptor located on locus coeruleus NE neurons. The latter contention seems the less likely for the following reasons. Throughout the CNS, an inhibitory role, not an excitatory, for the 5-HT_{1A} receptor has been described. Moreover, as stated above, there is lack of 5-HT_{1A} receptor binding in the locus proper as well as the lack of mRNA signal coding for this receptor in the locus coeruleus. Finally, microiontophoretic applications of 8-OH-DPAT do not alter the firing activity of locus coeruleus NE neurons (Gorea *et al.* 1991). The two former possibilities will thus be considered further starting with a possible role of the somatodendritic 5-HT_{1A} autoreceptors. It seems indeed plausible that, by inhibiting the firing activity of 5-HT neurons, 5-HT_{1A} agonists would reduce the small serotonergic tonic inhibition and thereby would lead to an increase in the firing activity of

locus coeruleus NE neurons. Consistently with what has been observed in various terminal regions (with respect to the raphe system), 8-OH-DPAT indeed reduces the extracellular concentration of 5-HT in the locus coeruleus, as determined by push-pull cannula experiments in freely moving rats (Singewald *et al.* 1997). An indirect support to this contention is that the degree of increase of firing activity of locus coeruleus NE neurons induced by either 5-HT_{1A} agonist or 5-HT₂ antagonist is similar. Indeed, as is covered below, there is evidence supporting that the serotonergic inhibitory input would be mediated by the 5-HT₂ receptors. Another crucial evidence supporting the role of the somatodendritic 5-HT_{1A} autoreceptors in mediating the stimulatory effect of 8-OH-DPAT on the firing activity of locus coeruleus NE neurons is that it is prevented by a 5, 7-DHT lesion (S. Szabo, personal communication). It has also been shown that the acute systemic administration of the selective and potent 5-HT_{1A} antagonist WAY 100635, in contrast to agonists, suppresses the firing activity of locus coeruleus NE neurons (Haddjeri *et al.* 1997). That this effect is mediated by an effect on the 5-HT system is shown by its complete elimination by prior serotonergic lesion with 5, 7-DHT. That an increase of endogenous output is involved is also supported by the fact that this suppression was shown to be 5-HT₂-mediated, presumably postsynaptic (Fornal *et al.* 1996). An enhancement of the firing activity of 5-HT neurons by WAY 100635, thereby enhancing 5-HT output in the locus coeruleus, would provide explanation for this phenomenon. However, whereas in the awake cat, the systemic administration of WAY 100635 induces a clear increase in firing activity of 5-HT neurons (Fornal *et al.* 1996), its effect in the anaesthetized rat is much more variable (Forster *et al.* 1995; Martin *et al.* 1999). Electrophysiological recordings *in vivo* in the guinea-pig and *in vitro* in the rat have reported a small increase of firing activity following WAY 100635 (Mundey *et al.* 1995; Corradetti *et al.* 1996). Recordings *in vivo* in the rat have reported variable results whereby, generally, the compound does not alter firing activity (5-100 µg/kg) although some neurons show modest increases (up to 25 %; Forster *et al.* 1995; Martin *et al.* 1999). It is still unknown how does a small increase in firing activity of 5-HT neurons translates in the output of 5-HT in the locus coeruleus. As an indication, some *in vivo* microdialysis studies have shown that the systemic administration of WAY 100635 by itself can enhance the output of 5-HT in the amygdala (Bosker *et al.* 1997a and 1997b). Therefore, it is possible that a small increase of firing activity of specific 5-HT neurons (presumably those that would project to the locus coeruleus) would enhance the release of 5-HT in a manner sufficient to induce a total suppression of firing of

NE neurons. The third possibility for the location of the 5-HT_{1A} receptors involves a postsynaptic 5-HT_{1A} receptors located on a non-NE neuronal element (thus participating in a polysynaptic circuit). Indeed, the excitatory action of 8-OH-DPAT as well as the inhibitory action of WAY 100635 on the firing activity could be mediated by a 5-HT_{1A} receptors located for instance on some GABAergic neurons. Therefore the inhibition of these neuronal elements by 5-HT_{1A} receptor stimulation would enhance the firing activity of locus coeruleus NE neurons whereas, given that it would be tonically inhibited, the administration of WAY 100635 would increase its activity thus leading to a suppression of the firing activity of locus coeruleus NE neurons. This is speculative as no evidence toward such an interpretation is available as of yet.

A 5-HT_{1A}-mediated modulation of the firing activity of locus coeruleus NE neurons of a totally different nature has also been documented. First, a 5-HT mediated decrease of sensory evoked activity in the locus coeruleus has been described (Chouvet *et al.* 1988). This observation was complemented by the demonstration that 5-HT_{1A} receptor activation reduces the glutamate-induced excitation of locus coeruleus NE neurons' firing activity (Aston-Jones *et al.* 1991; Charléty *et al.* 1991). The physiological role of this is however unclear. Indeed, the authors have suggested a presynaptic mechanism in that 5-HT_{1A} receptor activation would reduce the release of glutamate. However, they have observed a reduction of the response of microiontophoretically-applied glutamate thereby suggesting a postsynaptic action. More compelling, however, are the observations of Bobker and Williams (1989). Indeed, in a diligent pharmacological analysis using the *in vitro* locus coeruleus slice preparation, these authors demonstrated the presence of a functional 5-HT_{1A} and 5-HT_{1B} receptors modulating the release of glutamate whereas only activation of the 5-HT_{1B} receptor modulates the release of GABA. That these 5-HT receptors are located presynaptically (but on non-serotonergic nerve terminals) was further shown by the incapacity of these receptors' activation to alter the locus coeruleus NE neurons' responses to exogenous GABA or glutamate. These results are thus incongruent with those of Aston-Jones *et al.* (1991) and Charléty *et al.* (1991) suggesting a postsynaptic action. The reasons for this are unclear. However, it is likely that these mechanisms are not involved in the excitatory effect of systemic administration of 5-HT_{1A} agonists, as an excitation, and not a suppression of firing activity is observed.

8.4.2. Mediation by the 5-HT₂ receptors

The systemic administration of LSD, mescaline, DOI and other psychedelic hallucinogens induces a facilitation of the activation of locus coeruleus neurons by sensory stimuli whereas, paradoxically, it decreases spontaneous firing activity. The suppressant effect of these hallucinogens on the firing activity of locus coeruleus NE neurons is reversed by low doses of 5-HT₂ antagonists such as ritanserin (Rasmussen and Aghajanian 1986a; Rasmussen *et al.* 1986b; Gorea and Adrien 1988; Chiang and Aston-Jones 1993). Moreover, antipsychotic drugs reverse the actions of hallucinogens with doses that correlate with their affinity for 5-HT₂ receptors (Rasmussen *et al.* 1986b). There have been efforts in trying to elucidate the location of the 5-HT₂ receptor that mediates these effects. The microiontophoretic application of 5-HT₂ compounds does not alter firing activity of locus coeruleus neurons (Chiang and Aston-Jones 1993; Gorea *et al.* 1991) thereby suggesting that the receptors are not located on the locus coeruleus NE neuron's cell body. The 5-HT₂ receptor could simply be located away on the dendritic tree in a way similar to that described above regarding the lack of effect of microiontophoretic applications of 5-HT on locus coeruleus NE firing activity. This outcome is however unlikely given that mRNA for the 5-HT₂ receptor are not present in the locus coeruleus. Studies in slice preparations have generated equivocal results in the attempt to determine the cellular location of the 5-HT receptor activation on locus coeruleus NE neurons (see above). However, there is convincing evidence suggesting that the decrease of spontaneous firing activity of locus coeruleus NE neurons by 5-HT₂ receptor reflects the activation of GABAergic neurons (Chiang and Aston-Jones 1993). Activating these neurons inhibits firing activity of locus coeruleus NE neurons through GABA_A receptors. The presence of mRNAs coding for the GABA_A receptor subtype in the locus coeruleus has been demonstrated (Luque *et al.* 1994). Furthermore, such a scheme is fully consistent with the general excitatory effect, and not inhibitory, that has been attributed to 5-HT₂ receptor activation in various cell types including GABAergic neurons in various regions of the brain (Araneda and Andrade 1991; McCormick and Wang 1991; Shen and Andrade 1998; Willins *et al.* 1997). In addition, there is evidence from lesioning experiments that suggest that the 5-HT₂ receptors located in the prepositus hypoglossal nucleus are part of the network responsible for the 5-HT₂-mediated inhibition of spontaneous firing activity in the locus coeruleus (Gorea *et al.* 1991). This is consistent with the observations that, first, a GABAergic innervation to the locus coeruleus arises from the prepositus hypoglossal nucleus (6749, 6789) and second, that electrical stimulation of this nucleus leads to a GABA_A-mediated suppression

of firing activity of locus coeruleus NE neurons (Aston-Jones *et al.* 1991). Taken together, these experimental evidence are more congruent with the notion that the inhibitory actions on the firing activity of locus coeruleus NE neurons induced by 5-HT₂ receptor activation is mediated by activation of a GABAergic neurons than that of a direct action on locus coeruleus NE neurons *per se*.

It has further been suggested that the enhancement of phasic sensory responses induced by 5-HT₂ receptor activation reflects the activation of glutamatergic excitatory inputs to the locus coeruleus (Chiang and Aston-Jones 1993). Again, this mechanism is consistent with what has been described elsewhere (medial prefrontal cortex) regarding 5-HT₂ receptor mediated enhancement of glutamate release (Marek and Aghajanian 1999). The exact location of this 5-HT₂ receptor is however still unsure. It is noteworthy that a glutamatergic input from the nucleus paragigantocellularis to the locus coeruleus has been well described both anatomically and physiologically (Aston-Jones *et al.* 1991). Moreover, it has been suggested that the sensory evoked increase in firing activity of locus coeruleus NE neurons is mediated by this nuclei as its electrical activation induces predominantly a non-NMDA receptor-mediated excitation of locus coeruleus NE neurons (Aston-Jones *et al.* 1991; Bobker and Williams 1989). However, in the study of Chiang and Aston-Jones (1993), the 5-HT₂-mediated excitation was blocked by an NMDA receptor antagonist.

The serotonergic system exerts a tonic suppression on the firing activity of locus coeruleus NE neurons (see above). Several lines of evidence support the notion that it would be mediated by tonic activation of the 5-HT₂ receptor. First, systemic administrations of 5-HT₂ antagonists increases the firing activity of locus coeruleus NE neurons (Gorea and Adrien 1988; Rasmussen and Aghajanian 1986a; Aghajanian *et al.* 1990). This is also observed in *in vivo* voltammetry studies showing that DOPAC level (an index of NE cellular activity) is enhanced in the locus coeruleus following systemic administration of ritanserin (Clement *et al.* 1992). *In vivo* microdialysis experiments have shown that the administration of 5-HT_{2B/C} antagonist, but not of the selective 5-HT_{2A} antagonist MDL 100, 907, enhances the output of NE in the hippocampus and frontal cortex (Gobert and Millan 1999), which phenomenon most likely reflects an enhanced locus coeruleus NE neuron's firing activity. These observations, combined with those showing that MDL 100, 907 does not alter the firing activity of locus coeruleus NE neurons (S.T. Szabo, personal communication), provides evidence for the involvement of the 5-HT_{2C} receptor. Taken together it appears likely that the tonic inhibition

of locus coeruleus NE neurons by the the serotonergic system would be carried out a tonic *activation* of GABAergic neurons in the prepositus hypoglossal nucleus.

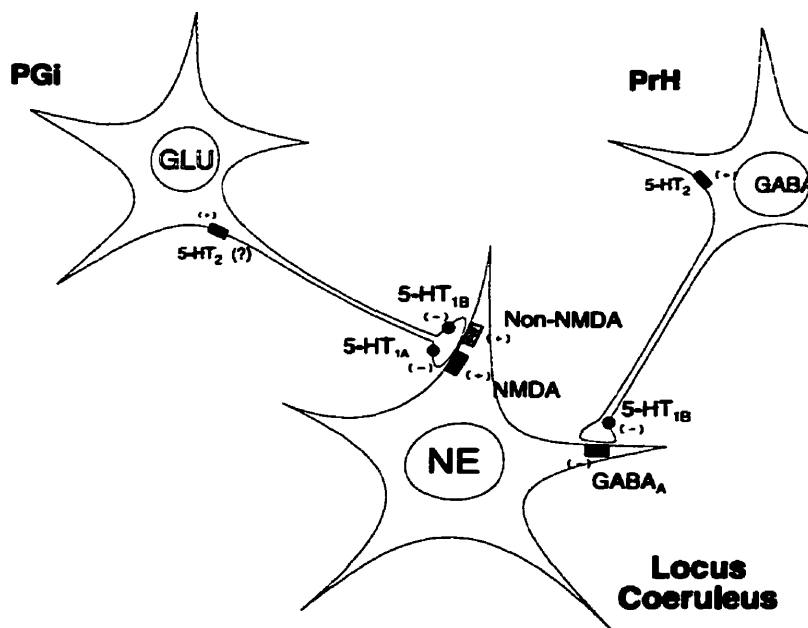


Figure 6. Schematic representation depicting the major inputs to the Locus Coeruleus with a focus on serotonergic modulations. The serotonergic input from the raphe nuclei have been left out for clarity.

Since the GABAergic innervation to the locus coeruleus

plays an important role and has been extensively referred to in this section, some notion pertaining to this will be covered. Microiontophoretic application of GABA directly onto locus coeruleus NE neurons suppresses their firing activity which effect is prevented by bicuculline thus demonstrating the role of the GABAA receptor in mediating this effect (Ennis and Aston-Jones 1989). Consistent with this, large number of mRNA encoding for the GABAA receptor have been found in the locus coeruleus (Luque *et al.* 1994). GABAergic innervation to the locus coeruleus has been reported to arise from neurons located in the preoptic and posterior hypothalamic areas, the ventrolateral part of the periaqueductal gray and the dorsal paragigantocellular nucleus. These findings are particular relevant to the firing pattern expressed by locus coeruleus NE neurons over the course of the sleep-wake cycle. Indeed, whereas during wakefulness locus coeruleus NE neurons fire at *ca.* 2 Hz, the firing activity decreases during slow wave sleep and are nearly quiescent during paradoxical sleep. Accordingly, recordings in freely moving rats have shown that the microiontophoretic application of the GABA_A antagonist bicuculline restored firing activity of locus coeruleus NE neurons during slow wave sleep and during paradoxical sleep (Gervasoni *et al.* 1998). These finding, in conjunction with those of Nitz and Siegel (1997) showing an increased GABA

release in the cat locus coeruleus during slow wave sleep and paradoxical sleep as compared with awake value, have prompted the authors to suggest that during wakefulness, there is a tonic inhibitory GABAergic action on the firing activity of locus coeruleus NE neurons which is enhanced during slow wave sleep and even more so during paradoxical sleep (Gervasoni *et al.* 1998). Whether this inhibitory tone is acting directly on NE neurons or on any excitatory inputs to the locus coeruleus remains however to be determined.

8.5. Terminal release of NE

8.5.1. Basic characteristics

The release of NE in terminal areas is impulse dependent as it is prevented by TTX (Carter 1997; Kalen *et al.* 1988; Itoh *et al.* 1990; Finberg *et al.* 1993; van *et al.* 1990). It is also dependent on an exocytotic mechanism since it is reduced by the omission of Ca^{2+} from the perfusion fluid (Carter 1997; L'Heureux *et al.* 1986; van *et al.* 1990; Dalley and Stanford 1995; Badoer *et al.* 1989). That the released NE in various terminal regions arises principally from fibers originating from the locus coeruleus is indicated essentially by three observations. 1) The electrical stimulation of the locus coeruleus enhances the synaptic concentration of NE. This was determined directly by the use of *in vivo* microdialysis (L'Heureux *et al.* 1986; Brun *et al.* 1993) or indirectly, by the use of *in vivo* electrophysiological paradigms (Curet and de Montigny 1988b; Segal and Bloom 1976b; Segal and Bloom 1976a; Rogawski and Aghajanian 1980b; Curet *et al.* 1992). 2) Application of TTX directly in the locus coeruleus greatly lowers NE levels in terminal area (Van *et al.* 1997) and 3) a 6-OHDA lesion reduces below the limit of detection the extracellular concentration of NE (Kalen *et al.* 1988).

The concentration of extracellular NE is determined by the difference between the amount of NE that is released and that which is actively taken up by the NE transporter. A determining role for the NE transporter in controlling the extracellular concentration of NE (and hence neurotransmission) has been demonstrated. Perhaps the best illustrative observation for this is the fact that the local administration (by reverse dialysis) of the NE reuptake inhibitor desipramine enhances the extracellular level of NE in terminal areas such as the hippocampus and frontal cortex (Dalley and Stanford 1995; Thomas and Holman 1991; Gustafson *et al.* 1991). These results thus demonstrate the active role played by the NE transporter for clearing NE from the extracellular space in physiological conditions. As for the release of NE *per se* in terminal areas, it is controlled by several mechanisms, two of which will be considered here: 1) the frequency of action potentials reaching the nerve terminals, *i.e.*, the frequency of firing of locus coeruleus NE neurons, and 2) the action of terminal α_2 -adrenergic autoreceptors.

8.4.2. Somatodendritic α_2 -adrenergic autoreceptors and terminal release

To determine the role and relative importance of the firing frequency of the locus coeruleus NE neurons on NE release in terminal areas, one of the strategy employed involved

assessing NE levels in terminal areas following pharmacologic interventions that either suppress the firing activity of locus coeruleus NE neurons or suppress the propagation of action potential reaching the release area. Thus, the local administration, directly in the locus coeruleus, of either clonidine or desipramine (and hence suppressing the firing activity of NE neurons through somatodendritic α_2 -adrenergic autoreceptors activation) decreases the extracellular concentrations of NE in terminal areas (Mateo *et al.* 1998; Van *et al.* 1997). Secondly, NE levels in terminal area are reduced by the application of TTX directly to the locus coeruleus (Van *et al.* 1997).

The systemic administration of NE reuptake inhibitors such as desipramine and amitriptyline enhances the extracellular concentration of NE not only in the locus coeruleus (Mateo *et al.* 1998) but also in various terminal areas such as the hippocampus (Seo *et al.* 1999), hypothalamus (Engleman and Wong 1996; Itoh *et al.* 1990) and cortex (Dennis *et al.* 1987; Seo *et al.* 1999; L'Heureux *et al.* 1986). This increase of NE levels is also observed in the hippocampus with the use of an electrochemical detection technique (*i.e.*, *in vivo* amperometry; Daws *et al.* 1998). However, in a manner similar to that observed for the 5-HT system, this increase is offset by the concurrent activation of the somatodendritic α_2 -adrenergic autoreceptor (and the consequent suppression of firing activity of NE neurons). In keeping with this, the systemic administration of idazoxan (an α_2 -adrenergic antagonist) potentiates the effect of desipramine on NE level in the hippocampus (D. Nutt, personal communication). Interestingly, there are regional differences to this effect as the systemic administration of desipramine induces a slight decrease in the extracellular concentration of NE in the cingulate cortex. The importance of somatodendritic activation in this effect is demonstrated by the fact that systemic administration of desipramine while an α_2 -adrenergic antagonist is applied in the locus coeruleus, will enhance the extracellular concentration of NE in the cingulate cortex (Mateo *et al.* 1998). It is noteworthy that despite inducing an enhancement of extracellular levels of NE, the systemic administration of desipramine does not alter the firing activity of dorsal hippocampus pyramidal neurons (Lacroix *et al.* 1991a; Curet *et al.* 1992). In contrast, the intravenous administration of amphetamine, which also enhances the extracellular concentration of NE, does. The effect of amphetamine was however shown to be, at least partly, consequent of its effect on 5-HT release (Curet *et al.* 1992).

8.4.3. The presynaptic α_2 -adrenergic autoreceptors

The presence of presynaptic adrenergic autoreceptors mediating the release of adrenaline and noradrenaline from adrenergic nerve terminals both in brain and in various tissues (heart, adrenal medullas, blood vessels) has been known for many years (Göthert 1985; Starke 1972a; Starke 1972b; Starke 1977; Reichenbacher *et al.* 1982; Langer 1980; Starke *et al.* 1975; Starke *et al.* 1974). As for the identification of the receptor subtypes mediating these effects, an anatomical model developed by Langer, (Curtis *et al.* 1997) suggested that the α_2 -adrenergic receptors would be located presynaptically whereas their counterpart of the α_1 subtype would be located postsynaptically. Subsequent studies have however shown that the α_2 -adrenergic receptors are also located postsynaptically. Studies on presynaptic α_2 -adrenergic autoreceptors were initially attempted using radioligand binding studies. However, this approach presented marked limitations due to the fact that nerve terminals represent only a small portion of the total tissue mass in such way that the number of presynaptic receptors is low compared to those that are present postsynaptically. The *in vitro* brain slices preparation has proven invaluable in the attempt to pharmacologically identify the receptor at hand, but also in the study of the mechanistic and operational functions of these receptors. These pharmacological studies demonstrated that the receptors mediating the inhibition of release of [³H]NE from preloaded brain slices belong to the α_2 -adrenergic receptor subtype (Frankhuyzen and Mulder 1982; Gobbi *et al.* 1993).

In vivo electrophysiological approaches have also been used to study the terminal α_2 -adrenergic autoreceptors modulating the release of NE. Curet and de Montigny (1988b) has shown that the electrical stimulation of the locus coeruleus induces the release of NE in the dorsal hippocampus. The release of NE was quantified by determining the duration of the inhibitory effect on the firing activity of CA₃ pyramidal neurons induced by the electrical stimulation. Using this paradigm, these authors, have shown that the effectiveness of the stimulation was reduced by both the systemic and local (by microiontophoresis) administration of clonidine, which effect was reduced by low dose of idazoxan (Curet and de Montigny 1989). Moreover, increasing the frequency of stimulation from 1 Hz to 5 Hz also reduced the effectiveness of the stimulation which effect was also reversed by idazoxan. This was interpreted by the authors as indicating that increasing the frequency of the stimulation results in an enhanced activation of the terminal α_2 -adrenergic autoreceptor (Curet and de Montigny 1989) thereby decreasing the net output of NE.

The α_2 -adrenergic nature of the autoreceptor controlling the release of NE from nerve

terminals is substantiated by *in vivo* microdialysis studies showing that the local (*i.e.*, by reverse dialysis) as well as the systemic administration of α_2 -adrenergic agonists (such as clonidine and UK 14, 304) decrease the extracellular levels of NE in terminal areas (Carter 1997; L'Heureux *et al.* 1986; Itoh *et al.* 1990; van *et al.* 1993; Dalley and Stanford 1995; Cuadra and Giacobini 1995; Meana *et al.* 1997; Abercrombie *et al.* 1988).

The pharmacology of α_2 -adrenergic receptors has developed over the years to the extent that multiple receptor subtypes have emerged and have thus prompted a parallel research endeavour seeking to identify which α_2 -adrenergic receptor subtypes controlled the release of NE. Investigations carried out prior to about 1991 only considered the α_{2A-C} receptors then known. Moreover, some of the classification schemes put forward were not necessarily followed by all authors. Such a situation has obviously generated confusion in the literature. Relatively early pharmacological studies carried out in rabbit and rat cortex have suggested that it is the α_{2A} -adrenergic receptors that mediate the inhibitory effect on NE release (Limberger *et al.* 1991; Limberger *et al.* 1989). A similar conclusion was also reached in synaptosomes (Gobbi *et al.* 1990b). The availability of newer ligands prompted however the reassessment of this receptor identification. It now appears that the presynaptic adrenergic receptor modulating the release of NE is of the α_{2D} -adrenergic receptor subtype in the rat (Kiss *et al.* 1995; Trendelenburg *et al.* 1993), guinea-pig (Trendelenburg *et al.* 1995), and mice brain (Limberger *et al.* 1995) whereas, in the human (Raiteri *et al.* 1992), pig (Trendelenburg *et al.* 1996) and rabbit (Trendelenburg *et al.* 1993) brain, it would be of the α_{2A} subtype. Indeed, these two receptors are considered being species homologues. Although beyond the scope of the present work, it is noteworthy that central and peripheral adrenergic autoreceptors are of different subtypes. In the context of pharmacological interventions, this situation provides obvious benefits.

The study of the presynaptic receptors mediating the release of various neurotransmitters has historically been the source of debate and, perhaps, also of confusion. Part of this stems from methodological considerations that makes the validity of some of the interpretations difficult to assess. Some of them have been described by (Starke *et al.* 1995). For example, the *in vitro* slice preparation enables the use of a great variety of experimental paradigms which, concurrently, generate different experimental conditions. By themselves, these different conditions can be the source of variability of results that renders interpretation more difficult. For instance, the presence (or absence) of a tonic activation of endogenous NE

(or even released [^3H]NE), on the presynaptic autoreceptor represents an illustration of this issue since the interpretations that are reached are dependent on the experimental conditions. Indeed, when exogenous ligands are administered, they compete with released NE and thus distort agonist EC_{50} and antagonist pK_A values. One can appreciate some of the variability that can emanate from this. There have been various efforts in order to circumvent this issue. For instance the use of synaptosomes with fast perfusion or the use of short trains of high-frequency of the pseudo-one pulse stimulation (POP) have been used to diminish the effect of the released endogenous ligand on presynaptic receptors. However, it is perhaps the use of *in vivo* microdialysis that has provided the most valid way to appraise the specific outcome of tonic activation of endogenous ligands on a specific autoreceptor. This approach has hence allowed for a general consensus that states that under physiological conditions, the terminal α_2 -adrenergic autoreceptor is indeed under tonic activation by endogenous NE. Such an interpretation derives from the observations that the local (as well as systemic) administration of various α_2 -adrenergic antagonists (atipamezole, idazoxan, piperoxane, BRL 44408, ARC 239 or yohimbine) enhances the level of extracellular NE in terminal areas (Carter 1997; L'Heureux *et al.* 1986; Itoh *et al.* 1990; Thomas and Holman 1991; Thomas *et al.* 1992; van *et al.* 1993; van *et al.* 1994; Dalley and Stanford 1995; Dennis *et al.* 1987; Cuadra and Giacobini 1995; Kiss *et al.* 1995; Abercrombie *et al.* 1988). A similar interpretation is derived from studies carried out using an *in vivo* electrophysiological paradigm in which the systemic administration of idazoxan enhances the amount of released NE per action potential (Curet and de Montigny 1989; Curet *et al.* 1992). Finally, again with the use of *in vivo* microdialysis, (van *et al.* 1994) have shown that the tonic activation of terminal α_2 -adrenergic autoreceptors is greater in the frontal cortex than in the amygdala or hippocampus.

Finally, it has been shown that the inhibitory effect of clonidine on the electrically-evoked release of [^3H]NE is greatly hampered by a pretreatment with NEM (Allgaier *et al.* 1986) therefore suggesting the role of a $\text{G}_{i/o}$ -protein.

9. ON THE EFFECTS OF NE ON POSTSYNAPTIC NEURONS

The electrophysiological effects of NE in the CNS are complex. For one the action carried out by the same adrenergic receptor varies with brain regions (the most prominent example being the α_1 -adrenergic receptors that ranges from being inhibitory to excitatory depending on brain structures). Moreover, the advent of intracellular recordings has allowed

for the delineation of more complex action of NE as it now appears that it serves in many system to modulate the responsiveness of neuronal excitability to other excitatory and inhibitory neurotransmitters. In the present section, an integrative perspective on the electrophysiological effects of NE will be attempted by exposing some representative data on the different receptor mediating those complex effects.

9.1. General characteristics

There has been a myriad of studies devoted to the understanding of the action of NE on postsynaptic cells in the brain. The hippocampus has been assuredly the target of most of these studies. One of the most prominent features of NE in various regions of the neuraxis is its modulation with the glutamatergic system. For instance in the cortex and in the hippocampus, the excitatory response of microiontophoretic applications of glutamate can be enhanced by bath or microiontophoretic applications of NE. In this regard, one needs to mention the pioneering work of the group of R. Nicoll (Madison and Nicoll 1982; Madison and Nicoll 1986a; Madison and Nicoll 1986b). This group has found that when applied by microiontophoresis, glutamate induces a small train of action potentials that last for less than 0.5 sec even though the cell is still sufficiently depolarized. This short train is greatly increased in the presence of NE which response is blocked by the β_1 antagonist atenolol. The same effect of NE is also observed when depolarizing pulses are given to the cell. Indeed, these pulses elicit a train of action potentials although there is also accommodation, *i.e.*, after a short period of time the cells do not fire anymore. This train of action potential elicited by depolarizing pulses is also increased in the presence of NE. These results thereby suggest that the effect of NE does not induce an action on a specific target of glutamate neurotransmission but well on a general electrophysiological parameter of the pyramidal cell. NE was further shown to increase excitability of hippocampal pyramidal neurons by reducing the Ca^{2+} -activated K^+ channel. Indeed, this well characterized current is present in pyramidal cells and involves a K^+ current that is turned on by Ca^{2+} that enters the cell during action potential discharge. This action of NE on this current is also blocked by atenolol. Therefore, the noradrenergic blockade of the AHP and its underlying current is mediated by β_1 adrenergic receptors. This is also supported by the observation that α -adrenergic antagonists are devoid of effects on this AHP while the latter is mimicked by β_1 agonist isoproterenol and dobutamine (Madison and Nicoll 1986b). Moreover, both these β_1 -adrenergic mediated responses, as one

might expect, are mediated by cAMP as they are mimicked by intrasomata injection of 8-bromo-cAMP. Altogether, these two actions of NE act in concert, not to have any effect on the resting membrane potential of the neuron, but instead to markedly alter the response of the neuron when it is activated. Finally, there have also been numerous demonstrations of NE enhancement of long-term potentiation in CA₃ and the dentate gyrus. Electrophysiological studies carried out *in vivo* have also demonstrated a β_1 -adrenergic receptor-mediated late excitation induced by release of endogenous NE (Curet and de Montigny 1988b).

In addition to the well-described β -receptor modulation of cellular excitability in the hippocampus, a role for α -adrenergic receptor activation has also been documented. Extracellular studies in the rat hippocampus suggest that whereas the suppressant effect of exogenous NE is mediated by the α_2 -adrenergic receptor, that induced by endogenous NE (elicited by locus coeruleus stimulation) is mediated by α_1 -adrenergic receptors thus suggesting that the former receptor would be located intrasynaptically, whereas the latter would be located extrasynaptically. Interestingly, (Bijak and Papp 1995) reports that the amplitude of population spikes elicited by stimulation of the Schaeffer's collaterals is depressed by α_1 -adrenergic receptor stimulation thereby suggesting that the intrasynaptic population of α_1 -adrenergic receptor can be activated by phenylephrine. Unfortunately, the signal transduction pathway involved in these responses has not yet been fully elucidated. Neither clonidine nor phenylephrine exerts any marked nor consistent action on membrane potential of hippocampal neurons (Segal, unpublished observations). It needs here to be mentioned that whereas in the hippocampus the α_1 -adrenergic response is mostly inhibitory, in other regions of the brain (such as the dorsal raphe, some hypothalamic nuclei, lateral geniculate neurons and spinal cord) it is mostly an excitatory one, mediated by the closing of a K⁺ channel (Rogawski and Aghajanian 1980a; Menkes *et al.* 1980; Freedman and Aghajanian 1987). In addition to the α_1 -adrenergic modulating the effect of released NE, its activation has been shown to lead to a slow depolarization of inhibitory interneurons resulting from the decrease of a K⁺ conductance (Bergles *et al.* 1996). This is consistent with the mRNA distribution of the α_{1A} -adrenergic receptor of the hippocampus which appears to be in non-pyramidal cells, in contrast to the other α_1 -adrenergic receptor subtype (Day *et al.* 1997). The relative importance of this in the effect of microiontophoretically applied NE (Curet and de Montigny 1988b) or perhaps more importantly of the electrical stimulation of the locus

coeruleus (Curet and de Montigny 1988a; Segal and Bloom 1976b), still needs to be fully elucidated.

9.2. Integrative aspect

One is rapidly confronted with the complexity of one single neurotransmitter action on membrane excitability which, in many cases, appears to be contradictory. There have been many integrative attempts in order to provide a unitary role of NE on cellular activity. Whereas NE can inhibit firing activity of a given neuron, it paradoxically augments evoked activity. These observations have led to the development of "the signal-to-noise ratio" hypothesis. As such, NE's action would be to enhance a cell response to its strongest input whereas to diminish it in presence of a low input or of basal activity. Of course, this scheme does not account for all of NE action in the brain, but it provides a general unifying mechanistic role for NE that has proven to be of significant value. A careful and elegant set of studies carried out by the group of Barry Jacobs have permitted to provide a very interesting correlate, at the behavioural level, to this general scheme attributed to NE. Indeed, they have shown that an increase NE tone lead to the amplification of the masseteric reflex. This was actually first demonstration that a behavioural output is potentiated by an enhance release of NE (reviewed in (Jacobs *et al.* 1991). These considerations led the authors to consider the question whether NE would be endowed with a general and global facilitatory effect on motor output. In any event, NE has oftentimes been assumed to accomplish a dual role, *i.e.*, one of a neuromodulatory function in addition to that of a classical neurotransmitter.

10. ON THE EFFECTS OF ANTIDEPRESSANT TREATMENTS ON THE NE SYSTEM

10.1 Somatodendritic α_2 -adrenergic autoreceptors

After long-term administration of desipramine, the effect of intravenous administration of clonidine is decreased, thus indicating that the somatodendritic α_2 -adrenergic autoreceptor is desensitized (Lacroix *et al.* 1991a). Interestingly, whereas the effect of microiontophoretically-applied clonidine is decreased (Scuvée-Moreau and Svensson 1982; Lacroix *et al.* 1991a), that for NE is not (Lacroix *et al.* 1991a) in those desipramine-treated animals. These results are not fully consistent with the notion of an α_2 -adrenergic receptor

desensitization and thus renders this issue intriguing. Several hypotheses can be put forward. The fact that clonidine, and not NE, unravelled this desensitization could rely in the fact that clonidine is a partial agonist on the α_2 -adrenergic receptor while NE is a full agonist. Indeed, it is possible that a modest desensitization would be unravelled only with a partial agonist as they often benefit from a smaller receptor reserve. This possibility (*i.e.*, the emergence of a partial desensitization) is congruent with the fact that following sustained administration of desipramine, locus coeruleus NE neurons do not recover their firing activity (see below). These results thus suggest that, if any, the desensitization of the α_2 -adrenergic autoreceptor does not bare profound consequences. Nonetheless, it is the imidazoline affinity of clonidine that has been suggested by the authors to be responsible for the discrepancy between the response to microiontophoretic NE and clonidine (Lacroix *et al.* 1991a). This hypothesis has later gained credence when it was observed that the long-term administration of imipramine induces a down-regulation of imidazoline₁ receptors in the locus coeruleus, but not of α_2 - adrenergic receptors. These findings were not fully replicated by Jimenez-Rivera *et al.* (1996) who reported a down-regulation of the α_2 -adrenergic receptors (using [³H]idazoxan) in the locus coeruleus following long-term administration of imipramine. Interestingly, a down-regulation (also with the use of [³H]idazoxan) was shown to occur in the locus coeruleus following a treatment with the NE reuptake inhibitor protriptyline as well as with clorgyline (Kovachich *et al.* 1993). Therefore, and unfortunately, there is not yet a clear and concise picture of the fate of the somatodendritic α_2 -adrenergic autoreceptors following prolonged treatment of drugs that enhance the synaptic concentration of NE. Nonetheless, one must appreciate the functional relevance of the desensitization, if any, of the somatodendritic α_2 -adrenergic autoreceptors. Indeed, assuming that a meaningful desensitization would ensue following long-term administration of a NE reuptake inhibitor, it could be expected that it would be accompanied by a parallel recovery of firing activity of NE neurons back to control values and this, in presence of a sustained enhancement of NE concentration in the locus coeruleus. This outcome is not observed following long-term administration of MAOIs (phenelzine, clorgyline, amiflamine and befloxatone) as the firing activity of locus coeruleus NE neurons remains suppressed throughout the duration of the treatment (Blier and de Montigny 1985; Blier *et al.* 1986a; Haddjeri *et al.* 1998b). In the case of “pure” NE reuptake inhibition, whereas some studies have failed to demonstrate any significant suppression of firing activity of locus coeruleus NE neurons after 21 days of sustained administration of

desipramine (Valentino *et al.* 1990; Lacroix *et al.* 1991a), others did (Huang *et al.* 1980). However, a careful scrutiny dictates that interpretation may have been somewhat hindered or mislead by methodological considerations. Indeed, in the earlier studies of Valentino (Valentino *et al.* 1990) and Lacroix *et al.* (Lacroix *et al.* 1991a) the mean firing activity of locus coeruleus NE neurons were determined 12 to 20 hours and 24 hours, respectively, after the last intraperitoneal injection of desipramine. Considering the important first-pass metabolism of the drug, one can wonder on the effective brain concentrations of the drug after such a delay and hence on the extent of the enhanced extracellular concentration of NE in the locus coeruleus of these rats. These considerations have been nonetheless more recently resolved as long-term administration of desipramine using a continuous perfusion apparatus (*i.e.*, osmotic minipumps) markedly suppressed firing activity of locus coeruleus NE neurons (Szabo *et al.* 1999). Taken together these studies indicate, first, that the extent of the desensitization of the somatodendritic α_2 -adrenergic autoreceptor, if any, is not accompanied by a significant functional consequence, as these receptors after a long-term administration of drugs that enhance the synaptic concentration of NE, still carry out effectively and fully their primary function, *i.e.*, control the firing activity of locus coeruleus NE neurons. Secondly, these results unravel the presence of a fundamental difference between the homeostatic mechanism controlling the firing activity of the neurons of the 5-HT and NE systems.

10.2. Serotonergic input to the locus coeruleus.

It is a striking lure that the serotonergic modulation of noradrenergic cellular activity has not been more studied in the context of antidepressant treatments. Nonetheless, lately, it has been observed that the subchronic administration of SSRI's does not alter the firing activity of locus coeruleus NE neurons, and this despite a presumably enhanced extracellular concentration of 5-HT in the locus coeruleus (or peri-locus coeruleus) area. Most interestingly, however, is the observation that the long-term administration of SSRI's suppresses the firing activity of the locus coeruleus NE neurons (Szabo *et al.* 1998; Szabo *et al.* 1999). It is thus tempting to consider this observation as an additional argument to the contention that supports that long-term antidepressant treatments enhance 5-HT neurotransmission. Indeed, since long-term administration of SSRI's leads to a desensitization of the somatodendritic 5-HT_{1A} autoreceptor thereby restoring normal firing activity of 5-HT neurons, it seems plausible to assume that 5-HT output to the locus coeruleus would be

increased, as was demonstrated in other regions. Moreover, the increase in firing activity of locus coeruleus NE neurons induced by 8-OH-DPAT is totally abolished in citalopram-treated animals (S.T. Szabo, personal communication). This provides further indication that the increase of firing activity of locus coeruleus NE neurons induced by 5-HT_{1A} receptor activation is indeed mediated by the somatodendritic 5-HT_{1A} receptors. Recall that following prolonged SSRI treatments, only somatodendritic, and not postsynaptic, 5-HT_{1A} receptors are desensitized (see section 5). It needs however to be mentioned that it is still unclear how can this phenomenon participate in the antidepressant action of these compounds. Indeed, the authors (Szabo *et al.* 1998; Szabo *et al.* 1999) have suggested that the suppression of locus coeruleus NE neurons would mostly contribute to the anxiolytic activity of SSRI's in light of the proposed hyperadrenergic state in anxiety disorders. Based on the important body of knowledge that suggests an overactive locus coeruleus-NE system during states of anxiety, it may appear peculiar that pharmacological interventions that reduce NE neurotransmission have not yet been able to produce anxiolytic effect. The efficacy of the 5-HT_{1A} agonist of the azapirone family as anxiolytic may thus appear peculiar in the context of noradrenergic activity. Indeed, these compounds *increase* NE neurotransmission, notably *via* increasing firing activity of locus coeruleus NE neurons. Therefore, given that two classes of serotonergic agents (*i.e.*, 5-HT_{1A} agonists and SSRI's), that are known to be anxiolytic, have two opposing effects on the firing activity of NE neurons, suggest that this effect is not involved in their anxiolytic effect.

10.3. Terminal α_2 -adrenergic autoreceptors

The fate, in terms of sensitivity, of the α_2 -adrenergic autoreceptor following long-term administration of NE reuptake inhibitors is somewhat equivocal in the literature. Indeed, *in vivo* electrophysiological studies have suggested first, that long-term administration of desipramine for 21 days (with a 24 hrs wash out period) induces a desensitization of this receptor (Lacroix *et al.* 1991b). Consistently, this phenomenon was accompanied by an enhanced efficacy of the electrical stimulation of locus coeruleus to suppress firing activity of CA₃ pyramidal neurons. However, using a different *in vivo* paradigm Mongeau *et al.* (1994b), whilst showing that a long-term treatment with nisoxetine, a NE reuptake inhibitor, desensitizes α_2 -adrenergic heteroreceptors, failed to detect any change of the sensitivity of the α_2 -adrenergic autoreceptors. Long-term administration of MAOI, such as befloxatone (Blier

and Bouchard 1994) or clorgyline (Campbell and McKernan 1986) does not alter the sensitivity of the α_2 -adrenergic autoreceptors. *In vitro* studies with desipramine have also generated conflicting results. For instance, Moret and Briley, have demonstrated that long-term administration of a high dose of desipramine *p.o.* induced a desensitization of the α_2 -adrenergic autoreceptors in hypothalamic slices (Moret and Briley 1994). It needs to be mentioned that the experiments were carried out with a 24-hour wash out period that proved to be insufficient as desipramine was still present in the slices, thus complicating the interpretation of the results. Other investigators have found that, whilst the output of [^{3} H]NE was enhanced in slices from rats treated with desipramine, the sensitivity of the α_2 -adrenergic autoreceptor was unaltered (Schoffelemeier *et al.* 1984; Schoffelemeier and Mulder 1982). The situation is unfortunately not clarified by the use of *in vitro* paradigms with other mixed 5-HT/NE reuptake inhibitors as, on the one hand, long-term treatment with milnacipran does not induce any changes in the sensitivity of the α_2 -adrenergic autoreceptor in hypothalamic slices (Moret and Briley 1994) nor in mesencephalic slices (Mongeau *et al.* 1998) whereas, on the other hand, that of duloxetine induced a desensitization the α_2 -adrenergic autoreceptor in hippocampal slices, but not in frontal cortex slices (Rueter *et al.* 1998b).

From the above, it would appear hazardous to conclude that “noradrenergic” antidepressants lead to a desensitization of the α_2 -adrenergic autoreceptors. Nonetheless, it is striking that in some studies an enhanced output of NE was observed in slices from desipramine treated animals in the absence of any alteration of the function of the α_2 -adrenergic autoreceptors. The mechanism underlying this outcome is not yet resolved.

10.4. Postsynaptic adrenergic receptors

10.4.1. α_1 -adrenergic receptors

Long-term administration of TCAs enhances the sensitivity of the amygdaloid neurons to NE (Wang and Aghajanian 1980). Similarly, TCAs were also shown to increase the sensitivity of the lateral geniculate and facial motor neurons to microiontophoretic application of NE (Menkes *et al.* 1980; Menkes and Aghajanian 1981). The response to microiontophoretic application of NE in these regions is mediated by α_1 -adrenergic receptors (Menkes *et al.* 1981; Marwaha and Aghajanian 1982; Menkes and Aghajanian 1981). This same group also reported an increase α_1 -adrenergic receptor sensitivity using the acoustic

startle reflex paradigm following long-term TCAs treatment (Menkes *et al.* 1983). This enhanced sensitivity has further been explained by the observation of an enhanced affinity of the α_1 -adrenergic receptor following long-term administrations of TCA's. This NE supersensitivity following TCA's treatment is however not universal as it stands in contrast to what has been reported in the hippocampus. Indeed, Lacroix *et al.* (1991a) have demonstrated that a long-term administration with desipramine, while inducing a functional desensitization of β -receptor function, does not induce any alteration of the sensitivity of either postsynaptic α_1 -adrenergic nor α_2 -adrenergic receptors. This was determined by assessing the effectiveness of locus coeruleus stimulation and microiontophoretic applications of NE, respectively. These results are congruent with the observation that prolonged, but not acute treatments, with various antidepressants (imipramine, citalopram and amitriptyline) enhance the number of α_1 -adrenergic receptors in the cortex but not in the hippocampus (Nowak and Przegalinski 1988; Maj *et al.* 1985). Nonetheless, an increase in the sensitivity of α_1 -adrenergic receptors following a long-term administration of imipramine has been observed in the hippocampus using an *in vitro* electrophysiological paradigm (*i.e.*, the ability of phenylephrine to reduce the population spike amplitude of CA₁ pyramidal neurons following electrical stimulation of Schaffer's collaterals; Bijak and Papp 1995). The exact reason for this discrepancy is not clear although it may be related to the fact that whereas the study of Lacroix *et al.* (1991a) was carried out in the CA₃ region of the hippocampus, that of Bijak and Papp (1995) was in the CA₁ region. In any event, these results underscore the presence of regional disparities with regards to antidepressant action on the α_1 -adrenergic receptor function.

10.4.2. α_2 -adrenergic receptors

The function of the α_2 -adrenergic receptors located postsynaptically has been assessed following long-term administration of various antidepressant treatments. The effect of microiontophoretic applications of NE in the hippocampus was thus found to be unchanged following long-term administration of desipramine, imipramine and amitriptyline (de Montigny and Aghajanian 1978; Gallager and Bunney, Jr. 1979; Marek *et al.* 1992; Lacroix *et al.* 1991a), repeated ECS (de Montigny 1984; Mongeau *et al.* 1994a), SSRI's (Mongeau *et al.* 1994b; Blier *et al.* 1984), nisoxetine (Mongeau *et al.* 1994b), biefloxatone, clorgyline, phenelzine and amiflamine (Mongeau *et al.* 1994b; Blier *et al.* 1986a; Blier *et al.* 1986b).

Since the response to microiontophoretic applications of NE in the hippocampus is mediated by α_2 -adrenergic receptors (Armah *et al.* 1990), these results argue that antidepressant treatments do not alter the function of postsynaptic α_2 -adrenergic receptors. These results also add credence to the notion that the function of the latter receptors appears to be immutable. Indeed, a 6-OHDA lesion does not induce any change in the function of these receptors in the hippocampus (de Montigny *et al.* 1980) nor in the amygdala (Wang *et al.* 1979). It is of note however that a relatively early study has suggested that a prolonged treatment (10 days) with amitriptyline or repeated ECS treatments reduce the number of α_2 -adrenergic receptors (Barturen and Garcia-Sevilla 1992). However this decrease would occur early in the midst of the treatment and gradually disappear in such way to be absent after three weeks of treatments (Barturen and Garcia-Sevilla 1992). Taken together, these results suggest that long-term administration of antidepressants does not alter the function nor the number of postsynaptic α_2 -adrenergic receptors to any significant and relevant extent.

10.4.3. β -adrenergic receptors

It is in 1975 when was published the first report that the long-term, but not the acute, administration of many types of antidepressant leads to a decrease in β -adrenergic mediated function (Vetulani and Sulser 1975). This study was to be soon confirmed by that of Banerjee *et al.* (1977). These reports have fostered tremendous interest to the extent that this phenomenon is perhaps the most extensively documented and has hence been reviewed extensively elsewhere (for example, Sulser *et al.* 1984b; Sulser 1984a; Potter 1996). Perhaps the most prominent feature of this phenomenon is the striking convergence that has been attained with the use of different experimental approaches, *i.e.*, from functional electrophysiological studies (Lacroix *et al.* 1991b) to biochemical studies (mostly assay of cAMP stimulation; Manier *et al.* 1987; Sapena *et al.* 1992; Nelson *et al.* 1991; Schoffelmeer *et al.* 1984) and of receptor number using radioligand binding studies and autoradiography (Maggi *et al.* 1980; Mogilnicka *et al.* 1987; Ordway *et al.* 1988; Nelson *et al.* 1990; Paetsch and Greenshaw 1993; Hosoda and Duman 1993). These studies show that antidepressant treatments such as ECS, imipramine, desipramine, maprotiline, zimelidine, iprindole, oxaprotiline, amitriptyline, tranlylcypromine, clorgyline, deprenyl, sertraline, nioxetine, bupropion or phenelzine leads to a decrease of β -adrenergic receptor function. There are some considerations that need to be nonetheless considered with regard to the reported down-

regulation of β receptors following antidepressant treatments. These will be considered in an epistemological context in which several conditions must be fulfilled for a given set of neurobiological observations to be correlated to a clinical observation (in our case, therapeutic efficacy). First, this purported down-regulation is not universal: indeed it is now well established that effective antidepressants such as fluoxetine, citalopram, paroxetine, mianserin and milnacipran do not induce a β -adrenergic receptor downregulation (Palvimäki *et al.* 1994; Saper *et al.* 1992; Nelson *et al.* 1990; Nèllet *et al.* 1996; Maggi *et al.* 1980). Secondly, in order for a neurobiological action to be validly transposed to any therapeutic efficacy, all drugs that share this action need to also display the clinical outcome. In this context, this is not fulfilled as there are some drugs that whilst inducing β -adrenergic downregulation are not effective antidepressants. The antipsychotic chlorpromazine, which does not show antidepressant activity, represents an illustrative example (Sulser *et al.* 1978; Schoffeleers *et al.* 1984). Third, it seems only legitimate that if this β -adrenergic downregulation were to be clinically relevant, the acute blockade with a β -blocker should demonstrate antidepressant activity. In this regard, not only β -blockers are not effective antidepressants, but their use can even be depressogenic (Paykel *et al.* 1982). In support of this, Avorn *et al.* (1986) have shown that in a population taking different classes of antihypertensive drugs, the ones who were using β -blockers were indeed showing a greater comorbidity with TCAs. Moreover, clinical studies have further suggested that some β -adrenergic receptor agonists such as salbutamol and clenbuterol display (albeit weak) antidepressant effects (Potter *et al.* 1990; Lecrubier *et al.* 1980; Widlocher *et al.* 1977). Finally, we will consider the temporal resolution. It will be mentioned that the β -adrenergic downregulation following antidepressant treatment has been shown to occur following sub-chronic regimens (Newman-Tancredi *et al.* 1996) which finding renders the temporal resolution of this phenomenon incongruent with that observed for antidepressant treatments in major depression.

Taken together, these observations most likely preclude the β -adrenergic downregulation to significantly contribute to the therapeutic activity of these drugs in major depression as this hypothesis is, at the least, not firmly convincing nor supported.

11. INTEGRATIVE ASPECT

Despite being a potentially very difficult and hazardous process, the transposition of experimental results obtained in the animal to human behaviour or therapeutic applications represents the *primum movens* of fundamental research. An epistemologically valid transposition process requires an adequate understanding of certain guiding principles. The search for the identification of the mechanism by which antidepressant treatments exert their therapeutic activity makes no exception. In the following lines, I will discuss some of the arguments that support the proposition that an increased 5-HT neurotransmission mediates the antidepressant response.

The initial discovery that effective antidepressant therapies such as iproniazid and imipramine altered the catabolic or the reuptake process of 5-HT in such a way to potentially influence the 5-HT-mediated synaptic event has been regarded as being suggestive that, indeed, the 5-HT system would have an active role in the antidepressant effect induced by these drugs. Of course, especially considering the wide spectrum of receptor affinities exhibited by TCA's, the effect of these antidepressants on 5-HT-related event could well represent an epiphenomenon to the antidepressant effect *per se*. It is in this context that the heuristic value of the demonstration of a remarkable commonality of actions of seemingly unrelated antidepressants converging to the 5-HT system is the most obvious. That this increase in 5-HT neurotransmission in the animal takes place with a time course that is compatible with that observed in a clinical setting represents a contention that is not only supportive of the hypothesis, but that is also mandatory.

If the antidepressant effect were indeed caused by an increase of 5-HT neurotransmission, any intervention that would significantly hinder the 5-HT neurotransmission in human should induce a relapse of depressive symptoms in patients treated with antidepressants. In humans, two strategies have been used to experimentally put this hypothesis to the test. The first was successfully used by Shopsin *et al.* The paradigm involved hindering 5-HT synthesis in patients effectively treated with imipramine or tranylcypromine with the use of the tryptophan hydroxylase inhibitor PCPA. The authors have observed a relapse of depressive symptoms in those patients. Although this outcome, by itself, is of great interest, they have also observed that the depressive symptoms that reappeared in these patients are strikingly similar to those reported before the onset of the initial antidepressant treatments. Essentially, these results suggest that an intact 5-HT

neurotransmission is a *sine qua non* prerequisite for antidepressants to express their therapeutic efficacy. This particular study, although not replicated, still provides one of the most pivotal evidence supporting a role for the 5-HT system in mediating the antidepressant effect. The second approach that has been used is the tryptophan depletion paradigm. In humans, a rapid but transient depletion of 80 to 90% of plasma tryptophan is accomplished in less than 5 hours after the oral ingestion of a tryptophan free amino-acid mixture (Young *et al.* 1989; Young *et al.* 1985). This paradigm was also shown to be accompanied by a decrease of 5-HT synthesis (Nishizawa *et al.* 1997). Thus, hindering 5-HT neurotransmission with the use of this paradigm in human was shown to cause a rapid but transient relapse of clinical depression in 50% to 70% of depressed patients treated with SSRI's although the incidence was lower in patients treated with desipramine (Delgado *et al.* 1990). Essentially, these results can be viewed as an adjunct to the results previously reported by Shopsin *et al.*, and thus, altogether, these observation constitutes robust indication that the 5-HT system is playing a determinant role in the antidepressant response.

Studies trying to provide an anatomical location of the antidepressant effect have not been able to propose a convincing scheme. However, one can ask whether there is correlation between the anatomical location of structures known to be innervate by 5-HT system and the functions that are altered in major depression. The fact that virtually all 5-HT neurons are located in the brain stem on or near the midline in the raphe cell and are clustered in distinct entities (as opposed to dispersed throughout the brain) in combination with a wide efferent system allows for an effective integration of various inputs that can easily results in a concerted modulation of a wide number of brain functions, including those of higher orders such as affect and cognition. It is here interesting to add the following. 5-HT itself is found in a variety of species including non-vertebrates such as the nematodes and *aplysia formica* and is thus a phylogenically old neurotransmitter. These observations suggest that first, the central 5-HT system is likely to subserve basic and fundamental functions within the CNS and second, that these functions must, to some degree, be common to the physiology and/or behaviour of various species such as fish, amphibians reptiles, and humans. In this respect, the involvement of this neurotransmitters in the treatments of disorders of affective nature such as major depression can at first sight seem unlikely. Nonetheless, the involvement of 5-HT in higher brain functions finds experimental support and thus suggest that this system has undergone a somewhat phylogenically parallel development with these brain functions. Altogether, the

concept that the pharmacological manipulation of such a system might be useful in the treatment of a disorder such as major depression seems assertable.

There are a myriad of functions for which the NE/locus coeruleus system has been suggested to be involved with. These include vegetative functions such as sleep and to functions of a cognitive nature such as different types of memory, selective attention and vigilance. A privileged window for the study of the physiological role subserved by the noradrenergic system has been the recording of locus coeruleus neurons of freely moving cats and this, in response of various stimuli and environmental conditions. Such experiments have permitted to delineate a role of the locus coeruleus in responses to various stressfull stimuli and to be involved in attention and orientation of the animal. One of the most prominent features of the pattern of firing activity of the cat locus coeruleus NE neurons, is its dependence on the behavioural state of the cat [to such an extent that it is employed as a criterion to confirm the noradrenergic nature of recorded neurons]. From a firing activity of *ca.* 2 Hz during active waking and arousal, the NE locus coeruleus neurons decline in activity across quiet waking, drowsiness and slow wave sleep and are completely silent during REM sleep. Interestingly, firing activity is little or not affected by various behaviours such as eating, grooming and various social interaction patterns as well as in response to non-stressful tactile stimuli. However, in responses to stressful-stimuli (presentation of a dog, prolonged restraint or exposition to a prolonged loud noise) the locus coeruleus NE neurons firing activity increased by 100-200% and this, in parallel with an increase of sympathetic nervous system activity index, *i. e.*, heart rate and plasma NE levels (Wilkinson and Jacobs 1988; Abercrombie and Jacobs 1987; Levine *et al.* 1990; Jacobs *et al.* 1991). In accordance with this, there is a considerable amount of studies that have demonstrated an increased level of NE in various regions of the brain that receives noradrenergic innervation in response to stress (usually foot shock; for example see Abercrombie *et al.* 1988; Tanaka *et al.* 1991; Tanaka 1999; Bremner *et al.* 1996). Further experiments have been able to refine this general scheme by showing that, in essence, the locus coeruleus NE neurons are best activated by stimuli possesses attributes of unexpectedness and novelty and have thus been suggested to be involved in vigilance (Aston-Jones *et al.* 1991).

Taken together, a general role for NE action of the brain has been perhaps best described by (Jacobs *et al.* 1991) that have suggested : “ the NE system of the CNS may act to enhance an organism’s reaction to internal or external stimuli, that is, the processing of

sensory information and of the motor response to it. This enhancement may occur during time of stress . . . or, in response to phasic alerting stimuli.” The nature of the responses elicited by NE/locus coeruleus activation, in conjunction with that of the stimuli that stimulates locus coeruleus activity has further prompted (Aston-Jones *et al.* 1991) to suggest that “. . . The locus coeruleus provides the cognitive complement to sympathetic function.” The cellular function of NE, combined with the very wide innervation pattern of locus coeruleus neurons provide the substrate necessary for such an extensive modulatory role of ones behaviour.

A hyperadrenergic tone has been associated with the expression of the anxiety state. In light of the function attributed to NE/locus coeruleus function depicted hereabove, the clinical manifestation of such a disorder (agitation, enhanced motor output etc,) easily finds a conceptual basis. Experimental evidence illustrative of this also exist. For example, administration of α_2 -adrenergic antagonists to rats and monkeys (which enhance both NE neurons firing activity and hence release) produces behaviours consistent with anxiety and fear (Bremner *et al.* 1996). In major depression, the picture is somewhat more obscure considering the heterogenous nature of the disorders. On the one hand, some depressive symptoms, *i.e.*, dysphoric arousal (anxiety), disturbed cognition, agitation and insomnia can be associated with an increased in NE output. On the other hand, symptoms such as anergia and anhedonia (the NE hypothesis of anhedonia (Van Praag *et al.* 1991) are more consistent with the presence of an abnormal reduction of noradrenergic function. An altered noradrenergic function in either direction could be involved in the manifestation or expression of different symptoms of major depression and hence account for perhaps part of the heterogenous nature of the disease.

What then does antidepressant do (or should do) to noradrenergic neurotransmission? There is considerable amount of data that needs to be integrated in order to provide a satisfactory answer to this. Some generalization can be attempted. As an illustrative example, we will consider first the formidable corpus of data showing that some, but not all, antidepressant treatments lead to down-regulation/desensitization of postsynaptic β -adrenergic receptor function. Although it seems clear that this does not participate in the antidepressant action (see section 10.3), conversely, it suggests that the system, during the course of treatment, is confronted to an enhanced noradrenergic output thus leading to a desensitization of postsynaptic β -adrenergic receptors. If we consider the general scheme depicted in Figure 7, one can appreciate that a desensitization (or down-regulation) of any given postsynaptic receptor need not necessarily to be accompanied with an overall decrease in

neurotransmission. Unfortunately, direct experimental evidence supporting (or refuting) this contention is unfortunately seldom found as most pharmacological studies involve paradigms of “challenging” and therefore do not assess the overall transmission. In any event, these considerations suggest that the down-regulation of β -adrenergic receptor function does not necessarily equate to a diminished NE neurotransmission. A similar reasoning can be applied to the general findings that drugs that enhance the synaptic concentration of NE leads to a desensitization of α_2 -adrenergic heteroreceptors located on 5-HT terminals. Indeed, the development of such a desensitization would be indicative that the system reacted to a sustained increase of NE tone. Therefore, as depicted by figure 7, it would appear hazardous to propose that the presence of such a desensitization would be accompanied by an enhanced 5-HT output. Indeed, it would appear more likely that the inhibitory tone exerted by the α_2 -adrenergic heteroreceptor would, in the course of a prolonged treatment that would increase NE output, desensitize in a way to exert a net effect not different from that of control level. In this respect, it must be underscored that there is no experimental evidence showing that, despite the desensitization of the α_2 -adrenergic heteroreceptors located on 5-HT terminals, the net 5-HT is actually increased.

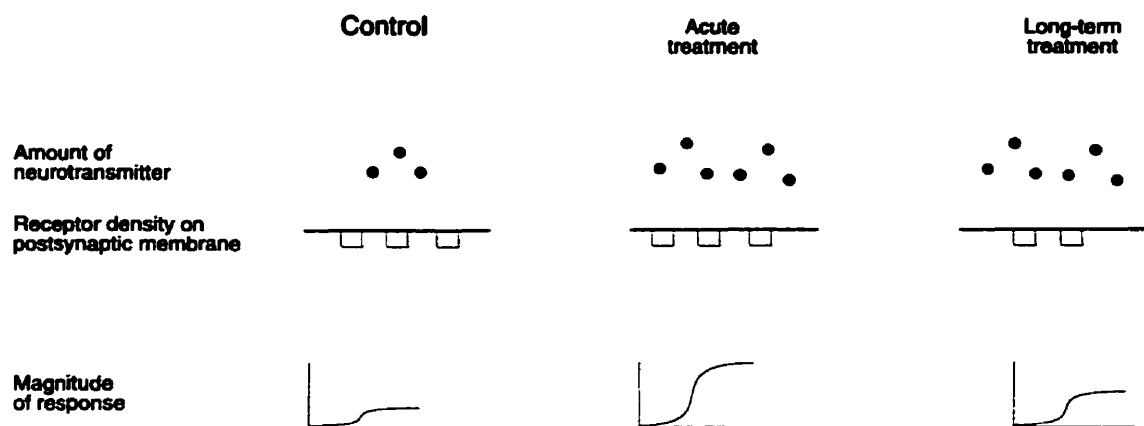


Figure 7. Schematized drawing illustrating a putative scheme expressing the magnitude of the overall neurotransmission of a system following the development of a down-regulation (and/or desensitization) of its receptors induced by sustained exposure to an increased amount of the neurotransmitter. This drawing is largely inspired from a model proposed by Duman *et al.* 1997

It has been covered that NE reuptake blockers, despite silencing locus coeruleus NE neurons, lead to enhanced level of extracellular NE in terminal areas. Long-term

administration of SSRI's, by reducing firing activity of NE neurons without a concurrent blockade of the reuptake of NE, therefore most probably reduces overall NE neurotransmission, yet is effective in major depression. Based on these results, it appears that there is lack of commonality of action of antidepressants on the NE system, in contrast to the 5-HT system suggesting that this system does not represent the primary system of antidepressant action. However, this by no means indicate that an action on this system does not any therapeutic benefits in major depression. Major depression is a heterogenous disorders. The clinical response to antidepressants is also heterogenous. As covered above, it is plausible that part of the heterogenous response to antidepressants reflects, perhaps only partly, their involvement with NE neurotransmission.

12. DISCUSSION

The introductory section of the present thesis was meant to present a comprehensive and critical review of current understandings of various aspect of the physiology and pharmacology of both the 5-HT and NE systems. Although implicated at different “levels”, both these systems have found implications in the mediation of the antidepressant effects and have posed as likely targets for the development of rational approaches articulated, of course, in a therapeutic perspective. The formulation of such rational approaches can however be significantly attempted (or appreciated) only in the presence of an adequate appraisal of the physiology and pharmacology of the two systems, hence the need for such a dissertation. These rational approach should be formulated in such ways as to provide likely avenue for the development of therapeutic approaches for a better antidepressant activity than that available in the present armamentarium. This contention should be expressed in essentially two clinical dimensions: a faster onset of therapeutic activity as well as efficacy in treatment-resistant depression. Formulated perhaps in a simplistic, although valid, fashion, there are two different propositions that will be considered here separately.

1- A better antidepressant response should be obtained with a combined action on both the 5-HT and NE system, and 2- A faster antidepressant response should be obtained by the combination of a 5-HT reuptake inhibitor and an antagonist of the somatodendritic 5-HT_{1A} autoreceptors.

The first hypothesis finds its rationale in the fact that effective antidepressant treatments are known to block the reuptake of 5-HT or of NE. However, a commonality of action of all effective treatment for major depression has been demonstrated for the 5-HT system, whereas such a demonstration for the NE system is lacking. Although this debate is still ongoing, clinical studies have been able to gain relevant insight by providing evidence that a combined pharmacological action on both these systems might bear synergistic therapeutic benefit. Indeed, several reports have suggested that some TCA's may be more effective than SSRI's in the treatment of severely depressed patients (Danish University Antidepressant group 1986; Danish University Antidepressant group 1990) and that this may be the result of their combined 5-HT and NE reuptake inhibition properties. Moreover, initial reports suggest that the combination regimen of the SSRI fluoxetine and the NE reuptake inhibitor desipramine resulted in a more robust antidepressant action than desipramine alone (Nelson *et al.* 1991; Nelson *et al.* 1998), while it was also suggested that such a combination therapy

was effective in treatment-resistant depression (Weilburg *et al.* 1989; Seth *et al.* 1992; Zajecka *et al.* 1995). The neurobiological substrate underlying the role of the recruitment of the NE system in mediating these clinical features is ill-defined. There exists however evidence from fundamental studies suggesting that blockade of the reuptake of NE leads to a greater output of 5-HT when the reuptake of 5-HT is already blocked. These results thus suggest that the beneficial effect of NE reuptake inhibition is ultimately mediated by an enhanced 5-HT output.

Based on these considerations, the development of compounds that would have a dual 5-HT and NE reuptake inhibiting properties finds a rational basis. To date, several such compounds have been developed although one has received extensive attention owing to its striking clinical features. Venlafaxine (1-[2-(dimethylamino)-1-(4-methoxyphenyl)-ethyl]cyclohexanol) is a phenylethylamine derivative which has demonstrated antidepressant properties (Schweizer *et al.* 1991; Guelfi *et al.* 1995; Guelfi *et al.* 1992; Mendels *et al.* 1993; Schweizer *et al.* 1994). Moreover, clinical studies have demonstrated that venlafaxine displays a positive dose-response relationship with regard to clinical efficacy (Schweizer *et al.* 1991; Mendels *et al.* 1993). Most interestingly, this relation is not observed with SSRIs. Venlafaxine displays the pharmacological profile of inhibiting, *in vitro*, the synaptosomal uptake of both 5-HT and NE and to a lesser degree of DA. Since venlafaxine inhibits the synaptosomal uptake of 5-HT with a greater potency than that of NE, suggests that venlafaxine would be a more potent inhibitor of 5-HT reuptake. The pharmacological profile of venlafaxine is further characterized by its lack of effect on MAO activity and by its lack of affinity at rat muscarinic, cholinergic, H_1 , α_1 -, α_2 -, and β adrenergic, DA_2 , benzodiazepine, opiate and 5-HT₁ and 5-HT₂ receptors (Muth *et al.* 1986) and of human H_1 , muscarinic, α_1 -, α_2 -, adrenergic, DA_2 , 5-HT_{1A} and 5-HT₂ receptors (Cusack *et al.* 1994).

In discussing the pharmacological profile of this compound, it is necessary to present the study of Moyer *et al.* (1992), who reported a rapid desensitization of β -adrenergic receptors following both acute (10 mg/kg, i.p.) and chronic (10 mg/kg, i.p.) administrations of venlafaxine. This desensitization is usually observed following chronic administration of certain antidepressants (See section 10.4.3). Interestingly, the combination of fluoxetine and desipramine, has also been demonstrated to induce a rapid down-regulation of β -adrenergic receptors (Baron *et al.* 1988). This observation led to the hypothesis that venlafaxine may show a rapid antidepressant effect. Nevertheless, this contention would be verified only if β

-adrenergic receptors desensitization were involved in the antidepressant efficacy of these drugs. This is however not supported by several findings (see section 10.4.3).

Notwithstanding this latter contention, the unique pharmacological profile of venlafaxine could nevertheless still give venlafaxine the propensity to display a more robust clinical efficacy. Indeed, as venlafaxine inhibits more profoundly 5-HT synaptosomal uptake compared to that of NE, it is therefore possible that when venlafaxine is administered at low dose, it would only block the 5-HT reuptake process thus acting as an SSRI, while as the dose is increased, there would be a gradual blockade of the NE uptake process. As both the 5-HT and the NE system influence one another, the simultaneous and sustained blockade of both the reuptake processes might have a synergistic effect and consequently increase the 5-HT neurotransmission to a greater extent and possibly more rapidly. This latter assertion might well represent the biological substratum responsible for the positive dose-response relationship observed clinically with venlafaxine (Mendels *et al.* 1993; Kelsey 1996) as well as for its reported faster onset of action (Derivan *et al.* 1995). Given these considerations it is crucial to assess the functional characteristics of venlafaxine where the interconnectivity and relative importance of both systems and with regard to how they are affected by a dual uptake inhibitor.

The goal of chapters I, II, III, IV and V was to provide a concise picture of the neurobiological actions induced by acute, short-term and long-term administration of venlafaxine on both the 5-HT and NE system. This research endeavour was undertaken with the overt goal of providing the neurobiological correlate of the apparent superior clinical efficacy of venlafaxine in the treatment of major depression.

2- A faster antidepressant response should be obtained by the combination of a 5-HT reuptake inhibitor and an antagonist of the somatodendritic 5-HT_{1A} autoreceptor.

For the present discussion, let's consider only the mechanism by which SSRI's increase 5-HT neurotransmission. The understanding of the series of event underlying the enhancement of 5-HT neurotransmission by antidepressant treatment has clearly shown that the early activation of the somatodendritic 5-HT_{1A} autoreceptors by SSRI's treatment offsets the enhancement of 5-HT transmission. This effect gradually disappears with time paralleling the recovery of firing activity of the 5-HT neurons. The evidence suggesting that the activation of postsynaptic 5-HT_{1A} receptors might represent a final common pathway to antidepressant

treatment has been covered elsewhere in this thesis. Therefore, attaining an earlier activation of this population of receptor is likely to accelerate the antidepressant response. In the case of 5-HT reuptake inhibitors, it thus seems legitimate to propose that circumventing the homeostatic inhibitory action of the somatodendritic 5-HT_{1A} autoreceptor represents a promising avenue. In support of this, it is well demonstrated that the combination of the mixed 5-HT_{1A}/β-adrenergic receptor antagonist pindolol (or any 5-HT_{1A} antagonists tested) with SSRI's induces a greater increase in the extracellular concentration of 5-HT than that induced by the SSRI alone (see chapter VI). Nonetheless, this hypothesis presents a major caveat in that it relies on the possibility to block selectively the somatodendritic 5-HT_{1A} receptor and not its postsynaptic congener. Indeed, should the postsynaptic 5-HT_{1A} receptors be blocked, an important and critical fraction of the 5-HT neurotransmission would prove to be concurrently blocked. Hence, these contentions imply that the SSRI treatment be accompanied by a selective blockade of the somatodendritic 5-HT_{1A} receptors. Although there is evidence showing that (-)pindolol shows antagonistic action of the somatodendritic 5-HT_{1A} receptors and not on postsynaptic 5-HT_{1A} receptors of the dorsal hippocampus, the effect of the coadministration of a 5-HT reuptake inhibitor and (-)pindolol on the activation of the postsynaptic 5-HT_{1A} receptors is yet to be determined. In chapter VI, we have carried out experimental series to assess directly this fundamental issue. To this end, we have described and validated a paradigm by which the activation of the postsynaptic 5-HT_{1A} receptors by a 5-HT reuptake inhibitor can be quantified and how this activation is potentiated by a pretreatment with (-)pindolol.

CHAPTER I.

AFFINITIES OF VENLAFAXINE AND VARIOUS REUPTAKE INHIBITORS FOR THE SEROTONIN AND NOREPINEPHRINE TRANSPORTERS⁴

JEAN-CLAUDE BÉIQUE*, NORMAND LAVOIE, CLAUDE DE MONTIGNY AND GUY DEBONNEL.

ABSTRACT

In vitro radioligand binding studies were carried out in rat brain membranes to assess the affinity of various reuptake inhibitors for the serotonin (5-HT) and the norepinephrine transporters using the selective ligands [³H]cyanoimipramine and [³H]nisoxetine, respectively. The selective 5-HT reuptake inhibitors paroxetine, indalpine and fluvoxamine displayed a high affinity for the 5-HT transporter whereas the norepinephrine reuptake inhibitor desipramine had a high affinity for the norepinephrine transporter. Duloxetine, a dual 5-HT and norepinephrine reuptake inhibitor, displayed a high affinity for both the 5-HT and the norepinephrine transporters. Interestingly, venlafaxine, a dual 5-HT and norepinephrine reuptake inhibitor, displayed only a moderate affinity for the 5-HT transporter ($K_i = 74$ nM) and a very low affinity for the norepinephrine transporter ($K_i = 1.26$ μ M). The relatively low affinities of venlafaxine contrast with its potent *in vivo* 5-HT and norepinephrine reuptake blocking properties. These results raise the possibility that the *in vivo* effects on the 5-HT and norepinephrine reuptake observed with venlafaxine may not be mediated solely by its binding to the [³H]cyanoimipramine and [³H]nisoxetine binding sites.

⁴The study presented in this chapter is published in *EUROPEAN JOURNAL OF PHARMACOLOGY* (1998), 349, 129-132. Reprinted with permission from Elsevier Science. Copyright 1998.

1. INTRODUCTION

Several lines of evidence point towards the involvement of the serotonergic (5-Hydroxytryptamine; 5-HT) and norepinephrine systems in the mechanism of action of antidepressant treatments (Blier and de Montigny, 1994). Hence, a new class of drugs known as dual 5-HT and norepinephrine reuptake inhibitors has been developed. For instance, venlafaxine blocks *in vitro* the synaptosomal uptake of both [^{3} H]5-HT and [^{3} H]norepinephrine (Muth et al., 1986; Bolden-Watson and Richelson, 1993). Clinical studies are now suggesting that not only venlafaxine displays antidepressant activity, but that it would also be more efficacious than fluoxetine for the treatment of major depression (Dierick et al., 1996), would be effective in the treatment of resistant depression (Nierenberg et al., 1994; de Montigny et al., 1996) and, finally, would show an early onset of action (Rickels, 1991). However, while venlafaxine shows an appreciable potency for inhibiting both 5-HT and norepinephrine reuptakes *in vivo* (Béïque et al., 1996), its potency to block *in vitro* the uptake of these monoamines is relatively low when compared to other reuptake inhibitors such as paroxetine for 5-HT uptake or desipramine for norepinephrine uptake (Bolden-Watson and Richelson, 1993). This latter observation, combined with the potentially unique therapeutic profile of venlafaxine, thus underscores the importance of a more accurate understanding of its pharmacological actions.

The present study was thus undertaken to directly determine the affinities of venlafaxine for both the 5-HT and norepinephrine transporters. To this end, the selective radioligands [^{3} H]cyanoimipramine and [^{3} H]nisoxetine, that bind selectively to the 5-HT and norepinephrine transporters, respectively (Burkard, 1980; Dumbrille-Ross and Tang, 1983; Tejani-Butt et al., 1990), were used. In order to provide a direct within study comparison, the affinities for the 5-HT and norepinephrine transporters of other monoamine reuptake inhibitors have also been determined as well as those of duloxetine, an antidepressant candidate which is also a dual 5-HT and norepinephrine reuptake inhibitor (Wong et al., 1993; Kasamo et al., 1996).

2. MATERIALS AND METHODS

2.1. Animals

Male Sprague Dawley rats (250-275 g) were used.

2.2. Radioligand binding procedures

Binding assays were performed on total brain minus cerebellum using procedures modified from Burkard (1980) and Dumbrille-Ross and Tang (1983) for [³H]cyanoimipramine binding and from Tejani-Butt *et al.* (1990) for [³H]nisoxetine binding. Brains were homogenized in 50 vol Tris-HCl buffer (50 mM, pH: 7.4). Following centrifugation (40,000 x g, 10 min at 4°C), the pellet was washed (3X) by resuspension and centrifugation. For [³H]cyanoimipramine binding, the final pellet was resuspended in 50 vol Tris-HCl buffer (50 mM, pH: 7.4) containing 120 mM NaCl and 5 mM KCl and immediately frozen at -70°C until used. For [³H]nisoxetine binding, it was resuspended in 300 mM NaCl and 5 mM KCl.

2.3. Saturation experiments

Saturation experiments were conducted in a final volume of 250 µl containing 8 different concentrations (0.003 to 4 nM) of either [³H]cyanoimipramine or [³H]nisoxetine and ~250 µg of proteins. Non-specific binding for [³H]cyanoimipramine was determined with 10 µM of paroxetine while that for [³H]nisoxetine with 10 µM of mazindol. After an incubation period of 1 h at 23°C for [³H]cyanoimipramine or 4 h at 4 °C for [³H]nisoxetine, the incubation was terminated by the addition of 4 ml of ice-cold buffer to each tube and rinsed 3 times with the same buffer. The contents were filtered under vacuum through Whatman GF/B glass fibre filters pre-soaked for 1 h in 0.3% polyethylenimine, using a Brandel cell harvester.

2.4. Competition assay

For the *in vitro* determination of K_i values, aliquots of membrane suspensions (≈ 250 µg protein) were incubated for 1 h at 23°C with 1 nM [³H]cyanoimipramine or for 4 h at 4°C with 2 nM of [³H]nisoxetine and with 19 to 22 concentrations (range 10⁻¹³- 10⁻⁵ M) of test molecules in a final volume of 250 µl. After incubation, 4 ml of ice-cold buffer were added and rapidly filtered, as described for the saturation experiments. The filters were subsequently

washed (3 times), dried and placed in scintillation vials with 5 ml scintillation cocktail. Radioactivity contained on the individual filters was determined by liquid scintillation spectrometry (Beckman LS 6000SE).

2.5. Statistics

Binding data were analysed by non-linear regression analysis using the "Receptor Fit Competition" program (Lundon Software, Chagrin Falls, OH). .

3. RESULTS

3.1. Saturation studies

Non-linear regression analysis of the saturation curves obtained following the incubation with different concentrations of [^3H]cyanoimipramine and [^3H]nisoxetine with rat brain homogenates indicated that each of these two radioligands labels a single class of saturable binding sites with a Hill coefficient close to unity. [^3H]Cyanoimipramine binding yielded a K_d of 0.61 ± 0.13 nM and a B_{max} of 225.5 ± 31.8 fmol/mg proteins whereas for [^3H]nisoxetine a K_d of 0.76 ± 0.042 nM and a B_{max} of 63.60 ± 5.91 fmol/mg protein were obtained.

3.2. Competition studies

Desipramine, duloxetine, fluoxetine, fluvoxamine, indalpine, paroxetine and venlafaxine were tested for their ability to displace [^3H]cyanoimipramine or [^3H]nisoxetine from the 5-HT and norepinephrine transporters, respectively. The competition curves are shown in Fig. 1. All tested compounds yielded monophasic competition curves with Hill coefficients close to unity for both radioligands used. The K_i values as well as the selectivity ratios (5-HT/norepinephrine) are shown in Table 1. Nineteen to 22 concentrations (10^{-13} M to 10^{-5} M) of unlabelled compounds were used for each value, expressed as mean $\pm$ S.E.M. from three experiments performed in triplicate.

4. DISCUSSION

In the present study duloxetine displayed a high and nearly equivalent affinity for both transporters, while venlafaxine, surprisingly, had only a moderate affinity for the 5-HT transporter and a low affinity for the norepinephrine transporter.

[³H]Cyanoimipramine has been shown to bind to a site that is associated with the 5-HT transporter and the reported K_d values are in keeping with the one reported in the present study (Burkard, 1980; Dumbrille-Ross and Tang, 1983). Similarly, [³H]nisoxetine labels a site known to be associated with the norepinephrine transporter, and the observed binding parameters are in keeping with those of previous studies (Tejani-Butt et al., 1990; Gehlert et al., 1995). However, regarding the competition studies with [³H]nisoxetine, all the K_i values obtained with the compounds used in the present study are lower than those reported by Cheetham *et al.* (1996). Nonetheless, the order of affinity of the common compounds for the [³H]nisoxetine binding site is similar in the present study with that of Cheetham *et al.* (*i.e.* desipramine >> paroxetine > fluoxetine > fluvoxamine).

Duloxetine is a dual 5-HT/norepinephrine reuptake inhibitor (Wong et al., 1993; Kasamo et al., 1996). Wong *et al.* (1993) have reported the affinities of duloxetine for both the 5-HT and norepinephrine transporters using [³H]paroxetine and [³H]tomoxetine, respectively. Interestingly, although the radioligands used in that latter study were different from those used in the present one, the reported K_i are in the same range as those reported herein, but with slightly different 5-HT/norepinephrine selectivity ratios (4 vs 1.7), both studies showing a preferential affinity of duloxetine for the 5-HT reuptake site. This preferential action of duloxetine on the 5-HT transporter has also been observed functionally *in vivo*, using an electrophysiological paradigm and also in *ex vivo* uptake studies carried on hippocampal slices (Kasamo et al., 1996).

The affinity of venlafaxine for the [³H]imipramine binding site has been previously assessed by Muth *et al.* (1986) and the reported K_i value falls into the same range as that found in the present study for [³H]cyanoimipramine (*i.e.* 90 in the former vs 74 nM in the present study). A most striking discrepancy emerges from the direct comparison of the affinities of venlafaxine for both transporters with its functional uptake blocking potencies when done in relation with other reuptake inhibitors. For instance, in the present study venlafaxine displays a near 2,000 folds lesser affinity than paroxetine for the 5-HT transporter whereas, *in vitro*, venlafaxine inhibits [³H]5-HT uptake with only a 53 times lesser potency than paroxetine

(Bolden-Watson and Richelson, 1993). The discrepancy is even more important when an *in vivo* electrophysiological paradigm is used for assessing 5-HT reuptake inhibition in the hippocampus and in the dorsal raphe nucleus, as venlafaxine and paroxetine are equipotent in inhibiting 5-HT reuptake (Béïque et al., 1996).

Similarly, the very low affinity of venlafaxine for the norepinephrine transporter which is 2,000 less than that of desipramine contrasts with the fact that, *in vitro*, venlafaxine is only 344 times less potent than desipramine to block [³H]norepinephrine uptake (Bolden-Watson and Richelson, 1993). Similarly to what was observed with 5-HT reuptake inhibition, the discrepancy is even more manifest when an *in vivo* electrophysiological paradigm is used as an index of norepinephrine reuptake inhibition, as venlafaxine was only slightly (~ 3 times) less potent than desipramine to block norepinephrine reuptake (Béïque et al., 1996).

In summary, the present study provides novel data on the binding affinities of duloxetine and venlafaxine for the 5-HT and norepinephrine transporters. Duloxetine binds with high affinity to both transporters in keeping with previous biochemical and electrophysiological studies. However the binding profile of venlafaxine to the two transporters reported herein would be predictive of a much weaker blockade of both the 5-HT and norepinephrine reuptake process by venlafaxine than that reported in functional studies (*in vivo* electrophysiological and *in vitro* uptake studies), as evidenced by the direct comparison with other reuptake inhibitors. This latter assertion thus raises the possibility that the effects observed in functional studies with venlafaxine could be mediated *via* a mechanism distinct from a sole binding to the [³H]cyanoimipramine and [³H]nisoxetine binding site. These important and crucial aspects of discrepancy and considerations, combined with the seemingly unique clinical profile of this drug, thus warrants further studies in order to clarify these issues.

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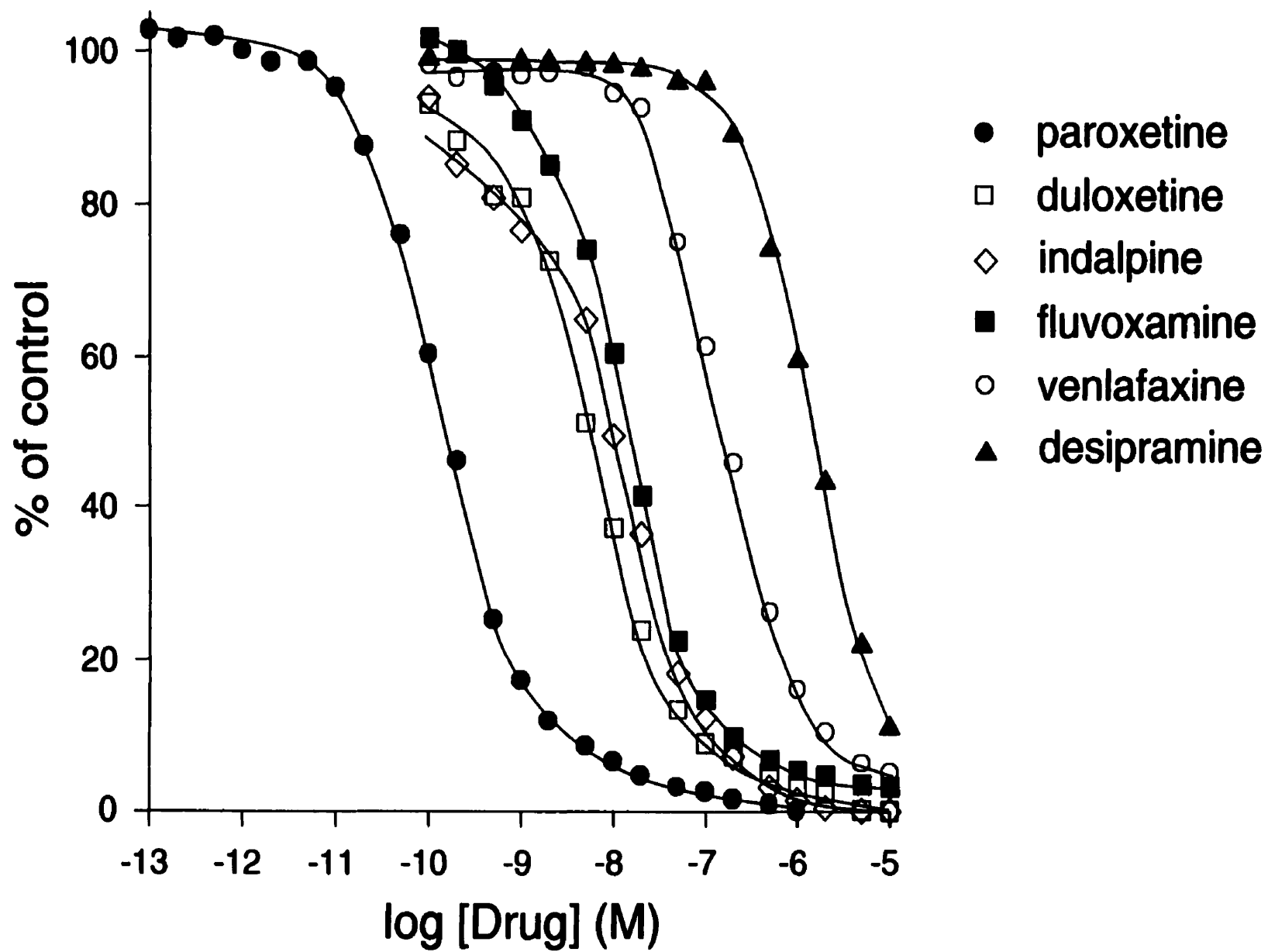
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Figure legends

Figure 1. Competition curves showing the displacement of [^3H]cyanoimipramine (1 nM; A) and [^3H]nisoxetine (2 nM; B) binding by reuptake inhibitors in rat brain (total brain minus cerebellum) membranes. The data are expressed as a percentage of specific binding. All competition curves yielded Hill coefficients close to unity.

Table 1. Inhibition, expressed as K_i values ($\pm$ S.E.M.), of [^3H]cyanoimipramine and [^3H]nisoxetine binding to rat brain homogenates by different monoamine reuptake inhibitors.



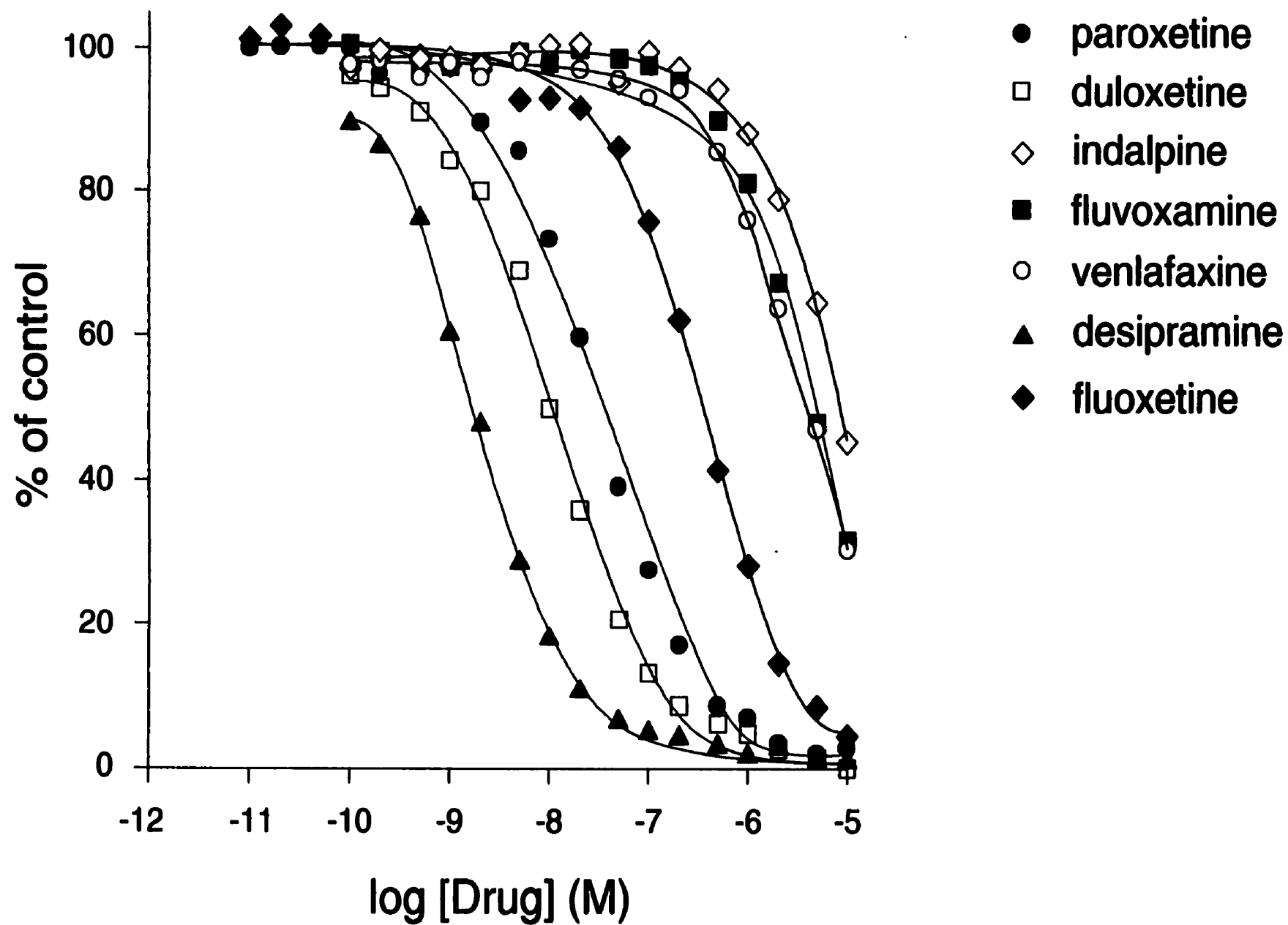


Table 1. Inhibition, expressed as K_i values ($\pm$ S.E.M.), of [^{3} H]cyanoimipramine and [^{3} H]nisoxetine binding to rat brain homogenates by different monoamine reuptake inhibitors.

	K _i (nM)		Selectivity ratio
	[^{3} H]CYANOIMIPRAMINE	[^{3} H]NISOXETINE	
Duloxetine	1.8 $\pm$ 0.1	3 $\pm$ 0.3	1.7
Venlafaxine	74 $\pm$ 1.9	1260 $\pm$ 144	17
Fluvoxamine	6.7 $\pm$ 0.2	1361 $\pm$ 36	203
Paroxetine	0.04 $\pm$ 0.004	11.8 $\pm$ 0.6	294
Indalpine	5 $\pm$ 0.3	2758 $\pm$ 245	557
Desipramine	575 $\pm$ 19	0.55 $\pm$ 0.04	0.0009
Fluoxetine	ND	119. $\pm$ 4	

CHAPTER II

PREFATORY REMARKS

In light of the previously published data on the potencies of venlafaxine to block the synaptosomal uptake of [³H]5-HT and [³H]NE, one could predict that it would bind with a greater affinity to the 5-HT transporter than to the NE transporter. Of course, strictly speaking, this is what has been observed. Nonetheless, we were confronted with the peculiar situation that the absolute affinities of venlafaxine for the two transporters are much lower than what could have been predicted. The results obtained from this first study were, if not surprising, at the least unexpected. At this point in time, one could illustrate the situation as thus: we were in the presence of a compound that blocked the uptake in an in vitro preparation with moderate potencies but yet, that shows remarkable low affinities for the two transporters. Confronted with this situation, it seemed mandatory to assess in a more physiologically relevant manner, the putative 5-HT and NE reuptake blockade properties of venlafaxine. To this end, we sought to determine the 5-HT and NE reuptake blocking properties of venlafaxine using an electrophysiological paradigm that presented the advantage to be carried out in vivo, in the whole animal. Altogether, in light of the confounding results presented in chapter I, this in vivo study was undertaken in order to provide an assessment of the in vivo reuptake blocking properties of venlafaxine. This was all the more so important considering that, at that time, there was no study assessing the reuptake blocking capacities of venlafaxine using an in vivo approach.

BLOCKADE OF 5-HYDROXYTRYPTAMINE AND NORADRENALINE UPTAKE BY VENLAFAXINE: COMPARATIVE STUDY WITH PAROXETINE AND DESIPRAMINE⁵.

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⁵The study presented in this chapter is published in *BRITISH JOURNAL OF PHARMACOLOGY* (1998)125, 526-532. Reprinted with permission from Nature Publishing Group, copyright 1998.

SUMMARY

1 Venlafaxine is an antidepressant agent which blocks *in vitro* the reuptake of both 5-HT and NA. The present *in vivo* electrophysiological studies were undertaken, in the rat, to compare the effects of venlafaxine on 5-HT and NA reuptake to those of the selective 5-HT reuptake inhibitor paroxetine and the selective NA reuptake inhibitor desipramine.

2 Administered acutely, venlafaxine dose-dependently prolonged the time required for a 50% recovery (RT_{50}) of the firing activity of dorsal hippocampus CA_3 pyramidal neurons from the suppression induced by microiontophoretic applications of 5-HT and NA. Venlafaxine and paroxetine increased with a similar potency the RT_{50} values for 5-HT, while desipramine was more potent than venlafaxine at increasing the RT_{50} values for NA. Moreover, venlafaxine demonstrated a greater potency at increasing the RT_{50} values for 5-HT compared to that of NA.

3 A two-day treatment with venlafaxine (delivered s.c. by osmotic minipumps) increased the RT_{50} values for both 5-HT and NA applications. The RT_{50} values for 5-HT were significantly increased at a dose of $10 \text{ mg kg}^{-1} \text{ day}^{-1}$, whereas those for NA were increased at a dose of $20 \text{ mg kg}^{-1} \text{ day}^{-1}$, consistent with the data obtained following the acute administration of venlafaxine.

4. Taken together, these results indicate that, *in vivo*, venlafaxine blocks both reuptake processes, with a potency greater for the 5-HT than for the NA reuptake process. This dual action, combined with the differential potency of venlafaxine, might constitute the biological *substratum* responsible for its apparent unique clinical efficacy in major depression.

INTRODUCTION

Several lines of evidence point towards an involvement of the serotonergic (5-HT) and noradrenergic (NA) systems in the pathophysiology of major depression (see Maes & Meltzer, 1995; Schatzberg & Schildkraut, 1995 for review). As for the therapeutic efficacy of currently available antidepressant agents, a considerable corpus of data suggests that it would be mediated *via* an increase of 5-HT neurotransmission (Blier & de Montigny, 1994). This latter increase has been documented with different classes of antidepressant drugs and shown to occur either through a direct action on the 5-HT system (de Montigny & Aghajanian, 1978; de Montigny, 1984; Blier *et al.* 1986; Chaput *et al.* 1986; Blier & de Montigny, 1990) or through indirect mechanisms *via* the noradrenergic system (Mongeau *et al.* 1994a; Mongeau *et al.* 1994b; Haddjeri *et al.* 1995). Consequently, it is conceivable that a drug that would act simultaneously on the two systems could increase 5-HT transmission to a greater extent, thus potentially providing a greater clinical efficacy and/or display an earlier onset of action.

Clinical evidence have suggested a potential therapeutic benefit of a dual blockade of the 5-HT and NA reuptake processes. Indeed, it was reported that the combination regimen of the selective 5-HT reuptake inhibitor (SSRI) fluoxetine and the NA reuptake inhibitor desipramine resulted in a more rapid antidepressant action than desipramine alone (Nelson *et al.* 1991), while it was also suggested that a similar combination therapy was effective in refractory depression (Weilburg *et al.* 1989; Seth *et al.* 1992; Zajecka *et al.* 1995). It is noteworthy, however, that these results have yet not been verified in double-blind, placebo controlled trials.

Venlafaxine (1-[2-(dimethylamino)-1-(4-methoxyphenyl)-ethyl]cyclohexanol) is a phenylethylamine derivative, which was shown to possess antidepressant properties (Schweizer *et al.* 1991; Guelfi *et al.* 1992; Samuelian *et al.* 1992). It was also reported to display an early onset of therapeutic action (Rickels, 1991; Derivan *et al.* 1995; Benkert *et al.* 1996) and to be effective in treatment-resistant depression when used at high doses (Nierenberg *et al.* 1994; de Montigny *et al.* 1995). This drug was shown to inhibit, *in vitro*, the synaptosomal uptake of both [³H]5-HT and [³H]NA (Muth *et al.* 1986; Bolden-Watson & Richelson, 1993) whilst showing no significant affinity for classical receptor subtypes in rat (Muth *et al.* 1986) and human brain (Cusack *et al.* 1994). In this regard, venlafaxine differs from all other antidepressants available and hence shows a unique pharmacological profile that

is predictive of a more favorable side-effect profile and, more important, that might confer a greater efficacy. However, a recent radioligand binding study has shown that venlafaxine binds to the 5-HT transporter with only a moderate affinity and to the NA transporter with a very low affinity (Béïque *et al.* 1998), thus raising important considerations with regard to the exact mechanism whereby venlafaxine exerts its reuptake blocking action.

Given the relative paucity of neurobiological studies that have examined the neurobiological actions of venlafaxine, it was deemed necessary to further characterize the purported reuptake inhibition properties of venlafaxine using an *in vivo* electrophysiological paradigm in the rat dorsal hippocampus. To this end, the effects of acute intravenous administration of venlafaxine were assessed on the recovery of firing activity of pyramidal neurons from microiontophoretic application of 5-HT and NA. The effects of a short-term treatment (2 days, s.c.) with venlafaxine, which allows the drug to reach steady state concentration, was also assessed. Moreover, in order to fully evaluate the dual 5-HT/NA reuptake inhibition potency of venlafaxine, its acute effect on the reuptake of 5-HT was compared to that of the SSRI paroxetine, and on NA reuptake with that of the NA reuptake inhibitor desipramine.

METHODS

Male Sprague Dawley rats (250-300 g; Charles River, St. Constant, Québec, Canada) were received one day before the experiments and housed three to four per cage. They were kept on a 12:12 h light/dark cycle, with access to food and water *ad libitum*.

Short-term treatments

Rats weighing between 250 and 275 g were anesthetized with halothane for the subcutaneous implantation of osmotic minipumps (Alza, Palo Alto, CA, U.S.A.) that delivered doses of venlafaxine of 10, 20 or 40 mg kg⁻¹ day⁻¹ or vehicle for 48 h. Venlafaxine was dissolved in a 50% ethanol/water solution, and control rats were implanted with minipumps containing vehicle. All experiments were carried out while the minipumps were still on board.

***In vivo* electrophysiological experiments**

Animals were anesthetized with chloral hydrate (400 mg kg⁻¹, i.p.) and mounted in a stereotaxic apparatus. Supplemental doses (100 mg kg⁻¹, i.p.) of chloral hydrate were given to maintain anesthesia, monitored by the absence of a nociceptive reaction to pinching of the tail or of a hind paw. Body temperature was maintained at 37 °C throughout the experiment and a catheter was installed in a lateral tail vein, prior to recording, for intravenous administration of drugs.

Extracellular unitary recordings of CA₃ dorsal hippocampus pyramidal neurons

Five-barrelled glass micropipettes were pulled in a conventional manner and their tips were broken back to 8-12 µm diameter under microscopic control. The central barrel, used for extracellular recordings, was filled with a 2 M NaCl solution containing fast green FCF. Three side barrels, used for microiontophoresis, were filled with the following solutions: 5-HT (20 mM in NaCl 200 mM, pH: 4), NA (20 M in NaCl 200 mM, pH: 4) or acetylcholine (ACh; 20 mM in 200 mM NaCl, pH: 5). The fourth barrel was used for automatic current balancing, and contained 2 M NaCl solution. The microelectrode was lowered 4 mm lateral and 4 mm anterior to lambda, according to stereotaxic coordinates of Paxinos and Watson (1986). CA₃ pyramidal neurons were recorded at a depth of 3.5 to 3.8 mm from the cortical surface and were identified by their characteristic large-amplitude (0.5-1.2 mV) and long-duration (0.8-1.2 ms)

single action potentials alternating with complex spike discharges (Kandel & Spencer, 1961). A leak or a small ejecting current of ACh (0 to + 5 nA) was used to activate silent or slowly discharging neurons to a physiological firing rate of 8-14 Hz (Ranck, 1973). 5-HT and NA were both retained with a current of -12 nA. The uptake activity following microiontophoretic applications of 5-HT and NA was assessed using the recovery time 50 (RT₅₀) values. RT₅₀ is defined as the time in seconds, from the termination of the microiontophoretic application, required by the neuron to recover 50% of its initial firing frequency. The RT₅₀ value has been shown to provide a reliable index of the *in vivo* activity of the NA reuptake process in the rat hippocampus (de Montigny *et al.* 1980; Gravel & de Montigny, 1987) and of the 5-HT reuptake process in the rat amygdala and lateral geniculate body (Wang *et al.* 1979) and in the hippocampus (Piñeyro *et al.* 1994). The RT₅₀ values were used to assess reuptake activity before and 2-5 minutes after the acute intravenous administration of venlafaxine and paroxetine on the 5-HT reuptake process and of venlafaxine and desipramine on the NA reuptake process. The effect of a short-term treatment (2 days) with venlafaxine on the reuptake inhibition of both these monoamines was also assessed using the RT₅₀ index.

DRUGS

Besides venlafaxine (Wyeth-Ayerst Research, NJ, U.S.A.) the following drugs were used: paroxetine (Smith Kline Beecham, West Sussex, U.K.), desipramine (RBI, Natick, MA, USA), 5-HT, NA and ACh (Sigma Chemical, St-Louis, MO, U.S.A.). Drugs were either prepared in physiological saline for i.v. administration or in a 200 mM NaCl solution for microiontophoretic applications.

Statistical analysis

Results are expressed throughout as means $\pm$ S.E.M., unless otherwise specified. When two means are compared, the statistical significance of the difference was assessed using either a paired or a non-paired Student's *t* test, as indicated in the legend to figures. Probability value of $p < 0.05$ was considered to be statistically significant.

RESULTS

Effect of the acute intravenous administration of venlafaxine and paroxetine on the recovery time from microiontophoretic applications of 5-HT.

The recovery time, from the suppression of hippocampus pyramidal neuron firing activity following microiontophoretic application of 5-HT, was assessed using the RT_{50} values before and after the acute intravenous administrations of venlafaxine and paroxetine. As the acute injection of venlafaxine and paroxetine (Piñeyro *et al.* 1994) decreased the firing activity of these pyramidal neurons, the current of ACh had therefore to be increased in order to maintain a rate of firing activity similar to that before the injection (Figure 1). Microiontophoretic applications of 5-HT in control rats resulted in a suppression of the firing activity which gradually recovered upon cessation of the application (Figure 1). Following the acute administration of 1 mg kg^{-1} i.v. venlafaxine, the RT_{50} values were significantly increased following application of 5-HT with a current of 5 nA but not with one of 1 nA. At the doses of 5, 10 and 20 mg kg^{-1} , the RT_{50} values for 5-HT with both currents used were significantly increased (Figure 2A and B). Successive intravenous administrations of 5 mg kg^{-1} paroxetine dose-dependently increased the RT_{50} value following 5-HT microiontophoretic application of 1 nA (Figure 2A) and of 5 nA (Figure 2B). With both currents used for 5-HT applications, venlafaxine and paroxetine did not significantly differ in their capacity to prolong the RT_{50} values.

Effect of the acute intravenous administration of venlafaxine and desipramine on the recovery time from microiontophoretic applications of NA

The effects of the acute administration of venlafaxine and desipramine on the NA reuptake process were assessed as for that of 5-HT. It is important to state that the effects of venlafaxine on the duration of microiontophoretic application of both 5-HT and NA were assessed on the same neurons. At the dose of 5 mg kg^{-1} i.v., venlafaxine did not modify the RT_{50} values following NA applications (Figure 3A). A significant increase was observed with the intravenous administration of 10 mg kg^{-1} , while with 20 mg kg^{-1} , an approximately two fold increase of the RT_{50} values was obtained.

The effect of venlafaxine on the RT_{50} values following NA applications was compared

to that of desipramine (Figure 3B). As previously observed (Lacroix *et al.* 1991), desipramine significantly and dose-dependently prolonged the RT_{50} values following NA applications at doses of 2 and 5 mg kg⁻¹ (Figure 3B).

Effect of a short-term treatment with venlafaxine on the recovery from microiontophoretic applications of 5-HT or NA

Rats were implanted with osmotic minipumps delivering doses of venlafaxine of 10, 20 or 40 mg kg⁻¹ day⁻¹ or vehicle. The electrophysiological experiments were carried out 48 h after the beginning of the treatment with the minipumps still in place. All three doses regimen of venlafaxine administration were individually tested in parallel with their own controls. The control rats and their matching venlafaxine-treated rats were tested with the same microiontophoretic pipettes (see figures 4, 5 and 6). The two-day treatment with a dose of 10 mg kg⁻¹ day⁻¹ of venlafaxine increased significantly the RT_{50} values obtained following microiontophoretic applications of 5-HT (1 and 5 nA), but not those following the applications of NA (3 and 10 nA), when compared to control rats (Figures 5 and 6). Following two-day treatments with doses of 20 mg kg⁻¹ day⁻¹ or 40 mg kg⁻¹ day⁻¹ of venlafaxine, the RT_{50} values obtained following microiontophoretic applications of 5-HT (1 or 5 nA) or NA (3 and 10 nA) were significantly increased (Figures 4, 5 and 6). Interestingly, the increases of the RT_{50} values for both 5-HT and NA did not appear to be dose-dependent, inasmuch as the high dose regimen did not further increase significantly the RT_{50} values for both 5-HT and NA applications compared to that obtained with the minimal effective dose.

Effect of the acute intravenous administration of desipramine following acute or short-term administrations of venlafaxine

The effect of acute intravenous administration of desipramine on the NA reuptake process was assessed in rats that had previously received either an intravenous dose of 5 mg kg⁻¹ venlafaxine. As before, the intravenous dose of 5 mg kg⁻¹ of venlafaxine did not modify the RT_{50} values obtained following microiontophoretic applications of NA with a 3 nA current (Figure 7A). The subsequent administration of a dose of 5 mg kg⁻¹ of desipramine induced an increase of the RT_{50} values (Figure 7A) which was identical to that produced by this dose of desipramine in naive rats (compare with Figure 3).

In a last series of experiments, the RT_{50} values obtained for NA prior to and following

the acute administration of 5 mg kg⁻¹ of desipramine were assessed in rats that had been treated for two days with doses of 10 or 40 mg kg⁻¹ day⁻¹ of venlafaxine. After both the 10 and 40 mg kg⁻¹ day⁻¹ regimen with venlafaxine, the acute administration of desipramine still significantly increased the RT₅₀ values following microiontophoretic applications of NA (Figure 7B).

Discussion

The results obtained in the present study indicate that venlafaxine displays both 5-HT and NA reuptake inhibition properties when assessed using an *in vivo* electrophysiological paradigm. Yet, venlafaxine demonstrated a greater potency to inhibit the 5-HT reuptake process over that for NA in these conditions. On the other hand, paroxetine and venlafaxine were equipotent to inhibit 5-HT reuptake, while desipramine was more potent than venlafaxine to inhibit NA reuptake when using this *in vivo* approach.

It has been well established that the RT_{50} value provides a reliable, quantitative, index to assess, *in vivo*, the uptake activity following microiontophoretic applications onto CA₃ dorsal hippocampus pyramidal neurons of both 5-HT (Piñeyro *et al.* 1994) and NA (de Montigny *et al.* 1980; Gravel & de Montigny, 1987). In the present study, the ability of venlafaxine to increase the RT_{50} values following 5-HT and NA microiontophoretic applications was assessed both acutely and following a 2-day treatment with venlafaxine. Following its acute intravenous administration, venlafaxine dose-dependently increased the RT_{50} values for both 5-HT and NA, thus confirming its purported dual reuptake blocking properties. However, whereas a dose of 1 mg kg⁻¹ was sufficient to induce a significant increase of the RT_{50} value for 5-HT, a dose of 10 mg kg⁻¹ was necessary to produce a significant increase of the RT_{50} values for NA (Figure 3). This suggests that venlafaxine is more potent in blocking the 5-HT transporter than the NA transporter.

The direct comparison of the RT_{50} values following acute intravenous administration of venlafaxine and paroxetine reveals that these two drugs displayed an equipotent inhibitory effect on the 5-HT reuptake process (Figure 2A and B). However, venlafaxine was less potent (about 5 times) than desipramine in inhibiting NA reuptake as shown by the higher dose of venlafaxine required to significantly increase the RT_{50} values following NA applications (Figure 3). Most interestingly, and perhaps even surprisingly, these relative potencies (*i.e.* venlafaxine vs paroxetine and desipramine for 5-HT and NA reuptake blockade, respectively) are incongruent with those previously reported from an *in vitro* uptake study done in rat brain synaptosomes (Bolden-Watson & Richelson, 1993), nor with those generated from a radioligand binding study (Béique *et al.* 1998). Indeed, Bolden-Watson *et al.* have reported that paroxetine is 53 times more potent than venlafaxine to inhibit 5-HT reuptake whereas we have reported, in a previous radioligand binding study, that paroxetine displayed a 2000 times greater affinity than venlafaxine for the [³H]cyanoimipramine binding site on the 5-HT

transporter (Béïque *et al.* 1998). As for the NA reuptake process, desipramine has been reported to be 344 times (Bolden-Watson & Richelson, 1993) and 4 times (Muth *et al.* 1986) more potent than venlafaxine to block the uptake of [^{3} H]NA from rat brain synaptosomes. The reasons for the discrepancy between these later studies is unclear considering that both studies were carried out with essentially similar protocols except that; 1) while total brain was used in the study of Muth *et al.*, only hippocampal tissue was used in that of Bolden-Watson and 2) the uptake assay in the study of Muth *et al.* was carried out with 100 nM [^{3} H]NA, while Bolden-Watson *et al.* used a concentration of 8 nM [^{3} H]NA. How such methodological factors may alter the relative potencies of venlafaxine and desipramine to inhibit [^{3} H]NA uptake however is obscure. Nonetheless, we have reported that desipramine displays an affinity for the [^{3} H]nisoxetine binding site on the NA transporter that is 2000 times greater than that of venlafaxine for this same site (Béïque *et al.* 1998). Altogether, the relative reuptake blocking potencies of paroxetine, desipramine and venlafaxine derived from the present study, when compared to those previously published, clearly expose a discrepancy between the functional 5-HT and NA reuptake blocking potencies of venlafaxine with its binding characteristics to both the 5-HT and NA transporters. Consequently, it can be argued that the sole existence of this discrepancy challenges the otherwise valid assumption that venlafaxine, as the other reuptake blockers do, would block the reuptake of both 5-HT and NA by its sole binding to the [^{3} H]cyanoimipramine and [^{3} H]nisoxetine binding site, respectively. The reasons underlying this very important and crucial peculiarity with regard to venlafaxine's mechanism of action is at present unresolved and thus remains to be elucidated.

The short-term treatments with venlafaxine (10, 20 and 40 mg kg⁻¹ day⁻¹ x 2 days), which permitted the drug to reach a steady state concentrations, given its short half-life in rodents (1 h; Howell *et al.* 1994), further corroborated the dual 5-HT/NA reuptake blocking properties of venlafaxine, because the RT₅₀ values for both 5-HT and NA were increased. Yet, while a dose of 10 mg kg⁻¹ day⁻¹ resulted in a significant increase of the RT₅₀ following 5-HT applications, 20 mg kg⁻¹ day⁻¹ was necessary to observe a similar effect following NA applications. Interestingly, with increasing doses, the effect of venlafaxine administered for 2 days displayed a plateau with regard to the increase of the RT₅₀ values and therefore was not dose-dependent. This observation is different from that reported with duloxetine, another 5-HT/NA reuptake inhibitor (SNRI), which, under the same experimental conditions, showed a clear dose-dependent relationship with respect to the RT₅₀ values for 5-HT and NA (Kasamo

et al. 1996). The reason for this apparent discrepancy between these two purportedly similar drugs (*i.e.* both are SNRI's) remains to be elucidated. Notwithstanding this latter observation, the data obtained with the short-term treatments with venlafaxine are congruent with those generated by intravenous administration of venlafaxine with regard to the preferential potency of venlafaxine to inhibit the 5-HT reuptake process over that of NA.

As shown in Figure 7A and B, when desipramine was acutely administered to rats that had received either an acute intravenous administration or a 2-day treatment of a maximal non-effective dose of venlafaxine on NA reuptake (5 mg kg^{-1} , i.v. and $10 \text{ mg kg}^{-1} \text{ day}^{-1}$, s.c. respectively), it was still able to exert its full effect (compare Figure 3 and 7A). These results indicate that venlafaxine did not prevent the effect of desipramine on the NA reuptake and that these two drugs do not exert additive effect. Assuming that these maximal non-effective doses of venlafaxine result in a significant transporter occupancy, this lack of additive effect of these two drugs on the reuptake of NA is compatible with the previous suggestion that they would block NA reuptake by acting on different sites (Lavoie *et al.* 1997; Béïque *et al.* 1998). On the other hand, the fact that desipramine (5 mg kg^{-1} , i.v.) was able to prolong the RT_{50} values for NA after a 2-day treatment with $40 \text{ mg kg}^{-1} \text{ day}^{-1}$ of venlafaxine and that these latter RT_{50} values are well below those obtained following acute administration of either venlafaxine (20 mg kg^{-1} , i.v.) or desipramine (5 mg kg^{-1} , i.v.), indicates that a 2-day treatment with $40 \text{ mg kg}^{-1} \text{ day}^{-1}$ of venlafaxine does not induce a maximal NA reuptake blockade. This latter finding is somewhat surprising since the $40 \text{ mg kg}^{-1} \text{ day}^{-1}$ regimen of venlafaxine appeared to induce a maximal effect on the NA reuptake as the RT_{50} values for NA were not further increased when the dose of $20 \text{ mg kg}^{-1} \text{ day}^{-1}$ was increased to $40 \text{ mg kg}^{-1} \text{ day}^{-1}$ (Figure 6), thus suggestive of a plateau. Whether this apparent incomplete NA reuptake blockade by the $40 \text{ mg kg}^{-1} \text{ day}^{-1}$ regimen is the result of adaptative changes that might have developed during the short-term treatment period or from a pharmacokinetic parameter that would limit the effective brain concentration of venlafaxine remains to be determined.

Taken together, the results presented herein provide functional evidence that venlafaxine exerts a more powerful action on the 5-HT reuptake process than that for NA, a finding that is consistent throughout the different studies that have directly assessed this matter. Indeed, the *in vitro* uptake study in rat brain synaptosomes of Bolden-Watson and Richelson (1993) shows a preferential inhibitory effect of venlafaxine on the reuptake of 5-HT over that of NA with a 3:1 ratio, while that of Muth *et al.* (1986) reported a 5:1 ratio. Given

the differential potencies of venlafaxine for these reuptake processes, it is conceivable that when a low dose of venlafaxine is administered to patients, it would act preferentially on the 5-HT reuptake process whereas when the dose is increased, a progressive recruitment of the noradrenergic system, through NA reuptake blockade, would ensue. This latter property may well represent the biological substratum responsible for not only the positive dose-response relationship observed clinically with venlafaxine which, most interestingly, does not occur with SSRI's (Kelsey, 1996), but also for the apparent remarkable clinical efficacy of high doses of venlafaxine. Indeed, clinical studies are now suggesting that venlafaxine, when used at high doses, demonstrates an early onset of action (Rickels, 1991; Derivan *et al.* 1995; Benkert *et al.* 1996) as well as therapeutic efficacy in treatment-resistant depression (Nierenberg *et al.* 1994; de Montigny *et al.* 1995).

In summary, the differential potencies of venlafaxine to inhibit *in vivo* the reuptake of 5-HT and NA demonstrated herein, provides a tenable and attractive hypothesis for the unique clinical properties of venlafaxine. Furthermore, the present data, by revealing a discrepancy between the functional *in vivo* potencies of venlafaxine to block 5-HT and NA uptake and its *in vitro* binding profile to the two transporters, suggest that venlafaxine might interact with the 5-HT and the NA transporters in a more complex manner than the classical 5-HT and NA reuptake blockers. Further studies are thus warranted in order to clarify this crucial aspect, given the seemingly unique clinical properties of venlafaxine.

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LEGENDS TO FIGURES

Figure 1. Integrated firing rate histogram of a CA₃ dorsal hippocampus pyramidal neuron showing the effects of microiontophoretic applications of 5-HT and NA, before and following the intravenous administration of 5 mg kg⁻¹ of venlafaxine. The solid bars above the trace indicate the duration of microiontophoretic application, for which the ejection current is given in nA. The two dots represent an interval of 2 min.

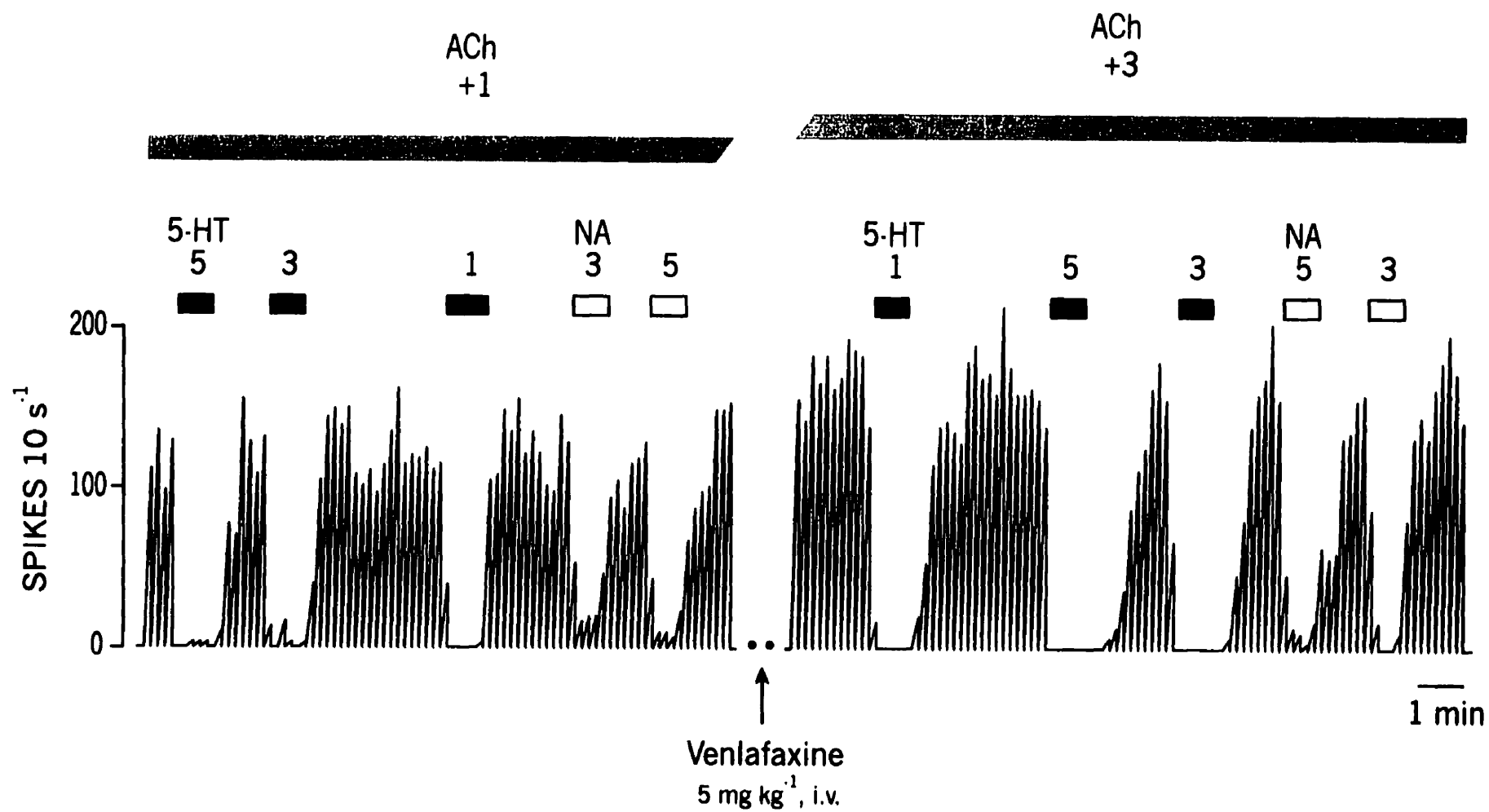
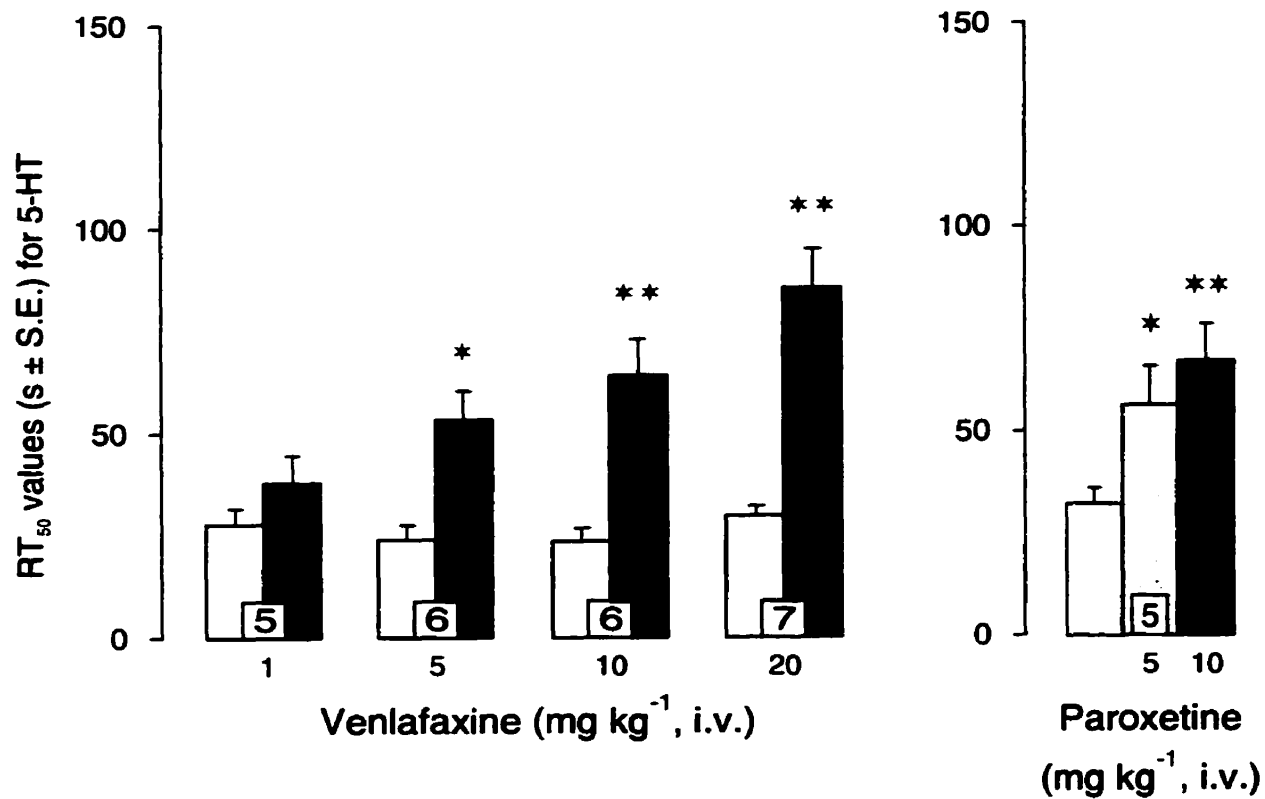


Figure 2. Recovery time, expressed as RT_{50} values (means $\pm$ S.E.M.), of CA₃ dorsal hippocampus neurons obtained with microiontophoretic applications of 1(A.) and 5 nA (B.) of 5-HT on ACh-induced firing activity, before and after the acute intravenous administration of venlafaxine or of 2 successive doses of 5 mg/kg (cumulative doses of 5 and 10 mg/kg, respectively) of paroxetine. * $p < 0.05$, ** $p < 0.01$ compared to control values using the paired Student's t -test. In this and subsequent figures, the numbers in squares represent the numbers of neurons tested.

A.

5-HT (1 nA)



B.

5-HT (5 nA)

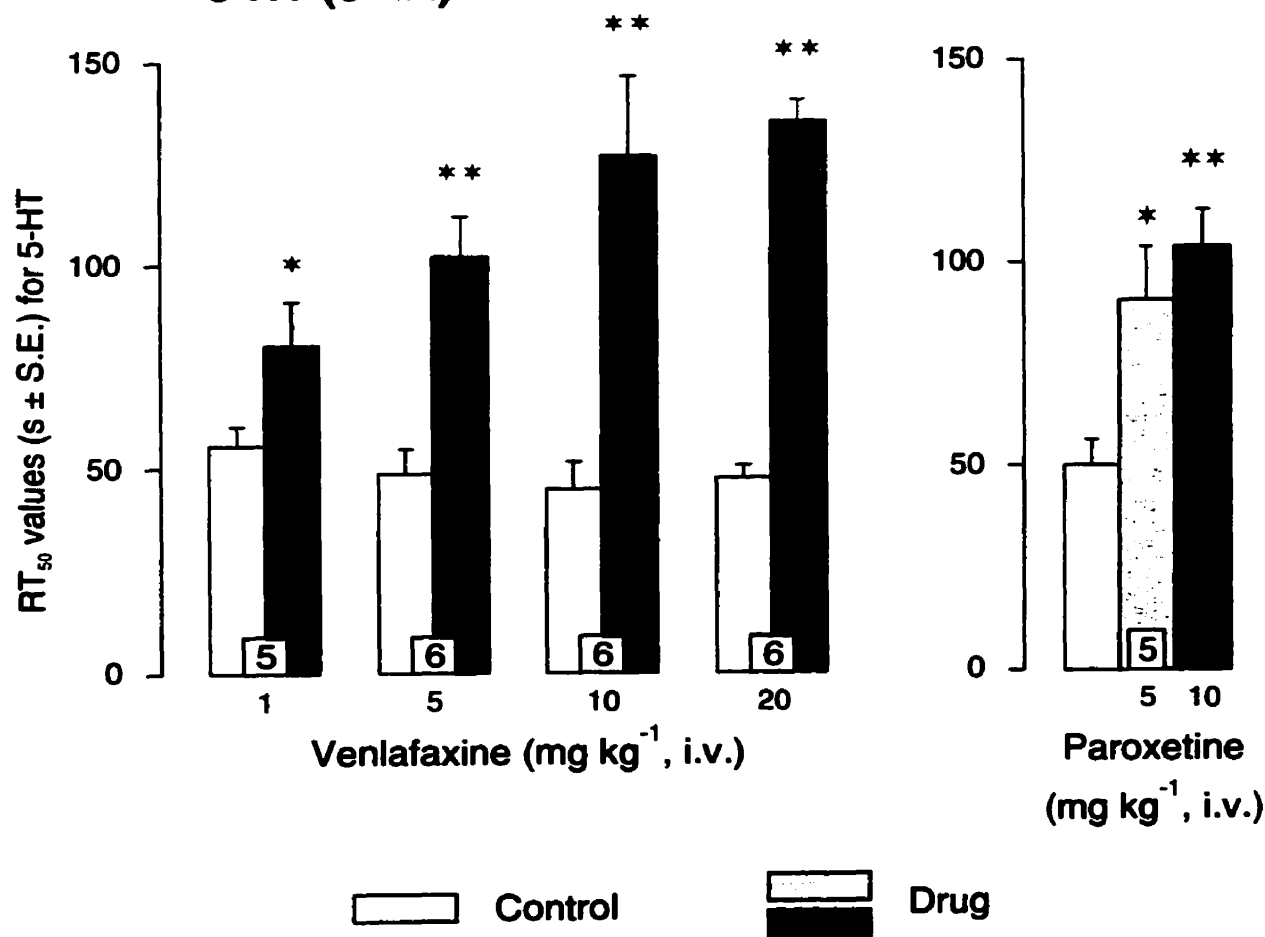
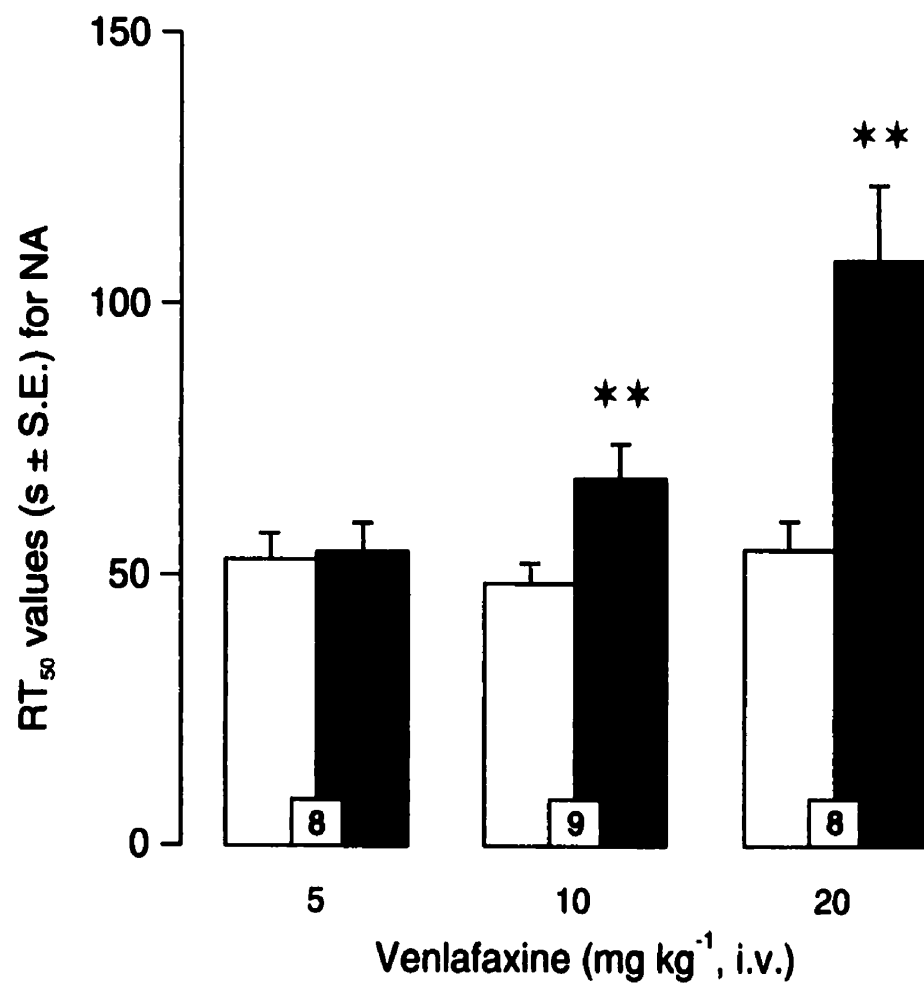
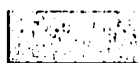


Figure 3. Recovery time, expressed as RT_{50} values (means $\pm$ S.E.M.), of CA₃ dorsal hippocampus neurons obtained with microiontophoretic applications of 3 nA of NA on ACh-induced firing activity, before and after the acute intravenous administration of venlafaxine (A) or of 2 and 3 mg/kg (cumulative doses of 2 and 5 mg/kg, respectively) of desipramine (B). * $p < 0.05$, ** $p < 0.005$ compared to control values using the paired Student's t test.

A. NA (3 nA)



Control



Drug

B. NA (3 nA)

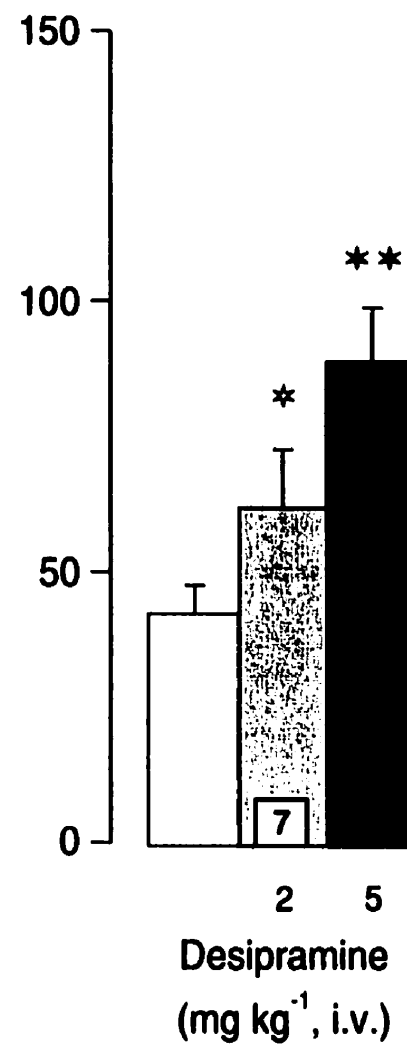
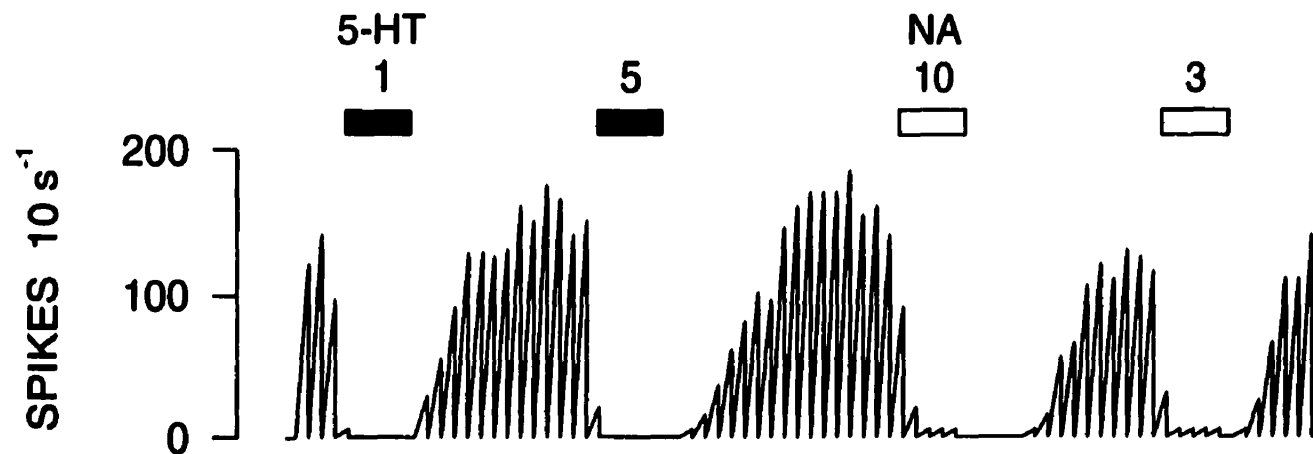


Figure 4. Integrated firing rate histograms of CA₃ dorsal hippocampus pyramidal neuron showing the effects of microiontophoretic applications of 5-HT and NA in rats following a two-day treatment with vehicle (A) or with 40 mg kg⁻¹ day⁻¹ s.c. of venlafaxine (B).

A. Control



B. Venlafaxine, 40 mg kg⁻¹ day⁻¹ x 2 days, s.c.

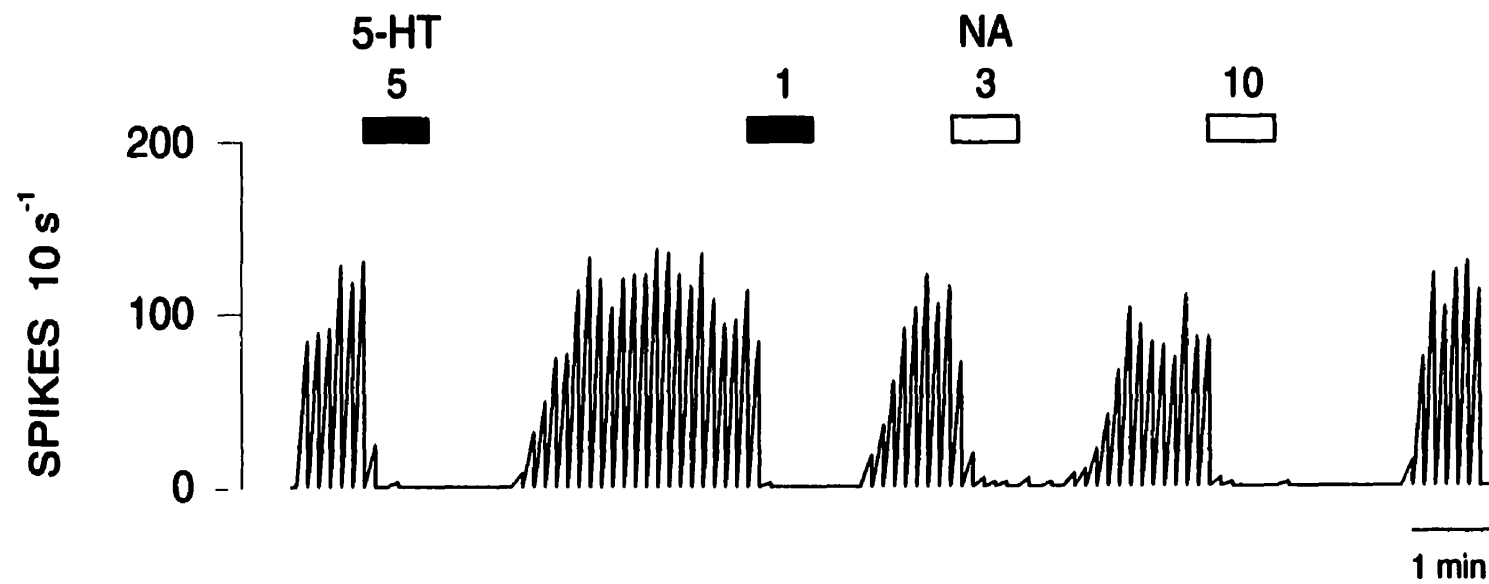
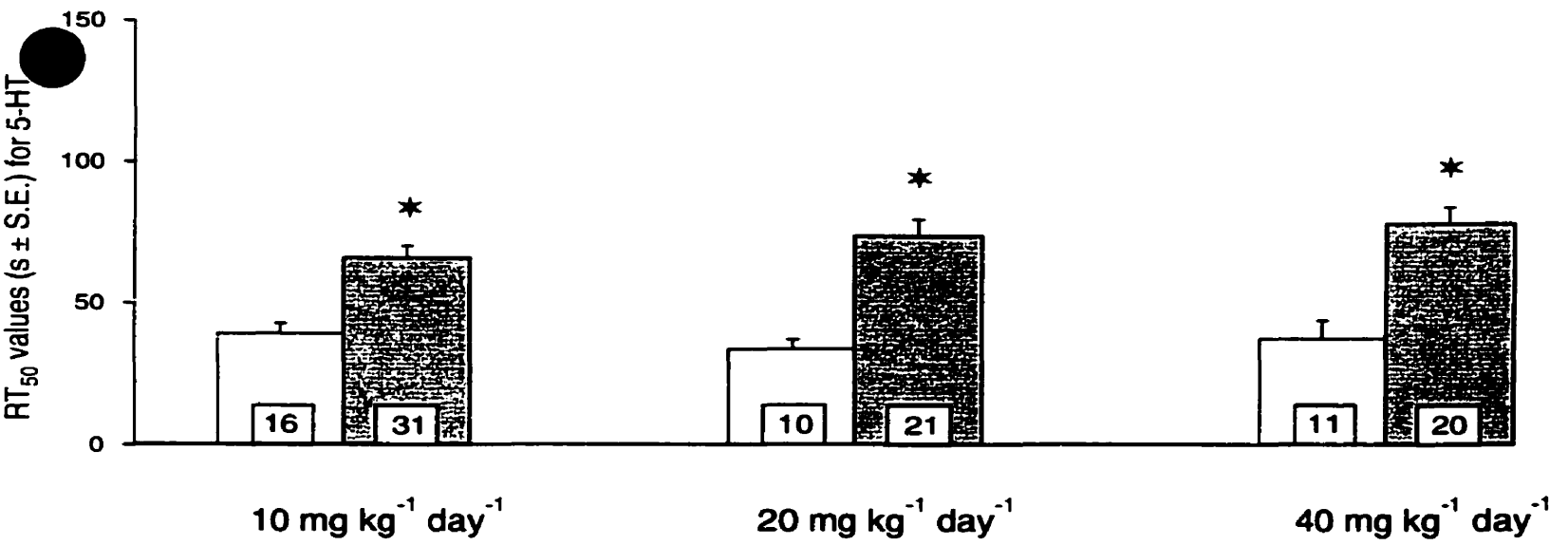
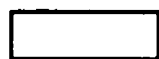
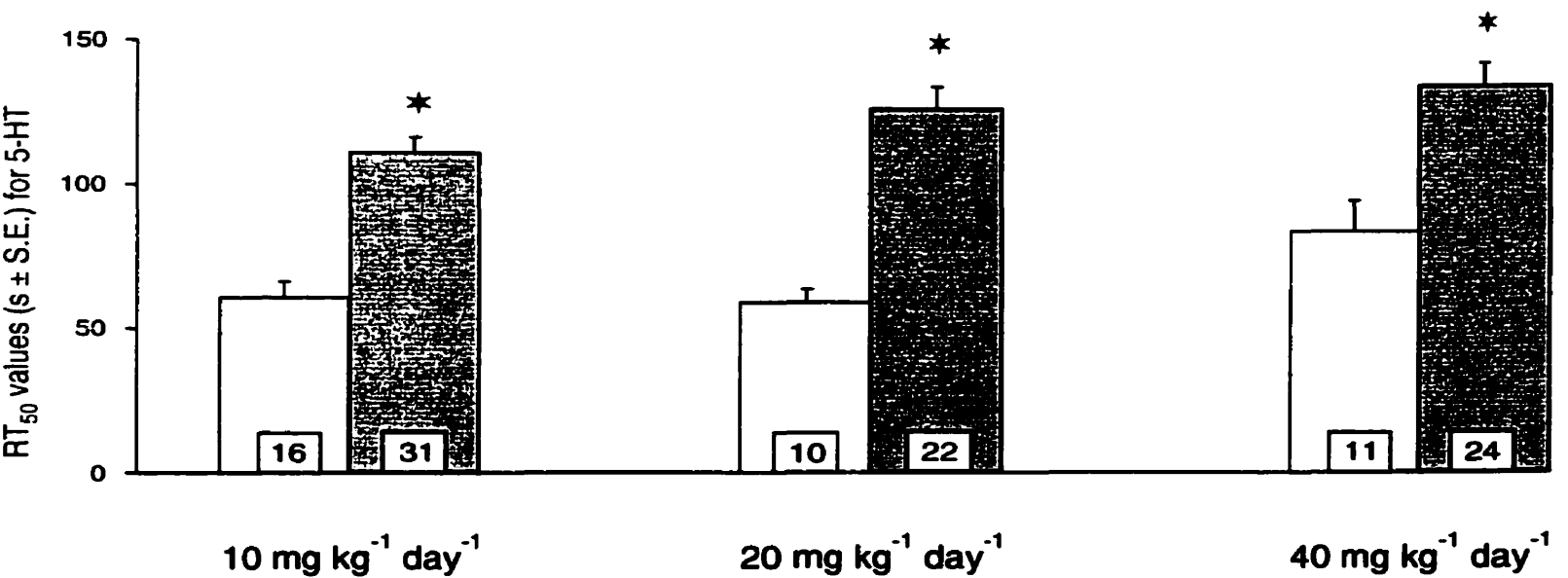


Figure 5. Recovery time, expressed as RT_{50} values (means $\pm$ S.E.M.), of CA₃ dorsal hippocampus neurons obtained with microiontophoretic applications of 1 nA (A) and 5 nA (B) of 5-HT on ACh-induced firing activity, in control rats , and following two-day treatments with 10, 20 or 40 mg kg⁻¹ day⁻¹ of venlafaxine. * $p < 0.005$ compared to control values using the two-tailed Student's t test.

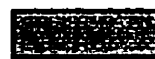
A. 5-HT (1 nA)



B. 5-HT (5 nA)



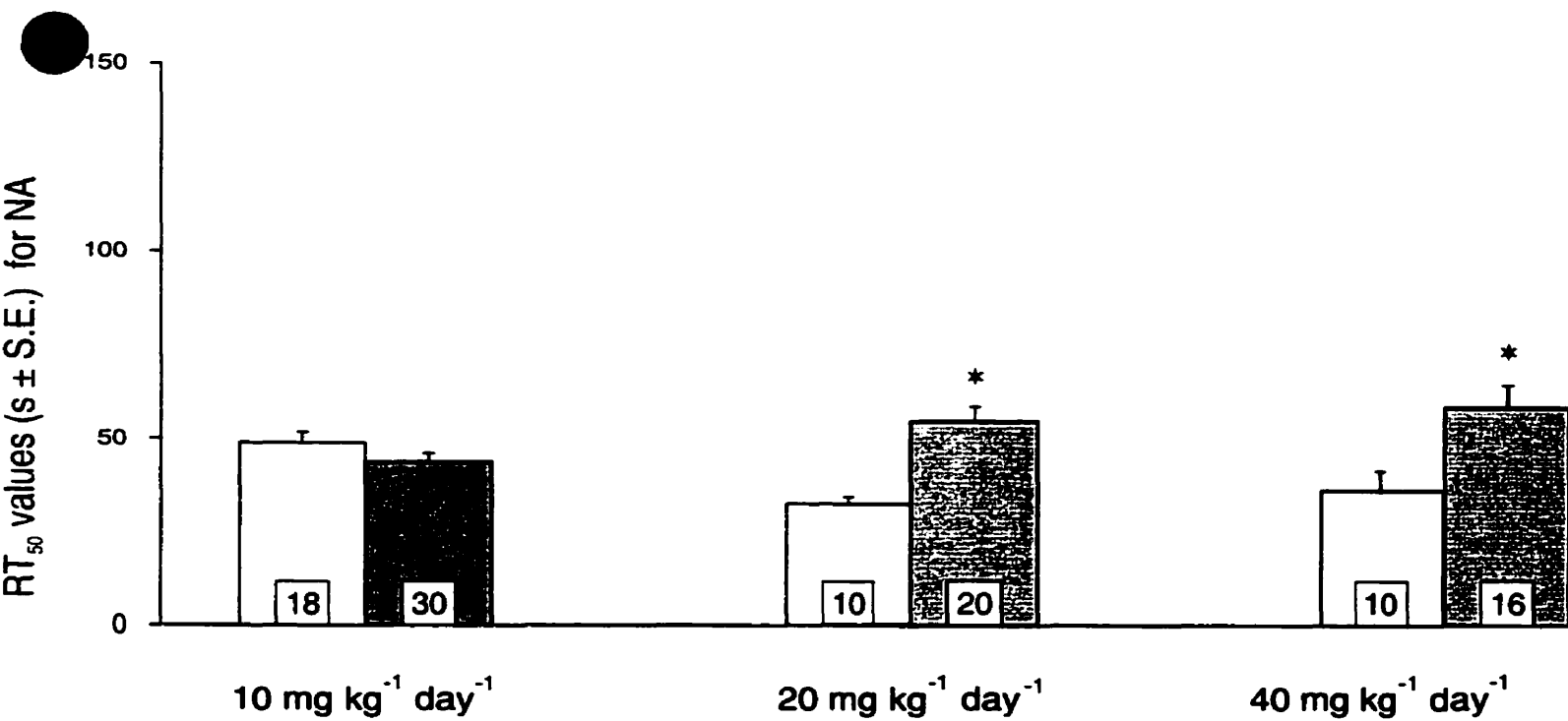
Control



Venlafaxine

Figure 6. Recovery time, expressed as RT_{50} values (means $\pm$ S.E.M.), of CA₃ dorsal hippocampus neurons obtained with microiontophoretic applications of NA (3 and 10 nA) on ACh-induced firing activity, in control rats, and following two-day treatments with 10, 20 and 40 mg kg⁻¹ day⁻¹ of venlafaxine. * $p < 0.005$ compared to control values using the unpaired Student's t test.

A. NA (3 nA)



B. NA (10 nA)

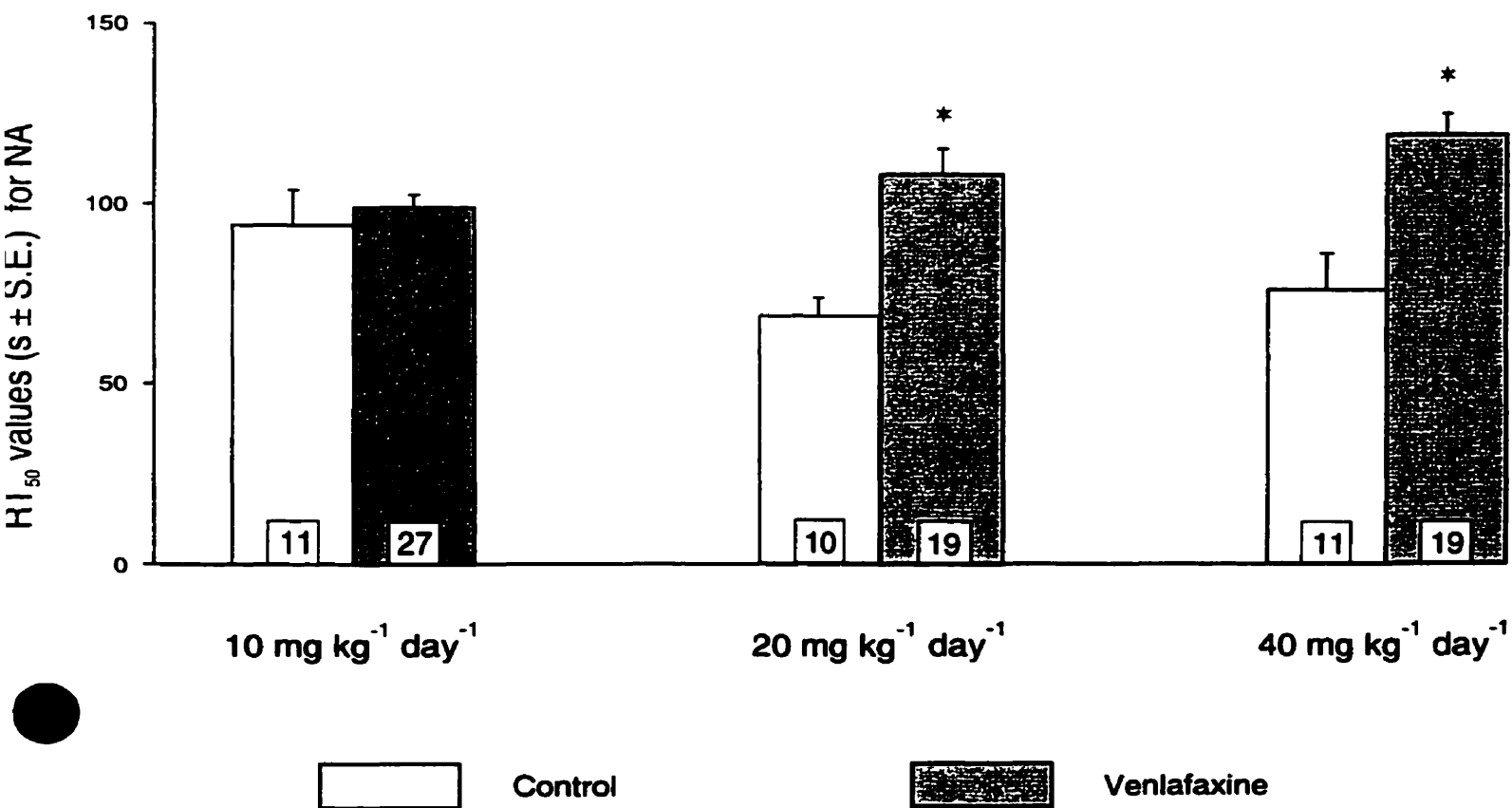
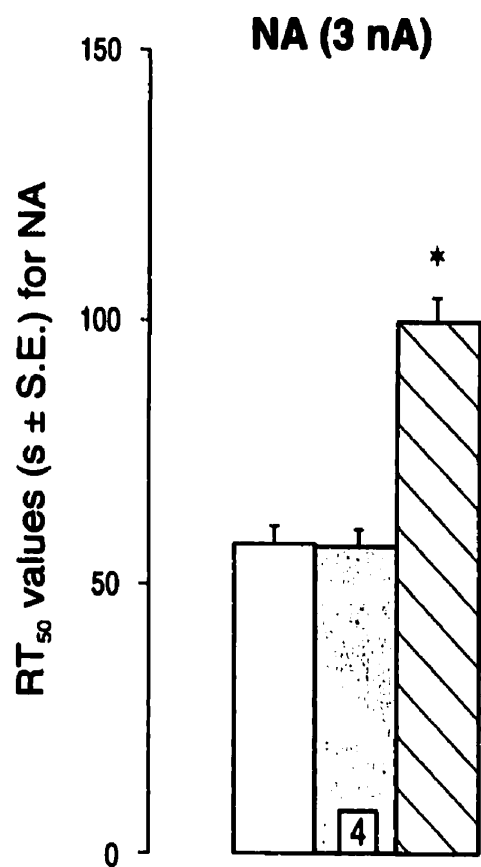


Figure 7. A Recovery time, expressed as RT_{50} values (mean $\pm$ S.E.M.), of CA₃ dorsal hippocampus neurons, obtained with microiontophoretic applications of 3 nA of NA on ACh-induced firing activity, before, and after the acute intravenous administration of venlafaxine (5 mg kg⁻¹), and following the subsequent administration of desipramine (5 mg kg⁻¹) column) to the same rats. * $p < 0.005$ compared to control values using the paired Student's t test.

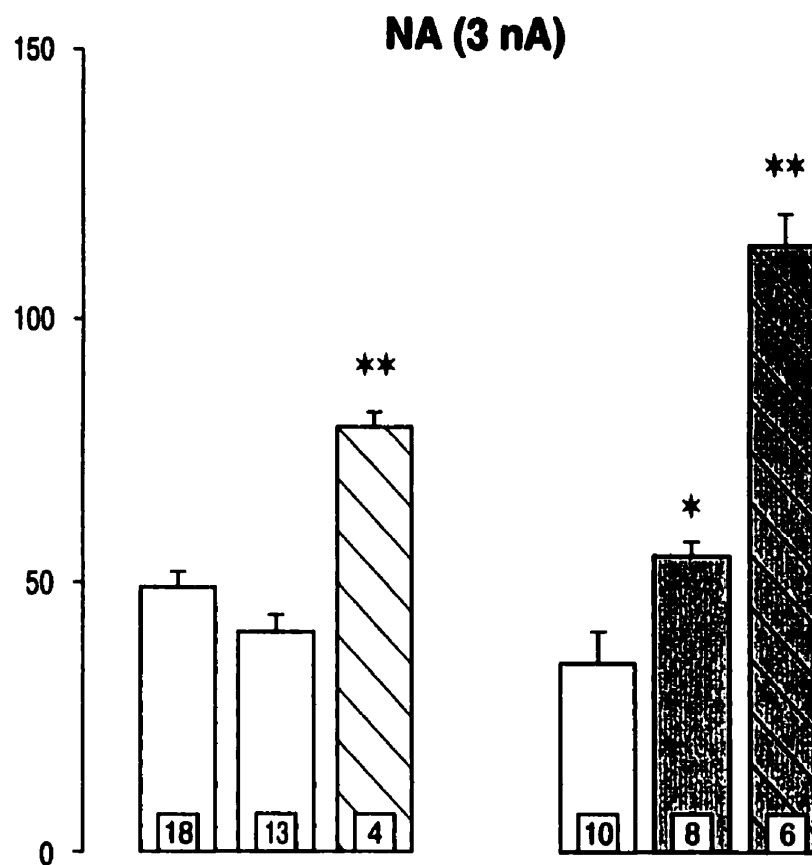
B Recovery time, expressed as RT_{50} values (means $\pm$ S.E.M.), of CA₃ dorsal hippocampus neurons obtained with microiontophoretic applications of 3 nA of NA on ACh-induced firing activity, before and after a two-day treatment with 10 or 40 mg kg⁻¹ day⁻¹ of venlafaxine and following the acute administration of desipramine (5 mg kg⁻¹, i.v.) to the same rats. * $p < 0.05$; ** $p < 0.001$, using the unpaired Student's t test.

A.



-  Control
-  Venlafaxine (5 mg kg⁻¹, i.v.)
-  Venlafaxine + desipramine (5 mg kg⁻¹, i.v.)

B.



-  Control
-  Venlafaxine (10 mg kg⁻¹ day⁻¹ x 2 day, s.c.)
-  Venlafaxine (40 mg kg⁻¹ day⁻¹ x 2 day, s.c.)
-  Venlafaxine + desipramine (5 mg kg⁻¹, i.v.)

CHAPTER III

PREFATORY REMARKS

The interpretation of this first in vivo study on the reuptake blockade potencies of venlafaxine is essentially two-fold. First, by showing that, in a physiological condition, venlafaxine blocks the reuptake of both 5-HT and NE, this study provided strong experimental evidence supporting the hypothesis that when used at low dose, venlafaxine would act as an SSRI, whereas as the dose would be increased, it would block the reuptake of both 5-HT and NE. The clinical implication of this latter contention has been discussed. Secondly, this study has sharpened the interrogations that were brought by the in vitro-radioligand binding study presented in chapter I by demonstrating that venlafaxine and paroxetine, on the one hand, blocked the reuptake of 5-HT equipotently, and, on the other hand, venlafaxine blocked the reuptake of NE about 3 times less potently than desipramine. Recall that these in vivo potencies are incongruent with the affinities of the compounds with the transporters per se.

These results, considered with those of chapter I suggests that there is a discrepancy between the in vivo reuptake blockade potencies of venlafaxine and its in vitro affinities for the two transporters, thus raising the possibility that the in vivo reuptake blockade of 5-HT and NE induced by venlafaxine would not be mediated by a classical binding to the 5-HT and NE transporter. Although this study provided a direct within study comparison with prototypical reuptake inhibitors (i.e., paroxetine and desipramine), there was a profound need to better express, in quantitative terms, the in vivo reuptake blocking potencies of venlafaxine if one was to expect to make a case of the in vivo-in vitro discrepancy suspected. This quantification was provided by assessing the potencies with which venlafaxine (as well as our two prototypical reuptake inhibitor paroxetine and desipramine) suppressed the firing activity of 5-HT neurons of the dorsal raphe nucleus and/or NE neurons of the locus coeruleus.

VENLAFAXINE: DISCREPANCY BETWEEN IN VIVO 5-HT AND NE REUPTAKE BLOCKADE AND AFFINITY FOR REUPTAKE SITES⁶

⁶The study presented in this chapter is published in Synapse (1998), 32, 198-211. A reprint of this publication is given in Annex C.

ABSTRACT

Using an *in vivo* electrophysiological paradigm, venlafaxine and paroxetine displayed a similar potency for suppressing the firing activity of dorsal raphe 5-HT neurons (ED_{50} : 233 and 211 $\mu\text{g/kg i.v.}$, respectively), while venlafaxine was three times less potent than desipramine (ED_{50} : 727 and 241 $\mu\text{g/kg i.v.}$, respectively) to suppress the firing activity of locus coeruleus NE neurons. The selective 5-HT_{1A} receptor antagonist WAY 100635 (100 $\mu\text{g/kg, i.v.}$) reversed the suppressant effect of venlafaxine and paroxetine on the firing activity of 5-HT neurons and the α_2 -adrenoceptor antagonist piperoxane (1 mg/kg, i.v.) reversed those of venlafaxine and desipramine on the firing activity of NE neurons. The ED_{50} of venlafaxine on the firing activity of 5-HT neurons was not altered (ED_{50} : 264 $\mu\text{g/kg}$) in noradrenergic-lesioned rats, while the suppressant effect of venlafaxine on the firing activity of NE neurons was greater in serotonergic-lesioned rats (ED_{50} : 285 $\mu\text{g/kg}$). Taken together, these results suggest that, *in vivo*, venlafaxine blocks both reuptake processes, its potency to block the 5-HT reuptake process being greater than that for NE. Since the affinities of venlafaxine for the 5-HT and NE reuptake carriers are not in keeping with its potencies for suppressing the firing activity of 5-HT and NE neurons the suppressant effect of venlafaxine on the firing activity of 5-HT and NE neurons observed *in vivo*, may not be mediated solely by its action on the [³H]cyanoimipramine and [³H]nisoxetine binding sites. In an attempt to unravel the mechanism responsible for this peculiarity, *in vitro* superfusion experiments were carried out in rat brain slices to assess a putative monoamine releasing property for venlafaxine. ($\pm$)Fenfluramine and tyramine substantially increased the spontaneous outflow of [³H]5-HT and [³NE], respectively, while venlafaxine was devoid of such releasing properties.

KEYWORDS: antidepressant; dorsal raphe; locus coeruleus; paroxetine; desipramine; fenfluramine; tyramine; releaser.

INTRODUCTION

Venlafaxine (1-[2-(dimethylamino)-1-(4-methoxyphenyl)-ethyl]cyclohexanol), a phenylethylamine derivative, has been shown to be effective in the treatment of major depression (Schweizer *et al.* 1991; Guelfi *et al.* 1992). Moreover, an increasing number of studies are suggesting that venlafaxine would display a unique clinical profile in that: 1- it would display an earlier onset of therapeutic action (Schweizer *et al.* 1991; Benkert *et al.* 1996); 2- it would display a positive dose-response relationship with regard to clinical efficacy (Kelsey, 1996); 3- it would be more efficacious than fluoxetine (Clerc *et al.* 1994; Dierick *et al.* 1996; Rudolph *et al.* 1997) and imipramine (Schweizer *et al.* 1994; Lecrubier *et al.* 1997) in treating major depression; and, finally, 4- it would show a significant efficacy in treatment-resistant depression (Nierenberg *et al.* 1994; de Montigny *et al.* 1998). Since *in vitro* studies carried out in rat brain synaptosomes have demonstrated that venlafaxine inhibits the uptake of both serotonin (5-hydroxytryptamine; 5-HT) and norepinephrine (NE; Muth *et al.* 1986a; Bolden-Watson and Richelson, 1993), these clinical properties have been suggested to be attributable to a dual 5-HT/NE reuptake inhibition. However, in a recent radioligand binding study, we have shown that venlafaxine displayed only a moderate affinity for the 5-HT transporter and low affinity for the NE transporter, as assessed with [³H]cyanoimipramine and [³H]nisoxetine competitive binding, respectively (Béique *et al.* 1998b). Accordingly, venlafaxine displayed a much lower affinity for both reuptake sites than prototypical reuptake inhibitors such as paroxetine and desipramine. These results thus put into evidence a clear discrepancy with regard to the mechanism of action of venlafaxine in major depression. In order to clarify this issue, it was deemed necessary to further assess the purported reuptake blocking properties of venlafaxine using a functional *in vivo* paradigm. In this regard, the suppression of the firing activity of dorsal raphe nucleus 5-HT and locus coeruleus NE neurons achieved by reuptake blockers is believed to provide a reliable and precise index of their potencies to inhibit 5-HT and NE reuptake as these two functions were shown to be correlated (Quinaux *et al.* 1982). We have thus assessed in the present study: 1) the potencies of venlafaxine and paroxetine for suppressing the firing activity of dorsal raphe 5-HT neurons; 2) the potency of venlafaxine, desipramine, paroxetine and fluoxetine for suppressing the firing activity of locus coeruleus NE neurons; 3) the effect of a 5-HT lesion and that of a NE lesion on the potencies of venlafaxine to suppress the firing activity of locus coeruleus NE and

dorsal raphe 5-HT neurons, respectively; 4) to compare the potencies of reuptake inhibitors with those already published with other reuptake inhibitors and to correlate these potencies with their respective affinities, taken from previous studies, for the 5-HT and NE transporters; and finally 5) the effects of fenfluramine, tyramine and venlafaxine on the spontaneous outflow of radioactivity from rat brain slices preloaded with [^3H]5-HT or [^3H]NE in order to determine a putative monoamine releasing properties for venlafaxine.

MATERIALS AND METHODS

Male Sprague Dawley rats (250-275 g; Charles River, St. Constant, Québec, Canada) were received at least one day before the experiments and housed three to four per cage. They were kept on a 12:12 h light/dark cycle, with access to food and water *ad libitum*.

***In vivo* electrophysiological experiments**

Electrophysiological experiments were performed on animals anesthetized with chloral hydrate (400 mg/kg, i.p.) and mounted in a stereotaxic apparatus. Supplemental doses (100 mg/kg, i.p.) were given to prevent any nociceptive reaction to pinching of a hind paw. Body temperature was maintained at 37 °C throughout the experiment and a catheter was installed prior to recording in a lateral tail vein for intravenous administration of drugs.

Extracellular unitary recordings of 5-HT neurons of the dorsal raphe nucleus and of NE neurons of the locus coeruleus.

Single-barrelled glass micropipettes were prepared in a conventional manner (Haigler and Aghajanian, 1974), with the tips broken back to 1-3 μm and filled with a 2 M NaCl solution saturated with Fast Green FCF. A burr hole was drilled on midline, 1 mm anterior to lambda for the dorsal raphe neurons recordings or 1 mm posterior to lambda and 1 mm lateral to midline for locus coeruleus neurons recordings. Spontaneously active dorsal raphe 5-HT neurons were encountered on a distance of 1 mm starting at the ventral border of the Sylvius aqueduct, and were identified using the criteria of Aghajanian (1978): slow, regular firing rate (0.5-2.5 Hz) and positive action potential of long duration (0.8-1.2 ms). Spontaneously active NE neurons of the locus coeruleus were identified using the criteria of Aghajanian (1978): regular firing rate (1-5 Hz) and positive action potential of long duration (0.8-1.2 ms) exhibiting a characteristic burst discharge in response to nociceptive pinch of the contralateral hind paw. 5-HT and NE neurons were recorded for at least 1 min to establish basal firing rate, before a single dose of the drug to be tested was administered intravenously in a volume of about 0.1 cc *via* a lateral vein of the tail. By giving a single dose to one rat, dose-response curves for the effects of venlafaxine, paroxetine, desipramine or fluoxetine were generated from which the dose required for a 50% suppression of the firing activity of the neuron (effective dose-50; ED₅₀ value) were determined.

6-Hydroxydopamine and 5,7-dihydroxytryptamine pretreatments

Rats weighing between 175 and 200 g were anesthetized with chloral hydrate (400 mg/kg, i.p.). Lesions of NE neurons were performed by injecting the NE neurotoxin 6-hydroxydopamine (6-OHDA; 120 μ g free base in 20 μ l of 0.9% NaCl and 0.1% ascorbic acid) intracerebroventricularly (1 mm lateral; 0.5 mm posterior to the coronal suture and 2- 2.5 mm below the surface of the brain) 1 h after the injection of fluoxetine (10 mg/kg, i.p.) in order to protect 5-HT neurons. The same protocol was applied for the injection of the 5-HT neurotoxin 5,7 dihydroxytryptamine (5,7-DHT; 200 μ g of free base in 20 μ l of 0.9% NaCl and 0.1% ascorbic acid, intracerebroventricularly) except that the rats received desipramine (25 mg/kg, i.p.), instead of fluoxetine, 1 h before the lesion to protect NE neurons. In both cases, the electrophysiological experiments were carried at least 14 days after the lesions were made.

Superfusion experiments

Rats were sacrificed by decapitation and the brain immediately removed and rapidly dissected on an ice-cold glass plate. Hippocampal or hypothalamic slices of 400 μ m of thickness were prepared using a McIlwain tissue chopper. The slices were then incubated for 30 min at 37°C in Krebs' buffer containing either 20 nM of [^{3} H]5-HT creatinine sulfate (specific activity 1017 GBq/mmol) or 20 nM of [^{3} H]NE (specific activity of 538.7 GBq/mmol) both purchased from NEN Life Science, Boston, MA and bubbled with a mixture of 95% O₂ / 5% CO₂. The composition of the Krebs solution was the following (in mM): NaCl 118, KCl 4.7, CaCl₂ 1.3, MgCl₂ 1.2, NaH₂PO₄ 1, NaHCO₃ 25, glucose 11.1, Na₂ EDTA 0.004 and ascorbic acid 0.11. At the end of the incubation period, the slices were transferred to glass superfusion chambers (2 slices of hippocampus or 1 of hypothalamus) and superfused at a rate of 0.5 ml/min with oxygenated Krebs' solution maintained at 37°C. Nineteen consecutive 4-min fractions were collected starting 90 min after the beginning of superfusion. One period of electrical stimulation, S₁, was carried out at 8 min after the end of the 90-min washing period. The electrical field was generated in the chambers between 2 platinum electrodes (30 mA, 2 ms, 3 Hz for 2 min for hippocampal slices; 20 mA, 2 ms, 3 Hz for 2 min for hypothalamic slices), positioned 2 cm apart. The stimulation period S₁ was carried out to ascertain the viability of the slices. Venlafaxine (3 or 10 μ M), ($\pm$)fenfluramine (10 μ M) or tyramine (3 μ M) were added to the perfusion medium at fraction # 14 for a period of 2 min while increasing the rate of superfusion to 1 ml/min. At the end of superfusion period, the slices were solubilized with

0.5 ml of Soluene 350 (Packard Instruments, Downers grove, IL, U.S.A.), and the radioactivity in the slices and superfusate samples was determined by liquid scintillation spectrometry. The results were expressed as the fraction of the tritium content present in the time at the onset of the respective collection periods. The overflow of tritium evoked by the electrical stimulation (S_1) as well as that induced by incorporation of the drug (venlafaxine, ($\pm$)fenfluramine or tyramine) to the perfusion medium (S_2), was calculated as the total increase in radioactivity released above the basal outflow of tritium determined in the sample immediately preceding the onset of the electrical or the drug-induced stimulation (i.e. at fraction #2 and #13, respectively).

Drugs

Venlafaxine and WAY 100635 were kindly provided by Wyeth-Ayerst Research (N.J., U.S.A.), paroxetine by Smith Kline Beecham (West Sussex, U.K.), fluoxetine by Lilly Research Laboratories (Indiana, U.S.A.), piperoxane by Rhône-Poulenc (Vitry, France), ($\pm$)fenfluramine by Servier (Courbevoie, France) while desipramine was purchased from RBI Natick (MA, U.S.A.), and tyramine from Aldrich (WI, USA). LSD was obtained from the Ministry of Health and Welfare (Ottawa, Canada).

Statistical analysis

In vivo electrophysiological experiments: Correlation coefficients (r values) for the dose-response relationship observed in the dorsal raphe were calculated using simple linear regression analysis and in the locus by polynomial regression analysis. The S.E.M. for the ED_{50} values for the dorsal raphe and the linear section of the locus coeruleus dose-responses curve were calculated by regression analysis, with the y value of 50 used as the regressor. Differences between two regressions were assessed by comparing their ED_{50} values using the confidence intervals method. The 95% or 99% confidence limit was determined from the Student's t distribution.

In vitro superfusion experiments: Results were expressed as means $\pm$ S.E.M. When two means were compared, the statistical significance of the difference was assessed using either a paired or a non-paired Student's t test, as indicated in the results section. Probability values of $p < 0.05$ were considered to be statistically significant.

RESULTS

Effect of the acute administration of venlafaxine and paroxetine on the firing activity of dorsal raphe 5-HT neurons.

The basal firing activity of the 33 dorsal raphe 5-HT neurons recorded was of 1.32 ± 0.11 Hz. A single intravenous dose of venlafaxine or paroxetine was administered to 14 naive rats *via* a lateral tail vein while recording a spontaneously active dorsal raphe 5-HT neuron. Both drugs induced a dose-dependent suppression of the firing activity of the dorsal raphe 5-HT neurons (Figure 1A, 1B, 2A and 2B). This suppressant effect was reversed by the subsequent acute intravenous administration of $100 \mu\text{g/kg}$ of the selective 5-HT_{1A} antagonist WAY 100635. Paroxetine displayed an ED₅₀ of $211 \pm 11 \mu\text{g/kg}$ while that of venlafaxine was of $233 \pm 12 \mu\text{g/kg}$ (Table I).

The suppressant effect of venlafaxine on the firing activity of 5-HT neurons was also assessed in rats pretreated with the noradrenergic toxin 6-OHDA. As was the case with naive rats, venlafaxine suppressed the firing activity of dorsal raphe 5-HT neurons in a dose-dependent manner. The ED₅₀ was calculated to be of $264 \pm 43 \mu\text{g/kg}$, a value not significantly different from that calculated in intact rats ($p > 0.1$; Table I).

Effect of the acute administration of venlafaxine, desipramine, paroxetine and fluoxetine on the firing activity of locus coeruleus NE neurons.

The basal firing activity of the 56 locus coeruleus NE neurons recorded was of 2.6 ± 0.18 Hz. The acute intravenous administration of venlafaxine or desipramine induced a dose-dependent suppression of locus coeruleus NE neurons firing activity (Figure 3A, 3B, 4A and B). From these dose-response curves, the ED₅₀'s of venlafaxine and desipramine to suppress the firing activity of NE neurons were calculated to be of $737 \pm 68 \mu\text{g/kg}$ and $240 \pm 54 \mu\text{g/kg}$, respectively (Table I). Low doses of venlafaxine did not induce the transient increase of the firing activity of NE neurons that had previously been reported (Haskins *et al.* 1985). It is noteworthy that the maximal suppressant effect of venlafaxine obtained at a dose of 2 mg/kg i.v. was of *ca.* 90%, *i.e.* a complete suppression of firing could not be attained even with doses of 3 and 4 mg/kg , i.v. In contrast, desipramine (Figure 3B and 4B) induced a total (*i.e.* 100%) suppression of firing activity. The subsequent intravenous administration of the α_2 -adrenergic antagonist piperoxane reversed the suppressant effect of both venlafaxine and desipramine

(Figure 3A and 3B). Following the restoration of the firing activity by piperoxane, an injection of the selective 5-HT_{1A} antagonist WAY 100635 (100 µg/kg, i.v.) was given in five rats. WAY 100635 induced a total suppression of firing in all neurons tested (Figure 3A). Thus, the suppressant effect of WAY 100635 appeared unimpaired since, by itself, when administered intravenously to naive rats, it induced, at this dose, a total suppression of the firing activity of NE neurons (data not shown), in keeping with the previous study of Haddjeri *et al.* 1997.

The effects of paroxetine and fluoxetine were also assessed on the firing activity of locus coeruleus NE neurons. Doses ranging from 0.5 to 10 mg/kg of paroxetine and 0.5 to 5 mg/kg of fluoxetine did not affect the firing activity of these neurons (Figure 5A and 5B). The intravenous administration of fluoxetine and paroxetine did not prevent the suppressant effect on the firing activity of the NE neurons induced by the subsequent intravenous administration of 100 µg/kg of WAY 100635 in 2 and 5 rats, respectively (Figure 5A and 5B).

The suppressant effect of venlafaxine on the firing activity of NE neurons was also assessed in rats pretreated with the serotonergic toxin 5,7-DHT. As was the case with naive rats, venlafaxine suppressed the firing activity of locus coeruleus NE neurons in a dose-dependent manner (Figure 3C and 4C). As was the case in naive rats, a full suppression of the firing activity of these neurons was never achieved in these pretreated rats (Figure 4C). Indeed, venlafaxine induced a suppression of around 80% of the firing activity. The ED₅₀ was calculated to be of 285 ± 49 µg/kg which was 2.6 fold lower than that obtained with intact rats ($p < 0.01$, using confidence intervals; Table I). The 5,7-DHT pretreatment did not alter the capacity of intravenously administered piperoxane (1 mg/kg) to reverse the suppressant effect of venlafaxine (Figure 3C). In keeping with previous findings (Haddjeri *et al.* 1997), the suppressant effect elicited by the acute intravenous administration of 100 µg/kg of WAY 100635 was abolished in 5,7-DHT rats. This lack of effect of WAY 100635 was taken as indirect evidence of the effectiveness of the lesion (Haddjeri *et al.* 1997).

Correlation between the potency of reuptake inhibitors to suppress the firing activity of 5-HT and/or NE neurons with their affinity for the 5-HT and/or NE transporters

In order to further evaluate the potencies of venlafaxine as a dual 5-HT/NE reuptake inhibitor, the potencies with which it suppressed the firing activity of both dorsal raphe 5-HT and locus coeruleus NE neurons were compared with those, taken from previous studies, of

other monoamine reuptake inhibitors. These potencies were further related to previously published affinities of these same reuptake inhibitors for the 5-HT and/or NE transporters.

The values for the suppression of firing activity of 5-HT and NE neurons (ED_{50} values) for reuptake inhibitors other than venlafaxine, desipramine and paroxetine, were taken from previously published electrophysiological studies that used an *in vivo* electrophysiological paradigm essentially similar to that used in the present study. As for the affinities of the various reuptake inhibitors for both transporters (K_i values), they were obtained from studies that have used either [^3H]cyanoimipramine or [^3H]paroxetine as a marker for the 5-HT transporter and of [^3H]nisoxetine for the NE transporter (for references for both the ED_{50} and K_i values, see legend to Figure 6). From these data, it was thus calculated, on the one hand, that the potencies, expressed as ED_{50} values, of eight 5-HT reuptake inhibitors (labelled 1 to 8, Figure 6A) for suppressing the firing activity of 5-HT neurons, are highly correlated with their affinities for the 5-HT transporter, expressed as K_i values ($r=0.89$). On the other hand, the potencies, expressed as ED_{50} values, of seven NE reuptake inhibitors (labelled 1 to 7, Figure 6B) for suppressing the firing activity of NE neurons, are also highly correlated with their affinities for the NE transporter, expressed as K_i values ($r=0.91$). However, and most interestingly, the affinities of both venlafaxine (VLX) and zimelidine (ZML) for the 5-HT transporters were out of keeping with their potencies for suppressing the firing activity of dorsal raphe 5-HT neurons. However, the affinity of norzimelidine (zimelidine's major metabolite; compound #6, Figure 6A) is well in keeping with its potency for suppressing the firing activity of dorsal raphe 5-HT neurons. Similarly, the affinity of venlafaxine for the NE transporter was out of keeping with its potency to suppress the firing activity of locus coeruleus NE neurons.

Effects of ($\pm$)fenfluramine and venlafaxine on the spontaneous release of tritium from hypothalamic slices preloaded with [^3H]5-HT

These *in vitro* experiments were carried out to rule out a possible 5-HT releasing property for venlafaxine by comparing its effect on the spontaneous release of [^3H]5-HT from preloaded hypothalamic slices with those of the 5-HT releaser ($\pm$)fenfluramine (Kannengiesser et al 1976; Carruba *et al.* 1977). In every experiments, the slices were first electrically stimulated to ascertain their viability. As shown in figure 7, the electrical stimulation of

hypothalamic slices induced a release of tritium (S_1 ; see table 2). The S_1 obtained from slices that were to received subsequently ($\pm$)fenfluramine was not statistically different from that obtained from slices that were to receive venlafaxine (see figure 7, A vs B, using the non-paired Student *t*-test). The 5-HT releasing agent ($\pm$)fenfluramine ($10 \mu\text{M}$), when superfused for 2 min (at 1 ml/min), induced a massive release of tritium, consistent with previous findings (Ramdine *et al.* 1989; Blier and Bouchard, 1993). The ($\pm$)fenfluramine-induced release of tritium was greater than that produced by the electrical stimulation of the same slices ($p < 0.001$, using the paired Student *t*-test; see figure 7A and table 2). Conversely, when venlafaxine ($10 \mu\text{M}$) was superfused in a fashion similar to that for ($\pm$)fenfluramine, only a minimal enhancement of tritium outflow over baseline could be detected, smaller than those induced by fenfluramine ($p < 0.001$ using the non-paired Student *t*-test) and by the electrical stimulation to the same slices ($p < 0.001$, using the paired Student *t*-test).

Effects of tyramine and venlafaxine on the spontaneous release of tritium from hippocampal slices preloaded with [^3H]NE

The existence of a NE releasing effect of venlafaxine was searched for by comparing the effect of venlafaxine on the spontaneous release of [^3H]NE from preloaded hippocampal slices with that of the NE releaser tyramine (Pylatuk and McNeil, 1976). In every experiments, the slices were first electrically stimulated to ascertain their viability. As shown in figure 8, the electrical stimulation of hippocampal slices induced the release of tritium (S_1 ; see table 2). The S_1 obtained from slices that were to received subsequently tyramine was not statistically different from that obtained from slices that were to receive venlafaxine (see figure 8, A vs B, using the non-paired Student *t*-test). The NE releasing agent tyramine ($3 \mu\text{M}$), when superfused for 2 min (at 1 ml/min), induced a massive release of tritium, consistent with the finding of Ramdine *et al.* (1989). The tyramine-induced release of tritium was greater than that produced by the electrical stimulation of the same slices ($p < 0.05$, using the paired Student *t*-test; see figure 7A and table 2). Venlafaxine ($3 \mu\text{M}$), when superfused to slices in a fashion similar to that for tyramine, produced only a minimal release of tritium over baseline, was smaller than those produced by tyramine ($p < 0.001$, using the non-paired Student *t*-test), and by the electrical stimulation to the same slices ($p < 0.001$, using the paired Student *t*-test).

DISCUSSION

The potencies of reuptake inhibitors for suppressing the firing activity of dorsal raphe 5-HT and locus coeruleus NE neurons can be taken as an *in vivo* index of 5-HT and NE reuptake blocking potencies, respectively (Quinaux *et al.* 1982). Hence, the results presented herein confirm and extend previous studies (Muth *et al.* 1986a; Bolden-Watson and Richelson, 1993; Béïque *et al.* 1998a; Béïque *et al.* 1998b) that have demonstrated the dual 5-HT/NE reuptake blocking properties of venlafaxine, its potency being greater to block the 5-HT reuptake process than that for NE. Venlafaxine and paroxetine were equipotent in suppressing the firing activity of dorsal raphe 5-HT neurons, while venlafaxine was about 3 times less potent than desipramine in suppressing that of locus coeruleus NE neurons (See Table I). The potencies of venlafaxine to suppress the firing activity of both dorsal raphe 5-HT and locus coeruleus NE neurons are nonetheless out of keeping with its affinities for both the 5-HT and NE transporters (Figure 6). This raises issues with regard to the exact mechanism by which venlafaxine exerts its *in vivo* 5-HT and NE reuptake blockade.

Venlafaxine dose-dependently suppressed the firing activity of dorsal raphe 5-HT neurons (Figure 2 and 4). It is well established that the acute administration of non-selective (Sheard *et al.* 1972; Scuvée-Moreau and Dresse, 1979), as well as selective 5-HT reuptake inhibitors (SSRI's) such as indalpine, citalopram, zimelidine or paroxetine (de Montigny *et al.* 1981; Blier *et al.* 1984; Chaput *et al.* 1986; Hajós *et al.* 1995) induces a suppression of the firing activity of dorsal raphe 5-HT neurons. Given that: 1) the suppressant effects of 5-HT reuptake inhibitors are dose-dependent; 2) neither paroxetine nor venlafaxine have appreciable affinity for any receptors known to modulate the firing activity of dorsal raphe 5-HT neurons (Muth *et al.* 1986; Thomas *et al.* 1987; Cusack *et al.* 1994); 3) and that the selective 5-HT_{1A} antagonist WAY 100635 reversed the suppressant effect of both paroxetine and venlafaxine, it can be assumed that the potency of the latter drugs for suppressing the firing activity of dorsal raphe 5-HT neurons is a reliable index to assess their potencies as 5-HT reuptake inhibitors. According to the latter assumption, the present results **suggest** that venlafaxine and paroxetine are equipotent (ED₅₀: 233 and 211 µg/kg, respectively; table I) in blocking 5-HT reuptake in the dorsal raphe. In support of this assertion, a previous study in the dorsal hippocampus using an *in vivo* electrophysiological paradigm have shown that paroxetine and venlafaxine inhibits the reuptake of 5-HT by 5-HT terminals with an equal potency (Béïque *et al.* 1998a). The suppressant effect of paroxetine and venlafaxine on the firing activity of

dorsal raphe 5-HT neurons is in keeping with previous studies (Hajós *et al.* 1995; Gartside *et al.* 1997).

The acute intravenous administration of NE reuptake inhibitors suppresses the firing activity of locus coeruleus NE neurons (Nyback *et al.* 1975; Scuvée-Moreau and Dresse, 1979; Haskins *et al.* 1985; Ceci and Borsini, 1996), which effect was shown to correlate with the NE reuptake blocking potencies of these compounds (Quinaux *et al.* 1982). Should this contention be also true for venlafaxine [*i.e.* that the potency of venlafaxine for suppressing the firing activity of locus coeruleus NE neurons can be taken as an index of its NE reuptake inhibition potency], the potency of venlafaxine obtained in this study (ED_{50} : 737 $\mu\text{g/kg}$) would suggest that it is three times less potent than desipramine (ED_{50} : 240 $\mu\text{g/kg}$) as a NE reuptake blocker, consistently with the ED_{50} values previously published (Nyback *et al.* 1975; Scuvée-Moreau and Dresse, 1979; Haskins *et al.* 1985; Ceci and Borsini, 1996). It is striking, however, that venlafaxine failed to induce a complete suppression of firing of NE neurons, unlike other NE reuptake inhibitors (Figure 4A and 4B).

The observations that, first, paroxetine and venlafaxine were equipotent in suppressing the firing activity of dorsal raphe 5-HT neurons, and second, that desipramine was three times more potent than venlafaxine to suppress that of locus coeruleus NE neurons is highly interesting given results from previous studies that have used different experimental approaches. For instance, a synaptosomal uptake study has shown that venlafaxine is 53 times less potent than paroxetine to inhibit the uptake of [^3H]5-HT and that venlafaxine is 344 times less potent than desipramine to inhibit that of [^3H]NE (Bolden-Watson and Richelson, 1993). Moreover, in a recent radioligand binding study, venlafaxine displayed an affinity for the 5-HT transporter about 2000 times lower than that of paroxetine (K_i of 74 and 0.04 nM, respectively), as assessed with [^3H]cyanoimipramine displacement, while venlafaxine also displayed a 2000 times lesser affinity for the NE transporter compared to desipramine (K_i of 1260 and 0.55 nM, respectively), as assessed with [^3H]nisoxetine displacement (Béïque *et al.* 1998b). The relative affinities and potencies derived from the latter *in vitro* studies are thus totally incongruent with the *in vivo* potencies reported herein. This discrepancy is nonetheless best demonstrated by the correlations depicted in Figures 6A and 6B: the potencies of reuptake inhibitors for suppressing the firing activity of dorsal raphe 5-HT and/or locus coeruleus NE neurons are tightly correlated with their respective affinities for both transporters. Of utmost interest, is the fact that the suppression of the firing activity of dorsal

raphe 5-HT neurons achieved by venlafaxine, as well as by zimelidine, does not conform to this correlation (Figure 6A). As for zimelidine, its suppressant effect on the firing activity of dorsal raphe 5-HT neurons is most probably mediated by its active metabolite, norzimelidine, since zimelidine itself displays a low potency to inhibit [^{3} H]5-HT uptake (Hyttel, 1982), and has low affinity for the 5-HT transporter (Kovachich *et al.* 1988; Marcusson *et al.* 1992), whereas norzimelidine (compound # 6 in Figure 6A) has potent 5-HT reuptake inhibition properties (Hyttel, 1982), and displays a high affinity for the 5-HT transporter that is well in keeping with its potency to suppress the firing activity of dorsal raphe 5-HT neurons (Figure 6A). Nonetheless, the most intriguing and perhaps the most convincing assertion derived from the present study is the fact that the potencies of venlafaxine for suppressing the firing activity of both 5-HT and NE neurons is out of keeping with its affinities for both transporters (Figure 6A and 6B). The underlying reasons for this discrepancy are yet to be resolved, although some possibilities can be readily excluded. For instance, venlafaxine's major metabolite, O-desmethyl-venlafaxine (WY-45,233; Howell *et al.* 1993), was shown to bear equal 5-HT reuptake blocking potencies *in vitro* (IC_{50} of 0.18 and 0.21 μ M, respectively) and lower NE reuptake potencies (IC_{50} of 1.6 and 0.64 μ M, respectively) than venlafaxine itself (Muth *et al.* 1986b), and thus suggest that this metabolite is not the mediator of the high *in vivo* potencies observed with venlafaxine, as is the case for norzimelidine's action on the firing activity of dorsal raphe 5-HT neurons.

Furthermore, given that venlafaxine is a dual 5-HT/NE reuptake inhibitor and that both systems are known to be functionally interconnected, it is possible that the effects of venlafaxine on the firing activity of both dorsal raphe 5-HT and locus coeruleus NE neurons may not be mediated by its sole and independent effect on these neurons *per se*, but well via an action on both these systems together. In order to assess the latter possibility, the effect of venlafaxine on the firing activity of dorsal raphe 5-HT neurons of rats treated with the noradrenergic toxin 6-OHDA was assessed given that an important noradrenergic input to the dorsal raphe has been described both anatomically (Anderson *et al.* 1977; Sakai *et al.* 1977) and functionally (Baraban and Aghajanian, 1980a; Baraban and Aghajanian, 1980b). Since the 6-OHDA lesion did not have any significant effect on the dose-response curve of the suppressant effect of venlafaxine on the firing activity of 5-HT dorsal raphe neurons (Figure 2A, 2C and Table I), it can be concluded that the suppressant effect of venlafaxine on the firing activity of dorsal raphe 5-HT neurons is solely attributable to a direct action on the 5-HT

system *i.e.* 5-HT reuptake blockade. Moreover, this contention is consistent with the fact that NE reuptake inhibition, achieved with the intravenous administration of desipramine, does not influence the firing activity of 5-HT neurons ($ED_{50} > 12$ mg/kg; Scuvée-Moreau and Dresse, 1979).

The 5-HT reuptake inhibition property of venlafaxine is probably not involved in its capacity to suppress the firing activity of locus coeruleus NE neurons for the following observations. First, 5-HT reuptake inhibition, achieved with the intravenous administration of the two prototypical SSRI's paroxetine and fluoxetine, did not induce any changes in the firing activity of locus coeruleus NE neurons (Figure 5A and B). Inasmuch as it is well documented that the acute systemic administration of 5-HT reuptake inhibitors induce an elevation in the extracellular concentration of 5-HT not only in the raphe area (Adell and Artigas, 1991; Rutter *et al.* 1995) but also in terminal areas such as the striatum, hypothalamus, hippocampus, diencephalon and frontal cortex (Invernizzi *et al.* 1995; Kreiss and Lucki, 1995; Rutter *et al.* 1995; Romero *et al.* 1996a; Romero *et al.* 1996b; Romero and Artigas, 1997), it is likely that the acute administration of paroxetine and fluoxetine would increase the extracellular concentration of 5-HT in the locus coeruleus. Although the exact nature of the serotonergic modulation of the NE locus coeruleus neurons has not been fully elucidated, nor has the exact nature of the receptors involved in the circuitry, several lines of evidence point towards an inhibitory role for 5-HT on the firing activity of NE neurons of the locus coeruleus (Segal, 1979; Rasmussen and Aghajanian, 1986; Aston-Jones *et al.* 1991; Haddjeri *et al.* 1997). In light of these considerations, the lack of effect of paroxetine and fluoxetine on the firing activity of locus coeruleus NE neurons is somewhat intriguing. At any rate, the lack of effect of these two SSRI's on the firing activity of locus coeruleus NE neurons supports the notion that the 5-HT reuptake inhibition property of venlafaxine cannot be held responsible for the unexpectedly high potency of venlafaxine for suppressing the firing activity of locus coeruleus NE neurons.

If the 5-HT reuptake blocking properties of venlafaxine were to be involved in the surprisingly high potency of venlafaxine to suppress the firing activity of locus coeruleus NE neurons, the lesioning of 5-HT neurons would have induced a shift to the right of the dose-response curve of the suppression by venlafaxine of the firing activity of locus coeruleus NE neurons, indicative of a dampened effect. Our findings, on the contrary, indicate that the dose-response curve of venlafaxine in the locus coeruleus was actually shifted to the left (ED_{50} : 285

vs 737 $\mu\text{g/kg}$; table I) in 5,7-DHT lesioned rats. This finding was unexpected given the presumed inhibitory role of 5-HT in the locus coeruleus. Based on the assumption that the 5-HT system would tonically suppress locus coeruleus NE neurons (Haddjeri *et al.* 1997), it is conceivable that compensatory homeostatic mechanisms have developed following the lesion with 5,7-DHT. In support of this assertion, it was observed in the amygdala that a 5,7-DHT lesion not only induced a supersensitivity to microiontophoretically applied 5-HT but also to NE (Wang *et al.* 1979). Given that venlafaxine, as well as other NE reuptake blockers, suppresses the firing activity of locus coeruleus NE neurons ultimately through activation of somatodendritic α_2 -adrenergic autoreceptors, the potentiation of the effect of venlafaxine on the firing activity of locus coeruleus NE neurons induced by the serotonergic lesion, may be attributable to a supersensitivity of these somatodendritic α_2 -adrenergic autoreceptors. This hypothesis seems at first incongruent with the study of Kasamo *et al.* (1996) who reported an unchanged capacity of duloxetine, another dual 5-HT/NE reuptake inhibitor, to suppress the firing activity of locus coeruleus NE neurons in 5,7-DHT treated rats. This discrepancy could nevertheless be explained, at least in part, by methodological considerations. Indeed, as the lesioning in that latter study were performed by injecting the toxin directly in the dorsal raphe, our lesioning were made intracerebroventricularly. Although the serotonergic input to the locus coeruleus has been originally believed to arise predominantly from the raphe nuclei (Pickel *et al.* 1977; Cedarbaum and Aghajanian, 1978), it has been more recently suggested that an important 5-HT input to the locus arises from the nucleus paragigantocellularis and from pericoerulear area (Pieribone *et al.* 1989; see Aston-Jones, 1991b for review). It is thus possible that, in the study of Kasamo *et al.* (1996), some relevant serotonergic input to the locus coeruleus may have been spared by the injection of the toxin in the dorsal raphe, which sole presence may have prevented the development of a NE supersensitivity.

The last possibility that was dealt with in the present study regarding a plausible mechanism that might have underlied the peculiar high *in vivo* potencies of venlafaxine was that of a putative 5-HT and/or NE releasing property. The rational being that releasers, by increasing the extracellular concentrations of 5-HT and/or NE would decrease the firing activity of 5-HT and/or NE neurons via somatodendritic autoreceptor activation. Indeed, as for the 5-HT system, while it was shown that the 5-HT releaser ($\pm$)fenfluramine suppressed, *in vivo*, the firing activity of 5-HT neurons in the dorsal raphe (Scuvée-Moreau and Dresse, 1990), the suppressant effect of 3,4-methylenedioxymethamphetamine (MDMA) on the firing

activity of 5-HT neurons, as assessed *in vitro* on mesencephalic slices, was shown to be mediated by the release of 5-HT (Sprouse *et al.* 1989). As for the NE system, the NE releaser amphetamine was shown to suppress the firing activity of locus coeruleus NE neurons and this, via an activation of α_2 -adrenergic autoreceptors (Rasmussen and Aghajanian, 1986). Therefore, in light of the fact that venlafaxine shares a phenylethylamine moiety with some of the monoamine releasers such as tyramine, amphetamine, methamphetamine or ($\pm$)fenfluramine, it was deemed necessary to investigate any putative 5-HT and/or NE releasing properties for venlafaxine. As illustrated in figures 7 and 8, venlafaxine was devoid of any 5-HT and/or NE releasing properties and that, in sharp contrast to ($\pm$)fenfluramine and tyramine, respectively. It is noteworthy, that superfusion of the slices with venlafaxine induced a small increase of the spontaneous outflow of both [^{3} H]5-HT and [^{3} H]NE. The latter most probably reflects the 5-HT and NE reuptake blocking properties of venlafaxine, rather than one of releasing. This is consistent with previous studies showing that superfusion with paroxetine and citalopram induce a small increase of the spontaneous outflow of [^{3} H]5-HT (Blier *et al.* 1990), while superfusion with desipramine induces a small increase of the spontaneous outflow of [^{3} H]NE (Mongeau *et al.* 1998). Therefore, present results rule out the possibility that venlafaxine might act via releasing 5-HT or NE.

In summary, if one assumes that suppression of firing activity of monoaminergic neurons by reuptake blockers can be taken as an index of their reuptake inhibition potencies, the present study suggests that venlafaxine has potent 5-HT and NE reuptake inhibition potencies when assessed using an *in vivo* electrophysiological paradigm. Since venlafaxine is more potent in inhibiting the reuptake of 5-HT compared to that for NE, it is possible that the seemingly unique clinical properties seen with high doses of venlafaxine may be mediated by the recruitment of the NE system by these higher doses. Nonetheless, the fact that the *in vivo* reuptake blocking properties of venlafaxine does not seem to be solely mediated by an action on the [^{3} H]cyanoimipramine or [^{3} H]nisoxetine binding sites on the 5-HT and NE transporters respectively, may cast doubt on the latter hypothesis and thus raises important considerations with regard to the exact mechanism by which venlafaxine exerts its antidepressant action.

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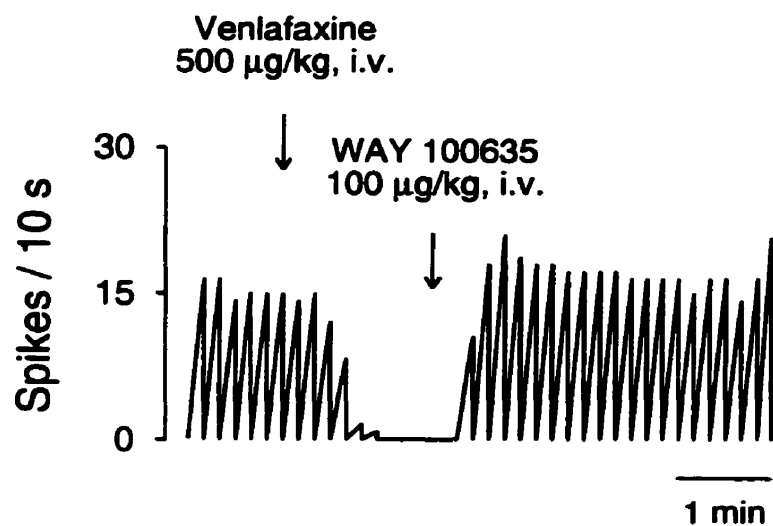
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LEGENDS TO FIGURES

Figure 1. Integrated firing rate histograms of serotonergic neurons of the dorsal raphe nucleus showing the effects of intravenous administration of 500 $\mu\text{g/kg}$ of venlafaxine (A) and 200 $\mu\text{g/kg}$ of paroxetine (B) on the spontaneous firing activity of the neuron. LSD (10 $\mu\text{g/kg}$) was sometimes injected intravenously in order to confirm the serotonergic nature of the neuron recorded. The effect of a subsequent intravenous administration of 100 $\mu\text{g/kg}$ of the selective 5-HT_{1A} antagonist WAY 100635 is also shown.

A.



B.

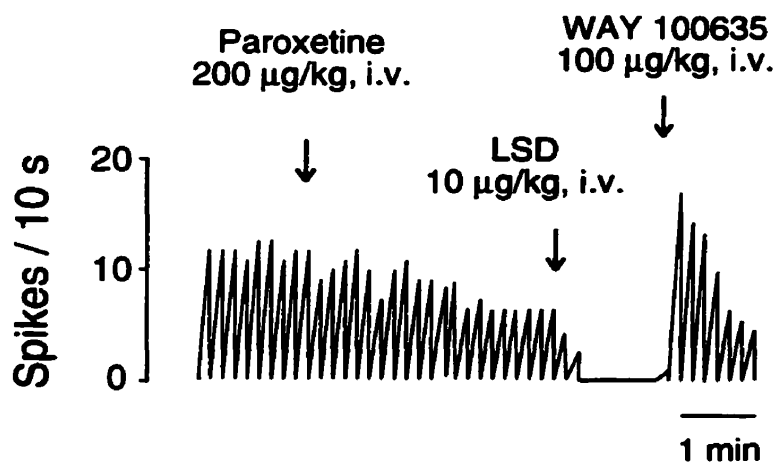


Figure 2. Dose-response curves showing the effect of intravenous administration of venlafaxine (A) and of paroxetine (B) on the firing activity of dorsal raphe 5-HT neurons. The dose-response curve of venlafaxine for suppressing the firing activity of dorsal raphe 5-HT neurons of rats that were treated i.c.v. with the neurotoxin 6-OHDA is shown in C. In these and subsequent dose-response curves, each dot represent the response of one neuron to a single dose in one rat while the dotted curved lines depict the 95% confidence interval of the regression.

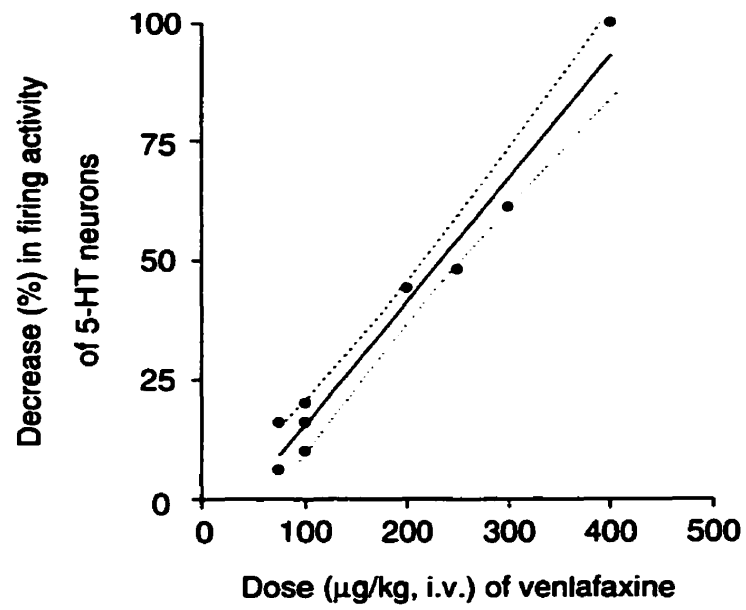
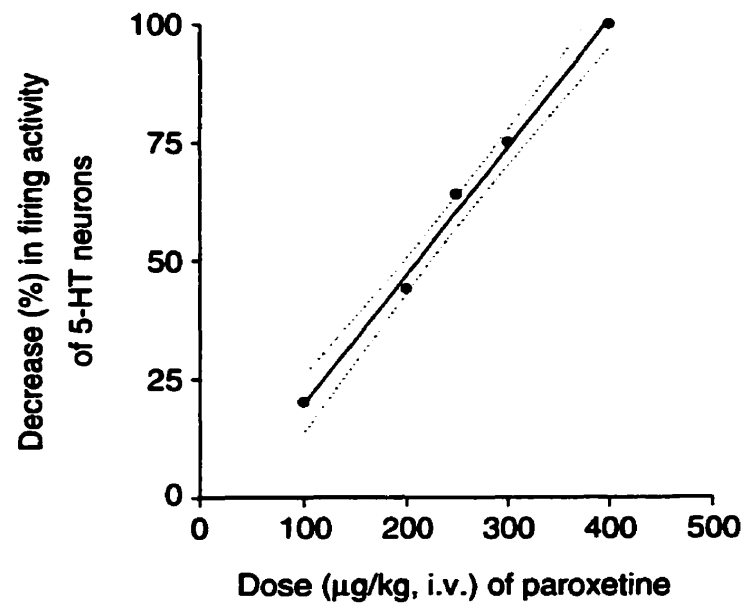
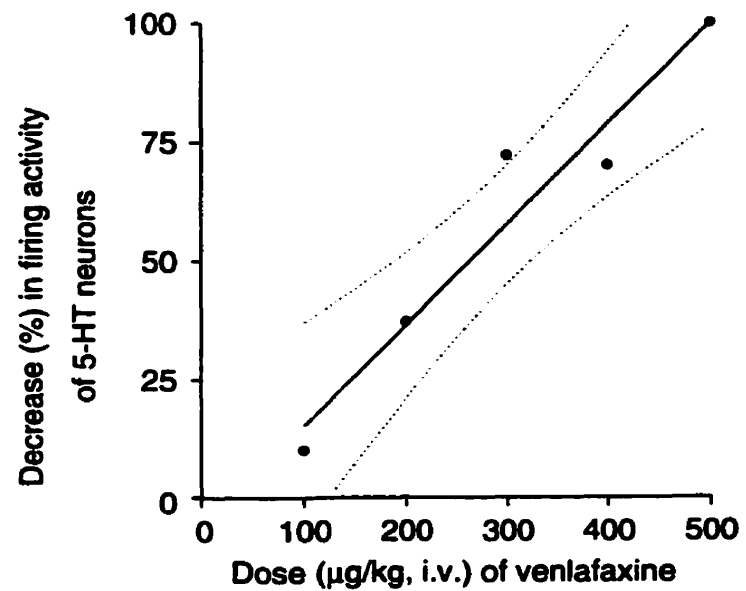
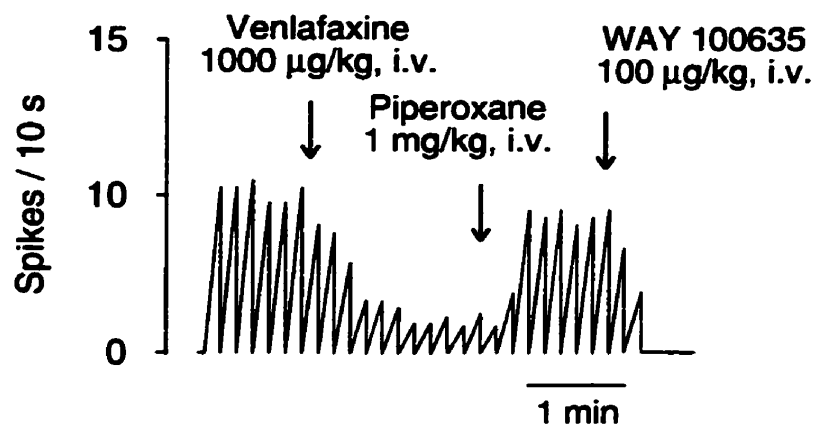
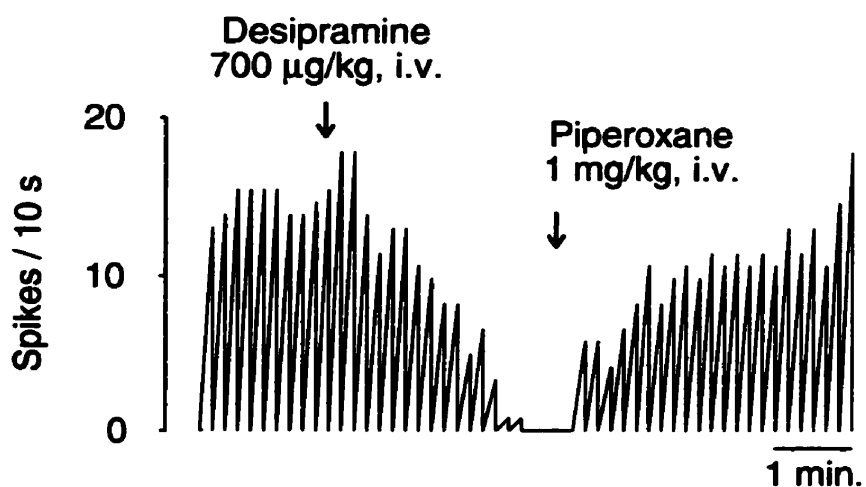
A.**B.****C.**

Figure 3. Integrated firing rate histograms of NE neurons of the locus coeruleus showing the effect of intravenous administration of 1 mg/kg of venlafaxine (A), 700 μ g/kg of desipramine (B) and multiple doses of venlafaxine in rats treated i.c.v. with the neurotoxin 5,7-DHT (C) on the spontaneous firing activity of the neuron. The effect of the subsequent intravenous administration of the α_2 antagonist piperoxane (1 mg/kg) and of WAY 100635 (100 μ g/kg) is also shown.

A.



B.



C.

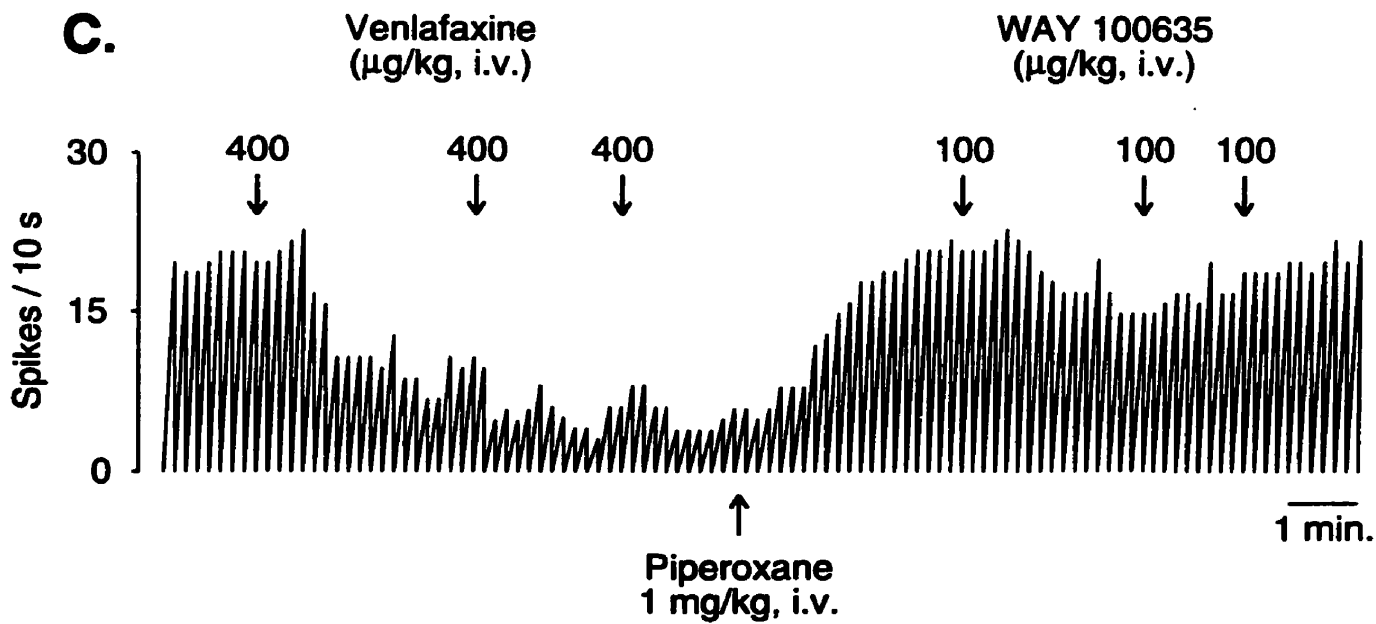


Figure 4. Dose-response curves showing the effect of intravenous administration of venlafaxine (A), desipramine (B) and venlafaxine in rats treated i.c.v. with the neurotoxin 5,7-DHT (C) on the firing activity of locus coeruleus NE neurons. The open circles in (C) represent the effect of venlafaxine on the firing activity of locus coeruleus NE neurons of rats treated i.c.v. with saline.

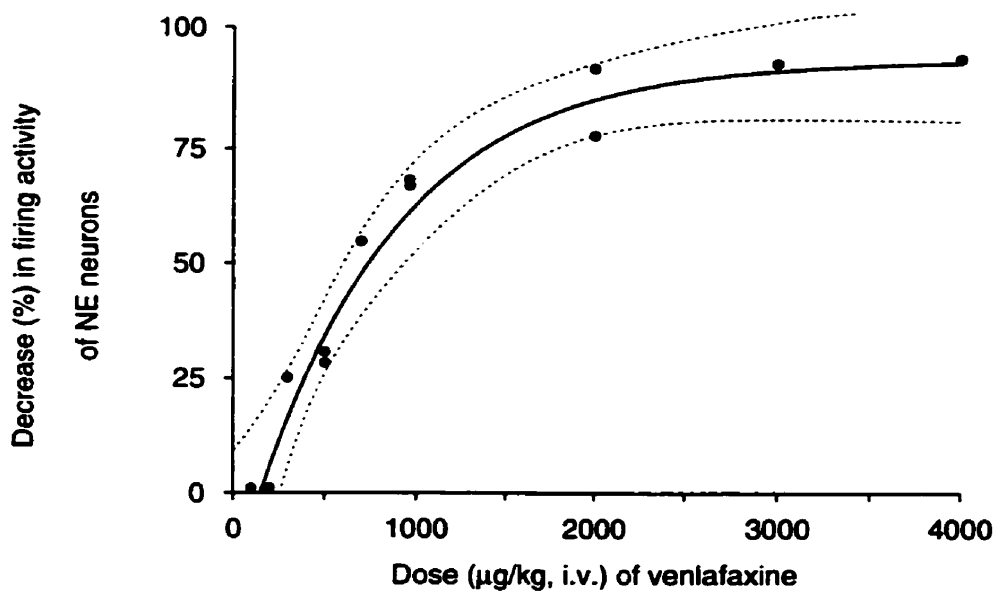
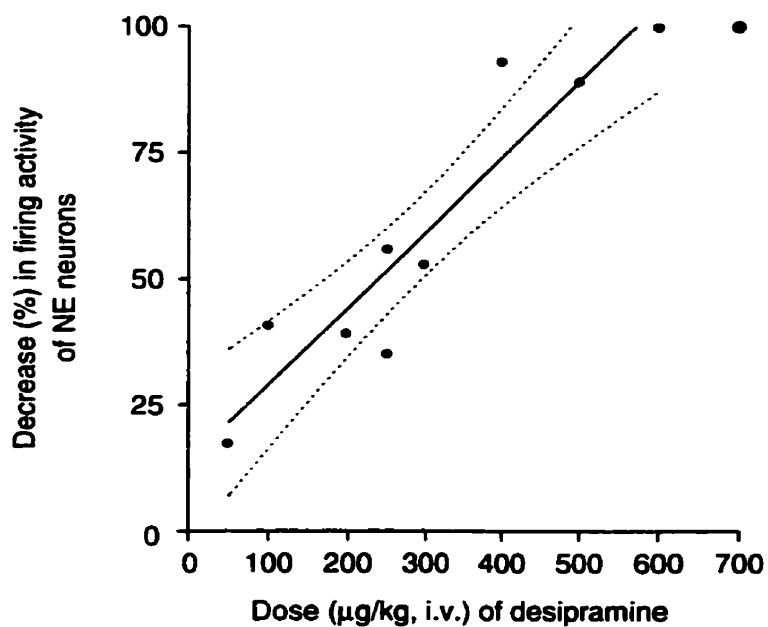
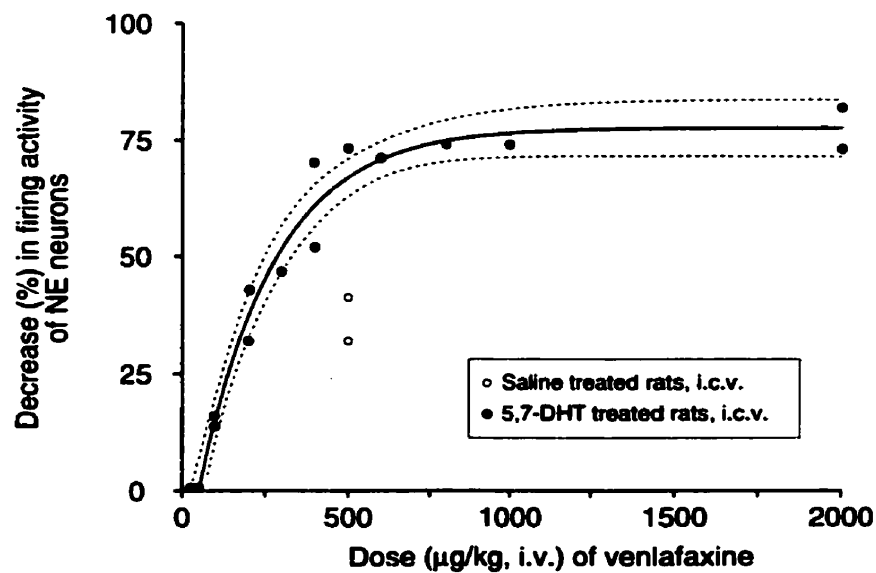
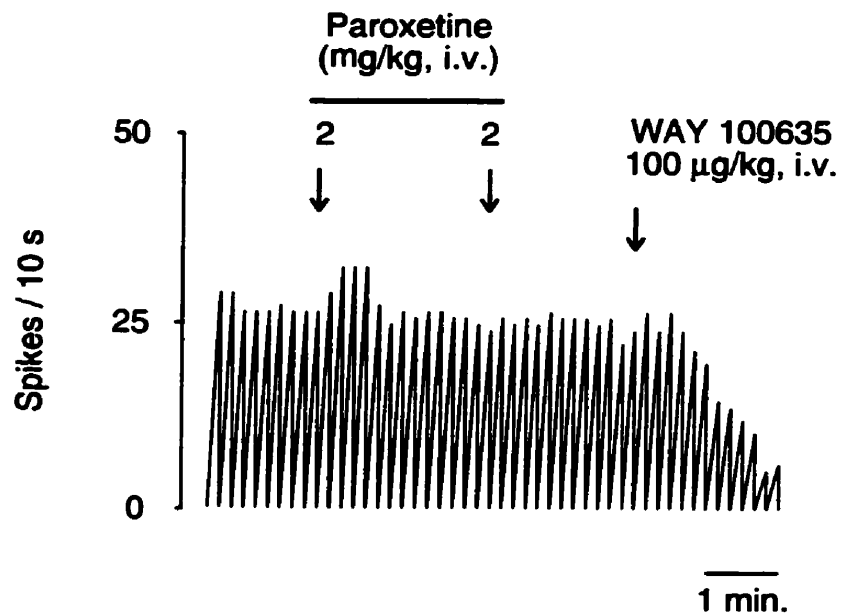
A.**B.****C.**

Figure 5. Integrated firing rate histograms of NE neurons of the locus coeruleus showing the effects of intravenous administration of paroxetine (A) and fluoxetine (B) on the spontaneous firing activity of the neuron. These two drugs did not induce any significant suppression of the firing activity of these neurons nor did they prevent the suppressant effect of WAY 100635 (100 μ g/kg, i.v.).

A.



B.

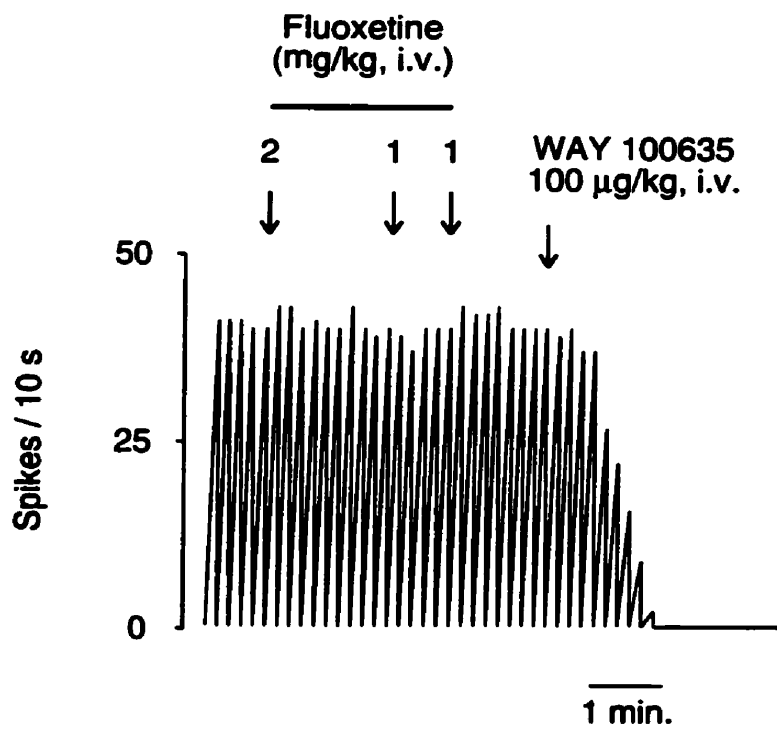


Figure 6. A. Correlation ($r = 0.89$) between the potency, expressed as ED_{50} values, of various compounds to suppress the firing activity of dorsal raphe 5-HT neurons and their affinity, expressed as K_i values, for the 5-HT transporter (taken from either [^3H]cyanoimipramine or [^3H]paroxetine displacement studies).

B. Correlation ($r = 0.91$) between the potency, expressed as ED_{50} values, of various compounds to suppress the firing activity of locus coeruleus NE neurons and their affinity, expressed as K_i values, for the NE transporter (taken from [^3H]nisoxetine displacement studies). The values reported for venlafaxine (VLX; A and B) and zimelidine (ZML; A) were not taken in account for the calculation of the correlation coefficient (r) of the two linear regression.

The numbers on figures 6A and 6B correspond to the following compounds followed by the study from which the data have been obtained (ED_{50} ; K_i). 6A: 1- Duloxetine (Kasamo *et al.* 1996; Béïque *et al.* 1998b); 2- Paroxetine (present study; Béïque *et al.* 1998b); 3- Citalopram (de Montigny *et al.* 1990; Cheetham *et al.* 1993); 4- Indalpine (Blier *et al.* 1984; Béïque *et al.* 1998b); 5- Chlorimipramine (Scuvée-Moreau and Dresse, 1979; Cheetham *et al.* 1993); 6- Norzimelidine (de Montigny *et al.* 1981; Kovachich *et al.* 1988); 7- Imipramine (Scuvée-Moreau and Dresse, 1979; Cheetham *et al.* 1993); 8- Amitriptyline (Scuvée-Moreau and Dresse, 1979; Cheetham *et al.* 1993); VLX- venlafaxine (present study; Béïque *et al.* 1998b); ZML- zimelidine (de Montigny *et al.* 1981; Kovachich *et al.* 1988).

6B: 1- Desipramine (present study; Béïque *et al.* 1998b); 2- Duloxetine (Kasamo *et al.* 1996; Béïque *et al.* 1998b); 3- Nortriptyline (Scuvée-Moreau and Dresse, 1979; Cheetham *et al.* 1996); 4- Tranylcypromine (Scuvée-Moreau and Dresse, 1979; Cheetham *et al.* 1996); 5- Imipramine (Scuvée-Moreau and Dresse, 1979; Cheetham *et al.* 1996); 6- Amitriptyline (Scuvée-Moreau and Dresse, 1979; Cheetham *et al.* 1996); 7- Chlorimipramine (Scuvée-Moreau and Dresse, 1979; Cheetham *et al.* 1996); VLX- Venlafaxine (present study; Béïque *et al.* 1998b).

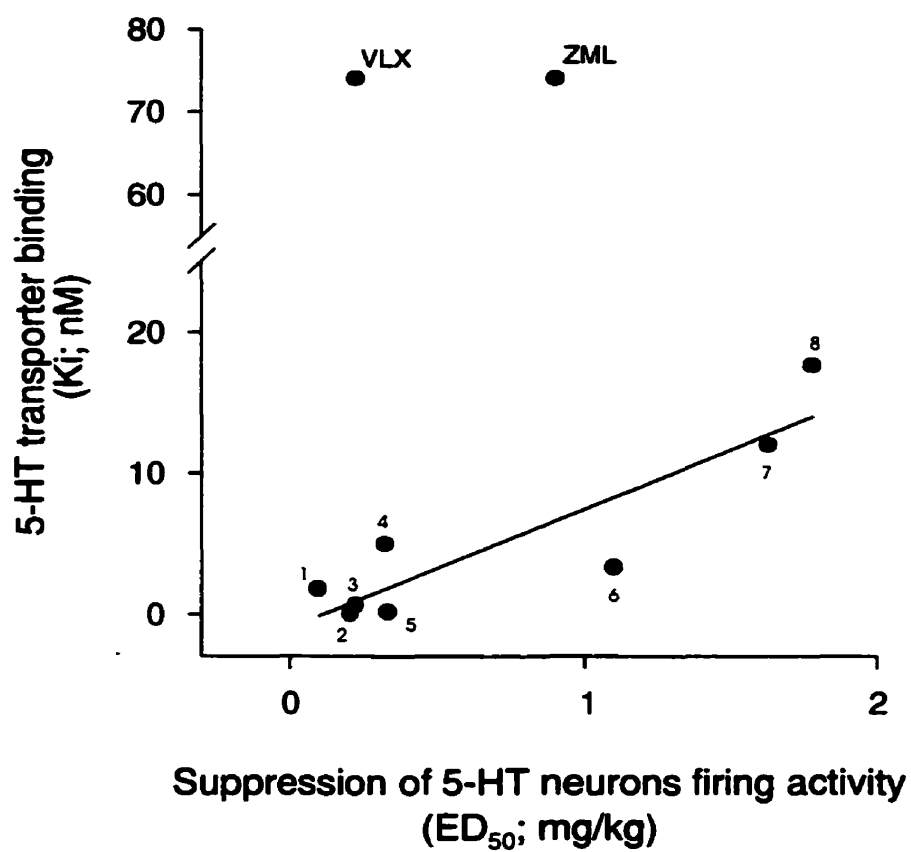
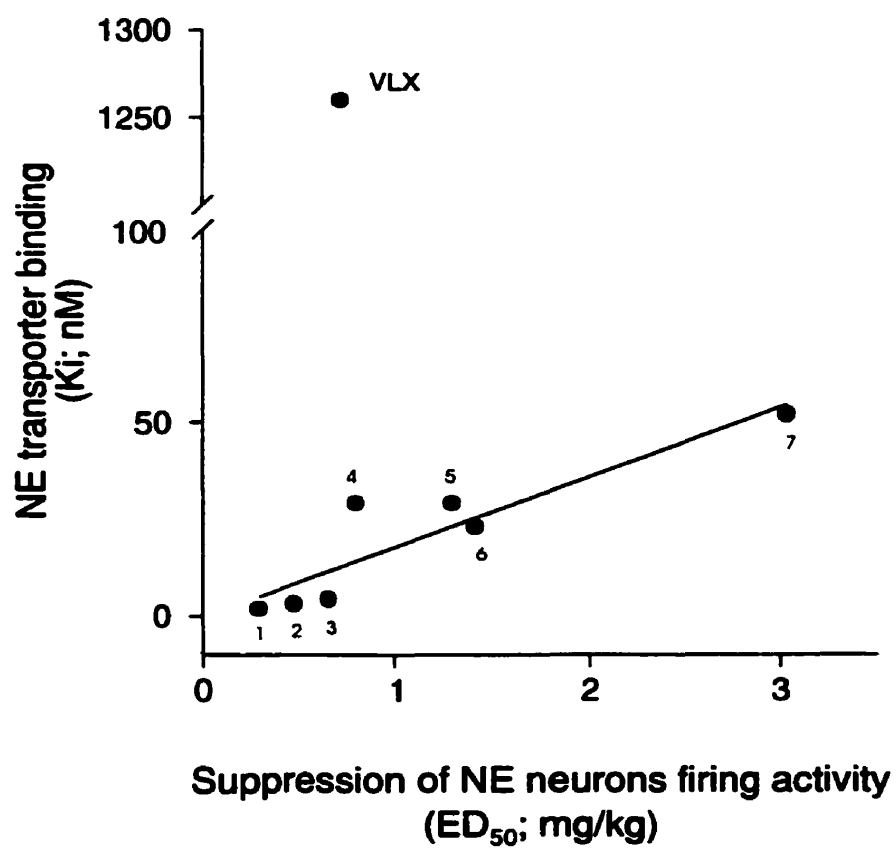
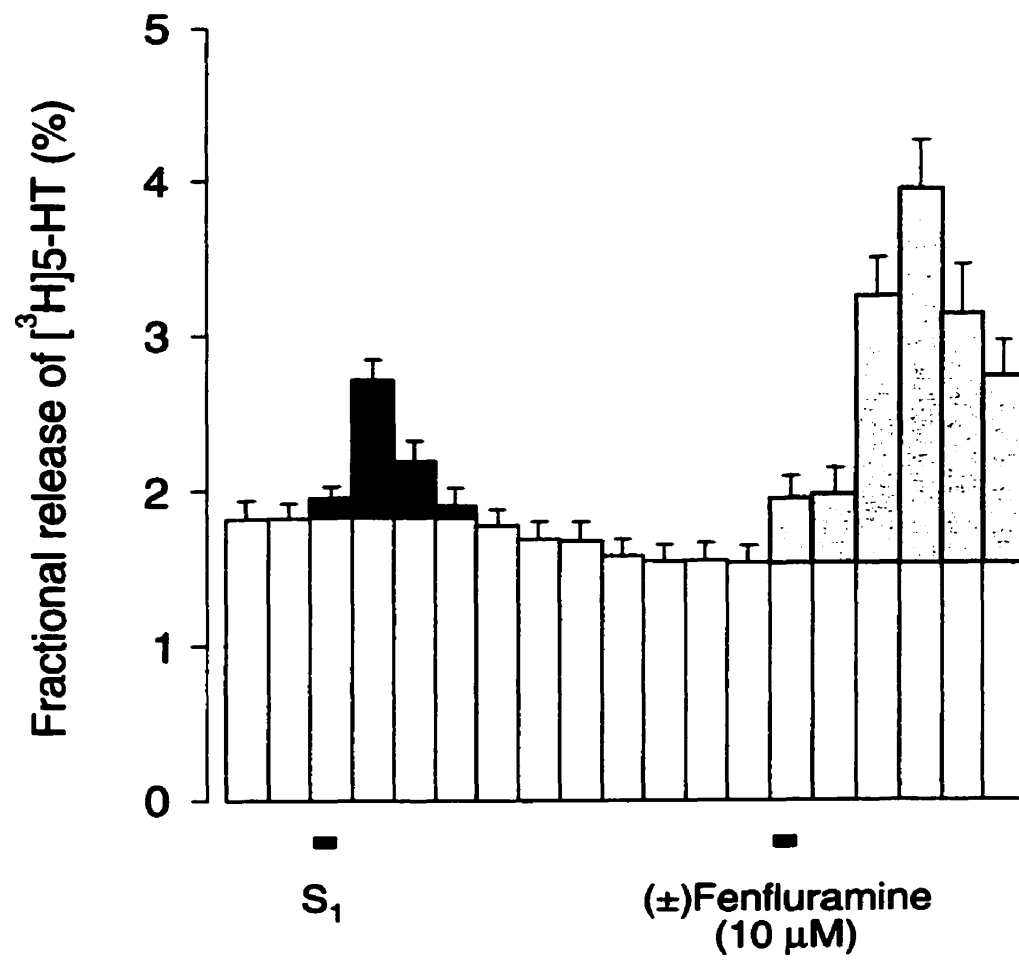
A.**B.**

Figure 7. Basal, electrically evoked (S_1) and drug-induced (S_2) release of tritium from hypothalamic slices preloaded with [^3H]5-HT. The electrical stimulation (S_1), was delivered at fraction #3 to ascertain the viability of the slices and consisted of 360 pulses delivered at an intensity of 20 mA, a duration of 2 ms, and a frequency of 3 Hz. The drug-induced (S_2) release of tritium was achieved by incorporating to the Krebs solution either ($\pm$)fenfluramine (A;10 μM) or venlafaxine (B;10 μM), and by perfusing the slices at 1 ml/min for 2 min. Ordinate scale: amount of tritium released per 4 min sample expressed as a fraction of total tissue tritium content of the slices at the start of the respective collection period. The gray area represents the proportion of tritium overflow evoked by either the electrical stimulation or drug superfusion. Values are expressed as means $\pm$ S.E.M. of 10 experiments.

A.



B.

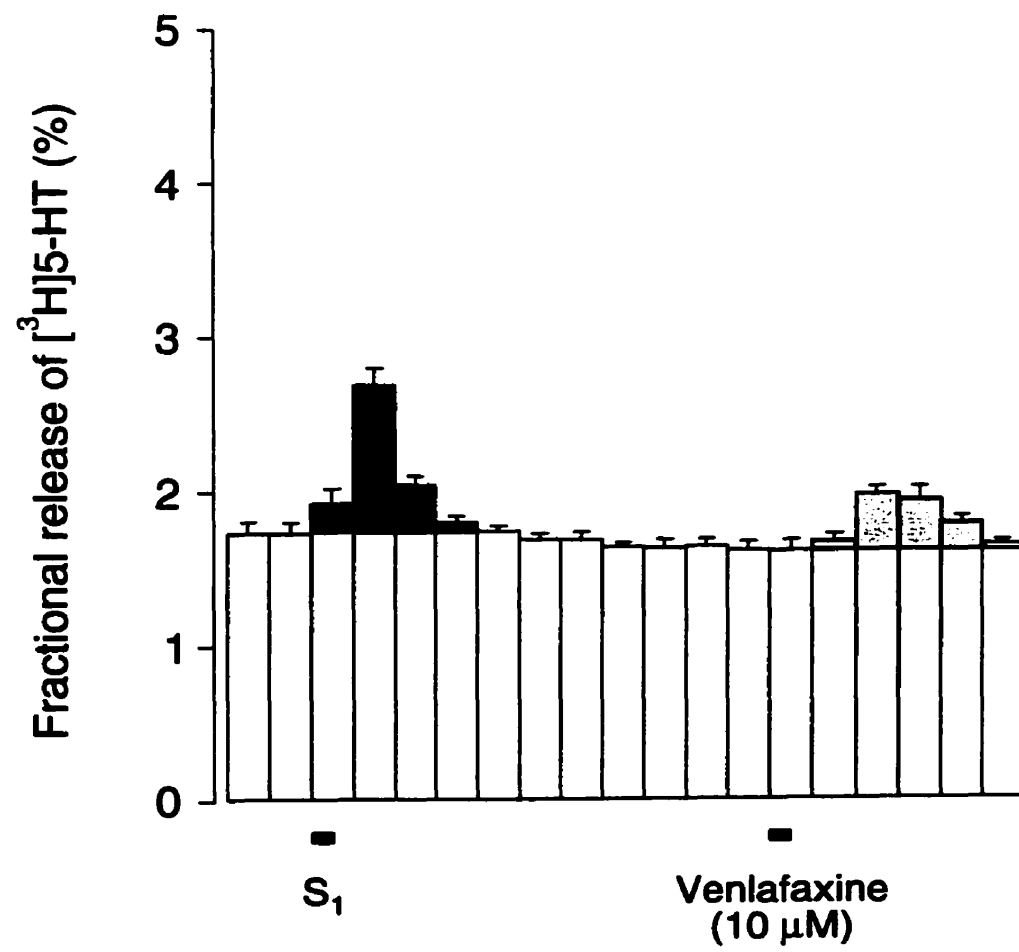
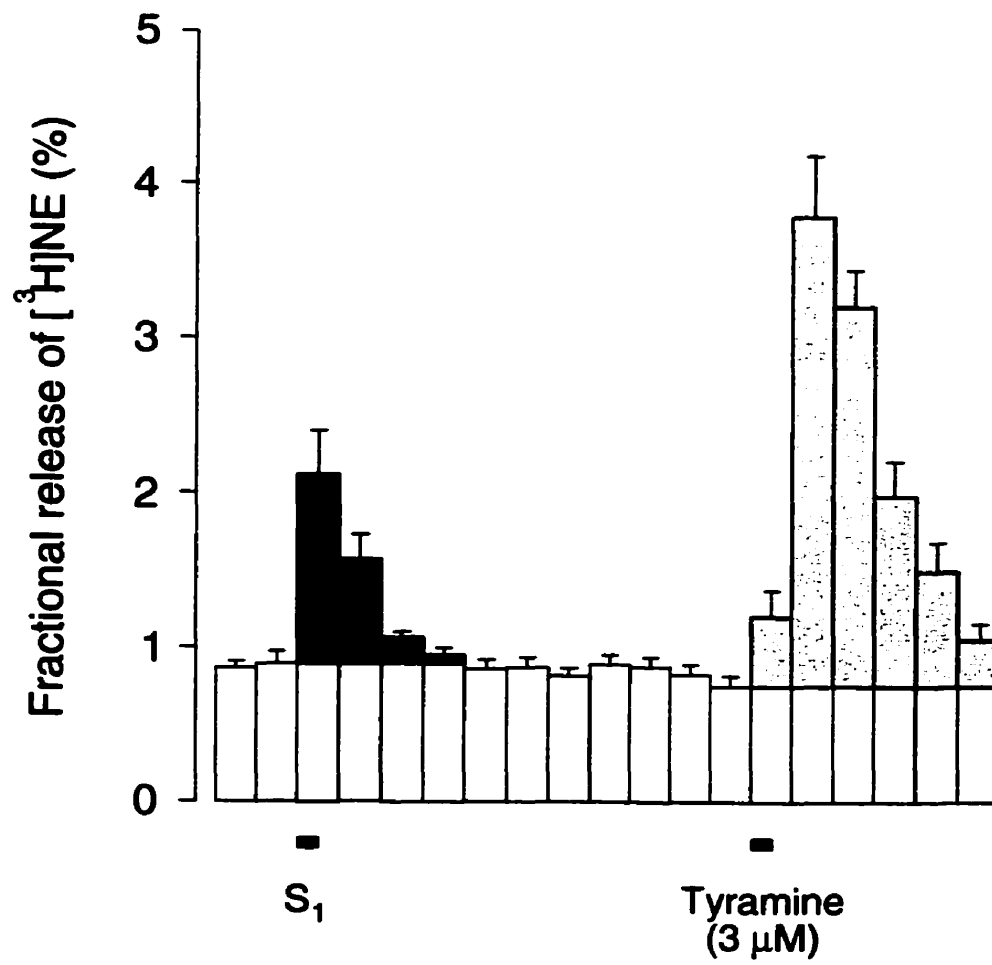


Figure 8. Basal, electrically evoked (S_1) and drug-induced (S_2) release of tritium from hippocampus slices preloaded with [^3H]NE. The electrical stimulation (S_1), was delivered at fraction #3 to ascertain the viability of the slices and consisted of 360 pulses delivered at an intensity of 30 mA, a duration of 2 ms, and a frequency of 3 Hz. The drug-induced release of tritium was achieved by incorporating to the Krebs solution either tyramine (A; 3 μM) or venlafaxine (B; 3 μM), and by perfusing the slices at 1 ml/min for 2 min. Ordinate scale: amount of tritium released per 4 min sample expressed as a fraction of total tissue tritium content of the slices at the start of the respective collection period. The gray area represents the proportion of tritium overflow evoked by either the electrical stimulation or drug superfusion. Values are expressed as means $\pm$ S.E.M. of 5 experiments.

A.



B.

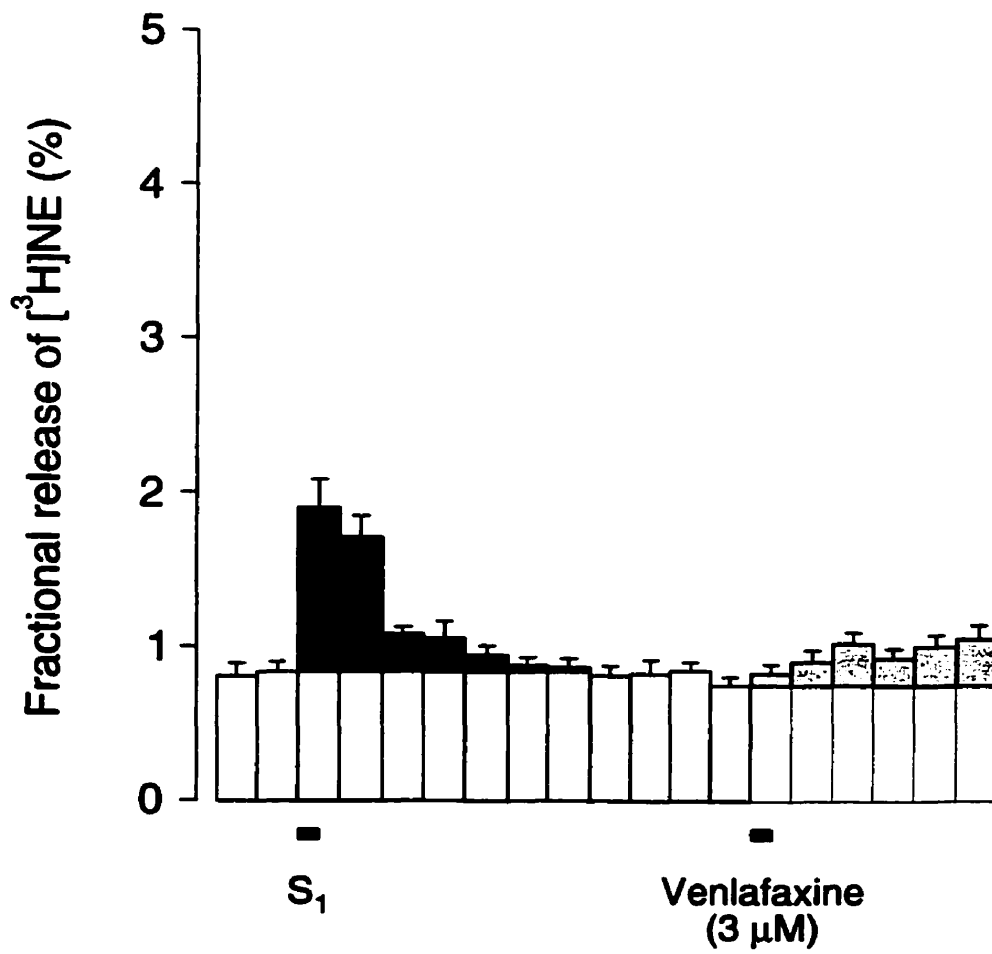


Table 1. Effects of venlafaxine, paroxetine, desipramine and fluoxetine on the firing activity of dorsal raphe 5-HT and/or locus coeruleus NE neurons¹

	ED₅₀ (μg/kg)	r	n
Dorsal raphe 5-HT neurons			
Venlafaxine	233 ± 12	0.98	9
Venlafaxine (6-OH-DA) ²	264 ± 43 ^{N.S.}	0.96	5
Paroxetine	211 ± 11	0.99	5
Locus coeruleus NE neurons			
Venlafaxine	737 ± 68	0.96	12
Venlafaxine (5,7-DHT) ²	285 ± 49*	0.98	15
Desipramine	240 ± 54	0.93	10
Paroxetine	> 10 000	~0	11
Fluoxetine	> 5 000	~0	5

¹ Data are the regression parameters of the dose-response curves shown in Figure 2,4 and 5. ED₅₀ = predicted dose that would elicit a 50% suppression of the firing activity of the neuron; r = regression coefficient; n = number of neurons; n.d. = not determined.

² The experiments were performed at least 14 days after the i.c.v. injection of the toxin.

* p < 0.01 using confidence intervals, compared to venlafaxine in untreated rats.

N.S. Not significant when compared to the ED50 value for venlafaxine in untreated rats.

Table II. Effects of venlafaxine, fenfluramine and tyramine on the spontaneous release of tritium from brain slices preloaded with either [^{3} H]5-HT or [^{3} H]NE ¹

Hypothalamic slices preloaded with [^{3}H]5-HT	S₁ Electrical stimulation	S₂ Venlafaxine (10 μM)	S₁ Electrical stimulation	S₂ Fenfluramine (10 μM)
	1.98 $\pm$ 0.24 (n=10)	0.64 $\pm$ 0.18	1.49 $\pm$ 0.16 ^{N.S.} (n=10)	7.7 $\pm$ 0.8 **
Hippocampal slices preloaded with [^{3}H]NE	S₁ Electrical stimulation	S₂ Venlafaxine (3 μM)	S₁ Electrical stimulation	S₂ Tyramine (3 μM)
	3.63 $\pm$ 0.56 (n=5)	0.35 $\pm$ 0.30	3.31 $\pm$ 0.5 ^{N.S.} (n=5)	6.4 $\pm$ 0.75 **

¹ Data represent the total increase in radioactivity released above resting outflow by either electrical stimulation (S1) or drug perfusion (S2), as calculated from figure 7 and 8.

^{N.S.} The electrically evoked release (S1) of tritium is not statistically different from that induced in slices that were subsequently exposed to venlafaxine (processed in parallel).

** p < 0.001 when compared to venlafaxine-induced release of tritium.

Annex to Chapter III

Following a suggestion by the external referee for the present thesis, we here present a revised version of figure 6.

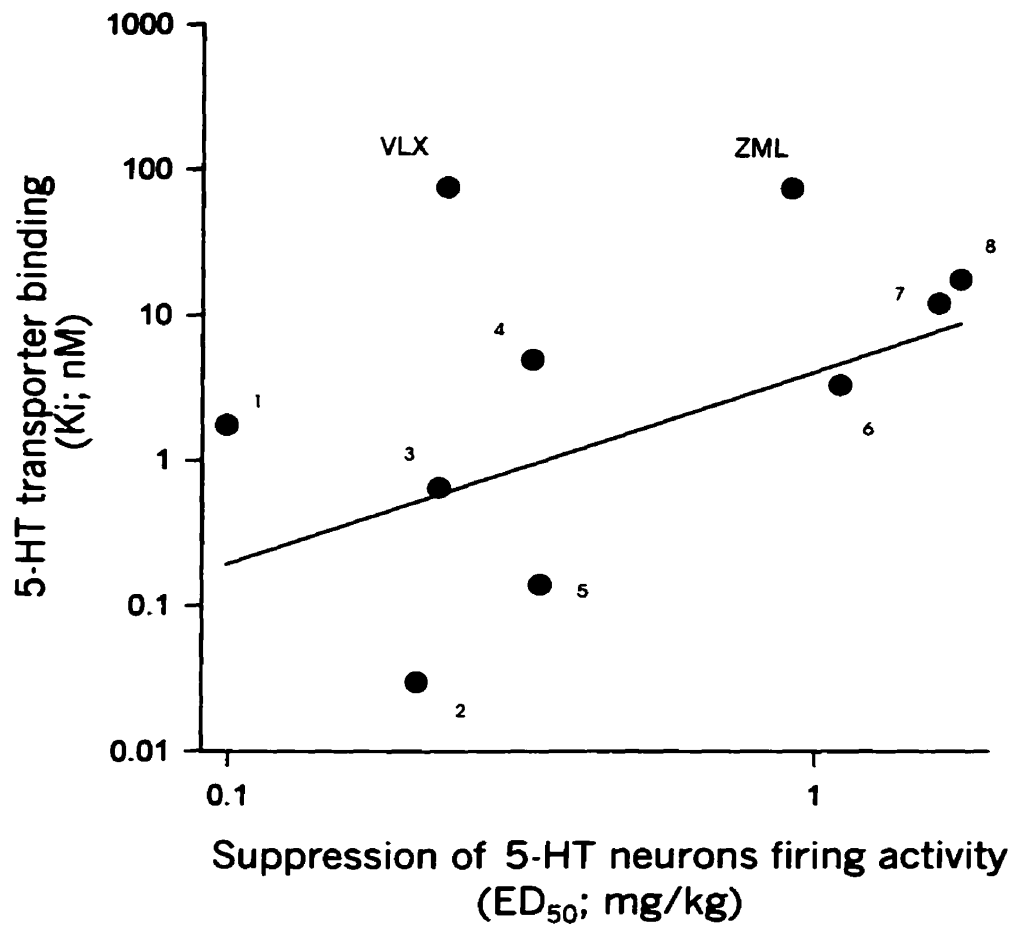
Figure 6. A. Correlation ($r = 0.63$) between the potency, expressed as ED_{50} values, of various compounds to suppress the firing activity of dorsal raphe 5-HT neurons and their affinity, expressed as K_i values, for the 5-HT transporter (taken from either [^3H]cyanoimipramine or [^3H]paroxetine displacement studies).

B. Correlation ($r = 0.92$) between the potency, expressed as ED_{50} values, of various compounds to suppress the firing activity of locus coeruleus NE neurons and their affinity, expressed as K_i values, for the NE transporter (taken from [^3H]nisoxetine displacement studies). The values reported for venlafaxine (VLX; A and B) and zimelidine (ZML; A) were not taken in account for the calculation of the correlation coefficient (r) of the two linear regression.

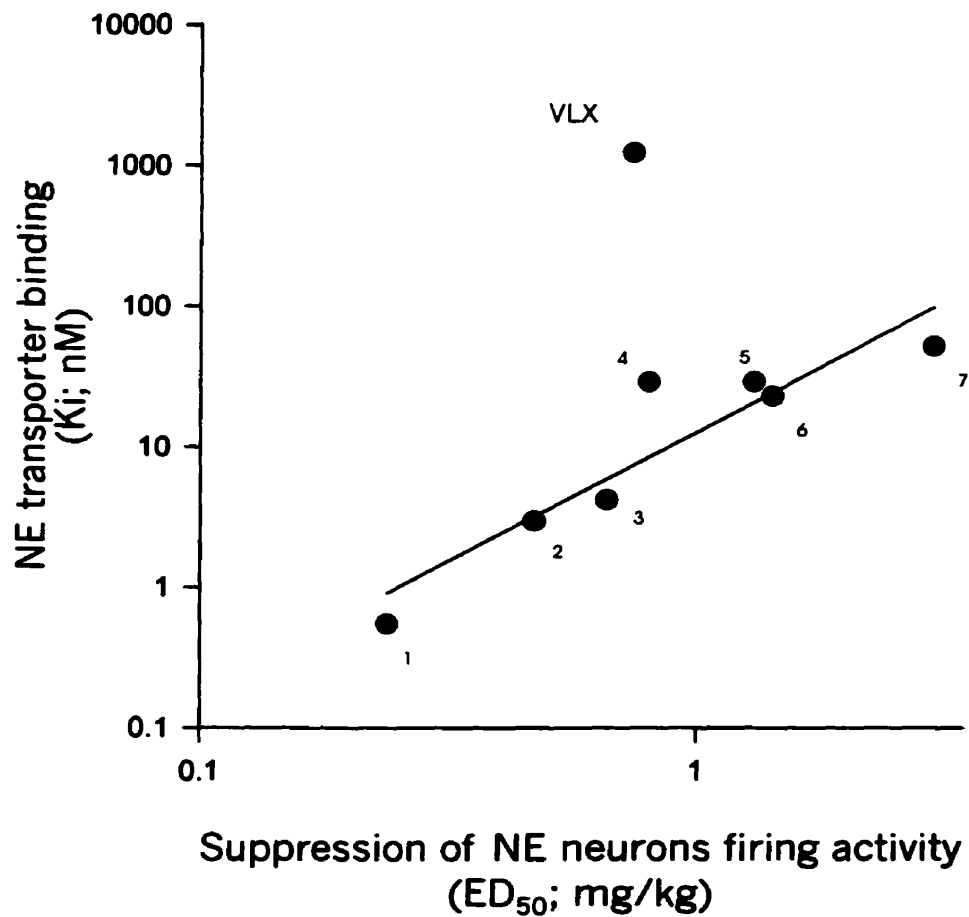
The numbers on figures 6A and 6B correspond to the following compounds followed by the study from which the data have been obtained (ED_{50} ; K_i). 6A: 1- Duloxetine (Kasamo et al. 1996; Béïque et al. 1998b); 2- Paroxetine (present study; Béïque et al. 1998b); 3- Citalopram (de Montigny et al. 1990; Cheetham et al. 1993); 4- Indalpine (Blieer et al. 1984; Béïque et al. 1998b); 5- Chlorimipramine (Scuvée-Moreau and Dresse, 1979; Cheetham et al. 1993); 6- Norzimelidine (de Montigny et al. 1981; Kovachich et al. 1988); 7- Imipramine (Scuvée-Moreau and Dresse, 1979; Cheetham et al. 1993); 8- Amitriptyline (Scuvée-Moreau and Dresse, 1979; Cheetham et al. 1993); VLX- venlafaxine (present study; Béïque et al. 1998b); ZML- zimelidine (de Montigny et al. 1981; Kovachich et al. 1988).

6B: 1- Desipramine (present study; Béïque et al. 1998b); 2- Duloxetine (Kasamo et al. 1996; Béïque et al. 1998b); 3- Nortriptyline (Scuvée-Moreau and Dresse, 1979; Cheetham et al. 1996); 4- Tranylcypromine (Scuvée-Moreau and Dresse, 1979; Cheetham et al. 1996); 5- Imipramine (Scuvée-Moreau and Dresse, 1979; Cheetham et al. 1996); 6- Amitriptyline (Scuvée-Moreau and Dresse, 1979; Cheetham et al. 1996); 7- Chlorimipramine (Scuvée-Moreau and Dresse, 1979; Cheetham et al. 1996); VLX- Venlafaxine (present study; Béïque et al. 1998b).

A.



B.



CHAPTER IV

PREFATORY REMARKS

Inasmuch as the suppression of firing activity of 5-HT and NE neurons provides a valid index of reuptake inhibition, the latter in vivo study was totally congruent with the general conclusion provided by the study carried out in the hippocampus presented in chapter II: First, venlafaxine blocks more potently the reuptake of 5-HT than that of NE and second, it blocks the reuptake of 5-HT with an equal potency than does paroxetine whereas it blocks the reuptake of NE ca. 3 times less potently than desipramine. In a corollary fashion, this study has demonstrated that the potencies with which various 5-HT and/or NE reuptake inhibitors inhibits the firing activity of 5-HT and NE neurons are correlated with their respective affinities for the 5-HT and NE transporters, respectively. Although the disclosure of this correlation appears to be intuitively obvious and although it is not derived from novel experimental data, it had never been reported before. In any event, but also most importantly, is the fact that the disclosure of this correlation has directly allowed for the unequivocal expression of the discrepancy between the in vivo reuptake inhibition potencies of venlafaxine and its in vitro affinities for both transporters. Essentially, this study was able to provide a quantitative dimension to the discrepancy that had been suspected on the ground of the experimental evidence provided in chapter I and II..

Independently of the in vivo-in vitro discrepancy documented with venlafaxine, it remains a fact that, although there are reports that venlafaxine would act faster than other antidepressant, its therapeutic activity in major depression shows a delay. For this reason alone, any research endeavour devoted to identify the mechanism of action responsible for the therapeutic activity of venlafaxine must consider the effects induced by its long-term administration. This has been the focus of chapter IV and V.

EFFECTS OF SUSTAINED ADMINISTRATION OF THE SEROTONIN AND NOREPINEPHRINE REUPTAKE INHIBITOR VENLAFAXINE: I-IN VIVO ELECTROPHYSIOLOGICAL STUDIES IN THE RAT⁷

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ABSTRACT

The effect of a 21-day treatment with the dual 5-HT and NE reuptake blocker venlafaxine (delivered s.c. by osmotic minipumps) was assessed on the time required for a 50% recovery (RT_{50}) of the firing activity of dorsal hippocampus CA_3 pyramidal neurons from the suppression induced by microiontophoretic applications of 5-HT and NE. The RT_{50} values for 5-HT were increased by both a 10 and 40 mg/kg/day regimens of venlafaxine, whereas those for NE were increased only by the 40 mg/kg/day regimen, indicative of a greater potency of venlafaxine in blocking 5-HT reuptake. The sensitivity of the postsynaptic 5-HT_{1A} and α_2 -adrenergic receptors was altered by neither regimen of venlafaxine. Using a paradigm by which the 5-HT_{1A} antagonist WAY 100635 can induce a disinhibition of firing activity of CA_3 pyramidal neurons, it was demonstrated that the high, but not the low, dose of venlafaxine led to an enhanced tonic activation of postsynaptic 5-HT_{1A} receptors in the dorsal hippocampus. The duration of the suppressant effect of the firing activity of CA_3 hippocampus pyramidal neurons produced by the electrical stimulation of the ascending 5-HT pathway was significantly reduced when the frequency of the stimulation was enhanced from 1 Hz to 5 Hz in control rats and in rats treated with 10 mg/kg/day, but not with 40 mg/kg/day of venlafaxine. Hence, venlafaxine induced a desensitization of the terminal 5-HT_{1B} autoreceptor only at the high dose. A 2-day treatment with 10 mg/kg/day of venlafaxine induced a suppression of the firing activity of 5-HT neurons of the dorsal raphe. The firing activity of these neurons was back to control level in rats that had been treated for 21 days with the same dose of venlafaxine. The suppressant effect of the intravenous administration of the 5-HT autoreceptor agonist LSD on the firing activity of dorsal raphe 5-HT neurons was reduced in

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rats that had been treated for 21 days with 10 mg/kg/day of venlafaxine. A 2-day treatment with 40 mg/kg/day of venlafaxine, unlike the 10 mg/kg/day regimen, induced a marked suppression of the firing activity of locus coeruleus NE neurons. However, in contrast to 5-HT neurons, NE neurons did not recover their firing activity after a 21-day treatment. Taken together, the results from this study indicate that the low dose of venlafaxine blocked selectively the reuptake of 5-HT, whereas the high dose blocked the reuptake of both 5-HT and NE. Moreover, an enhancement of serotonergic neurotransmission by venlafaxine was only achieved under conditions whereby the desensitization of the terminal 5-HT_{1B} autoreceptor is appended to that of the somatodendritic 5-HT_{1A} receptor.

KEYWORDS: antidepressant; major depression; *in vivo* electrophysiology; reuptake inhibitors; dorsal raphe; 5-HT neurons; locus coeruleus; NE neurons.

1. INTRODUCTION

The discovery that some tricyclic compounds and monoamine oxidase inhibitors were effective in the treatment of major depression has fostered the emergence of an historical debate on the role and relative importance of the serotonergic (5-HT) and the noradrenergic (NE) systems as the critical targets for antidepressant treatments to exert their therapeutic effect (Ross and Renyi, 1967; Carlsson, 1970; Maes and Meltzer, 1995; Schatzberg and Schildkraut, 1995). Although this debate is still ongoing, clinical studies have been able to gain relevant insight by providing evidence that a combined pharmacological action on both these systems might bear synergistic therapeutic benefit. Indeed, several reports have suggested that some TCA's may be more effective than selective 5-HT reuptake inhibitors (SSRI's) in the treatment of severely depressed patients (Danish University Antidepressant group, 1986; Danish University Antidepressant group, 1990) and that this may be the result of their combined 5-HT and NE reuptake inhibition properties. Moreover, initial reports suggest that the combination of the SSRI fluoxetine and the NE reuptake inhibitor desipramine results in an antidepressant effect more robust than that of desipramine alone (Nelson et al., 1991; Nelson *et al.* 1998). Furthermore, several studies have suggested that such a combination therapy is effective in treatment-resistant depression (Weilburg et al., 1989; Seth et al., 1992; Zajecka et al., 1995). Finally, and directly pertaining to the present study, there is evidence suggesting that the dual 5-HT/NE reuptake inhibitor venlafaxine (Muth et al., 1986; Béïque et al., 1998b) may display an earlier onset of therapeutic action (Schweizer et al., 1991; Benkert et al., 1996), have a greater efficacy and be effective in treatment-resistant depression; (Nierenberg et al., 1994; Schaeffer et al., 1998; de Montigny et al., 1999). The observation that these beneficial properties are seen only with high doses of venlafaxine is congruent and supportive of the hypothesis that, when used at low doses, venlafaxine acts as an SSRI and, as the dose is increased, a recruitment of the noradrenergic system by means of blocking the NE reuptake process would ensue (Béïque et al., 1998b). This hypothesis was formulated in light of the finding that whilst venlafaxine can block the reuptake of both 5-HT and NE, its potency to block 5-HT reuptake is greater than its potency to block NE reuptake.

Despite the presence of a reasonable congruence of the selectivity ratio of reuptake inhibition potencies (5-HT vs. NE; Muth et al., 1986; Bolden-Watson and Richelson, 1993; Owens et al., 1997; Tatsumi et al., 1997; Béïque et al., 1998b), an important discrepancy between the affinity of venlafaxine for both the 5-HT and NE transporters binding sites and

its *in vivo* reuptake inhibition potencies has been documented (Béïque et al., 1998c; Béïque et al., 1999). More specifically, it has been shown that the *in vivo* 5-HT and NE reuptake inhibition potencies of venlafaxine were much greater than could have been predicted from its affinities for the 5-HT and NE transporters. The disclosure of this peculiarity has thus shed doubt on the nature of the exact mechanism by which venlafaxine blocks both reuptake processes and ultimately exerts its antidepressant effect. It was thus deemed of interest to further investigate the mechanism of action of venlafaxine by assessing the effects of its long-term administration on different parameters involved in mediating both 5-HT and NE neurotransmissions.

Based on previous electrophysiological studies that have shown that a two-day treatment with 10 mg/kg/day of venlafaxine blocks selectively the reuptake of 5-HT, while that with 40 mg/kg/day blocks the reuptake of both 5-HT and NE, we have undertaken to assess the effects of these regimens of venlafaxine administered for 21 days. It was hypothesized that the long-term administration of the low dose of venlafaxine would induce the adaptative effects observed following long-term administration with SSRI's, while the high dose would append those seen with NE reuptake inhibitors. This report presents the results obtained using *in vivo* electrophysiological paradigms whereas a companion report presents those of studies carried out using *in vitro* superfusion and uptake paradigms. The latter report presents a synoptic discussion pertaining to the results presented in both papers.

Parts of the present work have previously been published in an abstract form (Béïque et al., 1998a)

2. METHODS

2.1. *Short- and long-term treatments*

Male Sprague Dawley rats (Charles River, St. Constant, Québec, Canada) weighing between 250 and 275 g or 100-150 g for short- and long-term treatment, respectively, were anesthetized with halothane for the subcutaneous implantation of osmotic minipumps (Alza, Palo Alto, CA, U.S.A.) that delivered doses of venlafaxine of 10 or 40 mg/kg/day or vehicle for two or 21 days. For the duration of the treatments, rats were housed three to four per cage and kept on a 12:12 h light/dark cycle, with access to food and water *ad libitum*. All experiments were carried out with the minipumps still in place. Rats treated with both doses regimens of venlafaxine were individually tested in parallel with their own controls. The control rats and their matching venlafaxine-treated rats were, usually, tested with the same microiontophoretic pipette.

2.2. *Electrophysiology: surgical procedures*

Electrophysiological experiments were performed on animals anesthetized with chloral hydrate (400 mg/kg, i.p.) and mounted in a stereotaxic apparatus. Supplemental doses (100 mg/kg, i.p.) were given to prevent any nociceptive reaction to pinching of a hind paw. Body temperature was maintained at 37 °C throughout the experiment and, prior to recording, a lateral tail vein was catheterized for intravenous administration of drugs.

2.3. *Extracellular unitary recordings of CA₃ dorsal hippocampus pyramidal neurons*

Five-barrelled glass micropipettes were pulled in a conventional manner and their tips were broken back to 8-12 μ m diameter under microscopic control. The central barrel, used for extracellular recordings, was filled with a 2 M NaCl solution containing fast green FCF. Three side barrels, used for microiontophoresis, were filled with the following solutions: 5-HT (5 or 20 mM in 200 mM NaCl, pH: 4), NE (20 mM in 200 mM NaCl, pH: 4) or acetylcholine (ACh; 20 mM in 200 mM NaCl, pH: 5). The fourth barrel was used for automatic current balancing, and contained a 2 M NaCl solution. The microelectrode was lowered 4 mm lateral and 4 mm anterior to lambda, according to stereotaxic coordinates of Paxinos and Watson (1986). CA₃ pyramidal neurons were recorded at a depth of 3.5 to 3.8 mm from the cortical surface and were identified by their characteristic large-amplitude (0.5-1.2 mV) and long-duration (0.8-1.2

ms) single action potentials alternating with complex spike discharges (Kandel and Spencer, 1961). A leak or a small ejecting current of ACh (0 to + 5 nA) was used to activate silent or slowly discharging neurons to a physiological firing rate of 8-14 Hz (Ranck, 1973). 5-HT and NE were both retained with a current of -12 nA and applied onto pyramidal neurons with ejecting currents of +1 and +5 nA for 50 s.

2.4. *Electrical activation of the afferent 5-HT fibers to the hippocampus*

A bipolar electrode (NE-110; David Kopf, Tujunga, CA, USA) was implanted on the midline with a 10° backward angle in the ventromedial tegmentum, 1 mm anterior to lambda and 8.3 mm below the cortical surface. A stimulator (S8800; Grass Instruments, Quincey, MA, USA) delivered two hundred square pulses of 0.5 ms at a frequency of 1 Hz and an intensity of 300 μ A. The stimulation pulses and the firing activity of the neuron recorded were fed to a computer equipped with a Tecmar interface. Peristimulus time histograms were generated on line, and the effects of 1 Hz and 5 Hz stimulations were determined for each neuron recorded.

2.5. *Extracellular unitary recordings of 5-HT neurons of the dorsal raphe nucleus and of NE neurons of the locus coeruleus*

Single-barrelled glass micropipettes were prepared in a conventional manner (Haigler and Aghajanian, 1974), with the tips broken back to 1-3 μ m and filled with a 2 M NaCl solution saturated with Fast Green FCF. A burr hole was drilled on midline, 1 mm anterior to lambda for the dorsal raphe neurons recordings or 1 mm posterior to lambda and 1 mm lateral to midline for locus coeruleus neurons recordings. Spontaneously active dorsal raphe 5-HT neurons were encountered over 1 mm from the ventral border of the Sylvius aqueduct, and were identified using the criteria of Aghajanian (1978): slow, regular firing rate (0.5-2.5 Hz) and positive action potential of long duration (0.8-1.2 ms). Spontaneously active NE neurons of the locus coeruleus were identified using the criteria of Aghajanian (Aghajanian, 1978): regular firing rate (1-5 Hz) and positive action potential of long duration (0.8-1.2 ms) exhibiting a characteristic burst discharge in response to nociceptive pinches of the contralateral hind paw. Systematic electrode descents were carried out through the dorsal raphe nucleus (*ca.* 1 mm each) and the locus coeruleus (*ca.* 0.5 mm each) and the encountered neurons were recorded for at least 30 s to determine their firing frequency. For the LSD dose-

response curves, 5-HT neurons were recorded for at least 1 min to establish a basal firing rate, before a single dose of the drug was administered intravenously in a volume of about 0.1 ml via a lateral vein of the tail. Dose-response curves for the effects of LSD were generated from which the dose required for a 50% suppression of the firing activity of the neuron (effective dose-50; ED₅₀ value) was determined.

2.6. *Drugs*

Venlafaxine and WAY 100635 were kindly provided by Wyeth-Ayerst Research (NJ, U.S.A.), and piperoxane by Rhône-Poulenc (Vitry, France). LSD was obtained from the Ministry of Health and Welfare (Ottawa, Canada). 5-HT, NE and ACh were purchased from Sigma Chemical (St-Louis, MO, U.S.A.). Drugs were prepared either in physiological saline for i.v. administration or in a 200 mM NaCl solution for microiontophoretic applications.

2.7. *Data analysis*

Microiontophoresis in the dorsal hippocampus. The uptake activity following microiontophoretic applications of 5-HT and NE was assessed using the recovery time 50 (RT₅₀) values. RT₅₀ is defined as the time in seconds, from the termination of the microiontophoretic application, required by the neuron to recover 50% of its initial firing frequency. The RT₅₀ value has been shown to provide a reliable index of the *in vivo* activity of the NE reuptake process in the rat hippocampus (de Montigny et al., 1980; Gravel and de Montigny, 1987) and of the 5-HT reuptake process in the rat amygdala and lateral geniculate body (Wang et al., 1979) and in the hippocampus (Piñeyro et al., 1994). The sensitivity of postsynaptic receptors to microiontophoretic application of either 5-HT or NE was assessed using the I•T₅₀ values where I is the current in nA, and T₅₀ the time required to suppress the firing activity by 50% (de Montigny and Aghajanian, 1978). Both the RT₅₀ and the I•T₅₀ values were calculated by an on-line computer.

Electrical activation of the afferent 5-HT fibers to the hippocampus. The duration of suppression of firing activity of a CA₃ pyramidal neuron by electrical stimulation of the ascending 5-HT pathway was measured in absolute silence value (SIL, in ms). This value is obtained by dividing the total number of events suppressed following the stimulation by the mean firing frequency of the neuron recorded (Chaput et al., 1986). Previous studies have demonstrated that the suppression of firing activity of CA₃ pyramidal neurons induced by the

stimulation of the ascending 5-HT pathway stems from the activation of postsynaptic 5-HT_{1A} receptors by endogenously released 5-HT (Chaput et al., 1986; Chaput and de Montigny, 1988). In order to determine changes of the function of the terminal 5-HT autoreceptor, two series of stimulations (1 and 5 Hz) were carried out while recording the same neuron. Indeed, it has been shown that increasing the frequency of stimulation reduces the efficacy of the stimulation resulting from terminal autoreceptor activation (Chaput et al., 1986) and that the degree of reduction of the silence value is thus a valid index of the function of the terminal autoreceptor following prolonged pharmacologic treatments (Blier et al., 1988; Chaput et al., 1991).

2.8. *Statistics*

Results are expressed throughout as means $\pm$ S.E.M., unless otherwise specified. The statistical significance of the difference between means was assessed using either a paired or a non-paired Student's *t*-test, a one-way or two-way ANOVA, or a single factor ANCOVA as indicated in the legend to figures. Probability value of $p < 0.05$ was considered to be statistically significant. Correlation coefficients values for the dose-response relationship observed in the dorsal raphe were calculated using simple linear regression analysis. The S.E.M. for the ED₅₀ values for the dorsal raphe LSD dose-response curve was calculated by regression analysis, with the y value of 50 used as the regressor. Differences between two regressions were assessed by comparing their ED₅₀ values using the confidence interval method. The 95% confidence limit was determined from the Student's *t* distribution.

RESULTS

3.1. *Effects of long-term treatments with venlafaxine on the RT₅₀ values for 5-HT or NE*

Microiontophoretic applications of 5-HT and NE, with currents of 1 and 5 nA, suppressed the firing activity CA₃ pyramidal neurons of the dorsal hippocampus. While the 21-day treatment with a dose of 10 mg/kg/day of venlafaxine failed to alter the RT₅₀ values obtained following microiontophoretic applications of 5-HT with an ejection current of 1 nA, the RT₅₀ values following microiontophoretic applications of 5-HT with a 5 nA current were increased by 111 % in these treated animals (Figures 1 and 2A). The RT₅₀ values following the applications of NE (1 and 5 nA), were not significantly increased when compared to control values (Figures 1 and 2B). Following a 21-day treatment with a dose of 40 mg/kg/day of venlafaxine, the RT₅₀ values obtained following microiontophoretic applications of 1 and 5 nA of 5-HT were increased by 126 % and by 163 %, respectively. At that dose, the RT₅₀ values obtained following microiontophoretic applications of 1 and 5 nA of NE were also increased, by 186 % and 90 %, respectively (Figures 1 and 2B).

3.2. *Effects of long-term treatments with venlafaxine on the sensitivity of postsynaptic 5-HT_{1A} and α_2 -adrenergic receptors*

From the experimental series illustrated in Figure 1, we have calculated the I•T₅₀ values (an index of the sensitivity of postsynaptic 5-HT_{1A} and α_2 -adrenergic receptors of the hippocampus, see Materials and Methods) calculated from the initial suppression of firing induced by the microiontophoretic applications of 5-HT and NE, respectively. The I•T₅₀ values for both 5-HT (data not shown) and NE (Figure 2C) were unchanged in rats treated with either treatment regimen of venlafaxine. In another experimental series, we have compared the effects of 5 and 20 mM solutions of 5-HT (see Figure 4). The results obtained from this experimental series are summarized in Table 1. With both microiontophoretic currents used (1 and 5 nA), the I•T₅₀ values were significantly greater when using the 5 mM concentration of 5-HT compared to a 20 mM concentration, and this in both control and treated animals ($p < 0.05$, using the non-paired Student's *t*-test). The sensitivity of postsynaptic 5-HT_{1A} receptors, as assessed using the I•T₅₀ values, was unchanged after a long-term treatment with 40 mg/kg/day of venlafaxine and this, irrespective of the concentration of 5-HT used (Table 1). From the inhibition produced by the microiontophoretic applications of 5-HT from the two different concentrations of 5-HT, the RT₅₀ values were re-assessed in control and in rats treated

with 40 mg/kg/day. The RT_{50} values were increased by the long-term administration of the high dose of venlafaxine, but this was only observed when the microiontophoretic application was made from the barrel containing 20 mM of 5-HT (Table 1).

3.3. *Effects of long-term treatments with venlafaxine on the disinhibition induced by the selective 5-HT_{1A} antagonist WAY 100635*

Dorsal hippocampus CA₃ pyramidal neurons were first brought to physiological range of firing activity by applying a small ejection current of ACh (Figure 5). The current of ACh used for this purpose was not significantly different in controls (2.1 ± 0.4 nA; n=6) than in rats treated with 10 mg/kg/day (2.5 ± 0.4 nA; n=6) and 40 mg/kg/day of venlafaxine (2.3 ± 0.5 nA; n=4). After reaching a stable baseline, the firing activity was subsequently reduced to *ca.* 3 Hz by reducing the current of ACh. Again, the current of ACh required for this purpose was not significantly altered by either of the treatment regimens of venlafaxine [0.4 ± 0.6 nA, 1 ± 0.4 nA and 0.8 ± 0.3 nA, for rats treated with vehicle (n=6), 10 mg/kg/day (n=6) and 40 mg/kg/day of venlafaxine (n=4), respectively]. In rats that had received the vehicle for 21 days, the acute intravenous administration of the selective 5-HT_{1A} antagonist WAY 100635 (four successive doses of 25 µg/kg; cumulative dose of 100 µg/kg) did not modify the firing activity of dorsal hippocampus CA₃ pyramidal neurons (Figures 5 and 6). The acute intravenous administration of the same dose of WAY 100635 was also devoid of any significant effect in rats treated for 21 days with 10 mg/kg/day of venlafaxine (Figure 5B and 6). In these same treated rats (10 mg/kg/day x 21 days), the effect of the microiontophoretic application of 5-HT was significantly reduced by the intravenous administration of 100 µg/kg of WAY 100635 (number of spikes suppressed by microiontophoretically-applied 5-HT before intravenous administration of WAY 100635: 707 ± 72 ; after WAY 100635: 235 ± 72 ; n=8; $p < 0.01$, using the paired Student's *t*-test) However, in rats treated for 21 days with 40 mg/kg/day of venlafaxine, the acute intravenous administration of the first dose (25 µg/kg) of WAY 100635 was sufficient to induce a marked enhancement of the firing activity of the CA₃ pyramidal neurons (Figure 5C and 6).

3.4. *Effects of long-term treatments with venlafaxine on the function of the terminal 5-HT autoreceptor*

In order to assess the function of the terminal 5-HT autoreceptor following long-term

administration of the two regimens of venlafaxine, an electrophysiological paradigm was employed whereby the afferent 5-HT fibers were electrically-stimulated at 1 and 5 Hz while recording the firing activity of a CA₃ pyramidal neuron. In control animals, increasing the frequency of stimulation from 1 Hz to 5 Hz induced a significant reduction of the silence value ($p < 0.01$ using the paired Student's t -test; Figure 7). The decrement of the silence value induced by increasing the frequency of stimulation from 1 Hz to 5 Hz was reduced only in rats treated with 40 mg/kg/day (Figure 7).

3.5. Effects of treatments with venlafaxine on the firing activity of dorsal raphe 5-HT neurons and on the sensitivity of the somatodendritic 5-HT_{1A} autoreceptor

A total of 184 serotonergic neurons were recorded during systematic multiple electrode descents in the dorsal raphe of control rats and of rats treated with 10 mg/kg/day of venlafaxine for two or 21 days while the minipumps were still in place (Figure 8). In control rats, spontaneously active 5-HT neurons displayed a mean firing activity of about 1 Hz (Figure 8A and D). The 2-day treatment with 10 mg/kg/day of venlafaxine reduced the spontaneous firing activity of 5-HT neurons by 47 % (Figure 8). Following 21 days of treatment with the same dose of venlafaxine, the firing activity of 5-HT neurons of the dorsal raphe had fully recovered (Figure 8D). The degree of suppression of the firing activity of 5-HT neurons induced by the acute intravenous administration of the 5-HT autoreceptor agonist LSD was determined. In control rats, LSD suppressed the firing activity of 5-HT neurons with an ED₅₀ of 4.8 µg/kg whereas, in rats treated with 10 mg/kg/day of venlafaxine for 21 days, LSD suppressed the firing activity of 5-HT neurons with an ED₅₀ of 13.2 µg/kg, ($p < 0.05$, using the confidence limit interval).

3.6. Effects of 2-day and 21-day treatments with venlafaxine on the mean firing activity of locus coeruleus NE neurons

A total of 219 noradrenergic neurons were recorded during systematic multiple electrode descents carried out in the locus coeruleus of rats treated for two or 21 days with the low (10 mg/kg/day), the high (40 mg/kg/day) dose of venlafaxine or with vehicle (Figure 10). As shown in Figure 10, the mean firing activity of spontaneously active NE neurons of the locus coeruleus of control rats was about 2.5 Hz. A 2-day treatment with the low dose (10 mg/kg/day) of venlafaxine induced a significant, albeit small (21 %), suppression of the

firing activity of locus coeruleus NE neurons. However, a 21-day treatment with the same low dose regimen (10 mg/kg/day) induced a 50 % suppression of firing activity of locus coeruleus NE neurons. This suppression was greater than that produced by the 2-day treatment with the same dose of venlafaxine ($p < 0.05$, one way ANOVA). A 2-day treatment with the high dose (40 mg/kg/day) of venlafaxine induced a marked suppression (61 %) of firing activity of locus coeruleus NE neurons while a 21-day treatment with the same the high dose of venlafaxine induced a suppression of 54 % of firing activity that was not different than that obtained following a 2-day treatment ($p > 0.05$, one way ANOVA).

3. DISCUSSION

In the present study, a 21-day treatment with 10 mg/kg/day (the low dose) of venlafaxine blocked the uptake of 5-HT whereas 40 mg/kg/day (the high dose) was required to append the blockade of the uptake of NE. None of the treatment regimens altered the sensitivity of postsynaptic 5-HT_{1A} and α_2 -adrenergic receptors. Using the paradigm whereby the 5-HT_{1A} antagonist WAY 100635 induces a disinhibition of firing activity of CA₃ pyramidal neurons, it was observed that the high, but not the low dose of venlafaxine, led to an enhanced tonic activation of postsynaptic 5-HT_{1A} receptors in the dorsal hippocampus. The observation that the high dose, but not the low dose regimen of venlafaxine induced a desensitization of 5-HT_{1B} autoreceptors suggest that the latter phenomenon might be required for a 5-HT reuptake inhibitor to lead to an enhanced activation of postsynaptic 5-HT_{1A} receptors. A 2-day treatment with the low dose reduced the firing activity of 5-HT neurons of the dorsal raphe and induced a desensitization of the 5-HT_{1A} somatodendritic autoreceptors allowing a full recovery of the firing activity after a 21-day treatment. The 2-day treatment with 10 mg/kg/day of venlafaxine had a minimal effect on the reuptake of NE, whereas the 40 mg/kg/day regimen for two days induced a clear NE reuptake blockade as it induced a marked suppression of firing activity of locus coeruleus NE neurons.

A 21-day treatment with the low dose of venlafaxine increased the RT₅₀ value following microiontophoretic applications of 5-HT, whereas a 40 mg/kg/day regimen was necessary to increase that following microiontophoretic applications of both 5-HT and NE (Figure 1, 2A and 2B). These findings are reminiscent of those observed, using the same paradigm, following two-day treatments with the same doses of venlafaxine: the low dose of venlafaxine blocked selectively the uptake of 5-HT whereas the high dose blocked the uptake of both 5-HT and NE (Béïque et al., 1998b). These sets of observations are fully consistent with previous studies showing that venlafaxine is more potent in blocking 5-HT uptake than that for NE (Muth et al., 1986; Béïque et al., 1998b; Béïque et al., 1999).

The present results demonstrate that the long-term administration with neither regimen of venlafaxine modified the responsiveness of CA₃ dorsal hippocampus pyramidal neurons to NE, thereby indicative of normosensitive postsynaptic α_2 -adrenergic receptors (Figure 3). It needs to be emphasized that the empirical validation of the RT₅₀ and of the I•T₅₀ methods for detecting 5-HT reuptake inhibition and postsynaptic 5-HT_{1A} receptor sensitivity, respectively, were achieved using slightly different methodological paradigms

pertaining to the concentrations of 5-HT used in the microiontophoretic side barrel (de Montigny and Aghajanian, 1978; Wang et al., 1979; Piñeyro et al., 1994). While changes in postsynaptic 5-HT receptor sensitivity have been documented with the use of 2 and 4 mM concentrations of 5-HT, no change in the 5-HT transporter function following treatments with fluoxetine or with the serotonergic toxin 5,7-dihydroxytryptamine was observed (de Montigny et al., 1980). Indeed, alterations of the 5-HT reuptake process could only be detected when the concentration of 5-HT was increased to 20 mM (Piñeyro et al., 1994). The systematic comparison (See Figure 4 and Table 1) of the effect of microiontophoretic applications of 5-HT using 5 and 20 mM concentrations were hence rendered necessary in order to have an adequate and valid appraisal of the sensitivity of postsynaptic 5-HT_{1A} receptors following the long-term administration of venlafaxine. The complementary sets of experiments (Figure 1, Figure 4 and table 1) thus demonstrate that, irrespective of the 5-HT concentration used, the sensitivity of postsynaptic 5-HT receptors was unaltered by a long-term treatment with venlafaxine, as no change in the $I \cdot T_{50}$ values was observed. As expected, when 5-HT was microiontophoretically applied using the side barrels containing a 5 mM concentration, the reuptake blockade induced by venlafaxine could not be detected, whereas it was put into evidence using the 20 mM concentration. This set of experiments gives further credence to the hypothesis of Piñeyro *et al.* (1994) which posits that the microiontophoretic application achieved from a more concentrated microiontophoretic 5-HT solution would ensure that 5-HT diffuses to the strata radiatum and oriens, where the majority of 5-HT terminals (Oleskevich and Descarries, 1990) and 5-HT transporters (Sur et al., 1996) are located, thus allowing the detection of any change in transporter function. Indeed, when 5-HT is microiontophoretically applied in the vicinity of the tip of the recording electrode (*i.e.*, at the level of the strata pyramidale), a blockade of the reuptake process escapes detection since this layer of the hippocampus is virtually devoid of 5-HT transporters (Sur et al., 1996).

It has been demonstrated, using various experimental approaches, that the long-term administration of 5-HT reuptake inhibitors, but not monoamine oxidase inhibitors (Blier et al., 1988), induces a desensitization of the terminal 5-HT autoreceptors (Chaput et al., 1986; Moret and Briley, 1990; Chaput et al., 1991; Blier and Bouchard, 1994) which are of the 5-HT_{1B} subtype in the rat (Göthert, 1986; Göthert et al., 1987; Middlemiss et al., 1988). In the present study, an electrophysiological paradigm whereby the effects of 1 vs 5 Hz stimulation

on the firing activity of CA₃ pyramidal neurons are measured was used to assess the function of the terminal 5-HT_{1B} autoreceptor following treatments with venlafaxine. Previous studies have shown that this paradigm provides a valid index of the function of the terminal autoreceptor. Using this paradigm, we found the decrement of the efficacy of the stimulation, induced by increasing the frequency from 1 Hz to 5 Hz, to be diminished only in rats treated with the high dose of venlafaxine (Figure 7). This suggests that the high dose, but not the low dose of venlafaxine, induced a functional desensitization of the terminal 5-HT_{1B} autoreceptors. These results are in perfect congruency with those obtained using an *in vitro* paradigm where the 40 mg/kg/day, but not the 10 mg/kg/day, regimen of venlafaxine administered for 21 days attenuated the effectiveness of the 5-HT_{1B} receptor agonist CP 93,129 to reduce the electrically-evoked release of [³H]5-HT from preloaded hippocampal slices (companion paper). Nonetheless, these results are intriguing considering the fact that a 10 mg/kg/day regimen of venlafaxine produced an efficient blockade of the 5-HT reuptake process (Figure 2). Thus, the latter treatment regimen could be expected to induce a desensitization of the terminal 5-HT_{1B} autoreceptor. The reasons underlying this apparent peculiarity are unresolved; this issue is addressed in the companion paper.

A previous *in vivo* electrophysiological study has demonstrated that treatments endowed with antidepressant activity increase the tonic activation of forebrain postsynaptic 5-HT_{1A} receptors (Haddjeri et al., 1998a). In light of the accumulating evidence suggesting a pivotal role of the latter receptor in mediating the therapeutic effect in major depression (de Montigny and Blier, 1991; Kurtz, 1992; Blier et al., 1997), it was deemed necessary to determine whether long-term administration of venlafaxine would enhance the tonic activation of postsynaptic 5-HT_{1A} receptors of the dorsal hippocampus. In keeping with previous observations (Haddjeri et al., 1998a), no tonic activation of postsynaptic 5-HT_{1A} receptors could be detected using this paradigm in control rats, as the intravenous administration of the 5-HT_{1A} antagonist WAY 100635 (Forster et al., 1995; Fletcher et al., 1996) failed to produce any disinhibition of the firing activity of the neurons recorded (Figure 5 and 6). However, an enhanced tonic activation of postsynaptic 5-HT_{1A} receptors was observed in rats treated with the high, but not with the low, dose regimen of venlafaxine. This finding is somewhat surprising considering that the low dose of venlafaxine not only efficiently blocked the reuptake of 5-HT (Figure 2A; Béïque et al., 1998b), but also desensitized the somatodendritic 5-HT_{1A} autoreceptor (Figure 9). Based on these

considerations, the fact that only the high dose of venlafaxine induced a desensitization of the terminal 5-HT_{1B} autoreceptors emphasizes the importance and physiological relevance of the desensitization of the terminal 5-HT_{1B} receptor in order for a 5-HT reuptake inhibitor to induce an enhanced tonic activation of the postsynaptic 5-HT_{1A} receptor.

The firing activity of dorsal raphe 5-HT neurons was markedly reduced by the 2-day treatment with the low dose of venlafaxine, which effect is deemed to result from an enhanced concentration of 5-HT in the raphe. The full recovery of the firing activity of 5-HT neurons after a 21-day treatment with venlafaxine (Figure 8) is fully congruent with previous observations with a variety of other 5-HT reuptake inhibitors (Blier and de Montigny, 1983; Blier et al., 1984; de Montigny et al., 1990). The fact that the dose-response curve of 5-HT autoreceptor agonist LSD for suppressing the firing activity of dorsal raphe 5-HT neurons was shifted to the right is indicative of a desensitization of the somatodendritic 5-HT_{1A} autoreceptor. This latter phenomenon is likely to underlie the recovery of firing activity of 5-HT neurons. The occurrence of the latter desensitization in the absence of an alteration of the terminal 5-HT_{1B} autoreceptor function raises the interesting possibility that the mechanism underlying the desensitization of the 5-HT_{1A} autoreceptor would be different from that for the terminal autoreceptor.

The 2-day treatment with the low dose of venlafaxine induced a small but significant suppression of the firing activity of locus coeruleus NE neurons, suggestive of a small degree of NE reuptake inhibition. The fact that evidence for a NE reuptake inhibition by the low dose of venlafaxine could not be obtained in the hippocampus using the RT₅₀ method (Béïque et al., 1998b; and Figures 1 and 3), most probably stems from the different sensitivities of the two approaches. In any event, it has to be emphasized that, from the small decrease of the firing activity of locus coeruleus NE neurons, the degree of NE reuptake inhibition at that dose is minimal. In this respect, the observation that the suppression of firing activity induced by the low dose of venlafaxine was greater after 21 days than after two days of treatment is intriguing. That the 5-HT reuptake inhibition property of venlafaxine is responsible for this phenomenon is suggested by the fact that the sustained, but not an acute, administration of SSRI's (Szabo et al., 1998; Béïque et al., 1999) induces a suppression of firing activity of locus coeruleus NE neurons.

The high dose regimen of venlafaxine, administered for two days, induced a marked suppression of the firing activity of NE neurons (Figure 10), thus confirming that it blocked

efficiently the reuptake of NE. Interestingly, with a prolonged treatment (21 days), there was no recovery of the firing activity of NE neurons which is in sharp contrast to what was observed for 5-HT neurons. Therefore, it might be asserted that NE neurons are devoid of an adaptative mechanism that would allow recovery of their firing activity upon prolonged administration of a NE reuptake inhibitor (in this case achieved with the high dose of venlafaxine). This hypothesis is congruent with the observations that in contrast to 5-HT neurons, NE neurons do not recover their normal range of firing activity following long-term administration of the monoamine oxidase inhibitors phenelzine, clorgyline, amiflamine and befloxatone (Blier and de Montigny, 1985; Blier et al., 1986; Haddjeri et al., 1998b). Similarly, the sustained administration of the NE reuptake desipramine for 21 days using a continuous perfusion with osmotic minipumps [similarly to the present study] markedly suppresses firing activity of locus coeruleus NE neurons throughout the treatment (S. T. Szabo, personal communication). Thus, as do NE reuptake inhibitors, venlafaxine induces a marked suppression of firing activity of locus coeruleus NE neurons when administered acutely (Béïque et al., 1999), and this is not followed by any recovery upon continuation of the treatment for 21 days (Figure 10). These results thus confirm and extend previous studies by demonstrating a fundamental homeostatic difference between the neurons of the 5-HT and NE systems as the former, and not the latter, are endowed with the capacity to recover their normal firing activity during prolonged and sustained reuptake inhibition.

The present study shows that long-term administration of a low dose of venlafaxine displays a good selectivity for the 5-HT reuptake process, whereas the high dose of venlafaxine blocks the uptake of both 5-HT and NE. These results support the hypothesis that, when administered at low doses, venlafaxine would act as an SSRI whereas, at higher doses, there would be a recruitment of the noradrenergic system through NE reuptake blockade. Interestingly, a dose of venlafaxine that induced a significant blockade of the 5-HT reuptake process was not accompanied by an enhanced tonic activation of postsynaptic 5-HT_{1A} receptors.

In order to present a more comprehensive appraisal of our understanding of the neurobiological changes that are induced by long-term treatment with venlafaxine, the present study was supplemented with *in vitro* experiments. The results of these experiments are presented in a companion paper.

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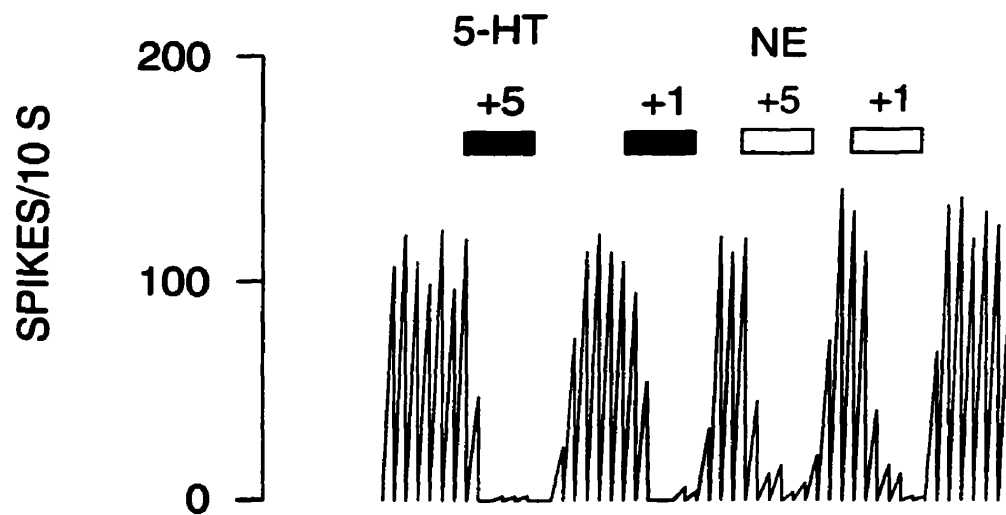
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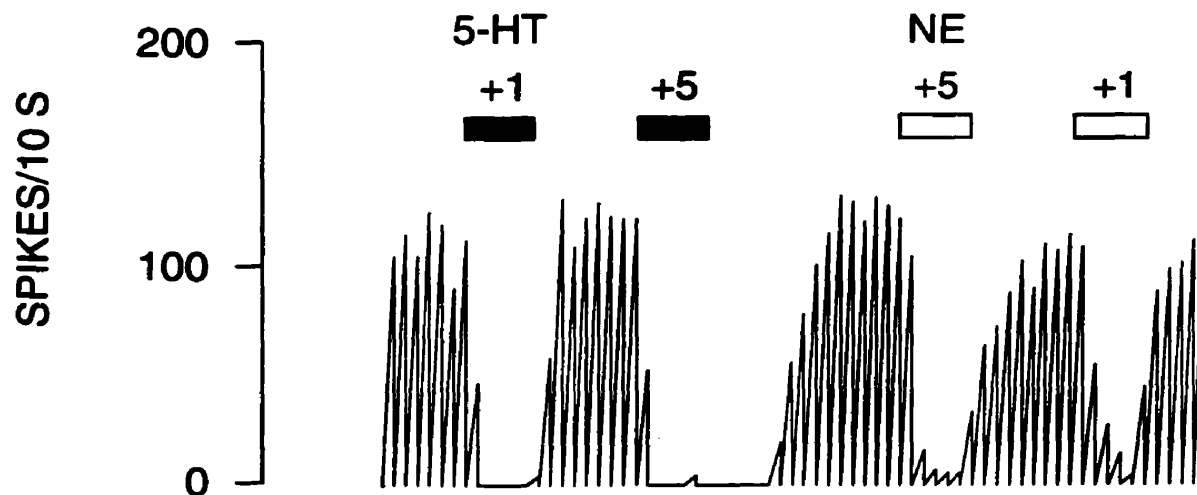
LEGENDS TO FIGURES

Figure 1. Integrated firing rate histograms of CA₃ dorsal hippocampus pyramidal neurons showing the effects of microiontophoretic applications of 5-HT and NE in rats following a 21-day treatment with vehicle (A), with 10 mg/kg/day, s.c. (B) and with 40 mg/kg/day, s.c. of venlafaxine (C).

A. Control



B. Venlafaxine, 10 mg/kg/day x 21 days, s.c.



C. Venlafaxine, 40 mg/kg/day x 21 days, s.c.

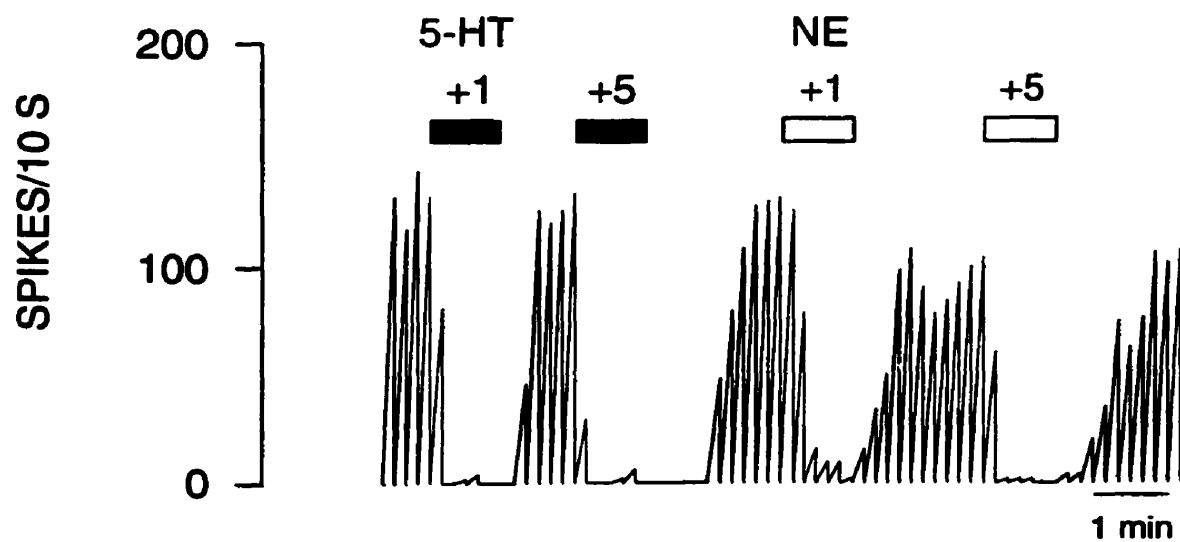
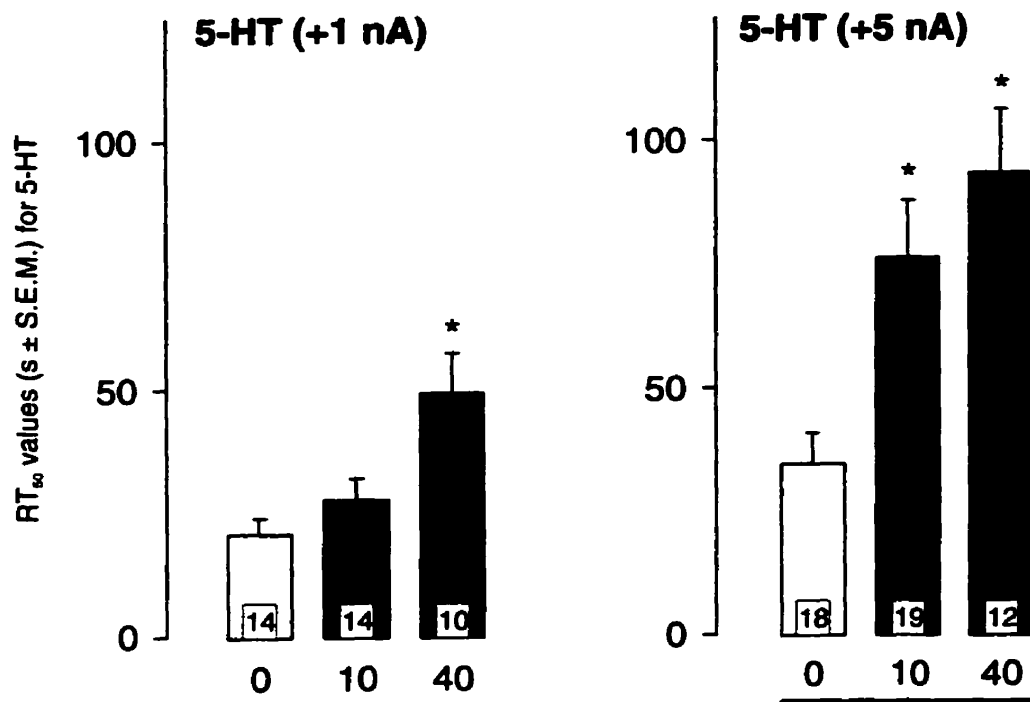


Figure 2. Recovery time, expressed as RT_{50} values, of CA_3 dorsal hippocampus neurons obtained with microiontophoretic applications of 1 nA and 5 nA of 5-HT in **A or of NE in **B** on ACh-induced firing activity following 21-day treatments with vehicle 10 or 40 mg/kg/day, s.c. of venlafaxine. * $p < 0.01$ compared to control values using the non-paired Student's t -test.**

A.



B.

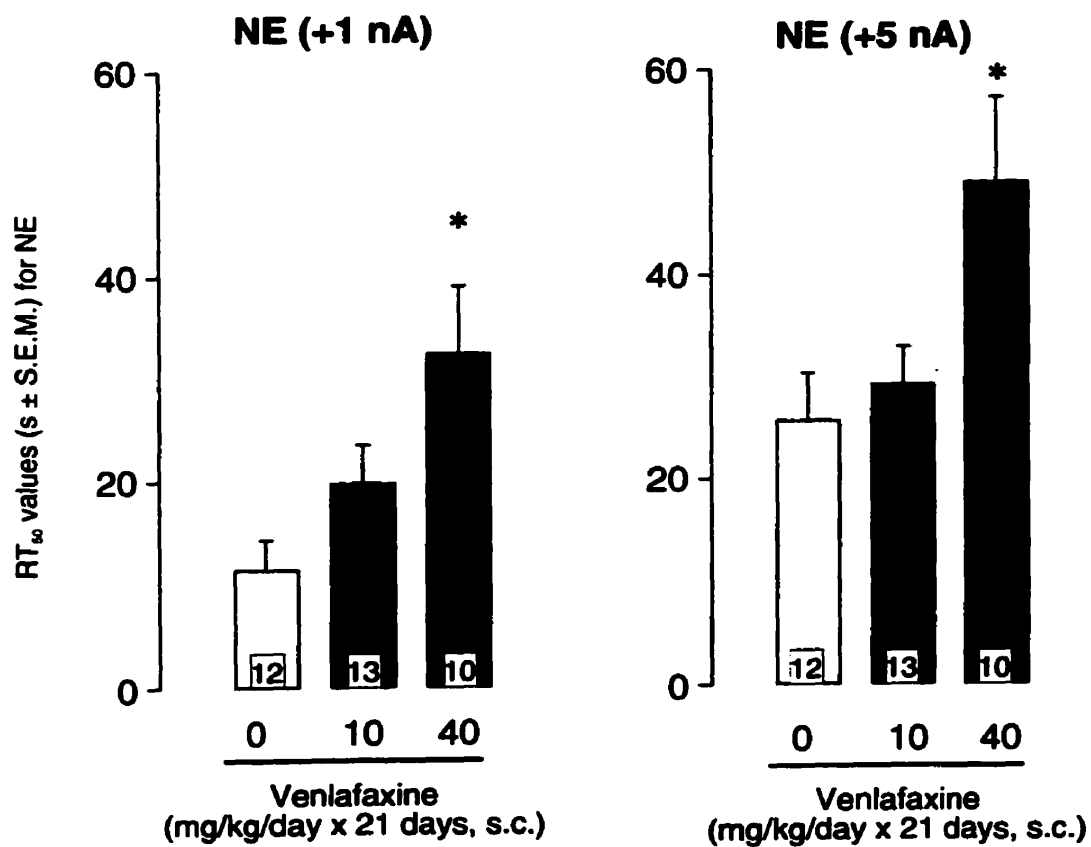


Figure 3. Responsiveness, expressed as $I \cdot T_{50}$ values of CA_3 dorsal hippocampus pyramidal neurons to microiontophoretic applications of 1 nA and 5 nA of NE on ACh-induced firing activity following 21-day treatments with vehicle, 10 and 40 mg/kg/day, s.c. of venlafaxine. None of the values in treated rats was significantly different from those obtained in controls ($p > 0.05$ compared to control values using the non-paired Student's *t*-test).

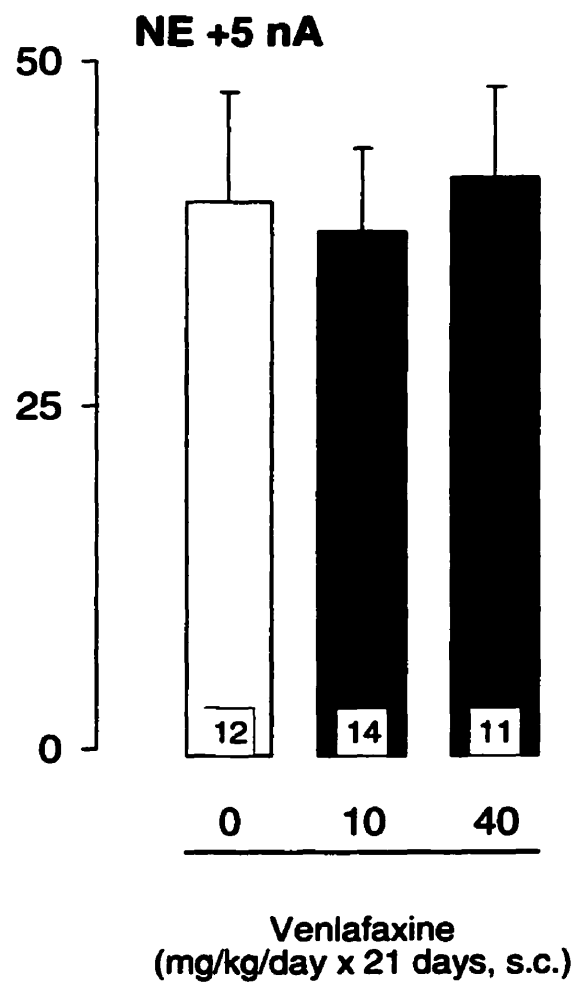
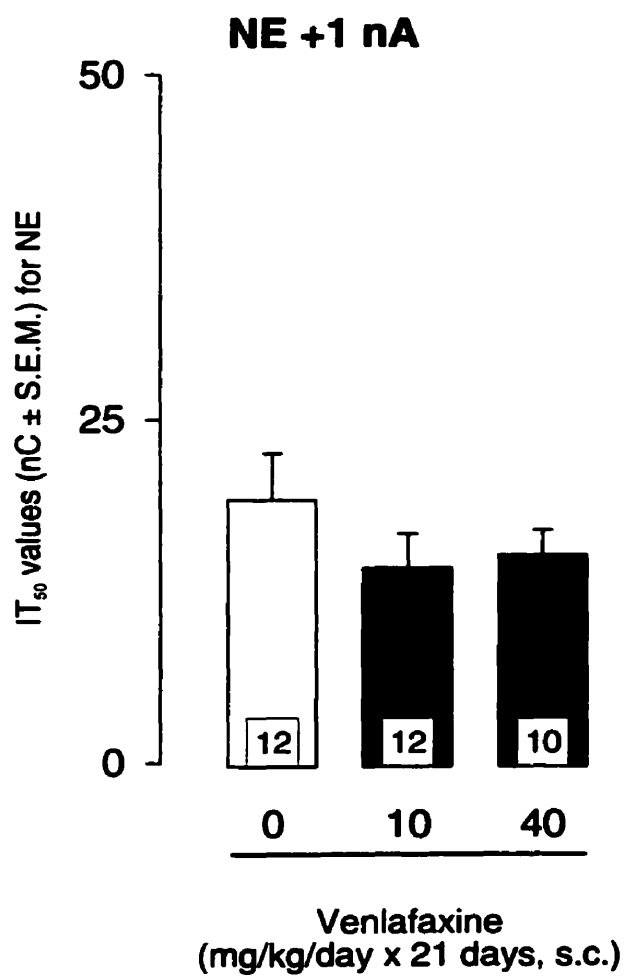
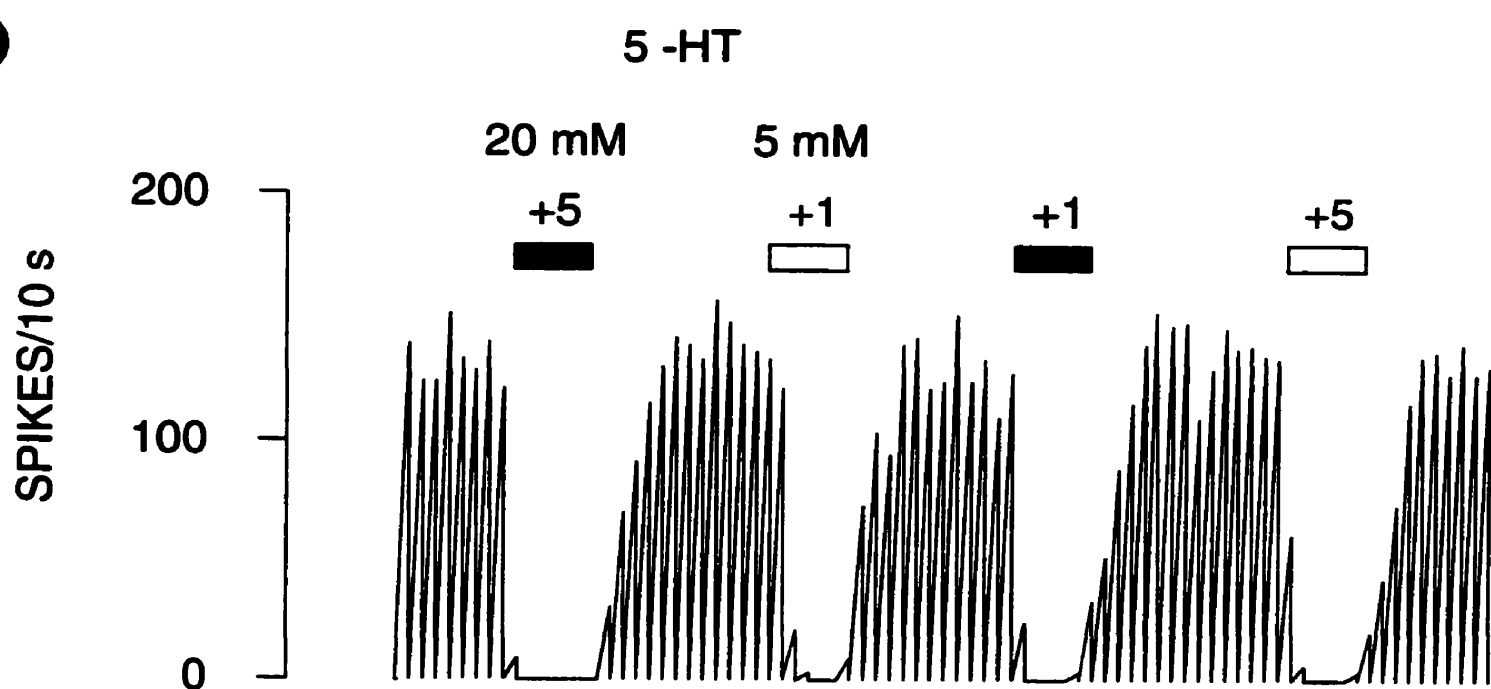


Figure 4. Integrated firing rate histograms of a CA₃ dorsal hippocampus pyramidal neurons showing the effects of microiontophoretic applications of two different side barrel concentration of 5-HT in rats following a 21-day treatment with vehicle (A) and with 40 mg/kg/day, s.c. of venlafaxine (B). The solid bars above the trace indicate the duration of microiontophoretic application for which the current is given in nA. The open and black bars indicate the use of two different side barrels for the microiontophoretic application of 5-HT for which the concentration of 5-HT is given in mM.

A. Control



B. Venlafaxine, 40 mg/kg/day x 21 days, s.c.

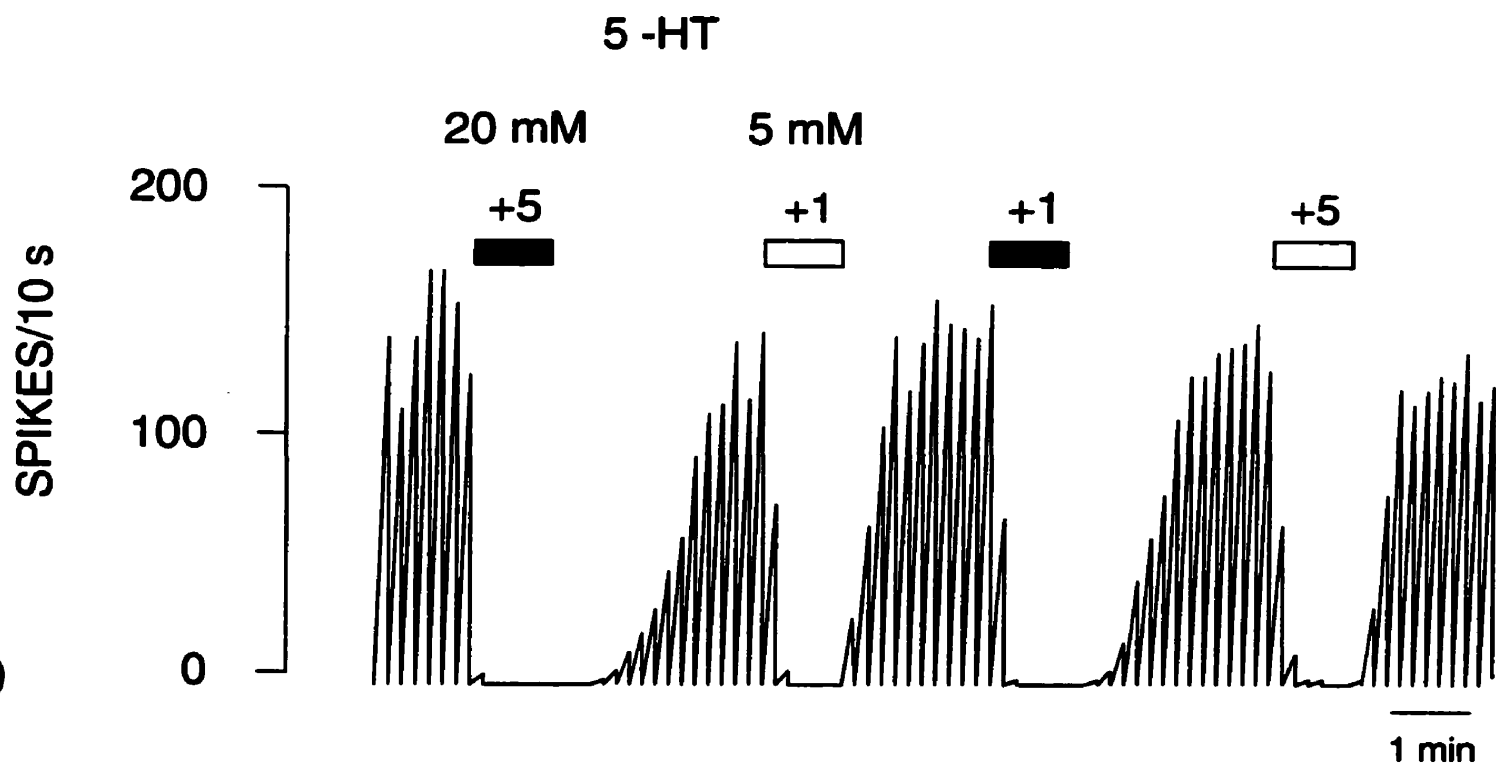
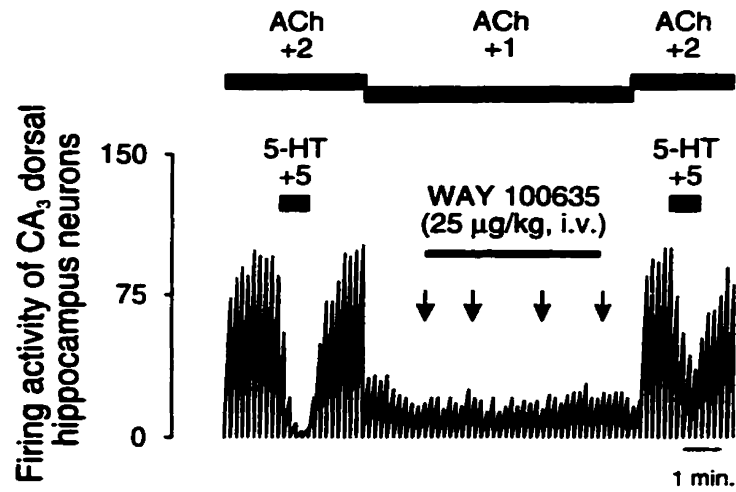
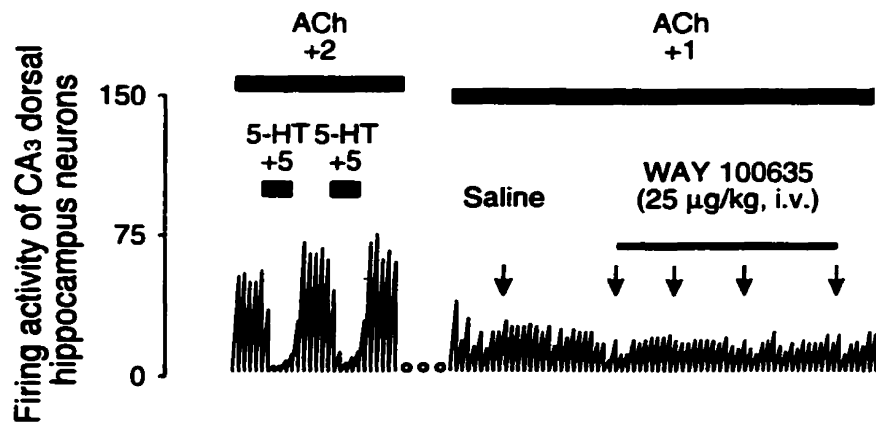


Figure 5. Integrated firing rate histogram of CA₃ dorsal hippocampus pyramidal neurons showing the effects of microiontophoretic applications of 5-HT before and after the intravenous administration of the 5-HT_{1A} antagonist WAY 100635 in controls (A) and in rats treated for 21 days with 10 (B) and 40 mg/kg/day, s.c. of venlafaxine. Note also the effects of intravenous administration of WAY 100635 on the ACh-activated firing activity of the neuron.

A- CONTROL



B- Venlafaxine 10 mg/kg/day x 21 days, s.c.



C- Venlafaxine, 40 mg/kg/day x 21 days, s.c.

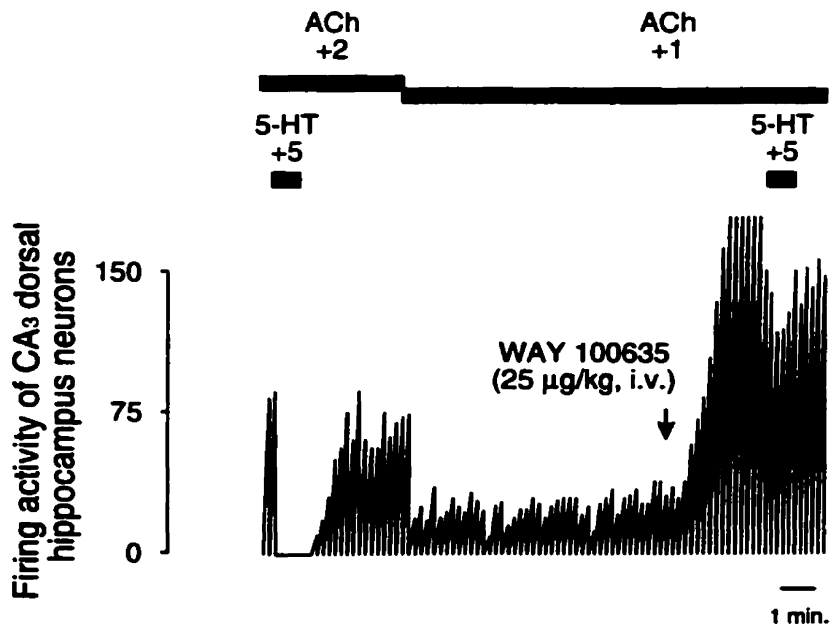


Figure 6. Effects of the acute intravenous administration of the 5-HT_{1A} antagonist WAY 100635 on ACh-induced firing activity of CA₃ dorsal hippocampus pyramidal neurons following 21-day treatments with vehicle, 10 and 40 mg/kg/day, s.c. of venlafaxine. ($p < 0.01$ compared to control values using the non-paired Student's t -test).

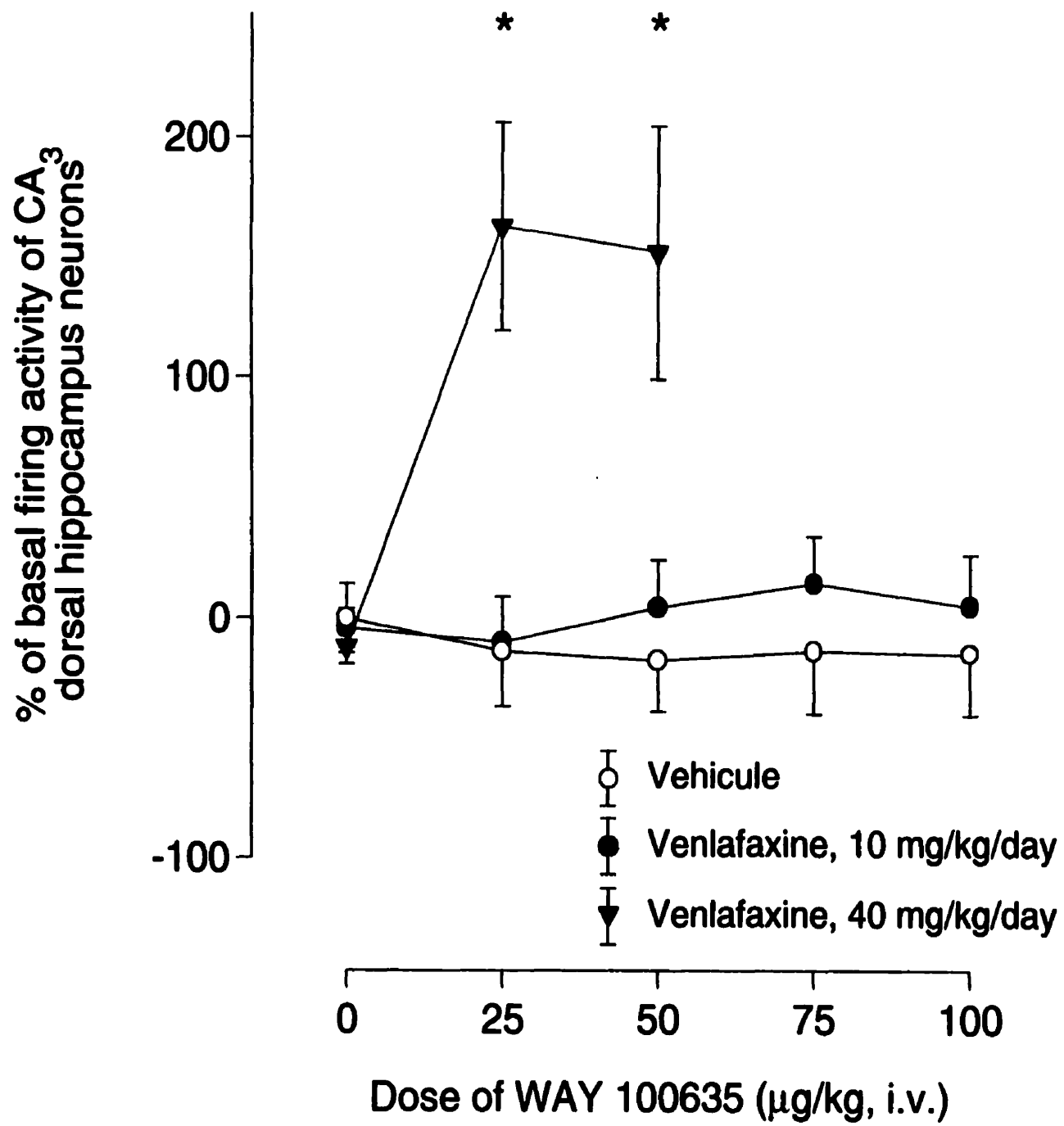
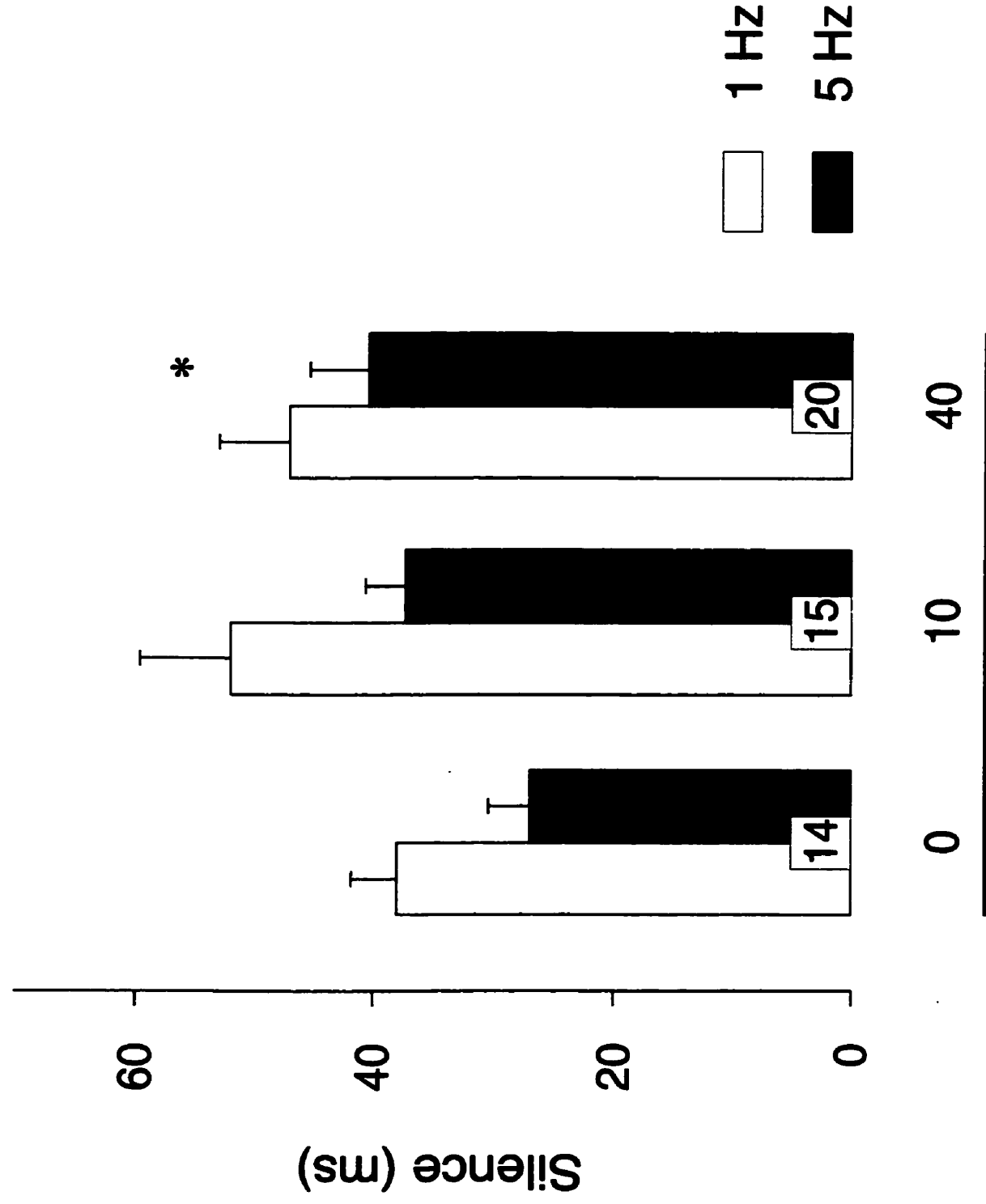


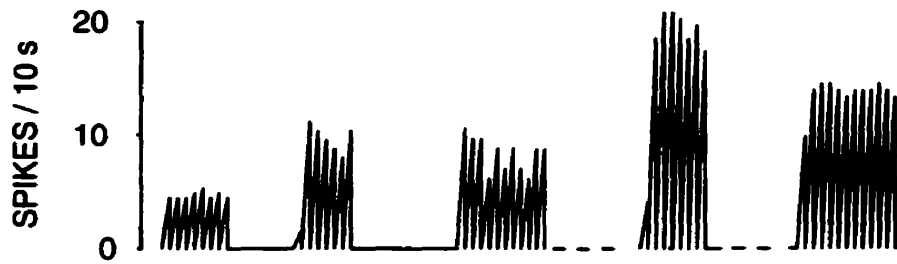
Figure 7. Inhibitory effect, expressed as the silence value (SIL; mean $\pm$ S.E.M.) of the electrical stimulation of the ascending 5-HT pathway on the ACh-induced firing activity of CA₃ dorsal hippocampus pyramidal neurons following 21-day treatments with vehicle, 10 and 40 mg/kg/day, s.c. of venlafaxine. The silence values were determined by delivering two hundred square pulses of 0.5 ms at a frequency of 1 or 5 Hz and an intensity of 300 μ A while recording the firing activity of the neuron. * $p < 0.05$, compared to the 1 Hz value obtained in control animals using a single factor ANCOVA, for which the SIL value obtained at 1 Hz was used as the covariate.



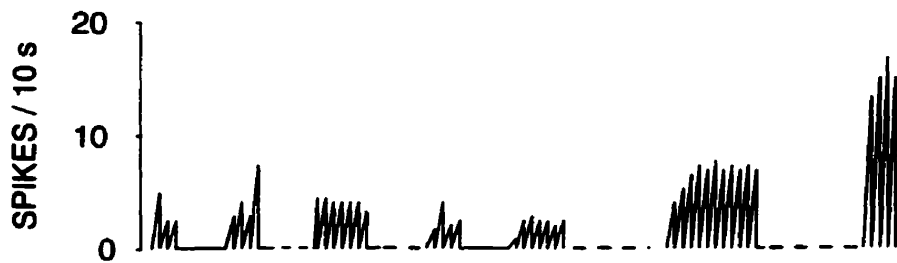
Venlafaxine
(mg/kg/day x 21 days, s.c.)

Figure 8. Integrated firing rate histograms of dorsal raphe 5-HT neurons recorded in one electrode descent in the dorsal raphe showing their spontaneous firing activity in rats treated with vehicle (A) or with 10 mg/kg/day of venlafaxine for two days (B) or for 21 days (C). The effects of a 2-day and a 21-day treatment with 10 mg/kg/day of venlafaxine on the spontaneous firing activity of dorsal raphe 5-HT neurons are depicted in D. The open horizontal bar represents the range (S.E.M. $\times$ 2) of the firing activity of these neurons in rats treated with vehicle. * $p < 0.01$ using a one-way ANOVA.

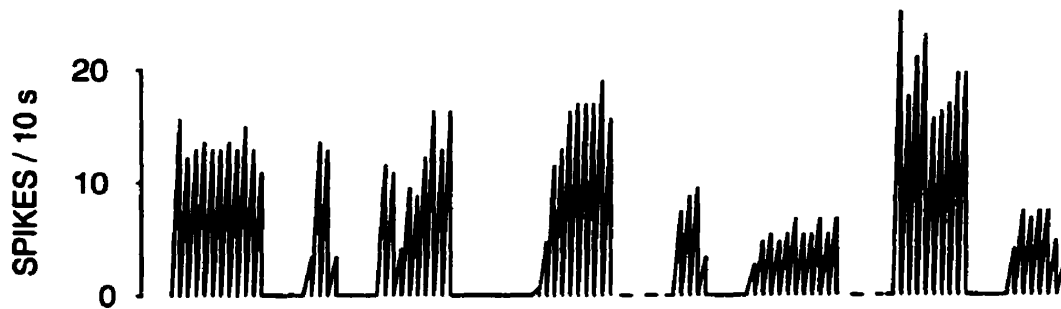
A. Control



B. Venlafaxine, 10 mg/kg/day x 2 days, s.c.



C. Venlafaxine, 10 mg/kg/day x 21 days, s.c.



D.

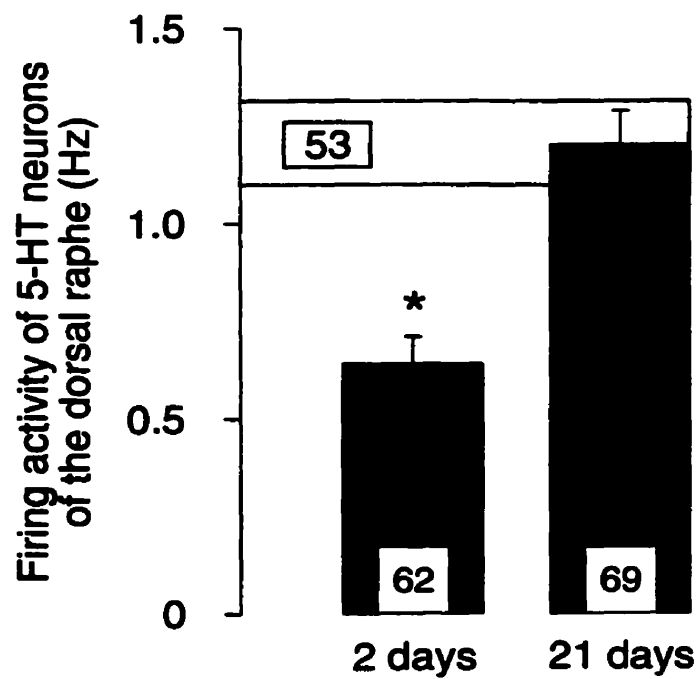
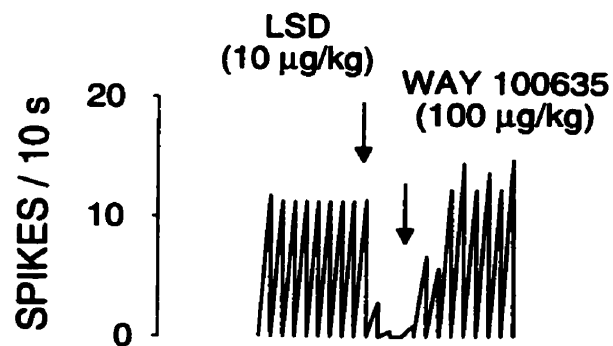
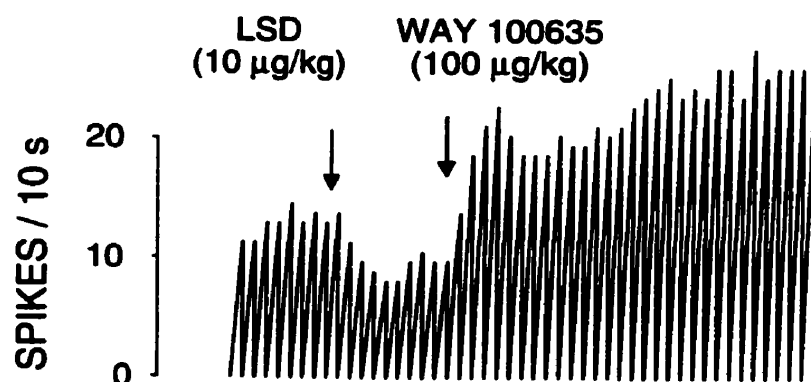


Figure 9. Integrated firing rate histograms of 5-HT neurons of the dorsal raphe nucleus showing the effects of intravenous administration of 10 $\mu\text{g/kg}$ of LSD in rats treated with vehicle (A) and with 10 mg/kg/day of venlafaxine for 21 days (B). The effect of a subsequent intravenous administration of 100 $\mu\text{g/kg}$ of the selective 5-HT_{1A} antagonist WAY 100635 is also shown. The dose-response curves showing the effect of intravenous administration of LSD on the firing activity of dorsal raphe 5-HT neurons of rats that were treated with vehicle (open circles) or with 10 mg/kg/day x 21 days of venlafaxine (filled circles) is shown in (C). Each dot represent the response of one neuron to a single dose in one rat. The curved lines depict the 95% confidence interval of the regression.

A. Control



B. Venlafaxine, 10 mg/kg/day x 21 days



C.

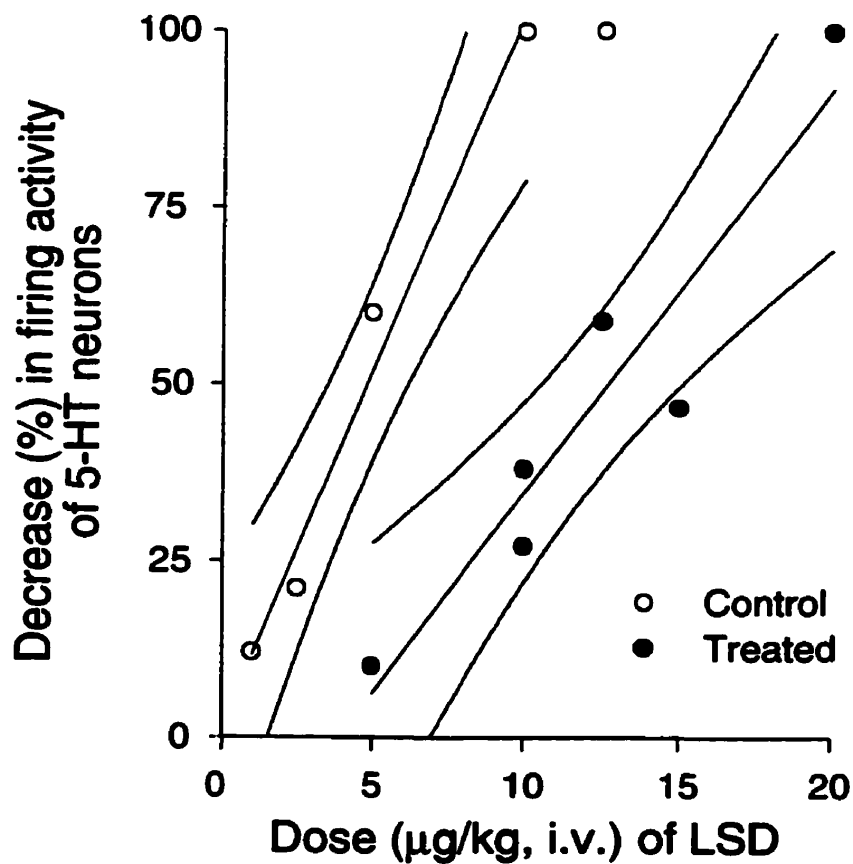
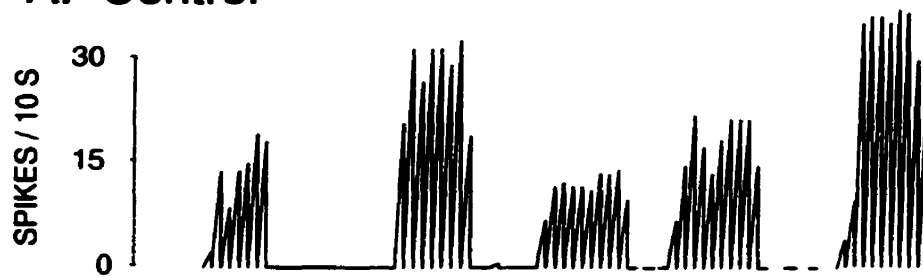


Figure 10. Integrated firing rate histograms of NE neurons recorded in one electrode descent in the locus coeruleus showing their spontaneous firing activity in rats treated for two days with vehicle (A) or with 40 mg/kg/day (B) of venlafaxine. The dots represent a pinch of the contralateral hind paw of the rat. Neurons that were activated only in this manner were considered as displaying a firing frequency of 0 Hz. The important recovery of firing activity following the acute intravenous administration of the α_2 -adrenergic receptor antagonist piperoxane confirmed the noradrenergic nature of the neuron recorded. In C, is shown the effects of a 2-day and a 21-day treatment with venlafaxine (10 and 40 mg/kg/day) on the spontaneous firing activity of locus coeruleus NE neurons. The open horizontal bar represents the range (S.E.M. x 2) of the firing activity of these neurons in rats treated with vehicle. * $p < 0.05$, ** $p < 0.01$, using one-way ANOVA.

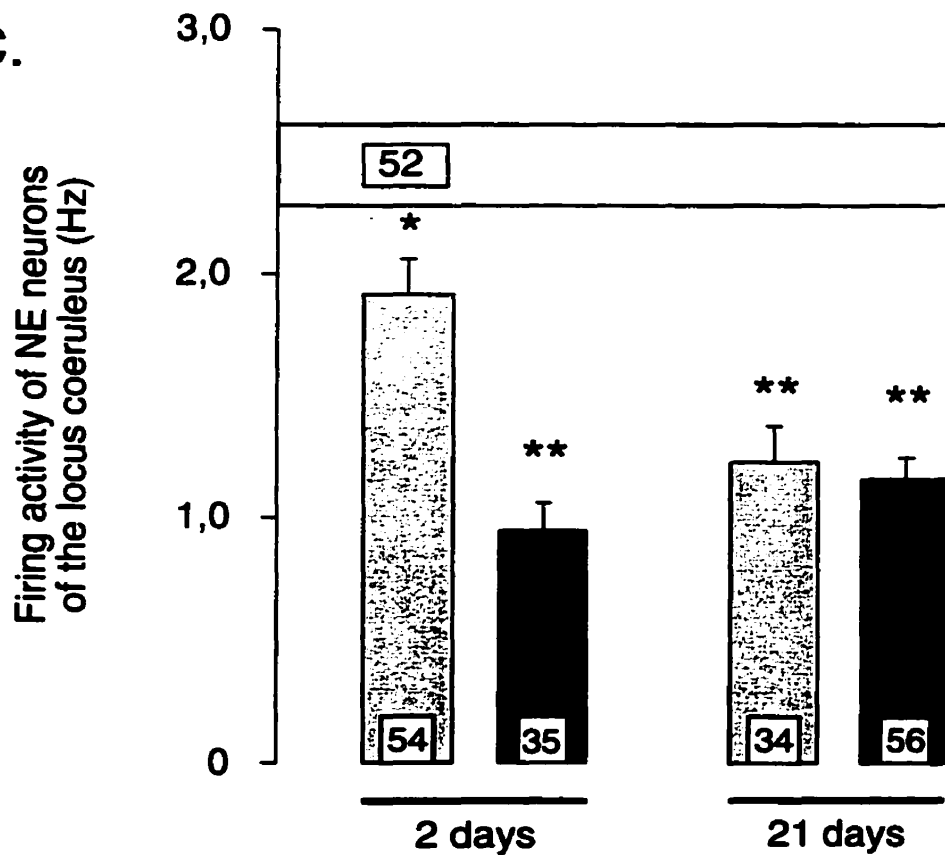
A. Control

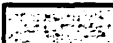


B. Venlafaxine, 40 mg/kg/day x 21 days, s.c.



C.



 Venlafaxine, 10 mg/kg/day x 21 days

 Venlafaxine, 40 mg/kg/day x 21 days

Table 1. $I\cdot T_{50}$ and RT_{50} values following microiontophoretic applications of 5-HT using different side-barrel concentrations of 5-HT¹.

$I\cdot T_{50}$ ²	5 mM		20 mM	
	+ 1 nA	+ 5 nA	+ 1 nA	+ 5 nA
Control	15 ± 3	74 ± 12	10 ± 1	37 ± 4
Venlafaxine 40 mg/kg/day	17 ± 3	54 ± 4	10 ± 1	34 ± 4

RT_{50} ²	5 mM		20 mM	
	+ 1 nA	+ 5 nA	+ 1 nA	+ 5 nA
Control	14 ± 2	24 ± 3	20 ± 3	44 ± 6
Venlafaxine 40 mg/kg/day	13 ± 2	27 ± 4	56 ± 8*	114 ± 13*

¹ Data were calculated from the suppressant effect on the firing activity of dorsal hippocampus neurons of microiontophoretically applied 5-HT for which the current is given in nA and the concentration in mM.

² $I\cdot T_{50}$ values are expressed in nC (± S.E.M), while RT_{50} values are expressed in sec (± S.E.M.).

* $p < 0.01$ when compared to control values.

CHAPTER V

PREFATORY REMARKS

Technical considerations dictates to a great extent the nature of the issues that can meaningfully be addressed experimentally. Hence, our investigation on the effects of long-term administration of venlafaxine involved essentially two experimental approaches that can be defined, in broad terms, as, on the one hand, in vivo electrophysiological approach and, on the other hand, in vitro superfusion and uptake approaches. Whereas the results obtained with the use of the former experimental approach have been the subject of the study presented in chapter IV, that of the latter are now reported in chapter V. We thought that presenting those results in two chapters divided in terms of technical approaches would be suitable for providing a clear and concise presentation of our investigation on the long-term effects of venlafaxine on parameters involved in the 5-HT and NE neurotransmission.

EFFECTS OF SUSTAINED ADMINISTRATION OF THE SEROTONIN AND NOREPINEPHRINE REUPTAKE INHIBITOR VENLAFAXINE : II. IN VITRO STUDIES IN THE RAT⁸.

JEAN-CLAUDE BÉIQUE, CLAUDE DE MONTIGNY, PIERRE BLIER AND GUY DEBONNEL

ABSTRACT

The effects of long-term administrations of a low (10 mg/kg/day) and a high (40 mg/kg/day) dose of the dual 5-HT and NE reuptake inhibitor venlafaxine (delivered s.c. by osmotic minipumps for 21 days) were assessed on the electrically-evoked release of tritium from hippocampal slices preloaded with either [³H]5-HT or [³H]NE, 48 h after the removal of the minipump. The high, but not the low, dose regimen of venlafaxine enhanced the electrically-evoked release of [³H]5-HT while treatment with the high dose of venlafaxine failed to alter the electrically-evoked release of [³H]NE. The inhibitory effect of the 5-HT_{1B} agonist CP 93,129 on the electrically evoked release of [³H]5-HT was unaltered by the low dose regimen of venlafaxine while it was attenuated in rats treated with the high dose of

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venlafaxine, indicative of a functional desensitization of the terminal 5-HT_{1B} autoreceptor. Unexpectedly, neither regimen of venlafaxine altered the inhibitory effect of UK 14,304 on the electrically evoked release of both [³H]5-HT and [³H]NE, indicating that neither the α_2 -adrenergic auto- nor heteroreceptors were desensitized. Finally, the function of the 5-HT and NE reuptake process were assessed. None of the treatment regimens altered the basal uptake of [³H]5-HT from hippocampal or mesencephalic slices nor that of [³H]NE from hippocampal slices. Finally, the enhancing effect of 1 μ M of paroxetine in the perfusion medium on the electrical release of [³H]5-HT was unaltered in hippocampal slices prepared from rats that had been treated for 21 days with 40 mg/kg/day of venlafaxine. Taken together, these results indicate that, in terms of alteration of the sensitivity of the terminal 5-HT_{1B} autoreceptor, α_2 -adrenergic auto- and heteroreceptors, the effects of long-term administration of venlafaxine are no different than those observed with classical SSRI's.

4. INTRODUCTION

The monoaminergic systems are endowed with homeostatic mechanisms that subserve an important function in controlling neurotransmission. For instance, the role of both serotonergic (5-HT) and noradrenergic (NE) terminal autoreceptors have been known for many years to tightly regulate the release of 5-HT and NE, respectively, thereby regulating the synaptic availability of the neurotransmitter (Langer, 1980; Göthert, 1986; Starke et al., 1989). There is a substantial corpus of data suggesting that most antidepressant treatments exert their primary pharmacological effects on monoaminergic systems. However, given that these effects, for example the blockade of the reuptake of either 5-HT and/or NE, supervene almost immediately upon initiating the treatment, they cannot *per se* be held responsible for the therapeutic response as it develops over the course of several weeks of treatment. On the basis of these contentions, a particular attention was thus paid to the effects of long-term antidepressant treatments on specific target receptors which modulate monoaminergic neurotransmission. Out of this endeavour, relevant findings have emerged. For instance, the long-term administration of selective 5-HT reuptake inhibitors (SSRI's) was shown to induce a desensitization of the terminal 5-HT_{1B} autoreceptors (Chaput et al., 1986; Moret and Briley, 1990; Chaput et al., 1991) thereby increasing the amount of 5-HT released per action potential. Moreover, the long-term administration of drugs that enhance the synaptic availability of NE were shown to induce the desensitization of the α_2 -adrenergic heteroreceptors located on 5-HT terminals (Mongeau et al., 1994; Yoshioka et al., 1995). Since the latter heteroreceptors inhibit the release of 5-HT (Göthert and Huth, 1980; Göthert et al., 1981; Maura et al., 1982; Mongeau et al., 1993), this also results in an enhanced release of 5-HT.

Venlafaxine (1-[2-(dimethylamino)-1-(4-methoxyphenyl)-ethyl]cyclohexanol) is an antidepressant for which clinical studies have suggested a superior clinical efficacy as it would display an earlier onset of action and show efficacy in treatment-resistant depression (Schweizer et al., 1991; Guelfi et al., 1992; Nierenberg et al., 1994; Kelsey, 1996; Benkert et al., 1996; de Montigny et al., 1999). Its pharmacological profile is characterized by the inhibition of the reuptake of both 5-HT and NE with a potency greater for blocking the reuptake of 5-HT than that of NE (Muth et al., 1986; Bolden-Watson and Richelson, 1993; Béïque et al., 1998b; Béïque et al., 1999). In light of the different adaptive changes induced

by either 5-HT or NE reuptake inhibitors, it was deemed important to determine whether the long-term administration of venlafaxine could induce some of the adaptative changes previously observed with either 5-HT and/or NE reuptake inhibitors. Thus, the sensitivity of the 5-HT_{1B} autoreceptors and the α_2 -adrenergic heteroreceptors located on 5-HT terminals and of the α_2 -adrenergic autoreceptors located on NE terminals were assessed following the long-term administration of a low (10 mg/kg/day) and a high (40 mg/kg/day) dose of venlafaxine for 21 days. To this end, an *in vitro* approach in which hippocampal slices were preloaded with [³H]5-HT or [³H]NE was employed. Finally, the function of the 5-HT transporter was assessed in mesencephalic and in hippocampal slices following long-term administration of venlafaxine by determining the effects of a challenging concentration of paroxetine on [³H]5-HT release.

Parts of the present work have previously been published in abstract form (Béïque et al., 1998a)

5. METHODS

2.1. Animals

Male Sprague Dawley rats (125-150 g; Charles River, St. Constant, Québec, Canada) were received one day before the implantation of minipumps and housed three to four per cage. They were kept on a 12:12 h light/dark cycle, with access to food and water *ad libitum*.

2.2. Treatments

Rats were anaesthetized with halothane and implanted with an osmotic minipump (Alza, Palo Alto, CA, U.S.A.) which delivered either 10 or 40 mg/kg/day of venlafaxine or vehicle for 21 days. The rationale for using these doses is based on previous studies that have shown that 10 mg/kg/day of venlafaxine given for two (Béïque et al., 1998b) or 21 days (companion paper) resulted in an effective uptake blockade of 5-HT while the 40 mg/kg/day regimen resulted in a effective blockade of both 5-HT and NE uptake process, as assessed using an *in vivo* electrophysiological paradigm. After the 21-day treatment, the minipumps were removed under halothane anaesthesia and the *in vitro* experiments were carried out 48 h later to allow elimination of venlafaxine.

2.3 Superfusion experiments

Rats were sacrificed by decapitation and the brain immediately removed and rapidly dissected on an ice-cold glass plate. Hippocampal slices of 400 μm of thickness were prepared using a McIlwain tissue chopper. The slices were then incubated for 30 min at 37°C in Krebs' buffer containing either 20 nM of [^3H]5-HT creatinine sulfate (specific activity 1017 GBq/mmol) or 20 nM of [^3H]NE (specific activity of 538.7 GBq/mmol) both purchased from NEN Life Science, Boston, MA and bubbled with a mixture of 95% O_2 / 5% CO_2 . The composition of the Krebs' solution was the following (in mM): NaCl 118, KCl 4.7, CaCl_2 1.3, MgCl_2 1.2, NaH_2PO_4 1, NaHCO_3 25, glucose 11.1, Na_2EDTA 0.004 and ascorbic acid 0.11. At the end of the incubation period, the slices were transferred to glass superfusion chambers (2 slices of hippocampus) and superfused at a rate of 0.5 ml/min with oxygenated Krebs' solution maintained at 37°C. Nineteen consecutive 4-min fractions were collected starting 90 min after the beginning of superfusion for the three structures. Two periods of stimulation, S_1 and S_2 , were carried out within the same experiment at 8 min and 52 min,

respectively, after the end of the 90-min washing period. The electrical field was generated in the chambers between 2 platinum electrodes (30 mA, 2 ms, 3 Hz for 2 min), positioned 2 cm apart. The first stimulation period (S_1) was used as control and the drugs were added 20 min before S_2 and remained present throughout the rest of the experiment. At the end of superfusion period, the slices were solubilized with 0.5 ml of Soluene 350 (Packard Instruments, Downers grove, IL, U.S.A.), and the radioactivity in the slices and superfusate samples was determined by liquid scintillation spectrometry. The results were expressed as the fraction of the tritium content present in the time at the onset of the respective collection periods. The fractional release evoked by electrical stimulation was calculated as the difference between the total amount of radioactivity released during stimulation and the basal outflow obtained in the sample preceding the onset of stimulation (sp_1 or sp_2). To assess the drug-induced changes of electrically evoked release of tritium from the slices preloaded with either [^3H]5-HT or [^3H]NE, the ratio of fractional release between the second and the first period of stimulation (S_2/S_1) was calculated. The sp_2/sp_1 ratios were also calculated to determine whether the drugs altered the basal outflow of radioactivity.

2.4 Determination of [³H]5-HT uptake

Hippocampal or mesencephalic slices were incubated in oxygenated Krebs' solution at 37°C to determine *in vitro* [³H]5-HT and/or [³H]NE uptake. After a 3-min period of stabilization, [³H]5-HT or [³H]NE was added to a final concentration of 100 nM in a total incubation volume of 2 ml. After 5-min of incubation, the uptake was stopped by transferring the slices into 5 ml of ice-cold Krebs' solution. The slices were then solubilized in 0.5 ml of Soluene 350. A parallel experiment was carried out at 0°C as a control for passive diffusion. The radioactivity in the media and in the tissues was determined by liquid scintillation spectrometry.

2.5 Statistics

All results are expressed as mean ± S.E.M. which were compared by the two-tailed Student's *t*-test. The concentration-effect curves were compared using two-way analysis of variance. In order to detect treatment effects, concentration-effect curves were constructed by studying simultaneously in the same superfusion apparatus slices prepared from a control and slices from a treated rat with the same drug solution. This experimental design was deemed optimal to minimize inter-experimental variations.

2.6 Drugs

Venlafaxine was kindly provided by Wyeth-Ayerst Research (Princeton, NJ, U.S.A.), CP 93,129 by Pfizer (Groton, CT, U.S.A.) and paroxetine by Smith Kline Beecham (West Sussex, U.K.). UK 14,304 was purchased from RBI Natick (MA, U.S.A.).

6. RESULTS

3.1 Effects of long-term administrations of venlafaxine on [³H]5-HT and [³H]NE overflow

Rats were treated for 21 days with either a low (10 mg/kg/day) or a high (40 mg/kg/day) dose of venlafaxine. Experiments were carried out 48 h after the removal of the minipumps. In the absence of any drugs in the perfusion medium, the electrically-evoked outflow of [³H]5-HT during the first electrical stimulation period (S₁) was significantly enhanced in slices of rats treated with 40 mg/kg/day of venlafaxine (*p* < 0.01, using the non-

paired student *t*-test), but not in those from rats treated with 10 mg/kg/day of venlafaxine when compared to controls processed in parallel (Figure 1). However, the electrically-evoked outflow of [³H]NE, obtained in S₁, in slices from rats treated with the 40 mg/kg/day dose of venlafaxine was not significantly different than that obtained from slices from control rats processed in parallel (Figure 1B).

3.2. Effects of long-term administrations of venlafaxine on the sensitivity of 5-HT_{1B} autoreceptors and α_2 -adrenergic heteroreceptors of 5-HT terminals and of terminal α_2 -adrenergic autoreceptors in the hippocampus

The effects of long-term treatments with both a 10 mg/kg/day and a 40 mg/kg/day dose of venlafaxine on the sensitivity of the terminal 5-HT_{1B} autoreceptor of the hippocampus were assessed by constructing concentration-effect curves for the inhibitory effect of the 5-HT_{1B} agonist CP 93,129 (Macor et al., 1990) on the electrically-evoked release of tritium from hippocampal slices preloaded with [³H]5-HT. CP 93,129 inhibited in a concentration-dependent manner (*p* < 0.01, using a one-way ANOVA) the electrically-evoked release of tritium in both groups (S₂/S₁ ratio; Figure 2A). The degree of inhibition by CP 93,129 was unaltered in rats treated for 21 days with 10 mg/kg/day of venlafaxine, indicating the presence of normosensitive terminal 5-HT_{1B} autoreceptors. In contrast, the inhibitory effect of CP 93,129 on the electrically evoked release of [³H]5-HT was significantly decreased in hippocampal slices of rats treated for 21 days with the 40 mg/kg/day of venlafaxine when compared with controls processed in parallel (*p* < 0.05, two-way ANOVA). These results indicate that the 40 mg/kg/day, but not the 10 mg/kg/day, dose regimen of venlafaxine induced a desensitization of the terminal 5-HT_{1B} autoreceptors.

The α_2 -adrenergic receptor agonist UK 14,304 (Loftus et al., 1984) inhibited in a concentration-dependent manner the electrically-evoked release of [³H]5-HT from hippocampal slices (*p* < 0.05; one-way ANOVA; Figure 3 A and B). Its effect was unaltered by either treatment regimen of venlafaxine (Figure 3 A and B). This suggests that the α_2 -adrenergic heteroreceptors located on 5-HT terminals are not desensitized by long-term administrations of venlafaxine.

The sensitivity of the terminal α_2 -adrenergic autoreceptor following the long-term administration of 40 mg/kg/day of venlafaxine was determined using the α_2 -adrenergic

receptor agonist UK 14,304. This compound induced a concentration-dependent inhibition of the electrically-evoked release of tritium from hippocampal slices preloaded with [^3H]NE (Figure 4). The concentration-response curves of the effect of UK 14,304 were similar in control rats and in rats treated with 10 or 40 mg/kg/day for 21 days of venlafaxine (Figure 4; $p > 0.05$; two-way ANOVA).

3.3. Effects of long-term administrations of venlafaxine on the function of the 5-HT and NE transporters

In order to assess the function of the 5-HT transporter, the basal uptake of [^3H]5-HT was first assessed in hippocampal and mesencephalic slices (the latter containing the raphe 5-HT cell bodies) prepared from control and from rats that had been treated for 21 days with 10 mg/kg/day or with 40 mg/kg/day of venlafaxine. As can be seen from Table 2, the basal uptake of [^3H]5-HT in hippocampal as well as in mesencephalic slices was not affected by either treatment regimen of venlafaxine. These results suggest the presence of an intact 5-HT reuptake process 48 h following a long-term administration of venlafaxine. The basal uptake of [^3H]NE was also assessed in hippocampal slices of control and treated animals. Neither treatment regimen (10 or 40 mg/kg/day) of venlafaxine altered the basal uptake of [^3H]NE from hippocampal slices ($p > 0.05$, using the non-paired Student's t -test).

The function of the 5-HT reuptake process following a treatment with venlafaxine was further assessed using the following paradigm. The electrically-evoked release of tritium from hippocampal slices prepared from controls and from animals treated with 40 mg/kg/day of venlafaxine was compared before (S_1) and after (S_2) the addition of $1\ \mu\text{M}$ of the selective 5-HT reuptake inhibitor paroxetine in the medium. In slices preloaded with [^3H]5-HT prepared from control animals, the presence of paroxetine in the perfusion solution elicited an increase of tritium overflow upon electrical stimulation resulting from 5-HT reuptake inhibition (Figure 5; S_2 compared to S_1 , $p < 0.01$, using the paired Student's t -test). The increase in the electrically-induced tritium overflow elicited by $1\ \mu\text{M}$ of paroxetine was also present in slices taken from rats treated with 40 mg/kg/day of venlafaxine (Figure 5; S_2 compared to S_1 , $p < 0.05$, using the paired Student's t -test). There was no significant difference in the S_2/S_1 ratio between slices prepared from control and from treated animals (S_2/S_1 ratio of 1.37 ± 0.04 and of 1.34 ± 0.09 for control and venlafaxine treated animals, respectively, $p < 0.05$ using the non-paired Student's t -test). These results thus further

corroborate that the 5-HT reuptake process is not modified by a long-term treatment with venlafaxine when assessed 48 h following the cessation of the treatment.

7. DISCUSSION

In the present study, a high (40 mg/kg/day), but not a low (10 mg/kg/day), dose of venlafaxine, administered for 21 days, elicited an increase of the electrically-evoked release (S_1) of tritium from hippocampal slices preloaded with [^{3} H]5-HT. The latter effect most probably stemmed from the desensitization of the terminal 5-HT_{1B} autoreceptor as inferred from the reduced efficacy of the 5-HT_{1B} agonist CP 93,129 to suppress the electrically-evoked release of [^{3} H]5-HT from preloaded hippocampal slices prepared only from rats treated with the high dose of venlafaxine. The increase of [^{3} H]5-HT release (S_1) cannot be attributed to a reduced function of the 5-HT transporter as it was not altered by the high dose regimen of venlafaxine. None of the treatment regimens of venlafaxine altered the function of either the α_2 -adrenergic hetero- or autoreceptors in the hippocampus, as indicated by the unaltered efficacy of the α_2 -adrenergic agonist UK 14,304 to inhibit the electrically-evoked release of [^{3} H]5-HT and [^{3} H]NE, respectively, from hippocampal slices.

The increase of the electrically-evoked release of [^{3} H]5-HT was obtained 48 h after the removal of the minipumps and after the slices had been wash-out for 90 min. Thus, it can be assumed that there was no venlafaxine present in the slices. Hence, an inhibition of the reuptake of 5-HT cannot account for the increase of [^{3} H]5-HT outflow observed in treated animals. It may however appear peculiar that this increment of 5-HT output in the hippocampus was not observed when an *in vivo* electrophysiological paradigm was used (Figure 6 of the companion paper). Indeed, the suppression of firing activity of CA₃ pyramidal neurons induced by the electrical stimulation of the ascending 5-HT pathway is, in essence, a measure of the same parameter as that presented in Figure 1, *i.e.*, the amount of 5-HT released by electrical stimulation. Hence, it needs to be emphasized that the sample size used in the *in vivo* study allowed for a valid pair-wise comparison (*i.e.*, effect of a 1 Hz vs a 5 Hz stimulation) but not a between-treatment comparison as is achieved herein.

The reduction of the efficacy of the 5-HT_{1B} agonist CP 93,129 to inhibit the release of [^{3} H]5-HT in slices prepared from rats treated with the high dose of venlafaxine provides strong evidence that the latter increase in [^{3} H]5-HT outflow stemmed from the desensitization of the 5-HT_{1B} terminal autoreceptor (Figure 2B). These results are in perfect congruence with those generated using our *in vivo* paradigm which have shown that increasing the frequency of stimulation of the 5-HT pathway from 1 to 5 Hz reduced its effectiveness in rats treated

with vehicle or with the low, but not with the high, dose regimen of venlafaxine for 21 days. These results were indeed interpreted as being indicative of a functional desensitization of the terminal 5-HT_{1B} autoreceptor following the long-term treatment with the high dose of venlafaxine. However, it may be alleged that these results are somewhat intriguing considering the fact that a 10 mg/kg/day regimen of venlafaxine produces an efficient blockade of the 5-HT reuptake process (Béïque et al., 1998b and companion paper) and could hence be expected to induce a desensitization of the terminal 5-HT_{1B} autoreceptor when given to rats for 21 days. Considering that following 5-HT reuptake blockade the rise in extracellular levels of 5-HT is of greater magnitude in the raphe area than that observed in terminals area (Adell and Artigas, 1991; Bel and Artigas, 1992), it is possible that the 10 mg/kg/day regimen of venlafaxine represents a dose sufficient to induce a desensitization of the 5-HT autoreceptor in the raphe and not of that in the hippocampus.

The presence of an α_2 -adrenergic heteroreceptor modulating the release of 5-HT from serotonergic terminals has been known for many years (Göthert and Huth, 1980; Göthert et al., 1981; Maura et al., 1982). Previous *in vivo* and *in vitro* studies have demonstrated a functional desensitization of the latter receptor by the long-term administration of drugs that enhance the synaptic concentration of NE such as NE reuptake inhibitor or monoamine oxidase inhibitors (Yoshioka et al., 1995). Therefore, in light of the fact that the high dose regimen of venlafaxine produced an efficient blockade of the NE uptake process (Béïque et al., 1998b and companion paper), it would be plausible that this treatment regimen of venlafaxine induces a desensitization of the α_2 -adrenergic heteroreceptors in the hippocampus. Nonetheless, as can be seen from figure 3, none of the treatment with venlafaxine altered the inhibitory effect of the α_2 -adrenergic agonist UK 14,304 on the release of [³H]5-HT from hippocampal slices. It thus appears that the enhanced electrically-evoked release of [³H]5-HT following the long-term administration of the high dose of venlafaxine is solely the result of the desensitization of the terminal 5-HT_{1B} autoreceptor. However, considering that the high dose of venlafaxine induced an efficient blockade of the NE reuptake process, the lack of α_2 -adrenergic heteroreceptors desensitization is intriguing. A likely explanation could be derived from a previous study that has shown that the addition to the perfusion medium of the SSRI citalopram attenuates the ability of the α_2 -adrenergic receptor agonist UK 14,304 as well as exogenous NE, to inhibit the release of [³H]5-HT from

hypothalamic slices (Blier et al., 1990). The latter study is thus suggesting the presence of an apparent link between 5-HT transporter occupancy and the function of the α_2 -adrenergic heteroreceptors. Given that the high dose regimen of venlafaxine induces an efficient 5-HT reuptake blockade, it could be speculated that the long-term administration of this dose would interfere with the mechanism whereby the α_2 -adrenergic heteroreceptors desensitises following sustained activation. However, using a similar paradigm, it was shown that the long-term administration of the 5-HT/NE reuptake inhibitor duloxetine, at a dose blocking both 5-HT and NE reuptake, induces a desensitization of the α_2 -adrenergic heteroreceptors and not of the 5-HT_{1B} autoreceptor in the hippocampus (Rueter et al., 1998). It thus seems that the desensitization of one of these receptors controlling the release of 5-HT (*i.e.*, 5-HT_{1B} and α_2 -adrenergic receptors) would occur at the expense of the other.

We have deemed necessary to assess the sensitivity of the α_2 -autoreceptors modulating the release of NE following the long-term administration of only the high dose of venlafaxine. As can be seen in figure 4, this treatment regimen of venlafaxine administered for 21 days did not alter the efficacy of the α_2 -adrenergic agonist UK 14,304 to suppress the release of [³H]NE from hippocampal slices, indicative of a normosensitive α_2 -adrenergic autoreceptor. The fate, in terms of sensitivity, of the α_2 -adrenergic autoreceptor following the long-term administration of NE reuptake inhibitor is somewhat equivocal in the literature. Indeed, *in vivo* electrophysiological studies have suggested first, that the long-term administration of desipramine for 21 days (with a 24 h wash-out period) induces a desensitization of the latter receptor (Lacroix et al., 1991). However, using a different *in vivo* paradigm, Mongeau *et al.* (1994), whilst showing that a long-term treatment with nisoxetine, a NE reuptake inhibitor, desensitises the α_2 -adrenergic heteroreceptors, failed to detect any change of the sensitivity of the α_2 -adrenergic autoreceptor. *In vitro* studies with desipramine have also generated conflicting results. For instance, whereas the long-term administration of a high dose of desipramine was shown to induced a desensitization of the α_2 -adrenergic autoreceptor in hypothalamic slices (Moret and Briley, 1994), other investigators have failed to detect it and this, despite an enhanced output of [³H]NE (Schoffelmeer and Mulder, 1982; Schoffelmeer et al., 1984). Inconsistencies also exist in the data obtained with other mix 5-HT/NE reuptake inhibitors: long-term treatment with milnacipran does not induce any changes in the sensitivity of the α_2 -adrenergic autoreceptor in hypothalamic slices (Moret

and Briley, 1994) nor in mesencephalic slices (Mongeau et al., 1998) whereas long-term treatment with duloxetine induces a desensitization the α_2 -adrenergic autoreceptor in hippocampal slices, but not in frontal cortex slices (Rueter et al., 1998).

The enhanced efficacy of the electrically-stimulated release of [^{3} H]5-HT (S_1 ; Figure 1) in slices from rats treated with the high dose of venlafaxine may have stemmed from a reduced function of the 5-HT transporter. To address this issue, we have used two experimental approaches that have proven valid to assess the function of the 5-HT transporter (Piñeyro et al., 1994; Blier and Bouchard, 1994a; El Mansari et al., 1995). First, the basal uptake of [^{3} H]5-HT is altered in neither hippocampal slices nor in mesencephalic slices (the latter comprising the raphe nuclei). Second, the unaltered capacity of paroxetine to enhance the electrically-evoked release of [^{3} H]5-HT from hippocampal slices further suggests the presence of a normal function of the 5-HT transporter following sustained administration of venlafaxine. It is possible that the lack of a strong binding of venlafaxine with the 5-HT transporter may account for its inability to alter 5-HT transporter function following long-term administration. Indeed, previous studies from our laboratory have shown that, when assessed in functional *in vivo* models, venlafaxine displayed a potent 5-HT reuptake inhibition that is incongruent with its rather modest affinity for the 5-HT transporter (Béïque et al., 1998c; Béïque et al., 1999).

The differential potencies of venlafaxine for blocking the 5-HT and NE reuptake processes prompted the elaboration of the hypothesis that a low dose of venlafaxine would act as an SSRI whereas, at higher doses, it would block the reuptake of both 5-HT and NE. This outcome has been verified in the present studies following long-term administration of the drug (see Table 2). The corollary working hypothesis was that long-term administration of a low dose of venlafaxine would induce adaptative changes observed with SSRI's whereas a high dose would append those observed following sustained NE reuptake inhibition. As can be seen from Table 2, long-term administration of venlafaxine does not stringently induce the adaptative changes one might predict on the basis of its reuptake inhibition potencies. In this regard, the lack of desensitization of the α_2 -adrenergic heteroreceptor following a treatment regimen that blocks the reuptake of NE is perhaps the most intriguing. Given the role of these receptors on 5-HT release, it is here interesting to highlight the fact that the 5-HT output was increased by the high dose of venlafaxine [as evidenced by the enhanced tonic

activation of postsynaptic 5-HT_{1A} receptors] and this, despite the presence of NE reuptake blockade and of normosensitive α_2 -adrenergic heteroreceptors. There is another point from the present studies that needs emphasis. Since the somatodendritic 5-HT_{1A} autoreceptor was desensitized by the low dose regimen of venlafaxine and that an enhanced tonic activation of postsynaptic 5-HT_{1A} receptors was only observed with the high dose regimen of venlafaxine, suggest that the latter outcome is dependent on the desensitization of both the somatodendritic and terminal 5-HT autoreceptor. However, one must also consider the results showing that the α_2 -adrenergic heteroreceptors, and not the 5-HT_{1B} autoreceptors, in the hippocampus are desensitized following long-term administration of the 5-HT/NE reuptake inhibitor duloxetine (Rueter et al., 1998). Taken together, these results therefore suggest that the desensitization of at least one of the autoreceptor regulating the release of 5-HT is a *sine qua non* condition for reuptake inhibitors to increase the net 5-HT neurotransmission. Given that an increase in 5-HT neurotransmission appears to provide the most likely substratum for the clinical efficacy of antidepressant treatments in major depression (Blier and de Montigny, 1994b), these results provide further support to the hypothesis stating that a better antidepressant response could be attained with the combination of a 5-HT reuptake inhibitor with an antagonist of either (or both) the somatodendritic or terminal 5-HT autoreceptors.

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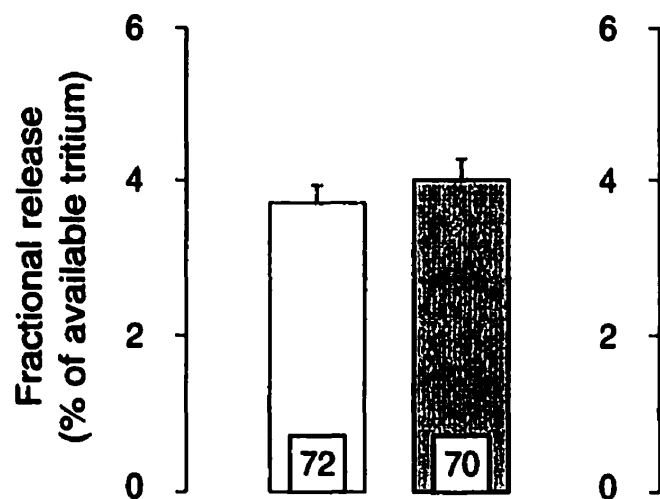
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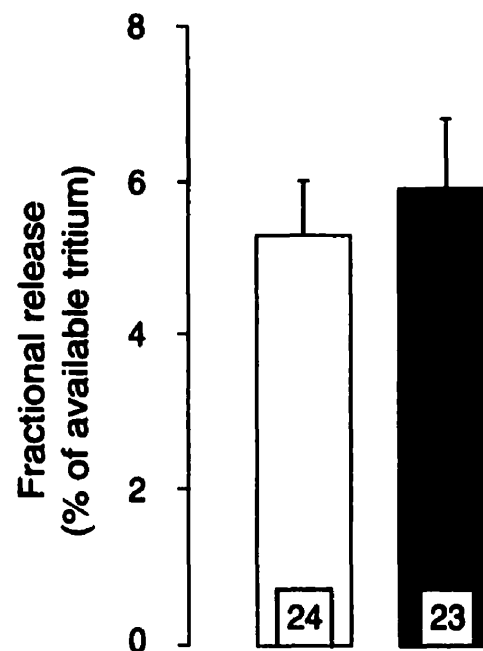
LEGENDS TO FIGURES

Figure 1. Effect of long-term administrations of a low and/or a high dose regimen of venlafaxine on the electrically evoked release of tritium from hippocampal slices preloaded with either [^3H]5-HT (A) or [^3H]NE (B). Venlafaxine was administered for 21 days with osmotic minipumps implanted subcutaneously and removed 48 h before the experiments to allow drug elimination. The ordinate is the fraction of the total tissue activity released by a 2-min period of electrical stimulation (S_1 ; 360 pulses, 30 mA, 2ms, 3 Hz) applied 8 minutes after the washing period. Experiments were always carried out in pairs of rats, slices from a control rat and slices from a treated one being processed in parallel in the same perfusion apparatus. The number of slices per group is given at the bottom of each column. Statistical significance was calculated using the non-paired Student *t*-test (* $p < 0.05$).

A- [³H]5-HT release



B- [³H]NE release



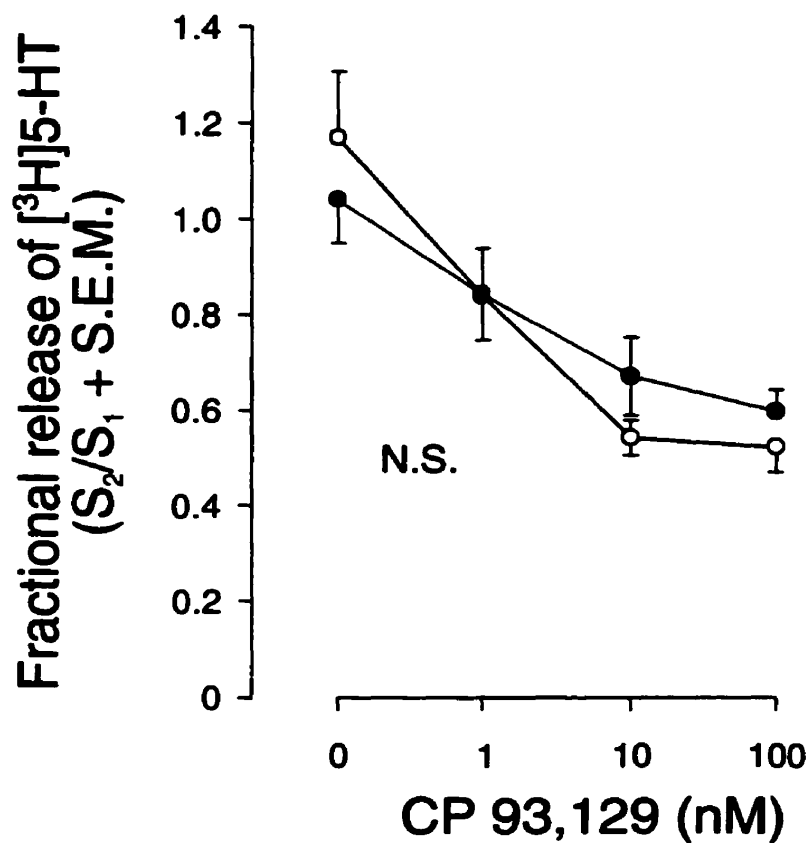
Control

Venlafaxine 10 mg/kg/day x 21 days

Venlafaxine 40 mg/kg/day x 21 days

Figure 2. Concentration-effects curves of the 5-HT_{1B} agonist CP 93,129 introduced 20 minutes before S₂, on the release of tritium ([³H]5-HT) elicited by the electrical stimulation of hippocampal slices prepared from control (open circles) and treated (filled circles) rats with 10 mg/kg/day (A) and 40 mg/kg/day (B) of venlafaxine for 21 days. Ordinate is the fraction of the total tissue radioactivity released by 360 pulses (30 mA, 2ms, 3 Hz for 2 minutes) expressed as the ratio (S₂/S₁) obtained between the second period of stimulation in the presence of CP 93,129 (S₂) and the first one done without this drug (S₁). Each point represent the mean ± S.E.M. of at least 3 experiments (usually 5 slices each) per group of pairs of control and treated rats processed in parallel. The level of statistical significance, calculated using two-way analysis of variance between the curves obtained in the control and the treated group is indicated in the graphs (NS: nonsignificant).

A. Venlafaxine 10 mg/kg/day x 21 days, s.c.



B. Venlafaxine 40 mg/kg/day x 21 days, s.c.

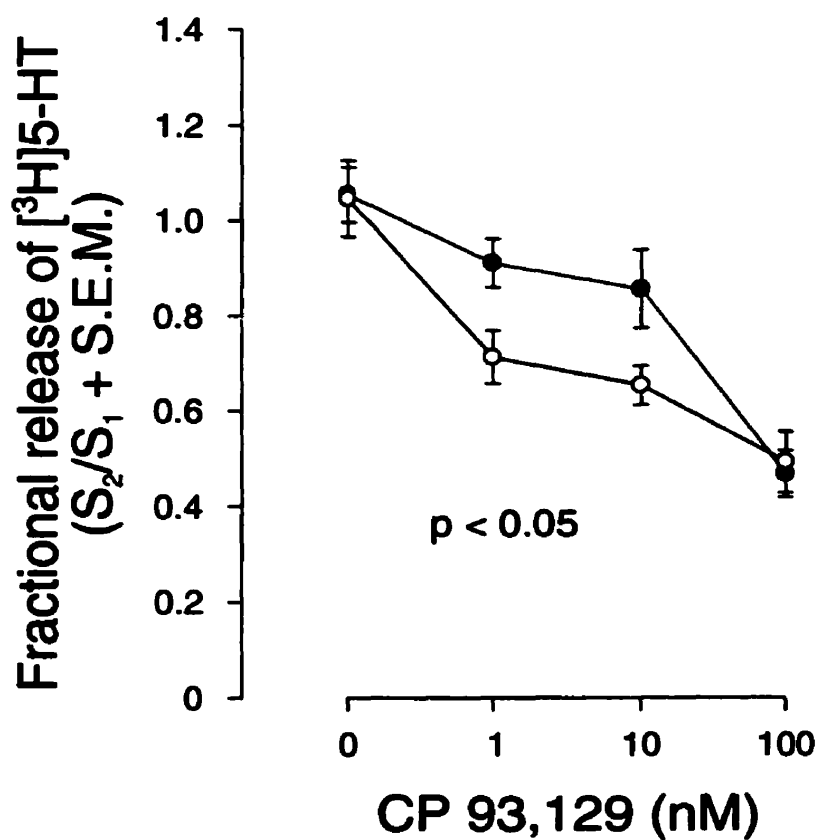
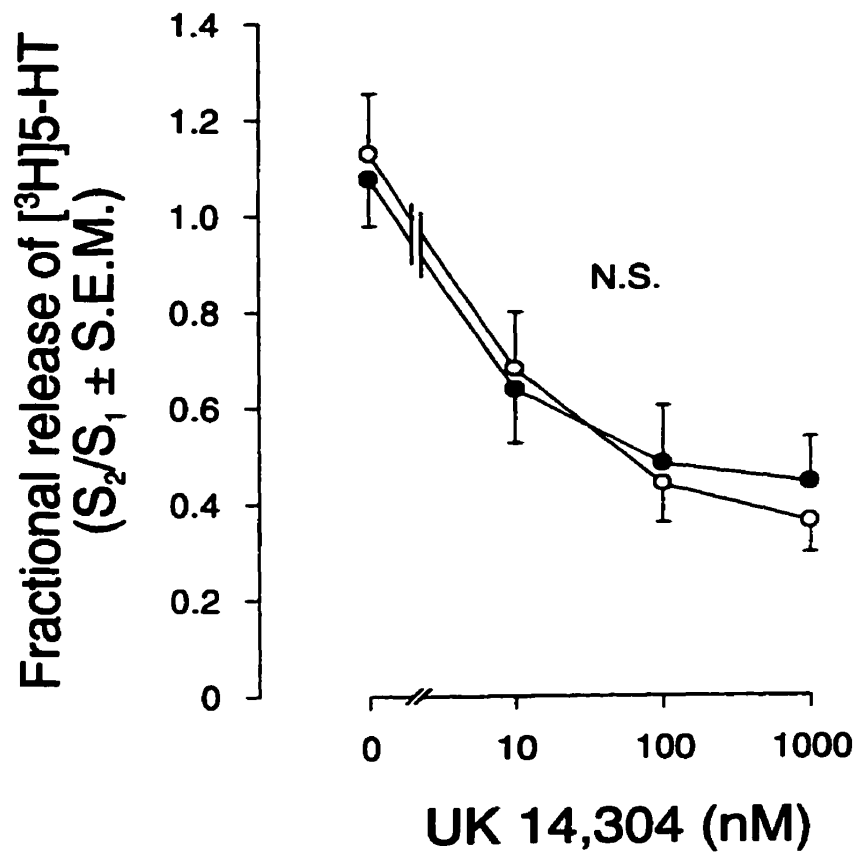


Figure 3. Concentration-effects curves of the α_2 -adrenergic receptor agonist UK 14,304 introduced 20 minutes before S_2 , on the release of tritium ($[^3\text{H}]5\text{-HT}$) elicited by the electrical stimulation of hippocampal slices prepared from control (open circles) and treated (filled circles) rats with 10 mg/kg/day (A) and 40 mg/kg/day (B) of venlafaxine for 21 days. Ordinate is the fraction of the total tissue radioactivity released by 360 pulses (30 mA, 2ms, 3 Hz for 2 minutes) expressed as the ratio (S_2/S_1) obtained between the second period of stimulation in the presence of UK 14,304 (S_2) and the first one done without this drug (S_1). Each point represent the mean $\pm$ S.E.M. of at least 3 experiments (usually 5 slices each) per group of pairs of control and treated rats processed in parallel. The level of statistical significance, calculated using two-way analysis of variance between the curves obtained in the control and the treated group is indicated in the graphs (NS: non significant).

A. Venlafaxine 10 mg/kg/day x 21 days, s.c.



B. Venlafaxine 40 mg/kg/day x 21 days, s.c.

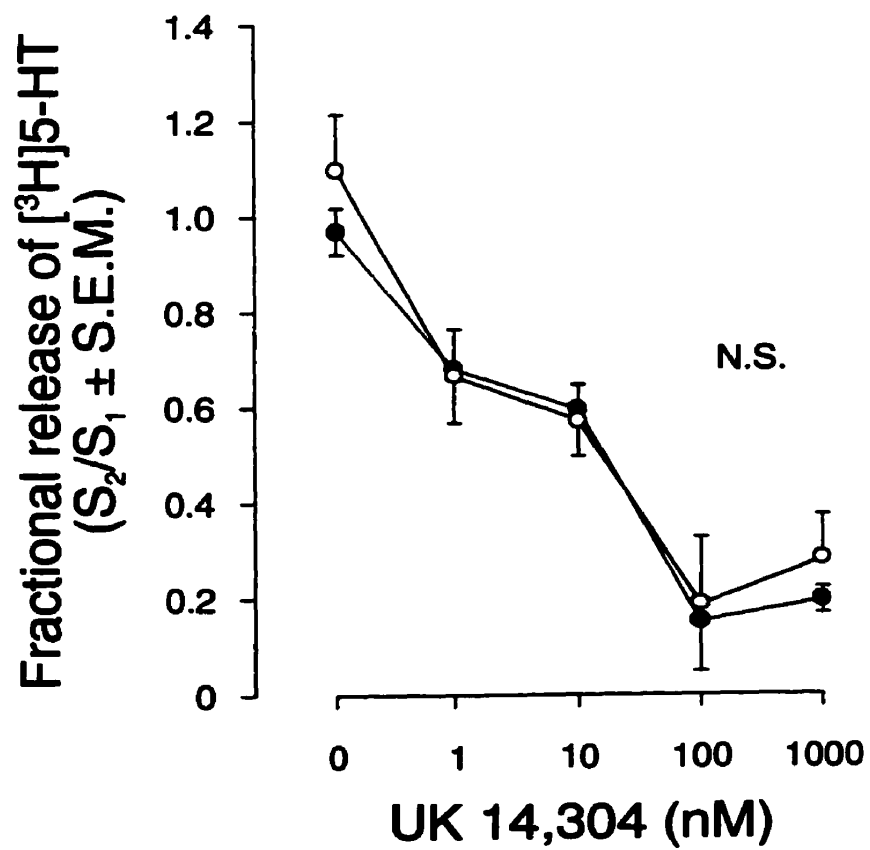


Figure 4. Concentration-effects curves of the adrenergic receptor agonist UK 14,304 introduced 20 minutes before S_2 , on the release of tritium ($[^3H]NE$) elicited by the electrical stimulation of hippocampal slices prepared from control (open circles) and treated (filled circles) rats with 10 mg/kg/day (A) and 40 mg/kg/day (B) of venlafaxine for 21 days. Ordinate is the fraction of the total tissue radioactivity released by 360 pulses (30 mA, 2ms, 3 Hz for 2 minutes) expressed as the ratio (S_2/S_1) obtained between the second period of stimulation in the presence of UK 14,304 (S_2) and the first one done without this drug (S_1). Each point represent the mean $\pm$ S.E.M. of at least 3 experiments (usually 5 slices each) per group of pairs of control and treated rats processed in parallel. The level of statistical significance, calculated using two-way analysis of variance between the curves obtained in the control and the treated group is indicated in the graphs (NS: non significant).

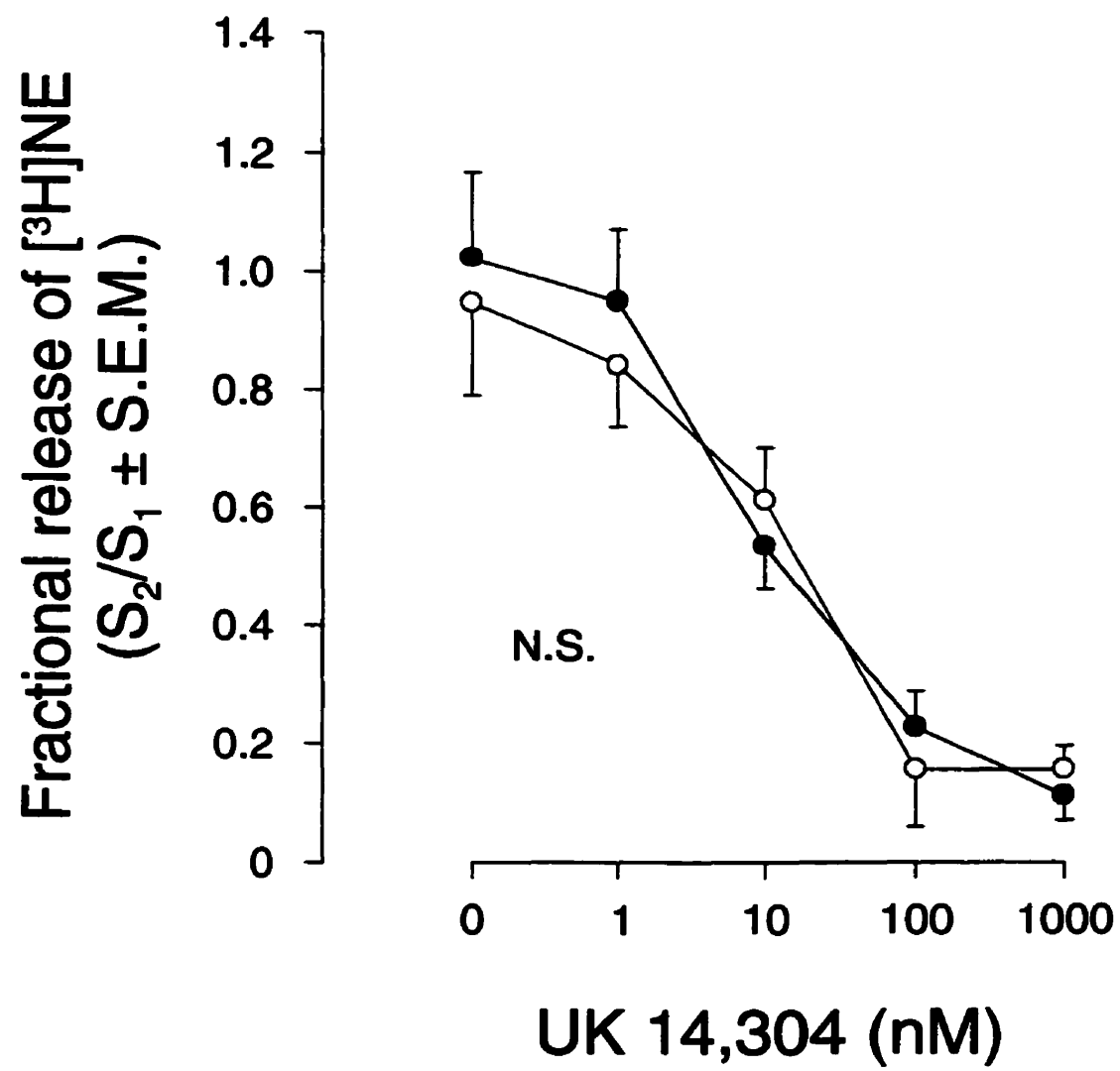
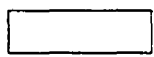
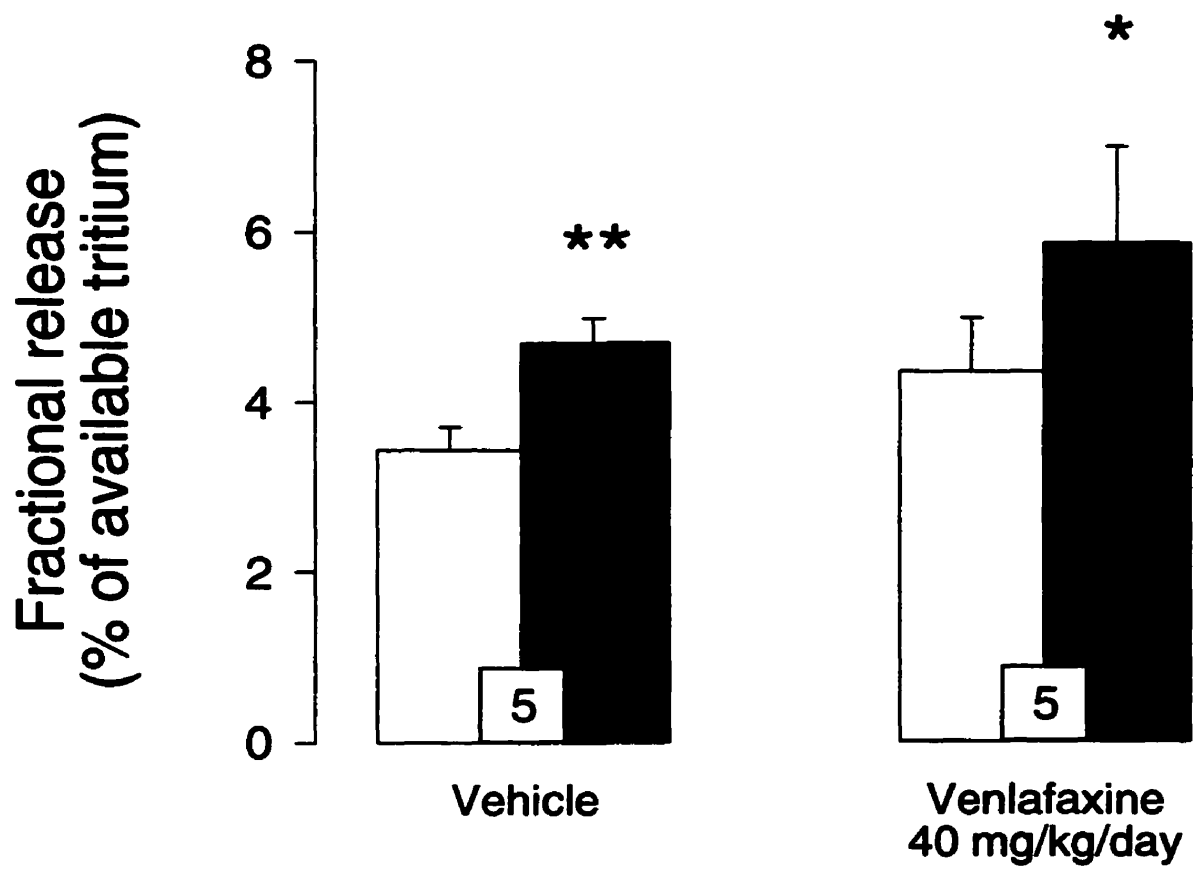


Figure 5. Effects of paroxetine ($1 \mu\text{M}$), introduced 20 minutes before S_2 , on the electrically evoked release of tritium from hippocampal slices preloaded with [^3H]5-HT prepared from controls and from rats treated with 40 mg/kg/day of venlafaxine for 21 days. Ordinate is the fraction of the total tissue radioactivity released by a 2 minute period of electrical stimulation (S_1 and S_2 ; 30 mA, 2 ms, 3 Hz). Experiments were carried out in pairs of rats, a control and a treated animal processed in parallel in the same perfusion apparatus. The number of slices is given at the bottom of each column. * $p < 0.05$, ** $p < 0.01$ using the paired Student's t -test)



S₁



S₂ in presence of
1 μM paroxetine

Table 1. Effect of long-term administrations of venlafaxine on the basal uptake of [^{3} H]5-HT from hippocampal slices or mesencephalic slices or of [^{3} H]NE from hippocampal slices¹

	Control	Venlafaxine 10 mg/kg/day	Control	Venlafaxine 40 mg/kg/day
Hippocampal slices				
Uptake of [^{3}H]5-HT	3.2 $\pm$ 0.2	2.7 $\pm$ 0.3	2.9 $\pm$ 0.1	3.2 $\pm$ 0.2
Uptake of [^{3}H]NE	2.8 $\pm$ 0.2	2.7 $\pm$ 0.2	3.2 $\pm$ 0.4	2.8 $\pm$ 0.2
Mesencephalic slices				
Uptake of [^{3}H]5-HT	6.2 $\pm$ 0.4	5.9 $\pm$ 0.3	5.4 $\pm$ 0.5	4.4 $\pm$ 0.3

¹ Data are expressed as the tissue/medium ratio of radioactivity

CHAPTER VI

PREFATORY REMARKS

Despite definite progress achieved with the newer antidepressants in terms of side effect profile and safety compared with the older tricyclic antidepressants (TCA's), all treatments currently used for major depression are still plagued by a delayed onset of therapeutic action, which is generally of about two weeks. Therefore, the impetus to develop therapeutic strategies endowed with a rapid onset of action has prompted intense research over recent years where serendipity has been replaced by rational approaches. In this regard the addition of the mixed β -adrenergic/5-HT_{1A} antagonist (-)pindolol to selected antidepressant has received tremendous attention. Indeed, there are now several reports that show that this drug combination would reduce the time to onset of therapeutic action. In this following chapter, we present a study in which we report data concerning the effects of a coadministration of (-)pindolol and venlafaxine on the 5-HT neurotransmission.

POTENTIATION BY (-)PINDOLOL OF THE ACTIVATION OF POSTSYNAPTIC 5-HT_{1A} RECEPTORS INDUCED BY VENLAFAXINE⁹.

JEAN-CLAUDE BÉIQUE, PIERRE BLIER, CLAUDE DE MONTIGNY AND GUY DEBONNEL

SUMMARY

The increase of extracellular 5-HT in brain terminal regions produced by the acute administration of 5-HT reuptake inhibitors (SSRI's) is hampered by the activation of somatodendritic 5-HT_{1A} autoreceptors in the raphe nuclei. The present in vivo electrophysiological studies were undertaken, in the rat, to assess the effects of the coadministration of venlafaxine, a dual 5-HT/NE reuptake inhibitor, and (-)pindolol on pre- and postsynaptic 5-HT_{1A} receptor function. The acute administration of venlafaxine and of

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the SSRI paroxetine (5 mg/kg, i.v.) induced a suppression of the firing activity of dorsal hippocampus CA₃ pyramidal neurons. This effect of venlafaxine was markedly potentiated by a pretreatment with (-)pindolol (15 mg/kg, i.p.) but not by the selective β -adrenoceptor antagonist metoprolol (15 mg/kg, i.p.). That this effect of venlafaxine was mediated by an activation of postsynaptic 5-HT_{1A} receptors was suggested by its complete reversal by the 5-HT_{1A} antagonist WAY 100635 (100 μ g/kg, i.v.). A short-term treatment with VLX (20 mg/kg/day x 2 days) resulted in a ca. 90% suppression of the firing activity of 5-HT neurons in the dorsal raphe nucleus. This was prevented by the coadministration of (-)pindolol (15 mg/kg/day x 2 days). Taken together, these results indicate that (-)pindolol potentiated the activation of postsynaptic 5-HT_{1A} receptors resulting from 5-HT reuptake inhibition probably by blocking the somatodendritic 5-HT_{1A} autoreceptor, but not its postsynaptic congener. These results support and extend previous findings providing a biological substratum for the efficacy of pindolol as an accelerating strategy in major depression.

KEYWORDS: *Major Depression; Antidepressant; Reuptake inhibitors; Dorsal raphe; Hippocampus; in vivo electrophysiology*

INTRODUCTION

It is becoming increasingly clear that the therapeutic efficacy of antidepressant treatments such as selective serotonin reuptake inhibitors (SSRI's) finds its substrate in an enhanced serotonergic (5-HT) neurotransmission (Blier and de Montigny 1994). At least in the rat, this occurs only in the midst of long-term administration of antidepressant treatments, which is necessary for the development of specific adaptative changes. Indeed, acute administration of SSRI's induces a reduction of firing activity of the 5-HT neurons of the raphe nuclei due to an increased activation of the somatodendritic 5-HT_{1A} autoreceptors resulting from the increased extracellular 5-HT (de Montigny *et al.* 1981; Blier *et al.* 1984; Chaput *et al.* 1986; Hajós *et al.* 1995; Béïque *et al.* 1999). However, in the course of a long-term administration of SSRI's, 5-HT neurons recover their normal firing activity as a consequence of the desensitization of these autoreceptors (Blier and de Montigny 1994).

A way by which the action of the somatodendritic 5-HT_{1A} autoreceptors can be circumvented has been delineated by the electrophysiological observation that a 2-day administration of the mixed β -adrenergic/5-HT_{1A} receptors antagonist (-)pindolol prevents the suppressant effects of paroxetine and of the 5-HT autoreceptor agonist LSD on the firing activity of dorsal raphe 5-HT (Romero *et al.* 1996). Although microdialysis studies cannot assess the postsynaptic impact, several such studies have corroborated the above by showing that the acute coadministration of (-)pindolol and an SSRI produces a greater elevation of extracellular levels of 5-HT in terminal regions than that achieved with the reuptake inhibitor alone (Dreshfield *et al.* 1996; Hjorth *et al.* 1996; Romero *et al.* 1996).

These observations have directly lead to the hypothesis which posits that the combination of 5-HT reuptake inhibitors with a blocker of the somatodendritic 5-HT_{1A} autoreceptor, by mean of preventing the initial decrease in firing activity of the 5-HT neurons, would lead to an earlier elevation of 5-HT levels and hence may shorten the delay of the antidepressant response (de Montigny *et al.* 1992; Artigas 1993). As a direct outcome of this research endeavour, it has been reported, in six of the seven double blind clinical studies assessing this issue to date, that the addition of pindolol to selected antidepressant agents is a successful strategy to shorten the delay of therapeutic action in major depression (Maes *et al.* 1996; Berman *et al.* 1997; Tome *et al.* 1997; Zanardi *et al.* 1997; Pérez *et al.* 1997; Bordet

et al. 1998; Zanardi *et al.* 1998).

Given that the postsynaptic impact of the enhanced concentration of extracellular 5-HT observed in terminals area, especially in limbic structures, following the combination of 5-HT reuptake blockers and (-)pindolol is undoubtedly of profound importance, it was deemed of interest to assess this aspect using an *in vivo* electrophysiological approach whereby the degree of activation of the postsynaptic 5-HT_{1A} receptor by endogenous 5-HT can be measured. Thus, it was first determined whether postsynaptic 5-HT_{1A} receptor activation could be induced by the intravenous administration of the dual 5-HT/norepinephrine (NE) reuptake inhibitor venlafaxine and, second, whether this activation could be potentiated by (-)pindolol.

MATERIALS AND METHODS

Male Sprague Dawley rats (250–300 g; Charles River, St. Constant, Québec, Canada) were received at least one day before the experiments and housed three to four per cage. They were kept on a 12:12 h light/dark cycle, with access to food and water *ad libitum*.

Short-term treatments

Rats were anesthetized with halothane for the subcutaneous implantation of osmotic minipumps (Alza, Palo Alto, CA, U.S.A.) that delivered for 48 h vehicle or venlafaxine (20 mg/kg/day) with or without (-)pindolol (15 mg/kg/day). Venlafaxine and (-) pindolol were dissolved in a 50% ethanol/water solution, and control rats were implanted with minipumps containing vehicle. All experiments were carried out while the minipumps were still present.

***In vivo* electrophysiological experiments**

Animals were anesthetized with chloral hydrate (400 mg/kg, i.p.) and mounted in a stereotaxic apparatus. Supplemental doses (100 mg/kg, i.p.) of chloral hydrate were administered to maintain anesthesia, monitored by the absence of a nociceptive reaction to pinching of the tail or of a hind paw. Body temperature was maintained at 37 °C throughout the experiment and a catheter was installed, prior to recording, in a lateral tail vein for intravenous administration of drugs.

Extracellular unitary recordings of CA₃ dorsal hippocampus pyramidal neurons

Five-barrelled glass micropipettes were pulled in a conventional manner and their tips were broken back to 8-12 μm under microscopic control. The central barrel, used for extracellular recordings, was filled with a 2 M NaCl solution containing fast green FCF. Two side barrels, used for microiontophoresis, were filled with the following solutions: 5-HT (20 mM in NaCl 200 mM, pH: 4), or acetylcholine (ACh; 20 mM in 200 mM NaCl, pH: 5). The third barrel was used for automatic current balancing, and contained a 2 M NaCl solution. The microelectrode was lowered 4 mm lateral and 4 mm anterior to lambda, according to stereotaxic coordinates of (Paxinos and Watson 1986). CA₃ pyramidal neurons were recorded at the depth of 3.5 to 3.8 mm from cortical surface and identified by their characteristic large-amplitude (0.5-1.2 mV) and long-duration (0.8-1.2 ms) single action potentials alternating with complex spike discharges (Kandel and Spencer 1961). A leak or a small ejecting current of ACh (0 to + 5 nA) was used to activate silent or slowly discharging neurons to a physiological firing rate of 8-14 Hz (Ranck 1973). The uptake activity following microiontophoretic applications of 5-HT was assessed using the recovery time 50 (RT₅₀) values. RT₅₀ is defined as the time in seconds, from the termination of the microiontophoretic application, required by the neuron to recover 50% of its initial firing frequency. The RT₅₀ value has been shown to provide a reliable index of the *in vivo* activity of the 5-HT reuptake process in the rat amygdala and lateral geniculate body (Wang *et al.* 1979) and in the hippocampus (Piñeyro *et al.* 1994). The RT₅₀ values following the microiontophoretic application of 5-HT was used to assess the extent of the lesioning of the 5-HT system induced by the pretreatment with the 5-HT toxin 5,7-dihydroxytryptamine (see below).

Extracellular unitary recordings of 5-HT neurons of the dorsal raphe nucleus

Single-barrelled glass micropipettes were prepared in a conventional manner (Haigler and Aghajanian 1974), with the tips broken back to 1-3 μm and filled with a 2 M NaCl solution saturated with Fast Green FCF. A burr hole was drilled on midline, 1 mm anterior to lambda for recording of dorsal raphe neurons. Spontaneously active dorsal raphe 5-HT neurons were encountered on a distance of 1 mm starting at the ventral border of the Sylvius aqueduct, and were identified using the criteria of (Aghajanian 1978): slow, regular firing rate (0.5-2.5 Hz) and positive action potential of long duration (0.8-1.2 ms). The mean firing

activity of dorsal raphe 5-HT neurons was determined by carrying out systematic microelectrode descents in the dorsal raphe while recording the firing activity of encountered 5-HT neurons. Usually, five microelectrode descents were carried out in the dorsal raphe of each rat.

5,7-Dihydroxytryptamine pretreatments

Rats weighing between 175 and 200 g were anesthetized with chloral hydrate (400 mg/kg, i.p.). Lesions of 5-HT neurons were performed by injecting the 5-HT neurotoxin 5,7 dihydroxytryptamine (5,7-DHT; 200 μ g of free base in 20 μ l of 0.9% NaCl and 0.1% ascorbic acid) intracerebroventricularly (1 mm lateral; 0.5 mm posterior to the coronal suture and 2- 2.5 mm below the surface of the brain) 1 h after the injection of desipramine (25 mg/kg, i.p.) in order to protect NE neurons. The electrophysiological experiments were carried out 14 to 16 days after the lesions were made.

Drugs

Venlafaxine and WAY 100635 were given by Wyeth-Ayerst Research, NJ, U.S.A., while paroxetine was given by Smith Kline Beecham, West Sussex, U.K.. 5-HT, ACh and metoprolol were purchased from Sigma Chemical, St-Louis, MO, U.S.A. (-) Pindolol was purchased from RBI chemicals, Natick, MA, U.S.A. Drugs were either prepared in physiological saline for i.v. administration or in a 200 mM NaCl solution for microiontophoretic applications. (-)Pindolol was first dissolved in a small volume of ethanol, then diluted to the appropriate concentration with saline for its acute i.p. administration in a volume of *ca.* 1 cc.

Statistical analysis

Results are expressed throughout as means $\pm$ S.E.M., unless otherwise specified. When means are compared, the statistical significance of the difference was assessed using either a paired, a non-paired Student's *t*-test or ANOVA, as indicated in the legend to figures. Probability was considered as statistically significant when the *p* was smaller than 0.05.

RESULTS

Effects of acute administration of venlafaxine and paroxetine on the firing activity of dorsal hippocampus CA₃ pyramidal neuron.

Hippocampal pyramidal neurons were brought to a physiological (8-14 Hz; Ranck 1973) firing frequency by the sustained leak or microiontophoretic application of ACh (0 to +5 nA) and recorded for at least 2 minutes to establish baseline firing activity. The acute intravenous administration of 5 mg/kg of venlafaxine (Figure 1A and 1C) or paroxetine (Figure 1B and 1D) resulted in a 73% and 80%, respectively, suppression of the firing activity of CA₃ pyramidal neurons. These effects were completely reversed by the subsequent intravenous administration (100 µg/kg) of the 5-HT_{1A} antagonist WAY 100635 (Forster et al., 1995; Fletcher et al., 1996; see Figure 1). Moreover, and as previously reported (Haddjeriet al. 1998a), the acute intravenous administration of WAY 100635 (100 µg/kg) did not significantly alter the firing activity of dorsal hippocampus CA₃ pyramidal neurons (data not show). However it significantly reduced the suppressant effect of the microiontophoretic application of 5-HT (number of spikes suppressed; $p < 0.01$ using the paired Student's *t*-test; Figure 2). These results indicate that the dose administered was sufficient to block postsynaptic 5-HT_{1A} receptors without altering the basal firing activity of the neuron.

Effect of a 5,7-dihydroxytryptamine lesion on the suppressant effect on the firing activity of dorsal hippocampus CA₃ pyramidal neurons induced by the acute administration of venlafaxine.

The effect of the acute intravenous administration of venlafaxine on the firing activity of CA₃ pyramidal neurons was assessed in rats that had been treated with the serotonergic neurotoxin 5,7-dihydroxytryptamine (5,7-DHT). First, the effectiveness of the lesion of the 5-HT system was determined by assessing the reuptake activity following the microiontophoretic applications of 5-HT. This was achieved using the RT₅₀ values method which provides an index on the reuptake activity of microiontophoretically applied 5-HT by serotonergic terminals (Piñeyroet al. 1994). These microiontophoretic applications of 5-HT were performed in the same control and 5,7-DHT-lesioned rats from which the effects of the intravenous administration of venlafaxine were to be subsequently determined . As

exemplified in figure 3A and 3B, the RT_{50} values following 5-HT applications in lesioned rats were greater than those obtained in control rats ($p < 0.01$, using the non-paired Student's *t*-test). *Post hoc* analysis revealed that, in all five lesioned-rats tested, the RT_{50} values following the microiontophoretic applications of 5-HT (mean value of 103 ± 15 seconds) were greater than the mean value obtained in control rats, which was of 44 ± 8 seconds. This confirmed that the 5-7-DHT lesion had destroyed 5-HT neurons in all lesioned animals. This lesion, as can be seen from figure 3C and 3D, prevented the suppressant effect of venlafaxine on the firing activity of CA_3 pyramidal neurons, even when venlafaxine was administered at cumulative doses up to 10 mg/kg, i.v..

Effects of pre-treatments with (-)pindolol and with metoprolol on the suppression of the firing activity of dorsal hippocampus CA_3 pyramidal neurons by venlafaxine

The acute intravenous administration of two successive doses of 500 μ g/kg of venlafaxine induced a dose-dependent suppression of the firing activity of dorsal hippocampus CA_3 pyramidal neurons (one-way ANOVA, $p < 0.05$; Figure 4A and 5) in rats that had received 20 min. before an injection of saline (1 cc, i.p.). The degree of suppression of firing activity by the same two successive doses of venlafaxine was significantly greater in rats pre-treated with 15 mg/kg i.p. of (-) pindolol (two-way ANOVA, $p < 0.01$; figure 4B and 5). However, a pretreatment with 15 mg/kg i.p. of the selective β -adrenergic antagonist metoprolol (Ablad *et al.* 1973) failed to alter the degree of suppression induced by the same two doses of venlafaxine (two-way ANOVA, $p = 0.29$; Figure 5). In all three groups, the acute administration of the 5-HT_{1A} antagonist WAY 100635 (100 μ g/kg, i.v.) reversed the suppressant effect of venlafaxine to or above baseline value (Figure 5).

Effects of two-day treatments with venlafaxine alone and with venlafaxine and (-)pindolol on the firing activity of dorsal raphe 5-HT neurons

Several studies from our laboratory have shown that, in the course of a sustained administration of a 5-HT reuptake blocking agent, the degree of suppression of firing activity of 5-HT neurons is maximal at two days of treatment (Blier *et al.* 1984; Chaput *et al.* 1986). We have thus made use of this paradigm to assess the ability of (-)pindolol to prevent the effect of venlafaxine on somatodendritic 5-HT_{1A} receptors. A total of 218 serotonergic neurons were recorded during multiple electrode descents (of *ca.* 750 μ m each) in the dorsal

raphe of control rats and of rats treated with 20 mg/kg/day of venlafaxine with or without (-)pindolol (15 mg/kg/day, s.c.) for 2 days while the minipumps were still in place (Figure 6A andB). In control rats, 5-HT neurons displayed a mean firing activity of about 1 Hz (Figure 6B). A two-day treatment with venlafaxine (20 mg/kg/day s.c. delivered by an osmotic minipump) reduced by 88 % the spontaneous firing activity of dorsal raphe 5-HT neurons ($p < 0.01$, using the Student's *t*-test; Figure 6B). In rats treated concomitantly with venlafaxine (20 mg/kg/day, s.c.) and (-)pindolol (15 mg/kg/day, s.c.), the mean firing activity of dorsal raphe neurons was no different than that obtained in control rats (Figure 6B).

DISCUSSION

The data presented herein show that the acute intravenous administration of venlafaxine and that of paroxetine, two 5-HT reuptake inhibitors, induced a suppression of firing activity of CA₃ dorsal hippocampus pyramidal neurons, which effect was reversed by the 5-HT_{1A} antagonist WAY 100635. Moreover, a pretreatment with the mixed β -adrenoceptor/5-HT_{1A} antagonist (-)pindolol, but not with the selective β -adrenoceptor antagonist metoprolol, potentiated the suppression of firing activity of CA₃ pyramidal neurons induced by venlafaxine. Finally, the observation that a two-day treatment with the coadministration of (-)pindolol and venlafaxine prevented the suppression of firing activity of dorsal raphe 5-HT neurons induced by a two-day treatment with venlafaxine alone, confirmed the antagonistic activity of (-)pindolol at the level of the somatodendritic 5-HT_{1A} autoreceptor.

The acute intravenous administration of 5-HT reuptake inhibitors suppresses the firing activity of the 5-HT neurons of the dorsal and median raphe nuclei (de Montigny *et al.* 1981; Blier *et al.* 1984; Chaput *et al.* 1986; Hajós *et al.* 1995) due to an enhanced activation of the somatodendritic 5-HT_{1A} autoreceptor by an increased level of extracellular 5-HT in the raphe (Adell and Artigas 1991; Bel and Artigas 1993; Hajós *et al.* 1995; Gartside *et al.* 1995; Rutter *et al.* 1995; Béïque *et al.* 1999). This is consistent with the fact that the potencies with which reuptake inhibitors suppress the firing activity 5-HT neurons are correlated with their affinities for the 5-HT transporter (Béïque *et al.* 1999). The functional outcome and relative importance of the suppression of firing activity of 5-HT neurons by the acute administration of 5-HT reuptake inhibitors were put into perspective by *in vivo* microdialysis studies which showed that the acute systemic administration of 5-HT reuptake inhibitors results in an enhanced level of 5-HT in terminal regions and this, despite the presence of presumably silent 5-HT neurons (Invernizzi *et al.* 1995; Kreiss and Lucki 1995; Malagie *et al.* 1995). In light of these data, it can be assumed that the suppression of the firing activity of pyramidal neurons of the CA₃ region of the dorsal hippocampus produced by the acute intravenous administration of venlafaxine and paroxetine, is mediated by the activation of the inhibitory postsynaptic 5-HT_{1A} receptors by the increased level of endogenous 5-HT. This is supported by the observation that this suppression was reversed by the selective 5-HT_{1A} antagonist

WAY 100635 (figure 1). These 5-HT_{1A} receptors, which also mediate the suppressant effect of microiontophoretically-applied 5-HT (Figure 2), are indeed known to have an inhibitory action on firing activity mediated by the hyperpolarization of the cells through opening of a potassium channel (Andrade *et al.* 1986). Moreover, the fact that the intravenous administration of the 5-HT_{1A} agonists flesinoxan, 8-OH-DPAT and flibanserin suppresses the firing activity of dorsal hippocampus CA₃ pyramidal neurons (Hadrava *et al.* 1995; Rueter *et al.* 1998) corroborates this conclusion. The observation that a lesion of the 5-HT system with 5,7-dihydroxytryptamine (5,7-DHT) prevented the suppressant effect of paroxetine (Piñeyro *et al.* 1994) as well as that of venlafaxine (Figure 3C and 3D) on the firing activity of CA₃ pyramidal neurons also provides strong credence to the idea that their suppressant effects result from increased levels of extracellular 5-HT subsequent to reuptake blockade. Given that venlafaxine blocks also the reuptake of NE, albeit with a weaker potency than that of 5-HT (Muth *et al.* 1986; Béïque *et al.* 1998a; Béïque *et al.* 1998b; Béïque *et al.* 1999), the possibility that this property could, at least in part, mediate the suppressant effect of venlafaxine on the firing activity of CA₃ pyramidal neurons has to be considered. The abolishment of this suppressant effect of venlafaxine by 5,7-DHT, combined with the observation that WAY 100635 completely reversed the effect of venlafaxine, strongly infers that the suppressant effect of venlafaxine was mainly the result of its effect on the 5-HT rather than on the NE system. This contention is further supported, first, by the lack of suppressant effect of intravenous administration of the NE reuptake inhibitor desipramine on the firing activity of hippocampal pyramidal neurons (Lacroix *et al.* 1991; Curet *et al.* 1992; Béïque *et al.* 1998a) and second, by the fact at the dose used in the present study (5 mg/kg, i.v.), venlafaxine does not block, *in vivo*, the uptake of NE in the hippocampus (Béïque *et al.* 1998a).

The suppression of firing activity of CA₃ pyramidal neurons induced by venlafaxine was potentiated by a pretreatment with (-)pindolol (Figure 5). Since this suppression was also totally reversed by the subsequent intravenous administration of the 5-HT_{1A} antagonist WAY 100635, these results suggest that (-)pindolol potentiates the degree of activation of postsynaptic 5-HT_{1A} receptors induced by venlafaxine. Several possibilities must be envisaged in the attempt to identify the pharmacological properties of (-)pindolol that underlie this potentiation. Some relevant issues will hence be discussed below.

The primary clinical indication of pindolol is for the treatment of mild to moderate

hypertension which is ought to its β -adrenergic receptor blockade. That the effects observed herein with (-)pindolol are not consequent of the latter property is shown by the observation that a pretreatment with metoprolol, a mixed $\beta_{1/2}$ -adrenergic antagonist devoid of significant affinity for 5-HT_{1A} receptors, failed to potentiate the effect of venlafaxine in the present paradigm (Figure 5). Importantly, this is fully consistent with the clinical observation of Zanardi *et al.* (1997), that metoprolol failed to accelerate the antidepressant response of paroxetine.

(-)Pindolol bears significant affinity for the 5-HT_{1B} receptor (Hoyer *et al.* 1985) which is known to negatively regulate the release of 5-HT in terminal areas of the rat brain (Engel *et al.* 1986). Given that in functional assays (-)pindolol acts as an antagonist at the latter receptor (Schoeffter and Hoyer 1989), it is plausible that the latter property could account, at least in part, for the potentiating effect of (-)pindolol on the suppressant effect of venlafaxine on the firing activity of hippocampal neurons that was observed in the present study. Nonetheless, while *in vivo* microdialysis studies suggest that a 5-HT_{1B} antagonism may increase the output of 5-HT elicited by 5-HT reuptake inhibitors, there is evidence that this would only be so in the presence of normal firing of 5-HT neurons, *i.e.*, in the presence of an adequate 5-HT_{1A} autoreceptor blockade (Hjorth 1993; Sharp *et al.* 1997). The 5-HT_{1A} antagonism of (-)pindolol thus appears to be a *sine qua non* condition for its 5-HT_{1B} antagonistic property to be of functional relevance. However, the involvement of the antagonism of the 5-HT_{1B} by (-)pindolol in mediating its potentiating effect cannot be readily ruled out in light of the data presented herein. Nevertheless, one must underscore the fact that the 5-HT_{1B} antagonism of (-)pindolol does not account, most probably, for the efficacy of (-)pindolol as an adjunct in major depression and this, for the following reason. In the human brain, it has been demonstrated that terminal 5-HT release is controlled by the human 5-HT_{1B} receptor (h5-HT_{1B}; Fink *et al.*, 1995; using the nomenclature put forward by Hartig *et al.* 1996). Even if the rat 5-HT_{1B} (r5-HT_{1B}) and h5-HT_{1B} receptor share high sequence homology (Voigt *et al.* 1991; Weinshank *et al.* 1992), they have important pharmacological differences one of which being that the h5-HT_{1B} does not bind (-)pindolol with high affinity (Hartig *et al.* 1992; Parker *et al.* 1993). Thus, these results indicate that the usefulness of (-)pindolol in major depression is unlikely to be due to an antagonistic activity at the r5-HT_{1B} receptor since the latter receptor does not have a pharmacological equivalent in humans (for

review, see Hartig *et al.* 1992; Hartig *et al.* 1996).

Previous studies have shown that the blockade of the somatodendritic 5-HT_{1A} autoreceptors potentiates the elevation of extracellular levels of 5-HT in terminal areas induced by 5-HT reuptake inhibitors (Hjorth *et al.* 1993; Gartside *et al.* 1995; Dreshfield *et al.* 1996; Hjorth *et al.* 1996; Romero *et al.* 1996). This contention has also been verified with venlafaxine in a recent report published during the preparation of this manuscript (Gur *et al.* 1999). These *in vivo* microdialysis studies do not however ascertain the overall 5-HT neurotransmission as they only can assess the level of extracellular 5-HT and not its effect on the postsynaptic target neurons. The importance of this is exemplified by WAY 100635 which possesses antagonistic properties both at the level of somatodendritic and postsynaptic 5-HT_{1A} receptors (Hajós *et al.* 1995; Béïque *et al.* 1999; present study). Indeed, while this drug potentiates the rise in extracellular level of 5-HT induced by SSRI's, it concurrently blocks postsynaptic 5-HT_{1A} receptors thereby partly cancelling out the effect of the enhanced 5-HT concentration on overall 5-HT neurotransmission (Figure 1 and 2). Given that activation of postsynaptic 5-HT_{1A} receptors in limbic areas may be involved in the antidepressant response (de Montigny *et al.* 1992), these results suggest that WAY 100635 most likely would not be an effective adjunct in the treatment of major depression, as previously suggested (Gartside *et al.* 1995). In this respect, (-)pindolol differs from WAY 100635. Indeed, whereas at the level of the dorsal raphe it prevented the suppression of the firing activity of 5-HT neurons by two-day treatment of venlafaxine (Figure 6), it did not block the effect of the venlafaxine-induced rise of endogenous on postsynaptic 5-HT_{1A} receptors of CA₃ pyramidal neurons of the dorsal hippocampus (Figure 4 and 5). WAY 100635, on the other hand, has been shown to antagonize, contrarily to (-)pindolol, the effects of endogenous 5-HT on 5-HT_{1A} receptors both in the hippocampus (Figure 1) and in the dorsal raphe (Béïque *et al.* 1999). These observations thus demonstrate functionally that (-)pindolol, but not WAY 100635, shows a discriminative antagonism between somatodendritic and hippocampal postsynaptic 5-HT_{1A} receptors, as it blocked the former and not the latter. Taken together, these results thus suggest that the antagonism of somatodendritic 5-HT_{1A} receptors in the absence of a blockade of their postsynaptic congeners by (-)pindolol is the most likely mechanism by which it potentiates the activation of postsynaptic 5-HT_{1A} receptors resulting from 5-HT reuptake inhibition. Moreover, the complementary use of the different paradigms in the present study should prove useful to

assess the properties of molecules with a putative differential antagonistic activities on somatodendritic vs postsynaptic 5-HT_{1A} receptors.

The discriminative pre- and postsynaptic 5-HT_{1A} receptor antagonism of (-)pindolol may appear surprising since there is not yet any definite evidence provided by molecular biology that these two populations of receptors are distinct entities. However, several lines of evidence suggest that the somatodendritic 5-HT_{1A} receptor and its postsynaptic congener have distinct physiological and pharmacological properties (reviewed in de Montigny and Blier 1992). Nonetheless, it needs to be mentioned that in the wake of the increasing interest aroused by the usefulness of pindolol in major depression, a considerable amount of apparent conflicting results has emerged. For instance, it has been shown that when administered acutely, (-) or (±)pindolol in the awake cat (Fornal *et al.* 1997) as well as (±)pindolol in the anesthetized rat (Haddjeri *et al.* 1998b), suppresses the firing activity of 5-HT neurons in the dorsal raphe. This effect appears to be mediated by an agonistic action of pindolol on somatodendritic 5-HT_{1A} receptors as, in both studies, it was reversed by the subsequent acute intravenous administration of WAY 100635. Moreover, the intravenous administration of pindolol, as well as its microiontophoretic application directly onto dorsal raphe 5-HT neurons, have been reported to induce a suppression of firing activity (Clifford *et al.* 1998). Overall, these results suggesting an agonistic activity of pindolol at somatodendritic 5-HT_{1A} receptors are in apparent discrepancy with the present ones showing that a two-day treatment of (-)pindolol produces an efficient antagonism on this population of receptors (Figure 6). Moreover, there is other evidence suggesting an antagonism of (-)pindolol on somatodendritic 5-HT_{1A} receptors. In particular, Romero *et al.* (1996) have observed that a two-day treatment with (-)pindolol prevents the suppressant effect on the firing activity of 5-HT neurons of 1) paroxetine (administered for two days), 2) of microiontophoretically applied 5-HT and 8-OH-DPAT and 3) of the systemic administration of the 5-HT autoreceptor agonist LSD. In keeping with these observations, when administered intravenously at low doses to rats, pindolol prevents the suppressant effect of intravenous administration of the 5-HT receptor agonist LSD on the firing activity of dorsal raphe 5-HT neurons (Haddjeri *et al.* 1998b). Although, this antagonism was not observed in the awake cats and in the anaesthetized rats with the use of systemic administration of the 5-HT_{1A} agonist 8-OH-DPAT (Fornal *et al.* 1997), *in vitro* electrophysiological studies in the rat have demonstrated that (-)pindolol antagonizes the inhibitory action of 5-CT and ipsapirone on the

firing activity of dorsal raphe 5-HT neurons in mesencephalic slices (Corradetti *et al.* 1998), thus giving further credence to the notion that pindolol possesses somatodendritic 5-HT_{1A} receptor antagonistic activity. Part of the answer to these inconsistencies may lie in the observation that a low dose of (-)pindolol is more effective than a higher dose in antagonizing the decrease of extracellular levels of hippocampal 5-HT induced by 8-OH-DPAT (Assié and Koek 1996). Although definite evidence remains to be provided, the most likely explanation for these apparent discrepancies is that pindolol would display a predominant agonistic activity at high concentrations and an antagonistic one at low concentrations. The latter contention is however questionable in light of the apparent discordant observation observed at the postsynaptic level also. Using the *in vitro* hippocampal slice preparation, it was shown that pindolol readily blocks the effect of 5-CT (Corradetti *et al.* 1998), whereas *in vivo* electrophysiological studies suggest that pindolol does not antagonize in the dorsal hippocampus microiontophoretically applied 5-HT (Romero *et al.* 1996) as well as endogenous 5-HT (present study) where the concentration of the drug is likely to be lower. Interestingly, using a biochemical assay (stimulation of [³⁵S]-GTPγS binding in Chinese Hamster Ovary cells) a recent study has shown that whilst (-)pindolol displayed intrinsic activity (although modest) on human-5-HT_{1A} receptors mediated function, it also blocked the action of 5-HT itself, thus showing a clear partial agonistic activity (Newman-Tancredi *et al.* 1998). Thus, the notion of a partial agonistic activity of pindolol may reconcile some of the apparently discrepant effects observed using different experimental conditions and approaches.

The results presented herein must also be considered in a clinical perspective. There is now a large body of evidence suggesting that the activation of the postsynaptic 5-HT_{1A} receptor plays a prominent role in the therapeutic action of antidepressants, thus validating the clinical significance and relevance of attaining its earlier activation (Kurtz 1992; Haddjeri *et al.* 1998a). Therefore, that (-)pindolol acts by circumventing the homeostatic activity of the inhibitory somatodendritic 5-HT_{1A} autoreceptor in a way to potentiate the activation of postsynaptic 5-HT_{1A} receptors provides a tenable and attractive hypothesis for its clinical effectiveness as an accelerating strategy in the treatment of major depression.

It has been suggested that venlafaxine is endowed with a faster onset of action in major depression and that it is effective for treatment-resistant depression (Nierenberg *et al.*

1994; Derivan *et al.* 1995; de Montigny *et al.* 1999). Since the latter phenomena supervene only when high doses are administered, it has been suggested that they would be attributable to the recruitment of the noradrenergic system through NE reuptake blockade as this occurs only when high doses are administered to rats (Béïque *et al.* 1998a). Thus, in light of the results presented herein, one can contemplate the possibility that the combination of pindolol and venlafaxine might prove a powerful strategy to hasten the antidepressant response as well to treat refractory depression.

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LEGENDS TO FIGURES

Figure 1. Integrated firing rate histograms showing the effects of acute intravenous administration of venlafaxine (A) and of paroxetine (B) on the firing activity of an ACh-activated dorsal hippocampus CA₃ pyramidal neuron. The effect of a subsequent acute intravenous administration of the selective 5-HT_{1A} antagonist WAY 100635 is also shown. The suppressant effects of venlafaxine (C) and of paroxetine (D) as well as their reversal by the intravenous administration of WAY 100635 (100 µg/kg) on the firing activity of CA₃ pyramidal neurons are expressed as a percentage (mean ± S.E.M.) of the basal firing activity. **p* < 0.01 compared to pre-administration values using the paired Student's *t*-test. In this and subsequent figures, the numbers in the columns represent the numbers of neurons tested.

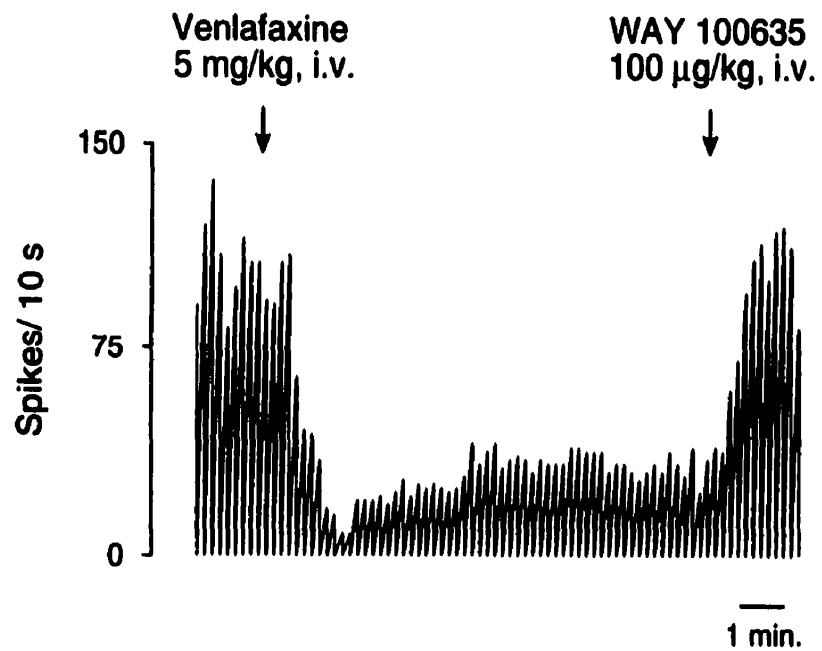
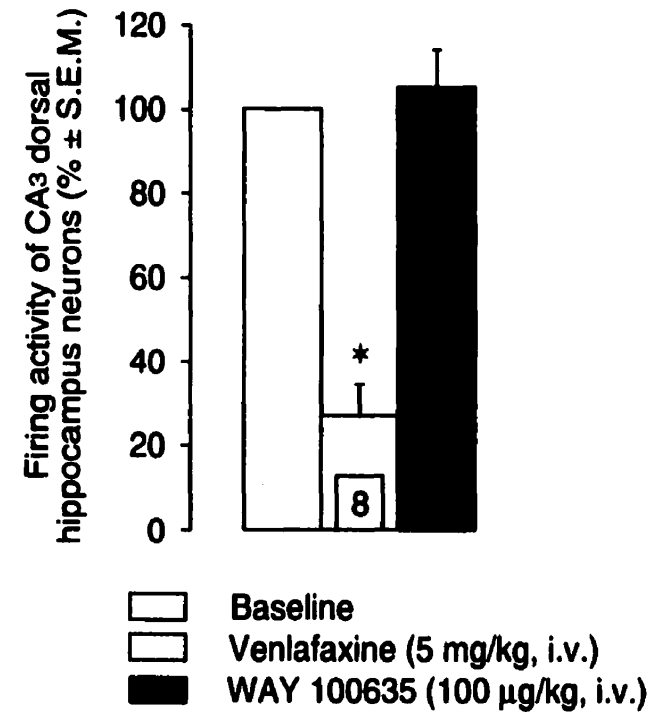
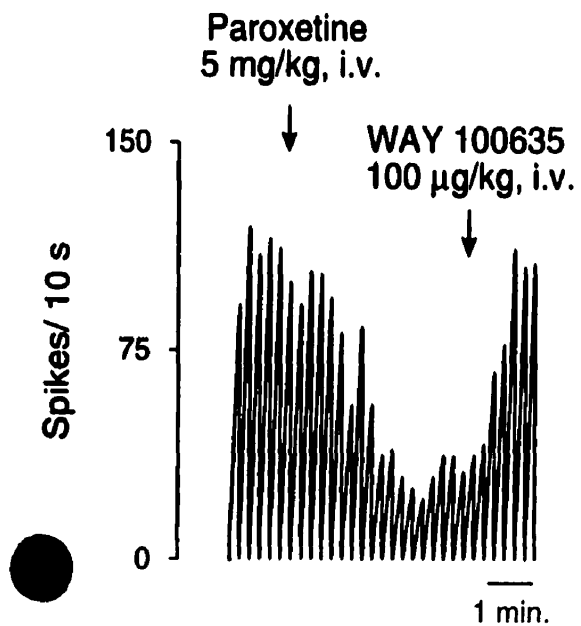
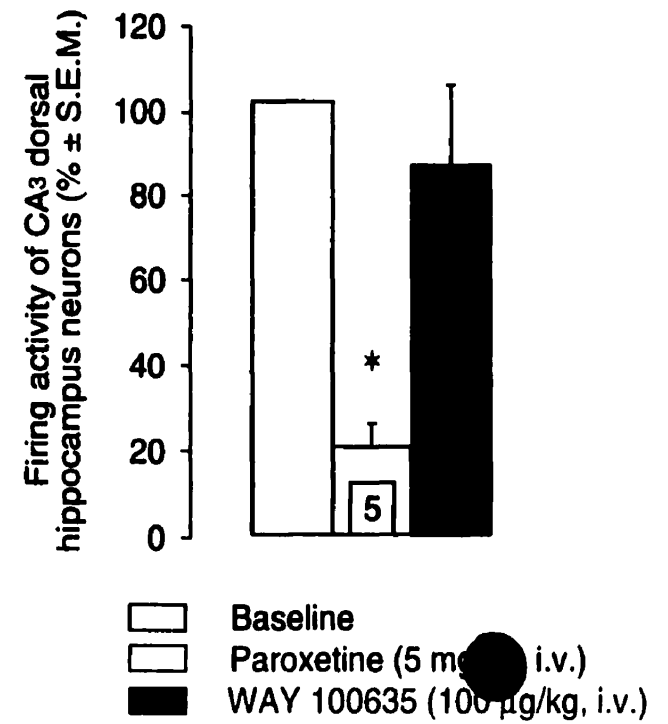
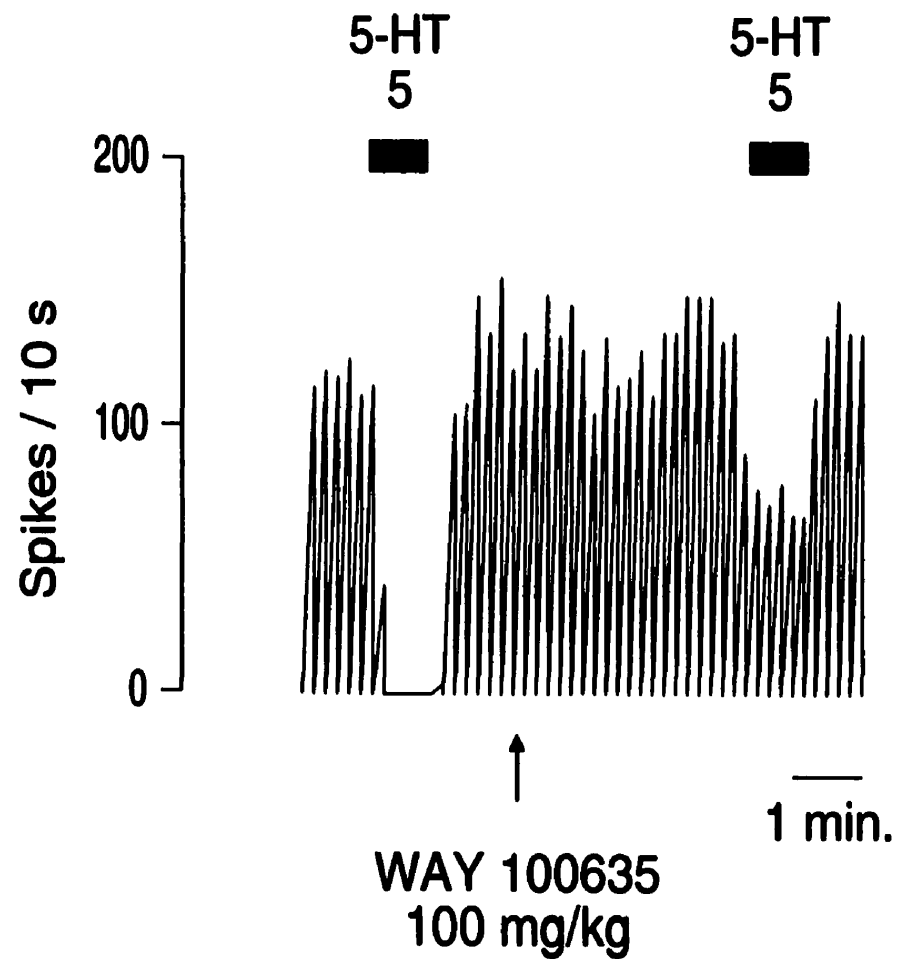
A.**C.****B.****D.**

Figure 2. (A) Integrated firing rate histogram of a CA₃ pyramidal neuron showing the effects of microiontophoretic applications of 5-HT (5 nA) before and after the intravenous administration of WAY 100635. The solid bars above the trace indicate the duration of the applications for which the ejection current is given in nA. (B) Effectiveness, expressed as the number of spikes suppressed, of microiontophoretic applications of 5-HT before and after the intravenous administration of WAY 100635. * $p < 0.01$ using the paired Student's t -test.

A.



B.

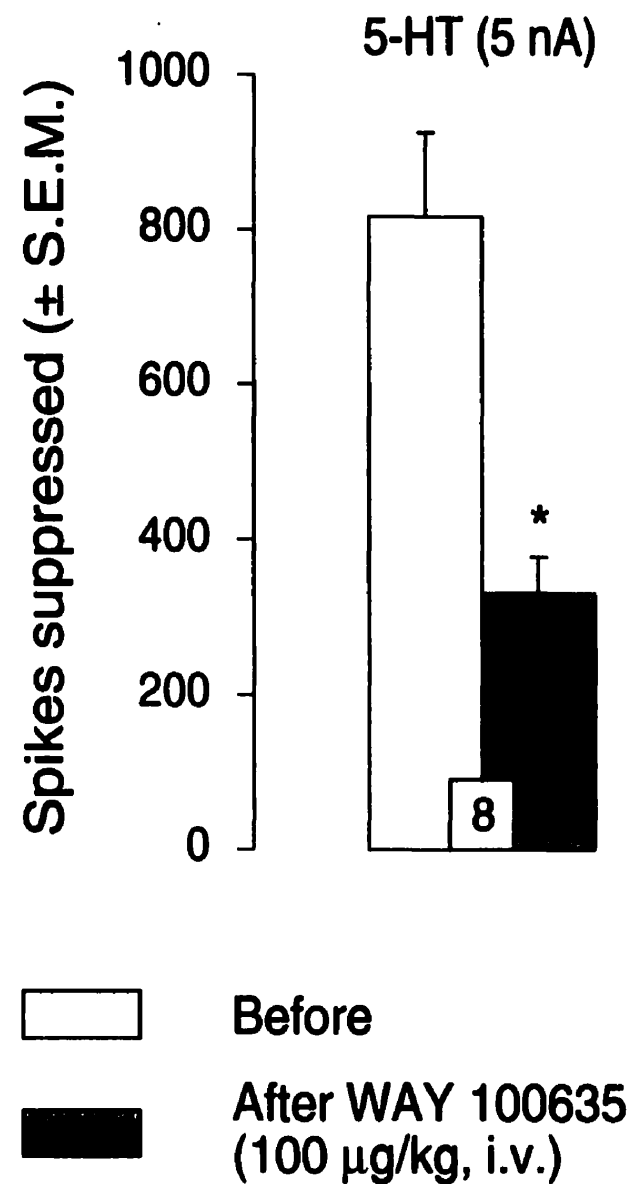


Figure 3. Integrated firing rate histograms of two CA₃ dorsal hippocampus pyramidal neurons showing the effects of microiontophoretic applications of 5-HT in control and in 5,7-DHT-lesioned rats (A). The solid bars above each trace indicate the duration of the applications for which the ejection current is given in nA. The recovery time, expressed as RT₅₀ values (means $\pm$ S.E.M.), of CA₃ dorsal hippocampus neurons obtained with microiontophoretic applications of 5 nA of 5-HT on ACh-induced firing activity in control and in 5,7-DHT-lesioned rats is shown in B. * $p < 0.01$, compared to control values using the non-paired Student's *t*-test. The integrated firing rate histogram in C shows the effect of acute intravenous administration of venlafaxine on the firing activity of an ACh-activated dorsal hippocampus CA₃ pyramidal neuron of a 5,7-DHT lesioned rat. The effect of a subsequent acute intravenous administration of the selective 5-HT_{1A} antagonist WAY 100635 is also shown. The two dots represent an interval of 2 min. The effect of venlafaxine (D), as well as that of the intravenous administration of WAY 100635 (100 μ g/kg), on the firing activity of CA₃ pyramidal neurons of 5,7-DHT-lesioned rats are expressed as a percentage (mean $\pm$ S.E.M.) of the basal firing activity.

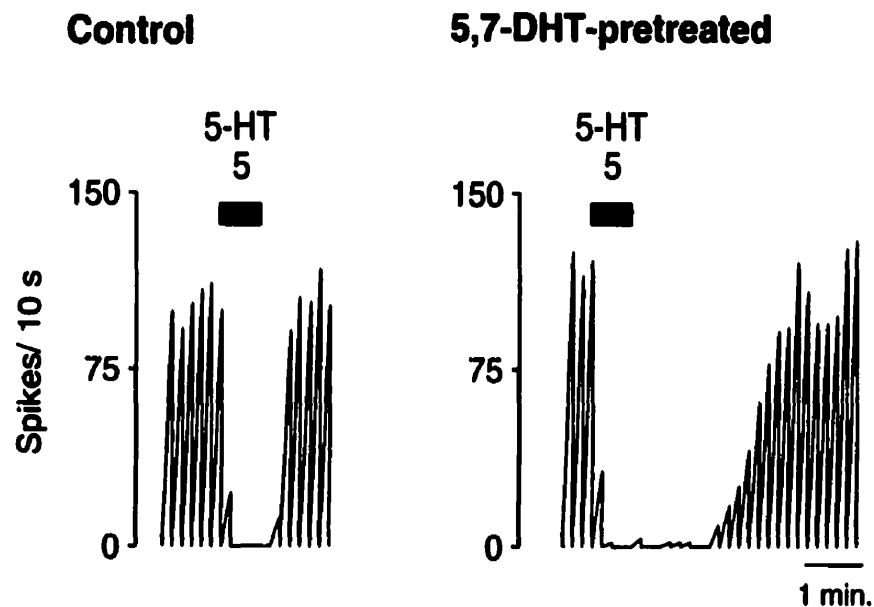
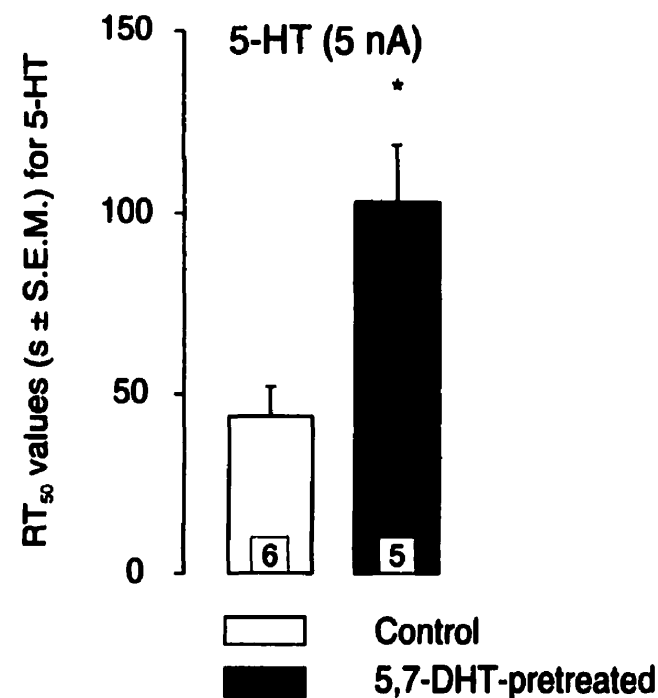
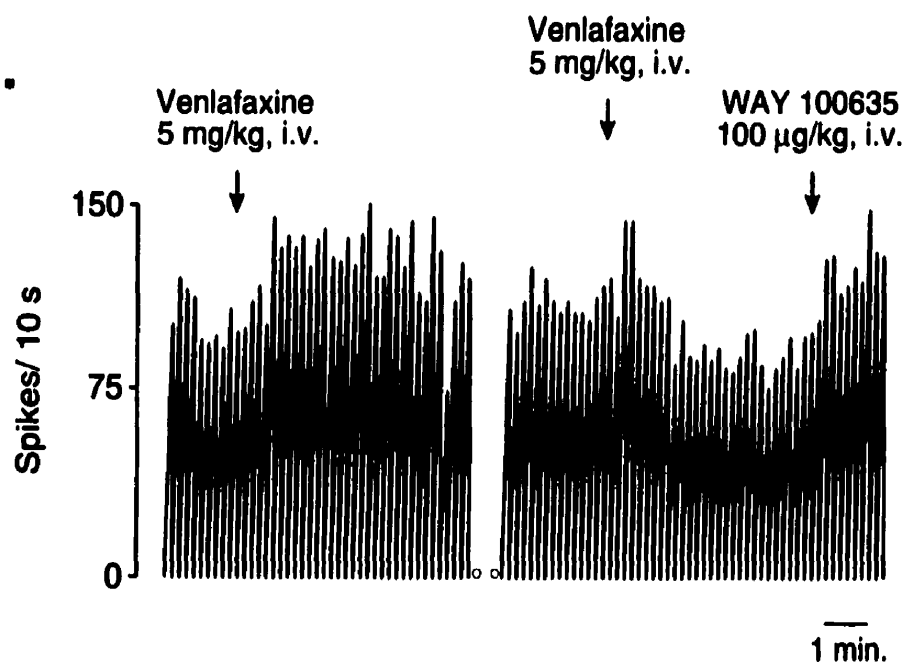
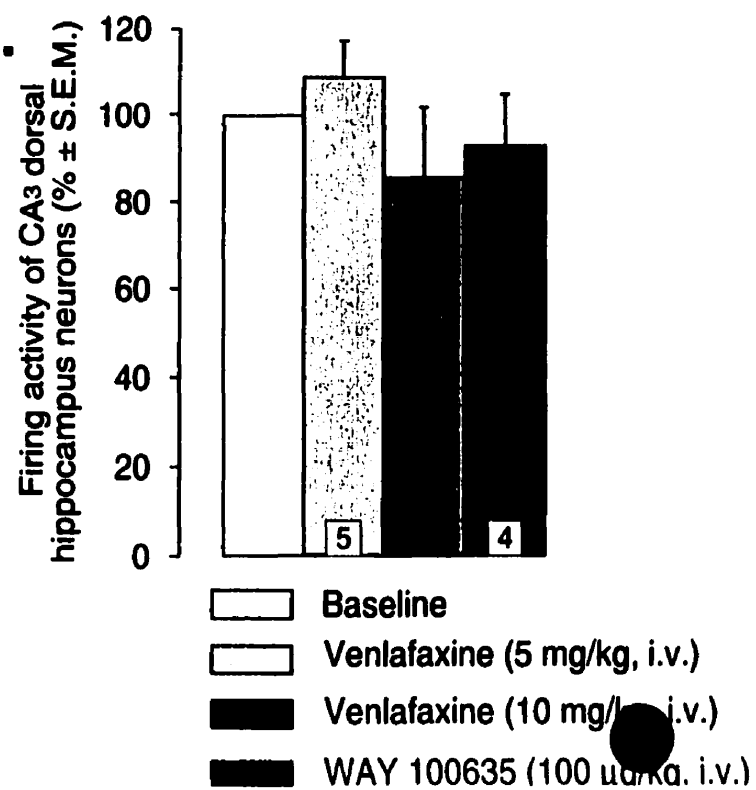
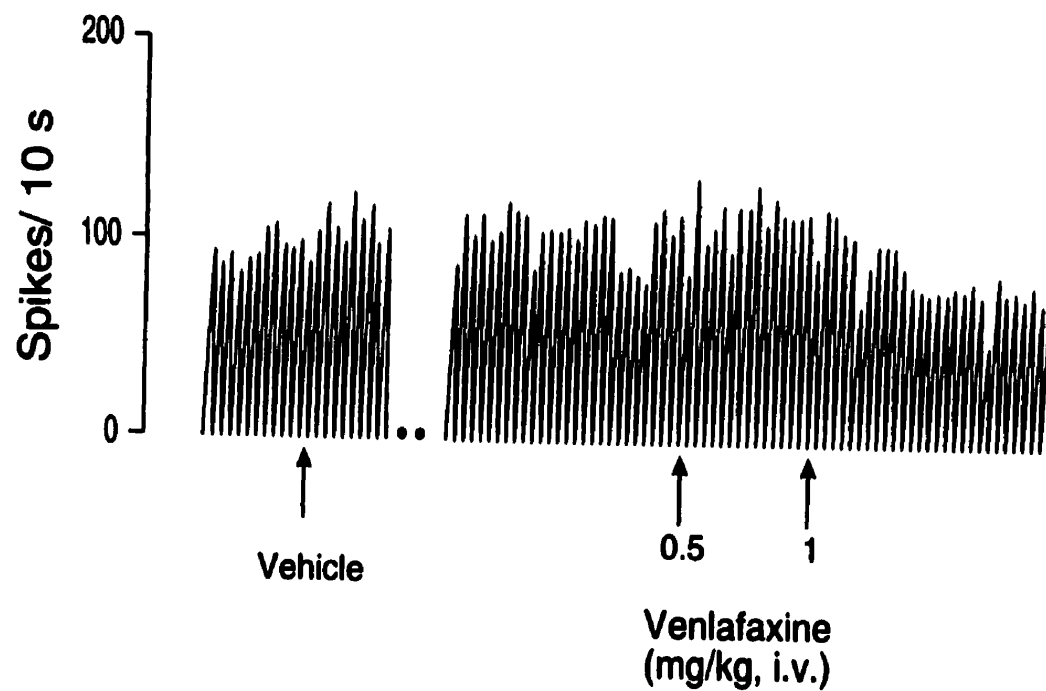
A.**B.****C.****D.**

Figure 4. Integrated firing rate histograms showing the response of dorsal hippocampus CA₃ pyramidal neurons to two successive acute intravenous administration of venlafaxine (of 500 μ g/kg each) in rats that had received 20 minutes prior either the vehicle (1 cc, i.p.; A) or (-)-pindolol (15 mg/kg, i.p.; B). The effect of a subsequent administration of WAY 100635 is also shown. The dots at the bottom of the histograms represent a period of *ca.* 20 min.

A.



B.

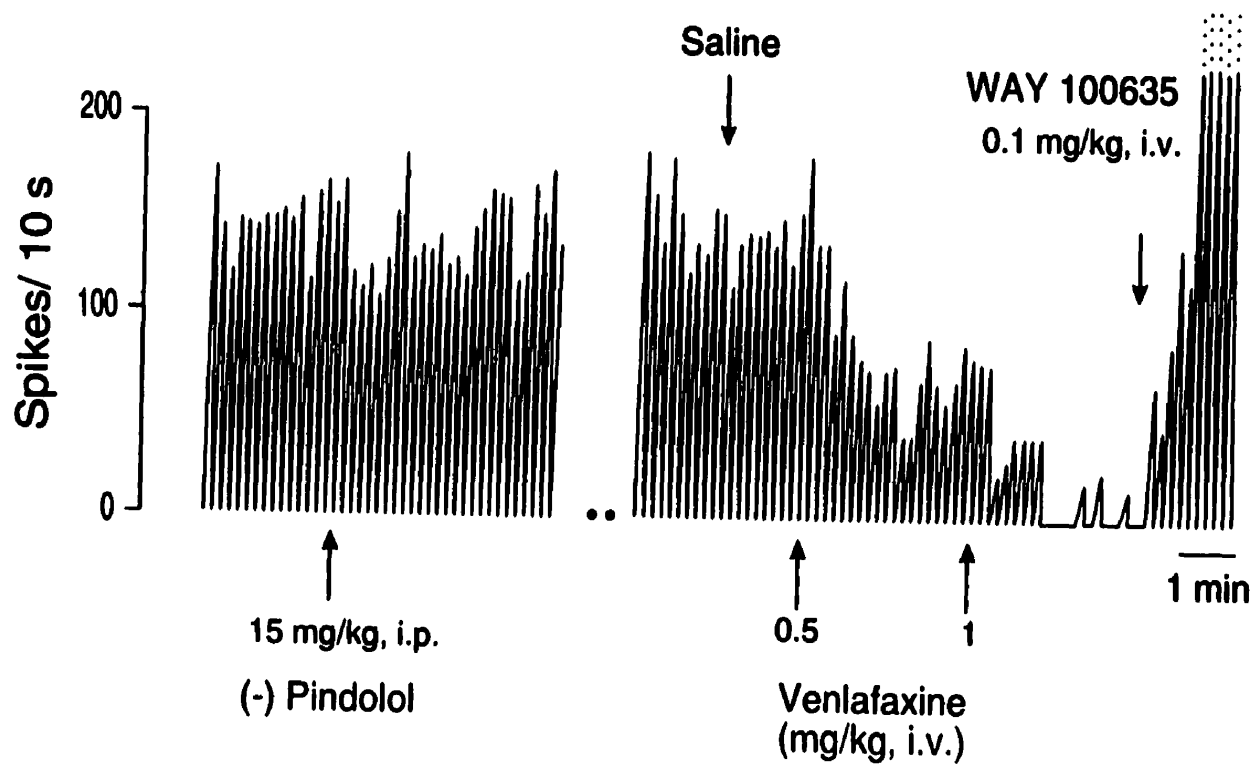
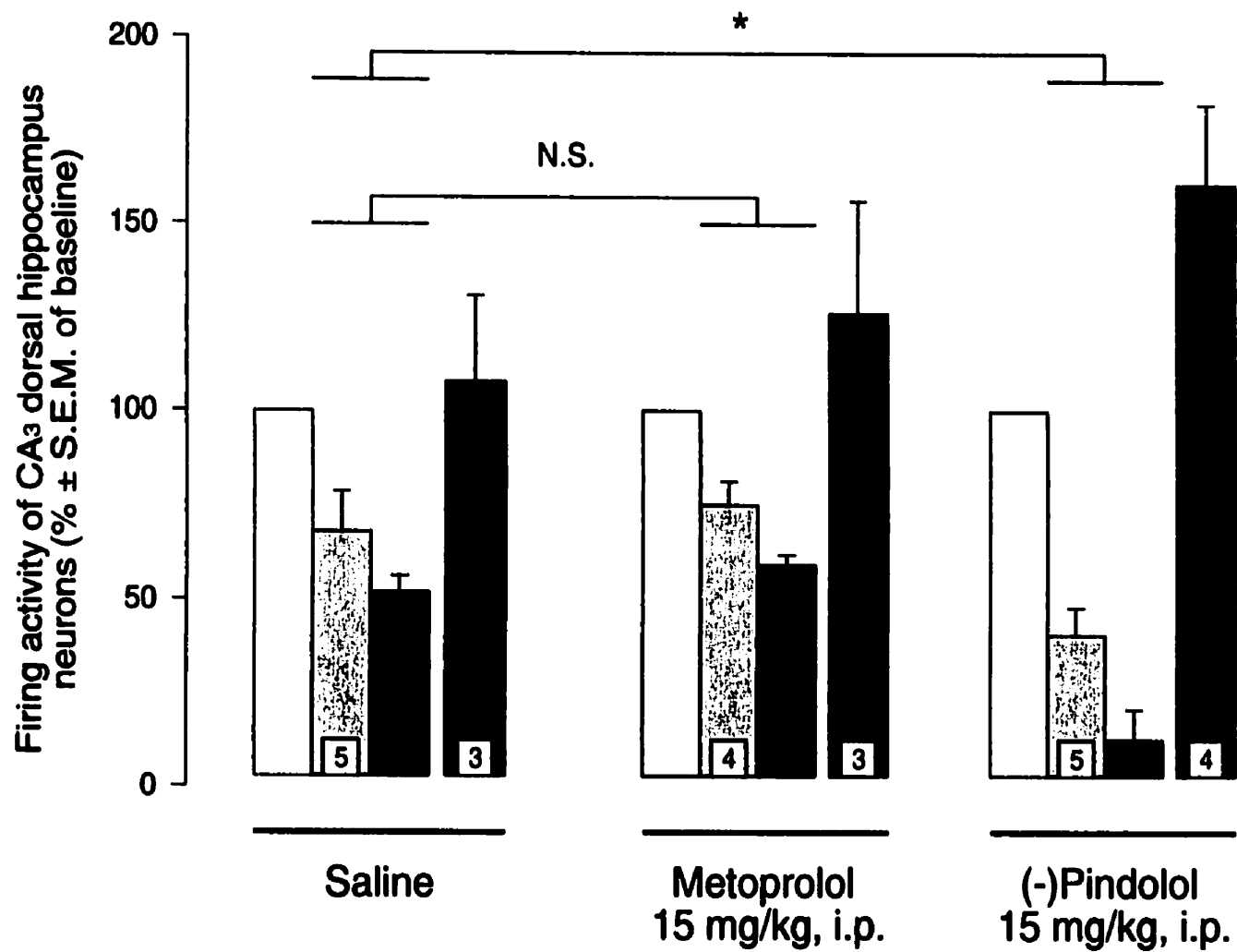


Figure 5. Firing activity of dorsal hippocampus CA₃ pyramidal neurons following the acute intravenous administration of two successive doses of 0.5 mg/kg of venlafaxine (cumulative doses of 0.5 mg/kg and of 1 mg/kg) expressed as the percentage of pre-injection (baseline) value in rats that were pre-treated 20 min prior with either the vehicle (1 cc, i.p.), metoprolol (15 mg/kg, i.p.), or (-) pindolol (15 mg/kg, i.p.). * $p < 0.01$, compared to the suppressant effect obtained in rats pre-treated with saline, using a two-way ANOVA.

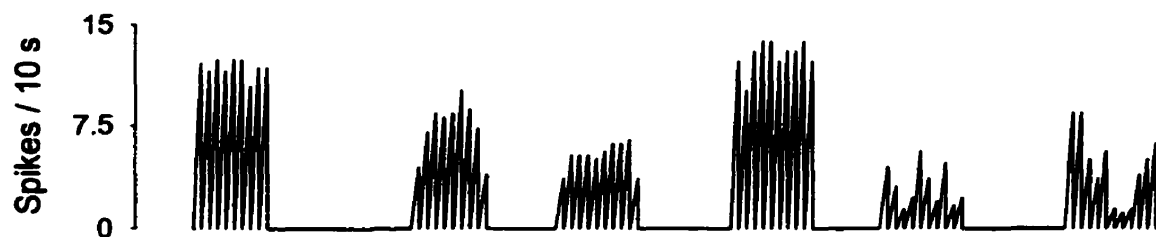


- Baseline
- Venlafaxine (500 µg/kg, i.v.)
- Venlafaxine (1 mg/kg, i.v.)
- WAY 100 635 (100 µg/kg, i.v.)

Figure 6. In A, integrated firing rate histograms of dorsal raphe 5-HT neurons recorded in the dorsal raphe showing their spontaneous firing activity in rats treated for two days with the vehicle, venlafaxine alone, or venlafaxine plus (-)pindolol. In B, are shown the effects of a 2-day treatment with venlafaxine alone and of a cotreatment with venlafaxine and (-) pindolol on the spontaneous firing activity of dorsal raphe 5-HT neurons. The open horizontal bar represents the range (S.E.M. x 2) of the firing activity of 5-HT neurons in rats treated with vehicle. * $p < 0.01$ using the non-paired Student's *t*-test.

A.

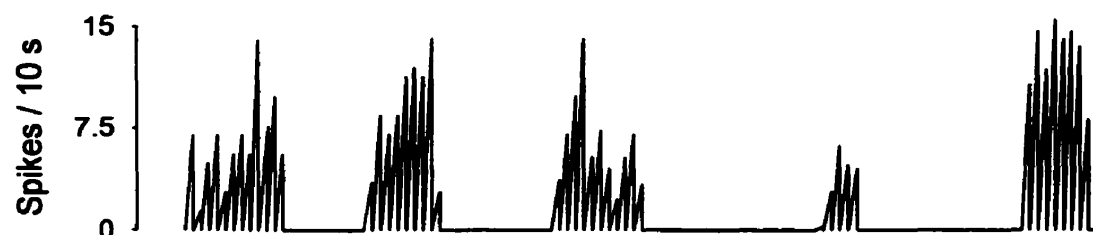
Control



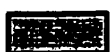
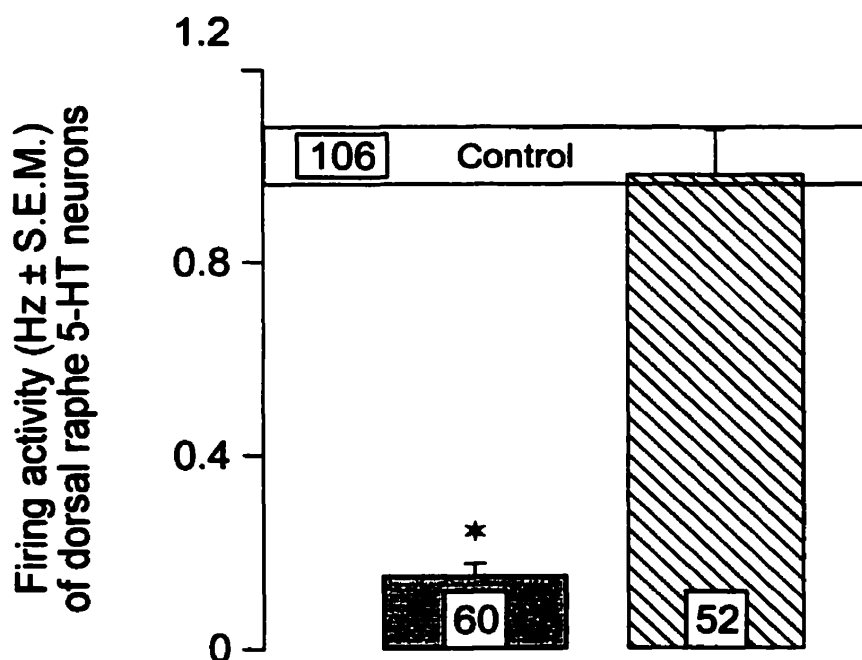
Venlafaxine (20 mg/kg/day x 2 days, s.c.).



Venlafaxine + (-)pindolol (15 mg/kg/day x 2 days, s.c.).



B.



Venlafaxine (20 mg/kg/day x 2 days, s.c.)



Venlafaxine + (-)pindolol (15 mg/kg/day x 2 days, s.c.)

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CONCLUSION

The fact that venlafaxine shows striking clinical features represented but one of the reasons that rendered its study interesting if not relevant and significant. Those clinical properties needed nonetheless to be accompanied by a unique pharmacological profile in order to maintain the relevance of any research endeavour devoted to the understanding of its mechanism of action. An illustration of this contention is provided by TCA's. Indeed, it may be claimed that the pharmacological profile of venlafaxine is not novel as there are several TCA's that shares with venlafaxine the ability to block the reuptake of both 5-HT and NE. The difference that makes the profile of venlafaxine to be fundamentally different than that of TCA's is that venlafaxine is devoid of a tricyclic moiety. This is of primordial importance in this context as there is evidence that the antidepressant activity displayed by TCA's is not necessarily related to their reuptake blocking potencies but well from another mechanism (possibly the sensitization of postsynaptic 5-HT_{1A} receptors) that would emanate directly from this tricyclic moiety. The best example illustrative of this contention involves the TCA iprindole. Indeed, although this compound does not block the reuptake of either 5-HT nor NE (Hyttel 1982), it is an effective antidepressant that sensitizes the postsynaptic 5-HT_{1A} receptors following prolonged administration. Based on these considerations, one can easily grasp part of the interest and value of studying the mechanism of action of venlafaxine.

The experimental results presented in chapter I, II and III allow to support the hypothesis stating that when used at low doses, venlafaxine would act as an SSRI and as the dose would be increased, there would be a parallel recruitment of the noradrenergic system through blockade of the NE reuptake process. This hypothesis is fully congruent with the various clinical observations that support an enhanced efficacy of venlafaxine when the doses are increased. Nonetheless, as best illustrated and expressed in chapter III, there is an important discrepancy between the *in vivo* 5-HT and NE reuptake blockade potencies of venlafaxine and its weak affinities for both the transporters. The unraveling of this *in vivo-in vitro* discrepancy sheds doubt on the exact nature of the mechanism by which venlafaxine blocks those reuptake processes, as it does not seem to be consequent of a classical binding to the transporters. In an attempt to unravel the mechanism responsible for this peculiarity, essentially two hypothesis were put forward and put to the test. We have first considered the

possibility that the effects of venlafaxine on the firing activity of both dorsal raphe 5-HT and locus coeruleus NE neurons may not be mediated by its sole and independent effect on these neurons per se, but well via an action on both these systems together. The involvement in the peculiar effects of venlafaxine of such an interplay was ruled out by the experiments carried out in 5, 7-DHT and 6-OHDA lesioned animals (see chapter III). Secondly, a putative monoamine releasing property of venlafaxine was considered. Again, this possibility was experimentally ruled out. These results were thus pointing to the possibility that the effects observed in functional studies with venlafaxine could be mediated via a mechanism distinct from a sole binding to the [³H]cyanoimipramine and [³H]nisoxetine binding sites. Many hypotheses can be formulated in an attempt to explain this assumption. For one, venlafaxine could act in a non-competitive fashion on both transporters. In the case of the binding of venlafaxine to the 5-HT transporter, this possibility would not however be supported by the fact that the current model of 5-HT reuptake inhibition indicates that the binding of 5-HT reuptake inhibitors (imipramine, paroxetine and citalopram) to the transporter share at least one common site i.e. that for 5-HT itself (Graham *et al.* 1989; Marcusson *et al.* 1989; Marcusson and Ross 1990). Nonetheless, the cocaine analog β -CIT has been shown to bind to the 5-HT transporter in an unique way with regard to the ionic and pH requirements for binding (Wall *et al.* 1993) and thus suggest the presence of a binding site complex on the transporter that could be functionally different from that bound to by 5-HT reuptake blockers, including cyanoimipramine. It is thus conceivable, however highly speculative, that venlafaxine would bind to the 5-HT transporter in a way to display an apparent affinity [assessed in the present case by [³H]cyanoimipramine competition] that would be out of keeping with its functional reuptake blocking potency.

As for the NE transporter, clinically effective antidepressant drugs that have NE reuptake inhibition property have been shown to bind to the nisoxetine site (see section 7.1). However, NE itself (NE > 1 μ M), in contrast to 5-HT for [³H]cyanoimipramine binding (Kovachich *et al.* 1988), is incapable of displacing [³H]nisoxetine from the transporter (Tejani-Butt 1992) thus suggesting that the binding site of nisoxetine, and presumably of other NE reuptake inhibitors, does not overlap the binding site for NE. Venlafaxine, in keeping with its phenylethylamine moiety, could thus block NE reuptake through a direct interaction at the NE site of the transporter without displaying significant affinity for the

[³H]nisoxetine binding site. Interestingly, venlafaxine blocks the reuptake of [³H]NE with a potency similar to that of NE itself in rat brain synaptosomes (Bolden-Watson and Richelson 1993). Of course these possibilities are highly speculative but nonetheless represent interesting and, importantly, testable avenues of research.

It is unfortunate that we have not been able to convincingly identify the neurobiological mechanism responsible for this *in vivo-in vitro* discrepancy. The disclosure of this distinct properties of venlafaxine poses two fundamental consequences. First, we are in the presence of an effective antidepressant that seems to block the reuptake process in a novel and unique way. Secondly, and which is corollary of the first issue, is that the primary interest in the study of venlafaxine has undergone a shift. Indeed, initially, the interest in studying venlafaxine was twofold: there was a genuine interest in the fact that the drug showed antidepressant activity but, secondly, this drug presented a potentially powerful and privileged tool to study the interconnectivity between the 5-HT and the NE system. Hence, the disclosure of this peculiarity with regard to the mechanism of action of venlafaxine belittled its value as a pharmacological tool but, in an inversely proportional manner, enhanced the value of its study in an heuristic context.

Regardless of the exact mechanism by which venlafaxine blocks the reuptake of both 5-HT and NE, it has been convincingly demonstrated that, in functional context, these two processes are blocked by venlafaxine. This outcome thus rendered necessary the study of the effects induced by its long-term administration. The relevance of such studies is of course justified by the fact that venlafaxine, not unlike all other effective antidepressants, shows a delay in the onset of their clinical efficacy. As stated in chapter IV and V, we have initially hypothesised, based on the differential potencies of venlafaxine to block the reuptake of 5-HT and NE, that the long-term administration of a low dose of venlafaxine would induce the adaptative changes observed with SSRI's, whereas that of a high dose (by blocking both reuptake processes) would append those occurring with NE reuptake inhibitors. Experimentation, in a very interesting fashion one would agree, has demonstrated that we have been, at best, only remotely right. Indeed, the complementary use of *in vivo* and *in vitro* paradigms to assess the sensitivity and/or function of various receptors has shown that the long-term administration of venlafaxine does not stringently induce the adaptative changes

one might have predicted.

The long-term administration of venlafaxine induces a tonic activation of the postsynaptic population of 5-HT_{1A} receptors. Although the mechanism underlying the appearance of this phenomenon (and of some peculiarity *i.e.*, present only following the high, and not the low dose of venlafaxine) has been discussed in chapter IV and V, this demonstration largely contributes to the heuristic value of the present research endeavour. Indeed, the demonstration by Haddjeri *et al.* that various antidepressant treatments increased the tonic activation of postsynaptic 5-HT_{1A} receptors found its pertinence in the fact that it proposed a neurobiological phenomenon that would be common to all antidepressant treatments hence representing a likely site for mediating the antidepressant response. In light of the now well demonstrated prominent role played by venlafaxine in the therapeutic armamentarium available for the treatment of major depression, one can easily grasp the importance of the demonstration that venlafaxine coalesced with this general consensus proposed by Haddjeri *et al.* (See Chapter IV).

This commonality of actions of antidepressant treatments represents one of the major arguments that forged the rationale behind the design of the study presented in chapter VI that involves the coadministration of venlafaxine with the mix β /5-HT_{1A} receptor antagonist pindolol. Indeed, if the activation of postsynaptic 5-HT_{1A} receptors were to be relevant in the antidepressant response, efforts for enhancing or accelerating the latter activation falls well within a rational approach aimed to either enhance or accelerate the antidepressant response. Interestingly, this precise outcome has seldom been the focus of investigations. Essentially, we have confirmed the idea that (-)pindolol blocks the dorsal raphe population of somatodendritic 5-HT_{1A} and not the postsynaptic 5-HT_{1A} receptors in the hippocampus. Most importantly, however, is the demonstration that the activation of postsynaptic 5-HT_{1A} receptors induced by venlafaxine is potentiated by pindolol which, most likely, represents the functional expression of this discriminative antagonism by pindolol. Therefore, we propose that (-)pindolol potentiates the activation of postsynaptic 5-HT_{1A} receptors by circumventing the homeostatic activity of the inhibitory somatodendritic 5-HT_{1A} autoreceptor.

These results must however be considered with caution in the process of transposition to a clinical dimension. Indeed, it is well probable that the antidepressant response is the

result of an extensive interplay between various systems and multiple receptors. The search of a holistic mechanism [*i.e.* activation of postsynaptic 5-HT_{1A} receptors] that would underlie this complex scheme may appear to encompass an overly reductionist approach. Indeed, more work is needed in order to fully grasp the intricacy of the response. Nonetheless, one is confronted to the growing body of evidence suggesting that the activation of the postsynaptic 5-HT_{1A} receptor has a prominent role to play in the therapeutic action of antidepressants. In light of these findings, it is very much likely that activation of postsynaptic 5-HT_{1A} receptors does not merely represent an epiphenomenon but a cornerstone in the orchestration of the antidepressant effect. Therefore, that (-)pindolol acts by circumventing the homeostatic activity of the inhibitory somatodendritic 5-HT_{1A} autoreceptor in a way to potentiate the activation of postsynaptic 5-HT_{1A} receptors provides a tenable and attractive hypothesis for its clinical effectiveness as a therapeutic adjunct in the treatment of major depression. Finally, it has been suggested that venlafaxine would be endowed with a faster onset of clinical action and therapeutic activity in treatment-resistant depression when used at high doses (Nierenberg *et al.* 1994; de Montigny *et al.* 1999). We have provided, in the present experimental work, fundamental evidence that allows to assert that the latter property of venlafaxine would be attributable to the recruitment of the noradrenergic system through NE reuptake blockade, phenomenon which would occur only with high doses of venlafaxine. Hence, when considering these observations in conjunction with the results obtained in chapter VI, one can contemplate the idea that the combination of pindolol and venlafaxine might perhaps represent one of the most powerful and flexible strategies in the armamentarium available for the treatment of major depression.

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