Antidepressant and Anxiolytic Action on the Serotonin$_{1A}$ Binding Site

Raymond Mongeau

A Thesis

in

the Department

of

Psychiatry

A thesis presented to the Faculty of Graduate Studies and Research in partial fulfillment of the requirement to obtain a Masters degree in Psychiatry from McGill University, Montréal, Québec, Canada.

November 1990

ABSTRACT

Antidepressant and Anxiolytic Action on the Serotonin$_{1A}$ Binding Site

Several lines of evidence suggest an involvement of the serotonergic system, and more particularly of serotonin$_{1A}$ (5-HT$_{1A}$) receptors in the regulation of emotions. Therapeutic manipulations which either increase or decrease the synaptic transmission of these systems have been reported to be beneficial in alleviating symptoms of depression and anxiety, respectively. In order to investigate the molecular basis of recent electrophysiological findings which implicated 5-HT$_{1A}$ receptors in the mechanism of action of antidepressants and anxiolytics, radioligand binding and autoradiographic studies using tritiated 8-hydroxy-2-(di-N-propylamino)-tetralin ([^{3}H]-8-OH-DPAT) were done in rat brain following various treatments. These included: the tricyclic antidepressant imipramine; the reuptake blockers paroxetine and indalpine; the monoamine oxidase inhibitor clorgyline; electroconvulsive shock; lithium; the classic benzodiazepine diazepam; and the 5-HT$_{1A}$ partial agonist gepirone. None of these treatments, nor the fluctuation in 5-HT availability provoked by the circadian cycle, gave any significant changes, with the exception of clorgyline which initially appeared to decrease the affinity of [^{3}H]-8-OH-DPAT for its receptor. A further series of studies *in vitro* and *in vivo* ascertained the possiblity that the 5-HT$_{1A}$ receptors may display two interconvertible affinity states and that, in fact, clorgyline induces a shift of the high to the lower affinity state. The findings from this second series of experiments suggested that labile changes, which may possibly be disrupted during membrane preparation, in the coupling between the 5-HT$_{1A}$ receptor and a guanine nucleotide binding protein (G-protein) may account for the effects that certain treatments have on 5-HT$_{1A}$ receptor responsiveness.

RESUME: Action des anxiolytiques et des antidépresseurs sur le site de liaison sérotonine$_{1A}$

De nombreux faits empiriques militent en faveur d'une implication des systèmes sérotonergiques, et plus particulièrement des récepteurs sérotonine$_{1A}$ (5-HT$_{1A}$), dans la régulation des émotions. Les manipulations thérapeutiques sur ces systèmes ayant pour but soit d'augmenter ou de diminuer leurs transmissions synaptiques sont reconnues comme étant bénéfiques pour combattre les symptômes de la dépression et de l'anxiété, respectivement. Dans le but de lier des bases moléculaires aux récentes études électrophysiologiques qui ont demontré l'importance des récepteurs 5-HT$_{1A}$ dans le mécanisme d'action de ces traitements, des études de radioliaisons et d'autoradiographies ont été effectuées à l'aide du 8-hydroxy-2-(di-N-propylamino)-tetraline tritié ([^{3}H]-8-OH-DPAT) dans le cerveau de rat suite aux traitements suivants; l'imipramine comme antidépresseur tricyclique, la paroxetine et l'indalpine comme bloqueurs de la recapture, la clorgyline comme inhibiteur de la monoamine oxidase, les électrochocs convulsifs, le lithium, le diazepam comme benzodiazepine classique, et enfin le gepirone comme agoniste partiel du récepteur 5-HT$_{1A}$. Ces traitements, ainsi que les fluctuations dans la disponiblité de la 5-HT telles que provoquées par le cycle circadien, n'ont eu aucun effet, à l'exception de la clorgyline qui semblait initialement diminuer l'affinité du [^{3}H]-8-OH-DPAT pour son récepteur. De plus amples études ont confirmé la possibilité que les récepteurs 5-HT$_{1A}$ puissent apparaître en fait sous deux états d'affinités interconvertibles, la clorgyline provoquant alors la conversion des sites de haute en basse affinité. Ces résultats suggèrent que des changements labiles, pouvant possiblement être perdus durant la préparation membranaire et se situant au niveau du couplage du récepteur 5-HT$_{1A}$ avec une proteine liant les nucleotides guaniniques (protéine G) pourraient expliquer les altérations de la réponse du récepteur 5-HT$_{1A}$ faisant suite à certains traitements.

Remerciements

J'aimerais ici exprimer ma gratitude à Barbara Suranyi-Cadotte qui m'a permis d'entreprendre ce travail, ainsi qu'à Sharon Welner et Rémi Quirion pour l'assistance et les conseils qu'ils ont su me donner.

Table of contents

Abstract ii

Résumé iii

Remerciements iv

Table of Contents v

List of Figures vi

List of Diagrams vi

List of Tables vii

Introduction 8

The Serotonergic System in Depression and Anxiety

- Anatomical considerations 12
- Treatments that decrease 5-HT transmission 14
- Treatments that increase 5-HT transmission 16
- The 5-HT receptor system 20

Aim of this Study 23

Materials and Methods 24

Results

I. [^{3}H]-8-OH-DPAT Scatchard analysis of *in vivo* treated rat brains using classic assay conditions 27

II. Further evidence for interconvertible affinity states of the 5-HT$_{1A}$ receptor in hippocampus 33

III. The 5-HT$_{1A}$ binding site and the circadian cycle 61

IV. [^{3}H]-8-OH-DPAT Scatchard analysis of *in vivo* treated rat brains using modified assay conditions 65

V. Autoradiographic analysis of treated dorsal raphe 69

General Discussion

The affinity and density of 5-HT_{1A} binding site following treatment 74

The 5-HT_{1A} receptor complex 80

Future Prospects 85

Conclusion 86

References 88

List of Figures

Figure 1. Saturation and Scatchard transformation of [^{3}H]-8-OH-DPAT binding to hippocampal, frontocortical and striatal membrane preparations. 40

Figure 2. Saturation binding of [^{3}H]-8-OH-DPAT to hippocampal membranes prepared in a Tris buffer containing $MnCl_2$ in the presence or absence of Gpp(NH)p. 44

Figure 3. Effects of NEM and DTT on [^{3}H]-8-OH-DPAT binding parameters to hippocampal membrane preparations. 46

Figure 4. Effects of inducing lipid peroxidation with ascorbate and $FeSO_4$ on [^{3}H]-8-OH-DPAT binding parameters, and the reversal by desferrioxamine 49

Figure 5. Effects of *in vivo* treatment with phenelzine, clorgyline and deprenyl on [^{3}H]-8-OH-DPAT binding parameters. 52

Figure 6. Typical autoradiograms of [^{3}H]-8-OH-DPAT labelling and its inhibition by Gpp(NH)p in the rostral and caudal parts of the dorsal raphe. 71

List of Diagrams

Diagram 1. Representation of the main projections of serotonin axons to the septo-hippocampal complex 13

Diagram 2. Putative 5-HT_{1A} affinity states in relation to its effector system 83

List of Tables

Table I. Effect of chronic *in vivo* treatment with paroxetine, electroconvulsive shock, imipramine, indalpine and clorgyline on the binding of [^{3}H]-8-OH-DPAT to rat hippocampal membranes.....31

Table II. Effect of chronic *in vivo* treatment with paroxetine, electroconvulsive shock, imipramine, indalpine and clorgyline on the binding of [^{3}H]-8-OH-DPAT of rat frontocortical membranes.....32

Table III. Comparative affinities of various competitors for the [^{3}H]-8-OH-DPAT binding site in hippocampal membrane preparations.....42

Table IV. Effect of lipid peroxidation on [^{3}H]-8-OH-DPAT specific binding.....48

Table V. *In vitro* effect of various concentrations of phenelzine on [^{3}H]-8-OH-DPAT binding parameters.....53

Table VI. Effect of varying preincubation time on [^{3}H]-8-OH-DPAT binding parameters.....54

Table VII. Lack of circadian rhythm variation of [^{3}H]-8-OH-DPAT binding.....64

Table VIII. Effect of electroconvulsive shock, imipramine, diazepam, lithium and gepirone on [^{3}H]-8-OH-DPAT binding parameters in the hippocampus.....68

Table IX. Effect of gepirone, imipramine and diazepam on dorsal raphe [^{3}H]-8-OH-DPAT binding measured by quantitative autoradiography.....73

INTRODUCTION

This study was motivated by the recent development of a new class of drugs, part of the pyrimidinylpiperazine family, which alleviate symptoms of mood disorders by apparently interacting directly with the serotonergic system. Representative members of this family include buspirone, gepirone and ipsapirone which are now known to act at the level of the serotonin$_{1A}$ (5-HT$_{1A}$) receptor (Traber and Glaser, 1987). Contrary to some other drugs which do not specifically act at the 5-HT$_{1A}$ site, those that do, such as gepirone are effective in treating both depression and anxiety. In fact many arguments favor the hypothesis of a major involvement of the serotonergic system and the 5-HT$_{1A}$ receptor in the action of antidepressants and anxiolytics.

An integrated interpretation of anxiety and depression is required in the context where 5-HT$_{1A}$ drugs are effective in the treatment of both disorders. In fact, clinically, these two affective states can hardly be dissociated. Symptoms of depression and anxiety are more often found to co-exist than occuring in either pure state (Roth et al., 1976). Gray (1982) proposed that anxiety and depression are at two poles of a single continuum, a postulate which is emergent, but contrasts with older efforts of classification which separated "reactive" and "endogenous" depressions. "Reactive" depression refers to a maladaptive adaptation of an individual to his environment, while an "endogenous" depression refers to a defective biological process. For Gray, this dichotomous classification is unsatisfactory considering the lack of convincing evidence that "reactive" depression requires more environmental stress than "endogenous" depression (Brown and Harris 1978). Nevertheless, a separation can somehow be made because depressed patients who are said to be "reactive" display several features of anxiety, including obsessional symptoms, irritability, restlessness, and difficulty in falling asleep, while

patients suffering from "endogenous" depression have motor retardation, early morning awakening, anhedonia, weight loss and feelings of hopelessness (Brown and Harris, 1978). According to Gray, these two groups of symptoms appear dissociated because they are at the two extremes of the anxiety-depression continuum. Therefore, etiological factors which precipitate the occurence of any affective disorder along the anxiety-depression continuum may belong to the external environment (e.g. psychosocial stress) or the internal environment (e.g. metabolic stress or inherited abnormal neural weakness).

Following the same logic, affective disorders may evolve from an anxious state to a depressed state with time. This is substantiated by the fact that affective disorders are more often of the agitated-anxious type in the young subjects, and more often of the retarded-depressed type in older individuals (Gray, 1982). The symptomatic expression of the dysregulations in psychoaffective diseases would therefore be dependent upon the psychophysiological capacity of an individual to deal with stress. This idea is consistent with the theoretical framework of Selye's well known "general adaptation syndrome" (1952) describing the reaction of an organism to stress; first there is an alarm reaction that mobilizes the reserves of the organism to struggle against the stressor; a stage of resistance follows during which additional reserves are mobilized to deal with the continuing stress; and finally a stage of exhaustion of the reserves if the stressor has not been eliminated. Depression resembles persistant exhaustion in the sense that an individual becomes hopeless and unable of coping with everyday life stress.

According to Laborit (1986), symptoms and maladaptation behaviors of depression are the morbid consequences of the learning processes of the inefficacy of action. Thereby, the nature of the pathophysiological processes are determined by the limiting role of the three principal axes of drive-oriented behaviors in normal physiology. The first one involves the previous experience (memory) of a gratifying

action; this action requires cathecholaminergic (noradrenergic and dopaminergic) pathways involved in the medial forebrain bundle (MFB). The second emerges from the instinctive responses elicited by nociception acting on the periventricular system, a mainly cholinergic system mediating flight behavior and agressive defensiveness. These behaviors are also kept in memory for future stimulus-responding depending upon their stress coping efficacy. Finally, if none of the above behaviors are effective in resolving the stress, the action inhibition system prevents exhaustion or further damages by the agressor. This system involves, among others, the septo-hippocampal pathways (see diagram 1) and the amygdala from which projections converge to the ventro-median hypothalamus that act as a common final inhibitory target. These pathways are both cholinergic and serotonergic and control the expression of stress via the hypothalamo-hypophyso-adrenal (HPA) axis, a system known to be perturbed in depression (Traskman, 1980). Therefore, some disturbances in depressions can be regarded as a consequence of the positive-feedback vicious-circle in action inhibition (Laborit, 1986). In anxiety, contrary to depression, hope still exists but there is an inability to resolve a stress by an appropriate behavioral pattern. The anguish resulting from this condition may arise also from the uninterrupted activation of the action inhibitory system which exacerbates morbid thoughts and elicits autonomous system hyperarousal.

The negative consequences of a persistent activation of the action inhibitory system leading to anxiety or depression can be alleviated by 5-HT_{1A} medications. Some other therapies can act only at one extreme or the other of the anxiety-depression continuum. One good example is electroconvulsive therapy, which is clearly more effective in "endogenous" depression than in any other affective illness (Gray, 1982). At the other end of the spectrum we have the classic benzodiazepines, like diazepam, which are effective for the anxious state, but not for the depressed state. Lying somewhere between, we have the monoamine oxidase inhibitors

(MAOI) which were introduced as antidepressants, such as phenelzine, and have provided little effect in "endogenous" depression, but have prominent beneficial effects in depression combined with anxiety (Robinson et al., 1973; Ravaris et al., 1976). Finally we have several tricyclic antidepressants (TCA), like imipramine, which have been shown to be effective in both depression and panic attack anxiety (Maier and Selingman, 1976).

The postulate that the serotonergic system is implicated in the efficacy of most of these treatments is now commonly upheld. The hypothesis that in depression and in anxiety 5-HT function is respectively decreased and enhanced are two popular assertions that will be examined. However, the focus on the serotonergic system in this thesis does not mean to underestimate the possible roles of other receptor systems in depression and anxiety. For example, in the 1960's, the cathecholamine hypothesis of mood dysregulation was the most prominent one (Schildkrault 1965; Bunney and Davis, 1965). The involvement of the dopaminergic system (Randrup, 1975) and the cholinergic system (Janowsky et al., 1972) has also received some attention. Further research may reveal whether the disturbances in the serotonergic system play an etiological role in depression and anxiety or are simply epiphenomena of the dysfunction in some other system. As will be seen, the only certainty to date is that drugs which act on the serotonergic system are effective in alleviating symptoms of psychoaffective diseases.

The Serotonergic System in Depression and Anxiety

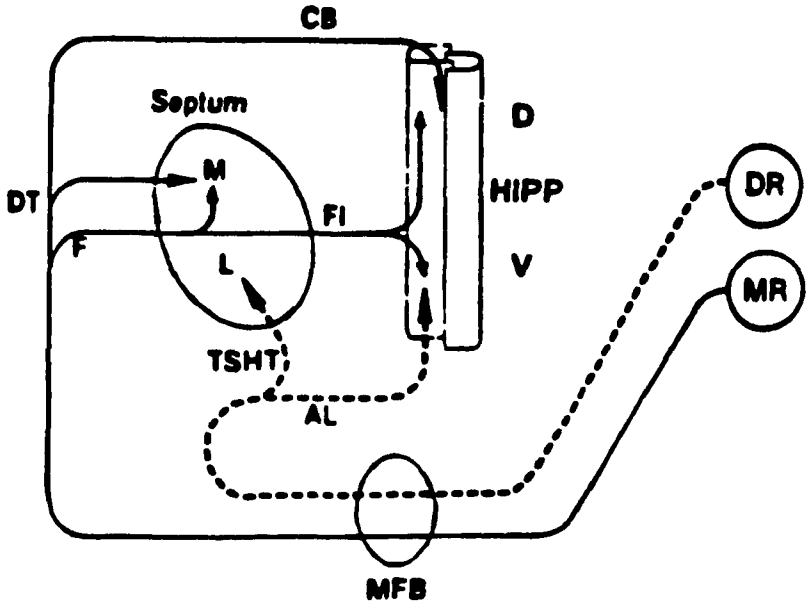
Anatomical Considerations

Important clues suggesting that serotonin may play a critical role in mood disorders were brought about by anatomical observations. The serotonergic pathways are among the most important neuronal networks underlying the function of the limbic system, the anatomical substratum for emotions originally described by Papez in 1937. A brief description of the structures of the serotonergic system will shed some light on its importance in mood regulation.

The cell bodies of the serotonergic projections that ascend to the forebrain are mainly located in the medial and dorsal area of the raphe nuclei in the brain stem. These neurons are the starting points of important pathways which find their postsynaptic targets throughout the brain; these areas include the cortex, hippocampus, hypothalamus, amygdala, thalamus, basal ganglia, substancia nigra, mamillary bodies, septum and tegmentum (Azmitia and Segal, 1978).

In our discussions we will put emphasis on the dorsal raphe (DR), especially in relation to its projection to the hippocampus and septum which are both intimately implicated in memory consolidation and the behavioral inhibition system (see diagram 1), as well as to the frontal cortex, an important area in the control of expression of emotions (Gray, 1982). The 5-HT_{1A} receptor appears to be frequently involved in the synaptic transmission of these neuronal networks, as supported by the fact that the highest densities of this receptor are found in DR, hippocampus, septum and frontal cortex (Palacios et al., 1987). The enthorinal cortex, an area from which the hippocampus receives inputs from higher areas, and the amygdala, a nuclei involved in agression and the action inhibition system (Gray, 1982), are also both densely endowed with 5-HT_{1A} receptors (Palacios et al., 1987).

Diagram 1. Representation of the main projections of the serotonin axons to the septo-hippocampal complex. AL, ansa lenticularis; CB, cingulum bundle; D, dorsal and V, ventral hippocampus; DR, dorsal raphe; DT, diagonal tract; F, fornix column; FI, fimbria; L, lateral and M, medial septal nucleus; MFB, medial forebrain bundle; MR, median raphe; TSHT, septohypothalamic tract. (From Azmitia, 1978, p. 81, in Elliot and Whelan.)



Like many other important nuclei, the serotonin producing neurons of the DR project to the thalamus, more precisely to the nonspecific midline nuclei of the thalamus which are implicated in the general cerebral awakening to emotion related stimulus (Kelly, 1985). The DR is also interconnected with the noradrenergic system via the locus coeruleus (LC), a nucleus which triggers the so called "alarm reaction" and its consequences on the autonomous nervous system (Mason and Fibiger, 1979). Noradrenergic terminals have been demonstrated in the DR (Sakai et al., 1977a; Svensson et al., 1975), while in turn the LC is innervated by the DR (Sakai et al., 1977b; Segal 1978).

Treatments that Decrease 5-HT Transmission

There is a general trend in animal and human studies demonstrating that a dimunition in the activity of the serotonergic system would be beneficial in treating anxiety, while aggravating depression. One strategy to reduce serotonergic function is to prevent the conversion of tryptophan into 5-hydroxytryptophan (5-HTP) the immediate precursor of 5-HT, by blocking the enzyme tryptophan hydroxylase with parachlorophenylalanine (PCPA). Some investigators (Shepard et al., 1982) made use of the PCPA strategy in behavioural suppression (Geller-Seifter model of anxiety) and found consistent release of the suppression of responding. Moreover, 5-HTP administration abolishes the anxiolytic action of PCPA. Interestingly, the 5-HT_{1A} agonists 5-methoxydimethyl tryptamine (5-MeODMT) (Shepard et al., 1982) and 8-hydroxy-2-(di-N-propylamino)-tetralin (8-OH-DPAT) (Engels et al., 1984) can replace 5-HTP.

On the other hand, neither 5-HTP nor PCPA had any significant effect on the performance deficits resulting from uncontrolable footshock in the learned helplessness animal model of depression (Anisman et al., 1979). In a clinical trial, however, Shopsin et al. (1975) induced a relapse of depression with PCPA in

patients who responded to tranylcypromine, a monoamine oxidase inhibitor, or to imipramine, a tricyclic antidepressant.

A more drastic way to deplete 5-HT brain content consists of specifically destroying serotonergic cells and their projections by injection of toxin, such as 5,7-dihydroxytryptamine (5,7-DHT) directly into the brain. Experiments in which application of 5,7-DHT were made into the ventral tegmentum (Tye et al., 1977) or in the DR (File et al., 1979), have shown an anxiolytic action in the Geller-Seifter model as well as in the novel environment model. Anatomical specificity is required; similar lesions in the median raphe had no anxiolytic effect, while lesions of the lateral septum, which is part of the septo-hippocampal action inhibition system, also produced an anxiolytic action. On the other hand, a similar depletion of catecholaminergic neurons had no effect on the expression of anxiety (Clarke and File, 1982).

The methods described above all reduce 5-HT release by acting at a presynaptic location. But a decrease in 5-HT transmission is also possible through a postsynaptic receptor blockade. Many animal studies on anxiety using nonspecific serotonergic antagonists failed to find any anxiolytic properties of drugs like cinanserin, cyproheptadine or metergoline. Consistent anxiolytic effects, nevertheless, have been obtained in many animal paradigms with the 5-HT_1 antagonist methysergide (conditioned emotional response, Graeff and Schoenfeld, 1970; Geller-Seifter model, Stein et al. 1975; punished drinking model, Petersen and Lassen, 1981). In clinical studies, ritanserin, a specific 5-HT_2 antagonist, was also found to be effective in patients with generalized anxiety disorders (Cooper and Desa, 1987) but not in patients with panic disorders (Westenberg and den Boer, 1989). Overall, these data indicate that blockade of the serotonergic system through 5-HT receptors is often an efficient way to reduce anxiety.

In clinical trials on depression, the antagonist methysergide was not found to alter mood in either normal or depressed people (Van Praag, 1978). Some antidepressant drugs, however, like amitryptiline and mianserin, also display antagonistic potencies on the 5-HT system (Willner, 1985). Their mechanism of action in the treatment of depression, however, is not likely due to their acute postsynaptic receptor blockade, but more probably to increased responsiveness of the post-synaptic receptors caused by their long term administration that outweighs the receptor blockade (see below).

Benzodiazepines (BZs), like diazepam, can also decrease serotonin turnover (Chase et al., 1970; Lidbrink et al., 1974; Lippman and Pugsley 1974; Jenner et al., 1975; Pratt et al., 1979; Saner and Pletscher, 1979; Soubrie et al., 1983; Pratt et al., 1985); this decrease in 5-HT correlates with anxiety reduction (Wise et al., 1972). However, whether or not the serotonergic system is critical in the efficacy of BZs remains unknown. The most accepted hypothesis is that BZs exert their anxiolytic action through the potentiation of the GABAergic system by an allosteric modification of GABA receptors (Costa and Greengard, 1975). However, BZs may also act via an indirect interaction with the 5-HT system. The report that anxiolytic activity of a 5-HT_{1A} partial agonist like gepirone is decreased by a previous addiction to BZs (Schweizer et al., 1986) supports this idea.

Treatments that Increase 5-HT Transmission

Several empirical facts show that increasing serotonergic transmission is an efficient therapeutic strategy for depression. A direct way to demonstrate this is by increasing 5-HT availability in the CNS by enhancing the concentration of 5-HTP. This was tested in clinical trials where Van Praag et al. (1982) found beneficial effects of 5-HTP, particularly in depressed patients with low pretreatment 5-HT turnover. Another reliable way to increase 5-HT availability is to block its

degradation by monoamine oxidase (MAO). This can be achieved by MAO A inhibition with phenelzine or with clorgyline, but not by MAO B inhibition with deprenyl since MAO B does not affect 5-HT monoamine oxidation (Campbell et al., 1979). Interestingly, MAO inhibitors are particularly effective in depressed patients with combined symptoms of anxiety (West and Dally, 1959; Robinson et al., 1973; Ravaris et al., 1976).

From an electrophysiological point of view, evidence suggests that 5-HT system activation might well be the common denominator that explains the diverse types of antidepressant action. The long-term adaptation observed in the 5-HT system following chronic regimens of antidepressant treatments are generally not seen in the NA system. This is exemplified by the differential effect of MAO inhibitors (MAOI) on the 5-HT and NA systems. First, both systems respond similarly to acute MAO A inhibition since they both possess somatodendritic autoreceptors which feedback promptly to any increase in the availability of their respective neurotransmitters. After a prolonged stimulation period, however, the 5-HT producing cells show the particular capacity to free themselves from the control of their somatodendritic autoreceptors; in other words they become desensitized. This recovery mechanism is not observed in NA neurons, suggesting an immutability of the noradrenergic somatodendritic autoreceptors (Blier & de Montigny, 1985).

Reuptake inhibitors are often preferred to MAOIs by psychiatrists, partly because most 5-HT in the synaptic cleft is removed by an uptake mechanism rather than via degradation by MAO. Some reuptake blockers in the category of tertiary tricyclic antidepressants (TCAs), like imipramine for example, can potently act at the level of serotonergic synapses, while some others, like nortriptyline, are active only at noradrenergic locations.

The idea that the efficacy of serotonergic reuptake blockers as antidepressants is directly based on their capacity to increase 5-HT by blocking the reuptake process in the synaptic cleft is commonly upheld, but this remains an assumption. In fact, this postulate faces many difficulties; first, the immediate effect of these drugs on reuptake can not be correlated with their delayed clinical efficacy and secondly, some TCAs, like trimipramine and mianserin, are completely devoid of reuptake properties on any of the monoamines in the synaptic cleft.

An alternative explanation for the antidepressant action of reuptake blockers has recently been suggested by the electrophysiological work of Blier and co-workers (1988) which suggests that the increased efficacy of 5-HT synaptic transmission which is observed after chronic treatment with these drugs is not directly caused by the 5-HT reuptake blockade, but rather by a reduced function of 5-HT terminal autoreceptors. The same authors suggested that the decreased function of terminal 5-HT autoreceptors might result from an allosteric modulation of this site caused by the long-term occupation of the reuptake site by the reuptake inhibitors. The long-term modification of the terminal 5-HT autoreceptors would then have nothing to do with the increased availability of 5-HT in the synaptic cleft *per se*. This theory is also substantiated by the fact that increases of 5-HT may also be provoked by other treatments, such as via MAO A inhibition with clorgyline, but such treatments are unable to reduce 5-HT terminal autoreceptor function in a way similar to 5-HT reuptake inhibitor, like fluoxetine. Nevertheless, it remains that clorgyline and fluoxetine are both effective antidepressants when administered chronically in as much as they both increase serotonergic transmission.

The therapeutic use of monoamine oxidase inhibitors and tricyclic antidepressants are not limited to depression. These treatments were found to be more effective than benzodiazepines in the treatment of panic disorders (Kelly et al., 1973). Other clinical trials conducted by Kahn et al. (1986) revealed that

imipramine was beneficial for patients with generalized anxiety disorders. On the other hand, electroconvulsive shock treatment (ECT), which has been reported to be superior to TCA in alleviating endogenous depression (Davidson et al., 1980), is not effective in anxiety disorders (Gray, 1982). Contrary to reuptake blockers and MAOIs, the effect of ECT appears to be postsynaptic since it increases the responsiveness of hippocampal pyramidal neurons to microiontophoretically-applied 5-HT (de Montigny, 1984), a phenomenon shared by many TCAs (de Montigny et al., 1988).

None of the methods to increase 5-HT transmission described thus far act directly at the serotonergic receptors. Receptor activation is possible by the use of synthetic serotonergic agonists or partial agonists. Many studies using this strategy revealed that mood reaction to 5-HT agonists depended upon the initial status of 5-HT receptor sensitivity. In this regard, Kahn et al. (1988) reported behavioral and neuroendocrine evidence for 5-HT receptor hypersensitivity in panic disorders. Their statement is based on challenge studies on human subjects with the putative 5-HT agonist, *m*-chlorophenylpiperazine (MCPP). An oral dose (0.25 mg/kg) of MCPP triggered acute panic attacks and anxiety in panic disorder patients. The occurence of panic attacks was positively correlated with an increase in plasma cortisol levels, a 5-HT-mediated endocrinological response. MCPP administered to normal controls or to depressed patients under the same condition was without any significant effect on any of the parameters tested. MCPP had a biphasic effect on the anxiety of panic disorder patients, aggravating their symptoms or inducing depressed mood in the first 10-14 days of treatment but creating clinical improvement as compared to the pretreatment status after 2-4 weeks (Kahn & Westenberg, 1985). This effect of MCPP was not reproduced, however, for other types of anxiety like obsessive-compulsive disorders (Charney et al., 1988).

All the arguments described above show strong support for the involvement of the serotonergic system in mood disorders. A major question that remains to be addressed now is whether or not a specific receptor subtype is intimately implicated in anxiety and depression.

The 5-HT Receptor System

Many binding studies indicate that chronic treatment with antidepressant drugs downregulate the 5-HT_2 receptor (see Willner, 1985, for a review). These drugs include typical TCAs, atypical antidepressants, and MAOIs. However, in the context of the increased 5-HT transmission that follows chronic antidepressant treatment, the relevance of this down regulation remains questionable. An enhanced receptor stimulation by 5-HT could hardly explain it, since it has been reported that chronic treatment with the selective and potent 5-HT uptake inhibitor citalopram did not affect the 5-HT_2 receptor density despite continued blockade of 5-HT uptake (Hyttel et al., 1984). A further discrepancy is the fact that, contrary to other antidepressant treatments, electroconvulsive treatment (ECT) increases rather than decreases the 5-HT_2 binding site capacity (Green et al., 1983; Kellar et al., 1981; Vetulani et al., 1981).

With the sole exception of MAOI treatment, the 5-HT_1 binding sites, as studied with [^{3}H]-5-HT, have generally been reported to be unaffected following antidepressant treatments (see Willner, 1985, for a review). Still, there remains the possibility that antidepressants may affect a specific subtype of the 5-HT_1 binding site. At this level, the status of research is much more incomplete since the development of specific drugs for the 5-HT_1 receptor subtypes is fairly recent. Nevertheless, the information accumulated so far favors the 5-HT_{1A} and the 5-HT_{1B} binding sites as critical loci.

The 5-HT_{1B} binding site is of particular interest in respect to its probable role as an autoreceptor on serotonergic terminals (Middlemiss et al., 1985). As mentioned earlier, this terminal autoreceptor seems implicated in the antidepressant efficacy of m antidepressant efficacy of many reuptake blockers. As binding studies supporting the idea that the electrophysiological observations may have their counterparts at the level of the 5-HT_{1B} receptor protein itself.

The involvement of the 5-HT_{1A} receptor in the mechanism of action of drugs used to treat anxiety and depression has recently been supported by the results of clinical trials as well as from electrophysiological data. For example, gepirone, a partial agonist at the 5-HT_{1A} receptor (Andrade & Nicoll, 1987), has recently been shown to possess both anxiolytic and antidepressant activity following chronic treatment (Amsterdam et al., 1987; Csanalosi et al., 1987). The therapeutic activity of gepirone was correlated with electrophysiological studies in rat brain (Blier & de Montigny, 1987) which have shown modification of the properties of the 5-HT neurons following chronic administration of this drug. At the presynaptic level, a sustained administration of gepirone initially decreases the firing activity of DR 5-HT neurons. This phenomenon is transient and is followed by a progressive recovery to normal after 14 days of treatment. This cascade of events is similar to that elicited by MAOIs in the sense that both treatments increase 5-HT neurotransmission via a desensitization of DR somatodendritic 5-HT_{1A} autoreceptors, combined with a direct activation of postsynaptic 5-HT_{1A} receptors by the agonist.

Contrary to reuptake blockers, 5-HT_{1A} agonists do not desensitize terminal 5-HT_{1B} autoreceptors, nor do they appear to change the postsynaptic 5-HT_{1A} receptor sensitivity. Nonetheless, other treatments such as lithium (used for manic-depressive patients), seem to involve an enhanced responsiveness of hippocampal 5-HT_{1A} receptors with no alteration of DR autoreceptors (Blier et al., 1987). The

increased responsiveness of 5-HT receptors observed with TCAs or ECT is probably also mediated through the 5-HT_{1A} receptor, since this subtype constitutes the most abundant 5-HT_1 receptor population in the hippocampus (Pazos & Palacios, 1985).

Many behavioral studies have also demonstrated an involvement of the 5-HT_{1A} receptor in anxiolytic and antidepressant actions. One interesting paradigm elaborated by Goodwin (1989) involves the 5-HT_{1A} agonist hypothermic effect of 8-OH-DPAT. This effect is most likely presynaptic since it can be abolished by a lesion of central 5-HT neurons. The authors have shown that this 5-HT_{1A}-mediated hypothermia can be attenuated by antidepressants like MAO inhibitors, ECT and lithium. Th', effect was interpreted as a tolerance of the 5-HT_{1A} autoreceptor to an increased release of 5-HT. Similar conclusions were drawn from the 8-OH-DPAT-induced 5-HT syndrome in which stereotyped behaviors are triggered by an overstimulation of the 5-HT_1 receptors with 8-OH-DPAT; these are decreased by antidepressants, MAO inhibitors, and ECT (Grahame-Smith, 1988).

The effect of 5-HT_{1A} agonists in animal models of anxiety are rather ambivalent, because, depending upon the circumstances and the type of test employed, researchers have found both anxiogenic or anxiolytic effects of these drugs. For example, in the elevated plus-maze, rats show anxious behavior in the large open field following 8-OH-DPAT administration (Critchley et al., 1987), while anxiolytic-like effects were observed in the social interaction model after ipsapirone administration (Schuurman and Spencer, 1987). According to Carli and Samanin (1988), the anxiolytic action of a 5-HT_{1A} agonists would be dependent upon the arousal state and the amount of stress the animal is exposed to. This hypothesis is supported by the fact that 5-HT_{1A} drugs have an optimal effect during the active period of the diurnal cycle in rats (Eison et al., 1986).

Different affinity states of the 5-HT_{1A} receptor could explain the apparent discrepancies raised by the different efficacies of the drugs acting at this site. This is illustrated by an experiment (Engel et al., 1984) in which the intrinsic activity of 8-OH-DPAT was dependent upon the sensitivity of the 5-HT_{1A} receptor. In the Vogel conflict paradigm, 8-OH-DPAT administrated to naive animals normally exerts an anticonflict action. Thus, in this "normal" situation, 8-OH-DPAT action is said to be antagonistic toward the 5-HT transmission. The anxiolytic effect of 8-OH-DPAT is, however, reversed by a pretreatment with PCPA (which depletes 5-HT availability by blocking its synthesis) rendering the 5-HT_{1A} receptors presumably supersensitive. As discussed earlier, PCPA alone has an anxiolytic action because it decreases 5-HT transmission. Therefore, 8-OH-DPAT would be considered as an agonist under these conditions since it is as efficient as 5-HT in blocking the anticonflict action of PCPA.

All this clinical, electrophysiological and behavioral evidence suggests a complex involment of the 5-HT_{1A} receptor in the efficacy of anxiolytic and antidepressant treatments.

Aim of this Study

In summary, the serotonergic system and 5-HT_{1A} receptors appear to be involved in mood disorders because 1) there is an anatomical implication of the serotonergic pathways with the limbic system which controls emotions, 2) there is a major 5-HT_{1A} receptor involvement in the neural transmission of these pathways, 3) blockade or depletion of 5-HT transmission appears to be beneficial in generalized anxiety disorders 4) chronic enhancement of 5-HT transmission appears to relieve depression while a dual effect on panic disorder symptoms is seen, first aggravating the symptoms and then being beneficial on a long term basis and 5) compared to other 5-HT receptor systems, the 5-HT_{1A} receptors appear to have

consistent clinical, electrophysiological and behavioral implications in various therapeutic actions.

Therefore, considering the accumulated evidence implicating the serotonergic pathways in anxiety and depression, and also the critical role the 5-HT_{1A} receptors may play in these, it is of importance to investigate whether different treatments that are effective in the treatment of mood disorders have some of their actions at the level of the 5-HT_{1A} binding site. In order to do so, radioligand binding studies and autoradiographic analysis were performed on certain brain areas following various antidepressant and anxiolytic treatments on rats (see results, section I, IV, V). In the ultimate elucidation of this problem, it was also necessary to address some questions about the fundamental nature of this receptor (see results, section II, III).

MATERIALS AND METHODS

Materials

Male Sprague-Dawley rats (275-350 g) were obtained from Charles River Breeding Farms (St. Constant, Quebec, Canada). Drugs and chemicals were obtained from the following sources: [^{3}H]-8-OH-DPAT (240 Ci/mmol) from Amersham (Arlington, USA); unlabelled 8-OH-DPAT, unlabelled 5-HT and deprenyl from Research Biochemicals Inc. (Walthman, MA, USA); Gpp(NH)p and DTT from Boehringer (Mannheim, West-Germany); phenelzine, clorgyline, pargyline, imipramine, malondialdehyde (MDA), thiobarbituric acic (TBA), polyethylimine (PEI), N-ethylmaleimide (NEM) and bovine serum albumin from Sigma Chemical Co. (St. Louis, MO, USA); diazepam from Sabex international (Boucherville, Quebec, Canada); gepirone from Bristol-Myers (Wellingford, CT, USA); desferrioxamine (DFX) from CIBA-Geigy (Mississauga, Ontario, Canada); Ecolite from ICN (Montreal, Quebec, Canada). All other chemicals were from

Fisher Scientific Co. (Montreal, Quebec, Canada). Autoradiographic film was from ultrofilm, LKB Instruments (Brommd, Sweden).

Membrane preparation and binding assay conditions

Receptor binding assays were adapted from the method of Peroutka and Snyder (1979). Rats were decapitated and their brains removed, frozen in 2-methylbutane and stored at -80°C until use. On the day of the assay, frontal cortex, hippocampus or striatum were thawed, dissected and homogenized in 20 vol of ice-cold 50 mM Tris-HCl buffer (pH 7.4 at 25°C) using a Brinkman Polytron (setting 5.5 for 15 s) and then centrifuged at 45,000 g for 10 min. The membrane pellet was resuspended in 20 vol of the same buffer and preincubated (preincubation step) at 37°C for 10 min in order to destroy endogenous amines. The final membrane pellet, obtained by recentrifugation as above, was resuspended in 90 vol of ice-cold 50 mM Tris-HCl buffer (pH 7.7 at 25°C) containing 10 uM pargyline, 1 mM $CaCl_2$ and 0.01% ascorbic acid.

In borosilicate tubes, 400 ul of membrane preparation (250-300 ug protein), 50 ul [^{3}H]-8-OH-DPAT (specific activity adjusted to 100-130 Ci/mmol; 0.05-60 nM), and 50 ul Tris-HCl buffer or 50 ul unlabelled 5-HT (10 uM; to define the non-specific binding at all ligand concentrations) in a final volume of 0.5 ml were incubated in a water bath at 25°C for 30 min under gentle agitation. At the end of that period, the incubation was terminated by rapid filtration through no. 32 glass filters presoaked in a 0.3% polyethylenimine solution to reduce non-specific binding using a Brandel cell harvester. Filters were then washed 3 times with 4 ml of Tris-HCl buffer. Radioactivity on filters was determined by liquid scintillation spectrometry using 5 ml of Ecolite at 48% efficiency using a Beckman LS 1800 counter. Proteins were determined according to the method of Lowry et al. (1951) using bovine serum albumin as the standard. Specific binding represented

approximately 85-95% in the hippocampus, 80-90% in the frontal cortex, and 70-80% in the striatum at radioligand concentrations smaller than 5 nM. Saturation binding parameters were determined by computer-assisted non-linear regression analysis using the McPherson (1983) and Munson and Robard (1984) programs. Curve fitting to a one site or two site model were compared using the F-test. The two site model was accepted if it was significantly better ($P<0.01$) than the one site model. Under normal assay conditions using a wide enough range of labelled ligand concentrations, the two site model always represented a better fit.

Autoradiographic assays

Sections (20 um) of rat brains were cut in the region of the DR, at -15^{o}C with a cryostat, thaw-mounted onto gelatin-coated glass slides kept at 4^{o}C, and finally stored at -80^{o}C until use. The sections were labeled *in vitro* for autoradiography according to the procedure of Rainbow et al. (1982), using 0.5 nM [^{3}H]-8-OH-DPAT (Amersham International U.S., 240 Ci/mmol). Briefly, tissue-mounted slides were preincubated for 30 min in 0.17 M Tris-HCl buffer, pH 7.6, and then one half of the slides were incubated at 20^{o}C for 60 min in the same Tris-HCl buffer supplemented with 4 mM $CaCl_2$, 1 mM $MnCl_2$, 0.01% ascorbate, 10 uM pargyline, and 0.5 nM [^{3}H]-8-OH-DPAT; the remaining half of the slides were incubated in the same supplemented buffer plus 100 uM Gpp(NH)p. After the incubation, sections were washed twice for 5 min each with ice-cold buffer supplemented with 4 mM $CaCl_2$ and 1 mM $MnCl_2$, quickly dipped in ice-cold pure deionized water, and dried with cold air. [^{3}H]-8-OH-DPAT autoradiographic levels of grey were almost completely absent when blanks were generated by adding 1 uM 5-HT to the incubation medium.

After drying, slices were opposed against a sheet of autoradiographic film, tightly pressed between two metal sheets (5 mm thick), and stored in the dark at room temperature for two weeks. Autoradiographic images of DR were analyzed by quantitative microdensitometry (Biocom System).

Methods particular to a certain set of experiments will be detailed as each set of experiments is described.

RESULTS

I. [^{3}H]-8-OH-DPAT Scatchard analysis of in vivo treated rat brains using classic assay conditions

The aim of this first set of experiments was to investigate changes in [^{3}H]-8-OH-DPAT binding parameters in brain areas from rats that had received various antidepressant treatments; these same rat brains, that we used for our assays, had previously demonstrated enhanced 5-HT transmission in an electrophysiological study. This was assessed by an increased efficacy of the stimulation of 5-HT pathways (de Montigny, Chaput and Blier, 1989). As mentioned earlier, the enhanced transmission takes different forms depending on the type of treatment. First an increased responsiveness of postsynaptic CA_3 neurons of the hippocampus to microiontophoretically-applied 5-HT and 8-OH-DPAT was observed following repeated electroconvulsive shock treatment (ECT) and following chronic imipramine, a common tricyclic antidepressant. Chronic administration of the 5-HT reuptake blockers paroxetine and indalpine, however, had no effect postsynaptically since they enhance 5-HT transmission through a desensitization of the presynaptic terminal autoreceptor. Chronic MAO A inhibition with clorgyline, on the other hand, decreased the responsiveness of the postsynaptic 5-HT_{1A} receptor to microiontophoretically-applied agonist. This effect is assumed to be outweighed by a

presynaptic somatodendritic autoreceptor desensitization which in turn enhances 5-HT transmission. We therefore decided to investigate, by [^{3}H]-8-OH-DPAT saturation analysis, the effect of these treatments on the 5-HT$_{1A}$ binding site in two postsynaptic areas, the hippocampus and the frontal cortex, in order to investigate whether these changes are evident at the molecular level of the 5-HT$_{1A}$ site.

Methods

[^{3}H]-8-OH-DPAT binding assays were performed as previously described for one population of high affinity binding sites, with the labelled ligand concentration ranging from 0.1 to 10 nM. Drugs dissolved in 0.9% saline were imipramine (10 mg/kg), paroxetine (5 mg/kg), indalpine (10 mg/kg) and clorgyline (1 mg/kg); they were injected i.p. for 21 days. Electroconvulsive shocks were administered 7 times over a period of two weeks.

Results and Discussion

In order to test the efficacy of the stimulation of the 5-HT pathways, de Montigny and colleagues used an antagonist of terminal 5-HT autoreceptors, methiothepin. Therefore, before making any comparison between the groups, it was necessary to check if this drug itself had any effect on [^{3}H]-8-OH-DPAT binding parameters. Methiothepin was previously shown to have some affinity for the [^{3}H]-8-OH-DPAT binding site (IC_{50}=80 nM, according to Hall et al., 1986). Therefore, we would expect this drug to increase the apparent K_d as would a competitive inhibitor (Munson & Rodbard, 1984) if a significant concentration of it was still present in the brain at the moment of the sacrifice 24 hours after its injection. However, no significant change was found in the binding parameters for [^{3}H]-8-OH-DPAT to hippocampal membranes from the control group (N=4): $K_d = 1.6 \pm 0.2$ nM, $B_{max} = 377 \pm 55$ fmol/mg, and the methiothepin group (N=8): $K_d = 2.2 \pm 0.2$

nM ($F=3.07$, $df= 1,12$; $P > 0.05$), $B_{max}=341 \pm 27$ fmol/mg ($F=0.44$, $df=1,12$; $P > 0.05$), or from frontocortical membranes between the control group ($N=4$): $K_d=1.2 \pm 0.1$ nM, $B_{max}=143 \pm 21$ fmol/mg, and the methiothepin group ($N=10$) $K_d=2.0 \pm 0.2$ nM ($F=3.96$, $df=1,10$; $P > 0.05$), $B_{max}=129 \pm 13$ fmol/mg ($F=0.33$, $df=1,10$; $P > 0.05$). Consequently we concluded that this drug has no interfering effect on the [^{3}H]-8-OH-DPAT binding assays to follow.

The results of the treatments on [^{3}H]-8-OH-DPAT binding in hippocampus and frontal cortex are presented in Tables I and II, respectively. One way analysis of variance on all the groups, including the control without methiothepin, revealed no change in receptor numbers for hippocampus ($F= 0.89$, $df= 6, 34$; $P > 0.05$), as well as for frontal cortex ($F= 0.72$, $df= 6, 36$; $P > 0.05$), but a significant main effect was found at the level of the K_d's for hippocampus ($F= 23.56$, $df= 6, 34$; $P < 0.001$), and for frontal cortex ($F= 19.38$, $df= 6, 36$; $P < 0.001$). Post hoc Student's t-tests revealed that both hippocampal and frontocortical [^{3}H]-8-OH-DPAT binding affinity from rats treated with clorgyline differed significantly from those of the saline group ($P < 0.001$). In the clorgyline group, the apparent K_d increased about 3 fold. This decrease in the affinity of the 5-HT_{1A} binding site may be correlated with the decreased responsiveness of hippocampal pyramidal neurons to microiontophoretically-applied 5-HT or 8-OH-DPAT following chronic clorgyline treatment. In the next experiment, we investigate whether the effect we observed above is really related to the chronicity of the treatment, and may therefore be related to its mechanism of antidepressant or anxiolytic action, or whether the effect is evident acutely.

None of the other groups showed any change in K_d compared to control. The absence of regulation of the postsynaptic 5-HT_{1A} binding site by the reuptake blockers, paroxetine and indalpine, may have been expected from their inability in electrophysiological studies to alter the postsynaptic 5-HT_{1A} receptor. However, the absence of up-regulation or increased affinity of the 5-HT_{1A} binding site following repeated ECS and chronic imipramine treatment contrasts with the increased responsiveness these treatments created at the level of the receptor when assessed *in vivo* by electrophysiological recording on the same group of animals. The reason for this discrepancy is not at present clear.

Complementary analysis from the binding experiments reported above showed that, using a range of concentrations commonly employed in studies from the literature, some of our Scatchard plots were fitted significantly better ($P < 0.05$) with two sites, while many others showed tendencies towards this. Therefore the characteristics of [^{3}H]-8-OH-DPAT binding may be more complex than first expected. Thus, it was necessary to investigate this aspect of 5-HT_{1A} binding further in a second set of experiments.

Table I: Effect of chronic treatment with paroxetine (5 mg/kg), electroconvulsive shock, imipramine (10 mg/kg), indalpine (10 mg/kg) and clorgyline (1 mg/kg) on rat hippocampal [^{3}H]-8-OH-DPAT binding site.

Treatment	K_d (nM)	B_{max} (fmol/mg)	N
saline	2.2 ± 0.2	341 ± 27	8
clorgyline	8.2 ± 1.3 *	425 ± 36	5
ECT	1.7 ± 0.3	336 ± 15	7
imipramine	1.6 ± 0.2	329 ± 49	7
indalpine	2.1 ± 0.1	361 ± 40	3
paroxetine	2.0 ± 0.2	328 ± 24	7

Each value is the mean ± S.E.M. of N determinations.
* $P < 0.001$ compared to control value.

Table II: Effect of chronic treatment with paroxetine (5 mg/kg), electroconvulsive shock, imipramine (10 mg/kg), indalpine (10 mg/kg) and clorgyline (1 mg/kg) on rat frontocortical [^{3}H]-8-OH-DPAT binding site.

Treatment	K_d (nM)	B_{max} (fmol/mg)	N
saline	2.0 ± 0.2	129 ± 13	10
clorgyline	6.2 ± 0.5 *	135 ± 17	4
ECT	1.7 ± 0.3	109 ± 34	7
imipramine	2.1 ± 0.3	129 ± 16	7
indalpine	2.1 ± 0.2	151 ± 5	3
paroxetine	2.2 ± 0.3	129 ± 5	8

Each value is the mean ± S.E.M. of N determinations. * $P < 0.001$ compared to control value.

II. Further evidence for interconvertible affinity states of the 5-HT_{1A} receptor in hippocampus.

The second set of experiments included in this thesis is a manuscript entitled as above by R. Mongeau, S. A. Welner, R. Quirion and B. E. Suranyi-Cadotte submitted to the Journal of Neurochemistry. These experiments aimed to clarify the nature of [^{3}H]-8-OH-DPAT curvilinear Scatchard plots prior to continuing experimentation with further antidepressant and anxiolytic treatments.

Abstract-The binding profile of [^{3}H]-8-hydroxy-2-(di-N-propylamino)-tetralin ([^{3}H]-8-OH-DPAT) to 5-HT_{1A} sites in rat hippocampal, frontocortical and striatal membranes has been compared. In these regions, [^{3}H]-8-OH-DPAT labels both a high and a low affinity binding site; the K_d values for each of the two sites are comparable in the different brain regions, but have different maximal capacity (B_{max}). By modifying the experimental conditions in a series of hippocampal membrane preparations, reciprocal changes in the proportion of the two sites were observed suggesting that they represent, at least in this region, different conformations or affinity states of a single receptor protein. In contrast to the lower affinity state, it appears that the high affinity state is stabilized by coupling with a G-protein. Evidence supporting this statement is provided by addition of the guanine nucleotide Gpp(NH)p, breakage of labile disulfide bonds using N-ethylmaleimide (NEM) and increasing membrane rigidity with ascorbate-induced lipid peroxidation, conditions which all reduced the high affinity state B_{max} values. Moreover, the high affinity state appears to be stabilized at the expense of the lower affinity state in the presence of Mn^{2+}. On the other hand, a complete shift to the low affinity binding state was observed after a 24 hour *in vivo* treatment with inhibitors of monoamine oxidase (MAO) A (phenelzine or clorgyline) but not of MAO B (deprenyl). This disappearance of the high affinity state with a concomitant increase in the binding capacity of the low affinity state was reproduced by inhibiting MAO A *in vitro*, as well as by reducing preincubation washout periods. Also, competitors of the [^{3}H]-8-OH-DPAT binding site, such as 5-HT and unlabelled 8-OH-DPAT, display two affinity sites while others like propranolol, tryptamine and spiperone recognize a single affinity component. These results suggest that the 5-HT_{1A} binding site may exhibit at least two interconvertible affinity states depending upon its microenvironment and the intrinsic activity of the ligand used.

Key words: 5-HT_{1A} Receptors - Modulation - Guanine nucleotide binding proteins - Sulfhydryl bonds - Lipid peroxidation - MAO inhibitors.

Abbreviations used: DFX, desferrioxamine; DTT, dithiothreitol; G-protein, guanine nucleotide binding protein; Gpp(NH)p, guanylylimidodiphosphate; 8-OH-DPAT, 8-hydroxy-2-(di-N-propylamino)-tetralin; 5-HT, 5-hydroxytryptamine; MDA, malondialdehyde; MAO, monoamine oxidase; NEM, N-ethylmaleimide; TBA, 2-thiobarbituric acid; TBAR, thiobarbituric acid-positive reactant.

In recent years, considerable progress has been made with respect to the classification of CNS serotonergic receptors. A distinction between 5-HT_1 and 5-HT_2 receptors is now commonly accepted on the basis of their respective affinities for [^{3}H]-5-HT and related agonists (5-HT_1 > 5-HT_2) and for certain 5-HT antagonists such as [^{3}H]-spiperone (5-HT_2 > 5-HT_1). The 5-HT_1 receptor class has been further divided into the 5-HT_{1A}, 5-HT_{1B}, 5-HT_{1C} and 5-HT_{1D} in the brain (for review, see Peroutka, 1988). The discovery in the early 1980's of a ligand, 8-hydroxy-2-(di-N-propylamino)-tetralin (8-OH-DPAT), which binds relatively selectively to 5-HT_{1A} sites (Hjorth et al., 1982; Gozlan et al., 1983; Middlemiss and Fozard, 1983; Hamon et al., 1984; Hall et al., 1985) has been invaluable for the characterization of this receptor class.

It has generally been accepted that [^{3}H]-8-OH-DPAT labels a single population of high affinity 5-HT_{1A} sites (Peroutka, 1986). It has also been suggested that 8-OH-DPAT may interact with another class of lower affinity binding sites located presynaptically in the striatum (Gozlan et al. 1983; Hall et al., 1985). However, recent data have shown the terminal presynaptic 5-HT autoreceptors to be of the 5-HT_{1B} subtype, at least in the rat brain (Engel et al., 1986; Hibert and Middlemiss, 1986). It has also been proposed that [^{3}H]-8-OH-DPAT may label a putative 5-HT reuptake site in striatum (Shoemaker and Langer, 1986; Alexander and Wood, 1988), as well as in platelets (Ieni and Meyerson, 1988).

It is also becoming increasingly clear that 5-HT_{1A} receptors are negatively coupled to adenylate cyclase via a guanine nucleotide binding inhibitory regulatory protein (G_i-protein) (De Vivo and Maayani, 1985; Weiss et al., 1986; Fargin et al., 1989). GTP is required for the uncoupling of the receptor from the G_i-protein in order for inhibition of adenylate cyclase to occur. Thus, this may suggest an alternate hypothesis based on the existence of interconvertible affinity states. The results reported here support this hypothesis and suggest that high affinity

[^{3}H]-8-OH-DPAT binding sites may correspond to a state stabilized by the regulatory protein since it is enhanced by Mn^{2+} and converted into a low affinity state by addition of guanylylimidodiphosphate (Gpp(NH)p), a nonhydrolysable GTP analogue. Moreover, the facts that both sulfhydryl bond cleavage and ascorbate-induced lipid-peroxidation reduced the high to low affinity ratio, and that agonists and antagonists have different saturation profiles, provide additional evidence for the existence of multiple affinity states for the 5-HT_{1A} receptor.

An additional objective of this study was to investigate the molecular basis for 5-HT_{1A} receptor desensitization observed following monoamine oxidase (MAO) inhibition. It is proposed that accumulation of endogenous neurotransmitters following the blockade of MAO A catabolic processes mediate alterations in the high to low affinity B_{max} ratio, as one would expect to be the case for interconvertible receptor states.

Methods

In vitro treatment

N-ethylmaleimide (NEM; 500 uM), dithiothreitol (DTT; 500 uM) and the MAO inhibitor phenelzine dose were added to the 50 mM Tris-HCl (pH 7.4) buffer prior to preincubation step while manganese chloride and Gpp(NH)p were added to the final incubation 50 mM Tris buffer (pH 7.7). To protect against disulfide bond breakage, DTT was added a few minutes before NEM (Stratford et al., 1988). Lipid peroxidation (Andorn et al., 1987) was induced by the addition of 1 uM $FeSO_4$ and 0.01% ascorbate to the membrane preparation prior to a 20 min preincubation at 37^{o}C. The reaction was stopped by centrifugation at 45,000 g for 10 min and the supernatant discarded. Two additional washouts were performed to prevent any direct action on the binding site of the lipid peroxidation blocker (desferrioxamine) or inducer (Fe^{2+}) which was added before the preincubation period. A thiobarbituric acid-positive reactant (TBAR) assay was used to assess the level of

lipid peroxidation. A standard curve using malondialdehyde (MDA) as the positive reactant was established by placing 0-16 nmol of this compound in a solution containing 2.2% trichloroacetic acid, 0.06 N HCl and 3 mM TBA in a final volume of 2.5 ml. The samples (1 ml) were prepared as for binding assays in 20 vol of 50 mM Tris buffer (pH 7.4), 1 μM $FeSO_4$ and various concentrations of ascorbate and then incubated at 37°C for 20 min, before adding TCA for a final concentration of 5% TCA. This homogenate was then centrifuged at 1700 x g for 10 min and the pellet discarded. A solution of TBA and HCl was added to the supernatant for final concentrations and volume as per the standard curve. The standards and samples were incubated at 100°C for 10 min and cooled on ice. The absorbance was then determined at 532 nm with a spectrophotometer.

In vivo MAO inhibition

Acute *in vivo* treatment with clorgyline (1 mg/kg), phenelzine (15 mg/kg), deprenyl (0.25 mg/kg) or saline were performed by i.p. injection of the drugs dissolved in a 50% propylene glycol and 50% saline (0.9%) solution, 24 hours prior to sacrifice. Statistical significance between the $B_{max}L$ of the treated animals was tested using a one way analysis of variance (ANOVA).

Results

[^{3}H]-8-OH-DPAT binding parameters in various brain regions

Using a broad range of [^{3}H]-8-OH-DPAT concentrations (0.05 to 60 nM), saturation analysis revealed that this ligand binds in a non-linear fashion to sites present in hippocampus (Fig. 1a) , frontal cortex (Fig. 1b) and striatum (Fig. 1c). The [^{3}H]-8-OH-DPAT binding profile fit significantly better ($P<0.01$) with two site than with a one site model. The apparent K_d of the high affinity component (K_dH) was between 15-100 fold higher than that of the low affinity one (K_dL) (Fig. 1). Such

a difference represents a good separation of the two binding components, especially since a high level of specific [^{3}H]-8-OH-DPAT binding is maintained at saturating concentrations (see insets, Fig.1 a,b,c).

In terms of maximal densities (B_{max}), the amount of sites in the high affinity state as compared to those in the low was found to vary among regions. The ratio of high to low affinity sites in the hippocampus was 0.79 $\pm$ 0.15, this being similar to that found in frontal cortex (0.98 $\pm$ 0.13), but different from striatum (0.11 $\pm$ 0.03) where a very small proportion of B_{max}H was found (Fig. 1). Thus it appears that high and low [^{3}H]-8-OH-DPAT binding components exist in different proportions in the three brain regions studied here.

fig.1

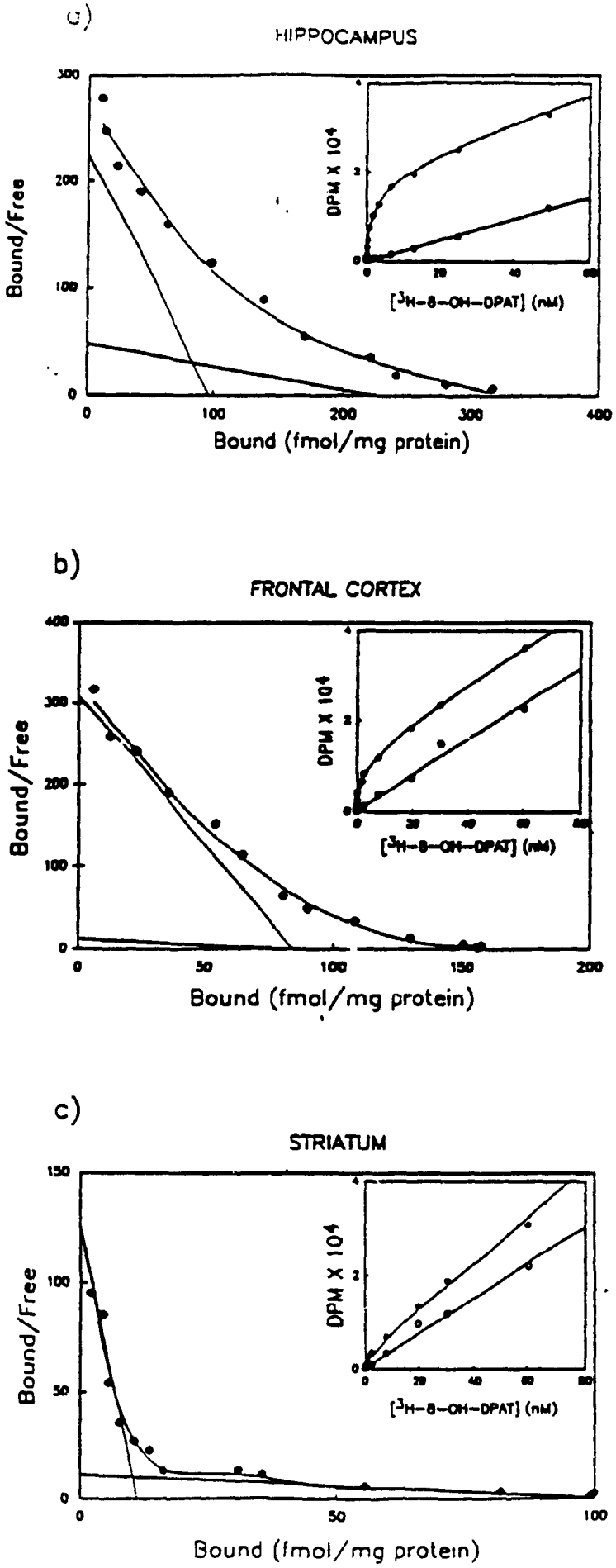

a)
HIPPOCAMPUS
Bound/Free
Bound (fmol/mg protein)
DPM X 10⁴
[³H-8-OH-DPAT] (nM)
b)
FRONTAL CORTEX
Bound/Free
Bound (fmol/mg protein)
DPM X 10⁴
[³H-8-OH-DPAT] (nM)
c)
STRIATUM
Bound/Free
Bound (fmol/mg protein)
DPM X 10⁴
[³H-8-OH-DPAT] (nM)

Fig. 1 Saturation (inset) and Scatchard transformation of [^{3}H]-8-OH-DPAT binding to **a)** hippocampal, **b)** frontocortical and **c)** striatal membrane preparations. Specific binding is represented by closed circles while non-specific binding is shown by open circles. Membranes were incubated for 30 min at 25^{o}C in 50 mM Tris-HCl buffer (pH 7.7), containing various concentrations of [^{3}H]-8-OH-DPAT (0.01-60nM). Mean $\pm$ SEM of 4-5 experiments each performed using 12 concentrations of ligand (each in triplicate): **Hippocampus:** K_dH = 0.51 $\pm$ 0.09 nM; $B_{max}H$ = 182 $\pm$ 33 fmol/mg protein; K_dL = 8.5 $\pm$ 2.4 nM; $B_{max}L$ = 225 $\pm$ 34 fmol/mg protein. **Frontal cortex:** K_dH = 0.55 $\pm$ 0.16 nM; $B_{max}H$ = 76 $\pm$ 4 fmol/mg protein; K_dL = 8.1 $\pm$ 1.5 nM; $B_{max}L$ = 80 $\pm$ 10 fmol/mg protein. **Striatum:** K_dH = 0.13 $\pm$ 0.07 nM; $B_{max}H$ = 13 $\pm$ 4 fmol/mg protein; K_dL = 13.5 $\pm$ 3.2 nM; $B_{max}L$ = 131 $\pm$ 20 fmol/mg protein.

Competition of [^{3}H]-8-OH-DPAT binding in membrane homogenates.

To further verify the existence of the two affinity states found in the hippocampus and to determine whether they have the 5-HT_{1A} receptor pharmacological profile, competition experiments were performed using some agonists and antagonists at this receptor. The K_d of the competitors for the site specifically bound by the radioactive ligand was calculated using saturation curve analysis; this showed that 8-OH-DPAT and 5-HT competition data were best fit with a two site model ($P < 0.001$) (Table III). In contrast, antagonists such as propranolol and spiperone displayed monophasic curves which were best fit with a single component of binding ($P < 0.001$) (Table III). Surprisingly, the binding profile of tryptamine, a weak agonist at [^{3}H]-8-OH-DPAT binding site, is similar to that for the two antagonists (Table III). Paroxetine, a serotonergic reuptake blocker, was evaluated in order to test if the low affinity component corresponded to a reuptake site (Shoemaker and Langer, 1986; Alexander and Wood, 1988). As shown in table III, paroxetine is unable to compete with the [^{3}H]-8-OH-DPAT site at up to micromolar concentrations.

Table III. Comparative affinities of various competitors for the [^{3}H]-8-OH-DPAT binding site in hippocampal membrane preparations.

compound	K_H (nM)	K_L (nM)	$B_{max}H$ %
8-OH-DPAT	0.66 ± 0.40	37 ± 9	33 ± 1
5-HT	1.8 ± 0.5	59 ± 26	42 ± 2
Propranolol	-	219 ± 7	
Tryptamine	-	397 ± 38	
Spiperone	-	513 ± 73	
Paroxetine	-	> 10 000	

Hippocampal membranes were incubated for 30 min at 25°C in 50 mM Tris-HCl buffer (pH 7.7), containing 1 nM [^{3}H]-8-OH-DPAT. Mean ± SEM of 3-4 experiments with at least 7 concentrations of competitor (each point in triplicate).

Modification of [^{3}H]-8-OH-DPAT binding parameters in hippocampus

Effect of $MnCl_2$ and Gpp(NH)p. As shown in Fig. 2, when assays are performed in the presence of 1 mM $MnCl_2$, [^{3}H]-8-OH-DPAT binds in a monophasic fashion, in contrast to data obtained in its absence (Fig. 1a). In fact, only the high affinity site is observed in the presence of Mn^{2+} (Fig 2); this effect is reversed by 100 uM Gpp(NH)p (Fig. 2). The B_{max} of the high affinity binding component decreased from 255 $\pm$ 24 fmol/mg protein in the presence of Mn^{2+} alone to 36 $\pm$ 9 fmol/mg protein when Gpp(NH)p was added; this loss was partially replaced by the appearence of 140 $\pm$ 23 fmol/mg protein of a low affinity component (Fig. 2).

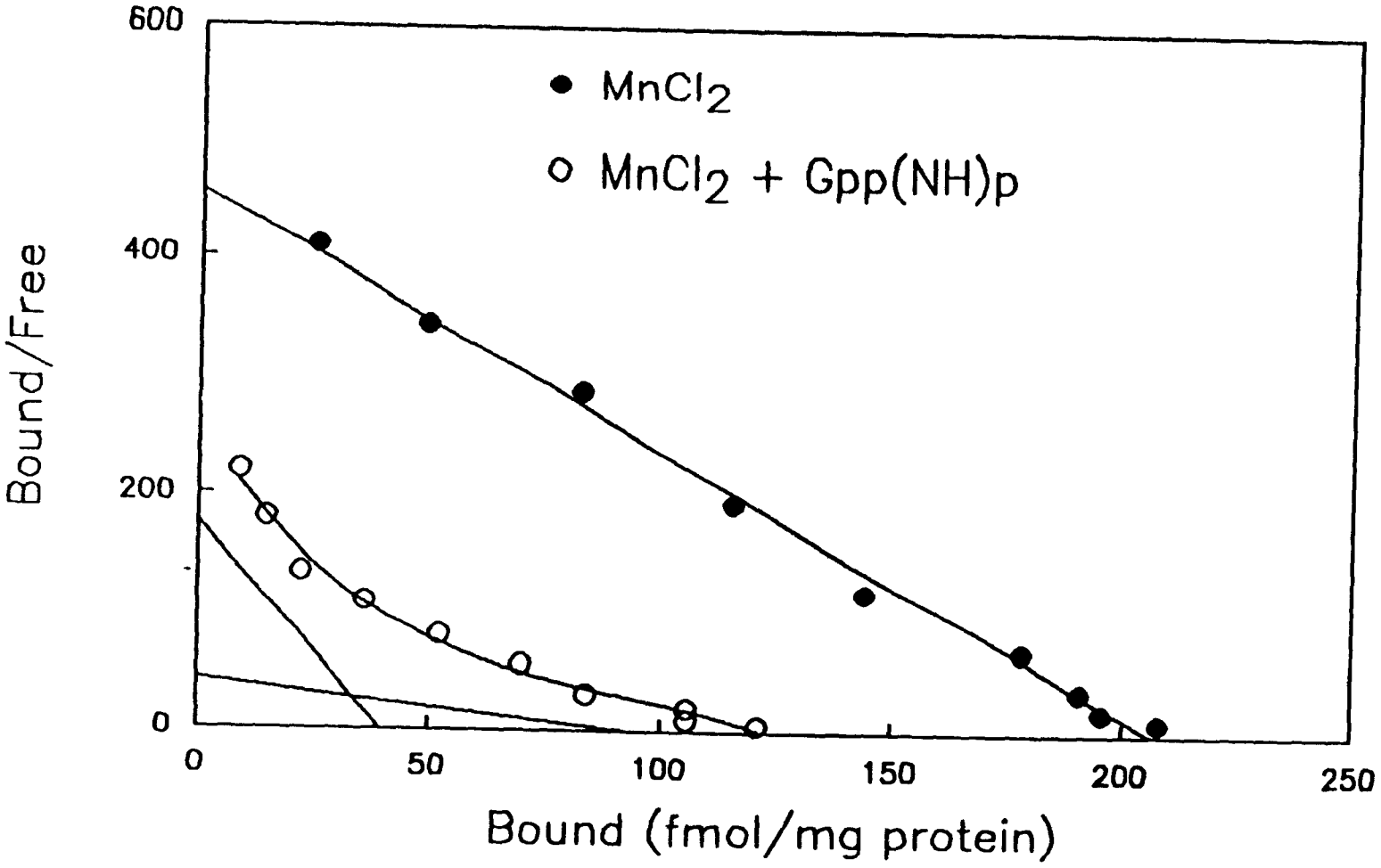


Fig. 2 Saturation binding of [^{3}H]-8-OH-DPAT (0.01-10 nM) to hippocampal membranes prepared in 50 mM Tris-HCl buffer (pH 7.7) containing 1 mM $MnCl_2$, and in the presence (open circles) or absence (closed circles) of 100 μM Gpp(NH)p. A single pool of membrane preparations was used for the two conditions in each experiment. A single class of sites was detected in absence of Gpp(NH)p. Mean $\pm$ SEM of 5 determinations using 10 concentrations of ligand each performed in triplicate: **without Gpp(NH)p:** K_dH = 0.69 $\pm$ 0.12 nM; $B_{max}H$ = 255 $\pm$ 24 fmol/mg protein, **with Gpp(NH)p:** K_dH = 0.23 $\pm$ 0.04 nM; $B_{max}H$ = 36 $\pm$ 9 fmol/mg protein; K_dL = 2.3 $\pm$ 0.3 nM; $B_{max}L$ = 140 $\pm$ 23 fmol/mg protein.

Effect of N-ethylmaleimide and dithiothreitol. Because G-proteins are usually coupled to receptor via sulfhydryl bridges, the effect of N-ethylmaleimide (NEM), a disulfide bond destroying agent (Riordan and Vallee, 1972), on [^{3}H]-8-OH-DPAT binding was investigated next. Addition of NEM (500 *u*M) before preincubation decreased the proportion of high affinity binding from 53 $\pm$ 3% to 30 $\pm$ 4% ($P < 0.001$; paired t-test performed on $B_{max}H$ values), while concurrently increasing the percentage of the low affinity binding from 47 $\pm$ 3% to 70 $\pm$ 4% ($P < 0.01$; paired t-test performed on $B_{max}L$ values) (Fig. 3). This change of ratio was mostly associated with decreases in $B_{max}H$ values (Fig. 3). In the presence of dithiothreitol (DTT; 500 *u*M) the total binding capacity increased slightly (8 $\pm$ 3%). Addition of NEM to membranes pretreated with an equimolar concentration of DTT reversed the deleterious effect of NEM alone (Fig 3), suggesting an action of NEM on disulfide bonds.

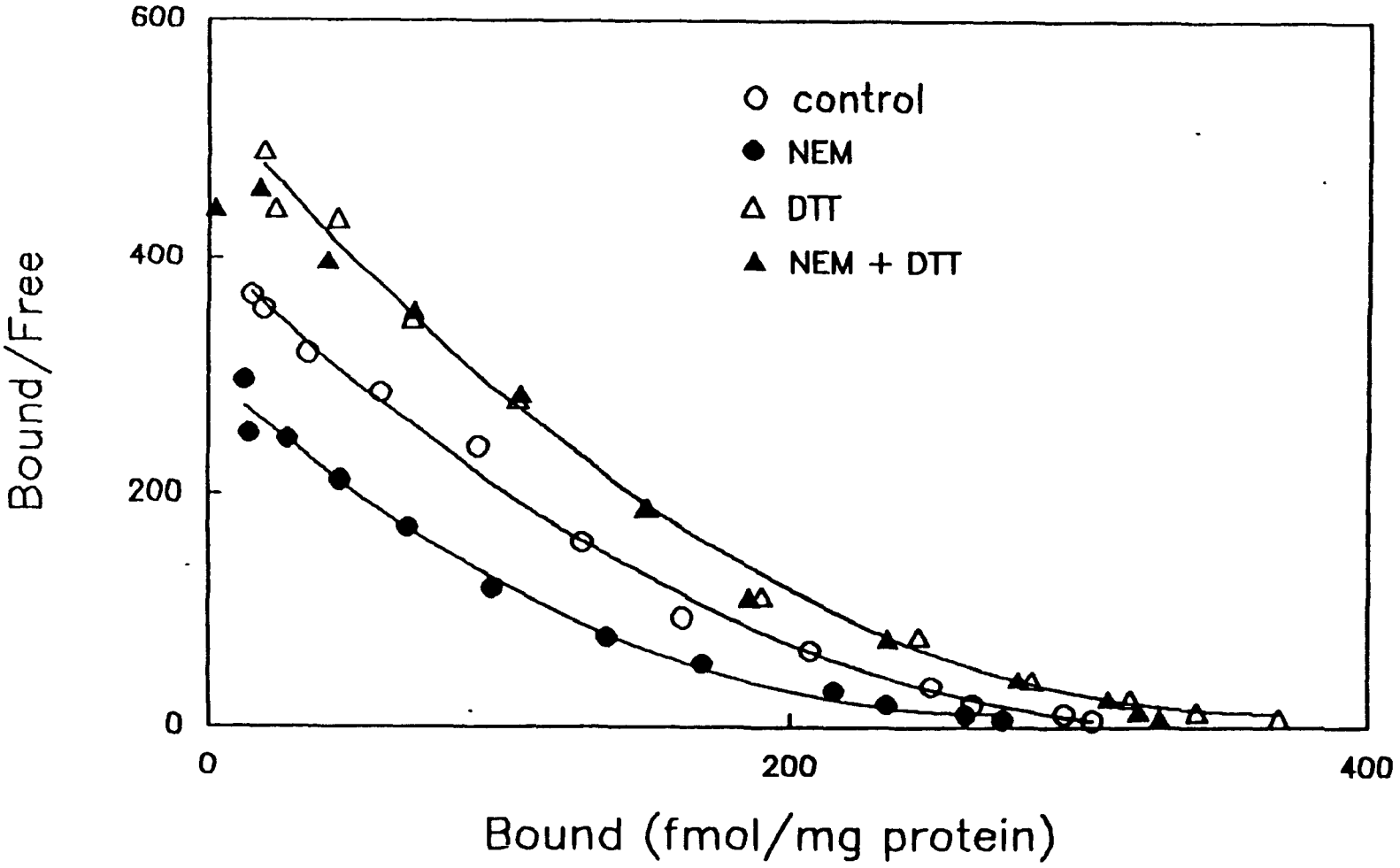


Fig. 3. Effects of NEM (500 *u*M) and DTT (500 *u*M) on [^{3}H]-8-OH-DPAT binding parameters to hippocampal membrane preparations. NEM and DTT were added to Tris-HCl buffer (pH 7.4) prior to preincubation at 37°C for 10 min. The effect of NEM was reversed by prior addition of DTT. Computer assisted analysis of the data gave the following estimates ± SE; **control:** K_dH = 0.46 ± 0.06 nM; $B_{max}H$ = 175 ± 25 fmol/mg protein; K_dL= 5.8 ± 2.7 nM; $B_{max}L$ = 148 ± 20 fmol/mg protein. **NEM:** K_dH = 0.39 ± 0.08 nM; $B_{max}H$ = 101 ± 25 fmol/mg protein; K_dL = 4.1 ± 1.4 nM; $B_{max}L$ = 187 ± 21 fmol/mg protein. **DTT and NEM + DTT:** K_dH = 0.37 ± 0.04 nM; $B_{max}H$ = 173 ± 24 fmol/mg protein; K_dL = 3.7 ± 0.3; $B_{max}L$ = 177 ± 20 fmol/mg protein. Data presented in the text are means of three assays, each performed in triplicate.

Effect of ascorbate-induced lipid-peroxidation. Since membrane fluidity is thought to be involved in regulating the level of coupling of G-proteins with receptors (Cooney et al., 1986), ascorbate-induced lipid-peroxidation which increases membrane rigidity (Dobretsov et al., 1977), was investigated next as a means to further verify the nature of [^{3}H]-8-OH-DPAT binding components. It has been shown that small concentrations of ascorbate in the presence of a metallic catalyst is a prooxidant and induces lipid peroxidation (Muakkassah-Kelly et al, 1982; Heikila, 1983; Andorn et al., 1987; May et al., 1988). Under our conditions, a decrease of [^{3}H]-8-OH-DPAT binding was negatively correlated ($r^2 = 0.93$) with the production of MDA, an index of lipid peroxidation (Table IV). Preincubation of membrane homogenates with 0.01% ascorbate and 1 uM $FeSO_4$ resulted in a decrease in the percentage of $B_{max}H$ from 45 $\pm$ 4% to 7 $\pm$ 6% while increasing $B_{max}L$ from 55 $\pm$ 4% to 93 $\pm$ 6% (Fig. 4).

Iron chelation by the agent desferrioxamine (DFX) has previously been shown to block ascorbate-induced lipid peroxidation (Andorn et al., 1987). As expected, DFX fully reversed the effect of ascorbate and Fe^{2+} (Fig. 4). Preincubation of homogenates in the presence of DFX (100 uM) alone increased [^{3}H]-8-OH-DPAT binding especially to the high affinity component (Fig. 4). However, the binding remained fully displacable with 5-HT, with no significant change in K_i values (1.7 $\pm$ 0.5 nM for control and 2.4 $\pm$ 0.8 nM for DFX treated).

Table IV. Effect of lipid peroxidation on [^{3}H]-8-OH-DPAT specific binding

Ascorbate (%)	[^{3}H]-8-OH-DPAT binding (dpm/ug wet tissue)	Malondialdehyde (nmol/ug wet tissue)
0	2677	0.23
0.01	835	3.84
0.05	1349	3.01
0.1	2074	0.48

Membranes were prepared in 50 mM Tris-HCL buffer (pH 7.4), plus 1 uM $FeSO_4$ and were exposed to various concentrations of ascorbate for 20 min at 37^{o}C. After this treatment, membranes were tested for either [^{3}H]-8-OH-DPAT binding or TBAR assay. Both sets of data are the mean of 2 experiments each performed in triplicate.

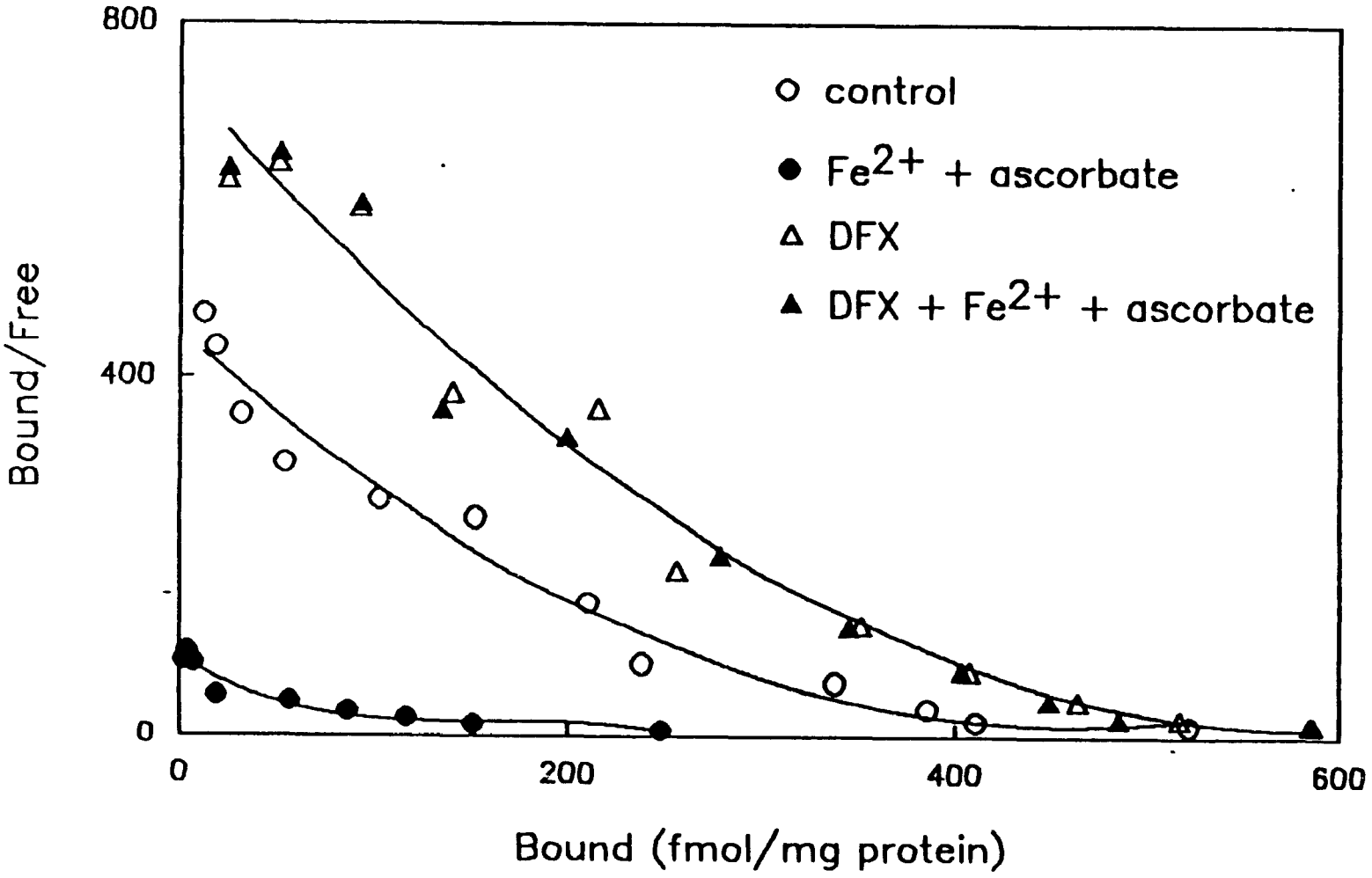


Fig. 4. Effects of inducing lipid peroxidation with ascorbate (0.01%) and $FeSO_4$ (1 *u*M) on [^{3}H]-8-OH-DPAT binding parameters, and the reversal by desferrioxamine (DFX; 100 *u*M). Ascorbate, $FeSO_4$ and DFX were added to Tris-HCl buffer (pH 7.4) prior to preincubation at 37°C for 20 min. After preincubation, membranes were washed two additional times to remove these substances. Computer assisted analysis of the data gave the following estimates ± SE; **Control:** K_dH = 0.59 ± 0.15 nM; $B_{max}H$ = 249 ± 61 fmol/mg; K_dL = 16.5 ± 18.5 nM; $B_{max}L$ = 352 ± 105 fmol/mg protein. **Ascorbate + $FeSO_4$:** K_dH = 0.19 ± 0.30 nM; $B_{max}H$ = 7 ± 6 fmol/mg; K_dL = 8.1 ± 2.7 nM; $B_{max}L$ = 258 ± 31 fmol/mg protein. **DFX and DFX + ascorbate + $FeSO_4$:** K_dH = 0.49 ± 0.09 nM; $B_{max}H$ = 341 ± 56 fmol/mg protein; K_dL = 15.7 ± 16.7 nM; $B_{max}L$ = 330 ± 88 fmol/mg protein. Data presented in the text are means of three assays, each performed in triplicate.

[^{3}H]-8-OH-DPAT binding parameters and monoamine oxidase activity

It has been proposed that inhibition of monoamine oxidase (MAO) A activity can desensitize postsynaptic 5-HT_{1A} receptors via an unknown mechanism (Sleight et al., 1988). In order to test if alterations of [^{3}H]-8-OH-DPAT binding to high and low affinity components could be correlated to this observation, we investigated the effect of MAO A and MAO B inhibitors on [^{3}H]-8-OH-DPAT binding in the hippocampus.

As shown in Fig. 5, the binding profile of [^{3}H]-8-OH-DPAT was profoundly altered in rat treated 24 hours prior to sacrifice with either phenelzine (15 mg/kg; i.p.) or the specific MAO A inhibitor clorgyline (1 mg/kg; i.p.), but not with the MAO B blocker deprenyl (0.25 mg/kg; i.p.). With both active agents, binding curves became linear with a complete loss of the high affinity component (Fig. 5). The $B_{max}L$ increased compared to the saline control (231 $\pm$ 28 to 326 $\pm$ 32 for phenelzine and 354 $\pm$ 18 fmol/mg protein for clorgyline; $P < 0.05$). Similar results can be obtained *in vitro* by adding various concentrations of phenelzine to membrane homogenates prior to preincubation. The optimal concentration required appears to be in the range of 1.0 uM, with higher concentrations not being more effective while 0.1 uM is found to be inactive (Table V). Phenelzine (1.0 uM) had no direct effect on [^{3}H]-8-OH-DPAT binding and had to be added before the preincubation to be effective. This may indicate that this effect is most likely related to a blockade of MAO A which may modify [^{3}H]-8-OH-DPAT binding parameters as a result of an accumulation of endogenous amines (Nelson et al., 1978).

This notion was further examined by varying the preincubation time since putative endogenous amines may not be fully destroyed by MAO A during short preincubation periods. Interestingly, similar data as following phenelzine or clorgyline treatment (Fig. 5) were obtained using a shortened preincubation period of 2 min (Fig. 6). Increasing the length of the preincubation period induced the

progressive appearance of the high affinity component (Fig. 6), as assessed by increases in $B_{max}H$ value (Table VI). However, no apparent alteration in K_dH and K_dL values were observed (Table VI).

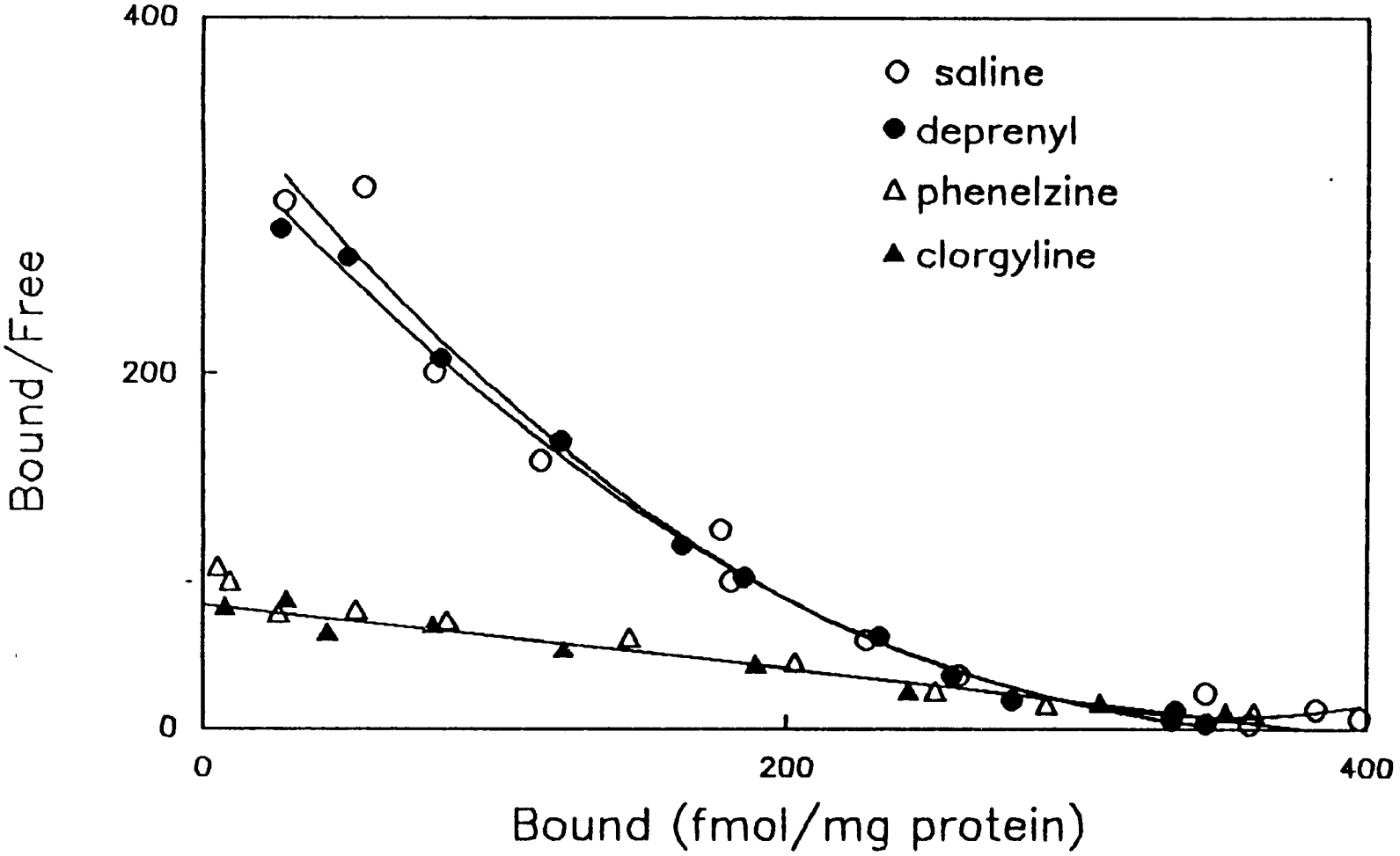


Fig. 5. Effects of *in vivo* treatment with phenelzine (15 mg/kg; i.p.), clorgyline (1 mg/kg; i.p.), and deprenyl (0.25 mg/kg; i.p.) on [^{3}H]-8-OH-DPAT binding parameters. The animals were sacrified 24 hours after the injection with one of the above-named drugs. Mean ± SEM of 3 determinations; **saline:** K_dH = 0.74 ± 0.38 nM; $B_{max}H$ = 149 ± 14 fmol/mg protein; K_dL = 6.9 ± 1.5 nM; $B_{max}L$ = 231 ± 28 fmol/mg protein. **phenelzine:** K_dL = 3.9 ± 0.4 nM; $B_{max}L$ = 326 ± 32 fmol/mg. **clorgyline:** K_dL = 4.0 ± 1.1 nM; $B_{max}L$ = 354 ± 18 fmol/mg protein. **deprenyl:** K_dH = 0.43 ± 0.13 nM; $B_{max}H$ = 131 ± 5 fmol/mg protein; K_dL = 5.7 ± 1.2 nM; $B_{max}L$ = 254 ± 12 fmol/mg protein.

TABLE V. *In vitro* effect of various concentrations of phenelzine on [^{3}H]-8-OH-DPAT binding parameters.

	High affinity state		Low affinity state	
Phenelzine (uM)	K_dH (nM)	$B_{max}H$ (fmol/mg protein)	K_dL (nM)	$B_{max}L$ (fmol/mg protein)
0	0.82 ± 0.12	264 ± 44	11.4 ± 8.0	216 ± 32
0.1	0.67 ± 0.09	280 ± 44	9.1 ± 5.6	226 ± 33
1	—	—	2.2 ± 0.1	441 ± 12
10	—	—	3.1 ± 0.2	439 ± 22
100	—	—	2.9 ± 0.2	453 ± 16

Phenelzine was added to 50 mM Tris-HCl buffer (pH 7.4) prior to the preincubation at 37°C for 10 min. Computer assisted analysis of the data gave the following K_d and B_{max} values ± SE. The assays, performed in triplicate, were reproduced three times.

TABLE VI. Effect of varying preincubation time on [^{3}H]-8-OH-DPAT binding parameters.

	High affinity state		Low affinity state	
Preincubation time (min)	K_dH (nM)	$B_{max}H$ (fmol/mg protein)	K_dL (nM)	$B_{max}L$ (fmol/mg protein)
2	—	—	2.2 ± 0.1	361 ± 8
4	0.02 ± 0.02	5 ± 2	1.7 ± 0.1	341 ± 6
7	0.22 ± 0.10	81 ± 43	2.2 ± 0.9	242 ± 37
10	0.27 ± 0.07	143 ± 43	3.9 ± 0.6	210 ± 35
20	0.30 ± 0.05	183 ± 29	7.0 ± 0.5	225 ± 27

Preincubation was carried out in 50 mM Tris-HCl (pH 7.4) at 37^{0}C and was terminated by cooling on ice. Computer assisted analysis of the data gave the following estimates ± SE. The assays, performed in triplicate, were reproduced three times.

Discussion

[^{3}H]-8-OH-DPAT binds to two affinity sites in various regions of the rat brain. In hippocampus, these sites appear to be affinity states of the 5-HT_{1A} receptor. This hypothesis is supported by the following observations: a) Mn^{2+} can shift the curvilinear binding curves to a homogenous population of the high affinity component, this being reversed by co-incubation with the nonhydrolysable guanine nucleotide Gpp(NH)p; b) NEM, a disulfide bond destroying agent, decreased the proportion of high affinity binding while markedly increasing the percentage of the lower affinity one, an effect that was reversed by DTT, a sulfhydryl stabilizing agent; c) ascorbate-induced lipid-peroxidation reduced the high to low affinity ratio, an effect reversed by DFX, a lipid peroxidation blocker; d) *in vivo* and *in vitro* inhibition of MAO A activity by phenelzine or clorgyline resulted in a complete loss of the high affinity component and a concomitant increase in the density of the low affinity binding state; e) both high and low [^{3}H]-8-OH-DPAT binding affinity components were apparent in membrane homogenates following lengthy preincubation periods, while only the low affinity state was seen following a short (2 min) preincubation period, and f) finally, competitors of [^{3}H]-8-OH-DPAT binding display biphasic or a monophasic saturation curve profiles depending upon their intrinsic activity at these sites. While agonists (5-HT and 8-OH-DPAT) and a divalent cation (Mn^{2+}) apparently shift [^{3}H]-8-OH-DPAT binding into the high affinity state, antagonists (propranolol and spiperone) and the low intrinsic activity agonists (tryptamine) as well as various incubation conditions (sulfhydryl bond alteration, lipid peroxidation, modulation of enzymatic activity) are able to promote equilibrium in favor of the lower affinity state. Thus relatively small changes in the 5-HT_{1A} receptor microenvironment may have profound effects on receptor affinity states and could partly explain discrepancies in the literature (Hall et al., 1985; Peroutka, 1986) concerning the existence of two [^{3}H]-8-OH-DPAT binding site in

various regions of the rat brain. Moreover, the use of broad range of [^{3}H]-8-OH-DPAT concentrations is certainly necessary for the detection of the low affinity binding components in hippocampus and frontal cortex. However, in the striatum where even at low concentrations both sites are easily apparent, the K_d of the low affinity state of the 5-HT_{1A} receptor may be confounded with the K_d of the non-5-HT_{1A} component of a similar value previously described in this region (Gozlan et al. 1983; Hall et al., 1985).

The effects of Mn^{2+} and Gpp(NH)p on [^{3}H]-8-OH-DPAT binding are in accordance with the theoretical model of De Lean et al. (1980) which is based on the *beta*-adrenergic receptor. Interpreting our results according to this model, Mn^{2+} may stabilize the high affinity state of the 5-HT_{1A} binding site by promoting the formation of a ternary complex consisting of the 5-HT_{1A} receptor, the ligand and a G-protein (G_i). Upon addition of Gpp(NH)p, the G-protein would uncouple from the 5-HT_{1A} receptor which would then adopt a lower affinity for the ligand.

It is well known that sulfhydryl groups are important for insuring adequate coupling between the receptor and its G-protein as demonstrated, for example, for *beta* (Stadel & Lefkowitz, 1979) and *alpha*$_2$ (Kitamura & Nomura, 1987) adrenergic receptors. For the 5-HT_{1A} receptors, Hall et al. (1986) have shown that binding of [^{3}H]-5-HT and [^{3}H]-8-OH-DPAT are differentially sensitive to NEM in certain brain regions. These authors reported a weak potency of NEM against [^{3}H]-8-OH-DPAT binding in the striatum while a very strong effect was seen in the hippocampus. These regional changes in NEM potency is thought to reflect the presence of a presynaptic non-5-HT_{1A} [^{3}H]-8-OH-DPAT binding component in the striatum, since contrary to hippocampus, the binding in this region is strongly affected by lesions of 5-HT neurons (Gozlan et al., 1983). However, in the postsynaptic [^{3}H]-8-OH-DPAT binding of the hippocampus, NEM may uncouple the 5-HT_{1A} receptor from a G_i protein by the cleavage of labile sulfhydryl bonds,

this in turn reducing the high affinity binding component. The low affinity state would not be as greatly affected by such a treatment since it is uncoupled from the regulatory protein. A recent report (Stratford et al., 1988) supports this interpretation with the high affinity [^{3}H]-8-OH-DPAT binding component altered by NEM being recoverd by the addition of a G_i/G_o mixture to the membrane preparation.

Lipid peroxidation negatively affects various receptors including D_2 (Andorn et al., 1987), beta-adrenergic (Heikila, 1983) and 5-HT (Muakkassah-Kelly et al., 1982; May et al., 1988). We observed here that ascorbate-induced lipid-peroxidation markedly decreased the B_{max} of the high affinity component of [^{3}H]-8-OH-DPAT binding. This effect was found to be completely reversed in the presence of DFX, a strong iron chelator known to block lipid peroxidation (Andorn et al., 1987). In addition, DFX improved binding conditions so that the high affinity state was increased, perhaps by a blockade of some naturally occurring lipid peroxidation in the absence of TBAR formation (Andorn et al., 1987). It is kown that lipid peroxidation increases membrane phospholipid rigidity, most likely through a transfer of oxidized fatty acids from the membrane depth to the outer layer (Dobretsov et al., 1977). In turn, membrane rigidity may decrease the rate of coupling between receptors and their relevant G-proteins. Thus, lipid peroxidation may hinder the formation of high affinity 5-HT_{1A} receptor G-protein complexes, this being reflected by a reduced $B_{max}H/B_{max}L$ ratio. It is important to emphasize that only the estimation of the ratio between these two sites gives us a reliable indication of interconversion for any of the *in vitro* treatments tested here. Our results can not exclude the possible existence of other intermediary affinity states which would explain apparent discrepancies in maximal binding capacities. Moreover, the effects of these manipulations on [^{3}H]-8-OH-DPAT binding may not be limited to the level of the affinity states. For instance, change in membrane

polarity caused by lipid peroxidation may cause the loss of some receptors into the membrane depth explaining the overall reduction in binding capacity, including $B_{max}L$. A similar phenomenom could explain the discrepancy in total receptor numbers following Gpp(NH)p treatment since Floyd and Lewis (1983) have shown that guanine nucleotide are strong potentiators of lipid peroxidation.

Estimation of the K_d of various competitors provided evidence that only agonists stabilize the 5-HT_{1A} receptor in its high affinity state, most likely through the stabilisation of the ternary complex with a G-protein. In accordance with previous reports using cloned 5-HT_{1A} receptors (Fargin et al., 1988; Albert et al., 1990), only 5-HT and 8-OH-DPAT, two agonists at the 5-HT_{1A} receptor, revealed the existence of the two affinity sites in hippocampus. Propranolol and spiperone, two antagonists at the 5-HT_{1A} receptor, bound to a single affinity component and with a much lower potency. Interestingly, tryptamine, which is identical to 5-HT except for the absence of a hydroxyl group in position 5, also revealed a single binding component. Dumuis et al. (1987) have shown that substitutions in position 5 of various tryptamine derivatives decreased agonist efficiency to inhibit VIP plus forskolin-stimulated cAMP production in hippocampal and cortical neurons in primary culture. Thus, it may be that such tryptamine derivatives do not possess full intrinsic activity at the 5-HT_{1A} receptor.

Sleight et al. (1988) reported that chronic MAO A, but not MAO B, inhibition decreases 8-OH-DPAT-mediated inhibition of forskolin-stimulated adenylate cyclase. This 5-HT_{1A} receptor "desensitization" was correlated with increased tissue levels of 5-HT. In our studies, *in vivo* and *in vitro* blockade of MAO A, but not MAO B, shifted [^{3}H]-8-OH-DPAT binding to the lower affinity component. This effect is mimicked by a shortened preincubation period. It seems logical to suggest that this is likely related to the presence and/or accumulation of endogenous amines in the preincubation media under these various assay

conditions. The indentity of the endogenous amines which produce this effect, however, remains to be established but possible substrates for MAO A include 5-HT itself, and trace monoamines like tryptamine. Furthermore, this effect is not likely due to simple competitive inhibition by 5-HT as we also found that by adding increasing concentrations of 5-HT to the final incubation medium the K_ds of both affinity states increased without alteration of the biphasic profile of the saturation curves (not shown). Furthermore interactions between these amines and [^{3}H]-8-OH-DPAT binding sites are not adequately represented by classical competitive inhibition models since changes in apparent K_d values and no change in B_{max} values are expected in theory with such a model (Munson & Rodbard, 1984). In our experiments, changes in either K_dH or K_dL values were not observed; however reciprocal modification in B_{max}H and B_{max}L values following various preincubation times were noted. An alternative explanation is based on the presence of increasing concentrations of endogenous amines during the preincubation period that could provoke the gradual uncoupling of the high affinity 5-HT$_{1A}$ receptor from its G_i-protein resulting in a shift to a lower affinity state, as observed after MAO A blockade or short preincubation periods. In this latter model, in contrast to the competitive inhibition one, the *in vitro* concentration of monoamines required to trigger a shift to the low affinity state may be rather low depending upon the extent of GTP binding sites occupied by endogenous GTP.

It has been suggested earlier that the lower affinity component of [^{3}H]-8-OH-DPAT binding represented binding to the high affinity 5-HT reuptake carrier (Shoemaker & Langer, 1985; Alexander & Wood, 1988). However, it seems very unlikely that the large proportion of the low affinity binding component observed in the hippocampus represents this uptake site since [^{3}H]-8-OH-DPAT binding was not competed by paroxetine at concentration up to 10 uM. Also, recent reports showing that the cloned 5-HT$_{1A}$ receptor displays both a high and a low affinity

components (Fargin et al., 1988; Albert et al., 1990) strongly argues that these two sites represent a single molecular entity, at least in the hippocampus. Further studies will be necessary to determine if such conclusion can be extended to other brain regions.

III. The 5-HT$_{1A}$ Binding Site and the Circadian Cycle

Wesemann et al. (1983) first reported that circadian rhythm and sleep deprivation may affect [^{3}H]-5-HT binding sites in rat brain. A diurnal change in the responsiveness of CA_3 hippocampal pyramidal neurons to 5-HT, being highest in the evening, was also reported by de Montigny (1981). This was consistent with the hypothesis of a major serotonergic involvement in the regulation of the sleep-waking cycle. This postulate is supported by the observation that lesion of the dorsal and central raphe, which decreases 5-HT availabilty, produces an insomniac state in the cat (Petitjean et al., 1978). This same group found similar results following inhibition of tryptophane hydroxylase with PCPA, the insomnia being abolished by restoring the 5-HT levels with its precursor 5-HTP (Petitjean et al., 1985).

An involment of the serotonergic system in the circadian rhythm takes on importance in regard of psychoaffective diseases in the sense that such diseases are often associated with major disturbances in sleep-wake parameters. For example, shortening of paradoxical sleep latency, as well as alterations of deep sleep, have been reported in depressed patients (Kupfer, 1981); the shortened paradoxical sleep latency is reversed by the antidepressant indalpine, a 5-HT reuptake blocker (Kafi-de St Hilaire et al., 1984). The same group also found consistent increases in paradoxical sleep latency following administration of 8-OH-DPAT, suggesting the involvement of the 5-HT$_{1A}$ receptors in this effect (Kafi-de St.Hilaire et al., 1987).

That the 5-HT$_{1A}$ receptor may be implicated in the sleep-waking cycle was also substantiated by a report by Akiyoshi et al. (1989) who found that the 5-HT$_{1A}$ binding site, as labelled by [^{3}H]-5-HT, was altered during the circadian cycle, being at a maximal density during the sleep light period and at a minimal density during the active dark period. This report is very interesting because it may account for the optimal active dark period anxiolytic effect of 8-OH-DPAT in the rat (Eison et al.,

1986). Also, if such a regulation in 5-HT_{1A} binding sites really occurs during the day, there may be optimal times for treatments in order to observe active regulation of the 5-HT_{1A} binding site. Therefore, before proceeding with further antidepressant or anxiolytic treatments, we decided to investigate frontal cortex [^{3}H]-8-OH-DPAT binding at various intervals during the day, [^{3}H]-8-OH-DPAT being a more reliable ligand for the 5-HT_{1A} binding site than [^{3}H]-5-HT used by Akiyoshi and co-workers.

Methods

A group of rats was sacrificed every 3 hours within a 15 min delay. The light period was from 8:00 to 20:00 h. Brains were rapidly removed from the skull, the frontal cortex dissected, frozen in 2-methylbutane, and kept at -80^{o}C until used. [^{3}H]-8-OH-DPAT binding assays were performed as previously described. To avoid confusion that may have arisen from putative reciprocal interconversion of the two [^{3}H]-8-OH-DPAT affinity states, we shifted all 5-HT_{1A} binding sites to the high affinity state with 1 mM $MnCl_2$ (see Results, section II). Thus, saturation curves performed with 6 concentrations points ranging from 0.05-10 nM were fitted significantly better with one site ($P < 0.01$).

Results and Discussion

Table VII shows that the sleep light period optima and the active dark period minima in [^{3}H]-5-HT binding capacity observed by Akiyoshi et al. (1989) is not present under our conditions using [^{3}H]-8-OH-DPAT. In fact, one way analysis of variance (ANOVA) revealed no significant difference for either affinity (K_d) ($F = 1.07$, $df = 7,53$; $P > 0.05$) or maximal receptor number (B_{max}) ($F = 1.63$, $df = 7,53$; $P > 0.05$) at any time during the day. The discrepancies between our results and theirs could arise from our use of a more specific ligand for the 5-HT_{1A} receptor or from our use of a more specific cortical area; frontal cortex instead of the whole cerebral cortex. Alternatively, the discrepancy may indicate that a 5-HT receptor subtype other than the 5-HT_{1A} is changed with the circadian cycle. However, as will be detailed in the general discussion, it is possible that alteration in the ratio of 5-HT_{1A} receptor affinity states resulting from change in 5-HT availabilty occuring *in vivo* is not observed *in vitro* because of the disappearance during membrane preparations of the putative changes in the receptor microenvironment. Nevertheless, it remains that for the purposes of our treatment experiments, the time of sacrifice during the day does not appear to be a confounding variable.

Table VII. Lack of circadian rhythm variation of [^{3}H]-8-OH-DPAT binding.

time (hour)	K_d (nM)	B_{max} (fmol/mg protein)	N
0	0.61 ± 0.08	197 ± 18	8
3	0.57 ± 0.06	211 ± 17	8
6	0.60 ± 0.05	214 ± 27	8
9	0.58 ± 0.06	258 ± 25	8
12	0.71 ± 0.13	186 ± 10	7
15	0.67 ± 0.09	189 ± 18	8
18	0.88 ± 0.19	224 ± 22	7
21	0.62 ± 0.06	185 ± 12	7

The light period was from 8:00 to 20:00 h and the dark period was from 20:00 to 8:00. The results presented are mean ± SEM of N determinations.

IV. [^{3}H]-8-OH-DPAT Scatchard Analysis of in vivo Treated Rat Brains using Modified Assay Conditions

With the exception of clorgyline which is acting *in vitro* during the membrane preincubation, none of the treatments of the first set of experiments were found to have any positive effect on [^{3}H]-8-OH-DPAT binding parameters. This was particularly astonishing because imipramine and ECT were previously found to increase the responsiveness of serotonergic target neurons (see introduction). Following the logic of our finding that 5-HT_{1A} receptors may display interconvertible affinity states, we hypothetized that any changes in 5-HT_{1A} receptor responsiveness which may take place *in vivo* may affect the two sites in a reciprocal fashion in such a way that it could lead to an apparent absence of regulation in the total binding capacity when assessed *in vitro* by Scatchard analysis. In other words, a one site fit of what is in fact a two site curvilenear saturation may have led to erroneous conclusions in the first set of experiments (see Results, section I).

For practical and economical reasons (labelled 8-OH-DPAT is expensive), an extended range of [^{3}H]-8-OH-DPAT concentrations for a two site fit could not have been used to analyse another set of treated brains despite our interest in finding reciprocal changes in the two affinity states. We therefore decided to shift all [^{3}H]-8-OH-DPAT binding to the high affinity state with $MnCl_2$ and use a limited range of concentrations (see Results, section II). This way, if treatments induce any observable change in the 5-HT_{1A} binding sites coupling with their G-protein, alteration in maximal binding capacity (B_{max}) could possibly be revealed in a linear Scatchard plot with the help of the stabilisation effect of Mn^{2+}.

We decided to apply this strategy to two treatments previously used in the first set, imipramine and ECT, in order to test whether there would be any change in the outcome of the binding data compared to that after the first set of treatments. We also used lithium, a treatment which has been shown to increase hippocampal

neuron responsiveness via the 5-HT$_{1A}$ receptor (Blier et al., 1987). Finally we used two anxiolytics, gepirone, which probably acts as an antagonist (or partial agonist) on the 5-HT$_{1A}$ postsynaptic receptor, and diazepam, which acts as potentiator of GABAergic neurotransmission via the benzodiazepine sites. We must also point out that besides being an anxiolytic, gepirone has been shown to possess antidepressant action (Amsterdam et al., 1987; Csanalosi et al., 1987; Robinson et al., 1989), probably through a desensitization of the DR 5-HT$_{1A}$ autoreceptors and by a direct agonist action on the 5-HT$_{1A}$ postsynaptic receptors (Blier and de Montigny, 1987).

Material and Methods

[^{3}H]-8-OH-DPAT binding assays were performed as previously described for one population of high affinity binding site, with the labelled ligand concentration ranging from 0.1 to 10 nM. The final incubation 50 mM Tris-HCl buffer contained 1 mM $MnCl_2$ to shift all the binding sites to the high affinity state. Drugs dissolved in 50% propylene glycol and 50% saline (0.9%) were imipramine (20 mg/kg), gepirone (10 mg/kg) and diazepam (2 mg/kg); they were injected i.p. for 21 days. ECT was administered 7 times over a period of two weeks. Lithium chloride (0.2%) was incorporated into the diet for one week. Blood levels ranging from 0.4-0.9 mEq/l were subsequently measured using flame photometry

Results and Discussion

One way analysis of variance of the results of Table VIII revealed no significant change in either affinity (K_d) ($F = 0.97$, $df = 5,30$; $P > 0.05$) or maximal receptor number (B_{max}) ($F = 1.19$, $df = 5,30$; $P > 0.05$). The conclusion reached in this second set of experiments in which treatment with imipramine and ECT was done is therefore similar to that of the first set. Taking into consideration the putative affinity states by adding $MnCl_2$ did not reveal anything new. Changes were not expected following direct activation of the postsynaptic 5-HT_{1A} receptor by gepirone because none were reported *in vivo* by electrophysiological recording (Blier and de Montigny, 1987). However increases may have been expected in [^{3}H]-8-OH-DPAT binding following lithium treatment since it has a prominent effect on the hippocampal cell sensitivity after i.v. injection of 8-OH-DPAT (Blier et al., 1987). Thus, on the basis of our results we can say that, at least postsynaptically, changes in [^{3}H]-8-OH-DPAT binding cannot explain changes seen in electrophysiological studies using the same treatments.

Table VIII. Effect of electroconvulsive treatment, imipramine (20 mg/kg), diazepam (2 mg/kg), lithium (0.2 %) and gepirone (10 mg/kg) on [^{3}H]-8-OH-DPAT binding parameters in hippocampus.

Treatment	K_d (nM)	B_{max} (fmol/mg protein)	N
control	0.69 ± 0.06	315 ± 22	10
diazepam	0.85 ± 0.02	330 ± 26	6
ECT	0.68 ± 0.09	293 ± 21	6
gepirone	0.79 ± 0.06	365 ± 16	6
imipramine	0.79 ± 0.04	308 ± 44	4
lithium	0.68 ± 0.14	292 ± 27	4

Final incubation 50 mM Tris buffer contained 1 mM $MnCl_2$. Each value is the mean ± SEM of N determinations.

V. Autoradiographic Analysis of Treated Dorsal Raphe

Since our previous data did not suggest that changes in the postsynaptic area were responsible for the efficacy of the treatments that we had under investigation, the aim of this last set of experiments was to investigate a possible alteration of 5-HT_{1A} autoreceptors on DR by quantitative autoradiography. One treatment that was chosen is a 5-HT_{1A} agonist, gepirone, which is known to desensitize DR after chronic administration. A sustained administration of gepirone initially decreases the firing activity of DR. This action is transient and is followed by a progressive recovery of firing activity on a long term basis (two weeks) where the autoreceptor is said to be desensitized to further exposure to gepirone, 5-HT or 8-OH-DPAT (Blier and de Montigny, 1987). It was also relevant to test a TCA, imipramine, which increases the efficacy of 5-HT neurotransmission by increasing responsiveness of the postsynaptic target (hippocampus) (Blier et al., 1987), but is devoid of effect on DR on a long term basis (Blier and de Montigny, 1980). Finally, diazepam was used as an active control which does not act, at least directly, on DR 5-HT autoreceptors.

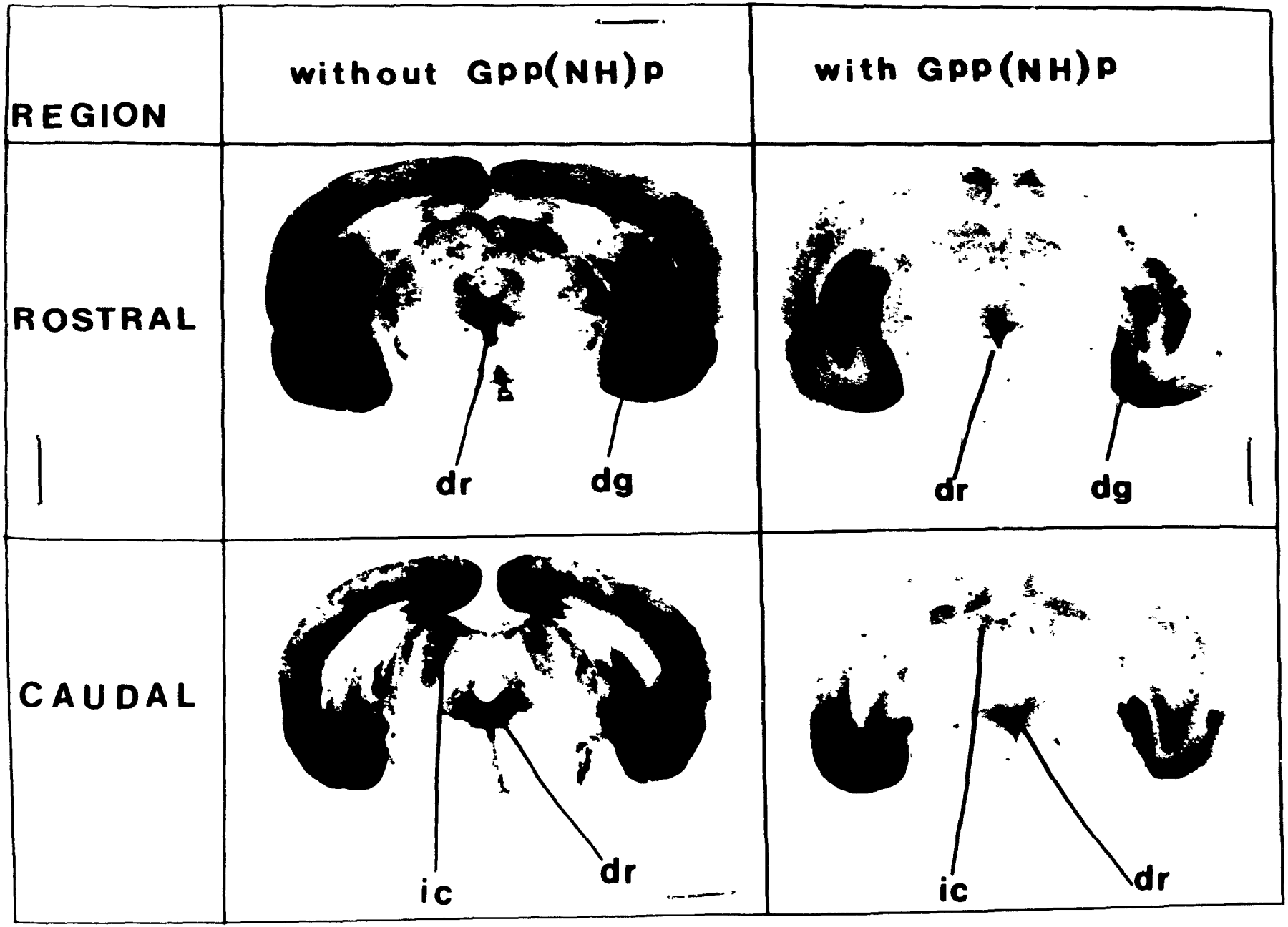
Methods

Autoradiography was performed as previously described in the general Method section. In order to compare similar DR areas between the different groups, autoradiograms were separated into the rostral part of DR, located in brain slices near the end of the dendate gyrus of the hippocampus and the caudal part of DR, located at the level where the inferior colliculus begins. The affinity states were also separated according to the stategy already described in the second section of this thesis. Briefly, all the [^{3}H]-8-OH-DPAT binding sites were first shifted to the high affinity state with the addition of 1 mM $MnCl_2$ to the final 170 mM Tris buffer. Another series of corresponding slices had the same buffer, supplemented with Gpp(NH)p (100 uM) which shifted all [^{3}H]-8-OH-DPAT binding sites to the low

affinity state. Representative autoradiograms from these two conditions are shown in Fig. 6.

Animals were treated with drugs dissolved in 50% propylene glycol and 50% saline (0.9%); these included imipramine (20 mg/kg), gepirone (10 mg/kg) and diazepam (2 mg/kg) and were injected i.p. for 21 days.

g. 6. Typical autoradiograms of [^{3}H]-8-OH-DPAT labelling and its inhibition by Gpp(NH)p in the rostral and the caudal part of the DR.



dr = dorsal raphe, dg = dendate gyrus, ic = inferior colliculus

Results and Discussion

One way analysis of variance of the results in Table IX revealed no change for any of the groups in the density of [^{3}H]-8-OH-DPAT binding under high affinity state conditions in the caudal ($F = 2.44$, $df = 3,8$; $P > 0.05$) and in the rostral area of DR ($F = 0.20$, $df = 3,8$; $P > 0.05$), as well under the low affinity state conditions in the caudal ($F = 1.51$, $df = 3,8$; $P > 0.05$) and in the rostral area of DR ($F = 2.28$, $df = 3,8$; $P > 0.05$). The efficacy of Gpp(NH)p to inhibit [^{3}H]-8-OH-DPAT binding was about 84% in all the groups and regions studied. The lack of alteration of DR observed with diazepam and imipramine correspond to what we expected since they have not been reported to desensitize the somatodendritic receptor of this nucleus. The results obtained with gepirone treatment, however, are surprising since a decreased density of the high affinity sites or a reduced ability of Gpp(NH)p to inhibit [^{3}H]-8-OH-DPAT binding (reflecting an uncoupling of the 5-HT_{1A} receptor from its G-protein) may have been expected to account for DR desensitization following chronic regiment of gepirone. These results suggest that the presynaptic 5-HT_{1A} binding site, similarly to postsynaptic 5-HT_{1A} binding site, does not appear to remain actively modulated *in vitro*.

Table IX. Effect of gepirone (10 mg/kg), imipramine (20 mg/kg) and diazepam (2 mg/kg) on dorsal raphe [^{3}H]-8-OH-DPAT binding measured by quantitative autoradiography.

Treatment	Region	[^{3}H]-8-OH-DPAT specific binding (fmol/mg protein) (mean ± SEM) H	L	% inhibition
saline	rostral	160 ± 9	22 ± 2	86
	caudal	136 ± 13	20 ± 3	85
diazepam	rostral	138 ± 26	22 ± 3	84
	caudal	108 ± 8	15 ± 5	86
gepirone	rostral	147 ± 18	31 ± 4	80
	caudal	143 ± 9	23 ± 3	84
imipramine	rostral	146 ± 26	29 ± 3	80
	caudal	128 ± 11	17 ± 2	87

A description of the rostro-caudal separation is given on fig. 6. H = high affinity state ($MnCl_2$ treated), L = low affinity state (Gpp(NH)p treated), % inhibition = (H-L)/H x 100. Values are mean ± SEM of 3 determinations.

GENERAL DISCUSSION

Electrophysiological and behavioral studies which showed alteration in the 5-HT_{1A} receptor system following antidepressant and anxiolytic treatment are unable to distinguish changes that occur directly at the receptor protein itself from those that occur at the effector and second messenger; these sites include the G_i-protein and adenylate cyclase which are linked to the 5-HT_{1A} receptor. Therefore, it is difficult to say that the 5-HT_{1A} receptor itself is involved in the clinical efficacy of drug treatment without direct demonstration of alteration in the binding site characteristics (K_d and B_{max}). The aim of these studies were therefore based on an interest to investigate the molecular correlates to functional findings that have been observed. To do so, [^{3}H]-8-OH-DPAT, a specific ligand for 5-HT_{1A} receptors, was used in saturation and autoradiographic analysis in rat brain. The results presented in this study do not indicate any observable direct modulation of the 5-HT_{1A} binding site by anxiolytic and antidepressant treatments known to be active on the serotonergic system *in vivo*. However, our finding that the 5-HT_{1A} receptor may display multiple affinity states raised the possibility that this discrepancy might result from our inability to observe *in vitro*, changes in the coupling of the 5-HT_{1A} receptor with its G-protein effector that had taken place *in vivo*. *In vitro* alteration of the 5-HT_{1A} receptor affinity state characteristics following monoamine oxidase inhibition with the antidepressants clorgyline and phenelzine have nevertheless been demonstrated here.

The affinity and density of 5-HT_{1A} binding sites following treatments

A lack of modulation of the 5-HT_{1A} receptor itself was somewhat surprising when one considers the numerous arguments, some of which were introduced in previous sections, that favor the 5-HT_{1A} receptor system as one of the major loci for the serotonergic aspect of anxiolytic and antidepressant action. However, conflicting findings are often reported in the literature. For example, paroxetine and

indalpine are two antidepressants in the family of 5-HT reuptake blockers which were used in the present experiments; we found no changes in the 5-HT_{1A} binding with these compounds. To our knowledge, the effect of these two reuptake blockers on the 5-HT_{1A} binding site has not been previously investigated but other drugs in this category including fluoxetine, citalopram and sertraline have previously been studied. Consistent with our results of no change in the hippocampus and frontal cortex following paroxetine or indalpine treatment, Welner et al. (1989) reported no change in the autoradiographic density of postsynaptic 5-HT_{1A} binding site in temporal cortex and in hippocampus following chronic fluoxetine treatment. However, following fluoxetine treatment,a significant reduction of [^{3}H]-8-OH-DPAT density at a presynaptic location in DR was noted. In a different report, chronic administration of sertraline was observed to decrease the 5-HT_{1A} receptor number in hippocampus by 18% (Reynolds et al., 1989). This contrasts with autoradiographic results of Hensler and Frazer (1989) which indicate no effect of sertraline (or citalopram) in dorsal or median raphe nuclei or regions of frontal cortex, hippocampus or hypothalamus.

The action of reuptake blockers is not likely to be at a postsynaptic location indicated by earlier electrophysiological studies. Sustained administration of citalopram, a specific 5-HT reuptake blocker, does not modify the responsiveness of serotonergic neurons to microiontophoretically applied 5-HT (Chaput et al., 1986). Citalopram does decrease, however, the somatodendritic 5-HT_{1A} autoreceptor sensitivity, as demonstrated by its capacity to reduce the inhibitory effect of LSD on the firing activity of DR. Welner et al. (1989) obtained results consistent with this finding using fluoxetine, while Hensler and Frazer (1989) did not find any change in DR [^{3}H]-8-OH-DPAT binding using citalopram (or sertraline). Therefore, considering these conflicting results, it is difficult to provide a unifying theory on how serotonin reuptake blockers are producing their antidepressant effects.

Tricyclic antidepressants (TCA), contrary to specific reuptake blockers, are known to induce a sensitization of postsynaptic serotonergic targets (de Montigny & Aghajanian, 1978). Hamon et al. (1987) using the TCA amitryptiline, found a significant increase in [^{3}H]-8-OH-DPAT binding in cerebral cortex and in septum that they claimed to be the molecular basis for the increased responsiveness of the 5-HT_{1A} receptor observed following long term amitriptyline treatment. Localizing these changes further, amitryptiline treatment was reported to cause changes in dentate gyrus and CA_1 region of hippocampus, but not in the CA_3 region nor the temporal cortex (Welner et al., 1989). However, in either frontal cortex or hippocampus in the present studies, no effect with the common TCA imipramine on 5-HT_{1A} binding was found. Further, Mizuta and Segawa (1988) have reported a significant decrease of [^{3}H]-8-OH-DPAT binding in frontal cortex and in hippocampus following prolonged imipramine treatment. Therefore, again, no unifying concept seems apparent for the mechanism of action of TCAs with regard to the 5-HT_{1A} binding site.

Our results also differ with those obtained by Mizuta and Segawa (1988) in the case of lithium treatment. In our hands, lithium chloride incorporated to rats food for one week produced no significant change in hippocampal 5-HT_{1A} receptor number (B_{max}). Following chronic (3 weeks) injection of lithium chloride (2 mEq/kg i.p.) Mizuta and Segawa found a significant decrease in [^{3}H]-8-OH-DPAT maximal binding capacity (B_{max}) in hippocampus, but not in frontal cortex. It is concievable that the different routes of administration (ie: oral vs intraperitoneal) could explain this discrepancy. But in fact, Blier et al. (1987) have shown that an oral treatment with lithium enhanced the responsiveness of hippocampal 5-HT_{1A} receptors to i.v. administered 8-OH-DPAT, without altering the DR 5-HT_{1A} autoreceptors or the dorsal hippocampus postsynaptic 5-HT_{1A} receptors to microiontophoretic application of 8-OH-DPAT. Therefore any effect induced by

lithium at this site, would likely result in either no change or an increased sensitivity of the hippocampal 5-HT_{1A} receptor but not the opposite. Seen in this context, Mizuta & Segawa's results seem inconsistent with these other findings.

A similar conclusion to that from lithium treatments has been previously drawn from electroconvulsive shock treatments (ECT) (de Montigny, 1984). Our results from two separate sets of ECTs indicated, again, no change in [^{3}H]-8-OH-DPAT binding capacity or affinity. We therefore conclude that the increased sensitivity observed *in vivo* following ECT is not necessarily reflected at the level of 5-HT_{1A} binding parameters *in vitro*.

With monoamine oxidase inhibitors, our results were complicated by the finding that the effect observed after chronic clorgyline treatment (increased K_d) was also observed following acute treatment. We also observed that this shift in receptor affinity states was reproducible by a simple addition of the MAOI *in vitro* or by a reduction of the preincubation time; this would indicate that the effect of clorgyline treatment likely resulted from a drug effect during the preincubation step. There is no direct way therefore to assess the *in vivo* change at the 5-HT_{1A} binding site after chronic MAOI treatment with a Scatchard analysis. Nonetheless, this does not exclude autoradiographic analysis, since the protocol differs greatly from that of membrane binding studies. In this respect, Hensler and Frazer (1989), in autoradiographic analysis of 5-HT_{1A} binding site showed that phenelzine or clorgyline do not alter [^{3}H]-8-OH-DPAT binding in dorsal or median raphe nuclei or regions of the frontal cortex, hippocampus or hypothalamus. Once again, this absence of positive results is particularly puzzling in the case of DR where somatodendritic autoreceptor desensitization by phenelzine and clorgyline have been demonstrated (Blier & de Montigny, 1985).

The lack of reliable observations with respect to the regulation of the 5-HT_{1A} binding site by typical antidepressant treatments is a situation similar to that found for the 5-HT_1 binding site using [^{3}H]-5-HT (see reviews by Willner, 1985, and Frazer et al., 1987). There are striking differences in agonist-induced regulation of the 5-HT_1 binding site, compared to other receptor systems which show comparable alterability in their responsiveness in both electrophysiological and behavioral studies. The nicotinic, the *beta*-adrenergic and the dopaminergic receptors, for instance, display an increased B_{max} values that can be correlated with an increased physiological responsiveness when presynaptic fibers of these systems are lesioned (Berg et al., 1972; Creese et al., 1977; Sporn et al., 1977). Agonist-induced down regulation (Noble et al., 1978; Harden, 1983) and antagonist-induced up regulation (Boyson et al., 1986) have also been consistently reported for these receptors. However, in the case of the 5-HT_1 binding site, positive results of that kind remain controversial.

For example, Nelson et al. (1978) found increased [^{3}H]-5-HT binding in the postsynaptic areas following 5-HT presynaptic fiber destruction with 5,7-DHT. Many other studies, however, have not reproduced these results (Benet & Snyder, 1976; Fillion et al., 1978; Segawa et al., 1979; Blackshear et al., 1981; Pranzatelli et al., 1986). More specifically at the level of the 5-HT_{1A} binding site, Hall et al. (1985) and Vergé et al. (1986) measured the binding of [^{3}H]-8-OH-DPAT after unilateral lesions of the medial forebrain bundle with 5,7-DHT. None of these studies showed 5-HT_1 binding site up-regulation in any of the postsynaptic targets analyzed. Similar findings were reported by Frazer and Hensler (1989) in an autoradiographic study in which none of the 18 postsynaptic brain areas examined displayed an up-regulation after 5,7-DHT lesion of serotonin neurons, yet [^{3}H]-8-OH-DPAT binding decreased in the serotonin producing neurons of the DR, as would be expected.

In keeping with the topic of 5-HT receptor modulation by neurotransmitter availability, Akiyoshi et al. (1989) reported a circadian rhythm for [^{3}H]-5-HT binding in cerebral cortex. Receptor B_{max} values were maximal during the light period and minimal during the dark period. The change in receptor sensitivity was said to correlate with low 5-HT levels during the light period and the high 5-HT levels during the dark period (Hery et al., 1972). Such an effect could have accounted for the fact that the optimal anxiolytic effect of 8-OH-DPAT in rat is during the active dark period (Eison et al., 1986). Indeed, logically, when the receptor number is at its lowest level, a partial agonist (8-OH-DPAT) has an intrinsic activity comparable to that of an antagonist when it competes for the endogenous neurotransmitter (5-HT), a full agonist at its site. Our results, however, demonstrated no significant cycle of the 5-HT_{1A} binding site as assessed by [^{3}H]-8-OH-DPAT labelling. Consistent with our results, Ohi et al. (1989) found no significative change in either [^{3}H]-8-OH-DPAT or [^{3}H]-5-HT binding that could be correlated with the hypersensitivity to 5-MeODMT observed after repeated foot shock, a stress adaptation phenomena most likely resulting from a decreased 5-HT availability. Considering these results, the 5-HT_{1A} receptor does not appear to be modulated by endogenous 5-HT.

The situation is similar with synthetic ligands which directly stimulate the 5-HT_{1A} receptors. We found no change in the binding capacity of hippocampus after a three week treatment with gepirone. In autoradiography, Welner et al. (1989) reported a similar finding in temporal cortex and in hippocampus with gepirone, while Hamon et al. (1989) found no effect of ipsapirone in hippocampus. A conclusion about the presynaptic 5-HT_{1A} receptor located on the DR, however, is not yet clear. Welner et al. (1989) have reported a significant decrease in DR [^{3}H]-8-OH-DPAT binding capacity following prolonged gepirone treatment; these results are consistent with the electrophysiological findings of Blier and de Montigny (1987)

where a tonic somatodendritic 5-HT_{1A} autoreceptor desensitization following sustained gepirone administration was demonstrated. These studies contrast with our findings in which no change in receptor density or in Gpp(NH)p inhibition of DR [^{3}H]-8-OH-DPAT binding was observed following gepirone administration.

Discrepancies in the various reports may arise from different assay conditions. For example, a concentration of 0.05 nM [^{3}H]-8-OH-DPAT was used in the present autoradiographic experiments while those of Welner et al. (1989) used 2 nM. In addition a cation which induces the high affinity state, Mn^{2+} was included in our incubation buffer as has been used previously by Hamon et al. (1987). Therefore, discrepancies in the literature could be explained by differences in experimental conditions, but it is not clear at which levels this might occur. Alternatively, the 5-HT_{1A} receptor and its binding parameters may be more complex than we, and other investigators, originally thought.

The 5-HT_{1A} receptor complex

The initial conception of the 5-HT_{1A} binding site was as a receptor which would exhibit simple kinetics with its ligands. This assumption was based on apparent linear Scatchard plots which describe, most often, a simple reversible bimolecular reaction between the receptor and the ligand which obeys the law of mass action. In the second part of our study, we demonstrated that the 5-HT_{1A} binding site displays complex binding phenomena. It became apparent at this point that the ternary complex model (de Lean et al., 1980), implicating a guanine-nucleotide-binding protein, could best explain the two affinity state B_{max} reciprocal shift that we observed. The arguments supporting this have been reviewed earlier and therefore, only the importance of this finding in regard to possible changes in receptor sensitivity will be emphasized here.

The most straightforward explanation to explain receptor regulation is a direct change in the level of expression of the protein unit representing the receptor. This solution is particularly attractive since it could account for the delayed onset of antidepressant and anxiolytic action at the 5-HT_{1A} receptor site. Indeed, it is generally assumed that a delay must occur before a drug can affect the nucleic acid machinery in the cell nucleus. Therefore, in the case of simple binding phenomena when the ligand and its receptor can be represented by the "key and static lock" model, the maximal number of labelled ligand that binds to the membranes (B_{max}) is a direct estimation of the number of receptor units in the tissue. The situation may be more complex, though, for non-linear binding phenomena, as is the case of [^{3}H]-8-OH-DPAT binding. Therefore, we would like to stress the fact that, even if we failed to find any changes at the 5-HT_{1A} binding site *in vitro* after any of our treatments, it does not necessarily imply a lack of regulation of this binding site *in vivo*.

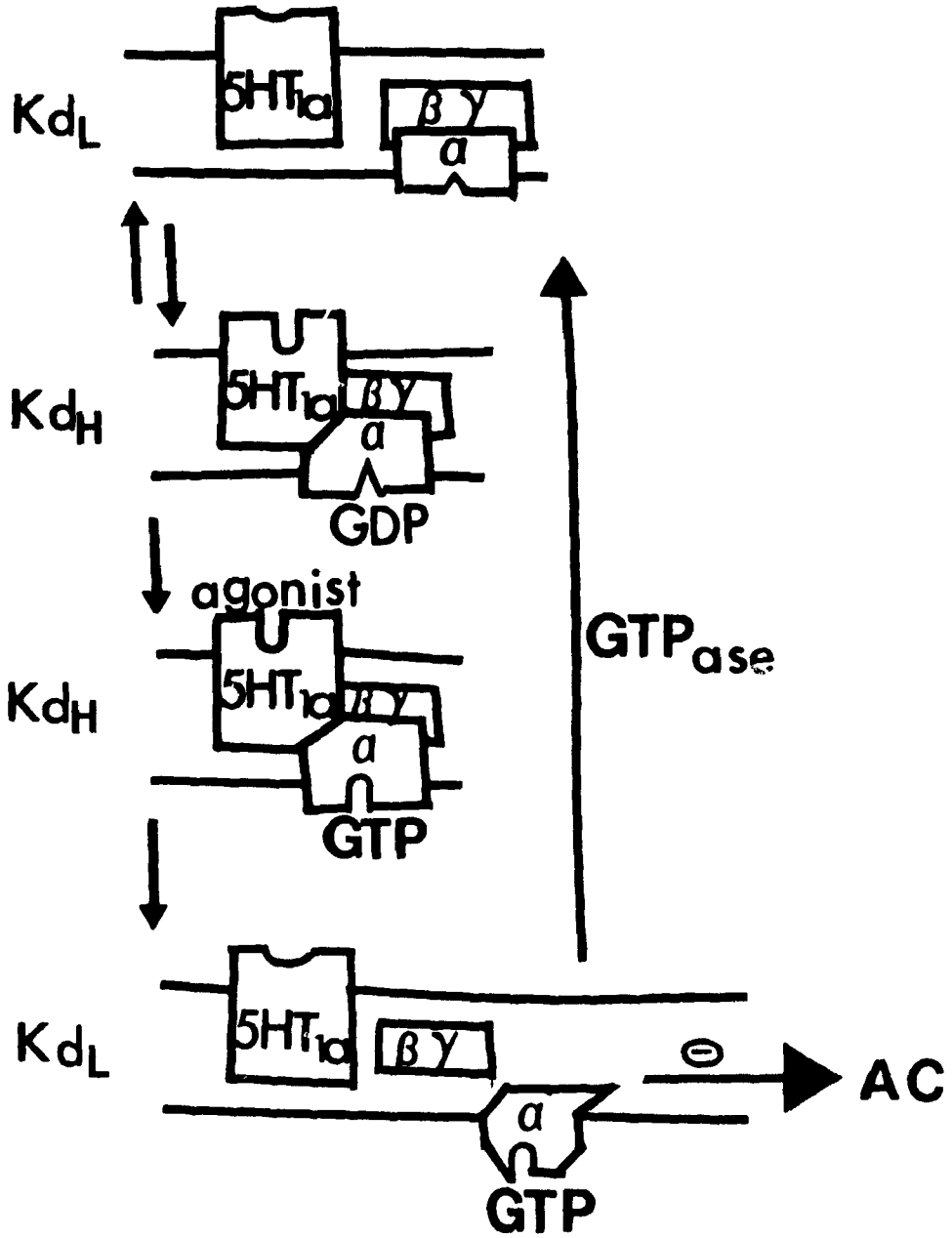
The 5-HT_{1A} receptor shares a high degree of genomic sequence homology with the *beta*-adrenergic (Fargin et al., 1988), which has a multiple affinity state profile (De Lean et al., 1980). But, contrary to the 5-HT_{1A} site, the *beta*-adrenergic binding site shows reliable B_{max} alterations following changes in its physiological responsiveness (for a review see Frazer et al., 1988). However, those changes cannot be ascribed to a change in the receptor genomic expression, since the increased *beta*-adrenergic receptor number induced by antagonist exposure or the decreased number resulting from an exposure to an agonist cannot be prevented by a protein synthesis inhibitor (Mukherjee & Lefkowitz, 1976). Consistent with this view, Chuang & Costa (1979) have not found any changes in RNA and protein synthesis that could be correlated with the decrease [^{3}H]-dihydroalprenolol binding induced by prolonged exposure to an agonist. Future research may also indicate that 5-HT_{1A} genomic expression is unaffected during receptor modulation.

If this is so, alternative investigations based on the putative affinity states of the 5-HT_{1A} site may prove to be more productive. For example, we may speculate that the molecular basis of somatodendritic 5-HT_{1A} autoreceptor desensitization is a permanent uncoupling of this receptor from its guanine nucleotide binding regulatory protein. We have shown that such an uncoupling, produced by Gpp(NH)p or NEM, induces the low affinity state for [^{3}H]-8-OH-DPAT binding. This induction of the low affinity state conformation could be an adaptative mechanism of the synapses to prevent 5-HT overstimulation. Thus, a delay would occur before the neurotransmitter molecule could again stimulate the 5-HT_{1A} receptor system; this would be dependent upon the recoupling of the receptor with the G-protein. Multiple steps may be required for this high affinity state reactivation, including hydrolysis of GTP into GDP by a GTPase (see schema 2 on the next page). Extensive increases in neurotransmitter availability, produced by MAOIs or reuptake blockers for example, would somehow hinder high affinity state reactivation. Such an adaptation mechanism may be possible, for instance, by the changing fluidity of the cell membrane lipid bilayer inasmuch as such a change provoked by ascorbate-induced lipid-peroxidation considerably altered the 5-HT_{1A} affinity state ratio in the direction of an increased low affinity state proportion (see Results, section II).

To be consistent, this interpretation must account for the differential alterability of the 5-HT_{1A} receptor observed in various brain areas. We have previously seen, from the work of de Montigny and colleagues, that treatments like ECT or lithium do not exert their effect on 5-HT transmission efficacy through a presynaptic action at the serotonergic neuron autoreceptors, like MAOIs, but rather through a postsynaptic action on pyramidal neurons. The increased responsiveness of the 5-HT_{1A} receptors contribute to an increased membrane hyperpolarization

DIAGRAM 2:

Putative 5-HT_{1a} Affinity States in Relation to its Effector System.



that reduces pyramidal cell firing. Such regional differences in 5-HT_{1A} high affinity state inducibility could well be accounted for by different types of G-protein stabilization.

The multiple affinity state receptor hypothesis could also explain our inability to observe the changes in the 5-HT_{1A} affinity state ratio that may be occuring *in vivo*. To be observed, the critical molecular change that prevailed *in vivo* would need the maintened *in vitro*. In other words, labile stabilization or destabilisation factors of the high affinity state could be disrupted during membrane preparation. For other receptors, such as the beta adrenergic, robust alterations such as phosphorylation and glycosylation (Cooney et al., 1986) may be preserved after membrane preparation. In contrast, the 5-HT_{1A} receptor may be a malleable element that would readopt its default conformation when the factors contributing to its alteration are removed.

Under milder assay conditions such as for the adenylate cyclase, the molecular alterations of the 5-HT_{1A} receptor system that prevailed *in vivo* seem to be maintained *in vitro*. We said earlier that the 5-HT_{1A} receptor is negatively coupled to adenylate cyclase (AC) in rat hippocampal membrane (De Vivo & Maayani, 1985). Therefore, a direct stimulation of AC with forskolin can be inhibited indirectly via G_i by an exposure of the 5-HT_{1A} receptor to 5-HT or 8-OH-DPAT. In relation to this, Newman & Lerer (1988) showed a decrease in the degree of inhibition of forskolin-stimulated AC activity by 5-HT in rat hippocampal membranes after administration of both chronic ECS and the TCA desipramine. Also consistent with this is the report of Sleight et al. (1988) who found a reduced ability of 8-OH-DPAT to inhibit forskolin-stimulated AC activity after chronic clorgyline treatment. This effect of MAO A inhibition was correlated with increased tissue levels of 5-HT. Therefore, the effect of clorgyline on the sensitivity of the 5-HT_{1A} in the latter experiment may be similar to the shift to the low affinity state

that was observed with this drug treatment in the saturation analysis of the present studies.

Future Prospects

The elements of discussion that we presented above bring our attention to the relationship between the 5-HT_{1A} receptor and the guanine nucleotide regulatory protein (G_i). It appears from our results that antidepressant and anxiolytic drugs do not act directly at the protein receptor itself. We hypothetize then that they may instead have their site of action at some critical element implicated in the coupling of the receptor protein with one or several G-protein transducer units; *alpha, beta,* or *gamma*. The fact that antidepressant drugs are known to have a very broad spectrum of action on many receptor systems also linked with a G-protein (e.g. cholinergic, dopaminergic, noradrenergic) favors such a hypothesis.

Some recent reports related to this subject also open new fields of investigation. Yamamoto et al. (1989) reported that antidepressants, but not benzodiazepines and neuroleptics, may directly affect G-protein GTP binding site *in vitro*. This change was reflected by an increased K_d and B_{max} of GTP binding that could be partly reversed by toxin-induced ribosylation of G_s, G_i or G_o. The authors suggest that an inhibition of basal GTPase activity by antidepressants may explain these alterations of GTP binding. From another perspective, Avissar et al. (1988) demonstrated that lithium inhibits the coupling of muscarinic cholinergic receptors and *beta*-adrenergic receptors to their respective G-protein, as assessed by a reduction of agonist-induced increase in GTP binding to those protein. Further studies by the same group (Avissar et al., 1989) suggest that the biochemical basis of lithium specificity for the CNS may stem from the heterogeneity of the *alpha* subunit. They have also shown (Schreiber et al., 1989) that chronic treatment with

imipramine, clomipramine and clorgyline alter the *beta*-adrenergic receptor coupling with its G-protein, while not affecting the coupling of the muscarinic receptor with its G-protein. These results indicate that the differential effects the antidepressant treatments have on the various receptor systems are probably in relation with different types of G-protein coupling. Further research will reveal if similar alteration in the coupling of the 5-HT_{1A} receptor with its G-proteins, which we hypothetize to differ depending upon neuronal cell type, is the molecular basis for anxiolytic and antidepressant drug action at the 5-HT_{1A} binding site.

CONCLUSION

We have reviewed several arguments implicating the serotonergic systems, and more specifically the 5-HT_{1A} receptors, in the therapeutic efficacy of antidepressants and anxiolytics. The absence of alteration of [^{3}H]-8-OH-DPAT binding site with any of the treatments representative of their class that we used is a surprising finding. The demonstration that [^{3}H]-8-OH-DPAT displays complex binding phenomena, compatible with a multiple affinity state model, precludes any straightforward conclusions about the apparent absence of treatment-induced modulation. We hypothesized that changes in the 5-HT_{1A} receptor affinity states resulting from labile molecular alteration in the coupling of the 5-HT_{1A} receptor with its G-protein induced by anxiolytic, antidepressant or 5-HT differential activation, may be disrupted during membrane binding preparation, explaining the apparent lack of change in [^{3}H]-8-OH-DPAT binding after our treatments. More work at the level of the 5-HT_{1A} affinity states will need to be done in order to test this speculation.

We believe, therefore, that new stategies of research taking into consideration the role of G-protein coupling in the 5-HT_{1A} receptor modulation may be more fruitful then concentrating on the [^{3}H]-8-OH-DPAT binding site alone. These experiments should be done in parallel with a functional assay, such as electrophysiology, in order to assess the consequences of the changes in the 5-HT_{1A} transmission.

REFERENCES

Akiyoshi J., Kuranaga H., Tsuchiyama K. and Nagayama H. (1989) Circadian rhythm of serotonin receptor in rat brain. *Pharmacol. Biochem. & Behav.* **32**, 491-493.

Albert P. R., Zhou Q-Y, Van Tol H. H. M., Bunzow J. R. and Civelli O. (1990) Cloning, functional expression, and mRNA tissue distribution of the rat 5-hydroxytryptamine$_{1A}$ receptor gene. *J. Biol. Chem.* **265**, 5825-5832.

Amsterdam J. D., Berwish N., Potter L. and Rickels K. (1987) Open trial of gepirone in the treatment of major depressive disorder. *Curr. Ther. Res.* **41**, 185-193.

Alexander B. S. and Wood M. D. (1988) [^{3}H]-8-OH-DPAT labels the 5-hydroxytryptamine uptake recognition site and the 5-HT$_{1A}$ binding site in the rat striatum. *J. Pharm. Pharmacol.* **40**, 888-891.

Andorn A. C., Bacon B. R., Nguyen-Hunh A. T., Parlato S. J. and Stitts J. A. (1987) Guanyl nucleotide interactions with dopaminergic binding sites labeled by [^{3}H]spiroperidol in human caudate and putamen: Guanyl nucleotides enhance ascorbate-induced lipid peroxidation and cause an apparent loss of high affinity binding sites. *Mol. Pharmacol.* **33**, 155-162.

Andrade R. and Nicoll R. A. (1987) Novel anxiolytics discriminate between postsynaptic serotonin receptors mediating different physiological responses on single neurons of the rat hippocampus. *Naunyn Schmiedebergs Arch. Pharmacol.* **336**, 5-10.

Anisman H., Irwin J. and Sklar L. S. (1979) Deficits of escape performance following catecholamine depletion: Implications for behavioural deficits induced by uncontrollable stress. *Psychopharmacology* **64**, 163-170.

Avissar S., Schreiber G., Danon A., Belmaker R. H. (1988) Lithium inhibits adrenergic and cholinergic increases in GTP binding in rat cortex. *Nature* **331**, 440-442.

Avissar S., Schreiber G., Aulakh C. S. and Murphy D. L. (1989) Lithium-selective alteration of brain G_S-protein function. *Soc. Neurosc. Abstr.* **15**, 174.1.

Azmitia E. C. and Segal M. (1978) An autoradiographic analysis of the differential ascending projections of the dorsal and median raphe nuclei in the rat. *J. Comp. Neurol.* **179**, 641-667.

Bennett J. P. and Snyder S. H. (1976) Serotonin and lysergic acid diethylamide binding in rat brain membranes: Relationship to postsynaptic receptors. *Mol. Pharmacol.* **12**, 373-389.

Berg D. K., Kelly R. B., Sargent P. B., Williamson P. and Hall Z. W. (1972) Binding of alpha-bungarotoxin to acetylcholine receptors in mammalian muscle. *Proc. Nat. Acad. Sci.* **69**, 147-151.

Blackshear M. A., Steranka L. R. and Sanders-Bush E. (1981) Multiple serotonin receptors: Regional distribution and effect of raphe lesions. *Eur. J. Pharmacol.* **76**, 325-334.

Blier P. and de Montigny C. (1980) Effect of chronic tricyclic antidepressant treatment on the serotoninergic autoreceptor: A microiontophoretic study in rat. *Naunyn-Schmiedebergs Arch. Pharmac.* **314**, 123-128.

Blier P. and de Montigny C. (1985) Serotoninergic but not noradrenergic neurons in rat central nervous system adapt to long-term treatment with monoamine oxidase inhibitors. *Neurosc.* **16**, 949-955.

Blier P., de Montigny C. and Tardif Danielle (1987) Short-term lithium treatment enhances responsiveness of postsynaptic 5-HT_{1A} receptors without altering 5-HT autoreceptor sensitivity: An electrophysiological study in the rat brain. *Synapse* **1**, 225-232.

Blier P. and de Montigny C. (1987) Modification of 5-HT neuron properties by sustained administration of the 5-HT_{1A} agonist gepirone: Electrophysiological studies in the rat brain. *Synapse* **1**, 470-480.

Blier P., de Montigny C. and Chaput Y. (1987) Modifications of the serotonin system by antidepressant treatments: Implications for the therapeutic response in major depression. J. Clin. Psychopharmacol. 7, 24-35s.

Blier P., Chaput Y. and de Montigny C. (1988) Long-term 5-HT reuptake blockade, but not monoamine oxidase inhibition, decreases the function of terminal 5-HT autoreceptors: an electrophysiological study in the rat brain. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **337**, 246-254.

Boyson S. J., McGonigle P., Reuter S., Schambron D. and Molinoff P. B. (1986) Quantitative autoradiography of D-1 and D-2 dopamine receptors following administration of neuroleptics. *Soc. Neurosci. Abstr.* **12**, 192.

Bressa G. M., Marini S. and Gregori S. (1987) Serotonin S2 receptors blockade and generalized anxiety disorders. A double-blind study on ritanserin and lorazapam. *Int. J. Clin. Pharmacol. Res.*, **7**, 111-119.

Brown G. W. and Harris T. (1978). In *Social origins of depression*. Tavistock, London.

Bunney W. E. and Davis J. M. (1965) Norepinephrine in depressive reactions. *Arch. Gen. Psychiatr.* **13**, 483-494.

Campbell J. C., Robinson D. S., Lovenberg W. and Murphy D. L. (1979) The effects of chronic regimens of clorgyline and pargyline on monoamine metabolism in the rat brain. *J. Neurochem.* **32**, 49-55.

Carli M. and Samanin R. (1988) Potential anxiolytic properties of 8-hydroxy-2-(Di-N-propylamino) tetralin, a selective serotonin$_{1A}$ receptor agonist. *Psychopharmacology* **94**, 84-91.

Chaput Y., de Montigny C. and Blier P. (1986) Effects of a selective 5-HT reuptake blocker, citalopram, on the sensitivity of 5-HT autoreceptors: Electrophysiological studies in the rat brain. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **333**, 342-348.

Charney D. S., Goodman W. K., Price L. H., Woods S. W., Rasmussen S. A., Heninger G. R. (1988) Serotonin function in obsessive-compulsive disorder. *Arch. Gen. Psychiatry*, **45**, 177-183.

Chase T. N., Katz R. I., Kopin I. J. (1970) Effect of diazepam on fate of intracisternally injected serotonin-C^{14}. *Neuropharmacology*, **9**, 103-108.

Chuang D. M. and Costa E. (1979) Evidence for internalization of the recognition site of beta-adrenergic receptors during receptor sensitivity induced by (-)-isoproterenol. *Proc. Natl. Acad. Sci. USA* **76**, 3024-3028.

Clarke A. and File S. E. (1982) Selective neurotoxin lesions of the lateral septum: Changes in social and aggressive behaviours. *Pharmacol. Biochem. Behav.* **17**, 623-628.

Cnanalosi I., Schweizer E., Case W. and Rickels K. (1987) Gepirone in anxiety: A pilot study. *J. Clin. Psychopharmacol.*, **7**, 31-193.

Costa E. and Greengard P. (eds.) (1975) *Mechanism of action of benzodiazepines*, Raven press, N. Y.

Cooney D., Holzhofer A., Boege F., Dees C., Jurss R., Keenan A. K., Hekman M. and Helmreich E. J. M. (1986) Receptor modification and receptor disposition in membranes. In *Receptor-receptor interactions: a new intramembrane integrative mechanism*, Fuxe K. and Agnati L. F. (eds.), Plenum press, N. Y. and London, pp.133-144.

Creese I., Burt D. R. and Snyder S. H. (1977) Dopamine receptor binding enhancement accompanies lesion-induced behavioral supersentivity. *Science* **197**, 596-598.

Critchley M. A. E., Njung'e K. and Handley S. L. (1987). 5-HT ligand effects in the social interaction test of anxiety. *Brit. J. Pharmacol.*, **92**, 659.

Davidson J., Turnbull C. D. and Miller R. D. (1980) A comparison of inpatients with primary unipolar depression and depression secondary to anxiety. *Acta Psychiatr. Scand.* **61**, 377-386.

de Lean A., Stadel J. M and Lefkowitz R. J. (1980) A ternary complex model explains the agonist-specific binding properties of the adenylate cyclase-coupled beta-adrenergic receptor. *J. Biol. Chem.* **255**, 7108-7117.

de Montigny C. and Aghajanian G. K. (1978) Tricyclic antidepressants: long-term treatment increases responsivity of rat forebrain neurons to serotonin. *Science* **202**, 1303-1306.

de Montigny C. (1981) Enhancement of the 5-HT neurotransmission by antidepressant treatments. *J. Physiol.* (Paris) **77**, 455-461.

de Montigny C. (1984) Electroconvulsive shock treatments enhance responsiveness of forebrain neurons to serotonin. *J. Pharm. Exp. Ther.* **228**, 230-234.

de Montigny C., Chaput Y. and Blier P. (1988) Lithium augmentation of antidepressant treatments: Evidence for the involvement of the 5-HT system? In *New concepts in depression*, vol. 2, Briley M., Fillion G. (eds), The MacMillan Press, pp. 144-160.

de Montigny C., Chaput Y. and Blier P. (1989) Long-term tricyclic and electroconvulsive treatment increases responsiveness of dorsal hippocampus 5-HT_{1A} receptors: An electrophysiological study in the rat. *Soc. Neuroscience* **15**, 324.21.

de Vivo M. and Maayani S. (1985) Inhibition of forskolin-stimulated adenylate cyclase activity by 5-HT receptor agonists. *Eur. J. Pharmacol.* **119**, 231-234.

Dobretsov G. E., Borschevskaya T. A., Petrov V. A. and Vladimirov Y. A. (1977) The increase of phospholipid bilayer rigidity after lipid peroxidation. *FEBS Lett.* **84**, 125-128.

Dumuis A., Sebben M. and Bockaert J. (1987) Pharmacology of 5-hydroxytruptamine-1A receptors which inhibit cAMP production in hippocampal and cortical neurons in primary culture. *Molec. Parmacol.* **33**, 178-186.

Eison A. S., Eison M. S., Stanley M. and Riblet L. A. (1986) Serotonergic mechanisms in the behavioural effects of buspirone and gepirone. *Pharmacol. Biochem. Behav.* **24**, 701-707.

Engel G., Gothert M., Hoyer D., Schlicker E. and Hillenbrand K. (1986) Identity of inhibitory presynaptic 5-hydroxytryptamine (5-HT) autoreceptors in the rat brain cortex with 5-HT_{1B} binding sites. *Naunyn-Schmied. Arch. Pharmacol.* **332**, 1-7.

Engel J. A., Hjorth S., Svensson K., Carlsson A., Liljequist S. (1984) Anticonflict effect of the putative serotonin receptor agonist 8-hydroxy-2-(Di-N-propylamino)tetralin (8-OH-DPAT). *Eur. J. Pharmacol.* **105**, 365-368.

Fargin A, Raymond J. R., Lohse M. J., Kobilka B. K., Caron M. G. and Lefkowitz R. J. (1988) The genomic clone G-21 which resembles a beta-adrenergic receptor sequence encodes the 5-HT_{1A} receptor. *Nature* **335**, 358-360.

Fargin A., Raymond J. R., Regan J. W., Cotecchia S., Lefkowitz R. J. and Caron M. G. (1989) Effector coupling mechanism of the cloned 5-HT_{1A} receptor. *J. Biol. Chem.* **264**, 14848-14852.

File S. E., Hyde J. R. and Macleod N. K. (1979) 5,7-Dihydroxytryptamine lesions of dorsal and median raphe nuclei and performance in the social interaction test of anxiety and in a home-cage aggression test. *J. Affective Dis.* **1**, 115-122.

Fillion G. M. B., Rousselle J. C., Fillion M. P., Beaudoin D. M., Goiny M. R., Deniau J. M. and Jacob J. J. (1978) High-affinity binding of ^{3}H-5-hydroxytryptamine to brain synaptosomal membranes: Comparison with ^{3}H-lysergic acid diethylamide binding. *Mol. Pharmacol.* **14**, 50-59.

Floyd R. A., Lewis C. A. (1983) Hydroxyl free radical formation from hydrogen peroxide by ferrous iron-nucleotide complexes. Biochemistry 22, 2645-2649.

Frazer A., Offord S. J. and Lucki I.(1988) Regulation of serotonin receptors and responsiveness in the brain. In *The Serotonin Receptors*, Sanders-Bush E. (ed.), The Humana Press, N.J. pp.319-362.

Frazer A. and Hensler J. (1989) Autoradiographic analysis of 5-HT_{1A} receptors after treatments that alter serotonergic neurotransmission. In *The neuropharmacology of serotonin, N. Y. Acad. Sc. Abstr.* 33.

Goodwin G. M. (1989) The effects of antidepressant treatments and lithium upon 5-HT_{1A} receptor function. *Prog. Neuro-Psychopharmacol. & Biol. Psychiat.* 13, 445-451.

Gozlan H., El Mestikawy S., Pichat L., Glowinski J. and Hamon M. (1983) Identification of presynaptic serotonin autoreceptors using a new ligand: ^3H-PAT. *Nature*, 305, 140-142.

Graeff F. G. and Schoenfeld R. I. (1970) Tryptaminergic mechanisms in punished and nonpunished behavior. *J. Pharmacol. Exp. Ther.* 173, 277-283.

Grahame-Smith D. G. (1988) Neuropharmacological adaptive effects in the actions of antidepressant drugs, ECT and lithium. In *New concepts in depression*, vol. 2, Briley M., Fillion G. (eds), The MacMillan Press, pp. 2-14.

Gray J. A. (1982) In *The neuropsychology of anxiety*, Oxford psychology series no. 1, Oxford University Press, Oxford, pp. 374-408.

Green A. R., Johnson P. and Nimgaonkar V. L. (1983) Increased 5-HT_2 receptor number in brain as a probable explanation for enhanced 5-hydroxytryptamine-mediated behaviour following repeated electroconvulsive shock administration to rats. *Br. J. Pharmacol.* **80**, 173-177.

Hall M. D., El Mestikawy S., Emerit M. B., Pichat L., Hamon M. and Gozlan H. (1985) [^{3}H]8-Hydroxy-2-(di-n-propylamino)-tetralin binding to pre- and postsynaptic 5-hydroxytryptamine sites in various regions of the rat brain. *J. Neurochem.* **44**, 1685-1696.

Hamon M., Bourgoin S., Gozlan H., Hall M. D., Goetz C., Artaud F. and Horn A. S. (1984) Biochemical evidence for the 5-HT agonist properties of PAT (8-hydroxy-2-(di-n-propylamino)tetralin) in the rat brain. *Eur. J. Pharmacol.* **100**, 263-276.

Hamon M., Emerit M. B., Mestikawy S. El, Verge D., Daval G., Marquet A. and Gozlan H. (1987) Pharmacological, biochemical and functional properties of 5-HT_{1A} receptor binding sites labelled by [^{3}H]8-hydroxy-2-(di-*n*-propylamino)tetralin in the rat brain, in *Brain 5-HT_{1A} Receptors*, Dourish C. T., Ahlenius S. and Hutson P. H., (eds), Ellis Horwood, Chichester, pp. 34-51.

Hamon M., Gozlan H., Schechter L. and Bolanos F. (1989) The 5-HT_{1A} receptor. In *The neuropharmacology of serotonin, N. Y. Acad. Sc. Abstr.* 9.

Harden T. K. (1983) Agonist-induced desensitization of beta-adrenergic receptor-linked adenylate cyclase. *Pharmacol. Res.* **35**, 5-32.

Heikkila R. E. (1983) Ascorbate-induced lipid peroxidation and binding of [^{3}H]dihydroalprenolol. *Eur. J. Pharmacol.* **93**, 79-85.

Hensler J. and Frazer (1989) Effect of chronic antidepressant treatments on serotonin$_{1A}$ (5-HT$_{1A}$) receptor density and responsiveness. *Soc. Neurosc.* Abstr. **15**, 274.13

Hery F., Rouer E. and Glowinski J. (1972) Daily variations of serotonin metabolism in the rat brain. *Brain Res.* **43**, 445-465.

Hibert, M and Middlemiss, D. N. (1986) Stereoselective blockade at the 5-HT autoreceptor and inhibition of radioligand binding to central 5-HT recognition sites by the optical isomers of methiothepin. *Neuropharmacology* **25**, 1-4.

Hjorth S., Carlsson A., Lindberg P., Sanchez D., Wikstrom H., Arvidsson L. E., Hacksell U. and Nilsson J. L. G. (1982) 8-Hydroxy-2-(di-n-propylamino) tetralin, 8-OH-DPAT, a potent and selective simplified ergot congener with central 5-HT-receptor stimulating activity. *J. Neural Transm.* **55**, 169-188.

Hyttel J., Overo K. F. and Arnt J. (1984) Biochemical effects and drug levels in rats after long-term treatment with the specific 5-HT-uptake inhibitor, citalopram. *Psychopharmacology* **83**, 20-27.

Ieni J. R. and Meyerson L. R. (1987) The 5-HT$_{1A}$ receptor probe [^{3}H]-8-OH-DPAT labels the 5-HT transporter in human platelets. *Life Sciences*, **42**, 311-320.

Janowsky D. S., El-Yousef M. K., Davis K. M. and Serkerke H. J. (1972) A cholinergic-adrenergic hypothesis of mania and depression. *Lancet*, **2**, 6732-6735.

Jenner P., Chadwick D., Reynolds E. H., Marsden C. D. (1975) Altered 5-HT metabolism with clonazepam, diazepam and diphhenylhydantoin. *J. Pharm. Pharmacol.* **27**, 707.

Kafi-de St Hilaire S., Merica H. and Gaillard J.-M. (1984) The effects of indalpine-a selective inhibitor of 5-HT uptake-on rat paradoxical sleep. *Eur. J. Pharmacol.* **98**, 413-418.

Kafi-de St Hilaire S., Hjorth and J.-M. Gaillard (1987) Effects of 8-OH-DPAT on the sleep-waking cycle in the rat, in *Brain 5-HT$_{1A}$ Receptors* (Dourish C. T., Ahlenius S. and Hutson P. H., eds), pp. 135-139. Ellis Horwood, Chichester.

Kahn R. S. and Westenberg H. G. M. (1985) 1-5-hydroxytryptophan in the treatment of anxiety disorders. *J. Affective Dis.* **8**, 197-200.

Kahn R. J., McNair D., Lipman R. S., Covi L., Rickels K., Downing R., Fisher S. and Frankenthaler L. M. (1986) Imipramine and chlordiazepoxide in depressive and anxiety disorder: Efficacy in anxious outpatients. *Arch. Gen. Psychiatry* **43**, 79-85.

Kahn R. S., Asnis G. M., Wetzler S and van Praag H. M. (1988) Neuroendocrine evidence for serotonin receptor hypersensitivity in panic disorder. *Psychopharmacology*, **96**, 360-364.

Kellar K. J., Cascio C. S., Butler J. A. and Kurtzke R. N. (1981) Differential effects of electroconvulsive shock and antidepressant drugs on serotonin-2 receptors in rat brain. *Eur. J. Pharmacol.* **69**, 515-518.

Kelly D. (1973) Progess in anxiety states. *Proc. Royal Soc. Med.*, **66**, 252-255.

Kelly J. P. (1985) Anatomical basis of sensory perception and motor coordination. In *Principles of neural science*, Kandel E. R. and Schwartz J. H. (eds.) Elsevier, NY, pp. 222-243.

Kitamura Y. and Nomura Y. (1987) Uncoupling of rat cerebral cortical alpha2-adrenoceptors from GTP-binding proteins by N-ethylmaleimide. *J. Neurochem.* **49**, 1894-1901.

Kupfer D. J. (1981) The application of EEG sleep in the treatment of depression. In Advances in Biological Psychiatry, Mendlewicz J. and van Praag H. M. (eds.), Plenum, NY, pp. 87-93.

Laborit (1986) In *L'inhibition de l'action*. Les presses de l'universite de Montreal, pp. 23-86, Masson, Paris.

Lidbrink P., Corrodi H. and Fuxe K. (1974) Benzodiazepines and barbiturates: Turnover changes in central 5-hydroxytryptamine pathways. *Eur J. Pharmacol.* **26**, 35-40.

Lippmann N., Pugsley T. A. (1974) Effects of bensoctamine and chlordiazepoxide on turnover and uptake of 5-hydroxytryptamine in the brain. *Br J. Pharmacol.* **51**, 571-575.

Lowry O. H., Rosebrough N. J., Farr A. L. and Randall R. J. (1951) Protein measurements with Folin phenol reagent. *J. Biol. Chem.* **193**, 265-275.

Maier S. F. and Seligman M. E. P. (1976) Learned helplessness: Theory and evidence. *J. Exp. Psychology* **105**, 3-46.

Mason T. S. and Fibiger H. C. (1979) Current concepts I. Anxiety: The locus coeruleus disconnection. *Life Sciences* **25**, 2141-2147.

May P. C., Morgan D. G., Salo D., Goss J. R. and Finch C. E. (1988) Effects of radioligand oxidation and ascorbate-induced lipid peroxidation on serotonin-1 receptor assay: Use of ascorbate and ethylenediamine tetraacetic acid buffers to prevent [^{3}H]-5-HT binding artifacts. *J. Neurosci.* **20**, 257-262.

McPherson G. A. (1983) A practical computer-based approach to the analysis of radioligand binding experiments. *Comput. Prog. Biomed.* **17**, 107-114.

Middlemiss D. N. and Fozard J. R. (1983) 8-Hydroxy-2(di-n-propylamino) tetralin discriminates between subtypes of the 5-HT$_1$ recognition site. *Eur. J. Pharmacol.* **90**, 151-153.

Middlemiss D. N. (1985) The putative 5-HT$_1$ receptor agonist, RU 24969, inhibits the efflux of 5-hydroxytryptamine from rat frontal cortex slices by stimilation of the 5-HT autoreceptor. *J. Pharm. Pharmacol.* **37**, 434-437.

Mizuta T. and Segawa T. (1988) Chronic effects of imipramine and lithium on postsynpatic 5-HT$_{1A}$ and 5-HT$_{1B}$ sites and on presynaptic 5-HT$_3$ sites in rat brain. *Japan. J. Pharmacol.* **47**, 107-113.

Muakkassah-Kelly S. F., Andresen J. W., Shih J. C., Hochstein P. (1983) Dual effects of ascorbate on serotonin and spiperone binding in rat cortical membranes. *J. Neurochem.* **41**, 1429-1439.

Mukherjee C. and Lefkowitz R. J. (1976) Desensitization of beta-adrenergic agonists in a cell-free sustem: Resensitization by guanosine 5"-(B-4-imino)triphosphate and other purine nucleotides. *Proc. Natl. Acad. Sci. USA* **73**, 1494-1498.

Munson P. J. and Rodbard D. (1984) Computerized analysis of ligand binding data: basic principles and recent developments, in *Computers in Endocrinology* vol. 14, Robard and Gianniforti, (eds), Raven Press, NY, pp. 117-145.

Nelson D. L., Bourgoin H. S., Glowinski J. and Hamon M. (1978) Characteristics of central 5-HT receptors and their adaptive changes following intracerebral 5,7-dihydrosytryptamine administration in the rat. *Mol. Pharmacol.* 14, 983-995.

Newman M. E. and Lerer B. (1988) Chronic electroconvulsive shock and desimipramine reduce the degree of inhibition by 5-HT and carbachol of forskolin-stimulated adenylate cyclase in rat hippocampal membranes. *Eur. J. Pharmacol.* 148, 257-260.

Noble M. D., Brown T. H. and Peacock J. H. (1978) Regulation of acetylcholine receptor levels by a cholinergic agonist in mouse muscle cell cultures. *Proc. Natl. Acad. Sci.* 75, 3488-3492.

Ohi K., Mikuni M. and Takahashi (1989) Stress adaptation and hypersensitivity in 5-HT neuronal systems after repeated foot shock. *Pharmacom. Biochem. Behav.* 34, 603-608.

Palacios J. M., Pazos A. and Hoyer D. (1987) Characterization and mapping of 5-HT_{1A} sites in the brain of animals and man, in *Brain 5-HT_{1A} Receptors*, Dourish C. T., Ahlenius S. and Hutson P. H., (eds), Ellis Horwood, Chichester, pp. 67-81.

Papez J. W. (1937) A proposed mechanism of emotion. *Arch. Neurol. Psychiatry.* 38, 725-743.

Pazos A. and Palacio J. M. (1985) Quantitative autoradiographic mapping of serotonin receptors in the rat brain. I. Serotonin-1 receptors. *Brain Res.*, **346**, 205-230.

Peroutka S. J. and Snyder S. H. (1979) Multiple serotonin receptors: differential binding of ^{3}H-serotonin, ^{3}H-lysergic acid diethylamide and ^{3}H-spiroperidol. *Mol. Pharmacol.* **16**, 687-699.

Peroutka S. J. (1986) Pharmacological differentiation and characterization of 5-HT_{1A}, 5-HT_{1B}, and 5-HT_{1C} binding sites in rat frontal cortex. *J. Neurochem.* **47**, 529-540.

Peroutka S. J. (1988) 5-Hydroxytryptamine receptor subtypes: molecular, biochemical and physiological characterization. *Trends Neurosci.* **11**, 496-500.

Petersen E. N. and Lassen J. B. (1981) A water lick conflict paradigm using drug experienced rats. *Psychopharmacology* **75**, 236-239.

Petitjean F., Buda C., Janin M., Sakai K. and Jouvet M. (1978) Patterns of sleep alterations following selective raphe nuclei lesions. *Sleep Res.* **7**, 44-52.

Petitjean F., Buda C., Janin M., Sallanon M. and Jouvet M. (1985) Insomnie par administration de parachlorophenylalanine: reversibilite par injection peripherique ou centrale de 5-hydroxytryptophane et de serotonine. *Sleep*, **8**, 56-67.

Pellow S., Johnston A. L. and File S. E. (1987) Selective agonists and antagonists for 5-hydroxytryptamine receptor subtypes and interactions with yohimbine and FG 7142 using the elevated plus-maze test int the rat. *J. Pharm. Pharmacol.* **39**, 917-928.

Pranzatelli M. R., Rubin G. and Snodgrass S. R. (1986) Serotonin-lesion myoclonus syndromes. I. Neurochemical profile and S-1 receptor binding. *Brain Res.* **364**, 57-66.

Pratt JF., Jenner P., Reynods E. H., Marsden C. D. (1979) Clonazepam induces decreased serotoninergic activity in the mouse brain. *Neuropharmacology*, **18**, 791-799.

Pratt J. A., Jenner P., Marsden C. D. (1985) Comparison of the effects of benzodazepines and other anticonvulsant drugs on synthesis and utilization of 5-HT in mouse brain. *Neuropharmacology*, 24, 59-68.

Rainbow T. C., Bleish W. V., Biegon A. and McEwen B. S. (1982) Quantitative densitometry of neurotransmitter receptors. *J. Neurosci. Method.* **5**, 127-138.

Randrup A., Munkvad J., Fog R., Gerlach J., Molander L., Kjellberg B. and Scheel-Kruger J. (1975) Mania, depression and brain dopamine. In *Current Developments in Psychopharmacology* Vol. 2, Essman W. B. and Valzelli L. (eds.), Spectrum, N. Y., pp. 206-248.

Ravaris C. L., Nies A., Robinson D. S., Ives J. O., Lambourn K. R. and Korson L. (1976) A multiple dose, controlled study of phenelzine in depression anxiety states. *Arch. Gen. Psychiatry.* **33**, 164-173.

Reynolds L. S., Connolly M. H., McLean S. and Heym J. (1989) Behavioral and biochemical evidence for downregulation of 5-HT_{1A} receptors by sertraline. *Soc. Neurosci.*, **15**, 342.22.

Riordan, J. F. and Vallee, B. L. (1972) Reactions with N-ethylmaleimide and p-mercuribenzoate. *Methods Enzymol.* **25**, 449-456.

Robinson D. S., Nies A., Ravaris C. L. and Lambourn K. R. (1973) The monoamine oxidase inhibitor phenelzine in the treatment of depressive anxiety states: A controlled clinical trial. *Arch. Gen. Psychiatr.* **29**, 407-413

Roth M., Gurney C., Mountjoy G. Q., Kerr T. A. and Schapira K. (1976). *In Monoamine oxidase and its inhibition.* Ciba Foundation Symposium 39 (New Series), Elsevier, Amsterdam, pp. 297-325.

Sakai K., Salvert D., Touret M., and Jouvet M. (1977a) Afferent connections of the nucleus raphe dorsalis in the cat as visualized by horseradish peroxidase technique. *Brain Res.* 137, 11-35.

Sakai K., Touret M., Salvert D., Leger L. and Jouvet M. (1977b) Afferent projections to the cat locus coeruleus as visualized by the horseradish peroxidase technique. Brain Res. **119**, 21-41.

Saner A. and Pletscher A. (1979) Effect of diazepam on cerebral 5-hydroxytryptamine synthesis. *Eur. J. Pharmacol.* **55**, 315-318.

Schreiber G., Avissar S., Auiakh C. S. and Murphy D. L. (1989) Antidepressant drugs alter beta-adrenergic receptor coupling to G_S protein. *Soc. Neurosc. Abstr.* **15**, 174.2.

Schildkrault, (1965) Catecholamine hypothesis of affective disorders: A review of supporting evidence. *Am. J. Psychiatr.* **122**, 509-522.

Schoemaker H. and Langer S. Z. (1986) [^{3}H]-8-OH-DPAT labels the serotonin transporter in the rat striatum. *Eur. J. Pharmacol.* **124**, 371-373.

Schuurman T. and Spencer D. G.(1987) Behavioral effects of 5-hydroxytryptamine$_{1A}$-receptor ligand ipsapirone TVX-Q-7821, a comparison with 8-hydroxy-2-di-N-propylaminotetralin and diazepam. *Psychopharmacology*, **89**, S54.

Schweizer T., Rickels K. and Lucki I. (1986) Resistance to the anti-anxiety effect of buspirone in patients with a history of benzodiazepine use. *New England J. Med.*, **314**, 719-720.

Selye, H. (1952) In *The story of the adaptation syndrome*. Acta, Montreal.

Segal M. (1978) Serotonergic innervation of the locus coeruleus from the dorsal raphe. *J. Physiol., Lond.* **286**, 401-415.

Segawa T., Mizuta T. and Nomura Y. (1979) Modifications of central 5-hydroxytryptamine binding sites in synaptic membranes from rat brain after long-term administration of tricyclic antidepressants. *Eur. J. Pharmacol.* **58**, 75-83.

Shephard R. A., Buxton D. A., Broadhurst P. L. (1982) Drug interactions do not support reduction in serotonin turnover as the mechanism of action of benzodiazepines. *Neuropharmacology* **21**, 1027-1032.

Shopsin B., Friedman E. and Gershon S. (1976) Parachlorophenylalanine reversal of tranylcypromine effects in depressed patients. *Arch. Gen. Psychiatry* **33**, 811-819.

Shopsin B., Gershon S., Goldstein M., et al. (1975) Use of synthesis inhibitors in defining a role for biogenic amines during imipramine treatment in depressed patients. *Psychopharmacol. Commun.* 1, 239-249.

Shreiber G., Avissar S., Aulakh C. S. and Murphy D. L. (1989) Antidepressant drugs alter beta-adrenergic receptor coupling to G_S protein. *Soc. Neurosc. Abstr.* **15**, 174.2

Sleight A. J., Marsden C. A., Palfreyman M. G., Mir A. K. and Lovenberg W. (1988) Chronic MAO A and MAO B inhibition decreases the 5-HT_{1A} receptor-mediated inhibition of forskolin-stimulated adenylate cyclase. *Eur. J. Pharmacol.* **154**, 255-261.

Soubrie P., Blas C., Rerron A., Glowinski J. (1983) Chlordiazepoxide reduces in vivo serotonin release in the basal ganglia of encephale isole but not anesthetized cats: Evidence for a dorsal raphe site of action. *J. Pharmacol. Exp. Ther.* **226**, 526-532.

Sporn J. R., Wolfe B. B., Harden T. K. and Molinoff P. B. (1977) Supersensitivity in rat cerebral cortex: Pre- and Postsynaptic effects of 6-hydroxydopamine at noradrenergic synapses. *Mol. Pharmacol.* **13**, 1170-1180.

Stadel J. M. and Lefkowitz R. J. (1979) Multiple reactive sulfhydryl groups modulate the function of adenylate cyclase coupled beta-adrenergic receptors. *Mol. Pharmacol.* **16**, 709-718.

Stein L., Wise C. D. and Belluzzi J. D. (1975) Effects of bendiazepines on central seronergic mechanisms. In *Mechanism of action of benzodiazepines*. Costa E. (ed), Raven press, NY.

Stratford C. A., Tan G. L., Hamblin M. W. and Ciaranello R. D. (1988) Differential inactivation and G protein reconstitution of subtypes of [^{3}H]-5-hydroxytryptamine binding sites in brain. *Mol. Pharmacol.* **34**, 527-536.

Svensson T. H., Bunney B. S. and Aghajanian G. K. (1975) Inhibition of both noradrenergic and serotonergic neurons in brain by the alpha-adrenergic agonist clonidine. *Brain Res.* **92**, 291-306.

Traber J. and Glaser T. (1987) 5-HT_{1A} receptor-related anxiolytics. *TIPS*, **8**, 432-436.

Traskman L., Tybring G., Asberg M., Bertilsson L., Lantto O. and Schalling D. (1980) Cortisol in the CSF of depressed and suicidal patients. *Arch. Gen. Psychiatry* **37**, 761-767.

Tye N. C., Everitt B. J. and Iversen S. D. (1977) 5-Hydroxytryptamine and punishment. *Nature* **268**, 741-742.

Van Praag H. M., (1978) Amine hypotheses of affective disorders. In *Handbook of psychopharmacology* Vol. 13. Iversen L. L., Iversen S. D. and Snyder S. H. (eds.), Plenum, NY, pp. 178-297.

Van Praag H. M. (1982) Serotonin precursors in the treatment of depression. In *Serotonin in biological psychiatry*, Ho B. T., Schoolar J. C. and Usdin E. (eds.), Raven press, NY, pp.259-286.

Verge D., Davel G., Marcinkiewicz M., Patey A., El Mestikawy S., Gozlan H. and Hamon M. (1986) Quantitative autoradiography of multiple 5-HT_1 receptor subtypes in the brain of control or 5,7-dihydroxytryptamine treated rats. *J. Neurosci.* **6**, 3474-3482.

Vetulani J., Lebrecht U. and Nowak J. Z. (1981) Enhancement of responsiveness of the central serotonergic system and serotonin-2 receptor density in rat frontal cortex by electroconvulsive shock treatment. *Eur. J. Pharmacol.* **81**, 85.

Weiss S., Sebben M., Kemp D. E. and Bockaert J. (1986) Serotonin 5-HT_1 receptors mediate inhibition of cyclic AMP production in neurons. *Eur. J. Pharmacol.* **120**, 227-230.

Welner S. A., de Montigny C., Desroches J., Desjardins P. and Suranyi-Cadotte. (1989) Autoradiographic quantification of serotonin$_{1A}$ receptors in rat brain following antidepressant drug treatment. *Synapse*, **4**, 347-352.

Wesemann W. W., Weiner N., Rotsch M. and Schulz E. (1983) Serotonin binding in rat brain: circadian rhythm and effect of sleep deprivation. *J. Neural. Transm.* **18**, 287-294.

West E. D. and Dally P. J. (1959) Effect of iproniazid in depressive syndromes. *Br. Med. J.* **1**, 1491-1494.

Westenberg H. G. M., den Boer J. A. (1989) Serotonin-influencing drugs in the treatment of panic disorder. *Psychopathology*, **22**(suppl 1), 68-77.

Willner P. (1985) In *Depression: a psychobiological synthesis*, John Wiley & Sons, NY, pp. 287-351.

Wise C. D., Berger B. D., Stein L. (1972) Benzodiazepines: Anxiety-reducing activity by reduction of serotonin turnover in the brain. *Science*, **17**, 181.

Yamamoto H., Mikuni M., Kagaya A., Kuroda Y. and Takahashi K. (1989) Antidepressants may directly affect GTP binding sites in vitro. *Soc. Neurosc. Abstr.* **15**, 215.12