NOTE TO USERS

POTENTIAL ANTIDEPRESSANT PROPERTIES OF SIGMA LIGANDS: ELECTROPHYSIOLOGICAL STUDIES IN THE RAT DORSAL RAPHE NUCLEUS AND HIPPOCAMPUS

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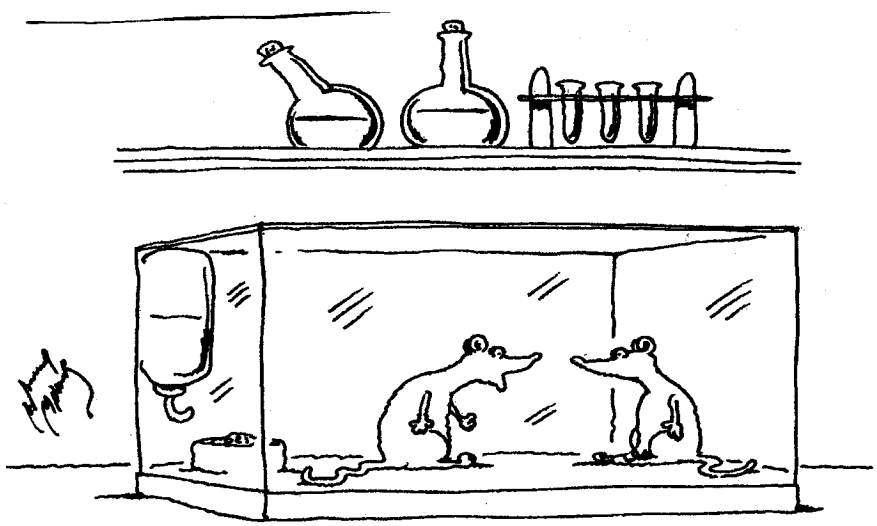

"I thought drug testing was a requirement for the job — I didn't realize it WAS *the job!"*

Statement of Contributions

I have written all the papers included in this thesis with the helpful guidance and thoughtful criticism of the co-authors. All the experimental work was carried out by me with the exception of; Chapter 2, Eric Dryver and Norman Lavoie performed the experiments and in Chapter 6, Dr. Nasser Haddjeri performed a small portion of the experiments. For all the manuscripts I was responsible for data collection, statistical analysis and manuscript writing.

Abstract

Behavioural models used to test antidepressants have shown that ligands that bind to sigma receptors possess "antidepressant-like" properties. The focus of this thesis has been to assess the action of various sigma ligands using *in vivo* extracellular recording in the dorsal raphe nucleus (DRN) and hippocampus. These two regions are heavily implicated in depression and in the mechanism of action of antidepressants. The hippocampus is also examined because it provides a model to distinguish sigma agonist and antagonist activity in the modulation of the NMDA response of CA_3 pyramidal neurons.

In the DRN, sigma ligands produced a significant increase in the firing activity of DRN 5-HT neurons after short-term treatments, which are maintained following chronic treatments. Most of the sigma ligands tested acted as sigma agonists and potentiated the NMDA response in the hippocampus. However, the sigma ligand 4-IBP produced an interesting profile that also involved inverse agonist activity, which was dependent on the response to NMDA prior to any drug administration. Overall, the results with the sigma ligands in the DRN and hippocampus suggest a modulatory role for sigma receptors on serotonergic and glutamatergic neurotransmission.

Lastly, the project focused on a combined sigma and 5-HT_{1A} ligand, OPC-14523. This ligand increased 5-HT firing activity after short-term treatments and modulated the NMDA response in the hippocampus. However, it also produced a desensitization of the 5-HT_{1A} autoreceptor but not the postsynaptic 5-HT_{1A} receptor after only 2 days of treatment. Further investigation with this compound

using microiontophoresis in the hippocampus and DRN showed that it acts as an agonist at both pre- and postsynaptic 5-HT_{1A} receptors.

In conclusion, sigma ligands have potential as antidepressant medications with a fast onset of action as they produce a rapid modulation of the 5-HT system in the DRN, and glutamatergic transmission in the hippocampus. In particular, the combined action at the 5-HT_{1A} and sigma receptors may increase the rate of desensitization of the 5-HT_{1A} autoreceptors. These actions of sigma ligands may produce antidepressant effects by completely novel mechanisms, which may provide an alternative to the antidepressants currently available, which would in turn benefit treatment-resistant patients.

RÉSUMÉ

Certains ligands sigma testés dans des modèles comportementaux chez l'animal, se sont révélés avoir un profil pharmacologique d'antidépresseur. L'objet de cette thèse a été d'évaluer l'effet de plusieurs ligands sigma en utilisant un modèle d'enregistrement unitaires extracellulaires obtenus dans le raphé dorsal (DRN) et l'hippocampe dorsal, chez le rat anesthésié. Ces deux régions ont été choisies du fait de leur application dans la dépression dans les mécanismes d'action des antidépresseurs. L'hippocampe a aussi été choisi car cette région a antérieurement servi à développer un modèle permettant de distinguer les agonistes des antagonistes sigma en fonction de leur effet sur l'activité neuronale induite par le NMDA dans la région CA_3.

Dans le DRN, les ligands sigma ont produit une augmentation du taux de décharge des neurones sérotonergiques (5-HT), que ce soit après des traitements à court ou à long-terme. La plupart des ligands sigma testés se sont comportés comme des agonistes en potentialisant la réponse au NMDA. Par contre, le 4-IBP a un profile pharmacologique original car il peut agir aussi comme agoniste inverse, en fonction de la réponse spontanée au NMDA. De manière globale, les résultats obtenus avec les ligands sigma dans le DRN et l'hippocampe suggèrent que ces récepteurs agissent comme modulateurs de la neurotransmission glutamatergique et sérotonergique.

Dans une dernière partie, le projet s'est concentré sur l'OPC-14523, un agoniste sigma ayant une haute affinité pour le récepteur $5\text{-}HT_{1A}$. Ce ligand a aussi augmenté le taux de décharge des neurones 5-HT après 48h de traitement.

Toutefois, il a aussi induit une désensibilisation des autorécepteurs 5-HT_{1A} sans modifier les récepteurs 5-HT_{1A} post-synaptiques. Des études complémentaires ont permis de démontrer que l'OPC-14523 agissait comme un agoniste des récepteurs 5-HT_{1A}, tant au niveau pré-synaptique que post-synaptique.

En conclusion, les ligands sigma semblent avoir un potentiel comme antidépresseur présentant un effet clinique plus précoce du fait de leur modulation rapide du système sérotonergique dans la DRN et glutamatergique dans l'hippocampe. Plus spécifiquement, l'effet combiné sur les récepteurs sigma et 5-HT_{1A} peut être responsable de la désensibilisation très rapide de ces derniers. Ces effets des ligands sigma pourraient conduire à la genèse de nouveaux antidépresseurs avec un effet totalement nouveau qui pourrait représenter une alternative aux antidépresseurs disponibles actuellement et aider les patients souffrant de dépression réfractaire.

Abbreviations

ACh-acetylcholine
ADX/CX-adrenalectomy/castration
AMPA-α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid
AP-7-2-amino-7-phosphonoheptanoic acid
2-APV-2-amino-5-phosphovaleric acid
AS-antisense
(-)Bay K8644-1,4-dihydro-2,6-dimethyl-5-nitro-4-[2-(trifluoromethyl)phenyl]pyridine-3-carboxylic acid methyl ester
BD-737-(+)-cis-N-[2,(3,4-dichlorophenyl)ethyl]-2-(1-pyrrolidinyl-cyclohexlamine)
BD-1047-N-[2-(3,4-dichlorophenyl)ethyl]-N-methyl-2-(dimethylamino)ethylamine
BD-1063-1-[2-(3,4-dichlorophenyl)ethyl]-4-methylpiperazine dihydrochloride
BDNF-brain derived neurotrophic factor
BMY-14802–α-(4-fluorophenyl)-4-(5-fluoro-2-pyrimidinyl)-1-piperazinebutanol
$[Ca^{+2}]_I$-intracellular Ca^{+2} concentration
cAMP-cyclic adenosine monophosphate
CMS- chronic mild stress
CNS-central nervous system
CPP- conditioned place preference
CSP-conditioned spatial preference
DA-dopamine
DAT- dopamine transporter
DHEA- dihydroepiandrosterone
DHEAS-dihydroepiandrosterone sulfate
DRN-dorsal raphe nucleus
DTG-1,3-di-(2-tolyl)guanidine
DuP 734-(1-(cyclopropylmethyl)-4-2′-oxoethyl)piperidine hydrobromide
E-5842-4-[4-fluorophenyl]-1,2,3,6-tetrahydro-1-[4-{1,2,4-triazol-1-il}butyl]pyridine citrate
ECT-Electroconvulsive shock therapy
EGTA-ethylene glycol-bis(β-aminoethyl ether)N,N,N′,N′ tetraacetic acid
EMD 57445- panamesine, (S)-(-)-[4-hydroxy-4-(3,4-benzodioxol-5-yl)-piperidin-1-yl methyl]-3-(4-methoxyphenyl)oxazolidin-2-one
ER-endoplasmic reticulum
FST-forced swimming test
GABA-γ-aminobutyric acid
Gö-6976-12-(-cyanoethyl)-6,7,12,13-tetrahydro-13-methyl-5-oxo-5H-indolo[2,3 a]pyrrolo(3,4-c] carbazole
Gpp(NH)p-5′ guanylylimidodiphosphate
GTP-guanosine triphosphate
GTPγS-5′-O-(3-thiotriphosphate)
HAL-haloperidol
5-HIAA-5 hydroxyindole acetic acid

5-HT-serotonin
4-IBP-4-(N-benzylpiperidin-4-yl)-4-iodobenzamide
IC_{50}-50% inhibitory concentration
IP_3-inositol-1,4,5 trisphosphate
JO-1784-igmesine, (+)-N-cyclopropylmethyl-N-methyl-1,4-diphenyl-1-1-ethyl-but-3-en-1-ylamine hydrochloride
K^+-potassium
K_d-binding affinity
L-687-384-1-benzylspiro[1,2,3,4-tetrahydronaphthalene-1,4-piperidine
Lu 28-179-1′[4-[1-(4-fluorophenyl)-1-H-indol-3-yl]-1-butyl]spiro-iso-benzofuran-1(3H)- 4′piperidine
LY379196-5,21:12,17-dimetheno-18H-dibenzo[1,0]pyrrolo[3,4-1][1,8]diacyclohexadecine-18,20(19H)-dione,8-[(dimethylamino)methyl]-6,7,8,9,10,11-hexahydro-monomethane sulfonate (9Cl)
LY392098-N-2-(4-(3-thienyl)phenyl)propyl z-propane sulfonamide
MAOI-Monoamine oxidase inhibitor
MGluR-Metabotropic glutamate receptors
MK-801-(+)-5-methyl-10,11-dihydro-5H-dibenzo (a,d) cyclohepten-5-10 imine maleate
mPFC-medial prefrontal cortex
MRI-magnetic resonance imaging
mRNA-messenger ribonucleic acid
MS-377-(R)-(+)-1-(4-chlorophenyl)-3-[4-(2-methoxyethyl)piperazin-1-yl]methyl-2-pyrolidinone L-tartrate
NE-norepinephrine
NE-100- N,N-dipropyl-2-(4-methoxy-3-(2-phenylethoxy)phenyl)-thylamine
NGF-nerve growth factor
NMDA-N-methyl-D-aspartate
nNOS-neuronal nitic oxide synthase
NO- nitric oxide
NPC 16377-6-[6-(4-hydroxypiperidinyl)hexyloxy]-methylflavone
OBX-olfactory bulbectomy
8-OH-DPAT-8-hydroxy-2-(di-n-propylamino)tetralin
OPC-14523-OPC, 1-[3-[4-(3-chlorophenyl)-1-piperazinyl]propyl]-5-methoxy-3,4-dihydro-2-quinolinone monomethanesulfonate
OVX-overiectomy
PCP-phencyclidine
p-CPA-para(4)-chloroamphetamine
PFC- prefrontal cortex
PKC- protein kinase C
PLC-phopholipase C
(+)-3-PPP -3-(3-hydroxyphenyl)-N-(1-propyl)piperidine
PRE-084-2-(4-morpholinoethyl)-1-phenyl-cyclohexane-1-carboxylate hydrochloride
PREG-pregnenalone
PROG-progesterone

PS-pregnenalone sulfate
S-21377-2-[4-(4-methoxybenzyl)piperazin-1yl-methyl]-4-oxo[4H]-benzo-thiazolin-2-one
S-21378-2-[(4-benzylpiperazin-1-yl)methyl]naphthalene dichiorydrate
SA-4503-1-(3,4-dimethoxyphenethyl)-4-(3-phenylpropyl) piperazine dihydrochloride
SKF-10,047-N-allylnormetazocine
SR 31747A-(Z)-N-cyclohexyl-N-ethyl-3(3-chloro-4-cyclohexylpheny)-propen-2-ylamine hydrochloride
SSRI- selective serotonin reuptake inhibitor
TCA-tricyclic antidepressant
TCP-N-[1-(2-thienyl)cyclohexyl]piperidine
VDCC-voltage-dependent Ca^{+2} channel
WAY 100635-N-[2-(4-[2-methoxyphenyl]-1-piperazinyl)ethyl]-N-2-pyridinylcyclohexanecarboxamide

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Chapter 1: Introduction

1. Sigma Receptors-Introduction and Background

1.1 Historical Perspectives and Sigma Receptor Subtypes

Sigma receptors were first classified as a subtype of opioid receptors (Martin et al 1976). However, naltrexone, an opioid antagonist, failed to antagonize the behavioral and autonomic effects of the prototypical sigma ligand SKF-10,047 (N-allylnormetazocine), suggesting that their activity does not involve the opiate system (Vaupel 1983).

Later, the availability of newer, more selective sigma ligands established sigma sites as distinct from known neurotransmitter receptors (Quirion et al. 1987). First, sigma receptors were distinguished from phencyclidine (PCP) receptors when it was found that (+)-SKF-10,047 labels two sites in the brain which can be discriminated by biochemical and autoradiographic methods using the selective sigma ligand 3-(3-hydroxyphenyl)-N-(1-propyl)piperidine [(+)-3-PPP] and the selective PCP ligand N-[1-(2-thienyl)cyclohexyl]piperidine (TCP). The high affinity site corresponds to sigma receptors while the low affinity site represents PCP receptors. (Gundlach et al.1985, Largent et al. 1986).

Sigma receptors were then further classified into subtypes, denoted $sigma_1$ and $sigma_2$ (Quirion et al. 1992). It was found that haloperidol (HAL) and 1,3-di-(2-tolyl)guanidine (DTG) do not discriminate between $sigma_1$ and $sigma_2$ receptors, while (+)-pentazocine and (+)-SKF-10,047 demonstrate high (nM) affinity for $sigma_1$ sites and lower (μM) affinity for $sigma_2$ sites. In general, (+) isomers were found to be more potent at $sigma_1$ receptors, while the opposite was

true at $sigma_2$ receptors (Quirion et al. 1992). In addition, another difference between the subtypes was that the binding activity of $sigma_1$ receptors was more sensitive to the modulatory effects of guanosine triphosphate (GTP) versus $sigma_2$ receptors (Itzhak 1989, Itzhak and Stein 1990, 1991).

There is debate over whether a third subtype ($sigma_3$) or other $sigma_1$ subtypes exist. Binding studies with [^{3}H](+)-pentazocine demonstrated that the binding sites were heterogenous. Specifically, (+)-pentazocine labeled high and low affinity sites in most of the cell lines examined. The authors did not feel that the lower affinity site represented $sigma_2$ receptors (high K_d=1-7 nm, low K_d= 125-360 nM) (Vilner et al. 1995). In addition, a third intermediate site (K_d= 30-60 nM) was observed in human cell lines, suggesting a possible third type of (+)-pentazocine binding site (Vilner et al. 1995). In agreement, competition assays revealed the presence of high and low affinity sites for (+)-pentazocine (Basile et al. 1992).

In keeping with the binding data, various electrophysiological data in the model of the modulation of the N-methyl-D-aspartate (NMDA) response of the dorsal hippocampus from our laboratory suggest that the $sigma_1$ receptor does not represent a single entity. Specifically, pertussis toxin pretreatment, which inactivates $G_{i/o}$-coupled receptors, prevents the potentiation of the NMDA response induced by DTG, (+)-N-cyclopropylmethyl-N-methyl-1,4-diphenyl-1-1-ethyl-but-3-en-1-ylamine hydrochloride (JO-1784) but not by (+)-pentazocine (Monnet et al. 1994). This suggests that a G-protein-dependent $sigma_1$ receptor modulates DTG and JO-1784's effects, while a G-protein-independent $sigma_1$

receptor mediates (+)-pentazocine's effects (Monnet et al. 1994). In addition, the effect of (+)-pentazocine persists following colchicine pretreatment (destruction of the mossy fibers, the major afference to CA_3 pyramidal neurons), which indicates that the $sigma_1$ subtype mediating its effect is likely located postsynaptically on pyramidal neurons (Debonnel et al. 1996). Meanwhile, DTG and JO-1784's responses were abolished after clochicine treatment, suggesting that the receptor mediating DTG and JO-1784's response is likely located presynaptically on the mossy fiber system (Debonnel et al. 1996).

Further evidence following experiments with (+)-pentazocine in the model of the NMDA response suggests multiple binding sites for this ligand. Intravenous administration of (+)-pentazocine potentiates the NMDA response and this is reversed by the opioid antagonist naloxone but not by other opioid antagonists such as cyprodime hydrobromide (μ), dippa (κ) and naltrindole (δ). In contrast, the potentiation induced by JO-1784, 1-benzylspiro[1,2,3,4-tetrahydronaphthalene-1,4-piperidine (L-687-384) and (+)-cis-N-[2,(3,4-dichlorophenyl)ethyl]-2-(1-pyrrolidinyl-cyclohexlamine) (BD-737) was not reversed by naloxone (Couture and Debonnel 2001). These results suggest that (+)-pentazocine acts on a subtype of the sigma receptor that JO-1784, L-684,384 and BD-737 do not. Thus, (+)-pentazocine is likely acting on more than one type of sigma receptor as also suggested by data discussed previously. Moreover, Tsao and Su (1997) purified a naloxone-sensitive, haloperidol-sensitive (+)-SKF-10,047 binding protein, which bound (+)-pentazocine but not DTG, (+)-3-PPP and

progesterone (PROG) and actually resembles the sigma opioid site originally proposed by Martin et al. (1976).

Therefore, overall, the data clearly suggests the need to further examine the classification of sigma receptors. There is likely more diversity among the $sigma_1$ site or perhaps a distinct third sigma receptor subtype. An alternate suggestion is that the sigma receptor is a multi-subunit receptor, a hypothesis that will be elaborated upon in a further section of this Introduction.

1.2 Anatomical Distribution of Sigma Receptors in the Central Nervous System (CNS)

Early binding studies, using the nonselective sigma ligand (+)-3-PPP, found the highest densities of binding in the spinal cord, pons-medulla, cerebellum, central gray and red nucleus, and hippocampus with moderate densities detected in the hypothalamus and cerebral cortex and low densities in the basal ganglia and thalamus (Gundlach et al. 1986).

Using selective ligands for sigma receptors subtypes, it was shown that the binding profile is different for the sigma receptor subtypes (McCann et al. 1994, Bouchard and Quirion 1997). Using the $sigma_1$ ligand [^{3}H]-SKF-10,047 for labeling $sigma_1$ sites and the combined $sigma_{1/2}$ ligand [^{3}H]-DTG in the presence of [^{3}H]-SKF-10,047 to label $sigma_2$ sites, the results showed that the brainstem yields the highest level of $sigma_1$ binding and lowest levels of $sigma_2$ receptor binding, the reverse is true in the hippocampus (McCann et al. 1994, Inoue et al. 2000). $Sigma_1$ sites were most abundant in the dentate gyrus of the hippocampus,

facial nucleus, thalamic and hypothalamic nuclei and cerebellum, while $sigma_2$ sites were found enriched in the substantia nigra, central gray matter, occulomotor nuclei, cerebellum, nucleus accumbens and the motor cortex (Bouchard and Quirion 1997, Inoue et al. 2000). Further studies with the selective $sigma_2$ ligand [^{3}H]Lu 28-179 (1′[4-[1-(4-fluorophenyl)-1-H-indol-3-yl]-1-butyl]spiro-iso-benzofuran-1(3H)-4′piperidine) showed the highest density of binding in the cerebral cortex, hippocampal formations, habenula nuclei, paraventricular hypothalamic and suraoptic nuclei and some brainstem nuclei (Søby et al. 1997). More recent binding studies using immunolocalization techniques found $sigma_1$ receptors to be relatively high in density in the olfactory bulb, amygdala, hypothalamus, cortex, hippocampal pyramidal layer and moderate in the striatum, the cerebellum, the dorsal raphe nucleus (DRN) and locus coeruleus (Alonso et al. 2000). Studies of $sigma_1$ receptor mRNA (messenger ribonucleic acid) levels, found high levels of expression in all layers of the cerebral cortex, striatum, hippocampus and cerebellum (Mei et al. 1997).

An association was demonstrated between sigma receptors and dopamine (DA) neurons as, in the substantia nigra, 6-hydroxydopamine lesions, which destroy DA neurons, decrease sigma ligand binding (Gundlach et al. 1986). With respect to glutamatergic neurons, sigma receptors were found to have a distinct binding pattern as compared to NMDA receptors. In the CA_3 region of the hippocampus, NMDA receptors were found to be located postsynaptically since [^{3}H]-TCP binding is slightly reduced after lesion of glutamatergic terminals of the entorhinodentate pathway of the dentate gyrus mossy fibre system (Maragos et al.

1991, Bekenstein et al. 1990). In comparison, sigma receptors appear to be associated with pyramidal cells since binding of [^{3}H]-(+)-3-PPP decreased after quinolinic acid treatment (Gundlach et al. 1986).

1.3 Potential Endogenous Ligands for Sigma Receptors

Early studies found neurosteroids to bind to sigma$_1$ receptors (Su et al. 1988, Yamada et al. 1994, Ramamoorthy et al. 1995, Maurice et al. 1996a). For example, the neurosteroids PROG, pregnenalone sulfate (PS) and dihydroepiandrosterone sulfate (DHEAS) dose-dependently inhibit the *in vivo* binding of [^{3}H]-SKF-10,047 in various binding studies. PROG was found to be the most potent, however, it was less potent than HAL. Results with PS need to be interpreted with caution, as its affinity for sigma receptors is relatively low (K_d=300 μm) (Su et al. 1988, Yamada et al. 1994, Ramamoorthy et al. 1995, Maurice et al. 1996a).

These binding studies led to the suggestion that PROG might be the endogenous ligand for sigma$_1$ receptors, as in humans PROG (in the plasma) is approximately 30 nM during the late menstrual cycle and approximately 450 nM in late pregnancy, corresponding to an estimated 10-60% fractional occupancy of sigma$_1$ receptors labeled by (+)-SKF-10,047 (Su et al. 1988). However, Schwartz et al. (1989) argues that PROG is not the endogenous sigma ligand as the concentration of endogenous PROG, specifically the free serum concentration, is insufficient to occupy the sigma receptors in the brain, even in later pregnancy.

There are data suggesting that PROG binds to sigma receptors under physiological conditions as it has been demonstrated that endogenous hormone levels affect sigma ligand activity in many animal models. For example, adrenalectomy/castration (ADX/CX), overiectomy (OVX), pregnancy, and post-partum periods can influence sigma ligands' effects in the electrophysiological model of the modulation of the NMDA response in the hippocampus (Bergeron et al. 1996, 1999), and the "antidepressant-like" effects of sigma ligands in behavioural models of depression (Urani et al. 2001). In addition, PROG affects the *in vivo* binding to $sigma_1$ receptors, as a 30-40% decrease in [^{3}H]SKF-10,047 binding was seen during pregnancy in the mouse hippocampus. Moreover, suppression of peripheral steroids by ADX/CX enhanced [^{3}H]SKF-10,047 binding and subsequent treatment with finasteride, which increases PROG levels, and produced decreased [^{3}H]SKF-10,047 binding as well (Maurice et al. 1996, 1998

At this point it cannot be determined conclusively whether PROG is the endogenous ligand. Recent developments using sigma ligands as Positron Emission Tomography ligands provide the opportunity to examine sigma receptor binding activity *in vivo* (Kawamura et al. 2000), which will surely help uncover the identity of sigma receptors' endogenous ligand. At this point the data certainly suggest that the endogenous ligand is a neurosteroid. However, it remains to be determined if it is PROG or perhaps one of its metabolites.

2. Pharmacology and Molecular Biology of Sigma Receptors

2.1 Sigma Receptor Pharmacology

A biphasic bell-shaped dose response curve has been observed for sigma ligands in various behavioural and electrophysiological paradigms (Maurice et al. 1994 a, b, Bergeron et al. 1995, Monnet et al. 1996). According to the bell-shaped dose-response curve, in the electrophysiological paradigm of the modulation of the NMDA response, low doses of sigma agonists induce a potentiation of the NMDA response (Monnet et al. 1990, 1992b). In contrast, high doses of sigma agonists such as DTG and JO-1784 (1000 μg/kg) can act as antagonists by preventing and suppressing the potentiation induced by low doses of other sigma agonists (Bergeron et al. 1995). A bell-shaped dose-response curve has also been demonstrated with sigma ligands in other models by other laboratories. For example, in NMDA-evoked release of [^{3}H]NE from preloaded rat hippocampal slices, JO-1784 and (+)-3-PPP demonstrated bell-shaped dose-response curves (Monnet et al., 1996). In behavioural models using (+)-5-methyl-10,11-dihydro-5H-dibenzo (a,d) cyclohepten-5-10 imine maleate (MK-801)-induced amnesia, DTG and (+)-pentazocine yielded bell-shaped dose-response curves in reversing amnesia (Maurice et al. 1994 a, b).

The bell-shaped dose-response curve may be due to low doses of sigma ligands activating a subtype of sigma receptors for which they have high affinity and higher doses may activate another subtype of the sigma receptor for which they have lower affinity that counteracts their effects seen at lower doses (Bergeron et al. 1993, 1995, 1997a). In electrophysiological experiments of sigma receptor modulation of the NMDA response, it was determined that the biphasic

effect of sigma ligands is not related to a desensitization of sigma receptors because a second injection over 60 minutes later induced the same degree of potentiation. Thus, the shape of the dose-response curve is most likely due to concomitant actions of sigma ligands on distinct subtypes (Bergeron et al. 1995, 1997a)

Chronic treatments with sigma ligands lead to different results, depending on whether they exert agonist or antagonist activity at sigma receptors. Long-term treatments with the antagonist HAL induce a downregulation of sigma receptors sufficient to hinder the potentiating effects of sigma agonists on the NMDA response (Bergeron et al. 1997b). This may be related to the observation that, long-term HAL induces a decrease in affinity and number of sigma sites (Itzhak and Alerhand 1989, Reynolds et al. 1991, Itzhak and Stein 1991, Kizu et al. 1991, Ericson and Ross 1992, Inoue et al. 2000). This was found to be specific for $sigma_1$ sites as $sigma_2$ binding was unaffected (Inoue et al. 2000). The long-term effect of HAL on sigma sites is in contrast to the usually observed upregulation of receptors following long-term treatments with antagonists and downregulation following chronic treatments with agonists. For example, chronic HAL, a DA receptor antagonist, has been shown to upregulate DA receptors (Itzhak and Alerhand 1989, Itzhak and Stein 1991). When examining $sigma_1$ receptor mRNA levels, chronic treatment with HAL had no effect while chronic 4-[4-fluorophenyl]-1,2,3,6-tetrahydro-1-[4-{1,2,4-triazol-1-il}butyl]pyridine citrate (E-5842), a putative sigma antagonist, increased transcript expression in the prefrontal cortex and striatum (Zamanillo et al. 2000). These results suggest that

the decreased binding sites observed following chronic HAL treatments are not due to decreased gene transcription, but could be due to receptor internalization (Zamanillo et al. 2000, Inoue et al. 2000).

In contrast to the downregulation observed with chronic treatments with sigma antagonists, long-term treatments with sigma agonists induce a supersensitivity of sigma receptors with respect to the modulation of the NMDA response in the hippocampus (Bergeron et al. 1997b). Chronic treatments with agonists lead to conflicting results in binding studies, as chronic (+)-pentazocine was shown to produce no change in the number of sigma receptor binding sites or binding affinity (Weissman and De Souza 1991). Meanwhile, another study showed chronic (+)-pentazocine to produce a decrease in binding affinity but no change in the number of binding sites (Kizu et al. 1991). Both these studies used [^{3}H]HAL that could also be labeling sigma$_2$ sites, which could explain the discrepancies. Thus, with respect to the dose-response curve and effects of chronic treatments, sigma ligands produce a unique profile. Based on this, attention is needed when interpreting results, as different doses may exert agonist or antagonist activity at sigma receptors. With sigma ligands, long-term treatments induce the opposite effects as what would normally be expected, as usually long-term agonists, not antagonists, induce a downregulation of receptors.

2.2. Cloning of the Sigma$_1$ Receptor

A significant breakthrough in sigma receptor research was the cloning of the sigma$_1$ receptor. The sigma$_1$ receptor was cloned from guinea pig liver,

human placental cell line and brain, mouse kidney and brain and rat brain (Hanner et al. 1996, Kekuda et al. 1996, Prasad et al. 1998, Pan et al. 1998, Seth et al. 1997, 1998). The protein cloned was a 223 amino acid, 1 transmembrane protein with potent (+)-pentazocine, HAL, DTG and (+)-3-PPP binding, but did not couple with G-proteins (Seth et al. 1998, Kekuda et al. 1996). The amino acid sequence of the sigma receptor cloned from the rat brain was highly homologous to the sigma receptor cloned from guinea pig liver and human placental cell line but was not related to other known neurotransmitter receptors (Hanner et al. 1996). A more recent study investigating putative transmembrane segments based on homology identified two putative transmembrane segments for the sigma$_1$ receptor (Aydar et al. 2002). Therefore, the exact structure of the sigma$_1$ receptor has yet to be fully elucidated.

The sigma$_1$ receptor protein is similar to the fungal gene product ERG2, which encodes a sterol isomerase. Human C8-C7 isomerase and the mammalian sigma$_1$ receptor possess the ability to bind drugs with comparable affinity suggesting that sigma$_1$ receptors contain a sterol-binding domain (Moebius et al. 1993, 1996, 1997). Furthermore, the yeast sterol C8-C7 isomerase has a high affinity binding site similar to mammalian sigma receptors and in the yeast some sigma ligands inhibit sterol biosynthesis (Moebius et al. 1996). In addition, there are several binding sites for steroid receptors in the 5' flanking region of the gene encoding for the murine sigma$_1$ receptor (Seth et al. 1997). Combined with their high densities in sterol producing tissues, these data provide a further argument

for a steroid-sigma$_1$ interaction and the authors hypothesize that the sigma receptor may in fact be an enzyme involved in sterol biosynthesis (Moebius et al. 1993, 1996, 1997).

The human SR 31747A ((Z)-N-cyclohexyl-N-ethyl-3(3-chloro-4-cyclohexylpheny)-propen-2-ylamine hydrochloride) (a sigma$_1$ ligand) binding protein was found to be identical to the sigma$_1$ receptor cloned by Kekuda et al. (1996), with labeling signals associated with the endoplasmic reticulum (ER) and nuclear envelope which further suggests its role in cholesterol synthesis or its anchorage to membranes (Jbilo et al. 1997, Dussossoy et al. 1999).

At this point it is not known whether the cloned sigma$_1$ receptor is the ligand binding subunit of a multi-subunit complex or perhaps one subtype of the sigma$_1$ receptor. Regardless, the cloning has led to an important focus on the molecular biology and signal transduction mechanisms of sigma$_1$ receptors. However, given the one-transmembrane segment cloned, it is most likely that it does not represent the complete functional receptor, but more experiments using techniques such as the use of selective sigma$_1$ receptor gene antisense (AS) will elucidate the exact structure of the functional sigma receptor in the future.

The cloning of the sigma$_1$ receptor led to the development of sigma$_1$ receptor knockout mice (Langa et al. 2003). Preliminary studies of these mice determined that they display no significant phenotypic and no behavioural abnormalities under baseline conditions, however further studies using these knockout mice in various behavioural and electrophysiological paradigms will likely produce interesting insight into the functional role(s) of sigma$_1$ receptors.

2.3 Interaction with G-proteins

There has been a lot of debate over whether or not sigma receptors act through G-protein-dependant signaling cascades. In support of sigma receptors' association with G-proteins, GTP and 5′ guanylylimidodiphosphate [Gpp(NH)p] decreased the binding of [^{3}H]3-PPP and [^{3}H] DTG as did pertussis toxin suggesting sigma receptors may be coupled to $G_{i/o}$ proteins. (Beart et al. 1989, Connick et al. 1992, Itzhak 1989). Furthermore, the downregulation of sigma receptors observed after chronic treatments with HAL is accompanied by a decreased responsiveness to guanine nucleotides in sites labeled by (+)-3-PPP (Itzhak and Stein 1991). More selective sigma agonists (+)-pentazocine and 1-(3,4-dimethoxyphenethyl)-4-(3-phenylpropyl) piperazine dihydrochloride (SA-4503) were found to stimulate GTPase activity, which was blocked by the $sigma_1$ antagonist N,N-dipropyl-2-(4-methoxy-3-(2-phenylethoxy)phenyl)-thylamine (NE-100), in mouse prefrontal cortex membranes (Tokuyama et al. 1997). In addition, effects on potassium (K^+) currents in frog melanotrophs were found to be G-protein-dependent (Soriani et al 1998), and $G_{i/o}$ proteins were also found to mediate the effects of JO-1784, (+)-3-PPP and DTG on NMDA-evoked release of [^{3}H]NE (Monnet et al. 1992a).

In contrast, there is data suggesting no interaction between sigma receptors and G-proteins. For example, sigma ligands' effects on K^+ currents in rodent neurohypophysis were shown to be G-protein independent, as G-protein activators or non-hydrolysable adenosine triphosphate analogues did not prevent this

modulation (further discussion in the following section) (Wilke et al.1999, Lupardus et al. 2000). Moreover, there was no effect of Gpp(NH)p on the specific binding of [^{3}H]-SKF-10,047 (McCann et al. 1994). Similar effects were seen with more selective sigma ligands (+)-pentazocine and BD-737, as their binding curves were unaffected by guanosine 5′-O-(3-thiotriphosphate) (GTPγS), and neither stimulates GTPase activity or [^{35}S]GTPγS binding (DeHavens-Hudkins et al. 1992, Hong and Werling, 2000).

In conclusion, further experiments will be necessary to fully elucidate the interaction between G-proteins and sigma receptors, but most likely, one subtype of the sigma$_1$ receptor interacts with G-proteins while another subtype relies on G-protein-independent signal transduction mechanisms.

2.4. Modulation of K^+ Currents by Sigma Receptors

Sigma ligands DTG and (+)-pentazocine have been shown to exert positive control on the electrical activity of cultured frog melanotrophs by inhibition of K^+ currents through a G-protein-dependent pathway (Soriani et al. 1998). In agreement, sigma receptors have been shown to inhibit voltage-gated K^+ channels in rodent neurohypophysial nerve terminals (Wilke et al. 1999). The main implication of this reduction of K^+ currents in the neurohypophysis would be enhanced release of the neuropepeptide hormones oxytocin and vasopressin by prolonging individual action potentials and increasing the duration of action potential bursts (Lupardus et al. 2000).

Interestingly, G-proteins and cytoplasmic factors were found to not be required for the modulation of K^+ currents in the neurohypohysis, but the sigma receptors and K^+ channels must be in close proximity for this functional interaction to occur (Lupardus et al. 2000). It requires the presence of sigma receptors therefore is not due to sigma ligands binding directly to K^+ channels (Lupardus et al. 2000). The authors suggest that this mechanism of signal transduction is mediated by protein-protein interactions within the cell membrane, thus acting analogous to a channel-activity-modifying protein (Lupardus et al. 2000). Similar mechanisms have been proposed for neurotrophin receptor-mediated modulation of Na^+ channels (Kafitz et al. 1999).

Recent evidence supports the suggestion that protein-protein interaction is the mechanism of signal transduction by sigma receptors (Aydar et al. 2002). Recent results confirm Lupardus and colleagues (2000) findings that sigma ligands do not interact directly with K^+ channels, as K^+ channels alone without sigma receptors in *Xenopus* oocytes showed no modulation by sigma ligands, but the K^+ current was inhibited by sigma ligands when the channels and the receptors were both expressed (Aydar et al. 2002). Furthermore, surprisingly, sigma receptors were able to alter channel function in the absence of sigma ligands, suggesting that the sigma receptor is associated with K^+ channels in a manner that can affect current flow. Meanwhile, in the presence of sigma ligand, sigma receptors exert more significant effects on current flow (Aydar et al. 2002).

Sigma receptors were precipitated by antibodies against voltage-dependent K^+ channels in the plasma membrane confirming that the proteins are a complex,

which may involve more proteins as well (Aydar et al. 2002), such as ankyrin and inositol-1,4,5-trisphosphate (IP_3) receptors as suggested by Hayashi and Su (2001), discussed in Section 2.5. Overall, this data suggests sigma receptors serve as auxiliary subunits to voltage-gated K^+ channels (Aydar et al. 2002).

This data on K^+ modulation relates back to the cloning data and further suggests sigma receptors being a subunit of a complex of proteins. Furthermore, this modulation of K^+ channels may occur in brain regions other than those tested thus far. Therefore sigma receptors may affect the release of other neurotransmitters via this mechanism.

2.5. Modulation of Ca^{+2} Currents by Sigma Receptors

The first data suggesting an interaction between sigma receptors and Ca^{+2} signaling was the observation that sigma ligands modulate protein phosphorylation in rat forebrain synaptosomes. Specifically, (+)-pentazocine, DTG, HAL, and (+)-SKF-10,047 inhibit depolarization-dependent increases in the phosphorylation of synapsin I (modulates neurotransmitter release) and increase the dephosphorylation of dynamin (modulates vesicle recycling), while increasing basal levels of phosphorylation of synapsin I and dynamin. The study showed that this was not due to direct action of sigma ligands on protein kinases or phosphatases but rather was dependent on Ca^{+2} signaling (Brent et al. 1995) Furthermore, the addition of (+)-pentazocine inhibited the rise in Ca^{+2} levels induced by depolarization, and the sigma ligands decreased basal intracellular

Ca^{+2} concentration ($[Ca^{+2}]_i$), suggesting that sigma receptor activation alone affects $[Ca^{+2}]_i$ (Brent et al. 1996, 1997).

Later, it was demonstrated that the early phase of Ca^{+2} influx depends on voltage-dependent modulation of Ca^{+2} channels through the sigma receptor-mediated closing of K^+ channels (indirect Ca^{+2} modulation) (Soriani et al. 1999). Meanwhile, the late peak Ca^{+2} influx is supported by a direct intracellular pathway, suggesting a coupling of sigma receptors to Ca^{+2} channels, by which sigma ligands can stimulate voltage-activated Ca^{+2} conductances independent of the K^+ channel pathway (Soriani et al. 1999).

Recently, several lines of evidence constitute another argument for the involvement of $sigma_1$ receptors in Ca^{+2} signaling. Specifically, the $sigma_1$ ligands PS, (+)-pentazocine and 2-(4-morpholinoethyl)-1-phenyl-cyclohexane-1-carboxylate hydrochloride (PRE-084) can modulate Ca^{+2} signaling in NG108 cells via $sigma_1$ receptors by 2 different modes of action. First, intracellularly, perhaps on the ER, sigma ligands potentiate the bradykinin-induced increase in cytosolic free Ca^{+2} in a bell-shaped manner, which was blocked by $sigma_1$ receptor AS (Hayashi et al. 2000). A second mode of action at the plasma membrane was demonstrated as PS and (+)-pentazocine inhibit (in agreement with the effects observed in forebrain synaptosomes) (Brent et al. 1996, 1997), while PRE-084 potentiates the depolarization-induced changes in Ca^{+2}, blocked by $sigma_1$ receptor AS (Hayashi et al. 2000).

Interestingly, in the presence of $sigma_1$ ligands the relative levels of $sigma_1$ receptors in the plasma membrane increase (Hayashi et al. 2000). $Sigma_1$

receptors have been shown to exist in the ER and plasma membrane (McCann and Su 1990) and $sigma_1$ receptors are two times as enriched in the microsomal fraction than in the synaptosomal fraction (McCann et al. 1989, McCann and Su 1990). However, more recent studies found that this actually depends on the presence of sigma ligands. Specifically, without exogenously applied $sigma_1$ ligands, $sigma_1$ receptors are predominantly present in the microsomes and slightly detectable in the plasma and nuclear membranes. However in the presence of (+)-pentazocine the relative levels of $sigma_1$ receptors in the plasma and nuclear membranes are increased, suggesting that $sigma_1$ receptors are translocated to the plasma membrane and nuclear membrane in the presence of a $sigma_1$ ligand (Hayashi et al. 2000). This mechanism may explain how an intracellular ER protein such as the $sigma_1$ receptor can affect Ca^{+2} signaling at the plasma membrane, and the activation of the $sigma_1$ receptor may trigger its translocation (Hayashi et al. 2000, Morin-Surun et al. 1999).

In a more detailed study of the phenomenon, (+)-pentazocine treatments led to the accumulation of $sigma_1$ receptors in the plasma membrane and facilitated anterograde movements of $sigma_1$ receptor-containing vesicles in the processes of NG-108 cells (Hayashi and Su 2003). Moreover, $sigma_1$ agonists potentiate the dynamics of the vesicles in the processes of NG-108 cells (Hayashi and Su 2003). In agreement, immunofluorescent staining shows $sigma_1$ receptors in the cytoplasm shift to the membrane, suggesting a translocation, which results in the recruitment of Ca^{+2}-dependent phopholipase C (PLC)/protein kinase C (PKC) cascade (Morin-Surun et al. 1999).

In support of sigma receptors acting through a G-protein/PLC/PKC cascade, the $sigma_1$ ligand E-5842 after acute treatments increases GTPγS-stimulated PLC activity in the frontal cortex and striatum, as well as increasing immunoreactivity levels of the $G_{q/11\alpha}$ protein in the frontal cortex (Romero et al. 2000). In keeping with this, more recent data demonstrated that the $sigma_1$ ligands (+)SKF-10,047 and (+)-pentazocine induce an increase in Ca^{+2} influx in hippocampal slices, when co-perfused with glutamate (Monnet et al. 2003). This effect was blocked by the $sigma_1$ antagonist NE-100 and by 12-(-cyanoethyl)-6,7,12,13-tetrahydro-13-methyl-5-oxo-5H-indolo[2,3-a]pyrrolo(3,4-c] carbazole (Gö-6976), a conventional PKC inhibitor (Monnet et al. 2003). These findings also support a role for PLC/PKC signaling cascades in $sigma_1$ receptors' modulation of Ca^{+2} currents.

Recent results suggest that the modulation of Ca^{+2} signaling described thus far involves the formation of a multi-protein complex. Specifically, $sigma_1$ receptors have recently been found to anchor ankyrin (a cytoskeletal adaptor protein) to the ER membrane and modulate the function of ankyrin and IP_3 receptor-3 on the ER (Hayashi and Su, 2001). In the model, the presence of the sigma agonist (+)-pentazocine leads to the $sigma_1$ receptor/ankyrin complex dissociating from the IP_3 receptor-3 which leads to increased binding of IP_3, which in turn increases Ca^{+2} efflux. On the other hand, in the presence of the $sigma_1$ antagonist NE-100, the $sigma_1$ receptor dissociates from ankyrin, which remains coupled to IP_3 receptor-3 on the ER (Hayashi and Su, 2001).

The data on the modulation of Ca^{+2} signaling by sigma receptors, similar to that with K^+ current modulation, suggests that $sigma_1$ receptors form multiunit complexes which leads, in turn, to the modulation of ion channels. This formation of complexes with other proteins enables sigma ligands to exert a wide variety of actions in the CNS, such that depending on the different combination of proteins formed, it is likely that different effector systems are activated, which then lead to different results. This hypothesis could also explain some of the discrepancies with respect to different signaling pathways observed to be involved with the effects of $sigma_1$ ligands. Clearly, further determination of the exact structure of the sigma receptors and any complexes they form, will subsequently lead to further elucidation of the signal transduction mechanisms employed by this group of receptors.

3. Functional and Clinical Implications for Sigma Receptors

3.1 Sigma Receptors and Schizophrenia

The first suggestion of a connection between sigma receptors and schizophrenia was the observation that the sigma ligand SKF-10,047 induces delusions, hallucinations and depersonalization (Brady et al. 1982). Furthermore, HAL and other antipsychotics have high affinity for sigma receptors (Su 1982, Tam and Cook 1984, Ferris et al. 1986). Further evidence of a potential role of sigma receptors in schizophrenia came from binding studies showing a reduction in sigma receptor binding in schizophrenic brains versus controls in the dentate

gyrus, parietal and temporal cortices (Weissman et al. 1991, Helmeste et al. 1996). It was suggested that this might be caused by neuroleptic treatments since long-term treatments with HAL induce a downregulation of sigma receptors, which may in fact underlie its therapeutic effects (Itzhak and Alerhand 1989, Reynolds et al. 1991, Weissman et al. 1990, Schlyer et al. 1992). Recent genetic evidence further suggests a sigma receptor-schizophrenia connection, as the $sigma_1$ receptor gene is located at human chromosome 9p13, a region questioned for its role in schizophrenia (Nanko et al. 1993). In addition, an association between polymorphism of the $sigma_1$ receptor gene and schizophrenia has been documented (Ishiguro et al. 1998). However, results of the most recent meta-analysis show no association between the $sigma_1$ receptor gene and schizophrenia (Uchida et al. 2003)

The evidence suggesting sigma receptors role in schizophrenia and the possible therapeutic effects of antipsychotics led to the development of selective sigma ligands to be tested as novel antipsychotics in animal models. For example, the sigma ligand (R)-(+)-1-(4-chlorophenyl)-3-[4-(2-methoxyethyl)piperazin-1-yl]methyl-2-pyrolidinone L-tartrate (MS-377) inhibits PCP-induced head weaving, which is recognized as a model of schizophrenia and is not affected by typical antipsychotics (Takahashi et al. 1999, Karasawa et al. 2000). Other behavioural studies show that MS-377 attenuates the development of methamphetamine-induced behavioral sensitization in a dose-dependent manner, another animal model of schizophrenia (Takahashi et al. 2000). MS-377 also potentiates the inhibitory effects of HAL and sultopride (D_2 selective antagonist)

on apomorphine-induced climbing behaviours, widely used for the evaluation of antipsychotics (Karasawa et al. 2002). The authors suggest that MS-377 may increase the therapeutic window of D_2 antagonists without inducing extrapyramidal symptoms and may be beneficial if used in combination with D_2 antagonists (Karasawa et al. 2002). The effect of MS-377 on apormorphine-induced climbing behaviors was inhibited by (+)-SKF-10,047 and SA-4503, therefore is clearly mediated by $sigma_1$ receptors. The effects of MS-377 are questionable as sigma agonists reverse them, which would not be the case if MS-377 were an antagonist at sigma receptors. However, MS-377's activity at sigma receptors is not yet characterized.

Another example of a novel sigma ligand being tested as an antipsychotic is the putative $sigma_1$ antagonist E-5842, which is active in animal models predictive of antipsychotic activity and shows effects on DAergic neurons (Guitart and Farre 1998, Sanchez-Arroyos and Guitart 1999, Guitart et al. 2000).

Sigma ligands have been proven to be effective in animal models that predict antipsychotic activity but have given mixed results in clinical studies. Some sigma ligands have shown mixed clinical efficacy but these ligands have been shown to be relatively nonspecific for sigma sites, such as rimcazole and α-(4-fluorophenyl)-4-(5-fluoro-2-pyrimidinyl)-1-piperazinebutanol (BMY-14802) (Munetz et al. 1989, Taylor et al. 1991, Borison et al. 1992). More recent clinical trials of the relatively selective sigma ligand (S)-(-)-[4-hydroxy-4-(3,4-benzodioxol-5-yl)-piperidin-1-yl methyl]-3-(4-methoxyphenyl)oxazolidin-2-one (EMD 57445, panamesine) found it to be only modestly effective as an

antipsychotic (Frieboes et al. 1997, Huber et al. 1999a, Müller et al. 1999). Perhaps a role for moderate blockade of sigma receptors exists in antipsychotic therapy, but evidence thus far does not suggest sigma receptor blockade alone is sufficient to cause therapeutic efficacy.

Sigma ligand's putative antipsychotic effects may involve their interaction with the DA system. Electrophysiological studies have demonstrated that the sigma ligands (+)-3-PPP, (+)-pentazocine and DTG inhibit DA firing activity while BMY-14802 and (+)-SKF 10,047 increase DA neuronal firing activity in the substantia nigra. Some authors suggest this is an effect mediated by DA receptors (Clark et al. 1985, Hjorth et al. 1985), while others suggest it is mediated by sigma receptors, as it can be reversed by the sigma antagonist BMY-14802 (Matthews et al. 1986, Freeman and Bunney 1984, Steinfels and Tam 1989, Steinfel et al. 1989, Matsuno et al. 1995). Morever, some electrophysiological studies found no effects with sigma ligands or contrasting results (Meltzer et al. 1992, Zhang et al. 1992, 1993, Gronier and Debonnel 1999). For example, electrophysiological experiments with more selective sigma ligands have examined NMDA-mediated DAergic activity. In these studies the $sigma_1$ ligand JO-1784, showed no effect on A9/A10 DA neuronal firing activity. However the $sigma_1$ receptor ligands 2-[4-(4-methoxybenzyl)piperazin-1yl-methyl]-4-oxo[4H]-benzo-thiazolin-2-one (S-21377) and 2-[(4-benzylpiperazin-1-yl)methyl]naphthalene dichiorydrate (S-21378) both slightly increased the spontaneous firing rate and potentiated the NMDA-induced neuronal activation of DAergic neurons (Gronier and Debonnel 1999). None of these ligands had any

effect on the DA-induced suppression of firing. Postsynaptically, in the nucleus accumbens, JO-1784, (+)-pentazocine and DTG increased NMDA-induced neuronal activation and also increased suppression by DA of NMDA and kainate-induced activations. These results further suggest the existence of $sigma_1$ subtypes (discussed earlier) as JO-1784, S-21377 and S-21378 appear to act via different subtypes. Overall however, the responses of DA neuronal activity were smaller than those seen in the hippocampal regions (Gronier and Debonnel 1999).

In support of a modulatory role of sigma receptors on DAergic neurotransmission, release studies show that sigma receptors are present in DA nerve terminals (Gonzalez-Alvear and Werling 1995, Chaki et al. 1998). Furthermore, radioligand binding studies demonstrate that sigma receptors are present in the substantia nigra and that sigma ligand binding densities were decreased by lesioning of DA neurons (Gundlach et al. 1986, Graybiel et al. 1989).

In release experiments, (+)-pentazocine and DTG increase and (+)-3-PPP decreases DA release in the rat striatum (Kanzaki et al. 1992, Gudelsky 1995, Patrick et al. 1993). In addition, BD-737, PROG, PS, and (+)-pentazocine inhibited NMDA-stimulated [^{3}H]DA release from rat striatal and hippocampal slices, an effect antagonized by (1-(cyclopropylmethyl)-4-2′-oxoethyl)piperidine hydrobromide (DuP 734), HAL, DTG or NE-100 (Gonzalez-Alvear and Werling 1995, Chaki et al. 1998, Nuwayhid and Werling 2003). Furthermore, PROG and PS's effects were also reversed by the PKCβ inhibitor 5,21:12,17-dimetheno-18H-dibenzo[1,0]pyrrolo[3,4-1][1,8]diacyclohexadecine-18,20(19H)-dione,8-

[(dimethylamino)methyl]-6,7,8,9,10,11-hexahydro-monomethane sulfonate (9Cl) (LY379196) (Nuwayhid and Werling 2003). This suggests that the PLC/PKC signaling cascade may be involved in the modulation of DAergic activity by $sigma_1$ receptors, as also suggested in the modulation of Ca^{+2} signaling by $sigma_1$ receptors (Section 2.5).

Further evidence of an interaction between $sigma_1$ receptors and DA neurotransmission have come from studies demonstrating that the acute administration of the $sigma_1$ ligand SA-4503 increased DA and DA metabolite levels in the rat frontal cortex but not in the hippocampus, striatum, midbrain, cerebellum, pons/medulla or hypothalamus (Kobayashi et al. 1997). This effect appears to be mediated by $sigma_1$ receptors as it was reversed by NE-100 (Kobayashi et al.1997).

The evidence has shown a definite interaction between the DA system and sigma receptors, however the extent of the interaction remains to be determined. The effects of sigma ligands on DA neurotransmission may be direct or indirect via other neurotransmitter systems. Nonetheless, the ability of sigma ligands to modulate DA neurotransmission has implication towards sigma ligands' therapeutic potential in disorders in which DA is implicated such as schizophrenia and drug abuse.

3.2 Sigma Receptors and Drugs of Abuse

$Sigma_1$ receptors are implicated in many of cocaine's effects including locomotor activation, sensitization, convulsion and lethality (reviewed in Maurice

et al. 2002). The first connection between sigma receptors and cocaine came from the demonstration that cocaine has high affinity for sigma receptors (2-7 μM), and that chronic cocaine treatments can induce a supersensitivity of sigma receptors (Sharkey 1988, Ritz and George 1993, Ujike et al. 1996). Similarly, chronic treatments with methamphetamine also lead to a supersensitivity of sigma receptors, which is blocked by BMY-14802 and the putative $sigma_1$ antagonist MS-377 (Ujike et al. 1992, Takahashi et al. 2000).

As for the acute effects of cocaine, $sigma_1$ antagonists including BMY-14802, rimcazole, 6-[6-(4-hydroxypiperidinyl)hexyloxy]-methylflavone (NPC 16377), N-[2-(3,4-dichlorophenyl)ethyl]-N-methyl-2-(dimethylamino)ethylamine (BD-1047), and 1-[2-(3,4-dichlorophenyl)ethyl]-4-methylpiperazine dihydrochloride (BD-1063) block the locomotory stimulation induced by cocaine or methamphetamine (McCracken et al. 1999 a, b, Menkel et al. 1991, Witkin et al. 1993). In agreement, $sigma_1$ receptor AS treatments decrease the locomotor effects of methamphetamine or cocaine with correlations observed between the changes in $sigma_1$ receptor gene expression and drug-induced behaviour (Matsumoto et al. 2001a).

$Sigma_1$ receptors also appear to be involved it the reward properties of drugs, as they were found to be active in blocking conditioned spatial preference (CSP) or conditioned place preference (CPP), in animal models of drug reward. Specifically, the acquisition and expression of cocaine-induced CSP and CPP was decreased by pretreatment with the $sigma_1$ antagonists NE-100 and BD-1047 (Romieu et al. 2000, 2002). Meanwhile, the $sigma_1$ agonists PRE-084 and JO-

1784 did not induce CPP but potentiated cocaine-induced CPP. Among the neurosteroid sigma ligands, the $sigma_1$ antagonist PROG (systemic or endogenous via finasterade) decreased CPP induced by cocaine, while the $sigma_1$ agonists dihydroepiandrosterone (DHEA) and pregnenalone (PREG) potentiated cocaine-induced CPP. The effects of DHEA and JO-1784 were additive and a cross-pharmacology was observed between neuroactive steroids and sigma ligands. Furthermore, $sigma_1$ receptor antisense-treated animals failed to develop cocaine-induced CSP (Romieu et al. 2000, 2003). Overall the data clearly suggest that agonists at $sigma_1$ receptors potentiate the reward properties of drugs while $sigma_1$ antagonists attenuate or block drug-induced rewards.

With respect to cocaine's toxic effects, the $sigma_1$ antagonists, HAL, BMY-14802, BD-1063, BD-1047, NPC 16377, EMD 57445 and AS directed against the $sigma_1$ receptor attenuate cocaine-induced convulsions (Witkin et al. 1993, McCracken et al. 1999a, b, Matsumoto et al. 2001 a, b, c). A correlation was found for the ability of sigma compounds to attenuate cocaine-induced convulsions and their affinity for the $sigma_1$ receptor (McCracken et al. 1999a, Matsumoto et al. 2001b). In accordance, pretreatment with the sigma agonists DTG or SA-4503 exacerbate convulsions and shift the dose-response curve for cocaine-induced convulsions to the left (Matsumoto et al. 2001a, McCracken et al 1999a). With respect to cocaine-induced lethality, the $sigma_1$ antagonists HAL, BMY-14802, and BD-1047 had protective effects and some novel sigma ligands were found to prevent cocaine-induced lethality even if administered post-treatment (Matsumoto et al. 2001 a, b, c). Thus, the data from cocaine-induced

lethality and convulsion experiments, similar to the drug-induced reward paradigms, suggest a protective role for $sigma_1$ receptor antagonists.

It is unclear whether the $sigma_1$ receptor activation by cocaine involves a direct interaction of the drug with the receptor or indirect mechanisms through cocaine's primary effect on the DA transporter (DAT). Some authors suggest that $sigma_1$ receptor activation appears to be consequent to DAT inhibition (Maurice et al. 2002), and it is thus likely that $sigma_1$ receptors are activated within post-synaptic DA neurons, and may involve $sigma_1$ receptor's modulation of cellular Ca^{+2} fluxes within the postsynaptic neurons, as discussed earlier (Section 2.5). In agreement, moderate to intense labeling of $sigma_1$ receptors have been found in most DAergic structures in mice, including the caudate putamen, nucleus accumbens, amygdala, septum and superficial layers of the cortex (Alonso et al. 2000). Interestingly, cocaine conditioning increased $sigma_1$ receptor mRNA in the nucleus accumbens (Romieu et al. 2002). In addition, it is also possible that the mechanism of $sigma_1$ ligands' effects in drug reward and toxicity paradigms involves sigma receptors' presynaptic modulation of DA release, as discussed previously, sigma ligands modulate DA release (Section 3.1).

Overall, many convincing lines of evidence suggest $sigma_1$ receptors' involvement in the short- and long-term effects of drugs of abuse. More research is needed to understand the exact mechanisms involved, but in general, antagonism of $sigma_1$ receptor-mediated effects appears to be beneficial with respect to many aspects of drug abuse. To this end, it has been suggested that

sigma$_1$ receptors provide a new target for therapeutic strategies for cocaine addiction (Romieu et al. 2002, Maurice et al. 2002).

3.3 Sigma Receptors, Neuroprotection and Cognitive Function

Sigma$_1$ receptors have been suggested to be involved in protection against neurotoxicity. Specifically, in models of cerebral ischemia, the sigma ligands DTG, BMY-14802 and JO-1784 protect rat brain neurons against hypoxia/hypoglycemia-induced, but not NMDA-induced, neurotoxicity (Lockhart et al. 1995). However, other findings demonstrated that sigma ligands possess neuroprotective ability *in vitro* against NMDA-induced neurotoxicity (DeLoore et al. 1994, Sharp et al. 1992, Decoster et al. 1995). BMY 14802, NPC-16377 and JO-1784 reduce neuronal damage in gerbil models of cerebral ischemia (Contreras et al. 1992, Clissold et al. 1993, O'Neill et al. 1995). This was suggested to involve sigma ligand's ability to inhibit the presynaptic release of glutamate (Nakazawa et al. 1998, Lobner and Lipton 1990, Ellis and Davies 1994).

Sigma ligands have been shown to be active in models of amnesia. Specifically, in models of spontaneous alternation behaviour, passive avoidance tasks, or the elevated plus maze, DTG, (+)SKF-10,047, (+)-pentazocine, SA-4503 and PRE-084 attenuate learning impairment induced by MK-801, an effect blocked by BMY-14802 or NE-100 (Maurice et al. 1994 a, b, Maurice and Privat 1997, Kamei et al. 1996). In addition, sigma$_1$ ligands also have promnesic effects in models of amnesia not involving NMDA receptor-mediated neurotoxicity. For

example, PRE-084 attenuates memory impairment induced by nimodipine (a Ca^{+2} channel antagonist) (Maurice et al. 1995). Also, carbon monoxide-induced amnesia was reversed by DTG or (+)-SKF 10,047 (Maurice et al. 1994a). In both models, the sigma antagonist BMY 14802 blocked the anti-amnesic effects of the $sigma_1$ ligands tested; suggesting $sigma_1$ receptors are mediating the observed promnesic effects (Maurice et al. 1994a, 1995). Overall, the data suggests a role for conventional $sigma_1$ agonists as neuroprotective in animal models of amnesia.

$Sigma_1$ ligands were also tested in senescence-accelerated mice, a model for age-related learning impairments. In this model, pretreatment with $sigma_1$ agonists JO-1784 or PRE-084 improved deficits in spontaneous alteration, passive avoidance performance, place learning and retention in the water maze (Maurice et al. 1996b). In agreement, the $sigma_1$ ligand 1-[3-[4-(3-chlorophenyl)-1-piperazinyl]propyl]-5-methoxy-3,4-dihydro-2-quinolinone monomethanesulfonate (OPC) improved spatial memory deficits of aged rats but did not affect cognitive performance in younger or uncompromised rats, and the authors suggest that OPC may help the increasing number of elderly persons who suffer from both depression and cognitive impairments (Tottori et al. 2002).

Recent evidence on the role of sigma receptors in aging has been controversial as some groups have shown an increase in sigma ligand binding while others showed a decrease during aging. For example, when examining [^{3}H]DTG binding in aged rats (Wallace et al. 2000) or [^{11}C] SA-4503 in aged monkeys, an increase in binding was seen (Kawamura et al. 2003). However, postmortem studies of human cortices show a decrease in [^{3}H](+)-pentazocine

binding (Kornhuber et al. 1996). Furthermore, when examining $sigma_1$ receptor mRNA no change was seen during aging in mice (Phan et al. 2003). The discrepant results may be due to the different ligands used, their variable affinity for $sigma_1$ over $sigma_2$ receptors, and/or the different species examined. Thus, more research is needed to elucidate whether sigma receptors play a role in normal or just pathological aging, as suggested by the observation that $sigma_1$ receptor density is decreased in the hippocampus in postmortem brains of Alzheimer's victims (Jansen et al. 1993).

$Sigma_1$ ligands have also been tested in the scopolamine-induced amnesia model. The sigma ligands DTG, (+)-3-PPP, JO-1784 and SA-4503 reverse the amnesia induced by scopolamine (Earley et al. 1991, Matsuno et al. 1996) an effect that is blocked by HAL and NE-100 (Matsuno et al. 1997). Similarly, the $sigma_1$ ligand OPC reverses the scopolamine-induced inhibition of the passive-avoidance response with a bell-shaped dose-response curve (Tottori et al. 2002). The authors suggest that this effect of OPC may involve a modulation of acetylcholinergic (AChergic) neurotransmission, as OPC (10 mg/kg) increases the release of ACh in the hippocampus compared to controls. The increase in ACh release is blocked by pretreatment with NE-100, suggesting a modulation of ACh neurotransmission by $sigma_1$ receptors (Tottori et al. 2002).

Modulation of AChergic neurotransmission may be a mechanism underlying sigma ligands' anti-amnesic effect observed in many animal models. In support of this, other sigma ligands have been shown to modulate ACh release, specifically (+)-SKF-10,047 and JO-1784 were found to potentiate, while DTG

inhibited, potassium chloride-evoked [^{3}H]ACh release from hippocampal slices, in a HAL-reversible manner (Junien et al. 1991). *In vivo* microdialysis studies demonstrate that the sigma ligands DTG, SA-4503, (+)-pentazocine and (+)-SKF 10,047 increased extracellular ACh concentration in the rat frontal cortex and hippocampus, an effect antagonized by HAL (Matsuno et al. 1992, 1993, Kobayashi et al. 1996, Horan et al. 2002). Moreover, the order of the sigma ligands' ability to increase ACh in the frontal cortex was positively correlated with [^{3}H]SKF 10,047 binding affinity but not [^{3}H]DTG's, therefore suggesting an effect involving $sigma_1$ receptors (Matsuno et al. 1993).

Another hypothesis of a potential mechanism by which $sigma_1$ ligands manifest their neuroprotective capacity is through sigma-mediated modulation of neurosteroidal activity (reviewed by Maurice and Lockhart 1997). Neurosteroid $sigma_1$ ligands also have cognitive benefits, as shown in both spontaneous alternation and passive avoidance responses. The anti-amnesic effect of DHEAS was blocked in $sigma_1$ AS-treated animals, demonstrating that the $sigma_1$ receptor is a necessary target for the anti-amnesic effect of the neurosteroid (Maurice et al. 1997, 2001). In agreement, DHEAS has been shown to prevent or reduce the neurotoxic effects of NMDA exposure in primary hippocampal cultures (Kimonides et al. 1998).

Overall, sigma agonists have shown protective effects in animal models of amnesia. The modulation of AChergic neurotransmission discussed may be involved, however sigma ligands could also exert neuroprotective effects through the modulation of Ca^{+2} currents (Section 2.4) or glutamatergic neurotransmission

(Section 5.2.1). Therefore, more research is necessary in order to determine the mechanism underlying sigma ligands anti-amnesic effects.

4. Focus on Depression

4.1 Involvement of the Glutamatergic System

4.1.1 Introduction to the Glutamatergic System

Glutamate is the most widespread excitatory neurotransmitter in the CNS. Glutamate receptors have been pharmacologically classified as ionotropic and metabotropic receptors (reviewed by Bleich et al. 2003). Ionotropic glutamate receptors include NMDA, α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) and kainate families of receptors. For the purpose of this introduction we will focus on NMDA and AMPA receptors, as they have been linked to depression and/or antidepressant action (see below). NMDA receptors control channels that allow Na^+ and Ca^{+2} ion influx and AMPA receptors allow the passage of Na^+ and K^+ ions. Thus, both of these ionotropic glutamate receptors mediate excitatory neurotransmission, and their activation results in cell membrane depolarization (reviewed by Seeburg 1993).

Metabotropic glutamate receptors (mGluR) are divided into 3 groups based on their sequence homology, effector coupling and agonist selectivity (Pin and Duvoisin 1995, Conn and Pin 1997). Group I (mGluR1 and mGluR5) are coupled to phosphatidylinositol hydrolysis/Ca^{+2} signaling pathways. Their activation leads to release of Ca^{+2} from intracellular stores leading to an increase in neuronal excitation. In contrast, Group II and III mGluR's are negatively

coupled to adenylyl cyclase, which lead to decreased cyclic adenosine monophosphate (cAMP) production and decreased glutamate release (Pin and Duvoisin 1995, Conn and Pin 1997).

4.1.2. The Glutamatergic System and Depression

There is some data suggesting altered glutamatergic transmission in depression (reviewed by Sanacora et al. 2003). Specifically, reports suggest that glutamate metabolism differs significantly between depressed patients and controls when examining glutamate and/or glutamine levels in plasma, or cerebrospinal fluid and when examining platelet intracellular Ca^{+2} release in response to glutamate stimulation (Kim et al. 1982, Altamura et al. 1993, 1995, Levine et al. 2000, Berk et al. 2001). Furthermore, some of these differences have been resolved by chronic antidepressant treatments (Mauri et al. 1998, Maes et al. 1998). Magnetic resonance imaging (MRI) methods have shown reduced glutamate levels in the anterior cingulate cortex (Auer et al. 2000, Michael et al. 2003) and this reduction can be reversed with successful treatments (Pfleiderer et al. 2003, Michael et al. 2003). Thus, altered glutamatergic neurotransmission may play a role in the pathophysiology of depression, but more research is necessary to elucidate whether it represents a vulnerability to depression or a secondary effect of other abnormalities in the brains of depressed patients.

4.1.3. The Glutamatergic System and Antidepressants

Preclinical tests for antidepressant efficacy have shown "antidepressant-like" properties of NMDA receptor antagonists (Trullas and Skolnick 1990, Petrie et al. 2000), including functional antagonists and ligands at the glutamate, glycine, polyamine, bivalent cation and ionophore recognition sites of the NMDA receptor complex (Trullas and Skolnick 1990, Trullas et al. 1989, Layer et al. 1995, Yilmaz et al. 2002). Chronic treatments with NMDA receptor antagonists results in behavioral effects similar to those produced by classical antidepressants in many animal models including the chronic mild stress (CMS) (Papp and Moryl 1994), and olfactory bulbectomy (OBX) models (Kelly et al. 1997, Redmond et al. 1997). The "antidepressant-like" effects of NMDA antagonists likely involves NMDA receptors located in the hippocampus as the NMDA antagonist 2-amino-7-phosphonoheptanoic acid (AP-7), when injected directly into the hippocampus, decreases immobility time in the forced swimming test (FST) (Padovan and Guimaraes 2004).

In addition to NMDA receptor antagonists having potential antidepressant properties, classical antidepressant treatments, in turn, have been linked to changes in NMDA receptor properties. For example, antidepressant drugs produce a time- and dose-dependent change in the radioligand binding properties of the NMDA receptor (Mjellem et al. 1993, Kelly et al. 1997, Maj et al. 1991, 1992, Paul et al. 1993, Nowak et al. 1993, Paul et al. 1994, Pilc and Leguko 1995, Skolnick et al. 1996, Nowak et al. 1998). These changes are apparent within all antidepressant classes (Paul et al. 1994), and may involve an alteration in the expression of NMDA receptor subunits (Boyer et al. 1998, Watkins et al. 1998).

Chronic antidepressant treatments can also alter NMDA receptor function (Massicotte et al. 1993, Pallotta et al. 2001, De La Garza et al. 2002). For example, repeated treatments with tricyclic antidepressants (TCA's) or selective serotonin reuptake inhibitors (SSRI's) decrease the amplitude of the field potentials and the ratio of the NMDA:AMPA/KA receptor-mediated component in *ex vivo* cortical slices, indicating a decrease in glutamatergic synaptic transmission (Bobula et al. 2003).

In humans, NMDA receptor abnormalities have been observed in suicide victims (Nowak et al. 1995). A decrease in the expression of NMDA receptor subunit1 mRNA has also been documented in the hippocampus of depressed patients (Law and Deakin 2001). Clinical studies with the NMDA antagonists amantadine, memantine and ketamine have shown efficacy in depression (Moryl et al. 1993, Huber et al. 1999b, Berman et al. 2000, Kudoh et al. 2002, Stryjer et al. 2003). Therefore, together the data suggest that a dysfunction of the NMDA receptor complex is likely involved in the pathophysiology of major depression and drugs that modulate NMDA mediated neurotransmission may possess "antidepressant-like" properties.

mGluR's have also been implicated in antidepressant action. Specifically, TCA and repeated electroconvulsive therapy (ECT's) decrease the effects of MGluR1 agonists, suggesting a subsensitivity of mGluR1s (Pilc and Legutko 1995, Pilc et al. 1998, Palucha et al. 1997, Palucha and Pilc 2002). In accordance with the involvement of Group 1 MGluR's, mGluR5 antagonists display antidepressant-like activity in behavioral models (Tatarczynska et al. 2001, Pilc et

al. 2002, Wieronska et al. 2002). Furthermore, imipramine and ECT after chronic treatments have been shown to increase mGluR 1a and 5a subunits in the rat hippocampus, which the authors suggested may be in order to compensate for the previously demonstrated subsensitivity of MGluR1 receptors (Bajkowska et al. 1999, Smialowska et al. 2002). Thus, blockade of Group I mGluR's may lead to an overall decrease in glutamate receptor-mediated transmission, which could be underlying the mechanism of action of mGluR5 antagonists in the animal models of antidepressant activity (Palucha and Pilc 2002).

Group II and III MGluR's may also be involved in antidepressant action as chronic treatments with antidepressants increase the expression and function of mGluR2/3 receptors in hippocampal slices (Matrisciano et al. 2002). Furthermore, Group III MGluR agonists produce antidepressant effects after central administration in behavioural tests, an effect blocked by a mGluR3 receptor antagonist (Palucha et al. 2004). Therefore, combining these data suggest that an increase in mGluR2/3-mediated transmission and a decrease in mGluR1-mediated transmission, lead to a decreased glutamatergic neurotransmission as an end result that may be relevant to antidepressant treatments.

Recently, AMPA receptors have also been implicated in antidepressant action due to the observation that the AMPA receptor potentiator N-2-(4-(3-thienyl)phenyl)propyl z-propane sulfonamide (LY392098) possesses "antidepressant-like" properties in the FST and tail suspension test (Li et al. 2001). LY392098 alone does-dependently decrease immobility similarly to classical antidepressant, and at doses less than those that decreases immobility,

significantly increases the potency at which other antidepressants decrease immobility (Li et al. 2003). LY392098 also increase brain derived neurotrophic factor (BDNF) mRNA in primary neuronal culture (Legutko et al. 2001, Lauterborn et al. 2001), which is suggested to play a critical role in the action of antidepressants (Altar 1999, Duman et al. 1997, Coyle and Duman 2003). In addition, treatments with classical antidepressants can influence AMPA receptors. Specifically, chronic antidepressant treatments increase the expression of AMPA receptors in hippocampal membranes (Martinez-Turrillas et al. 2002), and increase the phosphorylation of AMPA receptor subunits (Svenningsson et al. 2002), which may indicate enhanced AMPA-mediated synaptic transmission. The data on AMPA receptors suggest that a potentiation of AMPA receptor-mediated transmission may be beneficial in depression. This is in contrast to the suggested antidepressant effects of decreasing NMDA or mGluR-mediated neurotransmission. Therefore, perhaps altering the balance between different glutamate receptor-mediated transmissions may be crucial for an antidepressant response.

Alternatively, the involvement of glutamate receptors in antidepressant action may be via a common downstream effect. For example, one downstream effect of activation of glutamate receptors is an increase in Ca^{+2} levels and, in turn, nitric oxide (NO) synthesis (Southam and Garthwaite 1993). NO may be involved in the relationship between antidepressants and glutamate transmission, as a potential role for NO in affective disorders has been proposed (Harvey 1996, Van Amsterdam and Opperhuizen 1999). Antidepressants can inhibit the activity

of neuronal NO synthase (nNOS) (Finkel et al. 1996, Wegener et al. 2003). Accordingly, NOS inhibitors are active in acute and chronic preclinical antidepressant screening paradigms (Jefferys and Funder 1996, Harkin et al. 1999, Karolewicz et al. 1999, Yildiz et al. 2000).

Thus, subcellular signaling processes linked to glutamate neurotransmission are suggested to be involved in antidepressant actions and modulation of this activity at one of multiple loci within this signaling cascade may result in "antidepressant-like" effects. Overall, the data strongly suggests a role of the glutamate system in antidepressant action, but clearly more research is needed to elucidate whether particular glutamate receptor families are involved or a more common downstream target of all these receptors.

4.2 Involvement of the Serotonergic System

4.2.1. Introduction to the Serotonergic System

The main nucleus of the 5-HT system is the DRN, which contains approximately 40% of the 5-HT neurons of the brain (Wiklund et al. 1981). The major sites of termination of DRN fibers include the central gray, substantia nigra, thalamus, amygdala, lateral septum, hippocampal formation, basal forebrain, striatum, prefrontal cortex (PFC), frontal cortex, piriform cortex, and entorhinal cortex. Afferents to the DRN include the PFC, spinal cord, cerebellum, rostral basal forebrain, lateral hypothalamus and habenular nuclei (Aghajanian and Wang 1977). Interestingly, of the 33 brainstem nuclei with direct projections to the cortex, the DRN ranked first in overall strength of cortical projections (Vertes

1991). However, the DRN is heterogenous, as 1/3-2/3 is 5-HT, while the rest is non-5-HT neurons, including DA, NE, Glutamate, γ-aminobutyric acid (GABA), and various neuropeptides (reviewed by Kohler and Steinbusch 1982, Jacobs and Azmitia 1992).

5-HT neurons were defined by their slow, spontaneous, rhythmic firing pattern (0.5-2.5 Hz), large afterhyperpolarization potential, gradual interspike depolarization, and high input resistance (Aghajanian 1978, Aghajanian and Vandermaelen 1982). 5-HT_{1A} receptors are of particular importance in the regulation of 5-HT neurons' activity. Activation of these receptors triggers the opening of K^+ channels, which induces a hyperpolarization of the neuron and decreases its firing activity (Aghajanian and Lakoski 1984). 5-HT_{1A} receptors on 5-HT neurons of the DRN have been denoted somatodendritic autoreceptors as they exert autoregulatory control of the firing activity of 5-HT neurons (Aghajanian et al. 1978). Activation of these receptors by 5-HT or 5-HT_{1A} agonists induces a suppression of the firing activity (Aghajanian et al. 1978, Wang and Aghajanian 1978, Vandermaelen and Aghajanian 1983, Blier and de Montigny 1987; Sprouse and Aghajanian 1987, Vandermaelen et al. 1986).

In addition to 5-HT_{1A} autoreceptors, high densities of 5-HT_{1A} receptors located postsynaptically are found in the hypothalamus, amygdala, hippocampus, lateral septum, and frontal cortex (Marcinkiewicz et al. 1984, Köhler 1984, Pazos and Palacios 1985, Kia et al. 1996). Activation of postsynaptic 5-HT_{1A} receptors in the hippocampus by 5-HT or 5-HT_{1A} agonists leads to membrane hyperpolarization and a suppression of the firing activity of hippocampal

pyramidal neurons (Andrade et al. 1986, Andrade and Nicoll 1987, Colino and Halliwell 1987, Chaput and de Montigny 1988, Beck 1989, Tada et al. 1999).

Accumulating evidence has shown that although both the postsynaptic 5-HT_{1A} receptor and autoreceptor originate from a single gene, they present different pharmacological profiles (reviewed by Hoyer and Martin 1996, Barnes and Sharp 1999). First, the effectiveness of 5-HT_{1A} agonists to inhibit the firing activity of DRN versus hippocampal neurons are different such that 5-HT_{1A} agonists appear to act as full agonists in the DRN while they act as partial agonists in the hippocampus (Blier and de Montigny 1987, 1990a, Sprouse and Aghajanian 1988, Blier et al. 1993, Dong et al. 1997). Second, chronic SSRI and 5-HT_{1A} agonist treatments desensitize the 5-HT_{1A} somatodendritic autoreceptor in the DRN (Chaput et al. 1986, 1991, Invernizzi et al. 1994, Hervas et al. 2001, Le Poul et al. 2000, Kreiss and Lucki 1995, Blier and de Montigny 1990b), but do not change the responsiveness of postsynaptic 5-HT_{1A} receptors in the hippocampus (Le Poul et al. 2000, Chaput et al. 1986, Blier and de Montigny 1987, Haddjeri et al. 1999). Third, agonist-induced internalization of 5-HT_{1A} receptors only occurs presynaptically in the DRN, but not postsynaptically in the hippocampus (Riad et al. 2001). These differences could be related to the difference in the G-proteins linked to the receptors (Lesch and Manji 1992, Hensler 2002, 2003). In addition, 3 mRNA's were detected for the 5-HT_{1A} receptor gene, which provides genetic evidence for the possibility of 5-HT_{1A} receptor subtypes (Albert et al. 1990).

These postsynaptic 5-HT_{1A} receptors, in addition to the 5-HT autoreceptors have been shown to play a role in the regulation of DRN 5-HT

firing activity, via a long feedback loop. This was demonstrated by experiments that showed that after transection of the frontal cortex and forebrain, the dose-response curve of the inhibitory effects of the 5-HT_{1A} agonist 8-hydroxy-2-(di-n-propylamino)tetralin (8-OH-DPAT) was shifted to the right (Ceci et al. 1994). This effect is thought to involve a population of 5-HT_{1A} receptors located postsynaptically, such as the medial PFC (mPFC) (Hajos et al. 1998, 1999, 2003).

More recent experiments confirm the involvement of the mPFC as 8-OH-DPAT excites mPFC neurons and there is a direct connection between the mPFC and the raphe such that when stimulated electrically this pathway has a strong inhibitory influence on DRN 5-HT neurons, thus forming a long feedback loop (Hajos et al. 1998, 1999, 2003). In support of the feedback loop, 5-HT innervation of the mPFC arising from 5-HT neurons of the DRN has been observed (O'Hearn and Molliver 1984), and a substantial number of mPFC neurons project monosynaptically to 5-HT neurons of the DRN (Hajos et al. 1998, 1999, Peyron et al. 1998, Varga et al. 2001). The most recent study shows that stimulation of the DRN inhibits mPFC neurons, which is blocked by 5-HT_{1A} antagonists, which proves that 5-HT_{1A} receptors on mPFC neurons are key players in this feedback loop (Hajos et al. 2003).

In summary, this feedback loop provides the DRN 5-HT system with direct input from key regions of the corticolimbic system, therefore one would expect that dysfunction of the mPFC would impact the 5-HT system. Supporting the role of mPFC dysfunction in mood disorders are findings from neuroimaging studies showing a dysfunction of the mPFC in patients with severe major

depression (Bench et al. 1992, Baker et al. 1997). There is also evidence of hypofunction and decreased tissue volume in the mPFC, which persists after antidepressant treatment (Drevets et al. 1997). This could reflect an abnormality in brain development related to the tendency to develop major depression or degenerative changes due to recurrent episodes (Drevets et al. 1997).

The 5-HT system is far more complex than described thus far but this outline provides the basis for understanding some of the key 5-HT circuits involved in depression and antidepressant action.

4.2.2 The Serotonin System and Depression

The 5-HT theory of depression originated in the 1960's and stated that low levels of 5-HT at certain brain receptors led to depression (Lapin and Oxenkrug 1969). This theory was then modified to the theory that a deficiency in brain 5-HT increases one's vulnerability to depression, suggested by the observations that interference with the 5-HT system or storage may induce depression in vulnerable individuals and antidepressants enhance central 5-HT neurotransmission (reviewed by Maes and Meltzer 1995).

The tryptophan depletion studies provided further evidence of the importance of the 5-HT system in antidepressant activity but not necessarily in the pathophysiology of depression. In remitted depressed patients receiving SSRIs, acute L-tryptophan depletion led to a rapid clinically significant return of depressive symptomatology. However, remitted patients maintained with TCA's were less prone to relapse following tryptophan depletion (Delgado et al. 1990,

Heninger et al. 1992). Interestingly, unmedicated depressed patients do not worsen following tryptophan depletion. Therefore, the authors suggest that decreased 5-HT activity may not be the limiting factor but rather a vulnerability factor (Delgado et al. 1990, Heninger et al. 1992). The lack of an effect in healthy volunteers may be due to adaptive changes due to antidepressants and thus, the tryptophan depletion studies suggest more that antidepressants may not be correcting underlying deficits but rather compensating for it (Delgado et al. 1990).

Another method employed to study the involvement of the 5-HT system in depression and/or antidepressant action involves using blood platelets as a model for 5-HT neurons. Overall, 65-75% of studies on 5-HT reuptake sites in platelets show a lower level of uptake in depressed patients versus controls (Meltzer and Arora 1991). However, experiments using a more selective 5-HT transporter ligand (paroxetine) have not found significant differences in the levels of binding of 5-HT to reuptake sites of platelets (Lawrence et al. 1993). Mixed results were also found when investigating differences in cerebrospinal levels of the 5-HT metabolite 5 hydroxyindole acetic acid (5-HIAA) and postmortem tryptophan, 5-HT and 5-HIAA levels (Maes and Meltzer 1995). Thus, indirect methods of measuring 5-HT activity in depressed patients have been shown to be rather inconclusive in determining a causal role for decreased 5-HT levels in depression.

A more recent theory is that a simple deficiency of 5-HT is not the sole cause of depression, and that depression likely involves dysfunction in the areas of the brain that are modulated by monoaminergic systems, such as the frontal cortex and hippocampus, (reviewed by Delgado and Moreno 2000, Delgado

2000). Recent focus has shifted to downstream effects of antidepressants that may represent final common pathways for the various antidepressant drugs and may include influences on second messenger systems, phosphorylation of protein kinases in postsynaptic neurons, activation of transcription factors and binding of these factors to promoter regions and effects on gene expression (reviewed in Hyman and Nestler 1996, Nestler et al. 2002, Manji et al. 2003).

4.2.3 The Serotonin System and Antidepressants

There are convincing lines of evidence suggesting a role of the 5-HT system in the mechanism of action of antidepressants. Overall, electrophysiological studies have demonstrated that all known antidepressants increase 5-HT neurotransmission following chronic treatments, via different mechanisms (Blier and de Montigny 1994)

TCA's enhance the responsiveness of postsynaptic neurons in the hippocampus to 5-HT following long-term treatments, an effect shown to be mediated by postsynaptic 5-HT_{1A} receptors (de Montigny and Aghajanian 1978, Chaput et al. 1991). Thus, TCA treatments lead to an increased 5-HT neurotransmission, without the activation of 5-HT_{1A} autoreceptors (Blier and de Montigny 1980, Kreiss and Lucki 1995).

ECT has effects similar to TCA's in that repeated ECT's increase responsiveness of the hippocampal neurons' response to 5-HT and 8-OH-DPAT, due to a sensitization of postsynaptic 5-HT_{1A} receptors (de Montigny 1984, Chaput et al. 1991). This is in keeping with the observation that an increase in the

density of postsynaptic 5-HT_{1A} receptors is detected after repeated ECT while presynaptic 5-HT_{1A} receptors are not affected (Nowak and Dulinski 1991, Blier and Bouchard 1992).

Monoamine oxidase inhibitors (MAOI's) following short-term treatments decrease the firing activity of DRN 5-HT neurons (Blier and de Montigny 1985). However, as treatments continue 5-HT neurons regain normal firing activity due to the desensitization of somatodendritic 5-HT_{1A} autoreceptors (Blier and de Montigny 1985).

SSRI's, similarly to MAOI's, following acute and short-term treatments decrease the firing activity of DRN 5-HT neurons (Chaput et al. 1986, Blier et al. 1984, Blier and de Montigny 1983). After chronic administration, SSRI's lead to a desensitization of 5-HT_{1A} somatodendritic autoreceptors in the DRN (Blier and de Montigny 1994, Kreiss and Lucki 1995, Le Poul et al. 2000). However, in the hippocampus, the sensitivity of postsynaptic 5-HT_{1A} receptor-mediated responses is not changed (Blier and de Montigny 1983, Chaput et al. 1986, Varrault et al. 1991, Le Poul et al. 2000). In accordance with electrophysiological studies, in microdialysis studies, acute SSRI administration produces a small, transient increase in extracellular 5-HT concentration in the rat frontal cortex, while 14-day continuous infusion produced a 6 fold increase in extracellular 5-HT concentration (Bel and Artigas 1993). The proportion of 5-HT neurons with desensitized autoreceptors was shown to increase progressively during the course of several weeks of SSRI administration (Le Poul et al. 1995). These adaptive changes in 5-HT neurons may explain the delayed enhancement of 5-HT-

mediated transmission, consistent with the clinical onset of action of SSRI's (Blier and de Montigny 1994).

Based on the theory that the delay in the onset of therapeutic action of antidepressants is due to the time needed for 5-HT_{1A} autoreceptors to desensitize, the effects of combined treatments were investigated in an attempt to decrease the delay in the onset of action of SSRI's. The combined treatment with the SSRI citalopram and a selective 5-HT_{1A} receptor antagonist resulted in an approximate doubling of the 5-HT concentration compared to that after SSRI alone (Hjorth 1993). In agreement, pindolol (a 5-HT_{1A} antagonist) augmentation appears to be effective in milder forms of depression (Artigas et al. 1994, Bakish et al. 1997, Blier and Bergeron 1998).

The desensitization of the 5-HT_{1A} autoreceptors, but not postsynaptic 5-HT_{1A} receptors, following antidepressant treatments may involve an alteration in G-protein function. Specifically, 8-OH-DPAT-stimulated [^{35}S]GTPγS binding was attenuated in the DRN following chronic fluoxetine but not amitriptyline treatments (Hensler 2002). Moreover, binding studies of 5-HT_{1A} receptors, found that the binding was not altered following treatments with fluoxetine nor amitriptyline. These results suggest that the observed changes in [^{35}S]GTPγS binding seen following fluoxetine treatments were not due to changes in receptor numbers. Thus, desensitization of 5-HT_{1A} autoreceptors following chronic SSRI treatments appears to be caused by a decreased ability of 5-HT_{1A} autoreceptors to activate G-proteins (Hensler 2002).

In contrast to the findings in the DRN, in forebrain areas 8-OH-DPAT-stimulated [^{35}S]GTPγS binding was not altered by either antidepressant treatment. Binding studies showed an increase 5-HT_{1A} receptor binding in the dentate gyrus and CA_1 hippocampus following chronic amitriptyline treatment. These results in the forebrain suggest that the regulation of the postsynaptic 5-HT_{1A} receptor sensitivity or function following chronic treatment with fluoxetine or amitriptyline is not at the level of receptor-G protein interaction, and appear to occur more distally, perhaps at the level of effector or involve changes in neuronal function at the system or circuit level (Hensler 2002). Thus, the capacity to activate G-proteins may underlie the desensitization of the autoreceptors in the DRN (Hensler et al. 2002).

In agreement with the suggestion that chronic antidepressant treatments affect pre- and postsynaptic 5-HT_{1A} receptors differentially, 14-day treatments with fluoxetine were shown to result in a selective uncoupling of 5-HT_{1A} receptors from G-proteins in the DRN, but not in the hippocampus (Pejchal et al. 2002). Thus, uncoupling may play an important role in the delayed therapeutic effects of SSRI's and more studies are needed to determine whether this is due to post-translational modification of the receptors, changes in the G-protein population or another interacting molecule (Pejchal et al. 2002).

Overall, antidepressants clearly produce adaptive changes in the 5-HT system following chronic treatments. Whether these changes are key to the therapeutic efficacy of antidepressants remains to be determined. It is possible that alterations in 5-HT neurotransmission may just be one step involved, and

downstream mechanisms may also be necessary. To this end, this brings about a similarity to the glutamatergic system's involvement in antidepressant action, and perhaps a downstream target common to both the glutamatergic and serotonergic systems is the common target of antidepressant medications. Regardless, changes in 5-HT transmission in electrophysiological and microdialysis models are predictive of antidepressant potential, therefore, can be used as indices to assess potential novel antidepressants.

5. Sigma Receptors and Depression

5.1. Sigma Ligands as Antidepressants

The first interest in sigma ligands as antidepressants came from the observation that the antidepressants fluvoxamine, sertraline, fluoxetine, citalopram, sertraline, clorgyline and imipramine all possess moderate to high affinity (K_i=36-343 nM) for $sigma_1$ sites (Narita et al. 1996, Schmidt et al. 1989, Itzhak and Kassim 1990). Furthermore, in the model of the modulation of the NMDA response in the dorsal hippocampus, the antidepressants sertraline and clorgyline potentiate the NMDA response in the hippocampus with a bell-shaped dose-response curve similar to other sigma agonists, which was reversed by HAL. In contrast, the antidepressants paroxetine and tranylcypromine had no effects on the NMDA response despite their similar monoaminergic profiles to sertraline. Thus, the observed effects of sertraline and clorgyline are likely mediated by sigma receptors (Bergeron et al. 1993).

Sigma ligands were then tested in various behavioral tests predictive of antidepressant activity. The $sigma_1$ ligands SA-4503, (+)-pentazocine, DTG, JO-1784 and OPC dose-dependently decrease immobility in the FST (Matsuno et al. 1996, Tottori et al. 1997, 2001, Kinsora et al. 1998). The effects in the FST were blocked by NE-100. In addition, SA-4503 and (+)-pentazocine also decreased immobility time in the Tail Suspension Test, an effect also antagonized by NE-100 (Ukai et al. 1998). Therefore, sigma ligands show clear "antidepressant-like" effects in animal models, which have been shown to be via activation of $sigma_1$ receptors.

Interestingly, repeated treatments with the TCA imipramine (14 days) cause a decrease in the total number of sigma receptor binding sites without affecting the affinity of [^{3}H]DTG binding to sigma sites in the striatum, hippocampus and cortex of the rat (Shirayama et al. 1993). Similar reductions were observed after chronic administration of fluoxetine but not desipramine. Depletion of brain 5-HT by para(4)-chloroamphetamine (p-CPA) blocks the ability of imipramine to decrease DTG binding therefore cerebral 5-HT transmission may play a role in the regulation of cerebral sigma binding sites in the rat. The authors suggest that part of the antidepressant activity of imipramine and fluoxetine could be mediated by the sigma-5-HT interaction and certain differences in the clinical effects of various antidepressants may in part be explained by their distinct influence on cerebral sigma sites (Shirayama et al. 1993).

Recently OPC, a combined sigma$_1$ and 5-HT$_{1A}$ receptor ligand yielded interesting results in the FST (Oshiro et al. 2000, Tottori et al. 2001). Single doses decreased immobility time in the FST, and the effect of OPC was enhanced by its daily administration for 7 days. Both the sigma$_1$ receptor antagonist NE-100 and the 5-HT$_{1A}$ receptor antagonist N-[2-(4-[2-methoxyphenyl]-1-piperazinyl)ethyl]-N-2-pyridinylcyclohexanecarboxamide (WAY 100635) antagonized the behavioural actions of a single dose of OPC in the FST (Tottori et al 2001). Also, a one-week pretreatment with p-CPA fails to diminish the antidepressant effects of OPC in the FST (Watanabe et al. 2000). These results suggest that sigma receptors alone can mediate the antidepressant effects produced by OPC, but perhaps the combination of the sigma and 5-HT$_{1A}$ receptor affinities of OPC contribute to produce a more potent or rapid "antidepressant-like" effect. In keeping with this, a potentiation of the "antidepressant-like" effects in the rodent FST has been obtained with the combined administration of sigma and 5-HT$_{1A}$ receptor agonists compared with their separate administration (Yamada et al. 2000).

Neurosteroids that are sigma ligands have also been shown to exert "antidepressant-like" effects in animal models, that are dependent on the endogenous neurosteroidal systems. For example, in ADX/CX animals the effect of JO-1784 in the FST was enhanced versus controls such that it was active at 10 mg/kg versus 60 mg/kg. Also, ADX/CX mice showed an antidepressant effect of PRE-084 (20 mg/kg), which was absent in control mice. All these augmented effects were blocked by the sigma$_1$ antagonist BD-1047 (Urani et al. 2001).

Furthermore, treatment with finasteride, which leads to the accumulation of PROG, blocked the $sigma_1$-mediated effects. Thus, as discussed in previous sections, circulating steroids appear to exert a tonic modulatory effect on the $sigma_1$ receptor and therefore on $sigma_1$ receptor-mediated "antidepressant-like" effects (Urani et al. 2001). To this end, the effects of $sigma_1$ agonists as antidepressants are highly dependent on the endogenous PROG levels and perhaps depressed patients with decreasing levels of neurosteroids, such as the elderly, may be particularly sensitive to such therapy, as PROG would not be tonically inhibiting sigma receptors to the same degree (Urani et al. 2001). However, recent electrophysiological studies in the rat have shown that PROG can modulate 5-HT neuronal activity in the DRN as during pregnancy there is an increase in the 5-HT firing activity in the DRN, which correlates with the increase in PROG during pregnancy (Robichaud et al. 2002). Therefore, PROG's effects in animal models of depression may be more complex and may involve a combination of effects at different types of receptors, in addition to sigma receptors.

Most of the data on sigma receptors and depression have focused on the $sigma_1$ receptor. However, the $sigma_2$ ligand, Lu 28-179 showed "antidepressant-like" activity in the CMS model of depression. Specifically, chronic treatments with antidepressants lead to a normalized sucrose intake, which reverses the decreased intake caused by stress in this model. Lu 28-179 did not affect sucrose intake in non-stressed controls at any dose, but the 1.0 mg/kg dose produced a significant increase in sucrose intake in rats exposed to CMS after 3 weeks of

treatment (Sanchez et al. 2000). Lu 28-179's *in vitro* binding affinity, has a selectivity ratio for $sigma_2$ over $sigma_1$ receptors of about 90 (Sanchez et al. 1997), and it was not tested with an antagonist. Therefore, it remains possible that the $sigma_1$ site may contribute to these "antidepressant-like" effects of Lu 28-179.

In trying to identify the mechanism by which sigma ligands exert their "antidepressant-like" effects, a recent study examined the role of sigma receptors' regulation of Ca^{+2} (Urani et al. 2002). The effects of JO-1784 in the FST were demonstrated to be Ca^{+2}-dependent as the extracellular Ca^{+2} chelator ethylene glycol-bis(β-aminoethyl ether)N,N,N′,N′ tetraacetic acid (EGTA) prevented the effect of JO-1784 in a dose-dependent manner. In addition, JO-1784 at a lower dose had no effect alone. However, if co-administered with the L-type voltage-dependent Ca^{+2} channel (VDCC) positive modulator 1,4-dihydro-2,6-dimethyl-5-nitro-4-[2-(trifluoromethyl)phenyl]pyridine-3-carboxylic acid methyl ester [(-)-Bay K8644], significantly reduces immobility. In agreement, the L-type VDCC antagonist verapamil and the N-type VDCC antagonist ω-conotoxin blocked the effects of JO-1784 (Urani et al. 2002). Therefore, $sigma_1$ receptors may be interacting with pre- or postsynaptic VDCC's to exert "antidepressant-like" effects in the FST (Urani et al. 2002).

In addition, bradykinin, which increases IP_3 levels, enhanced the effect of JO-1784 (Urani et al. 2002). Further confirming the involvement of IP_3 receptors, the IP_3 receptor antagonist xestospongin C blocked the effect of JO-1784, thus the mobilization of intracellular Ca^{+2} from IP_3 receptor-sensitive pools also participates in the behavioural effects mediated by $sigma_1$ receptors located on the

ER membranes (Urani et al. 2002). The $sigma_1$ receptor then putatively moves to the plasma membrane and interacts with VDCC's (Brent et al. 1997, Hayashi et al. 2000).

Furthermore, sigma receptors' neuroprotective effects (discussed in Section 3.3) may have significance in major depression as recent evidence has shown hippocampal atrophy in major depression, which can persist long after depression is resolved (Bremner 1999, Sheline et al. 1996, 1999, 2003). This atrophy could be due to regression of dendritic processes, inhibition of neurogeneration or the loss of hippocampal neurons (reviewed by Sapolsky 2000).

To this end, recent evidence has provided a role for $sigma_1$ receptors in the morphological changes of cells, specifically in the initiation of neurite outgrowth and sprouting (Hayashi and Su 2001, Takebayashi et al. 2002). Specifically, $sigma_1$ receptor's and ankyrins are highly concentrated in the growth cone of NG-108 cells, a region related to neurite sprouting, extension, and guidance (Hayashi and Su 2001). The $sigma_1$ agonist (+)-pentazocine, had no effect by itself on neurite sprouting but potentiated the neurite-sprouting induced by nerve growth factor (NGF) (Takebayashi et al. 2002). In contrast, neurite sprouting induced by cAMP in PC12 cells was not affected by (+)-pentazocine. The $sigma_1$ antagonist NE-100, regardless of the presence of NGF did not affect neurite sprouting, but antagonized the potentiation induced by (+)-pentazocine, thus clearly indicating mediation via $sigma_1$ receptors (Takebayashi et al. 2002).

This effect of $sigma_1$ agonist may be involved in their observed "antidepressant-like" effects, as similar to sigma agonists the antidepressants

imipramine and fluvoxamine, potentiated the effects of NGF in this model (Takebayashi et al. 2002). These effects of imipramine and fluvoxamine were antagonized by NE-100, and no concentration of 5-HT tested affected neurite sprouting induced by NGF (Takebayashi et al. 2002). All together, these data clearly demonstrate that these effects on NGF-induced neurite outgrowth of both $sigma_1$ agonists and antidepressants are mediated by $sigma_1$ receptors.

Further evidence of sigma receptors' mediation of NGF activity comes from the observations that treatment of cells with NGF, even without exogenously added $sigma_1$ receptor agonists, increases the level of $sigma_1$ receptors in a dose-dependent manner. This was time-dependent as well and occurred in 2 days, and the effects of (+)-pentazocine and NGF were additive (Takebayashi et al. 2002). In keeping with this, treatment of the cells with imipramine and fluvoxamine alone for 2 days also increases $sigma_1$ receptor expression, while 5-HT fails to upregulate $sigma_1$ receptors (Takebayashi et al. 2002). This upregulation suggests that the increase of $sigma_1$ receptors may play an important role in the neurite sprouting observed, as a strong correlation exists between the increase of sprouting and the increase of $sigma_1$ receptor expression (Takebayashi et al. 2002).

To further test the role of sigma receptors in NGF signaling pathways, a PC12 line stably over-expressing $sigma_1$ receptors was established (MT40, 4x higher $sigma_1$ receptor expression) (Takebayashi et al. 2002). In MT40 cells, NGF was more potent and more efficacious in inducing neurite sprouting. In agreement, treatments with $sigma_1$ receptor AS significantly reduced the degree of

neurite sprouting induced by NGF (Takebayashi et al. 2002). Together, this data suggest a primary role for sigma$_1$ ligands in enhancing NGF-induced neurite growth by increasing the level of sigma$_1$ receptors in the cell, and proves a causal role for sigma$_1$ receptors in the sprouting, likely as an intrinsic molecule involved in the signaling pathway(s) evoked by NGF (Takebayashi et al. 2002). In this model, the action site of the sigma$_1$ receptor is not known but the authors suggest it may be upstream of MAPK and Rap 1 on one of the NGF signaling pathways and may involve sigma$_1$ receptors modulation of Ca^{+2} signaling (Takebayashi et al. 2002).

Thus, recent research has led to convincing evidence towards sigma$_1$ ligands' potential as antidepressants in behavioural models. However, when investigating this potential, one must examine the effects of sigma ligands on neurotransmitter systems relevant to depression, such as 5-HT and glutamatergic transmission, two transmitters that, as previously discussed, are clearly implicated in depression.

5.2 Possible Mechanisms of Action for Sigma Ligands as Antidepressants

5.2.1. Sigma Receptors and Glutamatergic Neurotransmission

Numerous studies have shown interactions between sigma receptors and NMDA receptor-mediated responses. For example, sigma ligands, including

HAL, (+)-pentazocine, 4-(N-benzylpiperidin-4-yl)-4-iodobenzamide (4-IBP), (+)-3-PPP, (+)-SKF-10,047 and DTG, antagonize NMDA receptor currents in *Xenopus* oocytes (Whittemore et al. 1997). The effect of sigma ligands on NMDA receptors in this study is thought to be indirect. However, high doses (μM) and/or nonselective sigma ligands were used. Furthermore, there was no correlation between the potency of NMDA receptor inhibition and the affinity or stereoselectivity for sigma sites (Fletcher et al. 1995, Whittemore et al. 1997, Pontecorvo et al. 1991). Thus, it is difficult to assess whether these observations are based on sigma receptor-mediated actions or more nonspecific effects.

More recently, *In vitro* inhibition was shown using the ligands HAL, (+)-pentazocine, DTG, (+)-SKF-10,047 and (+)-3-PPP, which all inhibited [^{3}H]TCP binding to NMDA receptors in neuronal cells correlated to the affinity for DTG sites (Yamamoto et al. 1995, Hayashi et al. 1995).

In our laboratory's model of modulation of the NMDA response in dorsal hippocampal pyramidal neurons of the CA_3 region, it was found that low doses of the sigma ligands DTG, JO-1784, (+)-pentazocine, BD-737 and L-687,384 selectively potentiate the neuronal response of these neurons to microiontophoretic application of NMDA (Monnet et al. 1990, 1992, Bergeron et al. 1993, 1995). The potentiation induced by these sigma ligands (putative agonists) is suppressed by antagonists such as BMY-14802 and (+)-3-PPP (Monnet et al. 1990, 1992, Bergeron et al. 1993, 1995). The effects of $sigma_1$ agonists on the NMDA response produces a bell-shaped dose-response curve, as previously described (Bergeron et al. 1995, Section 2.1). Importantly, at higher

doses the degree of potentiation progressively decreases such that sigma agonists can act as antagonists by suppressing the potentiation induced by sigma agonists (Debonnel et al. 1992, Bergeron et al. 1995). Thus, the discrepancies observed for the action of sigma ligands on NMDA receptor-mediated responses, with respect to inhibition versus potentiation, may be due to high doses being used in *in vitro* studies that were acting as antagonists at $sigma_1$ receptors.

$Sigma_2$ receptors have also been shown to modulate NMDA-mediated responses. Doses of the $sigma_2$ ligands Lu 28-179 and BD-1008, despite high affinity for $sigma_2$ receptors, require doses 5-10 times higher than $sigma_1$ ligands in the model of the potentiation of the NMDA response (Couture and Debonnel 1998). Also, the responses are not blocked by the $sigma_1$ antagonists NE-100 and PROG, or the combined $sigma_{1/2}$ antagonist HAL, suggesting these effects are mediated through $sigma_2$ receptors (Couture and Debonnel 1998).

In vitro evidence also suggests a potentiating role for $sigma_1$ agonists on NMDA-mediated responses. For example, JO-1784, BD-737, (+)-pentazocine and (+)-3-PPP potentiate in a concentration-dependent manner NMDA-induced [^{3}H]NE release from preloaded hippocampal slices of rats (Monnet et al. 1992, 1995, 1996). DTG, acting as an inverse agonist, concentration-dependently inhibits the overflow of [^{3}H]NE evoked by NMDA. HAL and BD-1063 do not modify [^{3}H]NE release alone but completely prevent the effects of JO-1784, BD-737, (+)-pentazocine, DTG and (+)-3-PPP, thus presenting antagonist profiles in this model (Monnet et al. 1992, 1995, 1996). Inactivation of G-proteins abolished the potentiating effects of JO-1784 and (+)-3-PPP, suggesting that the $sigma_1$

receptors mediating these effects are coupled to $G_{i/o}$ proteins (Monnet et al. 1992, 1995, 1996).

Other studies found contrasting results with respect to agonist and antagonist action in the *in vitro* model of NMDA-stimulated [^{3}H]NE release. For example, (+)-pentazocine and BD-737 were shown to inhibit NMDA-stimulated [^{3}H]NE release and the sigma$_1$ antagonist DuP 734 completely blocked BD-737's effects while partially blocking (+)-pentazocine's effects (Gonzalez-Alvear and Werling, 1995).

Neurosteroids that are sigma ligands have also been shown to modulate NMDA receptor-mediated effects, as DHEA at low doses potentiates the NMDA response in electrophysiological recordings from the hippocampus. The effect of DHEA can be blocked by NE-100 and HAL, suggesting mediation by sigma$_1$ receptors (Bergeron et al. 1996, Debonnel et al. 1996). In this model neither PREG nor PS modulate the NMDA response or act as antagonists, which may be due to their lower affinity for sigma$_1$ receptors mentioned previously (Su et al. 1988, Maurice et al. 1996). Both PROG and testosterone act as antagonists and suppress the potentiation of the NMDA response induced by sigma agonists such as DTG, (+)-pentazocine and JO-1784. DHEA's response is blocked by pertussis toxin while PROG's is not, suggesting different sigma$_1$ subtypes may be involved in the modulation of the NMDA response by neurosteroids (Bergeron et al. 1996, Debonnel et al. 1996).

Neurosteroids are also active in the *in vitro* model of NMDA-induced [^{3}H]NE release from hippocampal slices. Specifically, DHEAS potentiates while

PS inhibits (inverse agonist effect) NE release (Monnet et al. 1995). Both effects are blocked by HAL, PROG, BD-1063 and by pertussis toxin pretreatment suggesting G-protein-dependent $sigma_1$ receptors are responsible (Monnet et al. 1995).

Furthermore, endogenous hormone levels affect sigma receptor's modulatory effect on NMDA-mediated responses. For example, 2 weeks following OVX the potentiation of the NMDA response induced by DTG was significantly greater than in control female rats, suggesting that sigma receptors may be tonically inhibited by endogenous PROG (Bergeron et al. 1996, Debonnel et al. 1996). In agreement, 10 times the dose of (+)-pentazocine and DHEA is needed in pregnant females to potentiate the NMDA response. This lack of effect of sigma agonists in late pregnancy may be due to occupation of sigma receptors by high levels of PROG, which also supports it being a potential endogenous ligand for $sigma_1$ receptors (Bergeron et al. 1999, Debonnel et al. 1996).

In keeping with the theory of PROG endogenously binding to sigma receptors, during post-partum periods, the degree of potentiation of the NMDA response by DTG, (+)-pentazocine and DHEA was significantly higher than that observed in control females, and at Days 10-15 the potentiation of the NMDA response returned to control values. The potential supersensitivity of sigma receptors observed post-partum might be due to the rapid drop of PROG levels after delivery (Pepe and Rothchild 1974, Klink et al. 2002).

In OVX rats treated for 3 weeks with PROG (1000 μg/kg/day), low doses of DTG, JO-1784, DHEA or (+)-pentazocine, did not induce any potentiation of

the NMDA response. The potentiation by these ligands returned to normal after a 5-day washout (Bergeron et al. 1999). The necessity of such a long washout before the NMDA response returns to normal following PROG treatments in OVX rats suggests that a longer lasting adaptive change in sigma receptors could be involved.

Overall, all sigma ligands have demonstrated the ability to modulate NMDA-mediated glutamatergic neurotransmission. As previously discussed (Sections 4.2.2 and 4.2.3), NMDA receptors may be involved in the mechanism of action of antidepressants. Furthermore, altered glutamatergic neurotransmission may underlie depressive pathology. Therefore, the ability of sigma receptors to modulate NMDA receptor-mediated responses may have implications towards sigma ligands potential as antidepressants.

5.2.2 Sigma Receptors and Serotonergic Neurotransmission

Previous evidence has suggested a 5-HT-sigma interaction peripherally as DTG, HAL and BMY-14802 have been found to inhibit the 5-HT-evoked contractions of the guinea pig ileum longitudinal muscle/myenteric plexus preparation in a manner showing high correlation with their potency to compete with DTG binding (Campbell et al. 1989). One other study investigated the effect of the sigma ligand EMD 57445 on the 5-HT system indirectly, using behavioural tests (Skuza et al. 1997). EMD 57445 does not show activity in 8-OH-DPAT-induced behavioural syndrome, m-chlorophenylpiperazine-induced hypothermia, or L-5-hydroxytryptophan-induced head twitches. In addition, biochemical studies

show no changes in 5-HT or 5-HIAA levels in various brain regions, suggesting overall that EMD 57445 exerts no effects on 5-HT receptor populations or 5-HT metabolism (Skuza et al. 1997). However, these behavioural tests may not be capable of showing modulatory effects of sigma receptors on 5-HT activity. Furthermore, EMD 57445 has been suggested to be a sigma antagonist, therefore, may not show effects on its own. Based on this, it would be interesting to assess the effects of sigma agonists in the same behavioural and biochemical paradigms.

No studies have examined an effect of sigma ligands on serotonergic neuronal activity, either *in vivo* or *in vitro*, thus presenting a huge range of possibilities for future research in this area, particularly given the capacity for sigma ligands to modulate other neurotransmitter systems. Moreover, the ability of sigma ligands to exert "antidepressant-like" effects in behavioural models of depression, suggests that perhaps they exert some effects on the serotonergic system.

6. Overall Summary

Sigma receptors, since first identified in 1976, have evolved into their own unique receptor type. With the advent of selective sigma ligands and advancements in understanding their molecular biology, sigma receptors and their ligands are now being examined in many areas of research. Sigma receptors are particularly interesting, as they appear to exert modulatory effects on many neurotransmitter systems including glutamate, DA and ACh. Also, in behavioural models sigma ligands have demonstrated therapeutic benefits in such areas as

depression, drug abuse, schizophrenia and cognitive impairments. More research is necessary to elucidate the mechanism by with sigma ligands produce these behavioural results, and whether a modulation of neurotransmission underlies their potential therapeutic effects. With respect to depression, behavioral tests support sigma ligands as potential novel antidepressants. However, the mechanism underlying the observed "antidepressant-like" effects of sigma ligands is not well understood. One hypothesis to be explored is whether sigma ligands' ability to modulate glutamatergic transmission may be involved, as the glutamatergic system is implicated in depression. Secondly, investigation of sigma ligands potential effects on 5-HT neurotransmission, a system heavily implicated in depression and antidepressant action, is essential in unraveling the potential antidepressant properties of sigma ligands.

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Chapter 2

Effects of sigma ligands on NMDA receptor function in the bulbectomy model of depression: A behavioral study in the rat

As discussed in the Introduction, it has previously been shown by our laboratory that selective sigma ligands, at low doses, potentiate the NMDA response of CA_3 pyramidal neurons of the dorsal hippocampus *in vivo*. Overall, in this model and in *in vitro* models, sigma receptors have been shown to play a modulatory role on NMDA-mediated glutamatergic transmission (See Introduction).

Interestingly, NMDA receptors have been implicated in depression and in the mechanism of action of antidepressants, such that many studies have shown NMDA antagonists to possess antidepressant activity. In addition, NMDA receptor function has been shown to be altered following chronic antidepressant treatments (See Introduction).

Sigma ligands have been suggested as potential novel antidepressant agents, as they show "antidepressant-like" effects in behavioural models that screen for antidepressant activity, such as the tail suspension test and the forced swimming test. However, these models demonstrate only acute effects, which do not correspond well to the chronic treatments known to be necessary in order for patients to show improvement. In contrast to other animal models, the OBX model of depression is a behavioural model that is based on a change in physiology due to the removal of the olfactory bulb. The OBX model, as opposed to other models, has requires chronic treatment to demonstrate antidepressant-like

effects. Therefore, the OBX model may be considered more relevant with respect to the timeframe of adaptive changes observed in depressed patients. Another advantage of the OBX model is that it is suggested to represent a model of "depressed or abnormal" behaviour versus the other behavioural models which are tested on "normal" animals.

OBX surgery produces alterations in NMDA receptor ligand binding, which suggests that NMDA receptor-mediated glutamatergic signaling may be involved in the OBX model. Given that sigma ligands have known effects on NMDA transmission, and the potential involvement of both sigma and NMDA receptors in depression, the first study set out to investigate the effect of short- and long-term treatments with sigma ligands on NMDA-mediated behavioural effects in the OBX model. This paradigm provides a way to examine sigma ligands' potential antidepressant-like effects.

EFFECTS OF SIGMA LIGANDS ON NMDA RECEPTOR FUNCTION IN THE BULBECTOMY MODEL OF DEPRESSION: A BEHAVIOURAL STUDY IN THE RAT

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ABSTRACT

Sigma (σ) ligands have been shown to modulate NMDA receptor activity. In the present study we used the olfactory bulbectomy (OBX) animal model of depression to assess the effects of the σ_1 ligand igmesine on OBX-induced behaviours. Behavioural experiments demonstrated OBX (saline treated) rats to have increased dizocilpine induced behavioural modifications, including locomotor and circling activity as compared to SHAM rats (saline treated). A short-term (7 days) treatment with low doses of igmesine (50-200 µg/kg/day s.c.) had no effect on dizocilpine-induced behaviours while long-term treatments (14 days) with low doses of igmesine reversed the effect of the bulbectomy such that the treated OBX rats' behaviours were not significantly different from SHAM saline rats. Short-term treatments with high doses of igmesine (500-1000 µg/kg/day) also reversed the increased locomotor and circling behaviours seen in OBX rats (saline treated) while long-term treatments with the same high doses did not. These results provide behavioural evidence for σ ligand's potential to reverse some OBX-induced behaviours. Moreover, they support the notion of a bell-shaped dose response curve previously reported for σ ligands.

INTRODUCTION

The existence of σ receptors was first reported by Martin et al. (1976) who initially classified them as belonging to the opiate receptor family. Sigma receptors were later divided into 2 subtypes σ_1 and σ_2 based on their different ligand affinity, stereoselectivity, and response to various treatments (Itzhak and Stein, 1991; Quirion et al., 1992). In 1996, σ_1 receptors were cloned from guinea pig liver, human placental cell line, mouse kidney and brain and rat brain (Hanner et al., 1996; Kekuda et al., 1996; Pan et al., 1998). In recent years, using an *in vivo* electrophysiological paradigm of unitary extracellular recordings from pyramidal neurons of the CA_1 or CA_3 region of the dorsal hippocampus, we have shown that acute intravenous administration of low doses of several high affinity σ agonists, including igmesine and

(+)-pentazocine, does not affect the spontaneous firing activity of CA_3 pyramidal neurons, but produces a marked and selective dose-dependent potentiation of NMDA-induced firing activity (Bergeron et al., 1993; Monnet et al., 1990, 1992). Sigma ligands such as haloperidol, NE-100 and progesterone act as antagonists, by not modifying NMDA-induced firing activity, but preventing and reversing the effects of the above mentioned σ agonists (Monnet et al., 1990, 1992).

The majority of data implicating sigma receptor's role in depression involves the σ_1 subtype. Several σ_1 ligands (eg. SA-4503, (+)-pentazocine, DTG, igmesine, and OPC-14523) have been shown to have antidepressant abilities in behavioural tests for antidepressants including the forced swimming test and tail suspension test, with NE-100, a selective σ_1 antagonist, blocking this effect (Ukai et al., 1998; Tottori et al., 1997; Matsuno et al., 1996; Kinsora et al., 1998).

Moreover, preliminary results in a clinical trial suggests that igmesine might have antidepressant properties (Pande et al. 1998)

Olfactory bulbectomy (OBX) is currently recognized as a valuable animal model of major depression and useful in the study of the mechanisms of action of antidepressant drugs (Jesberger and Richardson, 1988; Lumia et al., 1992; Kelly et al., 1997). OBX in rodents provokes a variety of neurochemical and behavioural alterations, which are not related to anosmia and are reversible by a wide range of antidepressants (Leonard and Tuite, 1981; van Riezen and Leonard, 1990; Jesberger and Richardson, 1985; Lumia et al., 1992; Kelly et al., 1997; Grecksch et al., 1997). It is thought to represent the neurochemical actions of antidepressants on depressive substrates relative to their actions on normal substates (reviewed in Jesberger and Richardson, 1986). The behavioural changes induced by OBX appear two to three weeks after the surgery and are characterized by hyperactivity, irritability, disruption of sexual behaviour (Reviewed in Leonard and Tuite, 1981), deficits in learning avoidance responses, spatial memory (Archer et al., 1984) and sleep disturbances (Sakurada and Kisara, 1977; Sakurada et al., 1976). Furthermore, the measurable behavioural and biochemical alterations are normalized by chronic, but not acute administration of clinically efficacious antidepressant drugs from a variety of families (Jesberger and Richardson, 1986).

Indeed, previous studies in our laboratory have shown that OBX induces a down-regulation of NMDA receptors as, following OBX, the hyperactivity induced by the acute administration of the non-competitive NMDA-antagonist dizocilpine was markedly decreased (Robichaud et al. In Press). In keeping with this finding, within one week following OBX, [^{125}I]iodo-dizocilpine binding was decreased in the frontal and piriform cortices, in the anteroventral thalamic

nucleus and in certain amygdaloid nuclei, whereas, after three weeks, this binding was also decreased in the posteromedial cortex, the hippocampus and the lateral hypothalamus (Robichaud et al. In Press). The present study investigates the effects of short and long-term treatments with the σ_1 agonist igmesine on dizocilpine-induced locomotor activity in OBX rats.

METHOD

Male Sprague-Dawley rats (180-275 g) were used. Animals were housed in temperature (25°C) and humidity controlled rooms with a 12 h light/dark cycle (lights on at 07:00 h) with food and water ad libitum. Rats were allowed 48 hours of adaptation before undergoing surgery. Ethical Committee approval was given by the McGill University Animal Ethical Care Committee and all their rules and regulations were followed.

1. Surgery

Six groups of animals were studied. Three groups underwent OBX surgery, while three groups were SHAM operated. For OBX surgery (Jesberger and Richardson, 1986), animals were anesthetized (choral hydrate, 400 mg/kg i.p.) and fixed in a stereotaxic frame. Bilateral burr holes were made in the skull surface at the following coordinates A: +5 mm (from Bregma) and L: +/- 2 mm. Olfactory bulbs were sectioned and removed by aspiration; the cavities being filled with haemostatic sponges. For SHAM surgery, animals were similarly operated on but the bulbs were left intact. Following surgery animals were given two weeks to recover and to permit the appearance of the "OBX syndrome". Two weeks after lesion, OBX and SHAM operated rats were randomly assigned to one of the 5 pharmacological treatments (saline, 50, 100, 200, 500 or 1000 µg/kg/day of igmesine) for either 7 or 14 days. Drugs and saline were administered via an Alzet osmotic minipump (Alza, CA) inserted s.c. under halothane anesthesia and aseptic conditions.

2. Behavioural experiments

All animals (SHAM-operated and OBX rats) received a single injection of dizocilpine (200 µg/kg, i.p.). Animals were then placed in 80 cm diameter, circular activity measuring wooden boxes with a 45cm high wooden wall. Floors and walls were painted in black and the only lighting was indirect and provided by a 40 watt bulb located 2 metres from the box. Locomotor activity was recorded with a video tracking system (Videotrack, France) for four minute time periods up to 40 minutes, encompassing the time-course for the maximal behavioural effects of dizocilpine (Löscher and Hönack, 1992). The different other behaviours measured: circling, head weaving and fall-over were measured manually. Rectal temperature was also measured at 5 and 45 min. For assessing the effect of the acute administration of dizocilpine on locomotor activity, the mean ambulatory distance per minute was compared for each period of 4 minutes from time 0 to time 40. As the pattern of changes in locomotion were similar in SHAM and OBX animals (Figure 1), for assessing the effects of the short- and long-term treatments with igmesine, the mean value of the ambulatory distance per minute during the whole periods of 40 minutes were compared.

3. Drugs

Dizocilpine was purchased from Research Biochemicals International (Natick, MA), igmesine was a generous gift from F. Roman from Institut de Recherche Jouveinal (Fresnes, France).

4. Histological verifications

Following behavioural experiments, the rats were sacrificed and all surgical procedures were verified. If any residual tissue of the main olfactory bulbs remained or if the frontal cortex
have been damaged during the surgical procedures, then the behavioural data were not included in the final analysis.

RESULTS

1. Exploratory Behaviour

Following their introduction in the open field, the rats presented the usual exploratory behaviour, which progressively disappeared within 5 to 10 minutes. In SHAM-operated animals, the effects of the i.p. administration of dizocilpine (200 µg/kg) appeared within 10 to 15 minutes. The first manifestation was a progressive increase of the locomotion followed by the appearance of stereotypies, head weaving, and circling behaviours and a marked decrease of rearing behaviours (Table 1, Figures 1, 2). Approximately thirty minutes following the administration of dizocilpine, the maximal behavioural effects were observed, the increased locomotion was generally reduced after that time since most of the animals spent most of their time in stereotypies, or were unable to move without falling over (Figures 1 and 2).

2. Effects of olfactory bulbectomy

OBX rats treated with saline ("OBX-saline") had significantly increased locomotor activity versus SHAM rats treated with saline ("SHAM-saline") in the 4-40 minute period following the injection of dizocilpine (Figure 1). In "OBX-saline" rats, the behavioural effects of dizocilpine measured from 20 to 40 minutes following its administration, showed a greater than 30% increase in locomotor activity as measured by the distance travelled per minute (Figure 1). The grooming activity was not significantly changed by the injection of dizocilpine nor by the bulbectomy (data not shown). Circling behaviour was increased in "OBX-saline" versus "SHAM- saline" rats, even if the difference was statistically significant only during the first 20 minutes (Figure 2). In contrast,

head weavings and fall over (Figure 3) were markedly reduced (between 50 and 80%) following OBX.

3. Effects of low doses igmesine

Short-term (7-day) treatments of OBX rats with a low dose (200 µg/kg/day) of igmesine failed to produce any effect on the dizocilpine-induced locomotor behaviours (Figure 4A). However, in OBX rats, long-term treatments (14 days) with low doses of igmesine (50-200 µg/kg/day) dose-dependently decreased dizocilpine-induced motor effects in OBX rats (Figure 4B). Long-term treatments with low doses of igmesine also markedly decreased the circling behaviour in OBX rats versus "OBX-saline" rats (Figure 5). In addition, long-term treatments with low doses of igmesine reversed the decrease in head weaving behaviour seen in "OBX-saline" rats (Figure 6A). In contrast, 14-day treatment with igmesine (200 µg/kg/day) did not reverse the decrease in fall over seen in OBX compared to "SHAM-saline" rats (Figure 6B).

4. Effects of high doses of igmesine

Short-term treatments with igmesine at higher doses (500-1000 µg/kg/day) induced a dose-dependent decrease in locomotor activity after dizocilpine injection in OBX rats, and therefore, a normalized response to dizocilpine versus "OBX-saline" rats, comparable to SHAM saline-treated rats (Figure 7A). Long-term treatment with igmesine in high doses (500-1000 µg/kg/day) produced no significant difference between "OBX-saline" and OBX igmesine treated rats regarding dizocilpine-induced behavioural modifications (Figure 7B). In agreement with these data, when the circling

behaviours were assessed, no effect of igmesine could be observed with long-term treatments with high doses (500 and 1000 µg/kg/day) (Figure 8). In SHAM operated animals treated for 2 weeks with either low or high doses of igmesine ("SHAM-JO"), the locomotor and circling behaviours (data not shown) induced by dizocilpine, were not significantly different from those obtained in "SHAM-saline" rats and thus were decreased versus "OBX-saline" rats (Figures 9A and B).

DISCUSSION

Five weeks after a bilateral olfactory bulbectomy, and following three weeks of saline treatment, the behavioural modifications such as ambulatory distance and circling behaviours induced by the acute administration of 200 µg/kg i.p of dizocilpine were drastically increased in "OBX-saline" compared to "SHAM-saline" rats (Figures 1 and 2). In contrast, head weaving was decreased in "OBX-saline" rats versus "SHAM-saline" rats. The hyper-locomotion induced by OBX as well as the marked behavioural effects and stereotypies induced by the acute administration of a low dose of dizocilpine are in keeping with previous studies (Deutsch and Hitri, 1993). The potentiation of the behavioural effects induced by the acute administration of dizocilpine is in agreement with the previous results of Redmond et al. (1997) who showed that acute treatment with dizocilpine (0.3 mg/kg) produced increased home cage locomotor activity while a lesser dose (0.1 mg/kg) attenuated home cage locomotor activity. Though this is a different setting than the "open field" used in the present study, these results do correspond to the increase in locomotor activity seen in the present study in the "open field" as we administered a dose of 0.2 mg/kg. Furthermore, chronic treatments with dizocilpine decreased the locomotor activity produced by OBX. This does not go against our observation as we administered only acute doses and the chronic dose could have different effects due to changes in receptor function that would not occur in our paradigm with the acute doses. As both the administration of dizocilpine and the OBX surgery alone increase locomotor activity, it is not surprising to find that their combined effect further increases locomotor activity.

The two-week treatment of OBX rats with low doses of igmesine (50-200 µg/kg/day), induced a reversal of the behavioural response to the injection of dizocilpine compared to what was observed in "OBX-saline" rats (Figure 4B). However, the short-term treatment with the same low doses did not lead to any significant difference in the dizocilpine -induced behavioural response compared to "OBX-saline" (Figure 4A). Conversely, a short-term treatment with the high doses of igmesine reversed the behavioral modifications induced by OBX, whereas a long-term treatment with the same doses was without any effect (Figures 7A and B).

We have previously reported that the dose-response curves of the potentiation of the NMDA response by sigma ligands has a bell-shaped aspect (Bergeron et al. 1995). More specifically, following an acute intravenous administration, the maximum potentiation of the NMDA response was observed with 50 µg/kg of (+)-pentazocine and 4 µg/kg of igmesine (Bergeron et al., 1995). When higher doses were administered, the potentiating effect would progressively decrease and finally disappear and at higher doses, sigma agonists were acting purely as sigma antagonists (Bergeron et al. 1995). Moreover, we have shown that long-term treatments with low doses of sigma agonists induces an upregulation of sigma receptors, as following a three-week treatment with low doses of DTG, (+)-pentazocine or igmesine, the neuronal activation induced by microiontophoretic applications of NMDA is markedly increased (Bergeron et al., 1997). Finally, it has been well established that long-term treatments with antagonists induce a down-regulation of sigma receptors (Itzhak and Alerhand, 1989, Riva and Creese, 1990, Jansen et al., 1992, Bergeron et al., 1997).

Therefore, during a long-term treatment with sigma agonists a certain accumulation is needed in order for the ligands to function in the active "agonist range". As the concentration of the ligand increases, its agonist effects increase as

well as the sensitivity of the sigma receptor. This continues until a peak after which the ligand begins to function as an antagonist and the effects of the ligand progressively decrease as the concentration increases and the sigma receptor desensitizes. Thus, in the present study it is likely that at the lower dose the longer treatment was necessary in order for the igmesine to reach the "agonist range". This explains why a shorter duration of treatment with a higher dose produced the same effects. Furthermore, the long-term treatment at high doses no longer produced any effect most likely due to the concentration of igmesine being in the "antagonist range". This dose-response curve thus involves a change in the properties of the sigma receptor and modify the NMDA response without affecting the sensitivity of the dizocilpine binding site.

Furthermore, igmesine is a highly selective sigma ligand, which has negligible affinity for other receptor subtypes including PCP, adrenergic, dopaminergic and serotonergic receptors (Roman et al. 1990), therefore, the effects observed in the present study are not likely mediated through activation of another receptor. Indeed, the present results suggest that, following a short-term treatment with a high dose and a long-term treatment with a low dose, igmesine is acting as an agonist and potentiates the response induced by the endogenous ligand for the NMDA receptor still functional. Therefore, in these conditions, it can be postulated that a chronic treatment with low doses of igmesine, by potentiating the effects of the down-regulated NMDA receptors produced by surgery in OBX, will compensate for this down-regulation, thereby normalizing the response to dizocilpine, and thus inducing smaller behavioural effects (Figures 4B, 5 and 7A).

Recent studies have demonstrated interactions between several antidepressant drugs and the NMDA receptor complex. For example, the acute administration of desipramine, imipramine and nortriptyline drastically reduces

NMDA-induced epileptiform response and LTP in rat hippocampal slices (Watanabe et al., 1993), whereas a 5-week treatment with desipramine, imipramine and amitryptiline inhibits the binding of [^{3}H]-dizocilpine in a concentration-dependent manner (Kitamura et al., 1991). Acute and chronic treatments with imipramine, amitriptyline, citalopram and fluoxetine potentiate the hyperactive behaviours induced by dizocilpine, an effect which is blocked by haloperidol, but not by the D_1 and the D_2 selective antagonists SCH-23390 or sulpiride, respectively (Maj et al., 1991, 1992), suggesting that the effect of haloperidol is likely due to its affinity for sigma receptors and not for DA receptors. It has also been reported that chronic treatments with fluoxetine or imipramine reduce [^{3}H]-(+)pentazocine binding in the rat brain (Shirayama et al., 1993). In addition, several antidepressants have been found to decrease NMDA-activated ion current (White et al., 1990; Sernagor et al., 1989).

Swim stress and exposure to unpredictable mild stress increase the potency of glycine to displace [^{3}H]-5,7-DCKA from the glycine site on the NMDA receptor in an imipramine reversible manner (Nowak et al., 1995). This was specific to antidepressants after repeated treatments as structurally related non-antidepressant molecules did not produce this effect (Nowak et al., 1995). Similarily, adaptive changes of the NMDA receptor have been observed to occur selectively in the mice cortex, where chronic SSRI treatments also decreased glycine-displaceable binding (Nowak et al., 1996, 1998). These data further strengthen the hypothesis that NMDA receptor modification could represent the final pathway of antidepressant action as already suggested by several groups (Paul et al., 1994; Skolnick et al., 1996). Combined with our experimental data, these different observations suggest that the NMDA and sigma receptors may be involved in the pathophysiology of depression and in the mechanism of action of antidepressant treatments. Thus, a sigma ligand that modulates NMDA receptor

function could be expected to have some potential interest as an antidepressant treatment.

The inability of igmesine to reverse the dizocilpine -induced headweaving and fall over in OBX rats (Figures 6A and B) is likely due to these behaviours being mediated by pathways different from those involved in ambulation or circling behaviours. We have previously reported that following OBX, the modifications of NMDA binding parameters differ in several brain regions (Robichaud et al., in press). It is therefore plausible that some areas are either less enriched in sigma receptors or have sustained greater modifications of NMDA binding parameters which cannot be compensated for. This could also explain why the different responses to OBX surgery, such as head weaving and fall over are decreased in OBX rats (Figure 3A and B). Nontheless, our results showed OBX to decrease MK-801-induced ataxia (fall-over) (Figure 3B). Following treatments with JO-1784 (200 µg/kg), there was a further decrease in ataxia in addition to an increase in locomotor activity (Figure 6B). Therefore, this increase in locomotor activity could, in part, be due to the decrease in ataxia.

The effects observed in the present studies with igmesine may be due to sigma ligand's modulation of NMDA receptors previously discussed, however, a direct interaction with the serotonergic system cannot be ruled out. Recently, we have demonstrated the sigma ligands (+)-pentazocine and 4-IBP to modulate serotonergic neurotransmission, as 2-day treatments (2mg/kg/day) induced a 35% increase in average basal firing rate of the serotonergic neurons of the dorsal raphe nucleus, while igmesine at the same dose produced no change after short- or long-term treatments, using an electrophysiological paradigm of extracellular recordings *in vivo* (Bermack and Debonnel, in press). Akunne et al. (2001) showed that chronic treatment with igmesine (15 mg/kg/day) produced no change in 5-HT_{1A} receptor densities, only minor reductions in tyrosine hydroxylase

activity, no effects on 5-HT,NE reuptake nor 5-HT synthesis. Their study, in agreement with ours, suggested the pharmacological actions of igmesine may be in part due to mechanisms not mediated by the monoaminergic system and may involve NMDA receptors based on their observation of igmesine treatment blocking NMDA-induced increases in cGMP. This is in agreement with the published data mentioned by the reviewer showing 16-day treatment with igmesine (3 mg/kg) produced no change in serotonin turnover (Song et al. 1997).

Effective antidepressant treatments are expected to reverse OBX-induced alterations, thus normalizing NMDA receptor binding levels. Therefore, our results suggest that igmesine could have antidepressant properties in the OBX model. However, this would require further investigation comparing sigma ligands to anitdepressants with respect to various OBX-induced alterations. These experiments establish the first behavioural model indicating a behavioural effect of long-term treatment with low doses of sigma ligands, most likely related to their affinity for sigma receptors, reversing OBX-induced alterations.

Acknowledgements

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FIGURE LEGENDS

Figure 1. Mean (± S.E.M.) ambulatory distance traveled expressed in cm/min in "OBX-saline" rats (light bars) versus "SHAM-saline" rats (dark bars) following the acute injection of dizocilpine (200 µg/kg i.p.) administered at time 0. *P<0.05 Student's t-test. Experiments were carried out in 14 OBX rats and 16 SHAM rats.

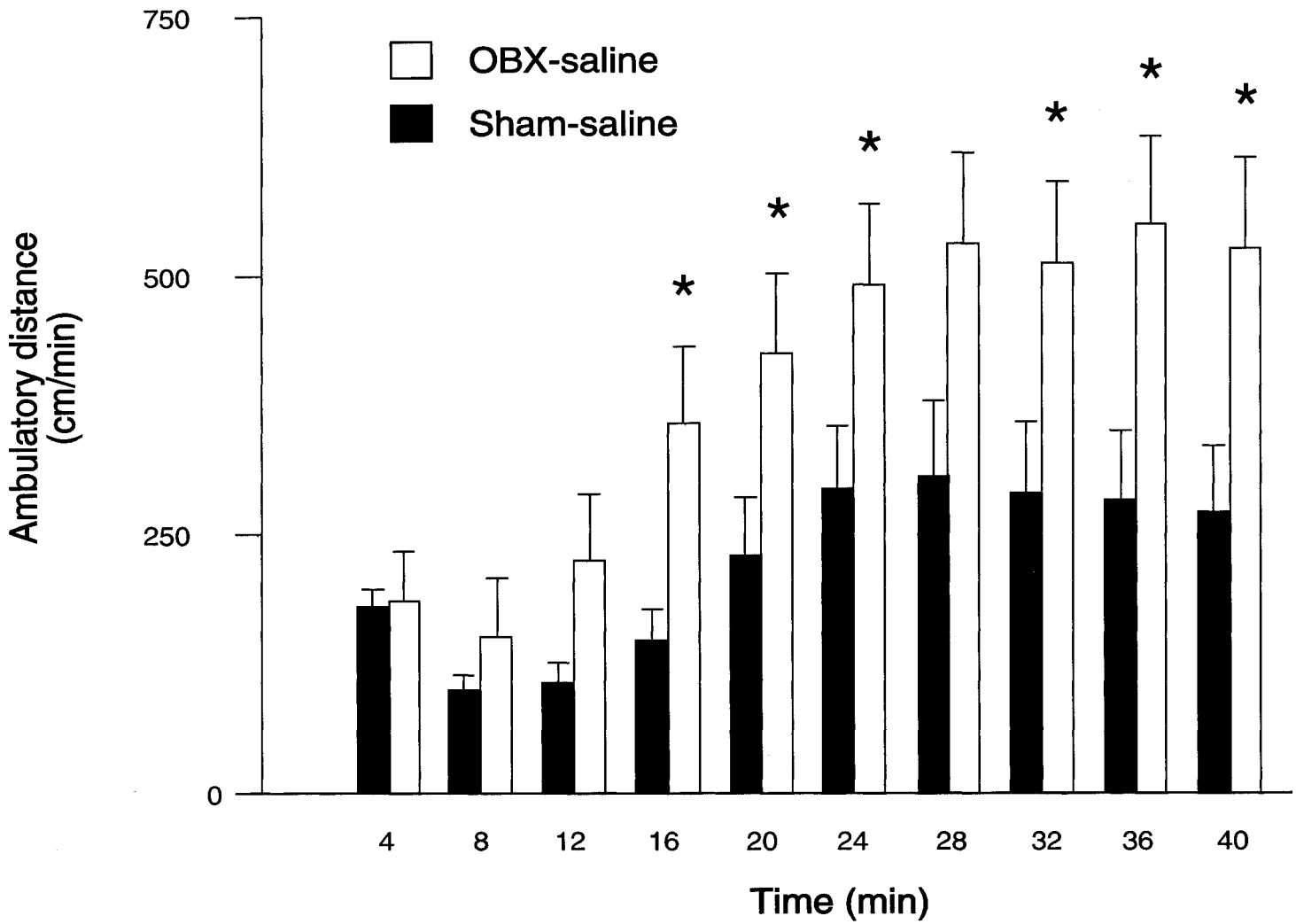
750
500
250
0
Ambulatory distance (cm/min)
OBX-saline
Sham-saline
*
*
*
*
*
*
4
8
12
16
20
24
28
32
36
40
Time (min)

Figure 1

Figure 2. Mean (± S.E.M.) number of circling behaviours observed in "OBX-saline" rats (light bars) versus "SHAM-saline" rats (dark bars) following the acute injection of dizocilpine (200 μg/kg i.p.) administered at time O. *P<0.05 Student's t-test. Experiments were carried out in 14 OBX rats and 16 SHAM rats.

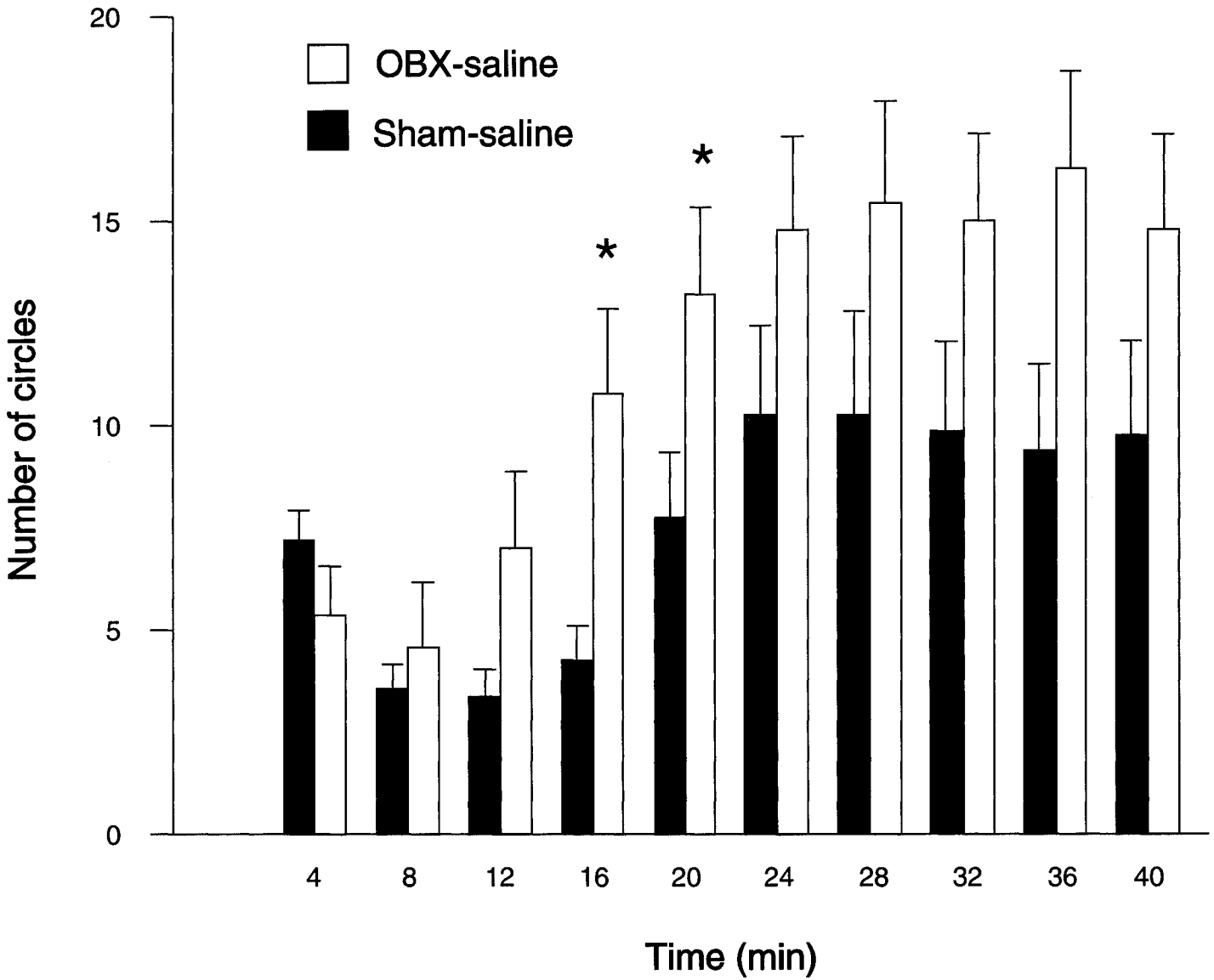
20
15
10
5
0
Number of circles
OBX-saline
Sham-saline
*
*
4
8
12
16
20
24
28
32
36
40
Time (min)

Figure 2

Figure 3. Mean (± S.E.M.) number of head weavings (A) or fall over (B) behaviours recorded in SHAM saline versus OBX saline rats observed for a period of 20 minutes, starting 8 minutes following the injection of dizocilpine (200 μg/kg). *$P<0.05$ Student's t-test. In this and the following figures the numbers at the bottom of the columns indicate the number of rats observed.

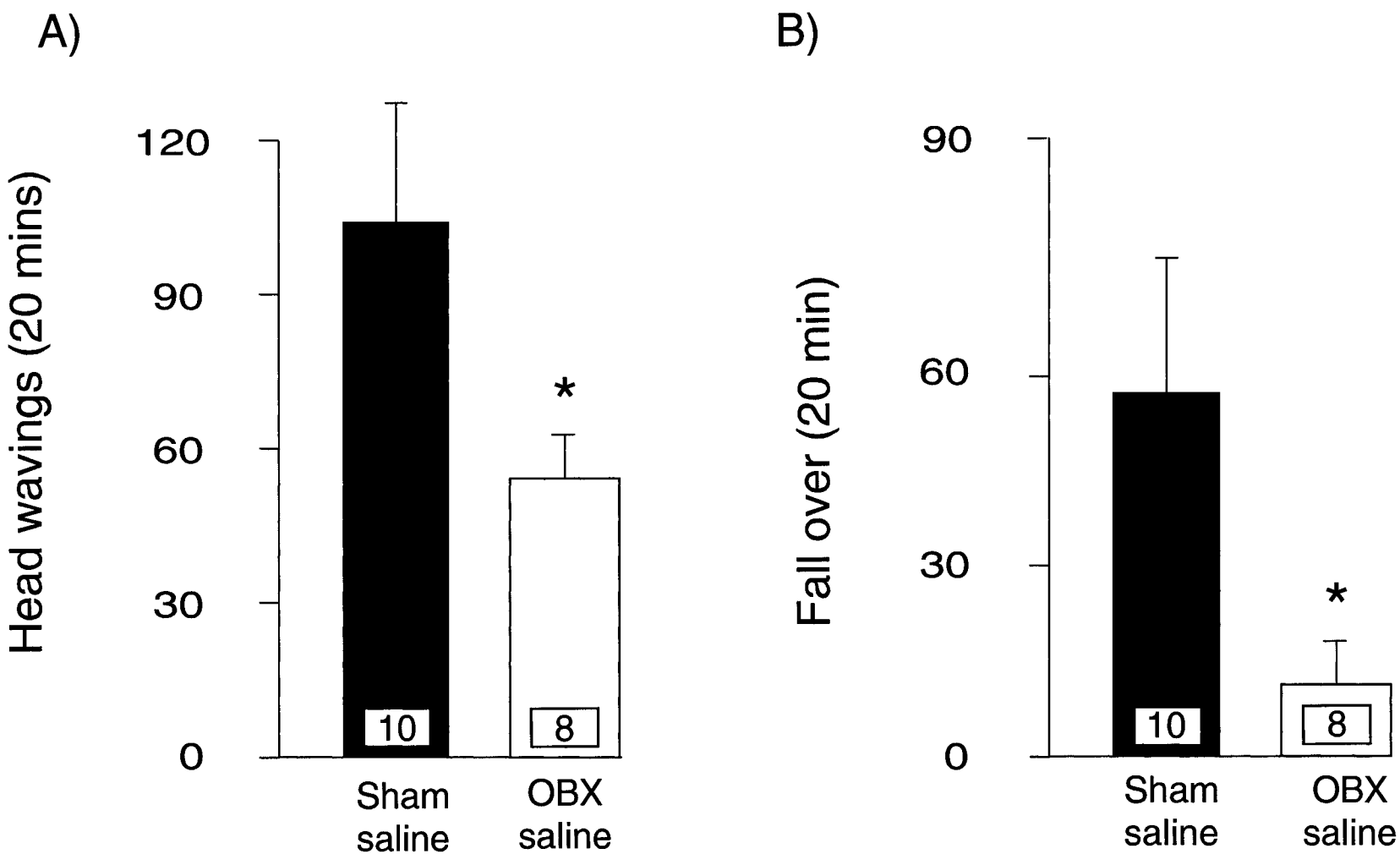
A)
Head wavings (20 mins)
120
90
60
30
0
*
10
8
Sham saline
OBX saline
B)
Fall over (20 min)
90
60
30
0
*
10
8
Sham saline
OBX saline

Figure 3

Figure 4. Ambulatory distance traveled by "SHAM-saline", "OBX-saline" and OBX rats treated for A) 7 days or B) 14 days with igmesine. Values represent the mean (± S.E.M.) distance expressed in cm/min measured during the 40 minutes duration of the experiment, following dizocilpine injection (200 μg/kg). *$P<0.05$ Student's t-test vs. "SHAM-saline"

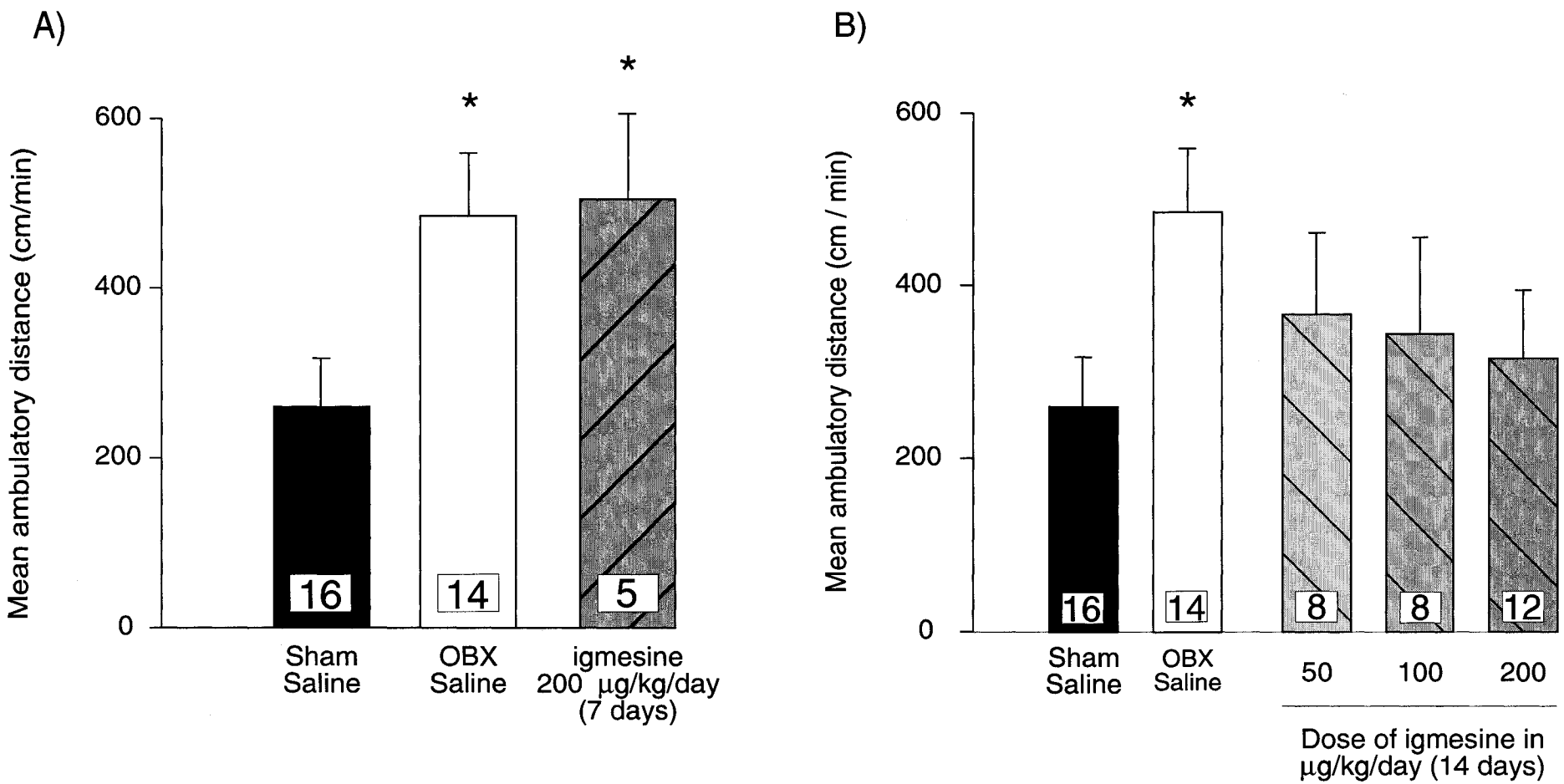
A)
Mean ambulatory distance (cm/min)
600
400
200
0
*
*
16
14
5
Sham Saline
OBX Saline
igmesine 200 µg/kg/day (7 days)
B)
Mean ambulatory distance (cm / min)
600
400
200
0
*
16
14
8
8
12
Sham Saline
OBX Saline
50
100
200
Dose of igmesine in µg/kg/day (14 days)

Figure 4

Figure 5. Mean (± S.E.M.) number of circling behaviours observed in "SHAM-saline", "OBX-saline", or OBX rats treated with 50-200 µg/kg/day of igmesine for 14 days, following the acute administration of dizocilpine (200 µg/kg i.p.) administered at time 0. *P<0.05 Student's t-test vs. "SHAM-saline".

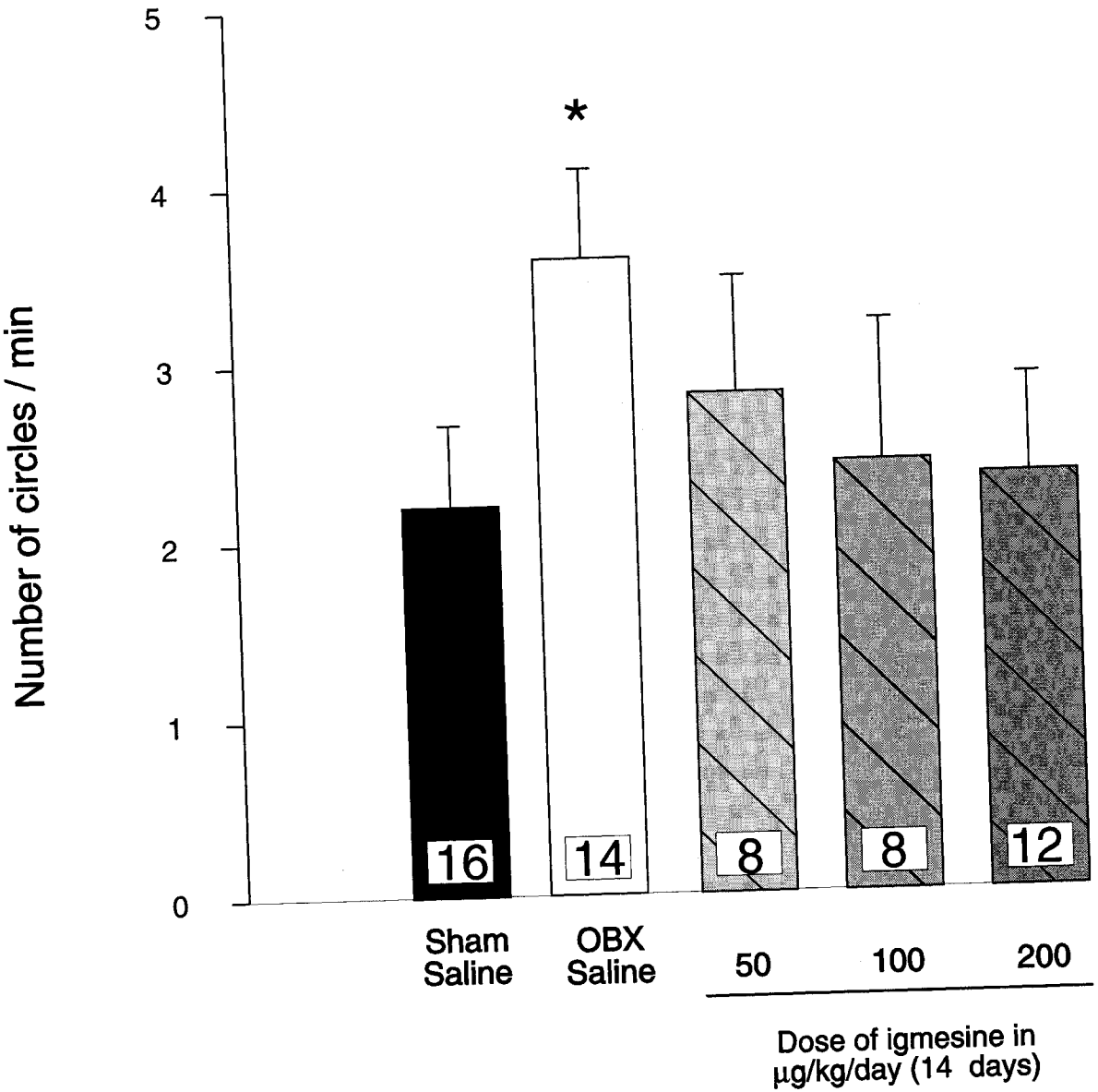
Number of circles / min
5
4
3
2
1
0
*
16
14
8
8
12
Sham Saline
OBX Saline
50
100
200
Dose of igmesine in µg/kg/day (14 days)

Figure 5

Figure 6. Mean (± S.E.M.) number of head wavings (A) or fall over behaviours (B) recorded in "OBX-saline", or OBX rats treated with 200 µg/kg/day of igmesine for 14 days. Rats were observed for a period of 20 minutes, starting 8 minutes following the injection of dizocilpine (200 µg/kg i.p.). *P<0.05 Student's t-test.

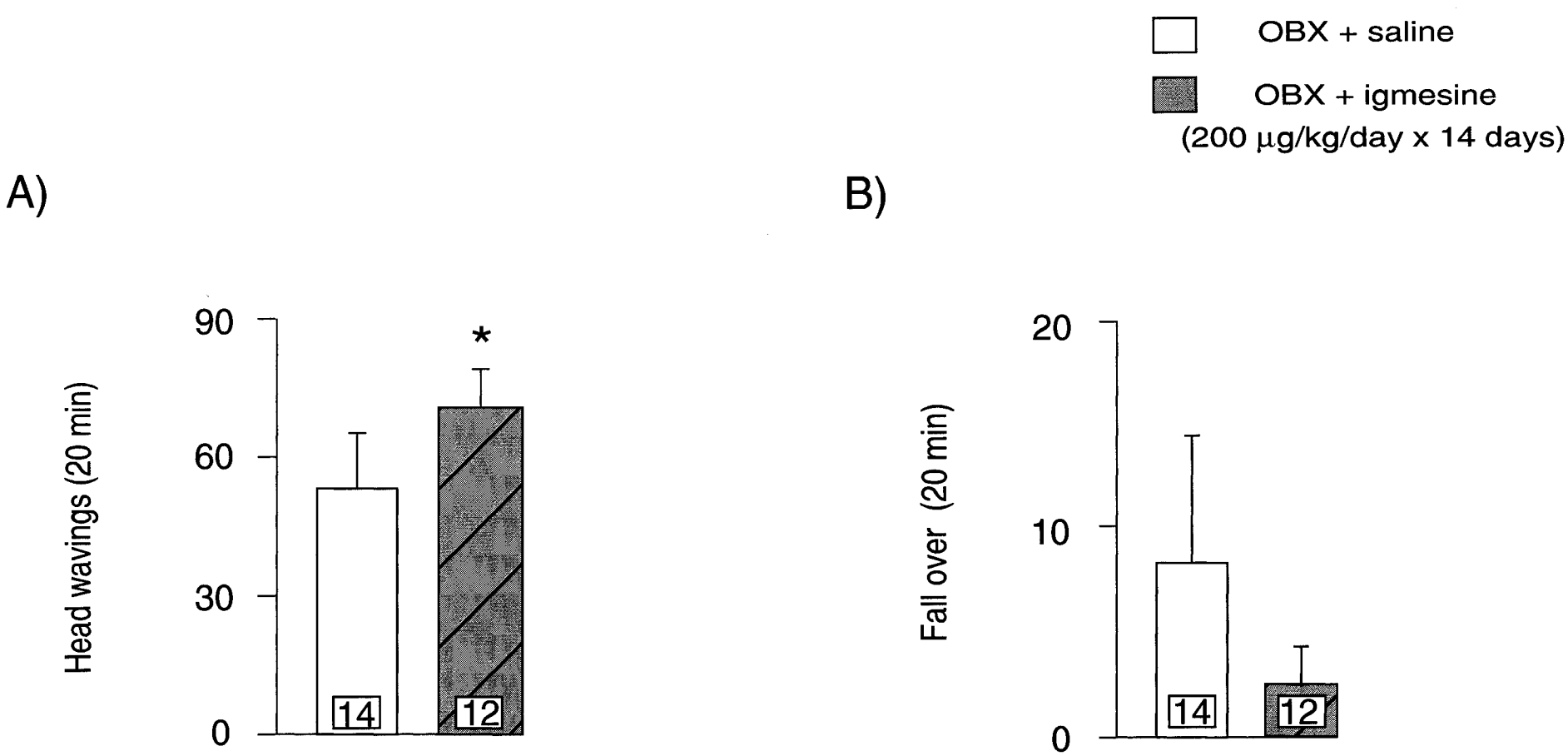
OBX + saline
OBX + igmesine
(200 μg/kg/day x 14 days)
A)
B)
Head wavings (20 min)
90
60
30
0
*
14
12
Fall over (20 min)
20
10
0
14
12

Figure 6

Figure 7. Ambulatory distance traveled by "SHAM-saline", "OBX-saline", or OBX rats treated with 500 or 1000 μg/kg/day of igmesine for A) 7 days or B) 14 days. Values represent the mean (± S.E.M.) distance expressed in cm/min measured during the 40 minutes duration of the experiments, following injection of dizocilpine (200 μg/kg i.p.). *P<0.05 Student's t-test vs. "SHAM-saline",

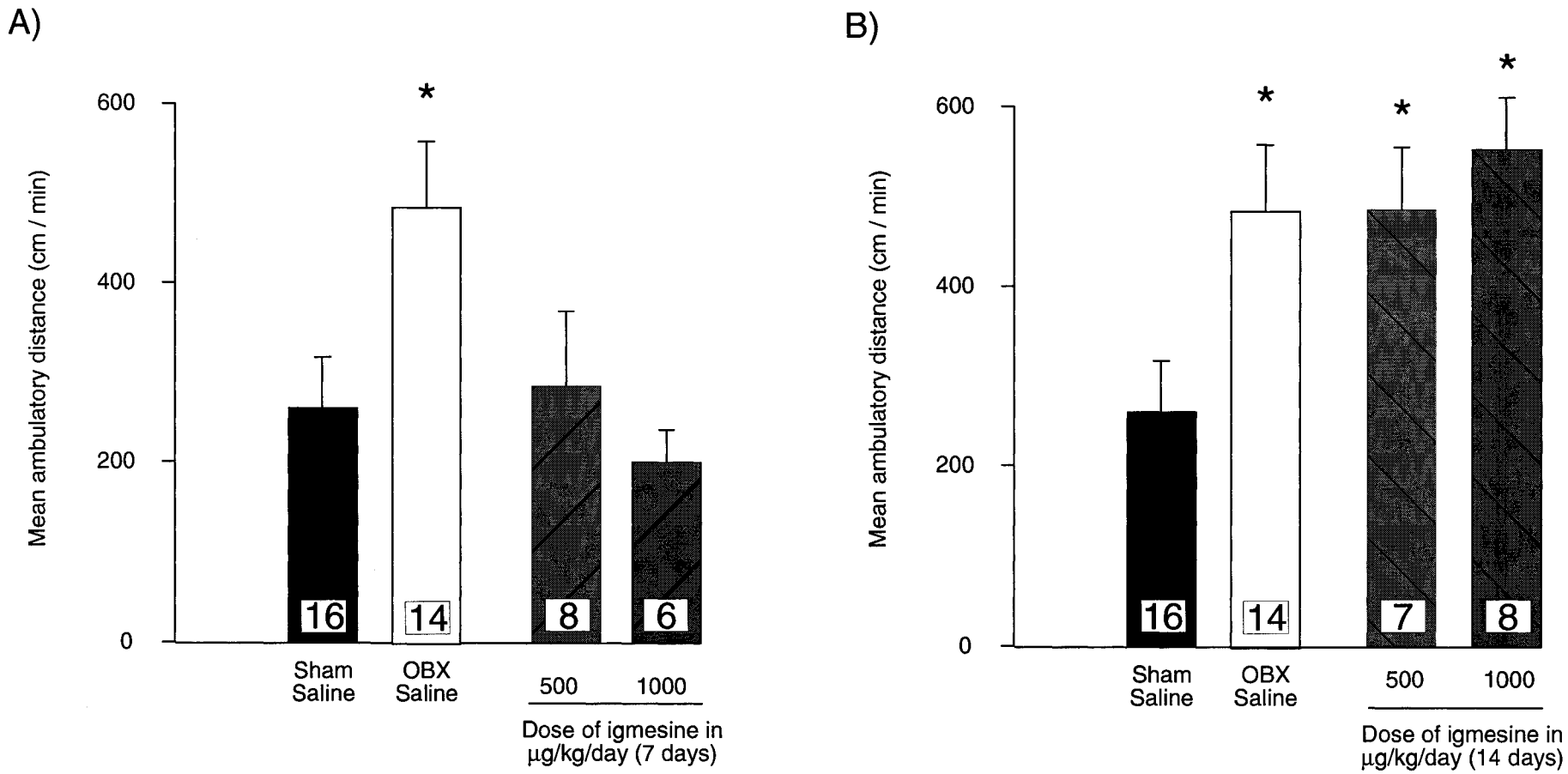
A)
Mean ambulatory distance (cm / min)
600
400
200
0
*
16
14
8
6
Sham Saline
OBX Saline
500
1000
Dose of igmesine in µg/kg/day (7 days)
B)
Mean ambulatory distance (cm / min)
600
400
200
0
*
*
*
16
14
7
8
Sham Saline
OBX Saline
500
1000
Dose of igmesine in µg/kg/day (14 days)

Figure 7

Figure 8. Mean (± S.E.M.) number of circling behaviours recorded in "SHAM-saline", "OBX-saline", or OBX rats treated with 500 or 1000 µg/kg/day of igmesine for 14 days, following the acute administration of dizocilpine (200 µg/kg i.p.) administered at time 0. * $P<0.05$ Student's t-test vs. "SHAM-saline".

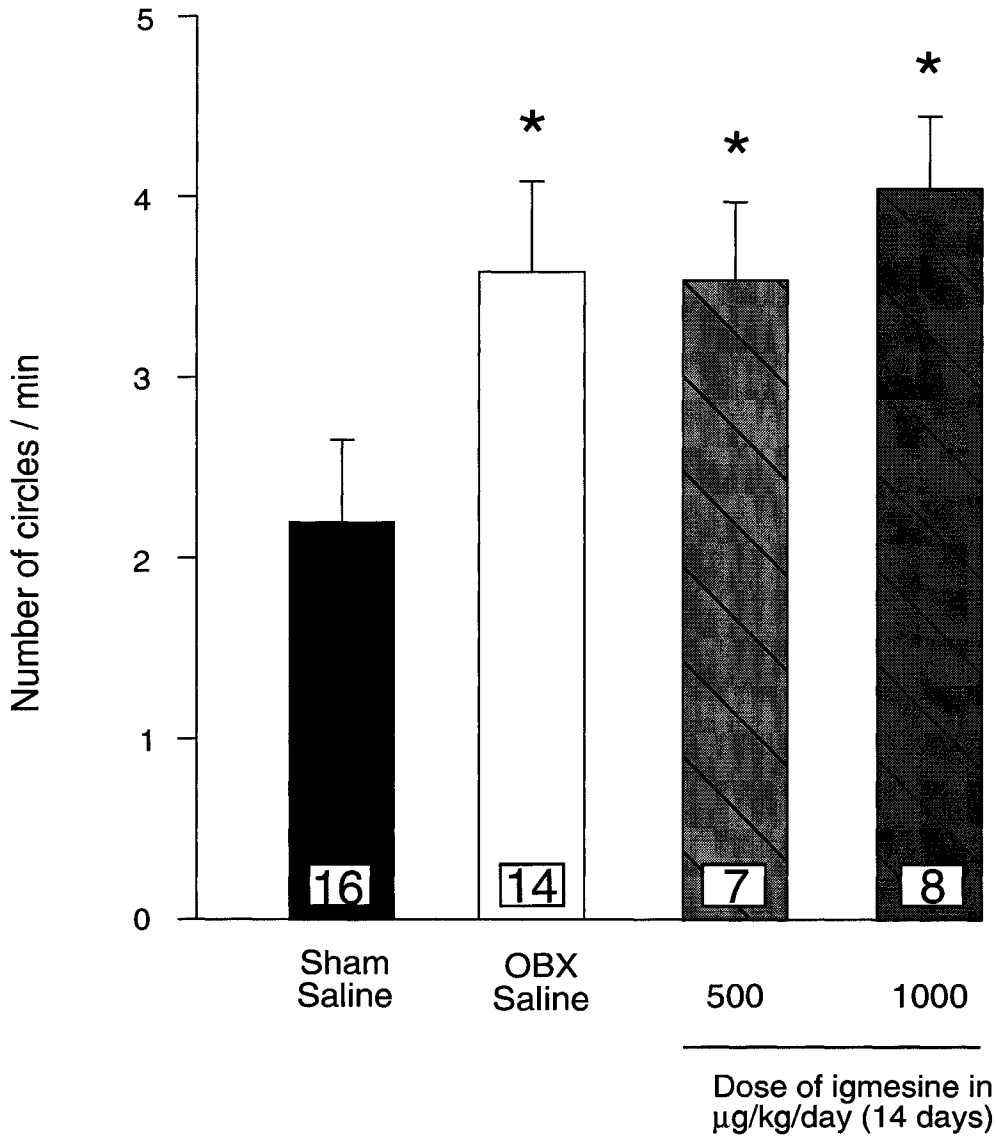
5
4
3
2
1
0
Number of circles / min
*
*
*
16
14
7
8
Sham
Saline
OBX
Saline
500
1000
Dose of igmesine in
µg/kg/day (14 days)

Figure 8

Figure 9. Ambulatory distance traveled by "SHAM-saline", "OBX-saline" and SHAM rats treated with igmesine for A) 7 days or B) 14 days. Values represent the mean (± S.E.M.) distance expressed in cm/min measured during the 40 minutes duration of the experiments, following the injection of dizocilpine (200 µg/kg i.p.).

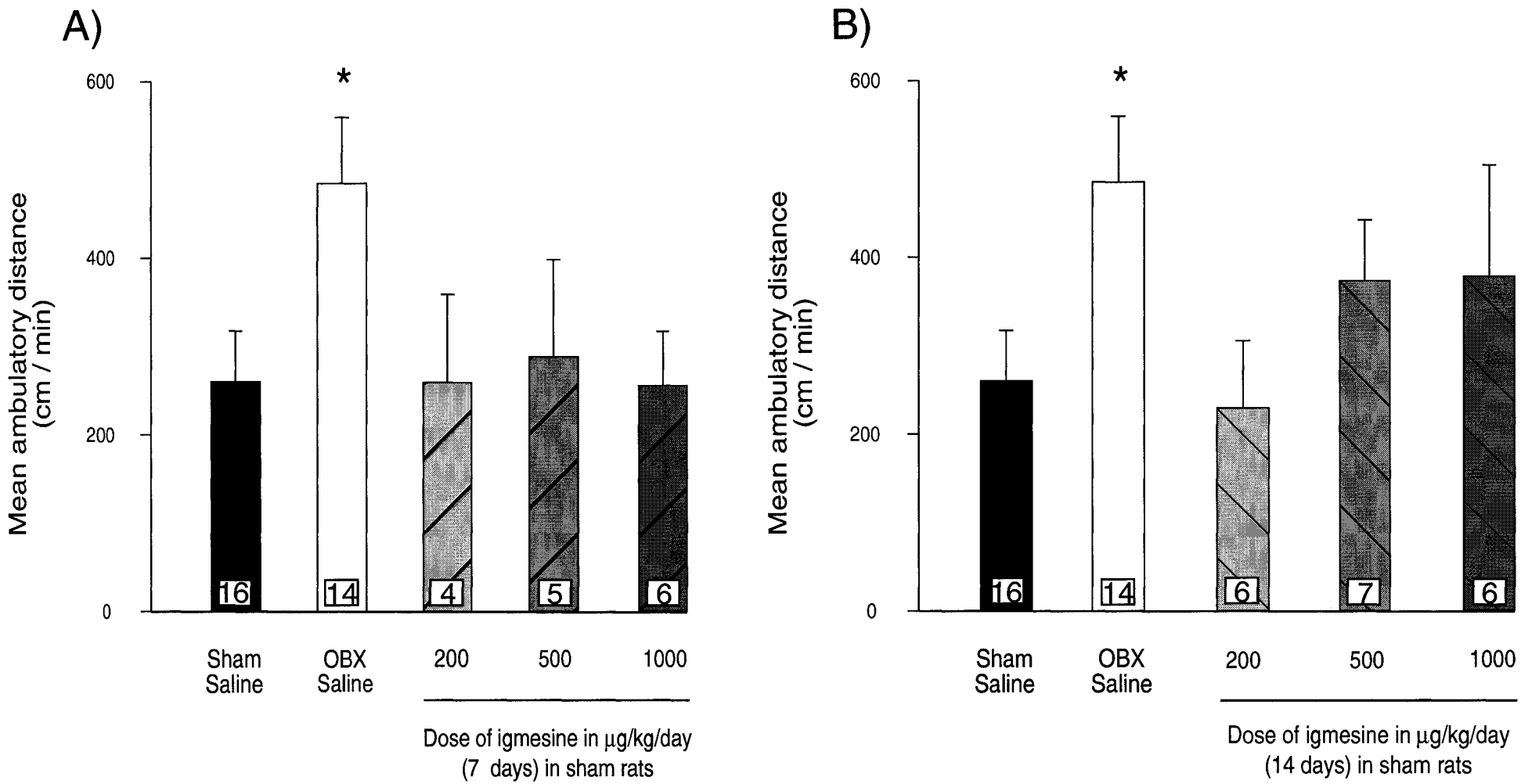
A)
600
400
200
0
Mean ambulatory distance
(cm / min)
*
16
14
4
5
6
Sham
Saline
OBX
Saline
200
500
1000
Dose of igmesine in µg/kg/day
(7 days) in sham rats
B)
600
400
200
0
Mean ambulatory distance
(cm / min)
*
16
14
6
7
6
Sham
Saline
OBX
Saline
200
500
1000
Dose of igmesine in µg/kg/day
(14 days) in sham rats

Figure 9

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Chapter 3

Modulation of serotonergic neurotransmission by short- and long-term treatments with sigma ligands

Chapter II demonstrated that, in addition to their "antidepressant-like" effects in acute models, sigma ligands also have "antidepressant-like" effects in animal models sensitive to long-term treatments. Furthermore, our first study suggested that the "antidepressant-like" effects of sigma ligands may involve a modulation of NMDA receptor-mediated transmission.

The majority of research on antidepressants has focused on the monoaminergic systems. As discussed in the Introduction, serotonin neurotransmission is particularly implicated in the pathophysiology of depression as well as in the mechanism of action of antidepressants. Our laboratory has done extensive electrophysiological studies showing that antidepressants, following chronic treatments, through various mechanisms, induce an increase in 5-HT neurotransmission. Given that sigma ligands are being investigated as potential antidepressants, it begs the question, as to whether sigma receptors can modulate 5-HT neurotransmission, an interaction yet to be investigated.

The *in vivo* electrophysiological paradigm of extracellular unitary recording from the DRN used in our laboratory is ideal for investigating whether sigma ligands can modulate 5-HT neurotransmission. The current study focuses on the effects of short- and long-term treatments with various sigma ligands on the average firing activity of DRN 5-HT neurons. We focus on the DRN because antidepressants such as SSRI's and MAOI's have been shown to induce an initial decrease in the firing activity of DRN 5-HT neurons after 2 days of treatment.

However, after chronic treatments, these antidepressants restore the firing activity of 5-HT neurons to its baseline level. Thus, it was important to identify what effects, if any, sigma ligands would have on the firing activity of 5-HT neurons and in what time frame. These data would provide a possible link between the sigma receptor and 5-HT system and provide another putative mechanism of action for the observed "antidepressant-like" effects of sigma ligands in behavioural models of depression and antidepressant activity.

MODULATION OF SEROTONERGIC NEUROTRANSMISSION BY SHORT- AND LONG-TERM TREATMENTS WITH SIGMA LIGANDS

J.E. Bermack & G. Debonnel

British Journal of Pharmacology 134: 691-699, 2001

Summary

1. Sigma receptors were first described in 1976 as opiate receptors but were later determined to be a distinct class of receptors with two subtypes, $sigma_1$ and $sigma_2$. Although the endogenous ligand is yet to be elucidated, the $sigma_1$ receptor has recently been cloned.
2. Behavioural models used to test potential antidepressants have shown sigma ligands to produce antidepressant effect but their mechanism of action is unknown.
3. The goal of the present study was to assess the effects of various $sigma_1$ ligands on the firing activity of serotonin (5-HT) neurons of the dorsal raphe nucleus (DRN) using extracellular *in vivo* recordings in anaesthetized rats.
4. The $sigma_1$ ligands (+)-pentazocine and 4-(N-benzylpiperidin-4-yl)-4-iodobenzamide (4-IBP) (2 mg $kg^{-1}day^{-1}$) increased markedly 5-HT firing activity after 2 days of treatment and maintain the same increased firing rate after long-term (21 days) treatments. Furthermore, the increased firing rate produced by 2 and 21 day treatment with (+)-pentazocine was prevented by the co-administration of N,N-dipropyl-2-(4-methoxy-3-(2-

phenylethoxy)phenyl)-thylamine (NE-100) (10 mg $kg^{-1}day^{-1}$) a selective $sigma_1$ antagonist, confirming the $sigma_1$ receptor's modulation of these effects. In contrast, the $sigma_1$ ligands (+)-N-cyclopropylmethyl-N-methyl-1,4-diphenyl-1-1-ethyl-but-3-en-1-ylamine hydrochloride (JO-1784) and 2-(4-morpholinoethyl 1-phenyl-cyclohexane-1-carboxylate hydrochloride (PRE-084) had no effect.

5. Following a 21-day treatment with (+)-pentazocine there was a marked reduction in the number of neurons found per track. This decrease was not seen after chronic treatment with 4-IBP and may represent a depolarization block.
6. These results suggest a modulation of serotonergic neurotransmission by some sigma receptors and provide a potential mechanism for the "antidepressant effects" reported and provide evidence toward $sigma_1$ ligands as potential antidepressants with a rapid onset of action.

Abbreviations: 4-IBP- 4-(N-benzylpiperidin-4-yl)-4-iodobenzamide, 5-HT-serotonin, 8-OH-DPAT- 8-hydroxy-2-(di-n-propylamino)tetralin, AF-DX116- 11-[[2-[(diethylamino)methyl]-1-piperidinyl]acetyl]-5-11-dihydro-6H-pyrido[2,3-6][1,4]benzodiazepine-6-one, BD-737- (+)-cis-N-[2,(3,4-dichlorophenyl)ethyl]-2-(1-pyrrolidinyl-cyclohexlamine, BMY-14802- α-(4-fluorophenyl)-4-(5-fluoro-2-pyrimidinyl)-1-piperazinebutanol, DRN- dorsal raphe nucleus, DTG- 1,3-di-(2-tolyl)guanidine, i.p.- intraperitoneal, GABA-γ-aminobutyric acid, JO-1784-(+)-N-cyclopropylmethyl-N-methyl-1,4-diphenyl-1-1-ethyl-but-3-en-1-ylamine

hydrochloride, L 687-384- 1-benzylspiro[1,2,3,4-tetrahydronaphthalene-1,4-piperidine, MAOI-monoamine oxidase inhibitor, MK-801 (dizocilpine)- (+)-5-methyl-10,11-dihydro-5H-dibenzo(a,d)cyclohepten-5-10-imine maleate, NE-100- N,N-dipropyl-2-(4-methoxy-3-(2-phenylethoxy)phenyl)-thylamine, NMDA-N-methyl-D-aspartate, OPC-14523- 1-[3-[4-(3-chlorophenyl)-1-piperazinyl]propyl]-5-methoxy-3,4-dihydro-2-quinolinone monomethanesulfonate, PRE-084- 2-(4-morpholinoethyl 1-phenyl-cyclohexane-1-carboxylate hydrochloride, SA-4503- 1-(3,4-dimethoxyphenethyl)-4-(3-phenylpropyl) piperazine dihydrochloride, SCH-50911- (25)(+)-5,5-dimethyl-2-morpholineacetic acid, SSRI- selective serotonin reuptake inhibitor, (+)SKF-10,047- (+)-N-allyl-normetazocine.

Introduction

Sigma receptors were first described by Martin et al., in 1976 as a subtype of opiate receptors. They were later distinguished from all known mammalian receptors by the development of selective sigma ligands and classified into $sigma_1$ and $sigma_2$ subtypes (Quirion et al., 1987, 1992). Their endogenous ligand is not known but the endogenous steroid progesterone has high affinity for $sigma_1$ receptors (Su et al., 1988). Many selective $sigma_1$ ligands have been synthesized including (+)-pentazocine, 4-IBP, (+)-N-cyclopropylmethyl-N-methyl-1,4-diphenyl-1-1-ethyl-but-3-en-1-ylamine hydrochloride (JO-1784) and 2-(4-morpholinoethyl 1-phenyl-cyclohexane-1-carboxylate hydrochloride (PRE-084). Recently, the $sigma_1$ receptor was cloned and found to be different from all known mammalian receptors (Hanner et al., 1996).

Our laboratory previously used an electrophysiological model to differentiate between sigma agonists and antagonists. Specifically, it was demonstrated that sigma ligands modulate NMDA (N-methyl-D-aspartate) receptors such that low doses (0.5-50μg) of sigma ligands have no effect on the spontaneous firing activity of hippocampal CA_3 neurons but dose-dependently and selectively modulate the response to NMDA (Monnet et al. 1990). In this model, sigma agonists (1,3-di-(2-tolyl)guanidine (DTG), (+)-pentazocine, JO-1784) potentiate the NMDA response and $sigma_1$ antagonists (N,N-dipropyl-2-(4-methoxy-3-(2-phenylethoxy)phenyl)-thylamine (NE-100), progesterone, haloperidol) have no effect on their own but block the effects of sigma agonists (Monnet et al., 1990, 1992 Bergeron et al., 1996).

Sigma$_1$ receptors have many potential functions one of which could be a role in the pathophysiology of depression or as antidepressants. Several sigma ligands have been shown to have antidepressant effects in behavioural models of depression such as the tail suspension and forced swimming tests (Matsuno et al., 1996, Tottori et al., 1997, Kinsora et al., 1998, Ukai et al., 1998). In addition, representatives from all classes of antidepressants have been shown to interact with sigma receptors (Bergeron et al., 1993, Narita et al., 1996, Shirayama et al., 1993). An enourmous corpus of evidence suggests the involvement of serotonin in the pathophysiology of depression (Delgado, 2000). Just as an example, electrophysiological studies have demonstrated that all long-term treatments with antidepressants, through various mechanisms, increase 5-HT neurotransmission (Chaput et al., 1991, Blier and de Montigny, 1994).

The purpose of this study was to assess the effect of short and long-term treatments with several sigma$_1$ ligands on serotonergic neurotransmission using an electrophysiological model of extracellular recordings of the firing rate of serotonin (5-HT) neurons from the dorsal raphe nucleus (DRN). Previous results using this model, demonstrated that acute and short-term treatments with SSRI's lead to a decreased firing activity of 5-HT DRN neurons, while long-term treatments lead to the restoration of 5-HT firing activity (Chaput et al., 1986, Blier et al., 1984, Blier & de Montigny, 1985). Therefore, we investigated the effects of short-term (2 days) and long-term (21 days) treatments with sigma$_1$ ligands to assess their effects on basal 5-HT firing rate.

Materials and Methods

Animals

Experiments were performed in male Sprague-Dawley rats (Charles River, St. Constant, Québec) weighing 250-300 g. They were housed under standard laboratory conditions including a 12-12h light-dark cycle with free access to food and water.

Treatments

For short-term treatments, rats 250-275g were anaesthetized with halothane and osmotic minipumps (ALZA Corporation, Palo Alto, CA, USA) were implanted subcutaneously. Minipumps contained either JO-1784, PRE-084, (+)-pentazocine, 4-IBP (4-(N-benzylpiperidin-4-yl)-4-iodobenzamide) (all 2 mg $kg^{-1}day^{-1}$) or saline for controls (50% saline, 50% dimethylsulfoxide for 4-IBP controls). A separate series of rats were implanted with 2 osmotic minipumps simultaneously, one containing (+)-pentazocine (2 mg kg^{-1} day^{-1}) and the other containing NE-100 (10 $mgkg^{-1}$ day^{-1}) for 2 days.

For long-term treatments, rats 125-150g were anaesthetized and implanted in a similar fashion to that done for 2-day treatments. Pumps contained either (+)-pentazocine, JO-1784 or 4-IBP (all 2 mg kg^{-1} day^{-1}) or saline for controls (50% saline 50% DMSO for 4-IBP controls). In addition, a separate series of rats were implanted with 2 osmotic minipumps simultaneously, one containing (+)-pentazocine (2 mg kg^{-1} day^{-1}) and the other containing NE-100 (10 mg kg^{-1} day^{-1}) for 21 days. The electrophysiology experiments were performed with the minipumps on board.

Electrophysiology

The experiments were performed on rats anesthetized with choral hydrate (400 mg kg^{-1} intraperitoneal (i.p.)) and mounted in a stereotaxic apparatus. Supplemental doses of choral hydrate (100 mg kg^{-1} i.p.) were administered as necessary to prevent any nociceptive reaction to pinching of the hind paw. The rat's body temperature was maintained at approximately 37°C by a thermistor-controlled heating pad.

Extracellular unitary recording of DRN 5-HT neurons was obtained with single-barelled glass micropipettes pulled in a conventional manner (Haigler & Aghajanian, 1974) with the tips broken back 1-3 μm and filled with 3% fast green solution. Electrode impedance ranged between 2 and 4 MΩ. A burr hole 4 mm in diameter was drilled 1 mm anterior to lambda on the midline. The electrode was then lowered along descents covering the DRN from 300 μm to approximately 1500 μm anterior of lambda. Spontaneously firing DRN 5-HT neurons were identified by their characteristic slow and regular rhythmical firing (Aghajanian & Vandermaelen, 1982). Following the experiments each rat was sacrificed with an intravenous injection of air (1 ml).

Data Collection

For each treatment group, the mean DRN 5-HT basal firing rate was determined by averaging the firing rate of all the neurons measured in the population (treatment). Each neuron was recorded for 90 seconds, and 5 descents

were performed per rat in the DRN of 3-6 rats with the total number of neurons averaged being greater than 40. Student's paired t-tests were done comparing treatments to controls using the program Sigmaplot 4.0. A value was considered significant if $P<0.05$.

Drugs

The following substances were used: JO-1784 (a gift from F. Roman, Institut de Recherche Jouveinal, Fresnes, France), (+)-pentazocine, 8-hydroxy-2-(di-n-propylamino)tetralin(8-OH-DPAT), (-)bicuculline methiodide (RBI Pharmaceuticals, Natick, MA, USA), PRE-084 (a gift from Dr. T-P. Su, NIDA/NIH, Baltimore, Maryland), NE-100 (a gift from Taisho Pharmaceutical Co.Ltd. Tokyo, Japan), 4-IBP and R(+)baclofen (Tocris Cookson Inc. Ballwin, MO, USA).

Results

The doses used in the present series of experiments were chosen according to data obtained previously. We have shown that doses of (+)-pentazocine and JO-1784 between 500-3000 $\mu g\ kg^{-1}$ induced the maximal agonistic effect on the potentiation of the NMDA response (Monnet et al., 1990, 1992). The same doses of PRE-084 and 4-IBP were used since these molecules possess very high affinity for $sigma_1$ receptors, similar to that of (+)-pentazocine and JO-1784 (Su et al., 1991, John et al., 1994, Steinfels et al., 1988, Roman et al., 1990).

In control animals, 5-HT neurons were encountered, starting at a depth of 5033 μm with an average of 2.8 neurons per track and a firing activity of 1.0 Hz. Figure 1 depicts a representative tracing of serotonergic neurons recorded in the DRN along a descent. In this example, the tracing is from a control rat or one treated for 2 days with (+)-pentazocine (2 $mg\ kg^{-1}\ day^{-1}$) showing an increased firing rate.

Treatment with JO-1784

The short-term (2 days) administration of JO-1784 (2 $mg\ kg^{-1}\ day^{-1}$) produced no significant change in the basal firing rate of DRN 5-HT neurons (Figure 2). In addition, a 21-day administration of the same dose of JO-1784 also did not affect the average firing rate of the 5-HT neurons of the DRN (Figure 2).

Treatment with PRE-084

The 2-day administration of PRE-084 (2 mg kg^{-1} day^{-1}) produced no significant change in the basal firing rate of the 5-HT neurons of the DRN (Figure 2).

Treatment with 4-IBP

A 2-day treatment with 4-IBP (2 mg kg^{-1} day^{-1}) produced a 35% increase in the basal firing rate of 5-HT neurons compared to saline-treated animals ($p=0.002$)(Figure 3). Furthermore, a 21-day treatment with 4-IBP maintained a 36% increase in firing rate as seen after 2 days (Figure 3). The co-administration of NE-100 (10 mgkg-1day-1) with 4-IBP (2 mgkg-1day-1) for 2 days did not modify the effect of 4-IBP and a significant increase in the average firing activity of 5-HT neurons was observed (Figure 3).

Treatment with (+)-pentazocine

As illustrated in figure 1, a 2-day treatment with (+)-pentazocine (2 mg $kg^{-1}day^{-1}$) produced a 33% increase in the basal firing rate compared to saline-treated rats ($p=0.001$) (Figure 4). Co-administration of NE-100 (10 mg kg^{-1} day^{-1}) with (+)-pentazocine (2 mg kg^{-1} day^{-1}) for 2 days completely prevented the increase of 5-HT firing activity caused by a 2-day treatment with (+)-pentazocine (Figure 4). (+)-Pentazocine treatment (2 mg kg^{-1}) for 21 days maintained a 43% increase in basal firing rate compared to saline-treated rats (Figure 5). Similarly, co-administration of NE-100 (10 mg kg^{-1} day^{-1}) with (+)-pentazocine (2 mg kg^{-1} day^{-1}) for 21 days produced no significant change in average firing rate nor neurons found per track compared to controls (Figure 5 and 6).

Neurons found per track following long-term treatments

As shown in Figure 6, in rats treated for 21 days with (+)-pentazocine (2 mg kg^{-1} day^{-1}), 94% less neurons were encountered per track. In rats treated for 21 days with 4-IBP there was no significant difference in the amount of neurons found per track compared to saline-treated rats (Figure 7). Various durations of treatment with (+)-pentazocine (2 mg kg^{-1} day^{-1}) did not significantly change the amount of neurons found per track versus controls until Day 21. Specifically, following 5- and 10-day treatments, increased firing rates were maintained (40% and 27% respectively) compared to controls, without decreasing the number of neurons found per track (Figures 5 and 6). In addition, when treated with a lesser dose of (+)-pentazocine (0.5 mg kg^{-1} day^{-1}) no change was seen for neurons yielded per track nor average firing rate compared to controls (Figure 8). To investigate the nature of the finding of decreased neurons per track, we injected animals treated with (+)-pentazocine for 21 days (2 mg kg^{-1} day^{-1}) with 8-OH-DPAT (4 μg kg^{-1} i.v.), bicuculline (375 μg kg^{-1} i.v.) or (+)baclofen (5-15 mg kg^{-1} i.v.). These different approaches did not restore the amount of neurons found per track to that of saline-treated rats (8-OH-DPAT 0.0 ± 0, bicuculline 0.2 ± 0.2 and baclofen 0.63 ± 0.24 versus (+)-pentazocine 0.41 ± 0.16, not significant).

Discussion

4-IBP is a selective sigma ligand with a high affinity for the $sigma_1$ receptor (K_i= 1.7 nM) and moderate affinity for the $sigma_2$ receptor (K_i=25.2 nM) (John et al., 1994). Short-term treatments with 4-IBP (2 mg kg^{-1} day^{-1}) for 2 days, produced a significant 35% increase in the basal firing rate of DRN 5-HT neurons (Figure 3). Similarly, the selective $sigma_1$ ligand (+)-pentazocine produced a 33% increase in the firing activity of 5-HT neurons of the DRN (Figures 1,4). This increase was not seen after treatment with the selective $sigma_1$ ligands PRE-084 and JO-1784 as their firing rates did not differ significantly from controls (Figure 2).

The increased firing rates observed after both short- and long-term treatments with (+)-pentazocine were completely prevented by co-administration with NE-100 (10 mg kg^{-1} day^{-1}), a selective $sigma_1$ antagonist (Figures 4 and 5). This confirms that the modulation of serotonergic firing activity demonstrated here is indeed mediated by $sigma_1$ receptors. However, as shown if figure 3, when NE-100 (10 mg kg^{-1} day^{-1}) was co-administered with 4-IBP for 2 days, the increase in the firing activity of the 5-HT neurons which was induced by 2 day treatments with 4-IBP was not prevented. Thus, the average firing activity remained significantly increased versus controls.

Various preclinical results for a variety of sigma ligands have already suggested that these compounds could produce antidepressant effects. Specifically, the sigma ligands 1-(3,4-dimethoxyphenethyl)-4-(3-phenylpropyl) piperazine dihydrochloride (SA-4503), (+)-pentazocine, DTG, JO-1784 and 1-[3-[4-(3-chlorophenyl)-1-piperazinyl]propyl]-5-methoxy-3,4-dihydro-2-quinolinone

monomethanesulfonate (OPC-14523) dose-dependently decreased immobility in mice in the forced swimming test and this decrease was antagonized by pretreatment with the sigma antagonist NE-100 (Matsuno et al., 1996, Tottori et al., 1997, Kinsora et al., 1998). In keeping with these data, the acute administration of SA-4503 and (+)-pentazocine decreased immobility in mice exposed to the tail suspension test, at doses that failed to influence motor activity and these effects were antagonized by NE-100 (Ukai et al., 1998).

A second line of evidence suggesting sigma receptor's potential involvement in the pathophysiology and/or the treatment of depression comes from many antidepressant's interaction with and/or high affinity for sigma receptors. For example, serotonin (5-HT) reuptake inhibitors (SSRI's) and monoamine oxidase inhibitors (MAOI's) prevent [^{3} H](+)3-PPP binding to sigma receptors in rat and mouse brains (Schmidt et al., 1989, Itzhak & Kassim, 1990). Furthermore, sertraline, an SSRI, and clorgyline, an MAOI, potentiate the NMDA response with a bell-shaped dose response curve, potentiation which are reversed by haloperidol (a $sigma_1$ antagonist). Paroxetine and tranylcypromine, with monoaminergic profiles similar to sertraline and clorgyline except that they are devoid of sigma affinity, did not affect the NMDA response, therefore, indicating that the effects of sertraline and clorgyline were not due to monaminergic effects (Bergeron et al., 1993). Thirdly, in rats, chronic treatments with imipramine or fluoxetine result in a down regulation of sigma receptors in the striatum, hippocampus and cerebral cortex, brain regions implicated in regulation of emotions. This down regulation involves a decrease in B_{max} and depends on

cerebral serotonergic transmission as it was reversed by p-chlorophenylalanine (Shirayama et al., 1993).

The significant increase in the firing activity of 5-HT neurons observed after only 2 days of treatment contrasts what has been seen up to now in electrophysiological studies assessing the effects of antidepressant medications. More specifically, electrophysiological data demonstrate that all antidepressant treatments after chronic treatments, through various mechanisms, increase 5-HT neurotransmission (Chaput et al., 1991, Blier & de Montigny, 1994). For example, acute treatments with MAOI's or SSRI's lead to decreased firing activity of 5-HT neurons in the dorsal raphe nucleus but as treatment continues the 5-HT neurons regain normal firing activity due to desensitization of the 5-HT_{1A} somatodendritic autoreceptors. This desensitization may be an adaptive change that explains the delayed enhancement of 5-HT mediated neurotransmission, which is consistent with the clinical onset of action of SSRI's (Chaput et al., 1986, Blier et al., 1984, Blier & de Montigny, 1985, 1994). In agreement with electrophysiological results, microdialysis experiments show that following a 14-day administration of an SSRI, there is a six fold increase in extracellular 5-HT concentration in the frontal cortex (Bel & Artigas, 1993). Indeed up to now only one antidepressant (mirtazapine) has been shown to induce an increase in the firing activity of DRN neurons following acute and long-term treatments (Haddjeri et al., 1998, Besson et al., 2000). Interestingly, mirtazapine was recently reported as showing a more rapid onset of action of its antidepressant properties (Wheatley & Kremer, 1997). Thus, the present data could suggest that not only sigma agonists might have

antidepressant properties, but also that their onset of action might be more rapid than those of classical antidepressants.

Following 21 days of treatment with both (+)-pentazocine and 4-IBP (2 mg kg^{-1} day^{-1}) the increase in firing activity seen after 2 days persists, suggesting this in not a transient effect. Interestingly, after 21 days of treatment with (+)-pentazocine but not with 4-IBP there was a drastic decrease in neurons found per track (Figure 6). This did not occur after shorter treatments of 10 or 14 days (Figure 6) nor after 21-day treatment with a lower dose of 0.5 mg kg^{-1} day^{-1} of (+)-pentazocine (Figure 7). Furthermore, the co-administration of NE-100 prevented the decreased neurons per track seen after 21 days of treatment with (+)-pentazocine (Figure 6). Therefore, this phenomenon appears to be selective for (+)-pentazocine and specific to long-term treatments over a certain dosage.

One possible explanation for the decrease in the number of neurons found per track after chronic (+)-pentazocine treatment is a decrease of spontaneously active 5-HT neurons, due to a depolarization blockade as seen in the dopaminergic neurons of the midbrain following chronic haloperidol administration (Grace & Bunney, 1986, Hollerman et al., 1992). Thus far, we have first investigated the reality of this potential depolarization blockade by testing if it could be reversed by a 5-HT_{1A} agonist. Following the intravenous administration of 8-OH-DPAT, a 5-HT_{1A} agonist at somatodendritic autoreceptors (Peroutka, 1985), the amount of neurons found per track was not changed. One would expect that the activation of the somatodendritic 5-HT_{1A} autoreceptor by 8-OH-DPAT would reverse a depolarization blockade since it repolarizes the neuron, as the depolarization blockade seen in dopaminergic neurons was

reversed by apomorphine, a dopamine autoreceptor agonist (Grace & Bunney, 1986, Hollerman et al., 1992). Thus, the lack of effect of 8-OH-DPAT suggests, either that the decreased number of neurons per track was not due to a depolarization blockade, or that higher doses of the 8-OH-DPAT were required. However, the latter appears unlikely as we used the dose previously shown to completely suppress 5-HT firing activity in the DRN (Blier et al., 1998). In a second attempt to repolarize the neurons, rats were injected with (+)baclofen (5-15 mg kg^{-1} i.v.), a -γ-aminobutyric $acid_B$ (GABA) agonist, which also, did not restore the number of spontaneously firing neurons suggesting that the silent neurons were not depolarized. However, a lack of repolarizing effect of the $GABA_B$ agonist could not be totally excluded based on recent findings suggesting that, under some circumstances, (+)baclofen might disinhibit DRN 5-HT neurons by preferentially activating $GABA_B$ autoreceptors (Abellan et al., 2000). Therefore, at present the possibility of a depolarization blockade cannot be completely ruled out and we will be further investigating this phenomenon as this would be the first report of such a phenomenon occurring in 5-HT neurons.

A second possible explanation for the decreased number of neurons found per track is an increased endogenous tonic GABA inhibition of the 5-HT neurons of the DRN (Hajos et al., 1999, Abellan et al., 2000). It has been suggested that the inhibitory effect of 8-OH-DPAT on firing activity of DRN neurons involves, in part, the activation of a $5\text{-}HT_{1A}$ receptor-mediated postsynaptic long feedback loop centered on the medial prefrontal cortex (Ceci et al., 1994, Hajos et al., 1999, Casanovas et al., 1999). This inhibition by the prefrontal cortex is thought to

involve activation of GABA interneurons by glutaminergic cortical input (Hajos et al., 1999, Haddjeri et al., 2000, Abellan et al., 2000). To test this possibility we injected (-)bicuculline (375 $\mu g\ kg^{-1}$ i.v.), a $GABA_A$ antagonist, but this did not restore the number of neurons found per track, suggesting overactive GABA tonic inhibition is not responsible.

It has also been shown that in addition to GABAergic modulation of neurons in the long feedback loop cholinergic and glutaminergic systems play key roles. This was demonstrated by the finding that the muscarinic antagonist atropine, the M_2 antagonist 11-[[2-[(diethylamino)methyl]-1-piperidinyl]acetyl]-5-11-dihydro-6H-pyrido[2,3-6][1,4]benzodiazepine-6-one (AF-DX116), the NMDA antagonist (+)-5-methyl-10,11-dihydro-5H-dibenzo(a,d)cyclohepten-5-10-imine maleate (MK-801) and $GABA_B$ antagonist (25)(+)-5,5-dimethyl-2-morpholineacetic acid (SCH-50911) all dampened the suppressant effect of 8-OH-DPAT on the firing activity of DRN 5-HT neurons while (-)bicuculline did not (Haddjeri et al., 2000). Therefore, the possible effect(s) of these other systems on the firing activity of neurons of the DRN and thus the neurons found per track should be assessed especially in light of the known interaction of sigma ligands with NMDA receptors discussed previously. Nevertheless, this dramatic decrease in the number of spontaneously active 5-HT neurons could suggest that the net effect of some sigma ligands will not always be beneficial from an "antidepressant" perspective.

The effects of (+)-pentazocine changed over the duration of the treatments, as shown in Figures 5 and 6. The level of increase in the average firing rate of

DRN 5-HT neurons progressively reduced in parallel to a progressive increase in the number of neurons found per track. We do not have any definite explanation for this phenomenon, however, if one assumes that the decreased number of spontaneously active neurons is due to a depolarization blockade, one possible explanation could be that, at the beginning of the treatment, spontaneously active neurons and silent neurons will see their firing activity progressively increase and then decrease before reaching the final stage of the depolarization blockade. In such a paradigm, Day 14 could represent the time with the maximum number of neurons firing, with some being already on the descending phase of the curve before the depolarization, while the initially silent ones have not yet reached their maximal firing activity. We are currently investigating the potential mechanisms responsible for this phenomenon.

The discrepancy between (+)-pentazocine and 4-IBP producing an increase in firing activity while PRE-084 and JO-1784 did not, is surprising. Firstly, like (+)-pentazocine, JO-1784 was shown to be a sigma agonist in our model of modulation of the NMDA response (Monnet et al., 1992). Secondly, although PRE-084 has not been tested in our model of NMDA modulation, it was found to act as an agonist in several behavioural models of learning and memory deficits. Specifically PRE-084 attenuated MK-801-induce learning impairments in mice similar to $sigma_1$ agonists (+)-pentazocine and (+)-N-allyl-normetazocine ((+)SKF-10,047) and was antagonized by the $sigma_1$ antagonist (BMY-14802) (Maurice et al., 1994a, b). Similarly, treatments with JO-1784 and PRE-084 (0.1-3 mg kg^{-1}) improved learning impairments in a BMY-14802 sensitive manner in senescense accelerated mice (Maurice et al., 1996)

This lack of effect of JO-1784 and PRE-084 on the firing activity of serotonergic neurons of the DRN may be explained by the existence of subtypes of $sigma_1$ receptors, which has been previously suggested by results from our laboratory. Specifically, potentiation of the NMDA response by DTG and JO-1784 is mediated by a subtype of $sigma_1$ receptor linked to a $G_{i/o}$ protein, whereas protentiation induced by (+)-pentazocine is mediated by another subtype of the $sigma_1$ receptor not linked to a $G_{i/o}$ protein, as only this response is pertussis toxin insensitive (Monnet et al., 1993). Furthermore, following colchicine pretreatment, which destroys the mossy fiber system, the neuronal response induce by DTG and JO-1784 was abolished while (+)-pentazocine's effect persisted, indicating the $sigma_1$ receptor subtype mediating (+)-pentazocine's effect is located postsynaptically on pyramidal neurons while the $sigma_1$ receptor subtype mediating DTG and JO-1784's effects is located presynaptically (Debonnel et al., 1996). Further evidence in support of the existence of subtypes of $sigma_1$ receptors was demonstrated recently as the potentiation of the NMDA response by (+)-pentazocine is reversed by naloxone, an opiate antagonist, while the potentiating effects of JO-1784, (+)-cis-N-[2,(3,4-dichlorophenyl)ethyl]-2-(1-pyrrolidinyl-cyclohexlamine (BD 737) and 1-benzylspiro[1,2,3,4-tetrahydronaphthalene-1,4-piperidine (L 687-384) were not (Couture & Debonnel, 2001). Thus, the modulation of serotonergic firing activity seen after a 2 day treatment with (+)-pentazocine and 4-IBP may be mediated by a specific subtype of $sigma_1$ receptor to which (+)-pentazocine and 4-IBP possess high affinity, while JO-1784 and PRE-084 may not.

(+)-Pentazocine and 4-IBP are not likely acting via the same receptors. Evidence for this includes the fact that (+)-pentazocine after chronic treatments induced a decrease in the number of neurons encountered per track while chronic treatment with 4-IBP did not. In addition, (+)-pentazocine's effect of increasing the 5-HT firing activity was reversed by the co-administration of NE-100 while 4-IBP's effect was not. These differences are likely due to effects mediated by different subtypes of the $sigma_1$ receptor. There has been previous evidence of multiple binding sites for (+)pentazocine in addition to the aforementioned results by Couture and Debonnel (2001), for example, saturation studies, in the presence of ions including Zn^{+2}, Ca^{+2}, Mg^{+2} and in Krebs-Ringer buffer have demonstrated multiple (+)-[^{3}H]-pentazocine binding sites in vivo (Basile et al., 1992). Further evidence showed [^{3}H]-pentazocine to label three different sites with different K_d values when various cell lines were tested (Vilner et al. 1995).

It is important to mention that JO-1784 or PRE-084's ability to modulate serontonergic neurotransmission cannot be completely ruled out. Maurice et al. (1994b), has shown that PRE-084 follows a bell-shaped dose-response curve, which has been previously described in the modulation of the NMDA response by sigma ligands, including JO-1784, in the hippocampus (Bergeron et al., 1995). Our doses were chosen based on those shown to produce an optimal response in the modulation of the NMDA response previously tested in our laboratory (Monnet et al., 1990, 1992). Thus, it is indeed possible that the dose of PRE-084 or JO-1784 tested may too low to reach the agonist range, or conversely, it may be at too high and functioning as an antagonist. After chronic treatments with sigma ligands in the NMDA model, our laboratory has shown that low doses of JO-1784

or DTG potentiate the response to NMDA however at high doses they function as antagonists having no effect on its own but blocking the effect of sigma agonists (Bergeron et al. 1997). Thus, the effect of these 2 ligands on serotonergic neurotransmission cannot be completely ruled out until a range of doses is tested.

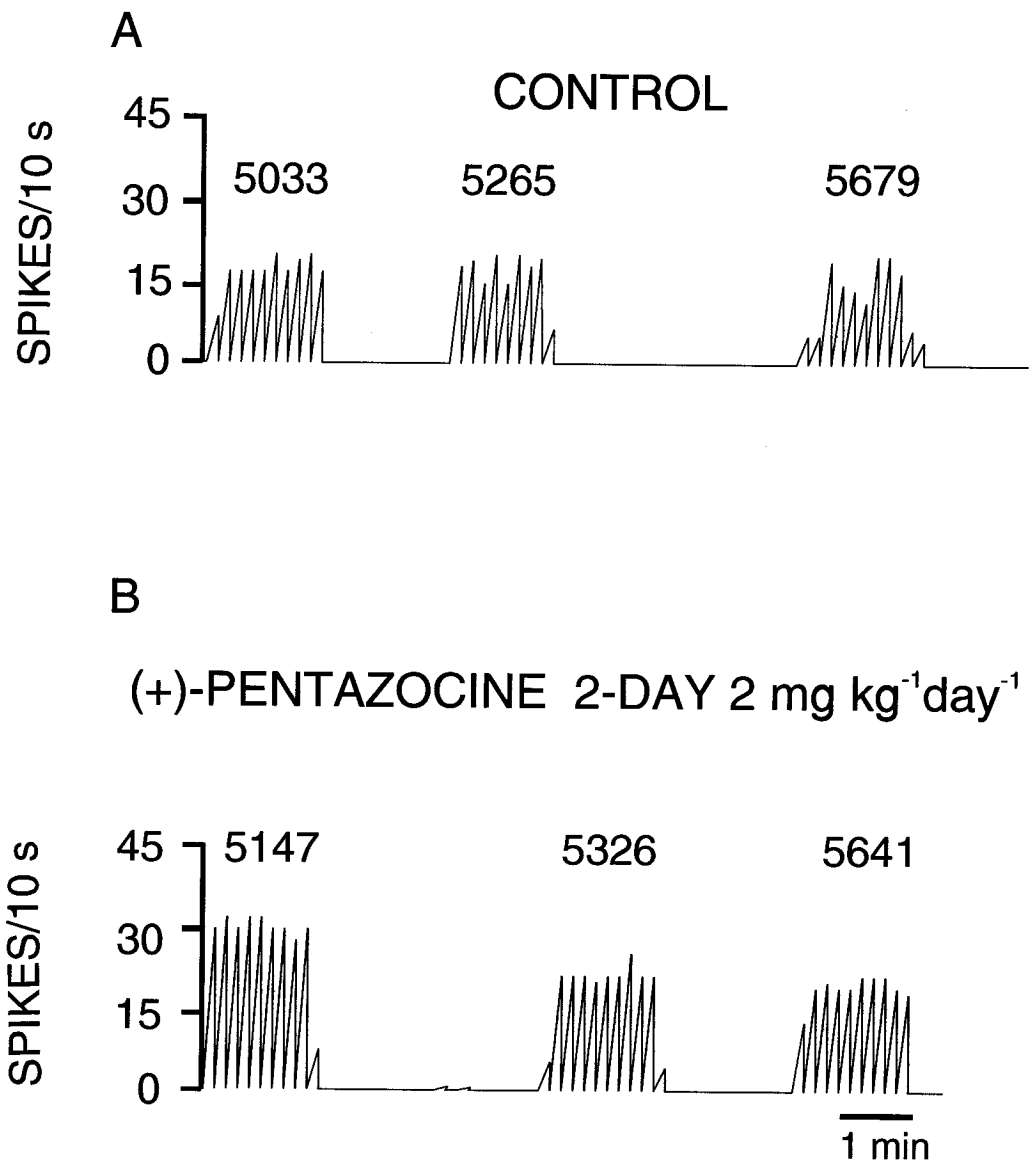
Even if this was not the case, it is also possible that PRE-084 and JO-1784 could possess some antidepressant properties but act via another mechanism. This may involve the modulation of NMDA receptors as other compounds that antagonize NMDA receptors have been shown to produce antidepressant effects in behavioural models of depression (Trullas and Skolnick, 1990, Maj et al., 1992, Papp and Moryl, 1994). In addition, an alternative theory is that these sigma ligands could be modulating noradrenergic activity.

The precise mechanisms underlying the modulation of serotonergic neurotransmission evidenced in the present study remain to be elucidated and are the focus of current investigation in our laboratory.

In conclusion, this series of experiments provides the first evidence of sigma receptor interaction with the 5-HT system. Thus, it strengthens the argument for sigma receptor's role in depression and provides a plausible mechanism of action to explain the antidepressant-like effects observed with some sigma ligands in behavioural models of depression. Importantly, these experiments show sigma ligands to produce an increase in 5-HT firing activity in just 2 days, a more rapid and robust effect that the vast majority of known antidepressant medications, thus, indicating its potential as an antidepressant with a rapid onset of action.

Figure Legends

Figure 1. Integrated firing rate histograms of dorsal raphe 5-HT neurons obtained in anaesthetized rats following 2-day treatment with saline (control) (A) or (+)-pentazocine (2 mg kg^{-1} day^{-1}) (B). The numbers above the histogram represent the depth at which the neuron was found.


A
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(+)-PENTAZOCINE 2-DAY 2 mg kg-1day-1
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5641
1 min

Figure 1

Figure 2. Mean firing activity expressed as spikes/10 seconds (mean ± S.E.M.) of dorsal raphe nucleus serotonergic neurons measured in anesthetized rats. Rats were treated with saline (control), JO-1784 (2 mg kg^{-1} day^{-1} for 2 days), PRE-084 (2 mg kg^{-1} day^{-1} for 2 days) or JO-1784 (2 mg kg^{-1} day^{-1} for 21 days). In this and the following figures, number in columns indicates the number of neurons tested.

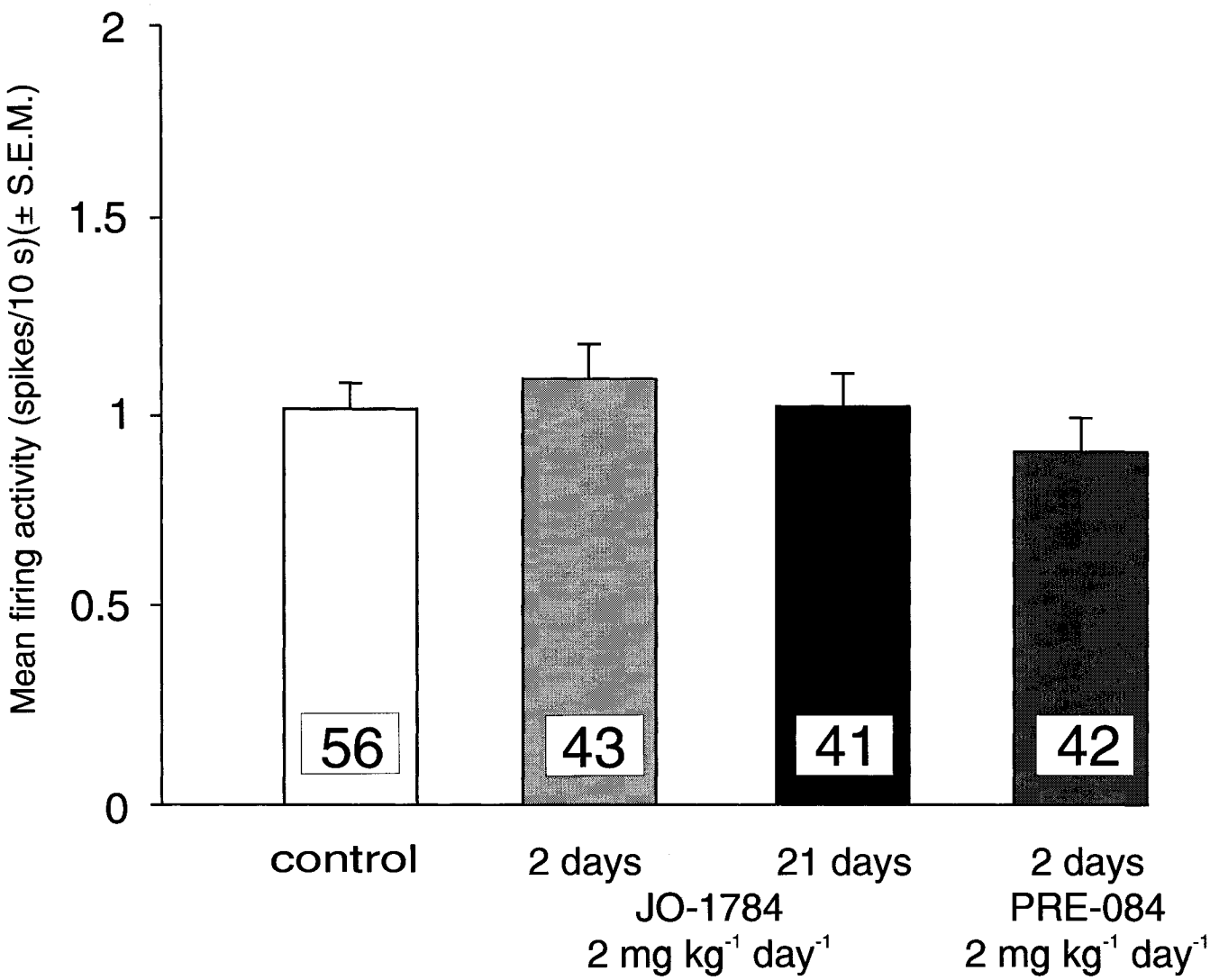
Mean firing activity (spikes/10 s)(± S.E.M.)
2
1.5
1
0.5
0
56
43
41
42
control
2 days
21 days
2 days
JO-1784
2 mg kg-1 day-1
PRE-084
2 mg kg-1 day-1

Figure 2

Figure 3. Mean firing activity expressed as spikes/10 seconds (mean ± S.E.M.) of dorsal raphe nucleus serotonergic neurons measured in anesthetized rats. Rats were treated with saline (control) for 2 days or 4-IBP (2 mg kg^{-1} day^{-1}) for 2, 10 or 21 days or co-administered 4-IBP (2 mg kg^{-1} day^{-1}) and NE-100 (10 mg kg^{-1} day^{-1}) for 2 days. *$P<0.05$.

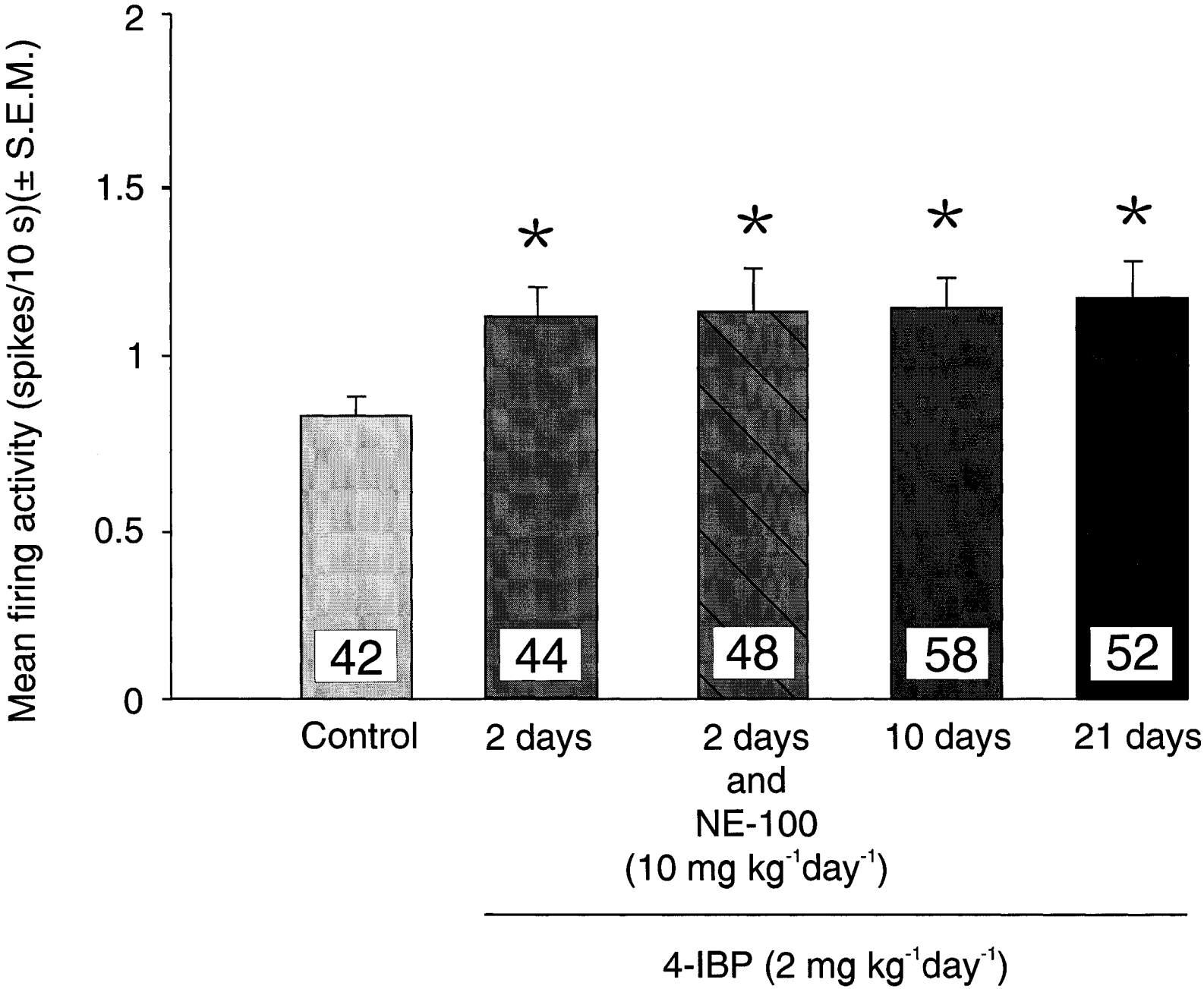
Mean firing activity (spikes/10 s)(± S.E.M.)
2
1.5
1
0.5
0
*
*
*
*
42
44
48
58
52
Control
2 days
2 days and NE-100 (10 mg kg-1day-1)
10 days
21 days
4-IBP (2 mg kg-1day-1)

Figure 3

Figure 4. Mean firing activity expressed as spikes/10 seconds (mean ± S.E.M.) of dorsal raphe nucleus serotonergic neurons measured in rats treated with saline (control), (+)-pentazocine (2 mg kg^{-1} day^{-1}) or co-administered (+)-pentazocine (2 mg kg^{-1} day^{-1}) and NE-100 (10 mg kg^{-1} day^{-1}) for 2 days. *$P<0.05$.

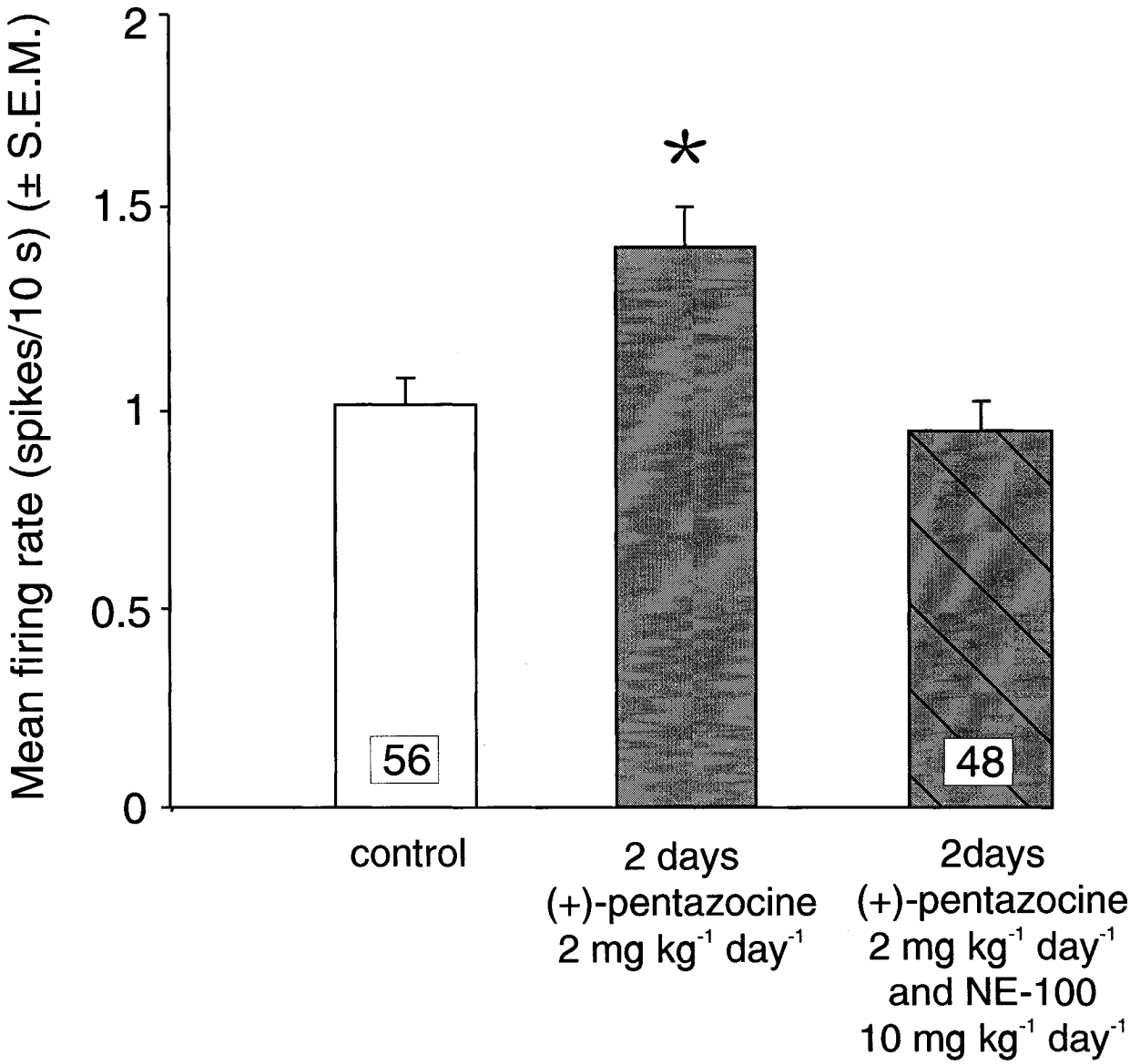
Mean firing rate (spikes/10 s) (± S.E.M.)
2
1.5
1
0.5
0
*
56
48
control
2 days
(+)-pentazocine
2 mg kg-1 day-1
2days
(+)-pentazocine
2 mg kg-1 day-1
and NE-100
10 mg kg-1 day-1

Figure 4

Figure 5. Mean firing activity expressed as spikes/10 seconds (mean ± S.E.M.) of dorsal raphe nucleus serotonergic neurons in rats treated with saline (control) or with (+)-pentazocine (2 mg kg^{-1} day^{-1}) for 5,10,14 or 21 days or co-administered (+)pentazocine (2 mg kg^{-1} day^{-1}) with NE-100 (10 mg kg^{-1} day^{-1}) for 21 days. *$P<0.05$.

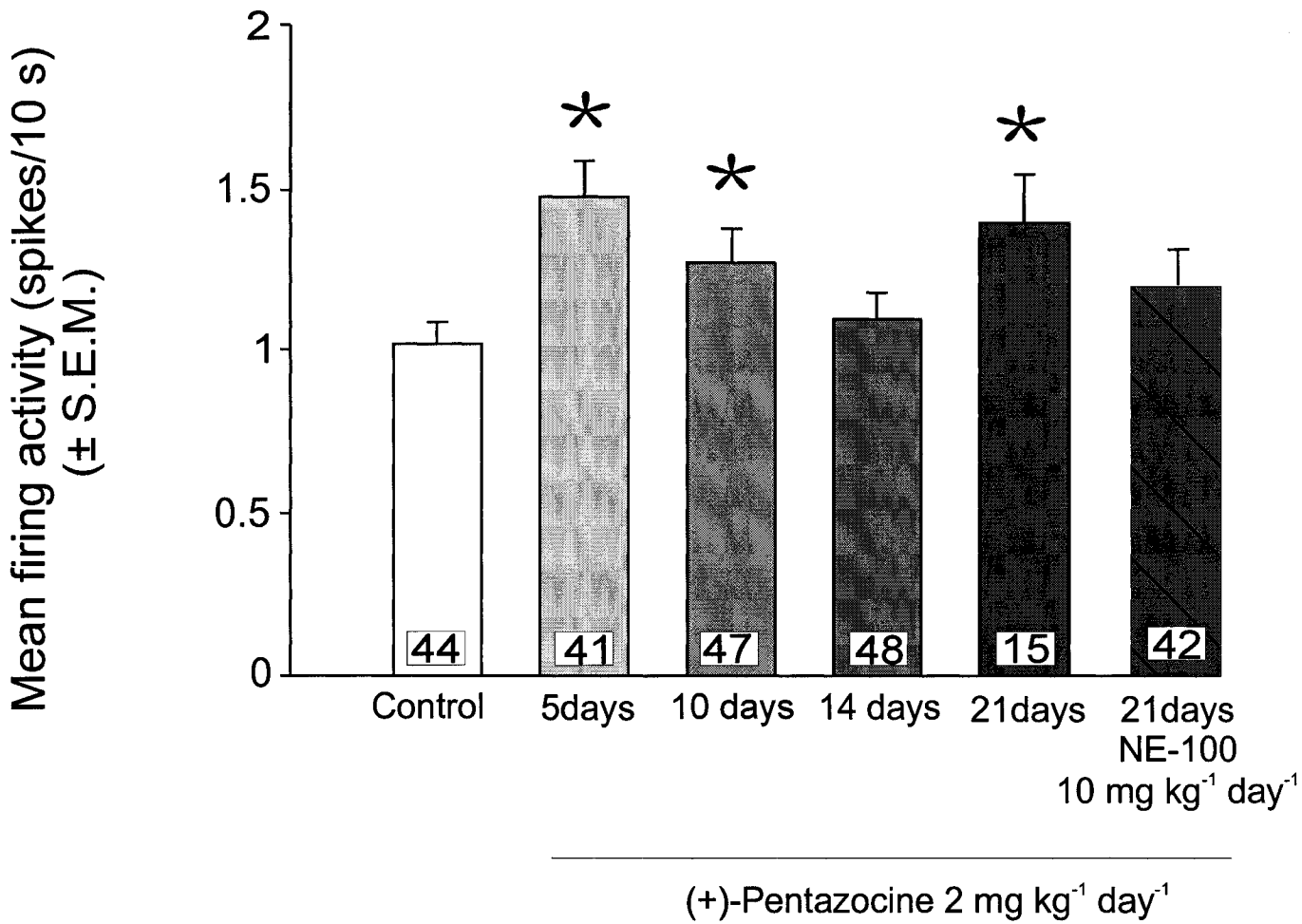
Mean firing activity (spikes/10 s)
(± S.E.M.)
2
1.5
1
0.5
0
*
*
*
44
41
47
48
15
42
Control
5days
10 days
14 days
21days
21days
NE-100
10 mg kg-1 day-1
(+)-Pentazocine 2 mg kg-1 day-1

Figure 5

Figure 6. Mean number of neurons per track (± S.E.M.) encountered in rats treated with saline (control) or with (+)-pentazocine (2 mg kg^{-1} day^{-1}) for 5,10,14 or 21 days or co-administered (+)pentazocine (2 mg kg^{-1} day^{-1}) with NE-100 (10 mg kg^{-1} day^{-1}) for 21 days or treated with 4-IBP (2 mg kg^{-1} day^{-1}) for 21 days *$P<0.05$.

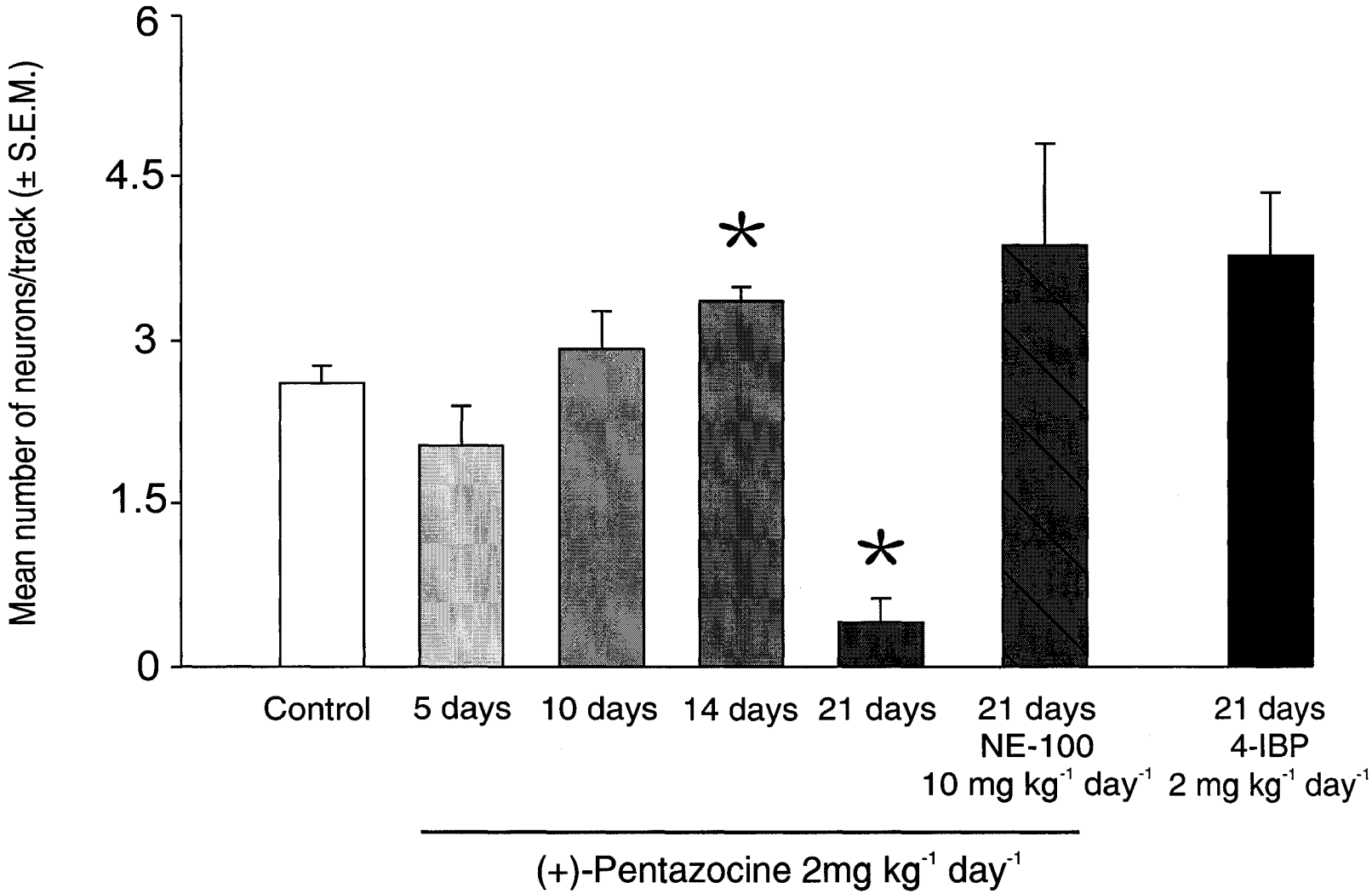
Mean number of neurons/track (± S.E.M.)
6
4.5
3
1.5
0
*
*
Control
5 days
10 days
14 days
21 days
21 days
NE-100
10 mg kg-1 day-1
21 days
4-IBP
2 mg kg-1 day-1
(+)-Pentazocine 2mg kg-1 day-1

Figure 6

Figure 7. Mean firing activity expressed as spikes/10 seconds (mean ± S.E.M.) of dorsal raphe nucleus serotonergic neurons in rats treated with saline (control) or for 21 days with (+)-pentazocine (0.5 mg kg^{-1} day^{-1}).

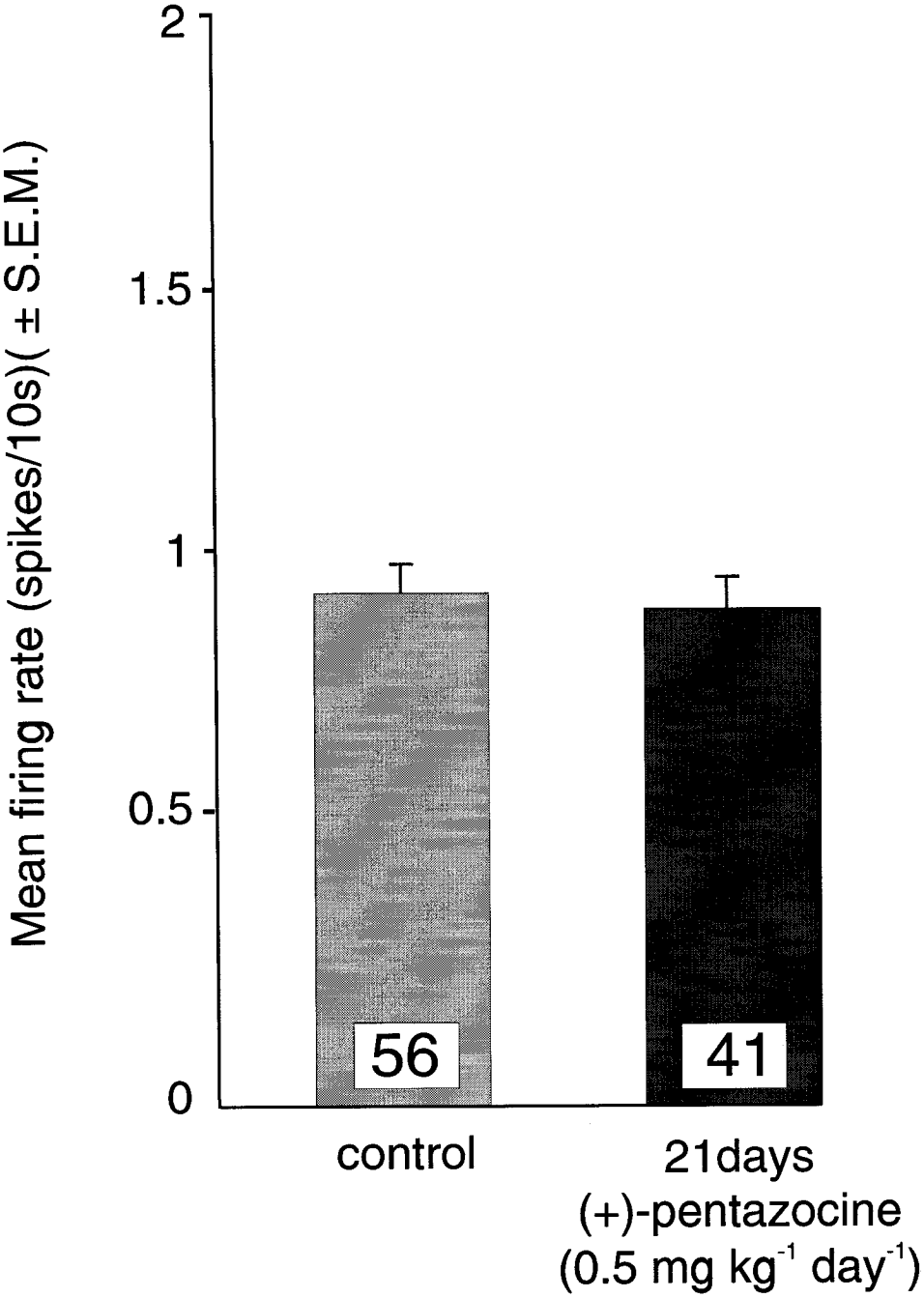
Mean firing rate (spikes/10s)(± S.E.M.)
2
1.5
1
0.5
0
56
41
control
21days
(+)-pentazocine
(0.5 mg kg-1 day-1)

Figure 7

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Chapter 4

Distinct Modulatory Roles of Sigma Receptor Subtypes on Glutamatergic Neurotransmission in the Dorsal Hippocampus

In Chapter III it was shown that the sigma ligand 4-IBP produces interesting effects on 5-HT neurotransmission in the DRN. Firstly, after short-term treatments 4-IBP induced a significant increase in the firing activity of 5-HT neurons of the DRN. Secondly, this increase of the firing activity was maintained with chronic (21 days) treatments. In this respect, 4-IBP produced effects similar to those of the sigma agonist (+)-pentazocine. However, (+)-pentazocine's effects were reversed by the $sigma_1$ antagonist NE-100, while 4-IBP's effects were reversed by the sigma antagonist haloperidol, not be NE-100. Thus, in some aspects, 4-IBP was presenting a different profile as compared to other sigma ligands.

Previous studies in our laboratory have used an electrophysiological paradigm to distinguish sigma agonists and antagonists. In this model, sigma agonists induce a potentiation of the NMDA response in CA_3 pyramidal neurons of the dorsal hippocampus. In accordance, antagonists have no effect on their own, but block the effect of sigma agonists. Given that 4-IBP's effects in the DRN are not blocked by the same antagonist as the other sigma agonists tested, we wanted to examine its action in our model of the modulation of the NMDA response in the hippocampus. In addition, as discussed previously, any modulation of NMDA-mediated neurotransmission has implications towards antidepressant potential. Thus, combined with its demonstrated modulation of 5-HT neurotransmission, we aim to uncover other potential properties of 4-IBP that

may contribute to its potential as an antidepressant as well as uncovering its pharmacological profile.

DISTINCT MODULATORY ROLES OF SIGMA RECEPTOR SUBTYPES ON GLUTAMATERGIC NEUROTRANSMISSION IN THE DORSAL HIPPOCAMPUS

Jordanna E. Bermack and Guy Debonnel

Synapse, *In Press*

ABSTRACT

Sigma ligands have been previously shown to modulate the N-methyl-D-aspartate (NMDA) response in the dorsal hippocampus, such that sigma agonists dose-dependently potentiate the NMDA-induced neuronal response. Recent studies on the sigma ligand 4-IBP found it to act differently from the sigma ligands (+)-pentazocine and DTG in the modulation of 5-HT firing activity in the dorsal raphe nucleus (DRN), as it was not antagonized by the same antagonists. Thus, this study set out to characterize 4-IBP's action as an agonist or antagonist at sigma receptors using the hippocampal paradigm of sigma ligand activity. Interestingly, we found that in 50% of the neurons encountered, 4-IBP produced a potentiation of both the NMDA- and quisqualate (QUIS)-induced responses. In the other 50% of neurons, 4-IBP produced an attenuation of both QUIS and NMDA responses. Only the attenuation induced by 4-IBP was blocked by the $sigma_1$ antagonist NE-100. In contrast, the non selective sigma antagonist haloperidol blocked both the attenuation and potentiation induced by 4-IBP for both QUIS and NMDA responses. These data suggest that 4-IBP may be acting as an agonist or inverse agonist activity at sigma receptors in this model. Furthermore, the initial responses to NMDA and QUIS were found to be higher in

the group in which 4-IBP induced an attenuation in the firing activity. This suggests a modulatory role for 4-IBP on glutamatergic neurotransmission in the hippocampus. This modulation appears to involve two distinct pathways, mediated by different $sigma_1$ receptor subtypes, one being an NE-100 and haloperidol-sensitive $sigma_1$ receptor, and the other a NE-100-insensitive, haloperidol-sensitive $sigma_1$ receptor. This modulatory role has many implications for disorders that have been shown to involve glutamatergic transmission in the hippocampus.

INTRODUCTION

Sigma receptors were first classified as a subtype of opiate receptors (Martin et al. 1976). They were later determined to be a distinct receptor, made up of two subtypes denoted $sigma_1$ and $sigma_2$ receptors (Quirion et al., 1987, 1992). Since then, much work has gone into determining their function, signal transduction mechanisms and endogenous ligand(s). Most of the focus has been on the $sigma_1$ receptor, which has been shown to play an important role in depression, cognitive impairment, and drug abuse (Reviewed in Maurice et al., 1997; Maurice et al., 2002; Debonnel et al., In Press).

Sigma ligands pharmacological activity has been assessed extensively in our laboratory using an *in vivo* electrophysiological paradigm of recordings from CA_3 pyramidal neurons of the dorsal hippocampus. This model distinguishes sigma agonists and antagonists, such that, acutely, low doses (0.5-50 μg/kg) of sigma agonists (DTG, JO-1784, (+)-pentazocine) have no effect on the spontaneous firing activity of hippocampal CA_3 neurons, but dose-dependently modulate the response to NMDA (Monnet et al., 1990, 1992). Accordingly, sigma antagonists, such as haloperidol, NE-100 and progesterone, have no effects on their own but block the effects of sigma agonists (Monnet et al., 1990, 1992, Bergeron et al., 1996).

Interestingly, in the hippocampus paradigm, the degree of potentiation induced by sigma agonists was found to present a bell-shaped (biphasic) dose-response curve. At higher does (>500 μg/kg) the potentiation progressively decreases and sigma agonists can act as antagonists by preventing the potentiation

induced by other sigma agonists (Bergeron et al., 1995). A bell-shaped dose-response curve has been obtained in other models of sigma receptor activity, for example, the NMDA-evoked [^{3}H]norepinephrine release from hippocampal slices (Monnet et al., 1996) and behavioural models of sigma ligands anti-amnesic effects (Maurice et al., 1994 a,b)

4-IBP is a selective sigma ligand with high affinity for the $sigma_1$ receptor (K_i=1.7nM) and $sigma_2$ receptors (K_i=25.2 nM) (John et al., 1994). When tested in the DRN, after only 2 days of treatment, 4-IBP produced a significant increase in serotonergic firing activity that was maintained after long-term (21 days) treatments. This is similar to the effects of the sigma ligands (+)-pentazocine and DTG in the DRN (Bermack and Debonnel, 2001). However, in the DRN paradigm 4-IBP showed a distinct difference compared to the other sigma ligands tested in that its effects were not blocked by the selective $sigma_1$ antagonist NE-100, while those of (+)-pentazocine and of DTG's were completely suppressed (Bermack and Debonnel, 2001).

Our laboratory has previously suggested the existence of $sigma_1$ receptor subtypes (Monnet et al., 1994; Debonnel et al., 1996; Couture and Debonnel, 2001; Bermack and Debonnel, 2001) and this has also been suggested by data from other laboratories (Basile et al., 1992; Vilner et al., 1995; Tsao and Su, 1997). Thus, 4-IBP may act on a subtype of the $sigma_1$ receptor other than or in addition to the NE-100-sensitive $sigma_1$ receptor. Therefore, the present experiments were carried out in the CA_3 region of the dorsal hippocampus recording extracellularly pyramidal neurons, to assess whether 4-IBP acts as a $sigma_1$ agonist or antagonist. This in turn will lead to a better understand of its

mechanism of action in the hippocampus and DRN. In addition, these experiments will lead to further characterization of the modulatory function of sigma receptors in the dorsal hippocampus.

MATERIAL AND METHODS

Animals

Experiments were performed in male Sprague-Dawley rats (Charles River, St.Constant, Québec) weighing 250-300g. Rats were housed under standard laboratory conditions including 12-12hr light-dark cycle and free access to food and water.

Electrophysiological experiments

Rats were anesthetized with urethane (1.25 g/kg, i.p.) and mounted in a stereotaxic apparatus. Five-barreled glass micropipettes were used for extracellular recordings of CA_3 pyramidal neurons and microiontophoretic applications. The central barrel, used for extracellular unitary recording, was filled with a 2M NaCl solution, saturated with Fast Green FCF. One of the side barrels, used for automatic current balancing, was filled with a 2M NaCl solution. Two of the remaining side barrels were filled with QUIS (1.5 mM in 400 mM NaCl, pH: 8) and NMDA (5 mM in 200 mM NaCl, pH: 8).

Recordings were obtained in the CA_3 region of the dorsal hippocampus. Hippocampal pyramidal neurons were identified by their long duration (0.8 to 1.5 ms), large amplitude (0.5 to 2 mV) action potentials and by the presence of "complex spike" discharges (Kandel and Spencer, 1961).

Activation by excitatory agents

Fifty-second alternate microiontophoretic applications of the excitatory agents NMDA and QUIS were carried out, and currents were adjusted to obtain similar and stable responses of the neuron to each agent. The degree of activation was calculated by determining the number of spikes generated/nanoCoulomb (nC).

Intravenous administration of sigma ligands

The effects of the acute intravenous administration of 4-IBP were measured by comparing the degree of activation induced by the abovementioned excitatory substances before, and five minutes after, the intravenous injection of 4-IBP (20 μg/kg). Three successive applications of each of the excitatory substances were averaged to determine the degree of activation induced by NMDA and QUIS. One injection was given to one rat while recording from one neuron.

We then tested whether two sigma antagonists, NE-100 and haloperidol, could antagonize any effects induced by 4-IBP. Five minutes following the administration of 4-IBP and 3 successive applications of each excitatory substance, an antagonist was administered intravenously. Determining the number of spikes generated/nC five minutes after its administration assessed the antagonist's effects.

Statistical Analysis

Statistical analysis was performed using the software SigmaStat for Windows Version 4.0 (Jandel Corporation). One-way ANOVA was used with alpha=0.05, followed by a post-hoc analysis using Student-Newman-Keuls method of comparison between groups for all data where more than 2 groups were compared. When only 2 groups were compared Student t-tests were used. $P < 0.05$ was considered statistically significant for all statistical tests.

Drugs

NMDA, QUIS and 4-IBP were purchased from Tocris Cookson Inc. (Ellisville, MO, USA), haloperidol was purchased from Sigma Aldrich Canada (Oakville, ON, Canada), NE-100 was a gift from Taisho Pharmaceutical Co. Ltd (Tokyo, Japan).

RESULTS

Effects of 4-IBP on the NMDA-induced Neuronal Response

4-IBP had no significant effect on the baseline firing activity of CA_3 pyramidal neurons (data not shown). In approximately 50% of the neurons encountered, 4-IBP (20 μg/kg i.v.) induced a significant potentiation of the NMDA-induced neuronal response in CA_3 pyramidal neurons (compared to controls 0.16 ± 0.03 nC vs. 0.07 ± 0.01 nC n=12 [F=4.36 P<0.05 q=3.18])(Group A) (Figure 1A and 2A). However, in the other 50% of neurons encountered, the administration of 4-IBP (same dose) induced a significant attenuation in the response of the CA_3 pyramidal neurons to NMDA (compared to controls 0.08 ± 0.01 nC vs. 0.22 ± 0.03 nC n=12 [F=9.41 P<0.001 q=6.10]) (Group B) (Figure1B and 2B).

Effects of 4-IBP on the QUIS-induced Neuronal Response

Following 4-IBP administration, the effects on the QUIS response followed a pattern very similar to that seen with the NMDA response. Group A neurons, which showed an increase in the response to NMDA following 4-IBP, also showed a trend towards an increase in the response to QUIS (compared to controls 2.55 ± 0.34 nC vs. 2.09 ± 0.18 nC n=12 n.s.) (Figure 1A and 3A). In contrast, Group B neurons, which showed a decrease in the response to NMDA following 4-IBP, also showed a significant decrease in the QUIS-induced

response following 4-IBP (compared to controls 2.73 ± 0.30 nC vs. 4.41 ± 0.65 nC n=12 [F=3.60 P<0.05 q=3.76]) (Figure 1B and 3B).

Effects of the selective sigma$_1$ antagonist NE-100

For Group A neurons, in which the 4-IBP administration produced a potentiation of NMDA- and QUIS-induced responses, the intravenous administration of NE-100 (100 μg/kg) following that of 4-IBP, did not reverse the effects of 4-IBP on NMDA responses (4-IBP vs. NE-100 0.16 ± 0.03 nC vs. 0.19 ± 0.04 nC n=12 n.s.) (Figure 2A), nor on QUIS-induced responses (4-IBP vs. NE-100 2.55 ± 0.34 nC vs. 2.41 ± 0.29 nC n=12 n.s.) (Figure 3A). For group B neurons, in which 4-IBP produced an attenuation in the QUIS and NMDA responses, the subsequent administration of NE-100 (100 μg/kg) was able to reverse the attenuation induced by 4-IBP on NMDA-induced responses (4-IBP vs. NE-100 0.08 ± 0.02 nC vs. 0.15 ± 0.02 nC n=12 [F=9.41 P<0.001 q=3.13]) (Figure 2B). Following NE-100 administration the QUIS response was not significantly different from controls (NE-100 vs. controls 3.31 ± 0.36 nC vs. 4.41 ± 0.65 nC n=15 n.s.) (Figure 3B). Thus, NE-100 was only able to reverse 4-IBP's attenuating effects.

Effects of the nonselective sigma antagonist haloperidol

In Groups A and B, the administration of haloperidol (100 μg/kg i.v.) following that of 4-IBP reversed either the increase or decrease of the NMDA response induced by 4-IBP (Group A 4-IBP vs. haloperidol 0.26 ± 0.04 nC vs.

0.16 ± 0.02 nC n=9 [F=7.83 P=0.001 q=3.62] Group B 4-IBP vs. haloperidol 0.17 ± 0.04 nC vs. 0.26 ± 0.06 nC n=9 n.s.) (Figures 4). For the QUIS-induced responses, in Groups A and B, haloperidol when administered subsequent to 4-IBP reversed either effect of 4-IBP (Group A 4-IBP vs. haloperidol 7.08 ± 0.68 nC vs. 5.03 ± 0.82 nC n=9 [F=5.38 P<0.05 q=3.12] Group B 4-IBP vs. haloperidol 3.47 ± 0.58 nC vs. 5.41 ± 0.51 nC n=9 [F=4.86 P<0.05 q=3.76]) (Figure 5).

Initial Responses to QUIS and NMDA

Upon further investigation, a significant difference was found in the initial responses to NMDA (prior to 4-IBP administration) between groups A and B. Specifically, in Group A, in which 4-IBP induced a potentiation of the NMDA response, the initial response to NMDA was significantly smaller than in Group B ($p<0.05$, Student's t-test) (Figure 6A).

A significant difference was also found in the initial responses to QUIS application between Groups A and B such that Group A had a significantly decreased response to QUIS compared to Group B, prior to 4-IBP administration ($p<0.05$, Student's t-test) (Figure 6B)

DISCUSSION

The present results show that 4-IBP, administered at a dose of 20 μg/kg (i.v.), induces two distinct responses in CA_3 pyramidal neurons of the dorsal hippocampus. In one group of neurons (A) a potentiation of the NMDA-induced neuronal response and a trend towards an increased QUIS-induced response was produced by the administration of 4-IBP (Figures 2A and 3A). In contrast, Group B neurons show a clear attenuation of NMDA- and QUIS-induced responses following the same dose of 4-IBP (Figures 2B and 3B). There was no significant difference in the basal firing rate following 4-IBP administration (data not shown) and the currents of QUIS and NMDA applied were kept constant throughout the experiments, therefore any responses seen were most likely due to 4-IBP's effect on NMDA and QUIS responses specifically. The direction of the response induced by 4-IBP appears to be dependent on the initial responses of the neurons to NMDA and QUIS, prior to 4-IBP administration (Figure 6), thus suggesting an important modulatory role for sigma receptors on hippocampal glutamatergic neurotransmission.

The selective $sigma_1$ antagonist NE-100 only reversed the attenuating effects of 4-IBP on the QUIS and NMDA-induced responses (Figures 2 and 3). However, the QUIS-induced responses were not significant in Group A, so antagonist properties of NE-100 could only be assessed on the NMDA-mediated responses in this group. In contrast, the nonselective sigma antagonist haloperidol successfully reversed both the attenuating and potentiating effects of 4-IBP on NMDA and QUIS-induced responses (Figures 4 and 5). These distinct effects of

the different antagonists and the lack of effect of NE-100 on some of 4-IBP's effects, suggest that distinct pathways, likely through different subtypes of the $sigma_1$ receptor, mediate these two modulatory effects. The differential effect of NE-100 and haloperidol follows the results previously obtained with 4-IBP in the DRN where short- and long-term treatments with 4-IBP induced an increase in serotonergic neurotransmission that was not reversible by NE-100 co-administration, but was reversed by haloperidol co-administration. In contrast, the effects of (+)-pentazocine and DTG in the dorsal raphe nucleus were blocked by NE-100 co-administration (Bermack and Debonnel, 2001).

There is limited data from studies using 4-IBP. Binding studies with 4-IBP in breast cancer cell lines, show that [^{125}I]BP binding was inhibited by haloperidol and DTG (John et al., 1994). The first studies of sigma ligands in a model of anoxic depolarization in rat neocortical slices showed that 4-IBP blocked spreading depression and decreased K^+-induced swelling, and these effects were blocked by the sigma ligands (+)-3-PPP and BD-1063 (Anderson and Andrew, 2002). Thus, due to the fact that 4-IBP has not been tested in the more recognized and established models of sigma ligand activity, its exact target with respect to sigma receptor subtypes is yet to be fully elucidated.

The current results with 4-IBP suggest that it may act on more than one subtype of the $sigma_1$ receptor, one that is sensitive to haloperidol and not NE-100, and one that is sensitive to both NE-100 and haloperidol. Our laboratory has, previously suggested the putative existence of subtypes of the $sigma_1$ receptor. For example, pertussis toxin blocked the effect of DTG and JO-1784, but not that of (+)-pentazocine, on the NMDA-induced neuronal response in the dorsal

hippocampus (Monnet et al., 1994). Moreover, mossy fiber lesioning blocked the effects of DTG and JO-1784, but not those of (+)-pentazocine (Debonnel et al., 1996). Combined, these data suggest that JO-1784 and DTG act through a subtype of the $sigma_1$ receptor different than the subtype that (+)-pentazocine acts through. Furthermore, (+)-pentazocine has been shown to induce effects in the hippocampus that are blocked by naloxone, an opioid antagonist, while, naloxone had no effect on other $sigma_1$ ligands' effects in the same model (Couture and Debonnel, 2001).

In addition to the electrophysiological data, binding studies have suggested numerous subtypes of the $sigma_1$ receptor as (+)-pentazocine was found to bind to 2 or 3 distinct binding sites *in vitro* (Basile et al., 1992; Vilner et al., 1995). Furthermore, Tsao and Su (1997) purified a naloxone-sensitive, haloperidol-sensitive (+)-SKF-10,047 binding protein, which bound (+)-pentazocine but did not bind DTG, (+)-3-PPP nor progesterone, also suggesting at least a second $sigma_1$ receptor subtype. Within the hippocampus several populations representing different subtypes of the $sigma_1$ receptor are present and their density may depend on the activity level of the neurons. As suggested by previous data showing that under the presence of sigma ligands, sigma receptors translocate from the endoplasmic reticulum to the plasma membrane (Morin-Surin et al. 1999, Takebayashi et al. 2002).

It could be possible that the effects of 4-IBP seen in the present study are mediated via $sigma_2$ receptors. However, this is unlikely because $sigma_2$ ligands when tested in the same paradigm in the dorsal hippocampus needed doses 5-10 times higher than the $sigma_1$ ligands to induce a similar potentiation of the

NMDA response (Couture and Debonnel, 1998). Unfortunately, there are no selective $sigma_2$ antagonists available as yet, for *in vivo* experiments to completely rule out this possibility.

Thus, it appears that 4-IBP could modulate the NMDA and QUIS responses by two distinct pathways, each likely modulated by a different $sigma_1$ receptor subtype. Thus, a NE-100-sensitive, haloperidol-sensitive subtype of the $sigma_1$ receptor mediates an attenuation in the neuronal responses induced by QUIS and NMDA and this pathway is activated when the initial response to these excitatory amino acids is high. In contrast, when the initial responses are low, a second modulatory pathway that induces an increase in the NMDA- and QUIS-induced responses is activated by sigma ligands, and this pathway is mediated by an NE-100-insensitive, haloperidol-sensitive $sigma_1$ receptor.

4-IBP produced an attenuation of the NMDA and QUIS responses, this is opposite to the potentiation induced by sigma agonists and is blocked by an antagonist, which suggests an inverse agonist activity of 4-IBP. Inverse agonism is thought to involve a preferential binding to the inactivated receptor complex and inhibition of basal receptor activity versus antagonists which bind equally well to all receptor states and have no effect on basal receptor activity but block the effect of agonists and inverse agonists (Reviewed in Milligan and Bond, 1997; Strange, 2002). Thus, an inverse agonist may yield a lesser response compared to the endogenous ligand or a low dose sigma agonist, thus appearing to antagonize sigma agonist's effects. Inverse agonists for $sigma_1$ receptors have yet to be described *in vivo* however the sigma ligand DTG showed inverse agonist effects

in vitro in the model of sigma receptor mediation of NMDA-induced [^{3}H]norepinephrine release in hippocampal slices (Monnet et al.,1992).

Inverse agonism is difficult to show in *in vivo* models as it involves constitutive activity of receptors, which is difficult to control *in vivo* as it is complicated by the presence of endogenous ligands for the receptors (Milligan and Bond, 1997; Strange 2002), and in the case of sigma receptors, the endogenous ligand(s) is unknown. The difference in the initial responses to NMDA and QUIS in this study, suggests that the inverse agonism activity induced by 4-IBP may be dependent on the level of tonic activation of sigma receptors. The identification of inverse agonists for sigma receptors has important implications in understanding the mechanism of action and functional role of sigma receptors.

Lastly, in the current experimental series, 4-IBP produces similar effects on the NMDA and QUIS responses, however in the data set assessing NE-100 as an antagonist the QUIS responses were not significant. Most sigma ligands tested previously in this paradigm selectively potentiated the NMDA response while having no effect on the QUIS response (Monnet et al. 1990, 1992; Bergeron et al., 1995). The sigma ligand BD-737 demonstrated effects on the QUIS response similar to those on the NMDA response (Bergeron and Debonnel, 1997). More recently, the combined sigma$_1$ and 5-HT$_{1A}$ receptor ligand OPC-14523 also showed effects on the NMDA and QUIS responses (Bermack and Debonnel, submitted). More studies are needed to further investigate the effects of sigma ligands on QUIS-induced response as past studies have shown this ability is not possessed by all sigma ligands. Moreover, since in the current study the effects on

the QUIS response were not consistently significant, it was more difficult to assess the affects of various antagonists and in turn the receptor subtype involved for the responses related to QUIS.

Thus, in conclusion, 4-IBP appears to exert a complex modulatory effect on glutamatergic transmission in the hippocampus. This modulation depends on the initial responses to excitatory agents and likely is mediated by two subtypes of the $sigma_1$ receptor. This modulatory role of $sigma_1$ receptors on glutamatergic transmission has implications for many disorders that $sigma_1$ receptors have been previously implicated in, such as cognitive impairment, drug abuse and depression (Reviewed in Maurice et al. 1997; Maurice et al., 2002; Debonnel et al., In Press).

FIGURE LEGENDS

Figure 1

Integrated firing rate histograms of CA_3 dorsal hippocampus pyramidal neurons illustrating the effect of intravenous administration of 4-IBP on QUIS- and NMDA-induced activations and the subsequent administration of the $sigma_1$ antagonist NE-100 in A) a Group A neuron in which 4-IBP induced a potentiation of the NMDA and QUIS responses, which is not reversed by NE-100; B) a Group B neuron, in which 4-IBP induced an attenuation of the NMDA and QUIS responses, which is reversed by NE-100. The bars indicate the duration of applications for which currents are given in nA, open circles (oo) indicate a 5 minute interruption of the histogram.

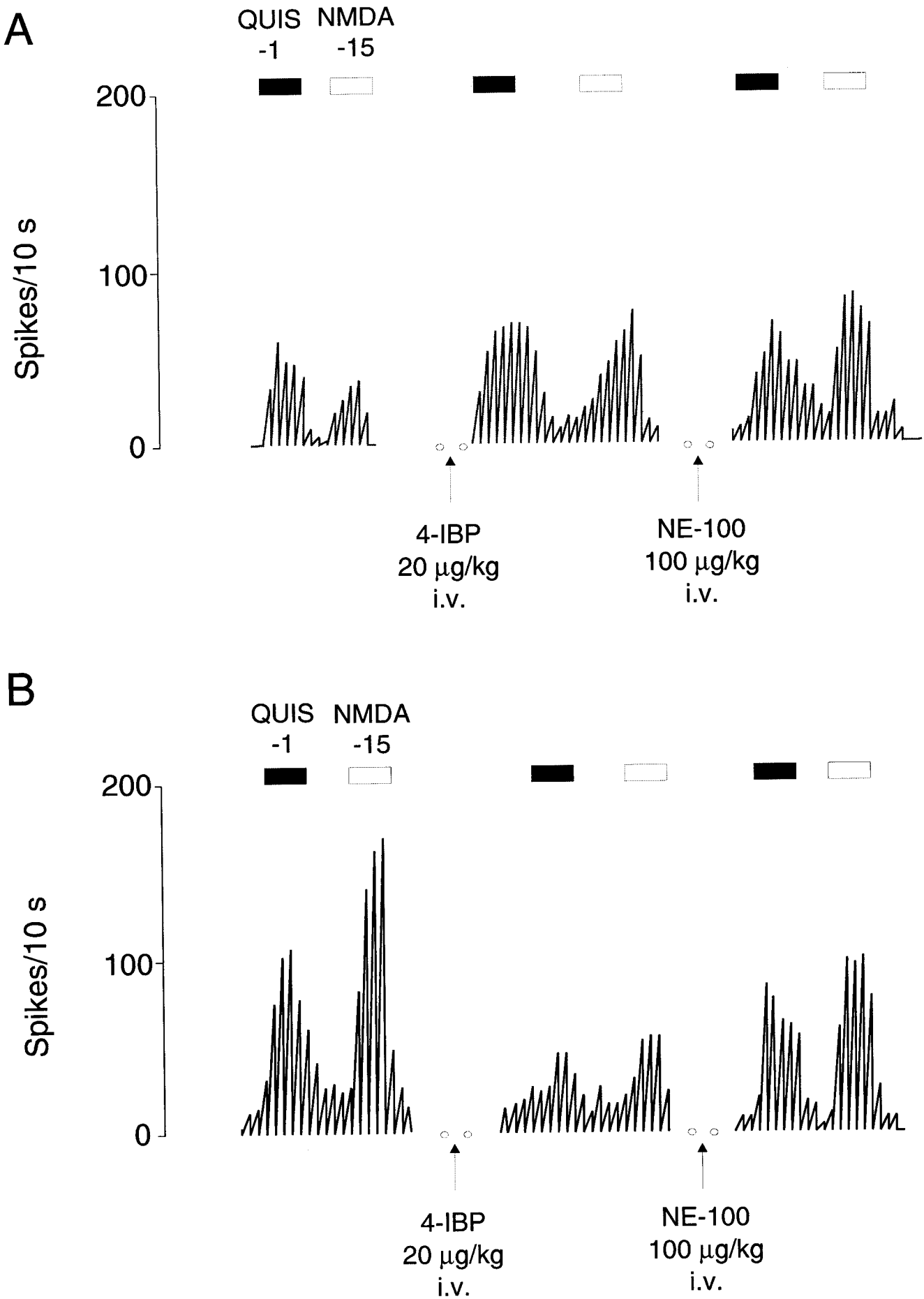
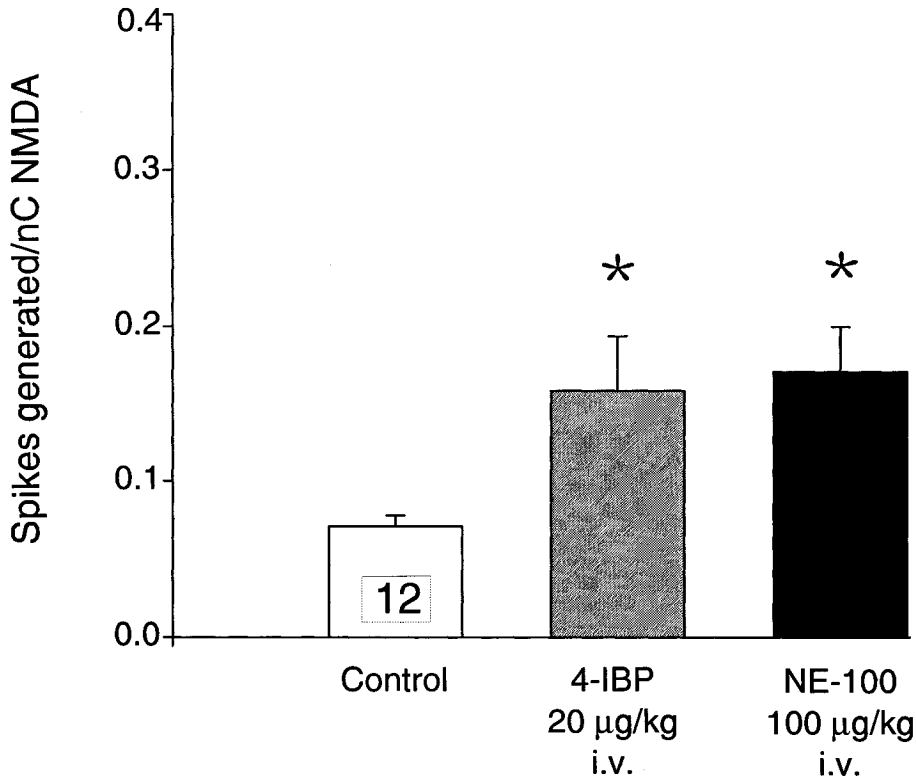

A
QUIS
-1
NMDA
-15
200
100
0
Spikes/10 s
4-IBP
20 µg/kg
i.v.
NE-100
100 µg/kg
i.v.
B
QUIS
-1
NMDA
-15
200
100
0
Spikes/10 s
4-IBP
20 µg/kg
i.v.
NE-100
100 µg/kg
i.v.

Figure 1

Figure 2

Effects of the acute administration of 4-IBP on the neuronal response to NMDA in CA_3 pyramidal neurons in A) Group A neurons, which show a potentiated response, not reversible by NE-100; B) Group B neurons, which shows an attenuated response, reversible by NE-100. Results are expressed as the mean number of spikes generated/nC (± S.E.M.) before (control, white columns), after the intravenous administration of 4-IBP (20 μg/kg) (grey columns) and after the subsequent administration of NE-100 (100 μg/kg) (black columns). The number in the box of each column in this and all subsequent figures indicate the number contributing to the average. *$p<0.05$.

A


Spikes generated/nC NMDA
0.4
0.3
0.2
0.1
0.0
*
*
12
Control
4-IBP
20 µg/kg
i.v.
NE-100
100 µg/kg
i.v.

B

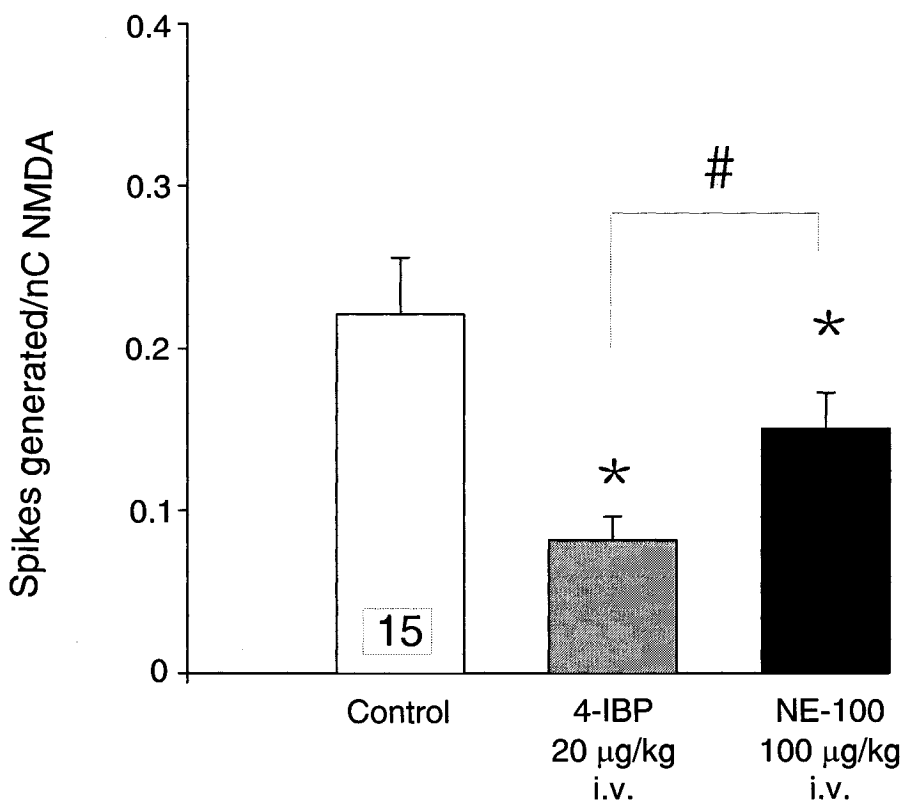
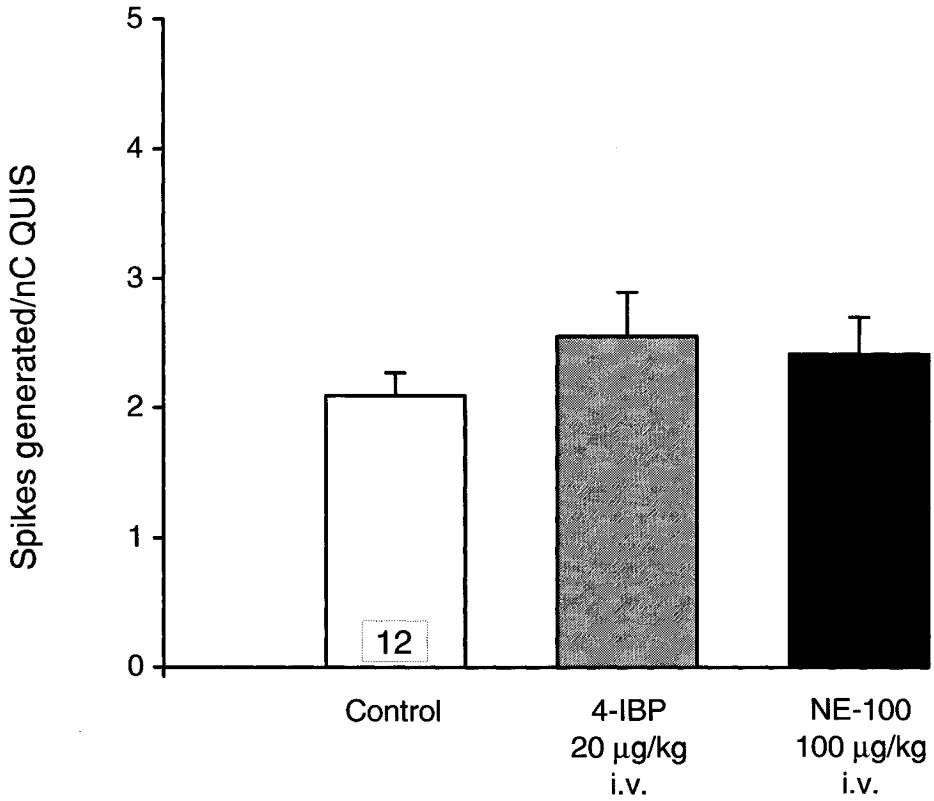

Spikes generated/nC NMDA
0.4
0.3
0.2
0.1
0
#
*
*
15
Control
4-IBP
20 µg/kg
i.v.
NE-100
100 µg/kg
i.v.

Figure 2

Figure 3

Effects of the acute administration of 4-IBP on the neuronal response to QUIS in CA_3 pyramidal neurons in A) Group A neurons, which show a potentiated response following 4-IBP administration, not reversible by NE-100; B) Group B neurons, which show an attenuated response following 4-IBP, reversible by NE-100. Results are expressed as the mean number of spikes generated/nC (± S.E.M.) before (control, white columns), after the intravenous administration of 4-IBP (20 μg/kg) (grey columns) and after the subsequent administration of NE-100 (100 μg/kg) (black columns).

A


Spikes generated/nC QUIS
5
4
3
2
1
0
12
Control
4-IBP
20 µg/kg
i.v.
NE-100
100 µg/kg
i.v.

B

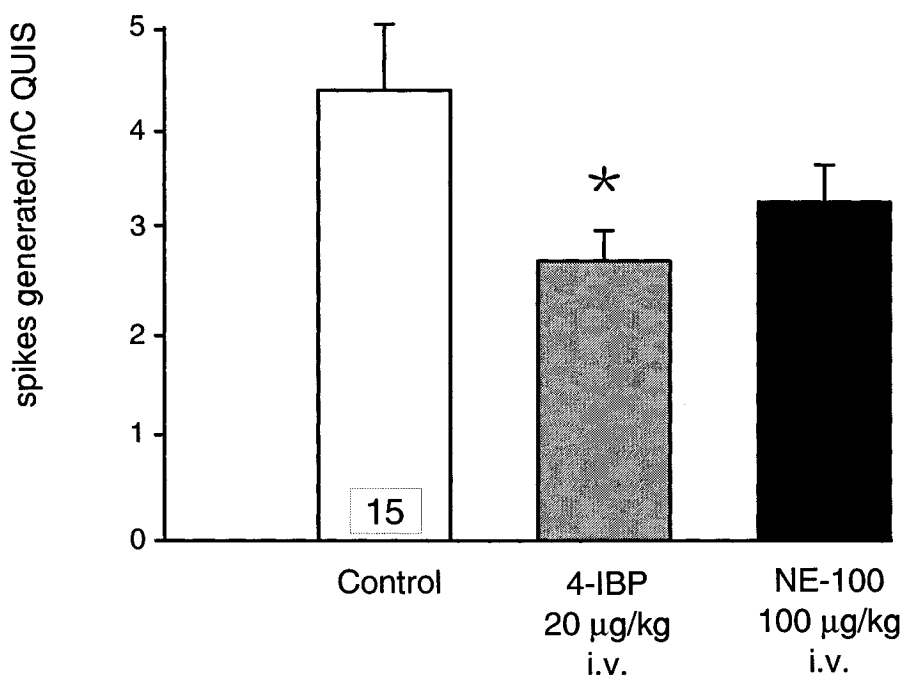
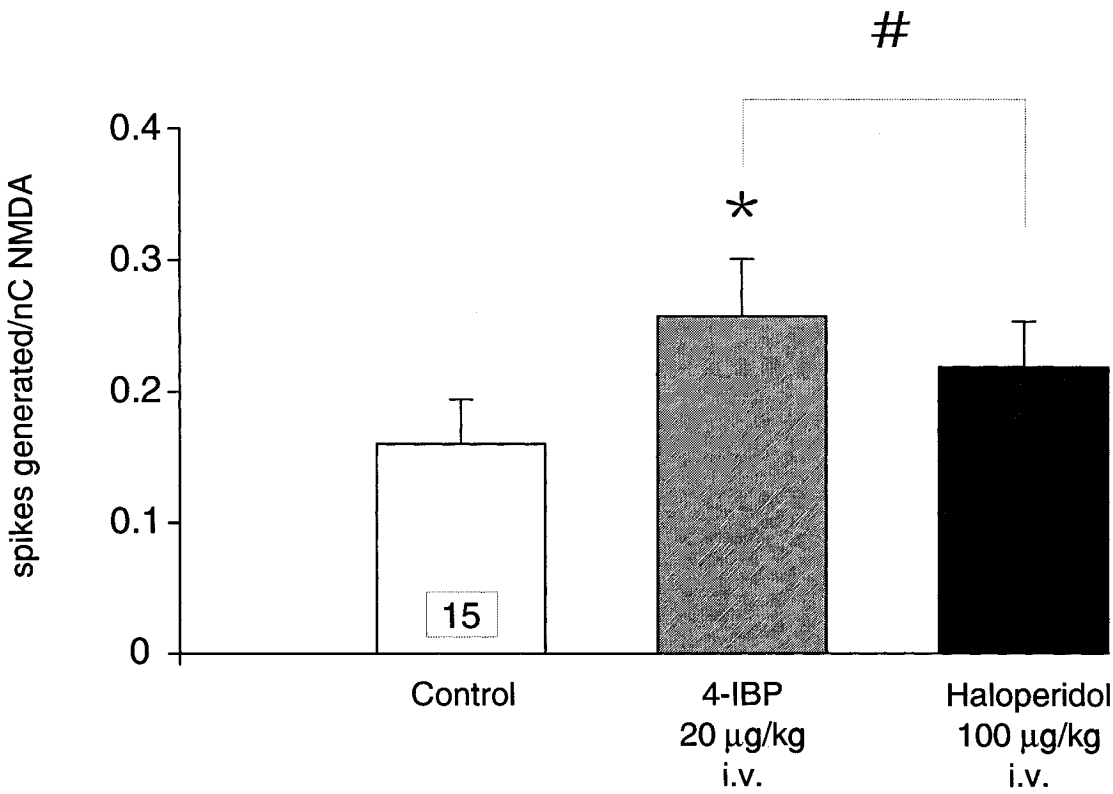

spikes generated/nC QUIS
5
4
3
2
1
0
*
15
Control
4-IBP
20 µg/kg
i.v.
NE-100
100 µg/kg
i.v.

Figure 3

Figure 4

Effects of the nonselective sigma antagonist haloperidol on the effects of the acute administration of 4-IBP on the neuronal response to NMDA in CA_3 pyramidal neurons. A) Group A neurons show a potentiated response following 4-IBP, which is reversed by haloperidol; B) Group B neurons show an attenuated response, which is reversible by haloperidol. Results are expressed as the mean number of spikes generated/nC (± S.E.M.) before (control, white columns), after the intravenous administration of 4-IBP (20 μg/kg) (grey columns) and after the subsequent administration of haloperidol (100 μg/kg) (black columns).

A


#
*
0.4
0.3
0.2
0.1
0
spikes generated/nC NMDA
15
Control
4-IBP
20 µg/kg
i.v.
Haloperidol
100 µg/kg
i.v.

B

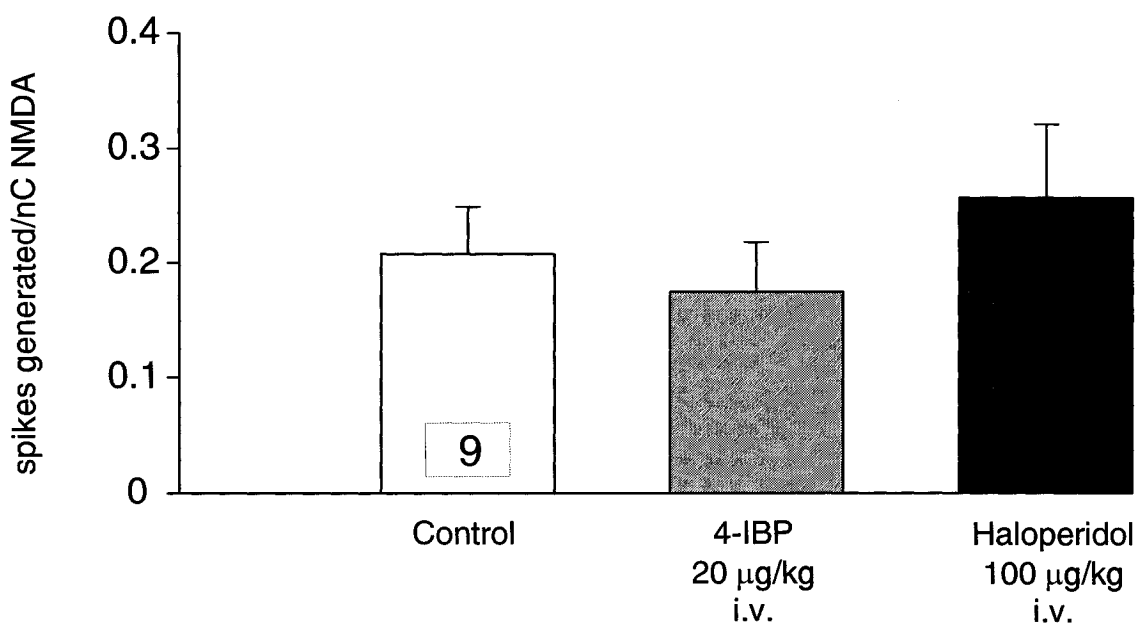
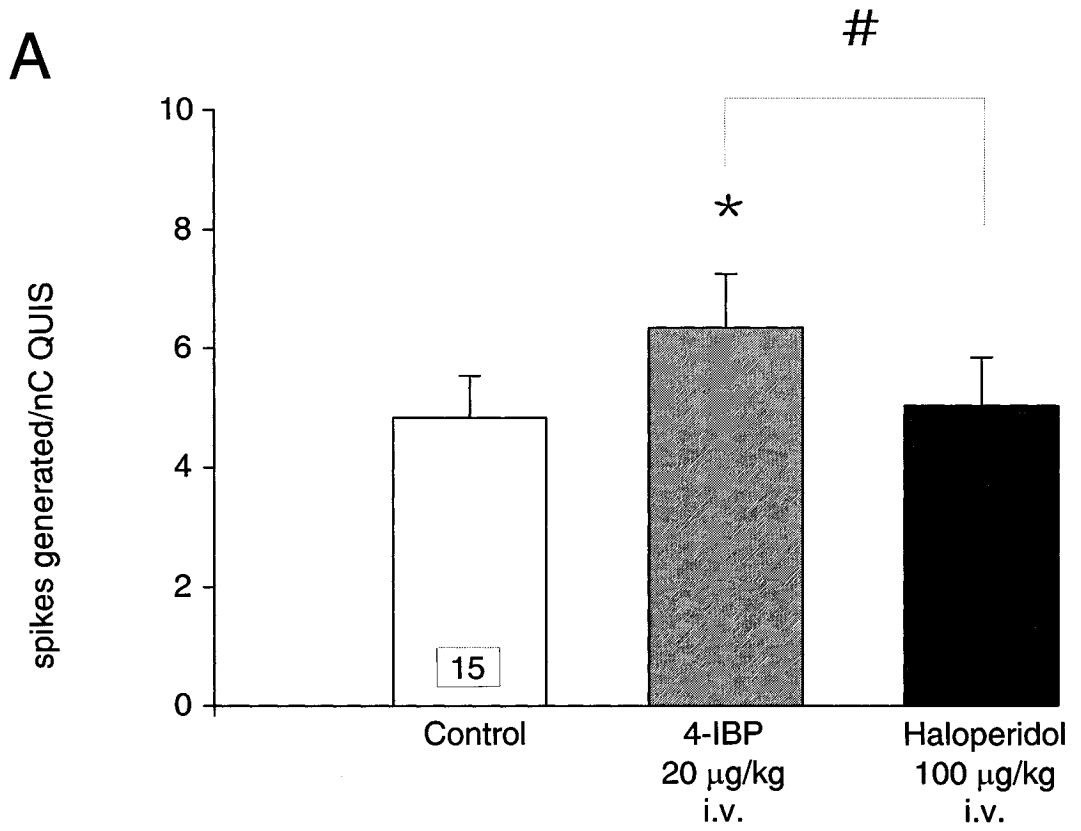

0.4
0.3
0.2
0.1
0
spikes generated/nC NMDA
9
Control
4-IBP
20 µg/kg
i.v.
Haloperidol
100 µg/kg
i.v.

Figure 4

Figure 5

Effects of the nonselective sigma antagonist haloperidol on the effects of the acute administration of 4-IBP on the neuronal response to QUIS in CA_3 pyramidal neurons. A) Group A neurons show a potentiated response to following 4-IBP, which is reversible by haloperidol; B) Group B neurons show an attenuated response following 4-IBP, reversible by haloperidol. Results are expressed as the mean number of spikes generated/nC (± S.E.M.) before (control), after the intravenous administration of 4-IBP (20 μg/kg) (grey columns) and after the subsequent administration of haloperidol (100 μg/kg) (black columns).


A
#
*
10
8
6
4
2
0
spikes generated/nC QUIS
15
Control
4-IBP
20 μg/kg
i.v.
Haloperidol
100 μg/kg
i.v.

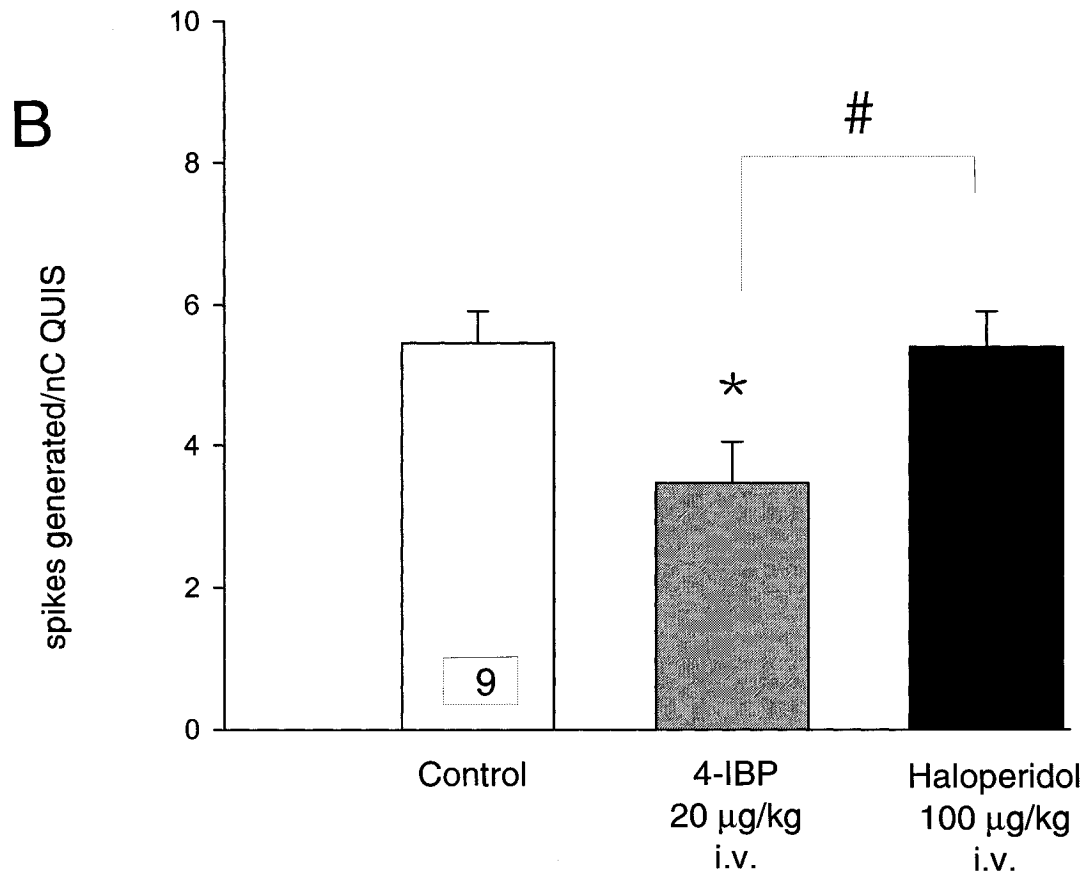
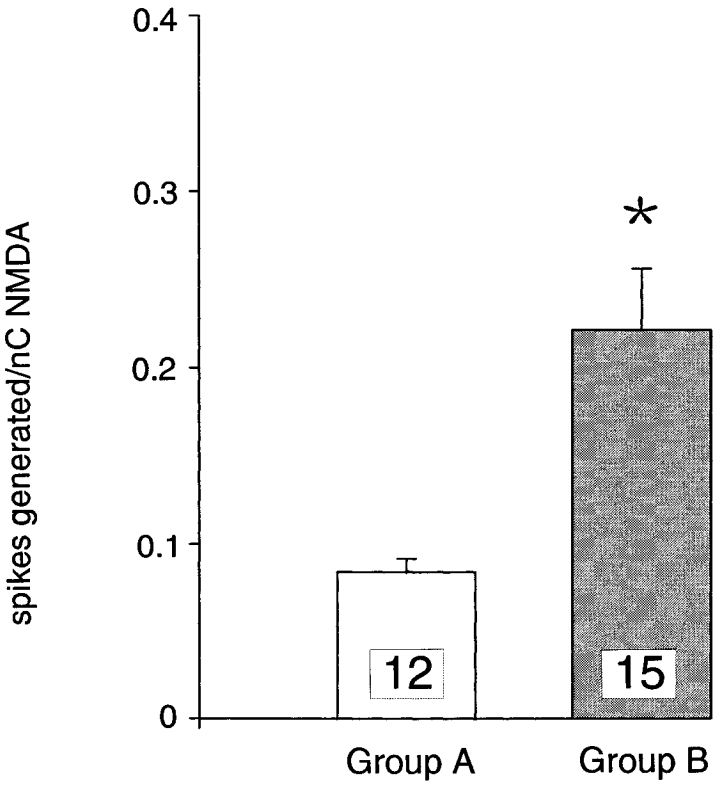

B
#
*
10
8
6
4
2
0
spikes generated/nC QUIS
9
Control
4-IBP
20 μg/kg
i.v.
Haloperidol
100 μg/kg
i.v.

Figure 5

Figure 6

Initial neuronal response to microiontophoretic applications of A) NMDA; B) QUIS in CA_3 pyramidal neurons before the administration of 4-IBP in both Groups A and B. Results are expressed as the mean number of spikes generated/nC (± S.E.M.).

A


0.4
0.3
0.2
0.1
0
spikes generated/nC NMDA
*
12
15
Group A
Group B

B

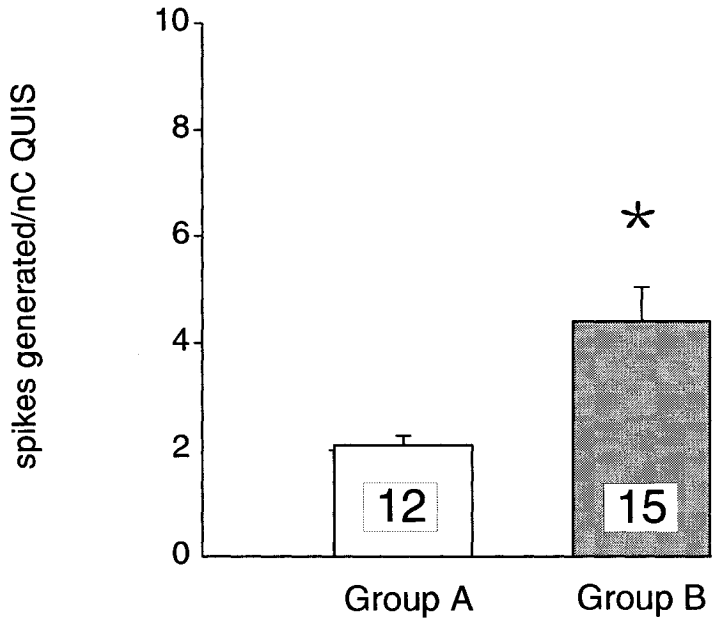

10
8
6
4
2
0
spikes generated/nC QUIS
*
12
15
Group A
Group B

Figure 6

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Chapter 5

The potential antidepressant OPC-14523 a combined sigma and 5-HT$_{1A}$ receptor ligand: Modulation of glutamatergic responses in the hippocampus

We have previously demonstrated in Chapters II and III, that sigma ligands which act as agonists by potentiating the NMDA response in the dorsal hippocampus can also modulate serotonergic neurotransmission in the DRN.

A new compound called OPC-14523 has affinity for sigma and 5-HT$_{1A}$ receptors in addition to 5-HT reuptake blocking properties. This compound has been shown in behavioural paradigms to have "antidepressant-like" effects, which were blocked by the sigma$_1$ antagonist NE-100 and the 5-HT$_{1A}$ antagonist WAY 100635. These results suggested that both OPC's sigma and 5-HT$_{1A}$ receptor affinities may contribute to its observed "antidepressant-like" effects.

Thus, the first series of experiments using OPC assessed its activity at sigma receptors using our paradigm of electrophysiological recording from the dorsal hippocampus. We assessed OPC's effects on both QUIS and NMDA-mediated responses, in turn, determining whether it would act as a sigma agonist or antagonist in this paradigm.

Assessing OPC's effects on glutamatergic neurotransmission has relevance to its potential as an antidepressant, as previously discussed, due to the interaction between NMDA-mediated transmission and depression and/or antidepressants.

THE POTENTIAL ANTIDEPRESSANT OPC-14523 A COMBINED SIGMA AND 5-HT_{1A} RECEPTOR LIGAND: MODULATION OF THE GLUTAMATERGIC RESPONSES IN THE HIPPOCAMPUS

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ABSTRACT

Rationale: OPC-14523 (1-[3-[4-(3-chlorophenyl)-1-piperazinyl]propyl]-5-methoxy-3,4-dihydro-2-quinolinone monomethanesulfonate, OPC) is a novel compound with high affinity for sigma and 5-HT (serotonin)$_{1A}$ receptors as well as for the 5-HT transporter. OPC has previously been shown to produce "antidepressant-like" effects in animal models of depression, which were attributed to its action on sigma and 5-HT_{1A} receptors. *Objective*: In this project we tested whether OPC presented a sigma agonist or antagonist profile in the modulation of glutamatergic responses. *Methods*: We used an electrophysiological model of *in vivo* extracellular unitary recording to assess the effect of OPC on the *N*-methyl-D-aspartate (NMDA)- and Quisqualic acid (QUIS)-induced responses in the hippocampus. *Results*: OPC (20 or 50 μg/kg) induced a potentiation of the NMDA- and QUIS-induced responses. This potentiation was not reversed by the $sigma_1$ antagonist N,N-dipropyl-2-(4-methoxy-3-(2-phenylethoxy)phenyl)-thylamine (NE-100) but was reversed by the non-selective sigma antagonist haloperidol. At higher doses (100 μg/kg), OPC had no effect alone but antagonized the potentiation induced by the sigma agonist 1,3-di-(2-

tolyl)guanidine (DTG). The 5-HT_{1A} antagonist WAY 100635 did not reverse the potentiating effects of OPC. Furthermore, the 5-HT_{1A} agonist 8-OH-DPAT, had an attenuating or no effect on the QUIS- or NMDA-induced neuronal responses, respectively. *Conclusions*: OPC presents an agonist effect and a bell-shaped dose-response curve similar to other sigma ligands and these effects in the hippocampus are mainly due to its action at sigma rather than 5-HT_{1A} receptors. These results suggest a modulatory effect of OPC on glutamatergic neurotransmission that may have implications towards its antidepressant potential.

1. INTRODUCTION

The existence of sigma receptors was first reported by Martin et al. in 1976 who initially classified them as belonging to the opiate receptor family. The racemic form of the prototypical ligand for these binding sites, the benzomorphan N-allyl-normetazocine (SKF-10,047), similarly to most of the earliest sigma ligands, possesses a relatively high affinity for both sigma and phencyclidine (PCP) receptors. A clear distinction between the physiological roles of these two receptors was thus long to establish. It is only later that data obtained with selective ligands have provided a better knowledge of the nature and the function of sigma receptors. The existence of at least two receptors, denoted $sigma_1$ and $sigma_2$ is now accepted, based on different pharmacological profiles and different responses to various treatments (Quirion et al. 1987, 1992).

In the last few years, we have used an *in vivo* electrophysiological paradigm whereby unitary extracellular recordings from pyramidal neurons from CA_3 regions of the dorsal hippocampus permitted the assessment of the modifications of their response to microiontophoretic applications or intravenous administrations of various drugs. Using this model, we measured the effect of several sigma ligands on the neuronal firing activity by comparing the responsiveness to microiontophoretic applications of the excitatory amino acids N-methyl-D-aspartate (NMDA), and quisqualate (QUIS).

We have found that the intravenous administration of low doses of several sigma ligands including 1,3-di-(2-tolyl)guanidine (DTG), (+)-pentazocine, 1-benzylspiro[1,2,3,4-tetrahydronaphthalene-1,4-piperidine (L-687-384), and (+)-N-

cyclopropylmethyl-N-methyl-1,4-diphenyl-1-1-ethyl-but-3-en-1-ylamine hydrochloride (JO-1784) did not affect the spontaneous firing activity of dorsal hippocampus CA_3 pyramidal neurons, but produced a marked and selective dose-dependent potentiation of NMDA-induced firing activity (Monnet et al. 1990; 1992; Bergeron et al. 1995; Debonnel et al. 1996; Bergeron and Debonnel 1997). However, the intravenous administration of low doses of the sigma ligands haloperidol, α-(4-fluorophenyl)-4-(5-fluoro-2-pyrimidinyl)-1-piperazinebutanol (BMY-14802), 3-(3-hydroxyphenyl)-N-(1-propyl) piperidine ((+)3-PPP), and N,N-dipropyl-2-(4-methoxy-3-(2-phenylethoxy)phenyl)-thylamine (NE-100) did not modify NMDA-induced firing activity, but prevented and reversed the effects of sigma agonists, thus presenting an antagonist profile (Monnet et al. 1992, Bergeron et al. 1995; Bergeron and Debonnel 1997).

We have also shown that the potentiating effect of these sigma ligands on the NMDA response, displayed a bell-shaped dose-response curve. At low doses, the potentiating effects increased progressively and then decreased at "high" doses and completely disappeared at doses higher than 500 μg/kg (Bergeron et al. 1995; Bergeron and Debonnel 1997). At these "high" doses, the sigma "agonists" acted as "antagonists", as they prevented the effect of low doses of other sigma "agonists" (Bergeron et al. 1995). Several laboratories have confirmed our data on the modulation of the NMDA response as well as the bell-shaped dose response curves of the effects of sigma ligands (Maurice et al. 1994a, b; Monnet et al. 1996).

1-[3-[4-(3-chlorophenyl)-1-piperazinyl]propyl]-5-methoxy-3,4-dihydro-2-quinolinone monomethanesulfonate (OPC) is a novel compound with high affinities for $sigma_1$ (IC_{50}=47nM), $sigma_2$ (IC_{50}=56nM) and 5-HT_{1A} (IC_{50}=2.3 nM) receptors as well as 5-HT reuptake inhibitory activities (IC_{50}=27 nM) (Tottori et al. 2001). We have previously demonstrated that short-term treatments with OPC modulate 5-HT neurotransmission in the dorsal raphe nucleus (Bermack et al. *In Press*). In the present series of experiments we assessed the effects of OPC in our electrophysiological model and established dose-response curves of its effect on the NMDA and QUIS-induced responses, at doses between 5 and 1000 μg/kg, and using a 5-HT_{1A} agonist and antagonist assessed whether its effects were mediated by the sigma and/or 5-HT_{1A} receptor.

2. MATERIAL AND METHODS

2.1. Animals

Experiments were performed in male Sprague-Dawley rats (Charles River, St.Constant, Québec) weighing 250-300g. Rats were housed under standard laboratory conditions including 12-12hr light-dark cycle and free access to food and water. Ethical committee approval was given by the McGill University Animal Ethical Care Committee and all their rules and regulations were followed.

2.2. Electrophysiological experiments

Rats were anesthetized with urethane (1.25 g/kg, i.p.) and mounted in a stereotaxic apparatus. Five-barreled glass micropipettes were used for extracellular recordings of CA_3 pyramidal neurons and microiontophoretic applications. The central barrel, used for extracellular unitary recording, was filled with a 2M NaCl solution, saturated with Fast Green FCF. One of the side barrels, used for automatic current balancing, was filled with a 2M NaCl solution. Two of the remaining side barrels were filled with QUIS (1.5 mM in 400 mM NaCl, pH: 8) and NMDA (5 mM in 200 mM NaCl, pH: 8).

Recordings were obtained in the CA_3 region of the dorsal hippocampus. Hippocampal pyramidal neurons were identified by their long duration (0.8 to 1.5 ms), large amplitude (0.5 to 2 mV) action potentials and by the presence of "complex spike" discharges in response to activation by excitatory agents (Kandel and Spencer 1961).

2.3. Activation by excitatory agents

Fifty-second alternate microiontophoretic applications of the excitatory agents NMDA and QUIS were carried out, and currents were adjusted to obtain similar and stable responses of the neuron to each agent. The degree of activation was calculated by determining the number of spikes generated/nanoCoulomb (nC).

2.4. Intravenous administration of OPC-14523

The effects of the acute intravenous administration of OPC were measured by comparing the degree of activation induced by the abovementioned excitatory substances before, and five minutes after, the injection of OPC. Results were calculated by averaging the effects of three successive applications of each of the excitatory substances. One dose was tested in one rat while recording from one neuron. The ratio (N_2/N_1) representing the number of spikes generated/nC by NMDA and QUIS (N_1), before and after (N_2), OPC's intravenous injection, was used to assess the effect of OPC. A dose-response curve for OPC (5-1000 μg/kg) was constructed for the NMDA- and QUIS-induced responses. We also assessed whether the effects of OPC (20 or 50 μg/kg) were antagonized by the $sigma_1$ antagonist NE-100 (100 μg/kg), the non-selective sigma antagonist haloperidol (100 μg/kg) or the 5-HT_{1A} antagonist WAY 100635 (100μg/kg).

We investigated the possibility that OPC could acts as a sigma "antagonist" by administering the sigma agonist DTG (5 μg/kg i.v.), which has previously been demonstrated to potentiate the NMDA response (Monnet et al.,

1990, 1992). DTG was administered and the response to NMDA and QUIS recorded similar to described above, OPC was then injected. The "antagonist" effects were assessed by determining the ratio (N_3/N_2), where N_2 represents the number of spikes generated/nC of an excitatory substance after the intravenous injection of the "agonist" and (N_3) represents the number of spikes generated/nC after the administration of OPC.

As a comparison, we assessed the effects of the 5-HT_{1A} agonist 8-OH-DPAT in this paradigm on both the NMDA- and QUIS-induced responses, using a dose of 4 and 20 μg/kg (i.v.) and assessed the number of spikes generated per nC, similarly to what was done with OPC, before and after the administration of the 5-HT_{1A} antagonist WAY 100635 (100 μg/kg i.v.).

2.6 Statistical Analysis

Statistical analysis was performed using the software SigmaStat for Windows Version 4.0 (Jandel Corporation). One-way ANOVA was used with alpha=0.05, followed by a post-hoc analysis using Tukey's Method of comparison versus controls. $P< 0.05$ was considered statistically significant for all tests.

A dose-response curve for OPC was constructed for the NMDA and QUIS-induced responses using the best-fit equation [log-normal]$y=a+bexp(-0.5(\ln(x/c)/d)^2)$ determined by the software TableCurve Version 4.

2.7 Drugs

NMDA and QUIS were purchased from Tocris Cookson Inc. (Ellisville, MO, USA), 8-OH-DPAT, WAY 100635 and haloperidol were purchased from Sigma Aldrich Canada Limited (Oakville, ON, Canada), OPC was provided by Otsuka Pharmaceutical Co. Ltd (Tokushima, Japan), NE-100 was a gift from Taisho Pharmaceutical Co. Ltd (Tokyo, Japan).

3. RESULTS

In this series of experiments, we compared the effects of the intravenous administrations of 5, 20, 50, 100, 250, 500 and 1000 μg/kg doses of OPC, to construct dose-response curves of its effects on NMDA- and QUIS- induced activations. Figure 1 depicts a representative tracing of a CA_3 pyramidal neuron recorded from the dorsal hippocampus recorded during subsequent intravenous injections.

A 5μg/kg dose of OPC had no significant effect on QUIS- or NMDA-induced activations. Doses of 20 or 50 μg/kg of OPC induced a significant increase of the NMDA response (compared to controls 0.35 ± 0.05 n=18 vs. 0.14 ± 0.02 n=18 [F=10.43 P< 0.001, Tukey's Test q=6.39]) (Figures 1 and 2). OPC also induced an increase of the QUIS-induced firing activity of the same magnitude (compared to controls 7.21 ± 1.00 n=18 vs. 3.78 ± 0.45 n=18 [F=5.74 P= 0.007, Tukey's Test q=3.94]) (Figures 1 and 2). When comparing the ratios N_2/N_1 of OPC on QUIS- and NMDA-induced activations, OPC induced the same level of potentiation of the two excitatory amino acids (Figure 3).

A dose-response curve for OPC (5-1000 μg/kg) was constructed for the NMDA and QUIS-induced responses (Figure 4). When higher doses of OPC were used (>100 mg/kg), the QUIS and NMDA responses were not significantly changed (Figure 4).

The potentiating effects of OPC (20 μg/kg) on NMDA and QUIS responses were not reversed by the subsequent administration of the $sigma_1$ antagonist NE-100 (100 μg/kg) (Figures 1 and 2), however, the nonselective

sigma antagonist haloperidol did reverse the potentiation of the NMDA but had no effect on the potentiation of the QUIS response induced by OPC (Figure 5).

We also assessed the effect of a "high dose" of OPC on the level of potentiation induced by the prior administration the sigma ligand, DTG. DTG, administered at a dose of 5 μg/kg induced a potentiation of the NMDA response (compared to controls 0.19 ± 0.02 n=6 vs. 0.07 ± 0.01 n=6 [F=19.20 P< 0.01, Tukey's Test q=8.15]). Following the subsequent administration of OPC, at a dose of 100 μg/kg, the potentiation of the NMDA response was reversed (compared to controls 0.09 ± 0.01 n=6 vs. 0.07 ± 0.01 n=6, n.s.) (Figure 6). Furthermore, a second injection of DTG following that of OPC was unable to potentiate the NMDA response as opposed to prior to OPC injection.

Finally, to assess the potential role of OPC's affinity for 5-HT_{1A} receptors in this paradigm, we tested whether the 5-HT_{1A} antagonist WAY-100635 could reverse the effects of OPC (50 μg/kg). WAY 100635 at a dose of 100 μg/kg did not reverse the effects of OPC, in contrast, the NMDA response was further potentiated (WAY 100635 versus controls 0.65 ± 0.09 n=15 vs. 0.26 ± 0.03 n=15 [F=8.15 P= 0.001, Tukey's Test q=5.39]), while there was no significant effect on the QUIS-induced response (WAY 100635 versus controls 10.20 ± 1.25 n=15 vs. 6.86 ± 0.73 n=15, n.s.) (Figure 7).

In addition, we tested the effects of the 5-HT_{1A} agonist 8-OH-DPAT on the NMDA- and QUIS-induced responses. A dose of 4 μg/kg had no effect on either the QUIS- or NMDA-induced neuronal responses (data not shown), however, at a dose of 20 μg/kg, 8-OH-DPAT did have a significant attenuating effect on QUIS-

mediated neurotransmission (compared to controls 2.28 ± 0.43 n=15 vs. 5.57 ± 0.70 n=15 [F=9.91 P< 0.001, Tukey's Test q=5.21]), which was reversible by the 5-HT_{1A} antagonist WAY 100635 (WAY versus controls 5.86 ± 0.73 n=15 vs. 5.57 ± 0.70 n=15, n.s.). There was no significant effect of 8-OH-DPAT on NMDA-mediated transmission, but subsequent WAY-100635 administration did induce a significant increase in the NMDA-induced response (WAY 100635 versus controls 0.76 ± 0.12 n=15 vs. 0.30 ± 0.07 n=15 [F=9.52 P< 0.001, Tukey's Test q=5.35]) (Figure 8). 8-OH-DPAT at either dose had no significant effect on the basal firing activity of CA_3 pyramidal neurons (data not shown).

4. DISCUSSION

In the CA_3 region of the dorsal hippocampus, OPC induced a potentiation of the NMDA- induced response at doses of 20 and 50 μg/kg. The dose-response curve of the effects of OPC at doses of 5-1000 μg/kg, presented a bell-shaped curve such that at "high doses" (>100 μg/kg) OPC had no effect by itself. This type of bell-shaped dose-response curve has previously been described for various sigma ligands by our laboratory, such that, when administered at doses high enough, sigma agonists including (+)-pentazocine, DTG and JO-1784 had no effect on their own (Bergeron et al., 1995, 1997). A bell-shaped dose-response curve has also been demonstrated with sigma ligands in other models by other laboratories. For example, in NMDA-evoked release of [^{3}H]-noradrenaline from preloaded rat hippocampal slices, JO-1784 and (+)-3-PPP demonstrated bell-shaped dose-response curves (Monnet et al., 1996). In behavioural models using 5-methyl-10,11-dihydro-5*H*-dibenzo [a,d] cyclohepten-5,10-imine (MK-801)-induced amnesia, DTG and (+)-pentazocine yielded bell-shaped dose-response curves in reversing amnesia (Maurice et al., 1994 a,b). As hypothesized previously, the most likely explanation of these types of dose-response curves is the existence of several subtypes of the $sigma_1$ receptor and that higher doses may lead to the activation of a subtype for which the ligand possesses lesser affinity (Bergeron et al., 1995). The fact that the effects of OPC were not reversed by the $sigma_1$ antagonist NE-100, and that NE-100 has been shown to reverse only some of the effects produced by $sigma_1$ ligands (Bermack et al. 2001), suggests that OPC is acting on a different subtype of the $sigma_1$ receptor compared to NE-100.

Alternatively, this could suggest that the potentiation of the NMDA- response was not mediated through sigma receptors. However, the nonselective sigma antagonist haloperidol did block OPC's effects suggesting more likely the involvement of a specific subtype of the $sigma_1$ receptor.

In agreement, there is already a large body of evidence suggesting the existence of several subtypes of $sigma_1$ receptors from our laboratory (Monnet et al. 1994; Debonnel et al. 1996; Bermack and Debonnel, 2001; Couture and Debonnel, 2001) as well as other laboratories (Vilner et al., 1995; Tsao and Su, 1997). For example, pertussis toxin blocked the effect of DTG and JO-1784, but not that of (+)-pentazocine, on the NMDA-induced neuronal response in the dorsal hippocampus (Monnet et al., 1994). Mossy fiber lesioning blocked the effects of DTG and JO-1784 but not those of (+)-pentazocine (Debonnel et al., 1996). In addition to the electrophysiological data, binding studies have suggested numerous subtypes of the $sigma_1$ receptor as (+)-pentazocine was found to bind to 2 or 3 distinct binding sites *in vitro* (Basile et al., 1992; Vilner et al., 1995). Furthermore, Tsao and Su (1997) purified a naloxone-sensitive, haloperidol-sensitive (+)-SKF-10047 binding protein, which bound (+)-pentazocine but did not bind DTG, (+)-3-PPP nor progesterone, thus also suggesting at least a second $sigma_1$ receptor subtype. The data suggests that NE-100, a selective $sigma_1$ antagonist, appears to be only able to block one subtype of the $sigma_1$ receptor. To this end, OPC's effects on the NMDA response are likely mediated by an NE-100-insensitive $sigma_1$ receptor.

At its maximum intensity, the potentiation in the NMDA response induced by OPC was about 100%. This is smaller than the results obtained previously with other sigma agonists including DTG, (+)-pentazocine and JO-1784, which could induce up to 300 % potentiation of the NMDA response (Monnet et al. 1990; Bergeron et al. 1995). However, OPC's effects are still significant, but the smaller degree of potentiation induced by OPC compared to other $sigma_1$ ligands may suggest a partial agonist profile for OPC. This could also explain the current results demonstrating high doses of OPC's ability to antagonize the effects of DTG. "High" doses of sigma ligands have previously demonstrated antagonist properties, having no effect on their own yet blocking the potentiating effects of low doses of sigma agonists (Bergeron et al., 1995, 1997).

The potentiating effect of OPC was also observed on the QUIS- induced response, an effect that has been found previously with the sigma ligands (+)-cis-N-[2,(3,4-dichlorophenyl)ethyl]-2-(1-pyrrolidinyl-cyclohexlamine (BD-737) (Bergeron and Debonnel, 1997) and 4-IBP (Bermack and Debonnel, 2002). These effects on QUIS-induced responses may be due to these particular sigma ligands' action at a particular subtype of the sigma receptor not yet fully characterized.

An increase in glutamatergic neurotransmission may be involved in the pathophysiology of depression, and NMDA receptors have been implicated as NMDA antagonists have been reported to produce antidepressant effects (Drevets, 1999; Krystal et al. 2002; Paul and Skolnick, 2003). Specifically, NMDA antagonists have shown antidepressant-like effects in preclinical models of depression and produced synergistic effects when combined with classical

antidepressants (Trullas and Skolnick, 1990; Papp and Moryl, 1993; Rogoz et al., 2002). Furthermore, antidepressants have been shown to alter NMDA-receptor mediated effects (Maj et al., 1992; Nowak et al., 1998), which may be a crucial step in their antidepressant action (Paul et al., 1994; Paul and Skolnick, 2003).

This interaction between antidepressants and the NMDA receptor is of particular significance for our data, as OPC with its NMDA-modulating activity, in addition to its effects on serotonergic neurotransmission (Bermack et al. *In Press*), may produce antidepressant-like effects beyond those obtained with an agent targeting either system alone. Therefore, if one assumes an increase in glutamatergic neurotransmission in depressive states the bell-shaped agonist profile of OPC may lead to a decrease in NMDA-mediated responses. Our experiments performed in anesthetized "normal" rats may not mimic the glutamatergic activity in depression and the effects of antidepressants (or sigma ligands), as the glutamatergic system might be better assessed under pathological conditions.

It might have been plausible that the effects on the NMDA- and QUIS-induced responses were due to OPC's affinity for the 5-HT_{1A} receptor and not the $sigma_1$ receptor. However this was ruled out by our results demonstrating that the 5-HT_{1A} antagonist WAY 100635 could not reverse the potentiations of the NMDA- and QUIS-responses induced by OPC. Furthermore, the lack of effect of the 5-HT_{1A} agonist 8-OH-DPAT and its inhibitory effect on QUIS-induced responses at higher doses further suggest the effects of OPC seen in this

experimental paradigm are mediated through $sigma_1$ receptors and do not involve the 5-HT_{1A} receptor.

Previous evidence suggests that 5-HT_{1A} agonists attenuate NMDA-receptor mediated transmission (Srkalovic et al., 1994; Strosznajder et al., 1996; Madhavan et al., 2003), in a WAY 100635-dependent manner (Pugliese et al., 1998). Therefore, in our paradigm, one would expect a decrease in the NMDA-induced response following 8-OH-DPAT, but this was not found, possibly due to the fact that 8-OH-DPAT was administered intravenously therefore could be exerting compensatory effects in other populations of 5-HT_{1A} receptors in brain regions other than the hippocampus. However, the activation of 5-HT_{1A} receptors on pyramidal neurons by OPC may explain its narrower dose-response curve and smaller potentiation induced as compared to other $sigma_1$ ligands.

An interesting finding with respect to the effect of 5-HT_{1A} agonists in these experiments was that the 5-HT_{1A} antagonist WAY 100635 (100 μg/kg) produced a significant increase in the NMDA- and QUIS-induced responses when administered subsequent to 8-OH-DPAT (20 μg/kg). The fact that an antagonist would induce a larger potentiation suggests a tonic activation of the postsynaptic 5-HT_{1A} receptors in the dorsal hippocampus in this paradigm. This is in contrast to previous results from our laboratory, which showed no tonic activation of these postsynaptic receptors (Haddjeri et al., 1998). However, there is one significant difference in the present experimental paradigm, that being the recurrent application of NMDA in our study (which is kept comparable to QUIS throughout the experiment). Thus, perhaps by repetitively applying NMDA, an endogenous

stimulation of 5-HT_{1A} receptors was produced by ascending 5-HT projections. Projections back from the hippocampus provide feedback and modulate 5-HT neurotransmission from the raphe nuclei. This involves glutamatergic excitatory connections from the hippocampus directly or indirectly via the lateral habenular nuclei (Levine and Jacobs 1992; Peyron et al., 1998; Lee et al., 2003). Evidence for this includes the observations that the 5-HT firing activity in the dorsal raphe nucleus increases in response to glutamate administration (Vandermaelen et al., 1986) and NMDA elicits increased 5-HT release from primary raphe cultures (Becquet et al., 1993). Moreover, tonic activation of postsynaptic 5-HT1A receptors in the dorsal hippocampus has been demonstrated in unanaesthetized rats (Fornal et al. 1996, Kasamo et al. 2001).

This interaction between 5-HT and NMDA receptors could provide an additional explanation as to why 8-OH-DPAT had no effect on the NMDA response, if the postsynaptic 5-HT_{1A} receptors are already activated, and thus explaining the effects of WAY 100635 as well. In addition, 5-HT_{1A} receptor activation can also attenuate AMPA (α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid) receptor-mediated currents (Cai et al., 2002), which could explain the reductions of QUIS-induced responses induced by 8-OH-DPAT and the subsequent potentiation induced by WAY 100635 in the current study. Thus, through both NMDA- and QUIS-induced responses, OPC generates a potentiation of glutamatergic transmission such that the sigma properties of OPC appear to counteract the attenuating effects of 5-HT_{1A} receptor activation, at least at lower doses (≤50 μg/kg). More studies are necessary to elucidate the role of endogenous

5-HT on modulating the NMDA- and QUIS-mediated neurotransmission in the hippocampus and the possible implications of this modulation. However, for the purpose of this study, it is clear that the effects of OPC on the glutamatergic neurotransmission are mediated primarily by $sigma_1$ not $5\text{-}HT_{1A}$ receptors.

In conclusion, OPC appears to exert, at low doses, an agonistic effect on $sigma_1$ receptors. This, in itself could be sufficient to produce an antidepressant effect since several $sigma_1$ agonists in addition to OPC have been shown to increase the firing activity of dorsal raphe 5-HT neurons (Bermack and Debonnel, 2001, Bermack et al. *In Press*). In addition, the current data supports OPC having a modulating effect on glutamatergic neurotransmission mediated by its sigma agonist properties, and when combined with its effects on serotonin neurotransmission (Bermack et al. *In Press*) may provide increased clinical potential.

FIGURE LEGENDS

Figure 1

Integrated firing rate histogram of a CA_3 dorsal hippocampus pyramidal neuron illustrating the potentiating effect of the intravenous administration of OPC on QUIS- and NMDA-induced activations, and the subsequent administration of the sigma antagonist NE-100. In this and the following histograms, bars indicate the duration of applications for which currents are given in nA. Open circles (oo) indicate a 5-minute interruption of the histogram.

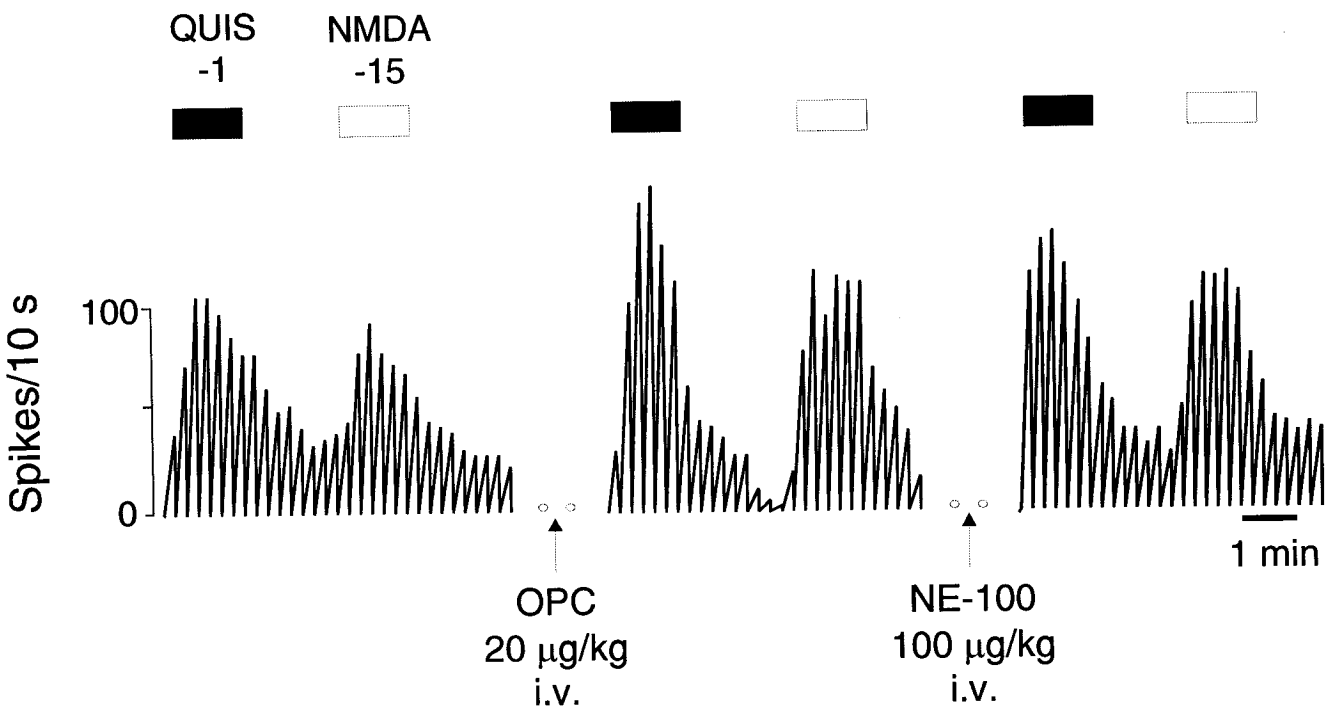
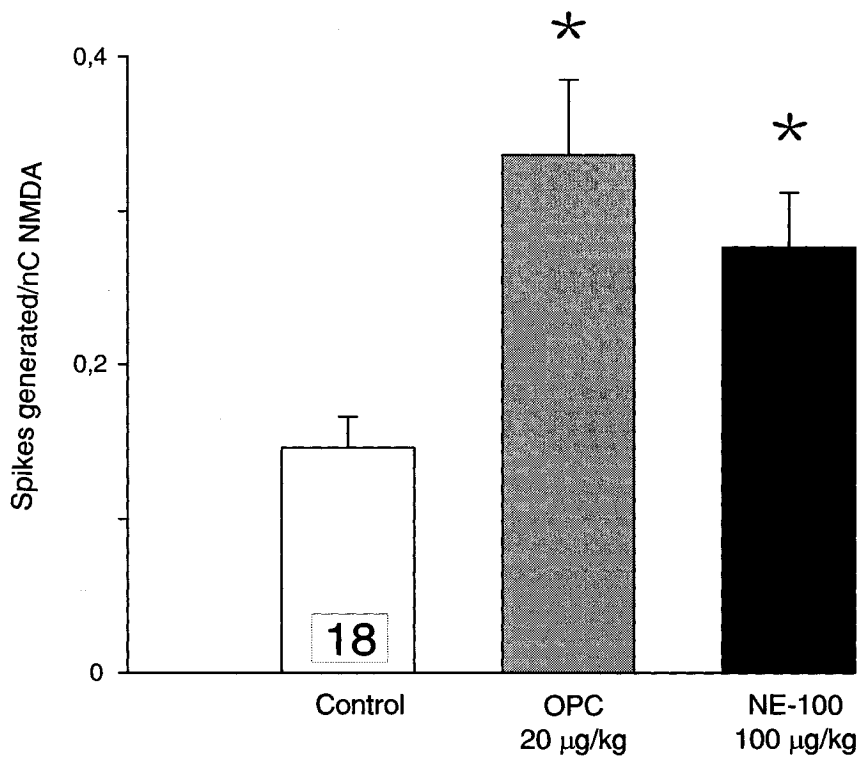

QUIS
-1
NMDA
-15
Spikes/10 s
100
0
1 min
OPC
20 µg/kg
i.v.
NE-100
100 µg/kg
i.v.

Figure 1

Figure 2

Effects of the acute administration of the sigma ligand OPC on the neuronal response to **A)** NMDA- or **B)** QUIS-induced firing activities of CA_3 hippocampal pyramidal neurons, expressed as the number of spikes generated/nC before (open column), after (grey column) the intravenous administration of 20 μg/kg of OPC, and after (black column) the subsequent intravenous administration of 100 μg/kg of the $sigma_1$ antagonist NE-100. In this and all subsequent bar graphs the number in the box at the bottom of each column represents the number contributing to the average * $p < 0.05$

A


Spikes generated/nC NMDA
0,4
0,2
0
*
*
18
Control
OPC
20 µg/kg
NE-100
100 µg/kg

B

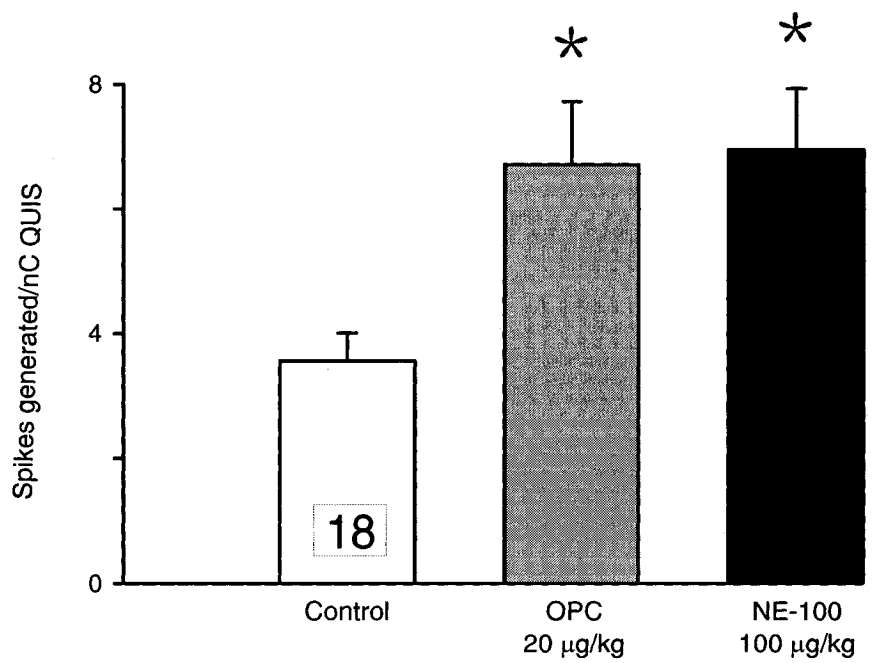

Spikes generated/nC QUIS
8
4
0
*
*
18
Control
OPC
20 µg/kg
NE-100
100 µg/kg

Figure 2

Figure 3

Effects of the acute administration of the sigma ligand OPC on the neuronal response to NMDA and QUIS-induced firing activities of CA_3 hippocampal pyramidal neurons expressed as the ratio N_2/N_1 of the number of spikes generated/nC before and after the intravenous administration of 20 μg/kg of OPC.

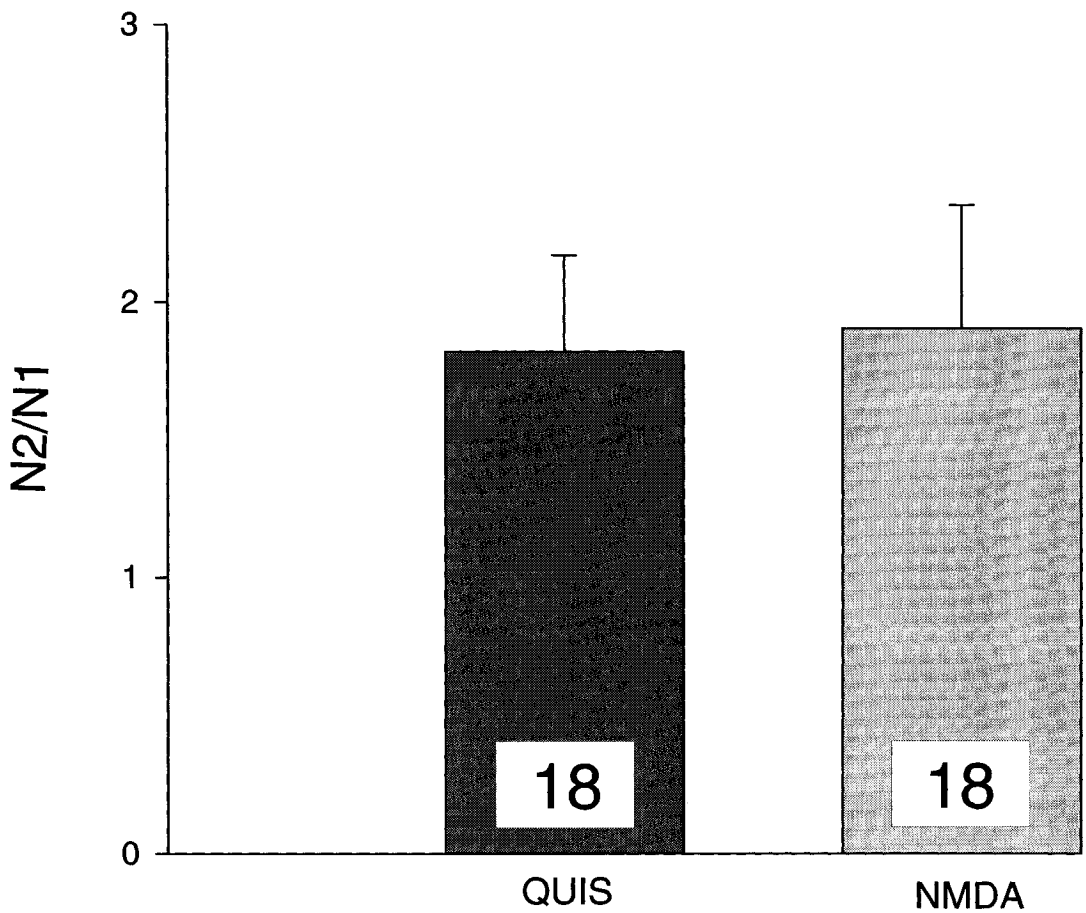
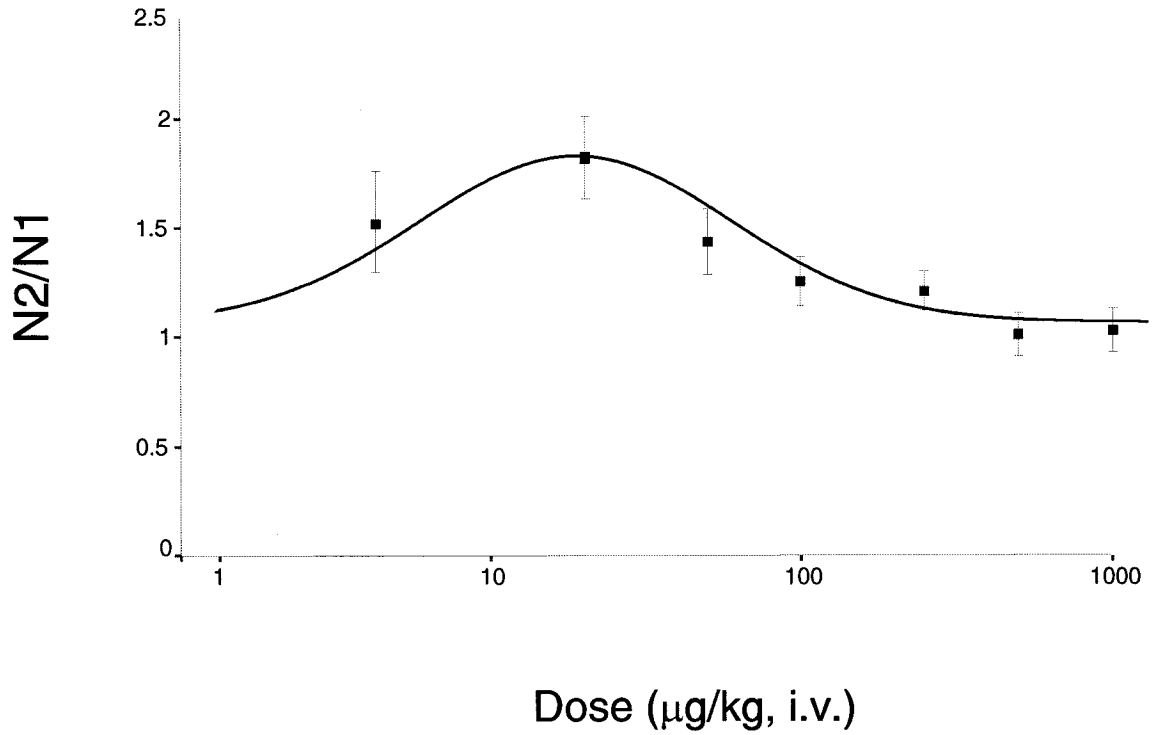


Figure 3

Figure 4

Dose-response curves of the effects of the acute administration of the sigma ligand OPC on the neuronal response to (**A**) NMDA- and **(B)** QUIS-induced firing activities of CA_3 hippocampal pyramidal neurons expressed as the ratio N_2/N_1 of the number of spikes generated/nC before and after the intravenous administration of OPC (20-1000 μg/kg).

A


N2/N1
2.5
2
1.5
1
0.5
0
1
10
100
1000
Dose (μg/kg, i.v.)

B

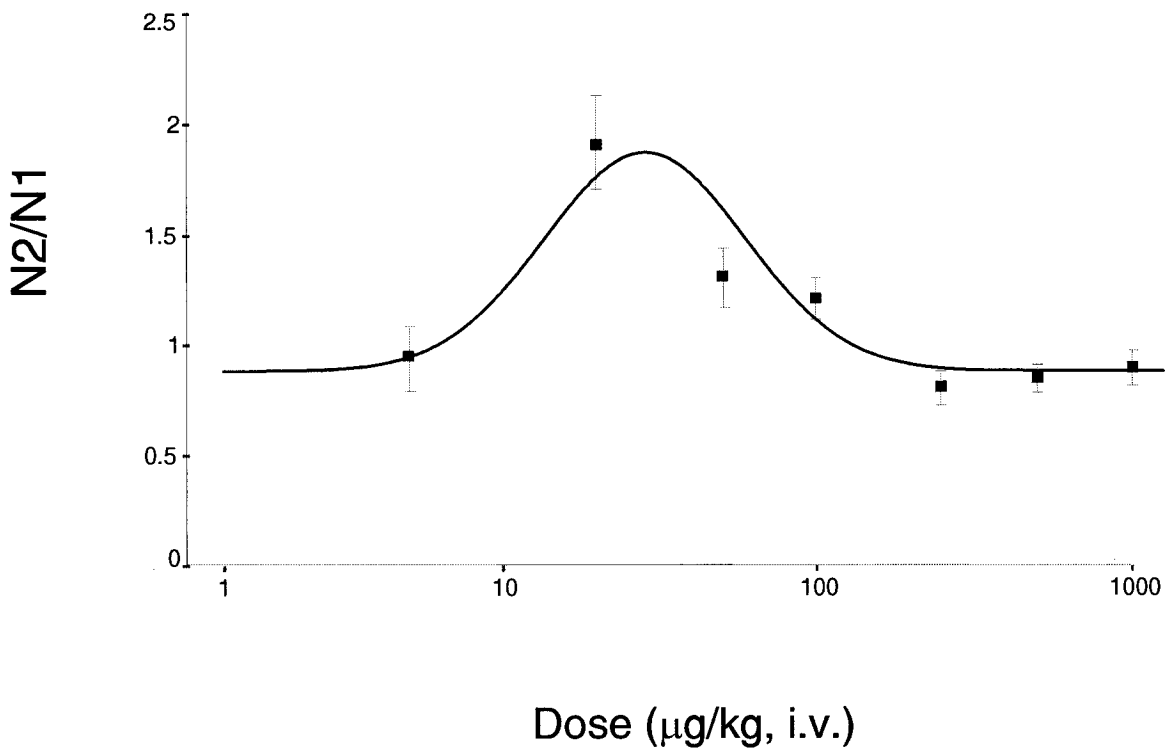

N2/N1
2.5
2
1.5
1
0.5
0
1
10
100
1000
Dose (μg/kg, i.v.)

Figure 4

Figure 5

Effects of the acute administration of the sigma ligand OPC on the neuronal response to **A)** NMDA- or **B)** QUIS-induced firing activities of CA_3 hippocampal pyramidal neurons, expressed as the number of spikes generated/nC before (open column), after (grey column) the intravenous administration of 20 μg/kg of OPC, and after (black column) the subsequent intravenous administration of 100 μg/kg of the nonselective sigma antagonist haloperidol. * $p < 0.05$.

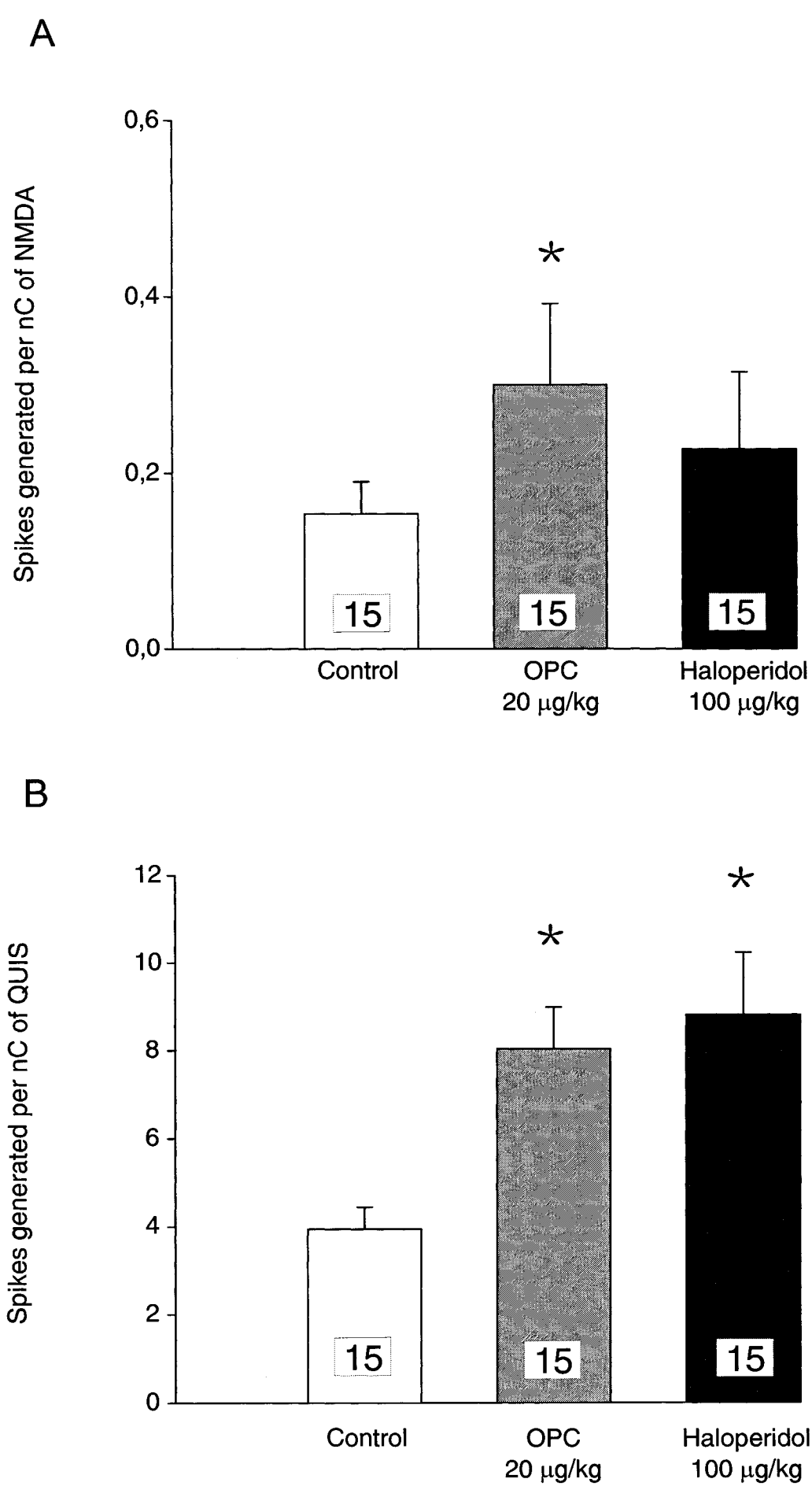
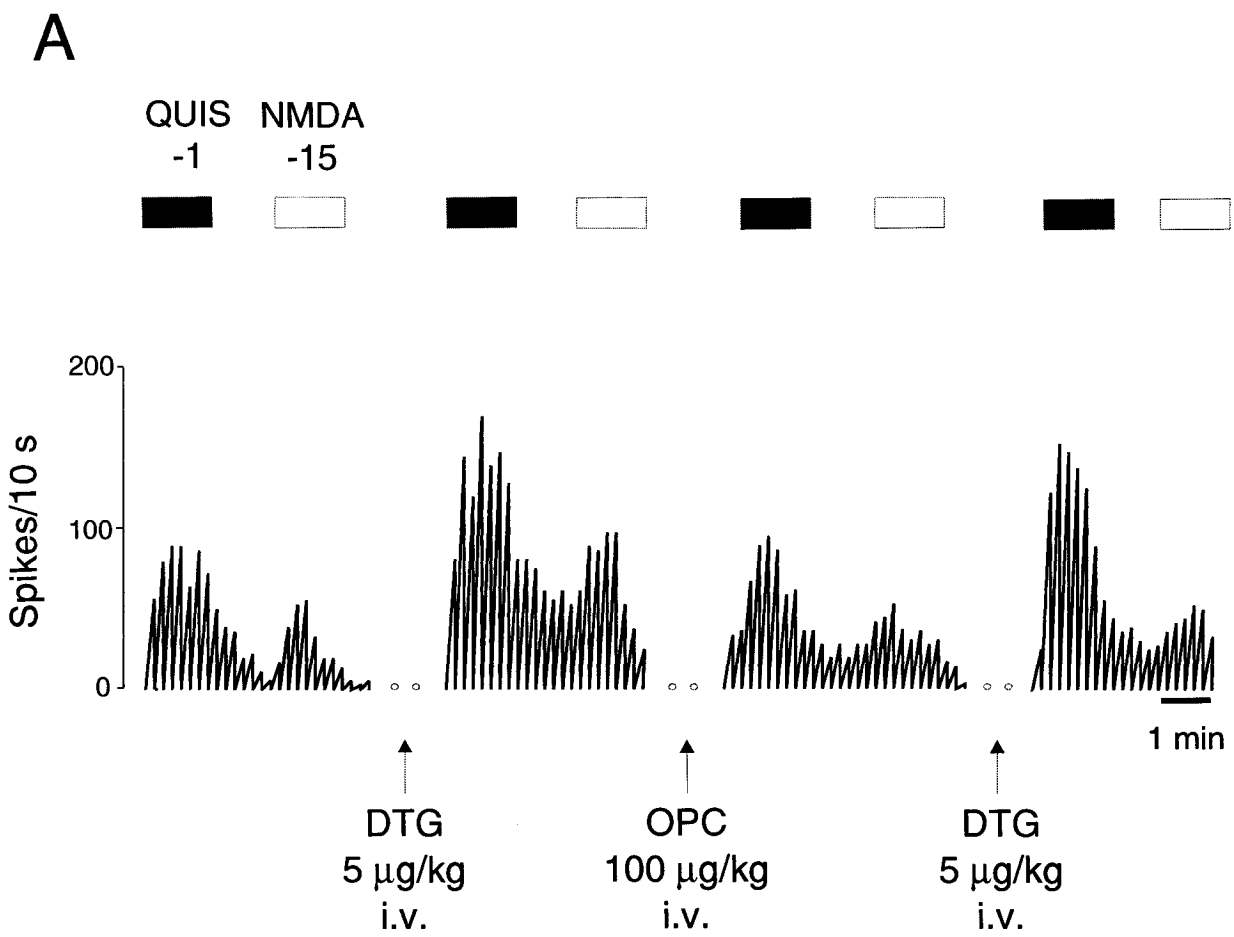

A
Spikes generated per nC of NMDA
0,6
0,4
0,2
0,0
*
15
15
15
Control
OPC
20 μg/kg
Haloperidol
100 μg/kg
B
Spikes generated per nC of QUIS
12
10
8
6
4
2
0
*
*
15
15
15
Control
OPC
20 μg/kg
Haloperidol
100 μg/kg

Figure 5

Figure 6

A) Integrated spontaneous firing activity of a CA_3 hippocampal pyramidal neuron illustrating the potentiation of the NMDA response by the intravenous administration of DTG as well as the reduction of this potentiation by the subsequent administration of OPC, which also prevents the effect of a further administration of DTG. **B)** Responsiveness expressed as the number of spikes generated per nC (mean ± SEM) of CA_3 dorsal hippocampus neurons to microiontophoretic applications of NMDA before (open columns) and after (grey columns) the intravenous administration of DTG in control rats, or following the subsequent administration of OPC. * $p < 0.05$.


A
QUIS
-1
NMDA
-15
200
100
0
Spikes/10 s
1 min
DTG
5 µg/kg
i.v.
OPC
100 µg/kg
i.v.
DTG
5 µg/kg
i.v.

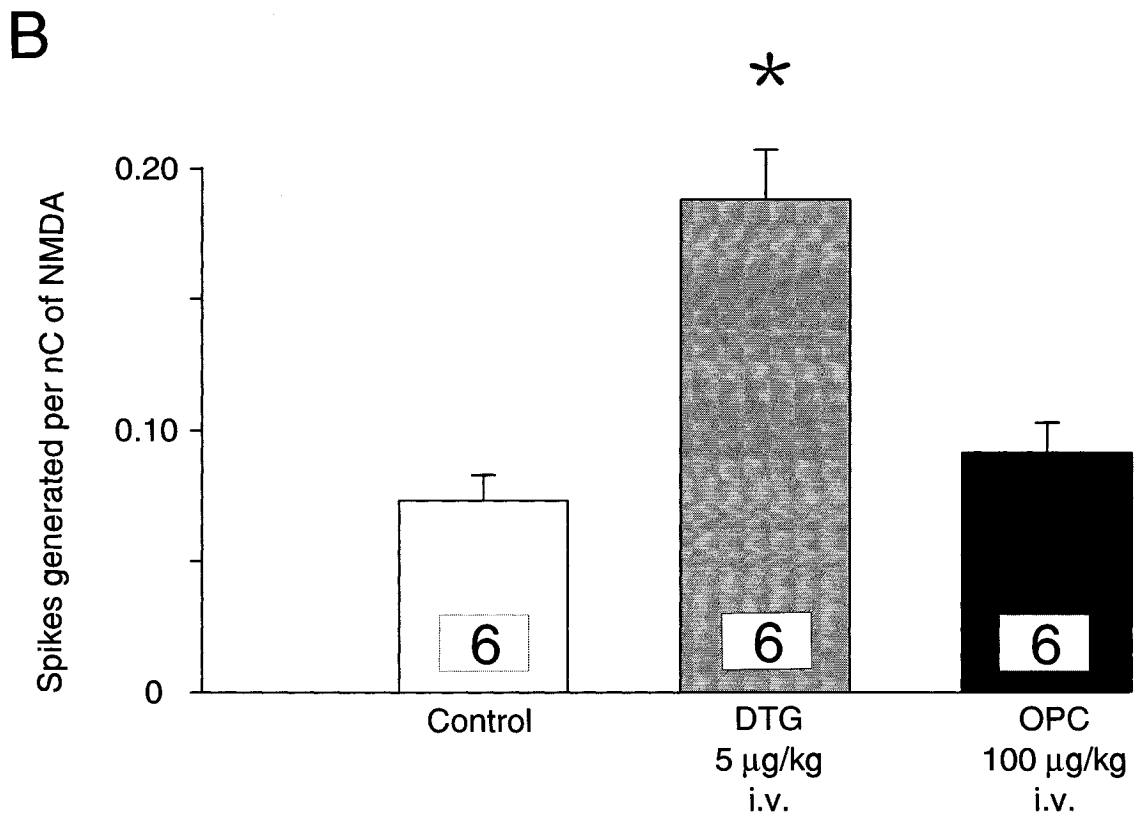
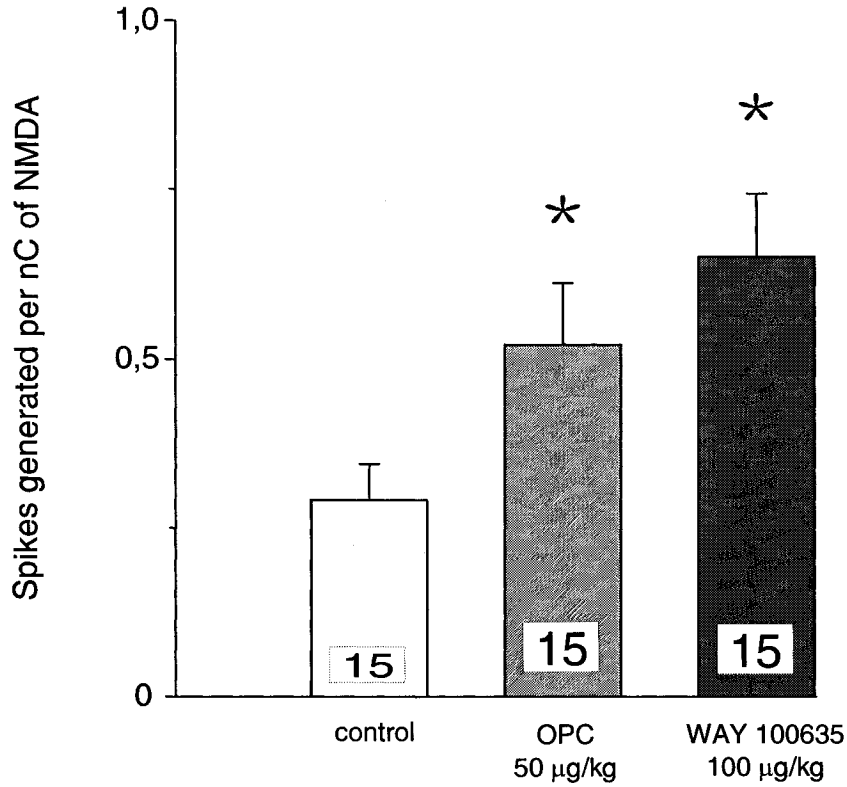

B
*
0.20
0.10
0
Spikes generated per nC of NMDA
6
6
6
Control
DTG
5 µg/kg
i.v.
OPC
100 µg/kg
i.v.

Figure 6

Figure 7

Effects of the acute administration of the sigma ligand OPC on the neuronal response to **A)** NMDA- or **B)** QUIS-induced firing activities of CA_3 hippocampal pyramidal neurons, expressed as the number of spikes generated/nC before (open column), after (grey column) the intravenous administration of 50 μg/kg of OPC, after (dark grey column) the subsequent intravenous administration of 100 μg/kg of the 5-HT_{1A} antagonist WAY 100635, and after (black column) the subsequent administration of 100 μg/kg of the nonselective sigma antagonist haloperidol. * $p < 0.05$.

A


1,0
0,5
0
Spikes generated per nC of NMDA
*
*
15
15
15
control
OPC
50 µg/kg
WAY 100635
100 µg/kg

B

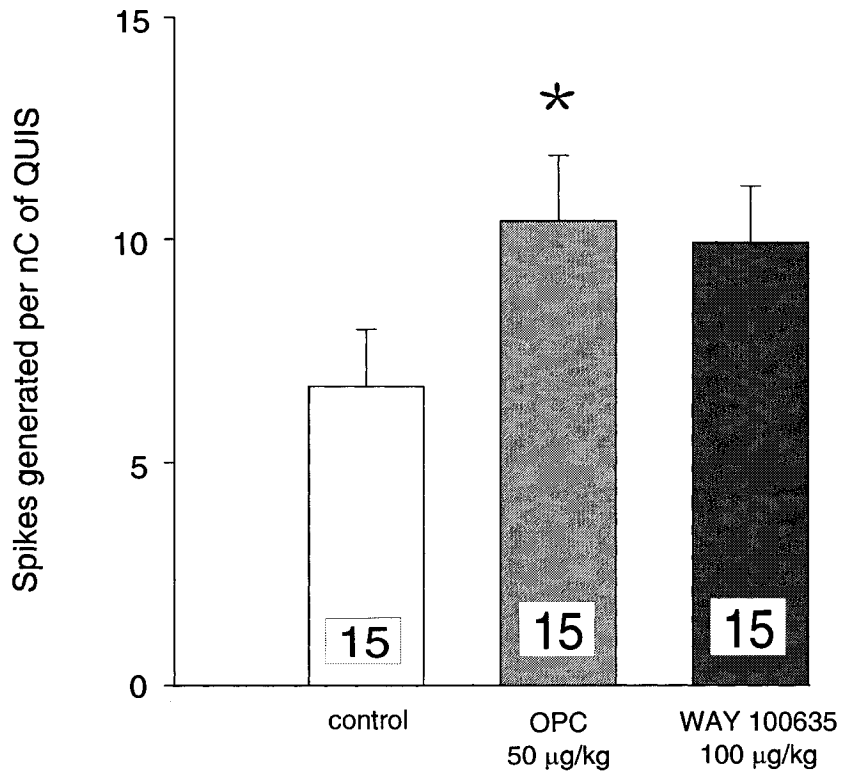

15
10
5
0
Spikes generated per nC of QUIS
*
15
15
15
control
OPC
50 µg/kg
WAY 100635
100 µg/kg

Figure 7

Figure 8

Effects of the acute administration of the 5-HT_{1A} ligand 8-OH-DPAT on the neuronal response to **A**) NMDA- or **B**) QUIS-induced firing activities of CA_3 hippocampal pyramidal neurons, expressed as the number of spikes generated/nC before (open column), after (grey column) the intravenous administration of 20 μg/kg of 8-OH-DPAT, followed by the subsequent administration of the 5-HT_{1A} antagonist WAY 100635 (100 μg/kg). * $p < 0.05$.

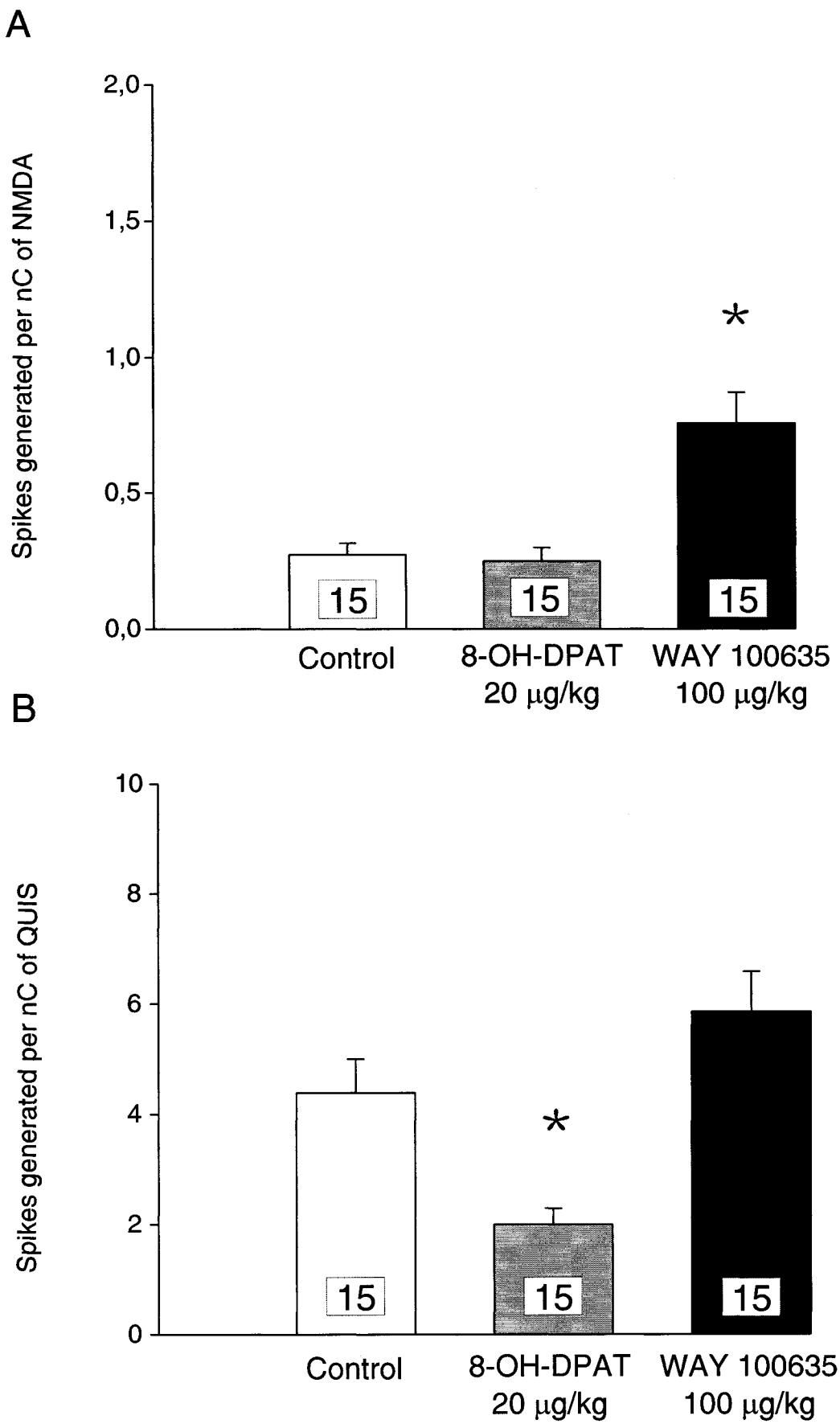

A
Spikes generated per nC of NMDA
2,0
1,5
1,0
0,5
0,0
15
15
15
*
Control
8-OH-DPAT
20 µg/kg
WAY 100635
100 µg/kg
B
Spikes generated per nC of QUIS
10
8
6
4
2
0
15
15
15
*
Control
8-OH-DPAT
20 µg/kg
WAY 100635
100 µg/kg

Figure 8

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Chapter 6

Effects of the potential antidepressant OPC-14523 a combined sigma and 5-HT_{1A} ligand: Modulation of 5-HT neuronal activity in the dorsal raphe nucleus

We have previously demonstrated that sigma ligands can induce an increase in 5-HT neurotransmission in the DRN after only 2 days of treatment (Chapter II). In contrast, most antidepressants, after 2 days of treatment, induce a decrease in DRN 5-HT firing activity. This decrease is due to the activation of 5-HT_{1A} autoreceptors and thus the activation of a negative-feedback loop that induces a suppression of the firing activity. Following chronic treatments, a restoration of the firing activity is seen, an effect thought to be due to the desensitization of the 5-HT_{1A} receptors. Accordingly, interaction of antidepressants with 5-HT_{1A} receptors, directly or indirectly, has important influences on the serotonergic system and hence a compound's antidepressant potential.

OPC has affinity for sigma and 5-HT_{1A} receptors, and we have previously shown that sigma receptor affinity alone can lead to a significant increase in 5-HT neurotransmission in the DRN. Thus it was particularly interesting to investigate how the combined 5-HT_{1A} and sigma receptor affinities would affect 5-HT neurotransmission. It is hypothesized that this combination may yield an additive effect on the net 5-HT neuronal activity. The current study assesses the function of OPC following short-term treatments on 5-HT firing activity in the DRN. In addition, the response of the 5-HT_{1A} autoreceptor is assessed to determine if OPC's 5-HT_{1A} affinity may be playing a role and more precisely whether this involves an antagonist or agonist effect at 5-HT_{1A} receptors. We then use a

selective sigma antagonist to assess if these effects are mediated via sigma or 5-HT_{1A} receptors.

EFFECTS OF THE POTENTIAL ANTIDEPRESSANT OPC-14523 A COMBINED SIGMA AND 5-HT_{1A} LIGAND: MODULATION OF NEURONAL ACTIVITY IN THE DORSAL RAPHE NUCLEUS

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Journal of Pharmacology and Experimental Therapeutics, *In Press*

ABSTRACT

OPC-14523 (OPC) is a novel compound with high affinity for sigma and 5-HT_{1A} receptors as well as for the 5-HT transporter. OPC has previously been shown to produce antidepressant-like effects in animal models of depression. This project set out to determine OPC's effect on serotonergic neurotransmission and to shed light on its mechanism(s) of action. In an electrophysiological model of *in vivo* extracellular recordings in anesthetized rats, a 2-day treatment (1 mg/kg/day), with OPC induced a significant increase in dorsal raphe nucleus (DRN) putative 5-HT neurons' firing activity. This increase was blocked by the co-administration of NE-100, a selective $sigma_1$ antagonist (10 mg/kg/day). Furthermore, after 2-day treatments with OPC, the 5-HT_{1A} autoreceptor response was altered, as demonstrated by the dramatically reduced response to an increase of endogenous 5-HT induced by the acute administration of paroxetine (500 μg/kg i.v.). However, the 5-HT_{1A} agonist 8-OH-DPAT (4 μg/kg i.v.) maintained its ability to decrease 5-HT firing activity, an effect which was reversible by the subsequent administration of the 5-HT_{1A} antagonist WAY 100635 (100 μg/kg i.v.). As 8-OH-DPAT has been shown to act preferentially through postsynaptic 5-HT_{1A} receptors, our data suggests that this effect of OPC is mediated primarily by the 5-

HT_{1A} autoreceptor. The decreased response of the 5-HT_{1A} autoreceptor to paroxetine was not blocked by the co-administration of NE-100, indicating that $sigma_1$ receptors are not involved in this effect. Thus, both sigma and 5-HT_{1A} receptors play a role in the "antidepressant-like" effects produced by OPC, which is in keeping with previously published behavioural data. In addition, the current series of experiments suggest that OPC might have potential as an antidepressant with a rapid onset of action, as compared to SSRI treatments, which initially suppress the firing activity of putative 5-HT neurons and require at least 2-3 weeks in order to restore the firing activity to baseline neuronal firing activity through a desensitization of the 5-HT_{1A} autoreceptor.

Abbreviations: 4-IBP- 4-(N-benzylpiperidin-4-yl)-4-iodobenzamide, 5-HT- serotonin, 8-OH-DPAT- 8-hydroxy-2-(di-n-propylamino)tetralin, DRN- dorsal raphe nucleus, DTG- 1,3-di-(2-tolyl)guanidine, GABA-γ-aminobutyric acid, JO-1784-(+)-N-cyclopropylmethyl-N-methyl-1,4-diphenyl-1-1-ethyl-but-3-en-1-ylamine hydrochloride, MAOI-monoamine oxidase inhibitor, NE-100- N,N-dipropyl-2-(4-methoxy-3-(2-phenylethoxy)phenyl)-thylamine, NMDA-N-methyl-D-aspartate, OPC-14523- 1-[3-[4-(3-chlorophenyl)-1-piperazinyl]propyl]-5-methoxy-3,4-dihydro-2-quinolinone monomethanesulfonate, p-CPA-chlorophenylalanine, SA-4503- 1-(3,4-dimethoxyphenethyl)-4-(3-phenylpropyl) piperazine dihydrochloride, SSRI- selective serotonin reuptake inhibitor, (+)SKF-10,047- (+)-N-allyl-normetazocine, WAY-100635-(N-[2-(4-[2-methoxyphenyl]-1-piperazinyl)ethyl]-N-2-pyridinylcyclohexanecarboxamide.

1. INTRODUCTION

An enormous amount of evidence suggests the involvement of the serotonin system in the pathophysiology of depression (Reviewed by Delgado, 2000). Electrophysiological data demonstrates that representatives from all classes of antidepressants, after long-term treatments and through various mechanisms, increase 5-HT neurotransmission (Chaput et al., 1991; Blier and de Montigny, 1994). For example, acute treatments with monoamine oxidase inhibitors (MAOI's) and selective serotonin reuptake inhibitors (SSRI's) lead to decreased firing activity of 5-HT neurons in the dorsal raphe nucleus (DRN), but as treatment continues, the 5-HT neurons regain normal firing activity due to desensitization of the 5-HT_{1A} somatodendritic autoreceptor. This desensitization has been proposed as the adaptive change that explains the delayed enhancement of 5-HT-mediated neurotransmission, which is consistent with the clinical onset of action of SSRI's (Chaput et al., 1986; Blier et al., 1988; Blier and de Montigny, 1994).

The existence of sigma receptors was initially reported by Martin et al., (1976). The existence of at least two receptors, denoted sigma_1 and sigma_2 is now accepted (Quirion et al., 1987, 1992). Sigma ligands have been implicated in the pathophysiology of depression or have been proposed as potential antidepressants. Sigma ligands such as SA-4503, (+)-pentazocine, DTG and JO-1784 have been shown to produce antidepressant-like effects in behavioural models of depression such as the Forced Swimming Test and Tail Suspension

Test and in clinical trials (Matsuno et al., 1996; Tottori et al., 1997; Kinsora et al., 1998; Ukai et al., 1998; Akunne et al., 2001; Pande et al., 1998).

We previously demonstrated that the sigma ligands 4-IBP and (+)-pentazocine produce an increase in the basal firing activity of 5-HT neurons of the DRN after both short-term (2 days) and long-term (21 days) treatments (Bermack and Debonnel, 2001). The effects of (+)-pentazocine were blocked by the co-administration of the selective $sigma_1$ antagonist NE-100, while those of 4-IBP were blocked by the co-administration of the non selective sigma antagonist haloperidol, but not NE-100 (Bermack and Debonnel, 2001).

OPC is a novel compound with high affinities for sigma and 5-HT_{1A} receptors and with 5-HT reuptake inhibitory activities (Tottori et al., 2001). Similar to other sigma ligands, OPC yielded antidepressant-like effects in animal models of depression (Tottori et al., 2001). Interestingly, the immobility time was reduced in the forced swimming test after a single dose of OPC and the daily administration for 7 days enhanced this effect (Tottori et al., 2001).

Thus, the purpose of this study was to assess the effect of acute and short-term treatments with OPC on DRN neurotransmission using an electrophysiological model of extracellular recordings of putative 5-HT neurons from the DRN. Furthermore, we assessed the effect of the treatments with OPC on the response of the 5-HT_{1A} somatodendritic autoreceptors since OPC has high affinity for 5-HT_{1A} receptors, and this could have relevance to its potential antidepressant properties.

2. MATERIAL AND METHODS

2.1. Animals

Experiments were performed in male Sprague-Dawley rats (Charles River, St. Constant, Québec) weighing 250-300g. Rats were housed under standard laboratory conditions including 12-12hr light-dark cycle and free access to food and water.

2.2. Acute Treatments

For the acute treatments, once a putative 5-HT neuron was identified and recorded for approximately 1 minute, saline or OPC were administered intravenously via the tail vein during electrophysiological recording. Five rats were tested for each dose studied, with one injection administered per rat.

2.3. Short-term Treatments

Animals were anesthetized with halothane for the subcutaneous implantation of osmotic minipumps (Durect Corporation, Cupertino, CA, USA) placed in the back of the animal. The minipumps delivered a dose of 1 mg/kg/day of OPC-14523 dissolved in 5% ethanol and distilled water. A separate series of rats were implanted with 2 pumps simultaneously; one containing OPC and the other containing NE-100 (10 mg/kg/day). The duration of all treatments were 2 days. The control groups were treated with minipumps filled with 5% ethanol and distilled water. Electrophysiological experiments were performed with the minipump(s) on board.

2.4. Electrophysiological experiments

Experiments were performed on rats anesthetized with chloral hydrate (400 mg/kg, i.p.). Supplemental doses of chloral hydrate (100 mg/kg i.p.) were

administered as needed to prevent any nociceptive reaction to pinching of the hind paw. The rat's body temperature was maintained at approximately 37 °C by a thermistor-controlled heating pad.

A 2 mm-diameter section of bone centered on 1 mm anterior to Lambda was removed from the skull. A glass micropipette, tip diameter 1-3 μm, filled with 1 M NaCl (impedance 2-4 MΩ) was lowered vertically, and 200 μm-spaced tracks covering the DRN were performed. Spontaneously active neurons of the DRN were encountered starting from the ventral border of the Sylvius aqueduct and down to 1 mm below. Putative 5-HT neurons, which constitute the vast majority of spontaneously active DRN neurons, were identified according to classical physiological parameters characterized by the simultaneous occurrence of triphasic, positive-going first, action potential waveform; spike duration >2 ms; slow (0.2-3.5 Hz) and clock-like discharge pattern (Aghajanian, 1978; Aghajanian et al., 1978). Following the experiments each rat was sacrificed with anesthetic overdose.

2.5. Data Collection

For acute treatments, the mean firing rate before and after the injection of OPC was compared to assess any acute effects of OPC on the firing activity. For each short-term treatment group, the mean DRN 5-HT firing rate was determined by averaging the firing activity of all the neurons per group. For each treatment group (OPC, OPC and NE-100), the total number of neurons contributing to the average was greater than 50 from a minimum of 4 rats. To assess the effect of

paroxetine or 8-OH-DPAT, the percentage of inhibition was calculated and the average for each of each drug dose was determined. Statistical analysis was performed with the software SigmaStat for Windows Version 4.0 (Jandel Corporation). One-way ANOVA was used with alpha=0.05, followed by a post-hoc analysis using Tukey's Method of comparison versus controls. Results (F) of statistical analysis are expressed in terms of degrees of freedom between groups (df) and number of groups compared (p). $P< 0.05$ was considered statistically significant for all tests.

2.6. Drugs

8-OH-DPAT (8-hydroxy-2-(di-n-propylamino) tetralin) and paroxetine HCl were purchased from Sigma Aldrich Canada Limited (Oakville, ON, Canada). OPC was provided by Otsuka Pharmaceutical Co. Ltd (Tokushima, Japan), NE-100 was a gift from Taisho Pharmaceutical Co. Ltd, (Tokyo, Japan).

3. RESULTS

The doses used were chosen based on results obtained in behavioural tests with OPC (Tottori et al. 2001) and on previous results with other sigma ligands in our electrophysiological paradigm (Bermack and Debonnel 2001). In control animals, putative 5-HT neurons were encountered starting at a depth of 4616 μm, with an average firing rate of 1.03 Hz.

3.1. Acute Treatments

To assess any potential acute effect of OPC on neuronal activity in the DRN, we compared the effect of an acute intravenous dose of 20 μg/kg of OPC to saline. This dose had no effect on the firing activity of putative DRN 5-HT neurons. Similarly, when administered at a dose of 50 μg/kg, OPC did not modify the firing activity (data not shown).

3.2. Average Firing Rate

We then assessed whether OPC would have any effects if administered for a short-term treatment. Following two-day treatments with OPC (at a dose of 1 mg/kg/day), the mean basal firing activity of putative 5-HT neurons was increased by 50% (as compared to controls 1.45±0.11 Hz n=51 vs. 1.02±0.07 Hz n=56 [$F(2,3)=5.80$ $P<0.05$ Tukey Test $q=4.81$]) (Figure 1). The co-administration during the two days of OPC and the $sigma_1$ antagonist NE -100 at a dose of 10 mg/kg/day prevented the increased firing activity observed with OPC alone such that the firing rate was not significantly different versus controls (1.21 ± 0.10 Hz n=54, vs. 1.02 ± 0.07 Hz n=56 [$F(2,3)=5.80$, n.s.]) (Figure 1).

3.3 Modulation of 5-HT Neuronal Activity by 5-HT_{1A} Autoreceptors

Since 5-HT_{1A} autoreceptors constitute a key element in the control of the firing activity of 5-HT neurons and since OPC has a high affinity for 5-HT_{1A} receptors we also assessed the function of the 5-HT_{1A} autoreceptor in rats treated for 2 days with OPC. To this end, we assessed the effect of the acute administration of the SSRI paroxetine (500 μg/kg, i.v.). Figure 2 depicts representative tracings of putative DRN serotonergic neurons recorded during administrations of paroxetine, a 5-HT_{1A} agonist (8-OH-DPAT) and a 5-HT_{1A} antagonist (WAY 100635). In control animals, paroxetine induced a near complete suppression of the firing activity of DRN neurons (Figure 2A). This effect was reversed by the subsequent administration of the 5-HT_{1A} antagonist WAY-100635 (Figure 2A). In animals treated with OPC-14523 for two days, the effect of paroxetine was drastically reduced (compared to controls 9.98 ± 4.47% n=5 vs. 99.4 ± 0.40% n=5 [$F(3,4)=123.18$ $P<0.05$ Tukey's test $q=23.51$]) (Figures 2C and 3). Increasing the dose of paroxetine by 3 folds (up to 1500 μg/kg i.v.), only increased the degree of suppression to about 16% compared to 99% with 500 μg/kg in the control animals (1500 μg dose compared to controls 16.40 ± 2.58% n=5 vs. 99.4 ± 0.40% n=5 [$F(3,4)=123.18$ $P<0.05$ Tukey's test $q=21.83$]) (Figure 3). In animals treated for two days with a combination of OPC-14523 and NE-100, the effect of paroxetine was identical to that observed in rats treated with OPC alone (500 μg dose compared to controls 3.94 ± 0.79% n=5 vs. 99.4 ± 0.40% n=5 [$F(3,4)=829.72$ $P<0.05$ Tukey's test $q=62.64$]) (Figures 2D and 3). In animals treated for 2 days with a dose of 1 mg/kg/day of OPC and following 3 doses of

paroxetine, a small dose of 8-OH-DPAT (4 μg/kg, i.v.) produced a drastic reduction of the firing activity of DRN neurons, very similar to what was observed in control animals (compared to controls 72.2 ± 4.31% n=5 vs. 68.2 ± 10.13% n=5 [F(2,3)=1.14 n.s.]) (Figures 2C and 4). The effect of 8-OH-DPAT was reversed by the subsequent administration of WAY-100635 (100 μg/kg, i.v.) (Figures 2C and 4).

Finally, in animals treated with the combination of OPC and NE-100 for 2 days, 8-OH-DPAT induced the same degree of inhibition of the firing activity of DRN neurons as in control rats or in animals treated with OPC alone (compared to controls 58.2 ± 4.04% n=5 vs. 68.2±10.13% n=5 [F(2,3)=1.14 n.s.]) (Figures 2D and 4).

4. DISCUSSION

OPC, in addition to being a sigma$_1$ ligand (IC_{50}=47-56nM), has affinity for the 5-HT_{1A} receptor (IC_{50}=2.3nM) and for the 5-HT transporter (IC_{50}=27 nM) (Tottori et al., 2001). The current experiments found that acute treatments with OPC (20 and 50 μg/kg) did not produce any change in the firing activity of 5-HT neurons. However, following 2-day treatments with OPC (1 mg/kg/day) the firing activity of putative 5-HT dorsal raphe neurons was increased by 50% (Figure 1). Interestingly, this effect of OPC on the basal firing activity DRN neurons was reversed by NE-100 (Figure 1).

The increase in firing rate observed after 2 days of treatment with OPC is in agreement with previous data from our laboratory showing that 2-day treatments with the sigma ligands 4-IBP and (+)-pentazocine to induce approximately a 35% increase in the firing activity (Bermack and Debonnel, 2001). The fact that this increase is prevented by the co-administration of NE-100, suggests an effect mediated via a subtype of sigma$_1$ receptors, presumably the same one mediating the effects of (+)-pentazocine observed previously, which induces an equal increase in the firing activity of dorsal raphe neurons following a 2-day treatment, an effect which was also suppressed by NE-100 (Bermack and Debonnel, 2001).

In addition to inducing an increase in the firing activity, OPC treatments also prevented the suppressant effects of paroxetine (500 μg/kg, i.v.) as shown by a dramatic decrease in the response to this SSRI (Figure 3). Interestingly, following 2-day treatments with OPC, the effect of the acute intravenous

administration of the 5-HT_{1A} agonist 8-OH-DPAT (i.e the reduction of the firing activity of 5-HT neurons) was still present (Figure 4). In contrast with the effects of a two-day treatment with OPC on the basal firing activity, its effects on paroxetine action was not prevented by a co-treatment with NE-100, suggesting that this effect is not mediated by the $sigma_1$ receptors but is rather due to OPC's affinity for 5-HT_{1A} receptors.

It could also be suggested that OPC's effects on paroxetine are due to a blockade of 5-HT reuptake by OPC. However, this appears unlikely since it would suggest a very potent blocking effect on the 5-HT transporter since 1 mg/kg/day would be as potent as 10-15 mg/kg/day of other SSRI's paroxetine, fluoxetine and citalopram. Whereas, in *ex vivo* experiments, OPC at doses of 100 and 300 mg/kg p.o. were required to inhibit H^3-5-HT reuptake in rats versus doses of 10 or 30 mg/kg of fluoxetine. Furthermore, fluoxetine (10 or 30 mg/kg) blocked the pCPA-induced decrease in 5-HT content in the hippocampus or frontal cortex while OPC required 400 mg/kg to produce the same effect (Tottori et al., 2001). Finally, if OPC was acting mainly via its reuptake properties one would expect it to have a similar profile as other SSRI's when administered acutely, ie. to induce a decrease in firing activity of 5-HT neurons, however the acute administration of OPC (20 or 50 μg/kg) produced no change in the firing activity.

Thus, our data suggest that OPC's effects on the paroxetine response are due to its affinity for 5-HT_{1A} receptors. 5-HT acts upon several subtypes of receptors (Reviewed in Hoyer and Martin, 1996; Barnes and Sharp, 1999), with

the 5-HT_{1A} receptors being particularly important in the regulation of 5-HT neurons' activity. Activation of these receptors triggers the opening of K^+ channels, which induces a hyperpolarization of the neuron and decreases its firing activity. The acute administration of SSRIs initially induces a moderate increase in the concentration of 5-HT in the vicinity of 5-HT cell bodies, thus triggering an activation of somatodendritic autoreceptors and a reduction of the firing activity of the 5-HT neurons, through a negative feedback mechanism (Gardier et al., 1996; Chaput et al., 1986; de Montigny et al., 1981; Aghajanian, 1978).

Numerous evidence has shown that the postsynaptic 5-HT_{1A} receptor, although very similar to the autoreceptor [since there is only one gene coding for the two types (Reviewed in Hoyer and Martin, 1996; Barnes and Sharp, 1999)] presents a different pharmacological profile. Some examples are; chronic SSRI and 5-HT_{1A} agonist treatments desensitize the 5-HT_{1A} somatodendritic autoreceptor in the DRN (Le Poul et al., 2000; Kreiss and Lucki, 1995; Blier et al., 1990), but do not change the responsiveness of postsynaptic 5-HT_{1A} receptors in the hippocampus (Le Poul et al., 2000; Chaput et al., 1986), and, agonist-induced internalization of 5-HT_{1A} receptors occured only presynaptically, but not postsynaptically (Riad et al., 2001). These differences could be related to the difference in the G-proteins linked to the receptors (Hensler, 2002).

Postsynaptic 5-HT_{1A} receptors also contribute to the control of the firing activity of 5-HT neurons through a long negative feedback loop (Pineyro and Blier, 1999). This system is not yet fully characterized but is initiated by the activation of medio-prefrontal-cortical postsynaptic 5-HT_{1A} receptors on glutamatergic neurons, leading to the activation of GABAergic interneurons in the

DRN (Celada et al., 2001; Martin-Ruiz and Ugedo, 2001; Haddjeri et al., 2000; Tada et al.,1999; Hajos et al., 1999,1998, 2003; Ceci et al., 1994). It has been shown that the inhibitory effect of the systemic administration of the 5-HT_{1A}agonist 8-OH-DPAT is mediated preferentially via this long feedback loop, through an activation of postsynaptic 5-HT_{1A} receptors (Celada et al., 2001; Hajos et al., 1998,1999; Ceci et al., 1994).

OPC does not appear to affect the postsynaptic 5-HT_{1A} receptors since 2-day treatments with OPC did not modify the effects of 8-OH-DPAT, which as stated above exerts preferentially its inhibitory effects of DRN firing activity through activation of 5-HT_{1A} postsynaptic receptors through the feedback loop just described. However, further investigation of OPC's effects, studied at postsynaptic sites would further clarify the pharmacological profile of OPC at postsynaptic 5-HT_{1A} receptors. Therefore, the effects of OPC on paroxetine-induced inhibition of firing appear to be mediated via the 5-HT_{1A} autoreceptor. They could either be the result of a very rapid desensitization of the receptor with an agonist or of the blockade of the receptor by an antagonist.

5-HT_{1A} agonistic properties of OPC have been previously reported, based on the ability of OPC to induce a flat body posture (Tottori et al., 2001). However, this effect was observed at a dose of 30 mg/kg whereas, the ED_{50} for the same effects with 8-OH-DPAT was of 0.05 mg/kg, when the two compounds present about the same affinity for the 5-HT_{1A} receptor (Oshiro et al., 2000). Thus, the behavioural effect observed could be due to weak agonist effects at postsynaptic receptors or to a non-selective effect of OPC as such high doses could involve activation of other types of receptors for which OPC has lower affinity. Such an

effect would be difficult to reconcile with a rapid desensitization. Moreover, if OPC was acting as an agonist on the 5-HT_{1A} autoreceptor one would expect a decrease of the firing activity of DRN neurons following its acute administration.

Conversely, an antagonist effect of OPC on 5-HT_{1A} autoreceptors could easily explain all the results observed in the present experiment. As, following the acute administration, as a 5-HT_{1A} antagonist it would have no effect on the spontaneous firing activity, moreover, through this antagonistic effect it would also prevent any inhibition of the firing due to an increase of endogenous 5-HT induced by the 5-HT transporter blocking properties of OPC. The same effect would be responsible for the decreased response to paroxetine following 2-day treatments.

Our results therefore suggest that besides its ability to block 5-HT uptake which was not assessed in the present series of experiments, OPC presents several aspects, each of them being by itself suggestive of a potential antidepressant effect. In conclusion, with the combination of all these aspects it could be expected that OPC, by inducing a rapid decreased response of the autoreceptor in addition to inducing an increase in the firing activity of DRN neurons, could represent a potent antidepressant with a rapid onset of action. Further studies into OPC's exact pharmacological effects on 5-HT_{1A} sites using microiontophoretic studies, which are now underway, will elucidate the mechanism underlying its observed effects on the autoreceptors. In addition, it remains to be established that OPC will keep a similar profile following a long-term treatment, which is what would be ultimately required with depressed patients.

FIGURE LEGENDS

Figure 1

(A) Representative spontaneous firing rate histograms of dorsal raphe 5-HT neurons in (1) control rats, (2) rats treated for 2 days with OPC (1 mg/kg/day) and (3) rats treated for 2 days with OPC (1 mg/kg/day) and NE-100 (10 mg/kg/day). Each group of spikes represents a 90 second recording of the firing activity, thus, for example A1, represents recordings from 3 separate neurons encountered in one descent in the DRN. The number on top of each neuron represents the depth at which the neuron was found in μm. Time calibration applies to all traces.

(B) Mean firing activity of dorsal raphe 5-HT neurons in control rats expressed in Hz ± SEM (open column), in rats treated for 2 days with OPC (1 mg/kg/day) (grey column) and in rats treated for two days with a combination of OPC (1 mg/kg/day) and the $sigma_1$ antagonist NE-100 (10 mg/kg/day) (black column). In this and all subsequent figures the number in the box in each column represents the number of neurons contributing to the average.* $p < 0.05$.

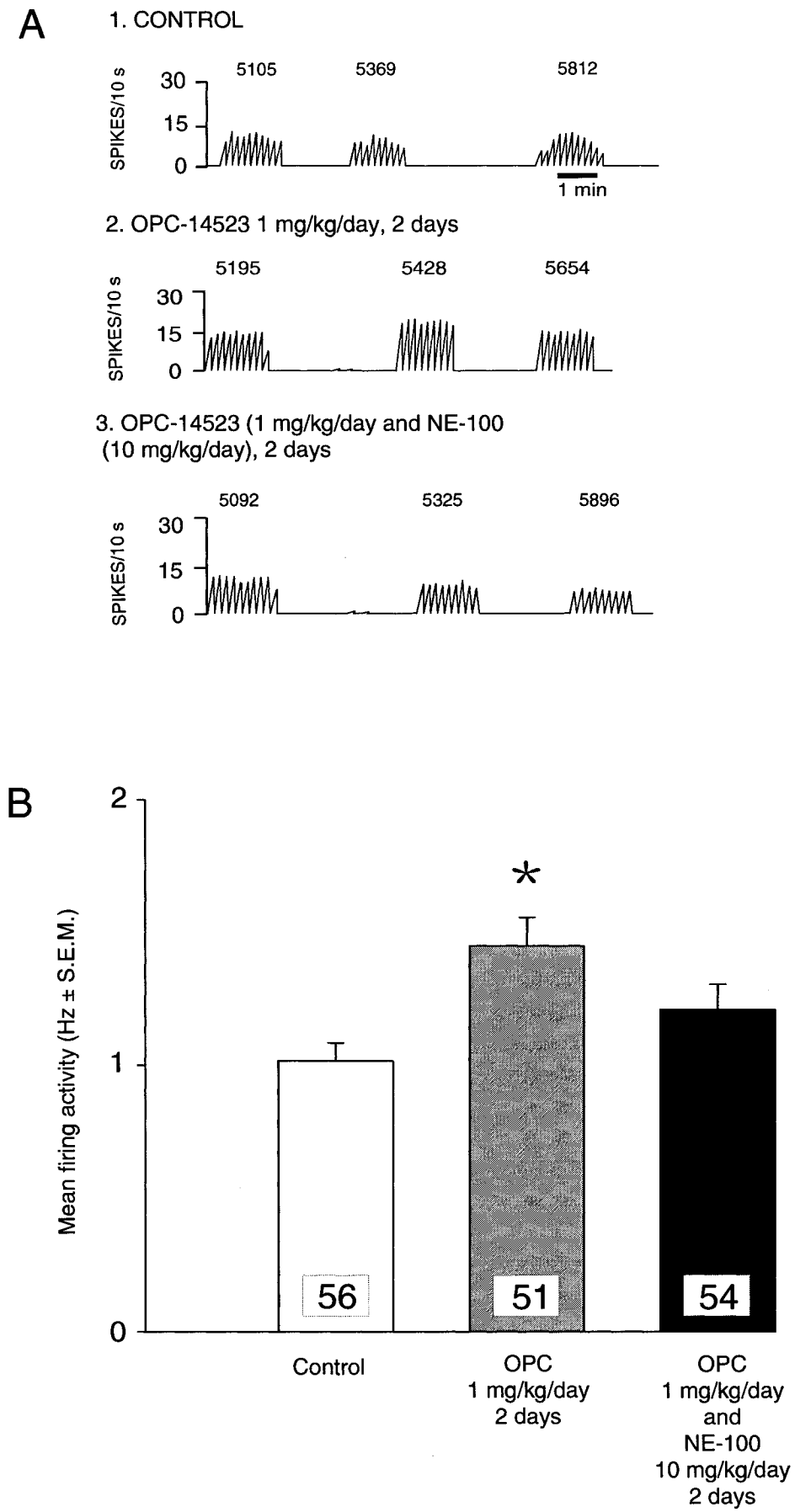

A
1. CONTROL
SPIKES/10 s
30
15
0
5105
5369
5812
1 min
2. OPC-14523 1 mg/kg/day, 2 days
5195
5428
5654
3. OPC-14523 (1 mg/kg/day and NE-100
(10 mg/kg/day), 2 days
5092
5325
5896
B
Mean firing activity (Hz ± S.E.M.)
2
1
0
*
56
51
54
Control
OPC
1 mg/kg/day
2 days
OPC
1 mg/kg/day
and
NE-100
10 mg/kg/day
2 days

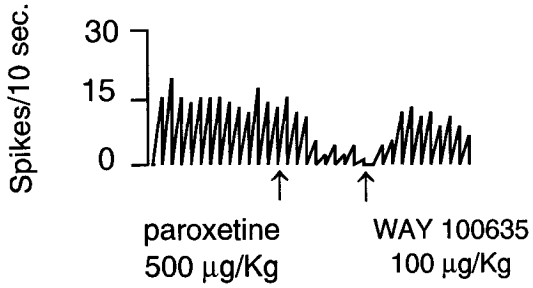
Figure 1

Figure 2

Spontaneous firing rate histograms of dorsal raphe 5-HT neurons in (A) control rats, illustrating the suppression of the firing activity by the intravenous administration of the SSRI paroxetine and the reversal of this suppression following the subsequent administration of the 5-HT_{1A} antagonist WAY-100635. (B) control rats, illustrating suppression of firing activity by the intravenous administration of the 5-HT_{1A} agonist 8-OH-DPAT and reversal of this suppression following the subsequent administration of WAY-100635. (C) Illustrates the reduction of the effect of paroxetine in a rat treated for 2 days with OPC (1 mg/kg/day) and that the effect of 8-OH-DPAT is maintained and reversed by the subsequent administration of WAY-100635. In (D), a two-day combined treatment with OPC and the $sigma_1$ antagonist NE-100 induces the same effects. Time calibration applies to all traces.

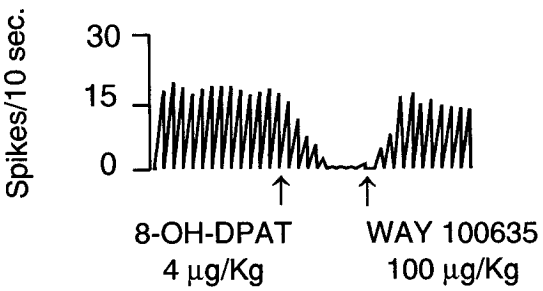
A

CONTROL-Paroxetine



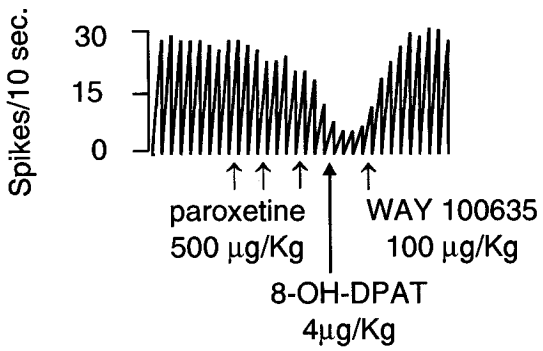
B

CONTROL-8-OH-DPAT



C

OPC 14523 1 mg/kg/day (2 days)



D

OPC 1 mg/kg/day + NE-100 10 mg/kg/day (2 days)

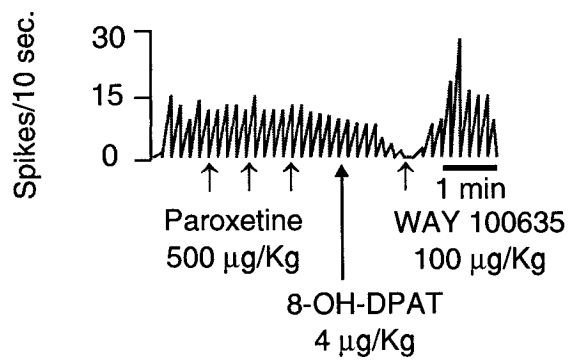


Figure 2

Figure 3

Responsiveness expressed as the degree of suppression of the mean firing activity of dorsal raphe 5-HT neurons by the acute intravenous administration of 500 μg/kg of paroxetine in control rats (open columns), and of successive doses of 500 μg/kg of paroxetine in rats treated for 2 days with OPC-14523 (1 mg/kg/day) (light grey columns), or rats treated for 2 days with OPC-14523 (1 mg/kg/day) and the $sigma_1$ antagonist NE-100 (10 mg/kg/day) (dark grey columns). * $p < 0.05$. Note that the number of neurons tested per dose when not shown due to space is the same for all doses.

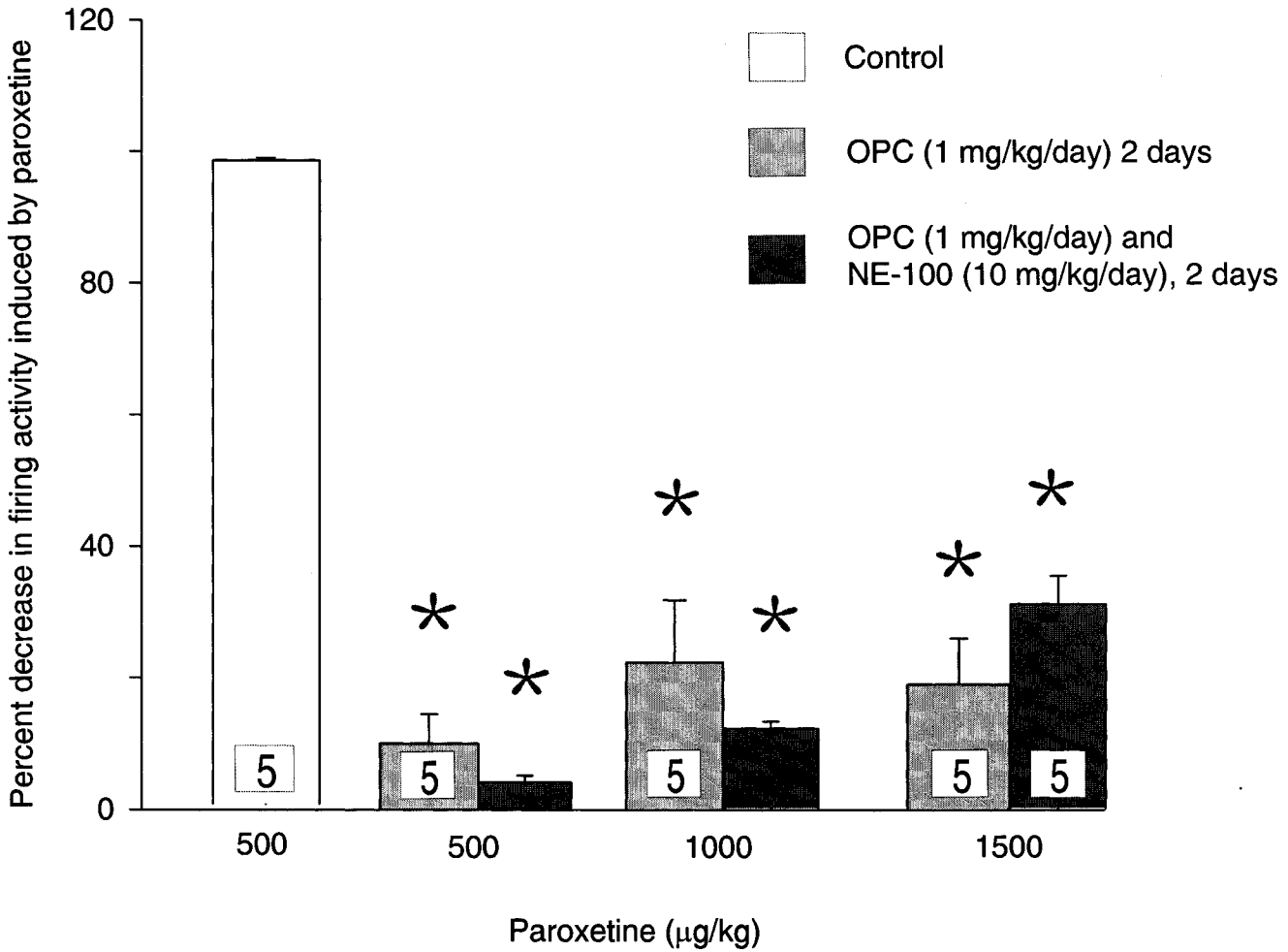

120
80
40
0
Percent decrease in firing activity induced by paroxetine
Control
OPC (1 mg/kg/day) 2 days
OPC (1 mg/kg/day) and
NE-100 (10 mg/kg/day), 2 days
5
5
5
5
5
5
*
*
*
*
*
*
500
500
1000
1500
Paroxetine (μg/kg)

Figure 3

Figure 4

Responsiveness expressed as the degree of suppression of the mean firing activity of dorsal raphe 5-HT neurons by the acute intravenous administration of 4 μg/kg of 8-OH-DPAT in control rats (open columns), and in rats treated for 2 days with OPC (1 mg/kg/day) (grey column) or OPC (1 mg/kg/day) and the $sigma_1$ antagonist NE-100 (10 mg/kg/day) (black column).

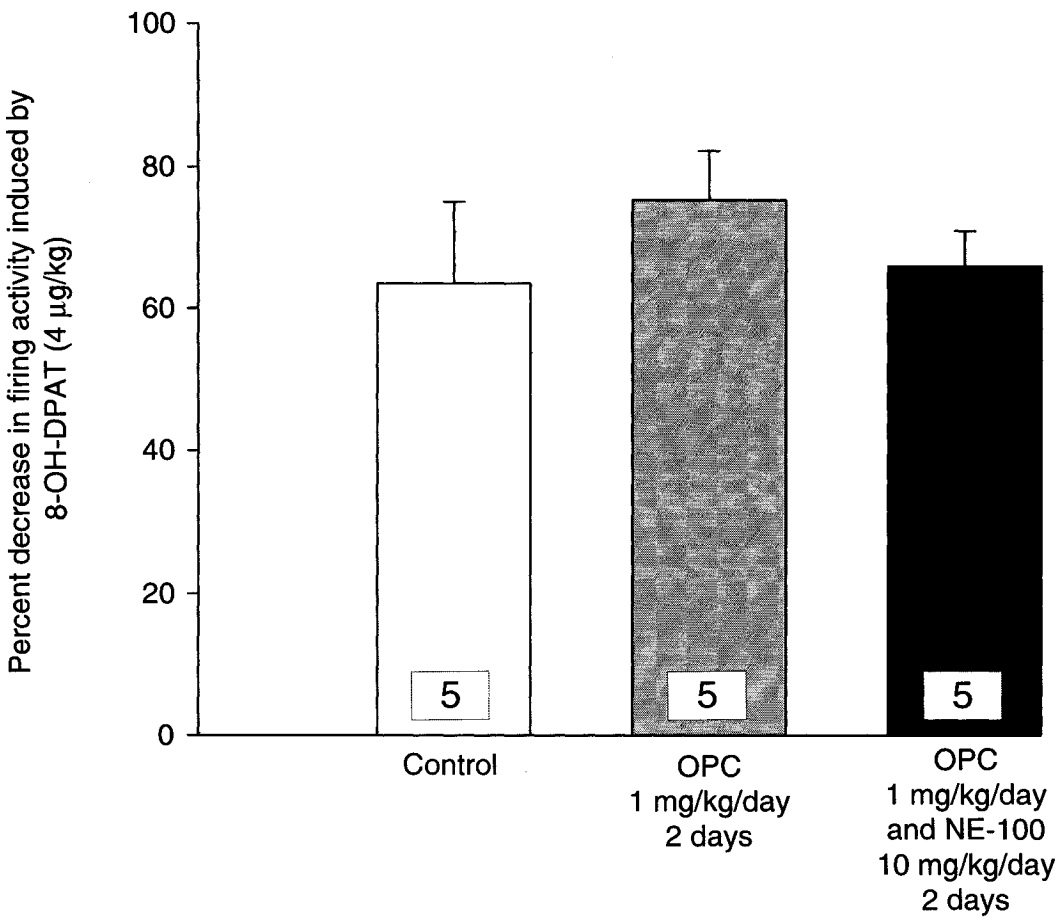

Percent decrease in firing activity induced by 8-OH-DPAT (4 µg/kg)
100
80
60
40
20
0
5
5
5
Control
OPC
1 mg/kg/day
2 days
OPC
1 mg/kg/day
and NE-100
10 mg/kg/day
2 days

Figure 4

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Chapter 7

Effects of the potential antidepressant OPC-14523 a combined sigma and 5-HT_{1A} ligand on pre- and post-synaptic 5-HT_{1A} receptors

Chapter 6 clearly demonstrated that short-term treatments with OPC induce a significant increase of the basal firing activity of 5-HT neurons of the DRN. This increase is blocked by the $sigma_1$ antagonist NE-100, and is very similar to that previously demonstrated in Chapter II by the sigma ligands 4-IBP and (+)-pentazocine. In addition, OPC following two-day treatments, induced a decreased responsesiveness of the 5-HT_{1A} receptor to the increase in synaptic 5-HT induced by the SSRI paroxetine. However, this effect on the autoreceptor was not blocked by NE-100. Thus, it appeared that the decreased response to paroxetine was due to OPC's affinity at 5-HT_{1A} receptors. At this point it was not known whether OPC acted at pre- or postsynaptic 5-HT_{1A} receptors, or whether it acted as a 5-HT_{1A} agonist or antagonist. Both pre- and postsynaptic receptors play important roles in the mechanism of action of antidepressants (see Introduction). Therefore, OPC's activity at either one or both 5-HT_{1A} receptor populations could provide support for OPC's potential as an antidepressant. Since the decrease in the response of the autoreceptor was induced in only 2 days of treatment with OPC, further investigation was needed, as this could be due to OPC acting as a 5-HT_{1A} antagonist or OPC could be a 5-HT_{1A} agonist that could somehow induce an extremely rapid desensitization. This has particular relevance to OPC's potential as an antidepressant with a rapid onset of action.

Thus, the current study uses microiontophoresis and extracellular recording to assess the effects of OPC on presynaptic 5-HT_{1A} receptors on 5-HT

neurons in the DRN and postsynaptic 5-HT_{1A} receptors on CA_3 pyramidal neurons in the dorsal hippocampus. This data will undoubtedly shed light on the mechanism by which OPC induces a rapid increase in the firing activity combined with a decreased 5-HT_{1A} autoreceptor response.

EFFECTS OF THE POTENTIAL ANTIDEPRESSANT OPC-14523, A COMBINED SIGMA AND 5-HT_{1A} LIGAND, ON PRE- AND POST-SYNAPTIC 5-HT_{1A} RECEPTORS

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ABSTRACT

OPC-14523 (OPC), a novel compound with high affinity for sigma and 5-HT_{1A} receptors as well as for the 5-HT transporter, shows "antidepressant-like" effects in animal models of depression. We have previously demonstrated that OPC produces an increase in 5-HT neurotransmission in the DRN after 2 days of treatment. In addition, OPC led to a decreased response of DRN 5-HT neurons to the acute administration of the SSRI paroxetine, an effect that appears to be mediated by OPC's 5-HT_{1A} receptor affinity. The current study sets out to investigate more specifically the effects of OPC on 5-HT_{1A} pre- and postsynaptic receptors, and to assess whether it acts as an agonist or antagonist. Using an electrophysiological model of *in vivo* extracellular recordings in anesthetized rats, the effects of OPC was assessed on presynaptic DRN 5-HT_{1A} autoreceptors and postsynaptically on hippocampal 5-HT_{1A} receptors of CA_3 pyramidal neurons. OPC applied by microiontophoresis, produced a significant decrease in the firing activity of 5-HT neurons of the DRN and of CA_3 pyramidal neurons of the dorsal hippocampus, similarly to the 5-HT_{1A} agonist 8-OH-DPAT. The effects of 8-OH-DPAT and OPC on 5-HT_{1A} pre- and postsynaptic receptors were significantly reduced by the co-application of the 5-HT_{1A} antagonist WAY-100635. In

addition, the effects of OPC were not blocked by the injection of the $sigma_1$ antagonist NE-100, nor by the nonselective sigma antagonist haloperidol. Therefore, OPC might have potential as an antidepressant with a rapid onset of action, as having affinity at 5-HT_{1A} autoreceptors may contribute to the rapid decrease of the autoreceptor response reported previously following OPC treatments. OPC's 5-HT_{1A} agonist properties postsynaptically may also contribute to its potential as an antidepressant.

INTRODUCTION

An enormous amount of evidence suggests the involvement of the serotonin (5-HT) system in the pathophysiology of depression and in the mechanism of action of antidepressants (Reviewed by Delgado, 2000; Stockmeier et al. 2003). Electrophysiological data demonstrate that after long-term treatments, representatives from all classes of antidepressants increase 5-HT neurotransmission (Chaput et al., 1991; Blier and de Montigny, 1994). For example, acute treatments with monoamine oxidase inhibitors (MAOI's) and selective serotonin reuptake inhibitors (SSRI's) lead to decreased firing activity of 5-HT neurons in the dorsal raphe nucleus (DRN), but as treatment continues, over several weeks the 5-HT neurons regain their normal firing activity due to a progressive desensitization of the 5-HT_{1A} somatodendritic autoreceptors (Chaput et al., 1986; Blier and de Montigny, 1994). This desensitization has been proposed as the adaptive change that explains the delayed enhancement of 5-HT-mediated neurotransmission, which is consistent with the clinical onset of action of SSRI's (Chaput et al., 1986; Blier and de Montigny, 1994; Invernizzi et al., 1994; Kreiss and Lucki, 1995; LePoul et al., 2000). In addition, postsynaptic 5-HT_{1A} receptors have also been shown to be sensitized or tonically activated by antidepressant drugs, suggesting postsynaptic 5-HT_{1A} receptor involvement in antidepressant action as well (Blier and de Montigny, 1994; Lucki et al., 1994; Haddjeri et al., 1998). Pre- and postsynaptic 5-HT_{1A} receptors, although genetically similar, present different pharmacological profiles (Blier and de Montigny, 1987; Sprouse

and Aghajanian, 1988; Blier et al., 1993; Dong et al., 1997), and therefore, each population may offer unique contributions with respect to antidepressant action.

The existence of sigma receptors were initially reported by Martin and colleagues (1976). Two receptor subtypes, denoted $sigma_1$ and $sigma_2$ are now recognized (Quirion et al., 1987, 1992). Sigma ligands such as 1-(3,4-dimethoxyphenethyl)-4-(3-phenylpropyl) piperazine dihydrochloride (SA-4503), (+)-pentazocine, 1,3-di-(2-tolyl)guanidine (DTG) and (+)-N-cyclopropylmethyl-N-methyl-1,4-diphenyl-1-1-ethyl-but-3-en-1-ylamine hydrochloride (JO-1784) have been shown to produce "antidepressant-like" effects in behavioural models of depression such as the Forced Swimming and Tail Suspension Tests as well as in clinical trials (Matsuno et al., 1996; Kinsora et al., 1998; Ukai et al., 1998; Akunne et al., 2001; Pande, 1998). Thus, sigma receptors have been implicated in the pathophysiology of depression and sigma ligands have been proposed as potential antidepressants. In addition, we previously demonstrated that the sigma ligands 4-(N-benzylpiperidin-4-yl)-4-iodobenzamide (4-IBP) and (+)-pentazocine produce an increase in the basal firing activity of DRN 5-HT neurons after both short-term (2 days) and long-term (21 days) treatments (Bermack and Debonnel, 2001).

OPC (OPC-14523, 1-[3-[4-(3-chlorophenyl)-1-piperazinyl]propyl]-5-methoxy-3,4-dihydro-2-quinolinone monomethanesulfonate) is a novel compound with high affinities for sigma and $5\text{-}HT_{1A}$ receptors and has 5-HT reuptake inhibitory activities (Tottori et al., 2001). Similar to other sigma ligands, OPC yielded antidepressant-like effects in animal models of depression (Tottori et al.,

2001). We previously reported OPC to induce an increase in serotonergic neurotransmission in the DRN that is dependent on its sigma receptor properties (Bermack et al., In Press). In addition, we showed that OPC induces a rapid (48 hours) and drastic decrease (90%) in the responsiveness of the 5-HT_{1A} autoreceptor to the increase in synaptic 5-HT concentration induced by the SSRI paroxetine. This decreased response of the autoreceptor was mediated by 5-HT_{1A} autoreceptors as opposed to postsynaptic 5-HT_{1A} or sigma receptors (Bermack et al., In Press).

Thus, the purpose of this study was to assess the effect of microiontophoretic applications of OPC on pre- and post-synaptic 5-HT_{1A} receptors using an *in vivo* electrophysiological model of extracellular recordings of 5-HT neurons of the DRN and CA_3 pyramidal neurons of the dorsal hippocampus. These experiments aimed at assessing the mechanism of action by which OPC produces the previously observed increase in 5-HT firing activity and rapid decrease in the response of the 5-HT_{1A} autoreceptor. Indeed, it was possible that OPC was acting as a 5-HT_{1A} autoreceptor antagonist, or it could have been acting as a 5-HT_{1A} autoreceptor agonist inducing an extremely fast desensitization. Therefore, it deemed relevant to determine OPC's effects at presynaptic 5-HT_{1A} autoreceptors as well as to elucidate OPC's potential activity at postsynaptic 5-HT_{1A} receptors, which may also contribute to its "antidepressant-like" effects.

METHODS

Animals

Experiments were performed in male Sprague-Dawley rats (Charles River, St. Constant, Québec) weighing 250-300g. Rats were housed under standard laboratory conditions including 12-12hr light-dark cycle and free access to food and water.

Electrophysiological experiments

For the experiments, rats were anesthetized with chloral hydrate (400 mg/kg, i.p.). Supplemental doses of chloral hydrate (100 mg/kg i.p.) were administered as needed to prevent any nociceptive reaction to pinching of the hind paw. The rat's body temperature was maintained at approximately 37 °C by a heating pad.

Dorsal Raphe Nucleus Recordings

A 2 mm-diameter section of bone centered on 1 mm anterior to Lambda was removed from the skull. A glass micropipette, tip diameter 1-3 µm, was lowered, and 200 µm-spaced tracks covering the DRN were performed. Spontaneously active neurons of the DRN were encountered starting from the ventral border of the Sylvius aqueduct and down to 1 mm below. 5-HT neurons, which constitute the vast majority of spontaneously active DRN neurons, were identified according to the simultaneous occurrence of several characteristics: triphasic, positive-going first, action potential waveform, spike duration >2 ms, slow (0.2-3.5 Hz) and clock-like discharge pattern (Aghajanian, 1978; Aghajanian et al., 1978). Five-

barrelled micropipettes were used. The central barrel, used for extracellular unitary recording, was filled with a 2M NaCl solution (impedance 2-4 MΩ). One of the side barrels, used for automatic current balancing, was filled with a 2M NaCl solution. The remaining side barrels were filled with one or more of: OPC (1 mM in 200 mM NaCl); 8-OH-DPAT (5 mM in 200 mM NaCl) and WAY 100635 (15 mM in 200 mM NaCl). The selective $sigma_1$ antagonist NE-100 (100 μg/kg) and the nonselective sigma antagonist haloperidol (100 μg/kg) were injected via the lateral tail vein. Following the experiments each rat was sacrificed with an anesthetic overdose.

CA_3 Pyramidal Neuron Recordings

Rats were anesthetized and mounted in a stereotaxic apparatus in a similar method as described in the DRN recordings, however the electrode was descended at 4.2 mm lateral and 4.2 mm anterior to Lambda into the CA_3 region of the dorsal hippocampus. Five-barreled glass micropipettes were used for extracellular recordings of CA_3 pyramidal neurons and microiontophoretic applications. The central barrel, used for extracellular unitary recording, was filled with a 2M NaCl solution. One of the side barrels, used for automatic current balancing, was filled with a 2M NaCl solution. The remaining side barrels were filled with quisqualic acid (QUIS)(1.5 mM in 400 mM NaCl) for activation of the CA_3 pyramidal neurons and any two of OPC (1 mM in 200 mM NaCl); 8-OH-DPAT (5 mM in 200 mM NaCl); and WAY 100635 (15 mM in 200 mM NaCl). Hippocampal pyramidal neurons were identified by their long duration (0.8 to 1.5

ms), large amplitude action potentials (0.5 to 2 mV), and by the presence of "complex spike" discharges (Kandel and Spencer, 1961). The sigma antagonists NE-100 (100 μg/kg) and haloperidol (100 μg/kg) were injected via the lateral tail vein. Following the experiments each rat was sacrificed with an anesthetic overdose.

Data Collection

The firing rate before and during the microiontophoretic application (OPC, WAY 100635 , and 8-OH-DPAT) or before and after the injection (NE-100, haloperidol) was compared. For each group, recordings were done from a minimum of 5 rats. The percent of baseline firing activity was calculated and averaged for each drug application.

Statistical analysis was performed using the software SigmaStat Version 4.0. One way ANOVA was used with alpha=0.05, followed by a posthoc analysis using Tukey's Method of comparison between groups. $P<0.05$ was considered significant for all groups

Drugs

8-OH-DPAT, WAY 100635, QUIS, haloperidol and paroxetine HCl were purchased from Sigma Aldrich Canada Limited (Oakville, ON, Canada). OPC was provided by Otsuka Pharmaceutical Co. Ltd., (Tokushima, Japan), NE-100 was a gift from Taisho Pharmaceutical Co. Ltd., (Tokyo, Japan).

RESULTS

Effect of OPC and 8-OH-DPAT in the DRN and hippocampus

Figure 1A depicts a representative tracing of a DRN 5-HT neuron recorded during several subsequent microiontophoretic applications. In the DRN, microiontophoretic application of the 5-HT_{1A} agonist 8-OH-DPAT induced a significant suppression of the firing activity of 5-HT neurons (67.26 ± 6.88% n=15 vs. control n=15 [F=12.71 P< 0.001 Tukey's Test q= 6.20]) (Figure 1B). Similarly, OPC induced a significant suppression of the firing activity of 5-HT neurons when applied directly to the DRN by microiontophoresis (61.03 ± 4.27% n=15 vs. control n=15 [F=26.48 P< 0.001 Tukey's Test q= 9.99]) (Figure 1C).

Figure 2A depicts a representative tracing of a CA_3 pyramidal neuron recorded from the dorsal hippocampus during subsequent microiontophoretic applications. When recording from CA_3 pyramidal neurons of the hippocampus, the microiontophoretic application of 8-OH-DPAT led to a significant attenuation of the firing activity (64.96 ± 6.43% n=15 vs. control n=15 [F=11.07 P< 0.001 Tukey's Test q= 6.59]) (Figure 2B). Similarly, OPC application led to a significant suppression of the firing activity of CA_3 pyramidal neurons (66.69 ± 4.06% n=15 vs. control n=15 [F=14.98 P< 0.001 Tukey's Test q= 7.64]) (Figure 2C).

Under our experimental conditions, OPC and 8-OH-DPAT induced similar levels of suppression when compared to each other, 39% and 33% respectively in the DRN and 36% and 34% in the hippocampus (Figures 1 and 2).

Effect of the 5-HT_{1A} antagonist WAY 100635

In the DRN, co-application by microiontophoresis of WAY 100635 with 8-OH-DPAT completely reversed the suppressant effects of 8-OH-DPAT on 5-HT firing activity (94.05 ± 2.46% n=15 vs. 67.26 ± 6.88% n=15 [F=12.71 P< 0.05 Tukey's Test q= 5.86]) (Figure 1B). When co-applied with OPC, WAY 100635 significantly reversed the suppressant effects of OPC (83.11 ± 3.30% n=15 vs. 61.03 ± 4.27% n=15 [F=26.48 P< 0.05 Tukey's Test q= 6.54]) (Figure 1C). In the hippocampus, similar to what was seen in the DRN, WAY 100635 co-applied with 8-OH-DPAT completely reversed the inhibitory effects of 8-OH-DPAT (85.19 ± 5.70% n=15 vs. 64.96 ± 6.43% n=15 [F=11.07 P< 0.05 Tukey's Test q= 3.96]) (Figure 2B). When co-administered with OPC, WAY 100635 significantly reversed OPC's effects leading to a smaller suppression of the firing activity (83.42 ± 4.47% n=15 vs. 66.69 ± 4.06% n=15 [F=14.98 P< 0.05 Tukey's Test q= 4.43]) (Figure 2C).

Effect of the selective $sigma_1$ antagonist NE-100

While recording 5-HT neurons' firing activity in the DRN, the intravenous administration of NE-100 (100 μg/kg) had no effect on the suppression of the firing activity induced by microiontophoretic application of OPC (63.96 ± 4.71% n=5 vs. 64.50 ± 5.52% n=5, n.s.) (Figure 3). When recording neurons subsequent to the injection of NE-100, OPC was still capable of inducing a significant suppression in the firing activity, similar to its effects prior to NE-100 administration (Figure 3). In the CA_3 region of the dorsal hippocampus, the

intravenous administration of NE-100 did not modify the inhibition induced by the application of OPC (68.14 ± 7.43% n=5 vs. 64.44 ± 5.08% n=5, n.s.) (Figure 4A). Furthermore, when recording from CA_3 pyramidal neurons subsequent to NE-100 injection, OPC was still able to induce a significant suppression in the firing activity (Figure 4A).

Effect of the non-selective sigma antagonist haloperidol

As the $sigma_1$ antagonist NE-100 had no significant effect on the response induced by OPC in either the DRN or the hippocampus, we assessed the effect of the less selective sigma antagonist haloperidol on OPC's effects in the hippocampus. The dose of haloperidol used has been previously shown to reverse the effects of sigma agonists in the model of the potentiation of the NMDA response in the dorsal hippocampus (Bergeron et al. 1995). Intravenous administration of haloperidol (100 μg/kg) had no significant effect on the suppressant effect of OPC on the firing activity in the dorsal hippocampus (78.68 ± 1.72% n=5 vs. 71.40 ± 7.02% n=5, n.s.). When recording from CA_3 pyramidal neurons after the injection of haloperidol, no significant difference was seen in OPC's inhibitory effects on the firing activity of CA_3 pyramidal neurons (Figure 4B).

DISCUSSION

OPC, in addition to being a sigma$_1$ ligand (IC_{50}=47-56 nM), has high affinity for the 5-HT_{1A} receptor (IC_{50}=2.3 nM) and for the 5-HT transporter (IC_{50}=27 nM) (Tottori et al., 2001). We have previously demonstrated, using electrophysiological recordings in the DRN, that 2-day treatments with OPC produce a significant decrease in the response of the 5-HT_{1A} autoreceptor (Bermack et al. In Press). This effect was not reversed by the co-administration of NE-100 suggesting that either OPC is acting as a 5-HT_{1A} autoreceptor antagonist or as an agonist capable of producing an extremely rapid desensitization (Bermack et al. In Press). Thus, the current experiments examined OPC's effects on 5-HT_{1A} receptors using *in vivo* microiontophoresis and electrophysiological recording in the DRN and dorsal hippocampus to assess whether it is an agonist or antagonist at pre- and post-synaptic 5-HT_{1A} receptors.

The main finding in the present study is that OPC acts as a 5-HT_{1A} agonist, similarly to 8-OH-DPAT, in both the hippocampus and DRN. In both regions, OPC's effects were significantly reversed by the 5-HT_{1A} antagonist WAY 100635, while the sigma antagonists NE-100 and haloperidol had no effect. These results suggest that the effects observed in the current experimental series are mainly due to OPC's affinity for 5-HT_{1A} receptors.

Thus, OPC presents a 5-HT_{1A} agonist profile. This is in agreement with a previous report that OPC possessed 5-HT_{1A} agonistic properties. These authors suggested that OPC is a 5-HT_{1A} autoreceptor agonist based on its ability to decrease 5-HT biosynthesis in the mouse forebrain. In addition, postsynaptic 5-HT_{1A} agonist properties were suggested based on the ability of OPC to induce a

flat-body posture (Tottori et al., 2001). However, both of these effects was observed at doses significantly higher than those required for the same effects with 8-OH-DPAT, whereas the two compounds present about the same affinity for the 5-HT_{1A} receptor (Oshiro et al., 2000). Given that these two methods were rather indirect assessments of 5-HT_{1A} receptor activity and that OPC and 8-OH-DPAT were administered subcutaneously or orally, more investigations were needed to determine if in fact these are 5-HT_{1A} receptor-mediated effects, and if so which brain regions are involved. The current findings clarify that OPC has 5-HT_{1A} agonist effects pre- and postsynaptically with comparable efficacy to 8-OH-DPAT, which discussed below, has implications towards its potential antidepressant effects.

5-HT_{1A} receptors are of particular importance in the regulation of 5-HT neurons' activity. Activation of these receptors triggers the opening of potassium channels, which induces a hyperpolarization of the neuron and decreases its firing activity (Aghajanian and Lakoski, 1984). 5-HT_{1A} receptors on 5-HT neurons of the DRN have been denoted somatodendritic autoreceptors as they exert autoregulatory control of the firing activity of these neurons (Aghajanian et al., 1978). The activation of these receptors by 5-HT or 5-HT_{1A} agonists induces a suppression of the firing activity (Blier and de Montigny, 1987; Sprouse and Aghajanian, 1987; Vandermaelen et al., 1986).

The fact that OPC acts as a 5-HT_{1A} autoreceptor agonist is relevant in light of the fact that these autoreceptors have clearly been implicated in antidepressant action. The acute administration of SSRI's initially induces a moderate increase in the 5-HT concentration in the vicinity of 5-HT cell bodies. This increase, in turn,

triggers the activation of somatodendritic autoreceptors and a reduction of the firing activity of 5-HT neurons, through a negative feedback mechanism (Gardier et al., 1996; Chaput et al., 1986; de Montigny et al., 1981; Aghajanian, 1978).

Long-term treatments with SSRI's or MAOI's have been clearly shown to decrease the functioning of the somatodendritic 5-HT_{1A} receptor, which leads to a restoration of the firing activity of DRN 5-HT neurons (Blier and de Montigny, 1985; Chaput et al., 1986, 1991; Blier et al., 1988; Invernizzi et al., 1994; Kreiss and Lucki, 1995; LePoul et al., 2000). Similarly, acute or short-term administration of 5-HT_{1A} agonists does not induce a desensitization of the 5-HT_{1A} autoreceptor, while chronic treatments does lead to a desensitization, similarly to that described after chronic SSRI treatments (Blier et al. 1990, Dong et al. 1997, 1998). The desensitization of the 5-HT_{1A} autoreceptor is believed to be crucial to the therapeutic efficacy of these antidepressant agents and to underlie the need for chronic treatments in order to see beneficial effects (Reviewed by Blier and de Montigny, 1994).

Thus, OPC's 5-HT_{1A} agonist activity at presynaptic 5-HT_{1A} receptors observed in the present study clearly suggests that the previously documented decreased 5-HT_{1A} autoreceptor response after only 48 hours of treatment (Bermack et al. In Press), is in fact a complete desensitization of the 5-HT_{1A} autoreceptor and not antagonism of this receptor. Furthermore, OPC acts much more rapidly in inducing a desensitization of the 5-HT_{1A} autoreceptors compared to classical antidepressants and 5-HT_{1A} agonists, as most of the studies have shown only a partial desensitization following a 7-day treatment and the necessity of at least two weeks of treatment to observe a complete desensitization (For

Review see Hensler, 2003). This further suggests that some 5-HT_{1A} agonists could be capable of inducing a rapid desensitization, as OPC's effects on the autoreceptor were not be mediated by its sigma receptor agonist activity (Bermack et al. In Press). Thus, by inducing a rapid desensitization, OPC may be able to induce a more rapid therapeutic effect.

In addition to 5-HT_{1A} autoreceptors, high densities of 5-HT_{1A} receptors located postsynaptically are found in the hypothalamus, amygdala, hippocampus, lateral septum, and frontal cortex (Pazos and Palacios, 1985; Kia et al., 1996). Activation of postsynaptic 5-HT_{1A} receptors in the hippocampus by 5-HT or 5-HT_{1A} agonists leads to membrane hyperpolarization and a suppression of the firing activity of hippocampal pyramidal neurons (Andrade et al., 1986; Colino and Halliwell, 1987; Tada et al., 1999).

Accumulating evidence has shown that the postsynaptic 5-HT_{1A} receptor and autoreceptor present different pharmacological profiles. Firstly, the effectiveness of 5-HT_{1A} agonists to inhibit the firing activity of DRN versus hippocampal neurons are different such that 5-HT_{1A} agonists appear to act as full agonists in the DRN while they act as partial agonists in the hippocampus (Blier and de Montigny, 1987; Sprouse and Aghajanian, 1988; Blier et al., 1993; Dong et al., 1997). Secondly, chronic SSRI and 5-HT_{1A} agonist treatments desensitize the 5-HT_{1A} somatodendritic autoreceptor in the DRN (Chaput et al., 1986, 1991; Invernizzi et al., 1994; Le Poul et al., 2000; Kreiss and Lucki, 1995), but do not change the responsiveness of postsynaptic 5-HT_{1A} receptors in the hippocampus (Le Poul et al., 2000; Chaput et al., 1986; Blier and de Montigny, 1987; Haddjeri et al. 1999). Thirdly, agonist-induced internalization of 5-HT_{1A} receptors only

occurs presynaptically in the DRN, but not postsynaptically in the hippocampus (Riad et al., 2001). These differences could be related to the difference in the G-proteins linked to the receptors (Lesch and Manji, 1992; Hensler, 2003). In addition, although the pre- and postsynaptic 5-HT_{1A} receptors originate from one gene, 3 mRNA's were detected for the 5-HT_{1A} receptor gene, which provides genetic evidence for the possibility of 5-HT_{1A} receptor subtypes (Albert et al., 1990). Thus, OPC's affinity at each population of 5-HT_{1A} receptors becomes important.

Therefore, in addition to OPC's agonist activity presynaptically, OPC's agonist activity postsynaptically on 5-HT_{1A} receptors has important consequences. Specifically, postsynaptic 5-HT_{1A} receptors contribute to the control of the firing activity of 5-HT neurons through a long negative-feedback loop (Blier and de Montigny, 1987; Sprouse and Aghajanian, 1988; Romero et al., 1994; Pineyro and Blier, 1999). This involves the activation of postsynaptic 5-HT_{1A} receptors, likely in the prefrontal cortex, leading through multiple synaptic connections, to the activation of γ–aminobutyric acid interneurons in the DRN, which in turn decreases 5-HT neurons' firing activity (Ceci et al., 1994; Haddjeri et al., 2000; Celada et al., 2001; Martin-Ruiz and Ugedo, 2001; Varga et al., 2001; Hajos et al., 1998,1999, 2003).

Postsynaptic 5-HT_{1A} receptors have also been shown to be involved in the action of antidepressants. Specifically, long-term treatments with antidepressants and repeated electroconvulsive shocks have been shown to enhance 5-HT neurotransmission through a sensitization or tonic activation of postynaptic 5-

HT_{1A} receptors in the hippocampus (de Montigny and Aghajanian, 1978; de Montigny, 1984; Haddjeri et al., 1998). This has led to the hypothesis that a selective postsynaptic 5-HT_{1A} agonist would exert a more rapid antidepressant effect by activating postsynaptic 5-HT_{1A} receptors without activating the autoreceptors (Blier and de Montigny, 1994).

Behavioural models of depression have shown that the activation of postsynaptic 5-HT_{1A} receptors produces antidepressant-like effects similar to those seen with classical antidepressants (Lucki et al., 1994). In addition, some clinical placebo-controlled studies have shown that treatments with the 5-HT_{1A} agonists gepirone, buspirone and ipsapirone in patients produce significant positive results for depression when used alone or in combination therapies (Robinson et al., 2003; Stahl et al., 1998, Dimitriou and Dimitriou, 1998, Blier and Ward, 2003). Therefore, the postsynaptic 5-HT_{1A} agonist profile of OPC also has implications towards OPC's potential effects in depression and the combination of effects on both 5-HT_{1A} receptor populations may be particularly beneficial.

Our results therefore suggest that OPC presents several aspects, each of them being by itself suggestive of a potential antidepressant effect. Firstly, it possesses an agonistic effect on $sigma_1$ receptors (Bermack and Debonnel, Submitted; Bermack et al., In Press). This, in itself could be sufficient to produce an antidepressant effect since it is responsible for the increase of the firing activity of DRN 5-HT neurons and is in keeping with the fact that selective sigma ligands have been reported to present an antidepressant-like profile in preclinical studies

(Urani et al., 2001; Matsuno et al., 1996; Kinsora et al., 1998; Ukai et al., 1998) or an antidepressant effect in humans (Pande et al., 1998). Secondly, OPC acts an agonist at somatodendritic 5-HT_{1A} autoreceptors in the DRN and induces a complete desensitization of the 5-HT_{1A} somatodendritic autoreceptor after only 2 days of treatment (Bermack et al., In Press). Also, as discussed, OPC possesses agonist properties at postsynaptic 5-HT_{1A} receptors, which could play a role in its potential antidepressant effects. In conclusion, with the combination of all these aspects, OPC could represent a potent antidepressant with a rapid onset of action. However, to further confirm this hypothesis, it remains to be established what effect OPC produces following chronic treatments and clinical trials.

FIGURE LEGENDS

Figure 1

(A) Representative spontaneous firing rate histogram of a dorsal raphe 5-HT neuron. In this and the following figures the boxes above the tracing represent the microiontophoretic applications of various compounds and the number refers to the current used in nA. (B) Percent of baseline firing activity of DRN 5-HT neurons (mean ± S.E.M.) following the microiontophoretic application of the 5-HT_{1A} agonist 8-OH-DPAT (PAT), and PAT and the 5-HT_{1A} antagonist WAY 100635 (WAY) together. (C) Percent of baseline firing activity of DRN 5-HT neurons (mean ± S.E.M.) following the microiontophoretic application of OPC, and OPC and the 5-HT_{1A} antagonist WAY together. In this and all subsequent figures the number in the box in the first column represents the number of neurons contributing to the average.* $p < 0.05$ compared to control; # $p<0.05$ between the two groups indicated.

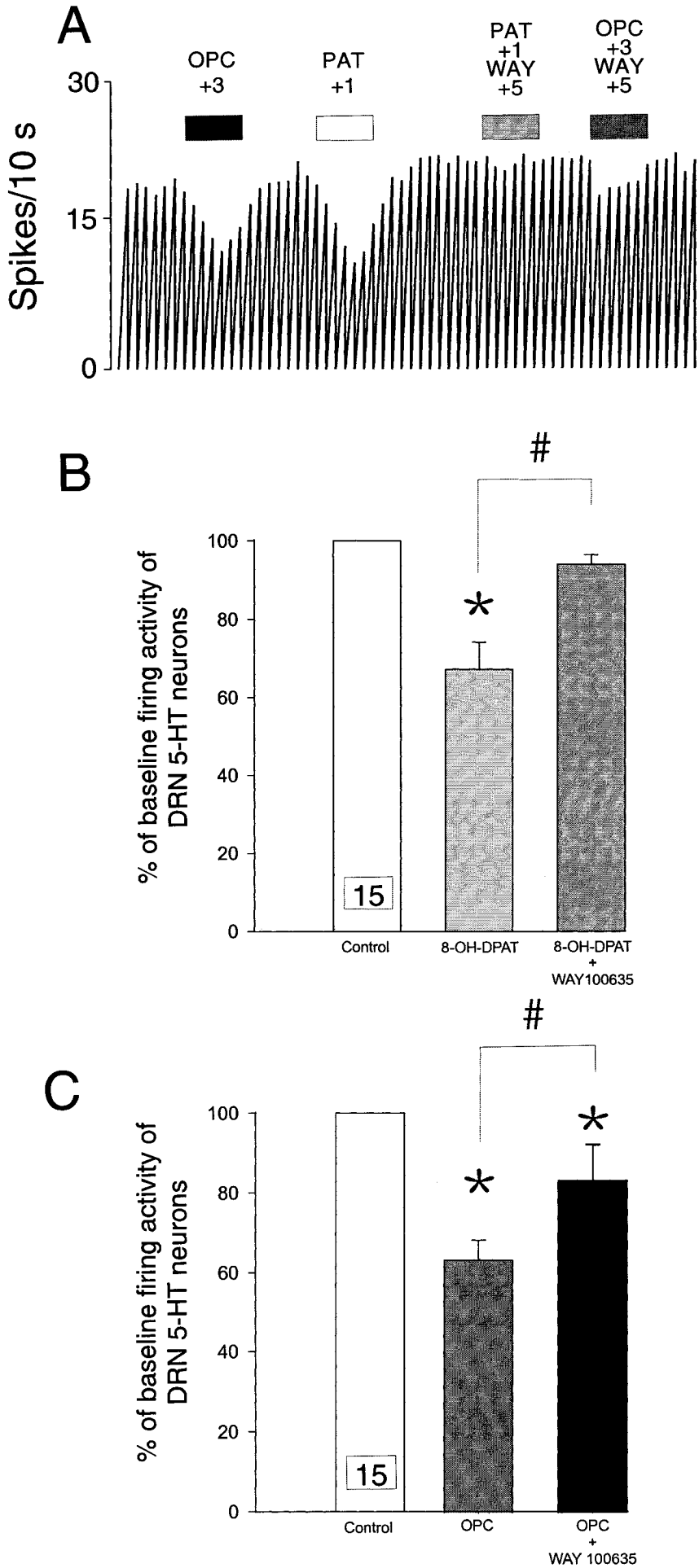

A
OPC
+3
PAT
+1
PAT
+1
WAY
+5
OPC
+3
WAY
+5
Spikes/10 s
30
15
0
B
#
*
% of baseline firing activity of
DRN 5-HT neurons
100
80
60
40
20
0
15
Control
8-OH-DPAT
8-OH-DPAT
+
WAY100635
C
#
*
*
% of baseline firing activity of
DRN 5-HT neurons
100
80
60
40
20
0
15
Control
OPC
OPC
+
WAY 100635

Figure 1

Figure 2

(A) Representative spontaneous firing rate histogram of a CA_3 pyramidal neuron of the dorsal hippocampus. The boxes above represent the microiontophoretic application of various compounds and the number refers to the current used in nA. (B) Percent of baseline firing activity of CA_3 pyramidal neurons (mean ± S.E.M.) following the microiontophoretic application of the 5-HT_{1A} agonist PAT, and PAT and the 5-HT_{1A} antagonist WAY together. (C) Percent of baseline firing activity of CA_3 pyramidal neurons (mean ± S.E.M.) following the microiontophoretic application of OPC, and OPC and the 5-HT_{1A} antagonist WAY together. * $p < 0.05$ compared to control; # $p<0.05$ between the two groups.

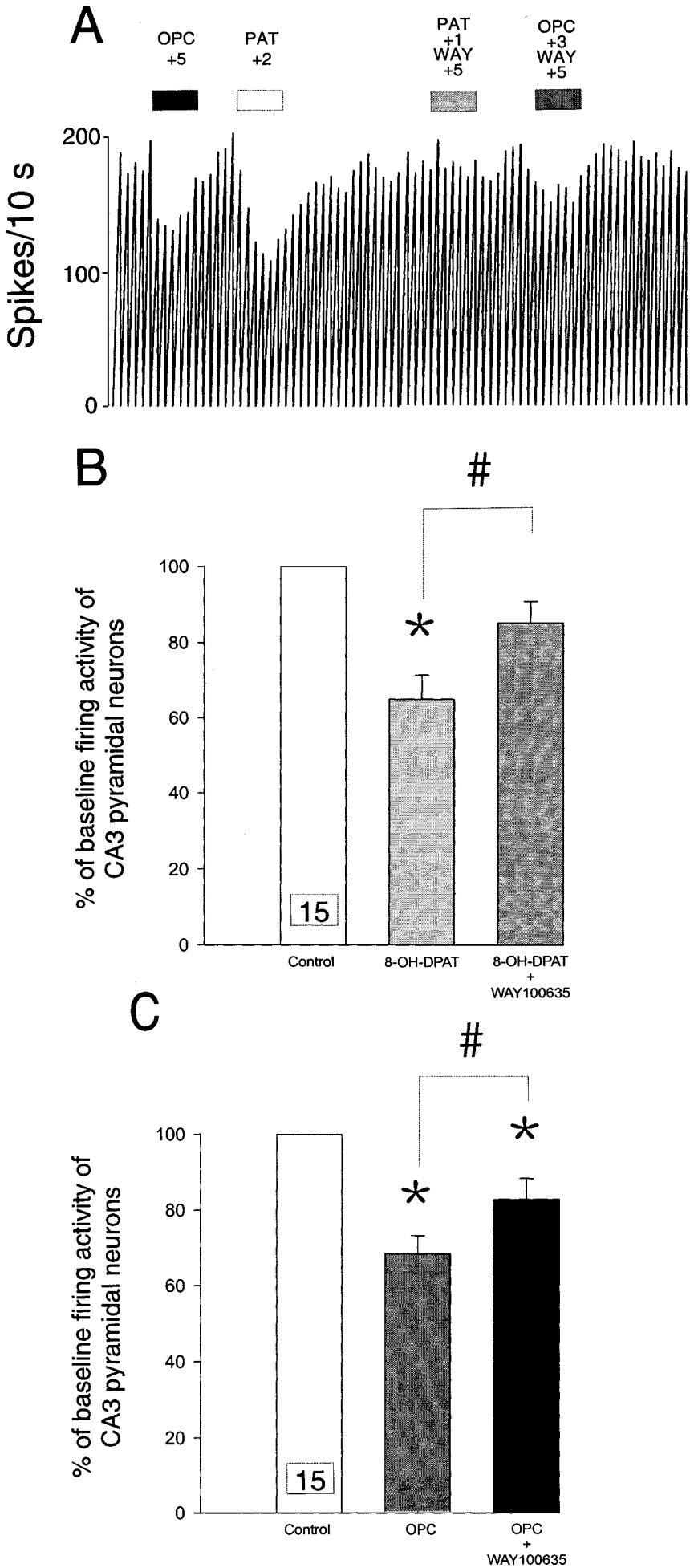

A
OPC
+5
PAT
+2
PAT
+1
WAY
+5
OPC
+3
WAY
+5
Spikes/10 s
200
100
0
B
#
*
% of baseline firing activity of CA3 pyramidal neurons
100
80
60
40
20
0
15
Control
8-OH-DPAT
8-OH-DPAT
+
WAY100635
C
#
*
*
% of baseline firing activity of CA3 pyramidal neurons
100
80
60
40
20
0
15
Control
OPC
OPC
+
WAY100635

Figure 2

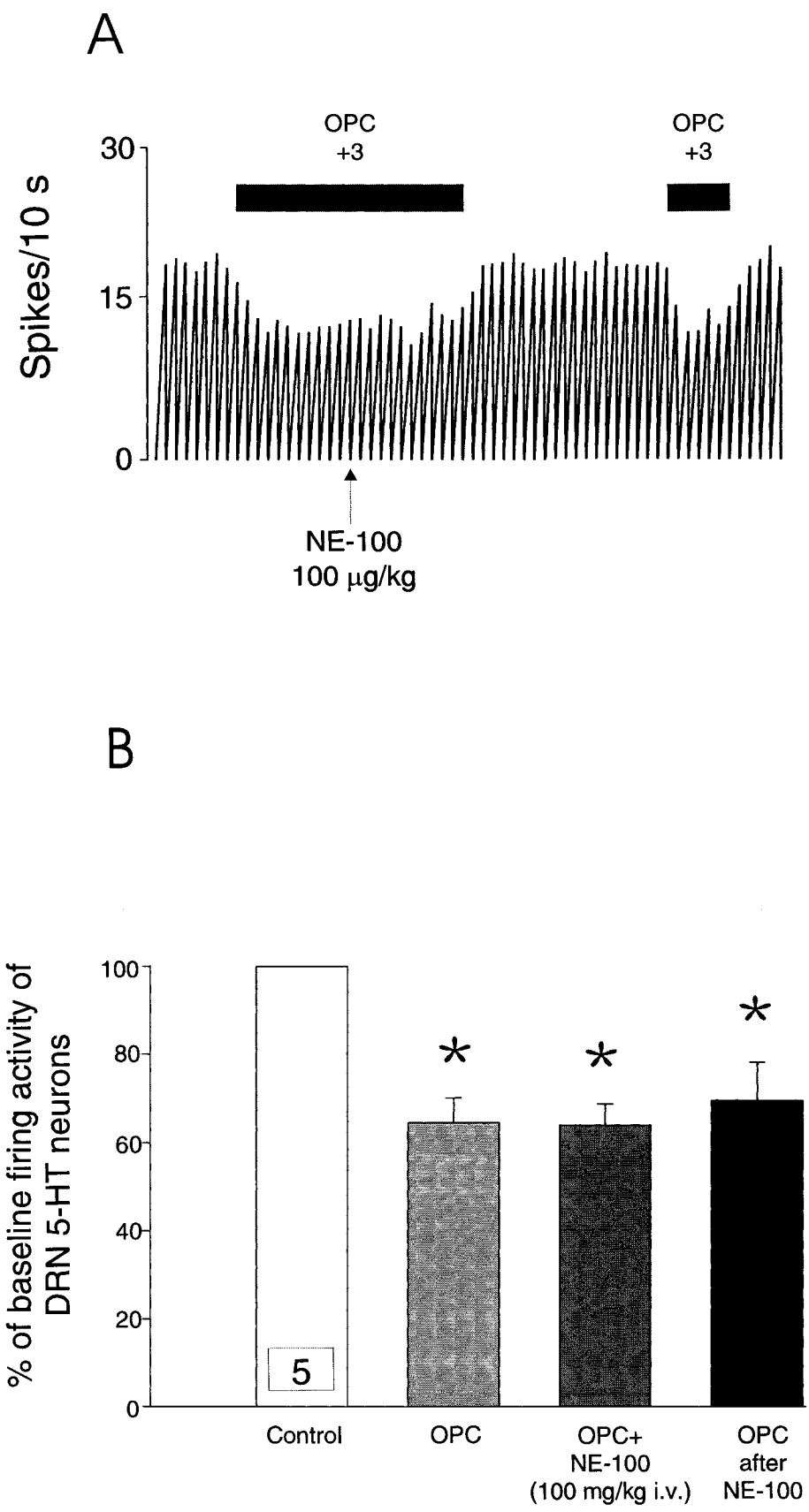

A
OPC
+3
OPC
+3
30
15
0
Spikes/10 s
NE-100
100 μg/kg
B
% of baseline firing activity of DRN 5-HT neurons
100
80
60
40
20
0
5
*
*
*
Control
OPC
OPC+
NE-100
(100 mg/kg i.v.)
OPC
after
NE-100

Figure 3

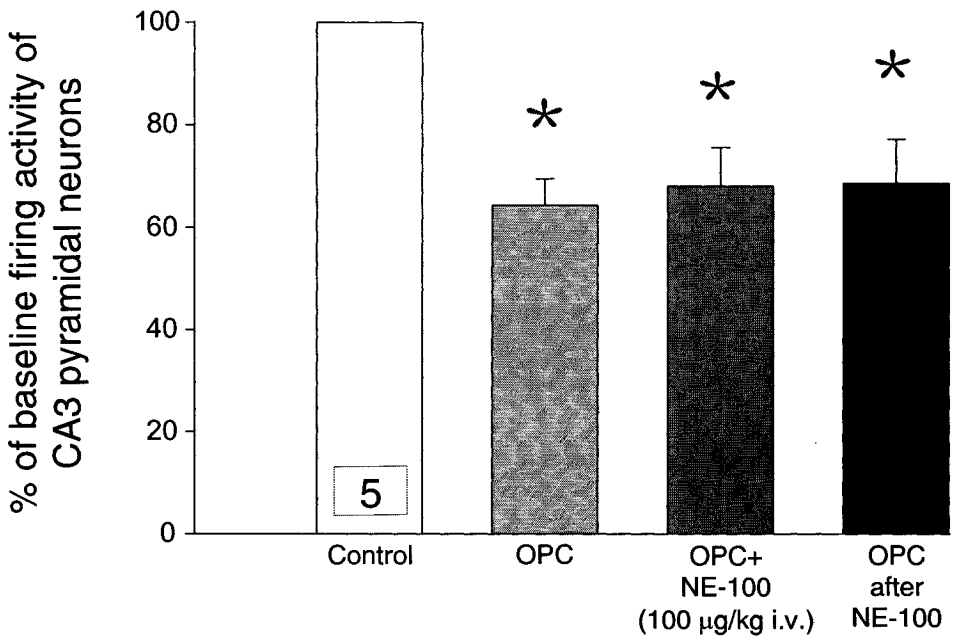
Figure 3

(A) Representative spontaneous firing rate histograms of DRN 5-HT neurons. The boxes above represent the microiontophoretic application of OPC using a current of +3 nA. (B) Percent of baseline firing activity of DRN 5-HT neurons (mean ± S.E.M.) following the microiontophoretic application of OPC, and during and following the intravenous administration of the $sigma_1$ antagonist NE-100 (100 μg/kg) * $p < 0.05$ compared to control.

Figure 4

(A) Percent of baseline firing activity of CA_3 pyramidal neurons of the dorsal hippocampus (mean ± S.E.M.) following the microiontophoretic application of OPC, and during and following the intravenous administration of the $sigma_1$ antagonist NE-100 (100 μg/kg) (B) Percent of baseline firing activity of CA_3 pyramidal neurons of the dorsal hippocampus (mean ± S.E.M.) following the microiontophoretic application of OPC, and during and following the intravenous administration of the sigma antagonist haloperidol (100 μg/kg) * $p < 0.05$ compared to control.

A


% of baseline firing activity of CA3 pyramidal neurons
100
80
60
40
20
0
5
*
*
*
Control
OPC
OPC+ NE-100 (100 µg/kg i.v.)
OPC after NE-100

B

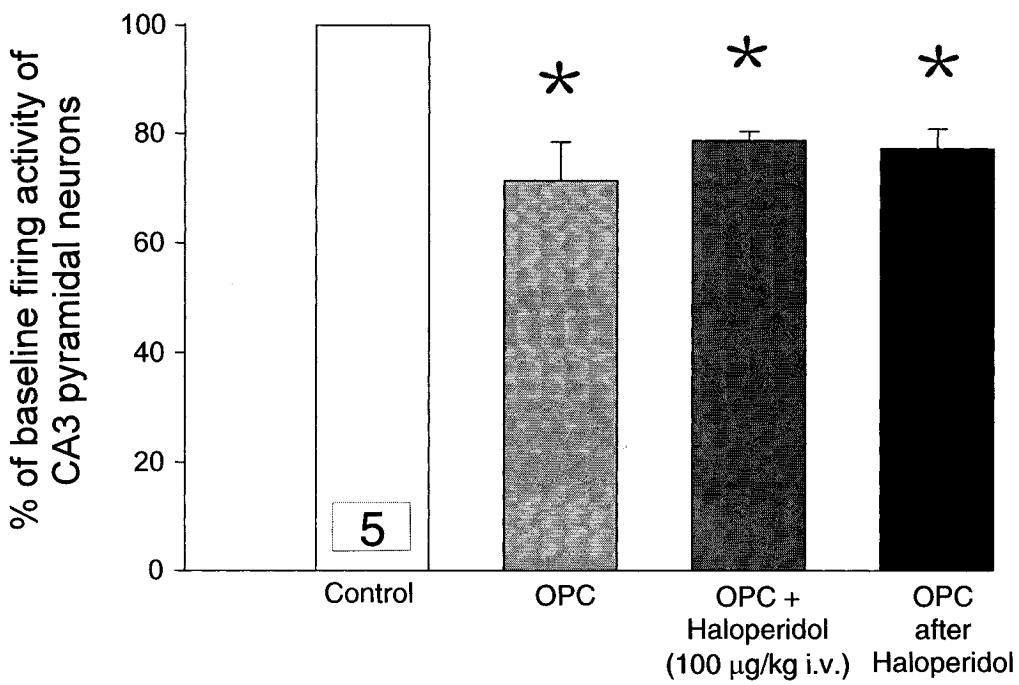

% of baseline firing activity of CA3 pyramidal neurons
100
80
60
40
20
0
5
*
*
*
Control
OPC
OPC + Haloperidol (100 µg/kg i.v.)
OPC after Haloperidol

Figure 4

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Chapter 7: Additional Comments

One discrepancy appeared between the two studies involving OPC and its acute effects in the DRN. In Chapter 6, when OPC was administered intravenously, it did not have any effect on the basal firing activity of DRN 5-HT neurons (doses of 20-50 μg/kg). In contrast, in Chapter 7 when OPC was applied directly into the DRN by microiontophoresis, OPC induced a decrease in the firing activity of 5-HT neurons. This decrease was significantly attenuated by the co-application of the 5-HT_{1A} antagonist WAY 100635. These results suggest that OPC possesses agonist activity at 5-HT_{1A} autoreceptors. However, if this was the case, one would also expect the intravenous administration of OPC to induce a decrease in the firing activity, as seen with other 5-HT_{1A} agonists, but this is not something that we observed in our experiments.

There are two issues that may be involved in this discrepancy. Firstly, is the issue of doses. The technique of microiontophoresis, which uses an electrical current to apply a compound directly to the brain region, makes it impossible to correlate the current applied with specific doses. However, as discussed in Chapter 7, OPC and 8-OH-DPAT have similar affinities for the 5-HT_{1A} receptors, and a dose of 4 μg/kg i.v. of 8-OH-DPAT is capable of inducing a complete cessation of the firing activity of DRN 5-HT neurons. Therefore, the doses of OPC tested should be sufficient to activate the presynaptic autoreceptors. In addition as discussed previously, 5-HT_{1A} agonists can also act postsynaptically through a long-feedback loop to induce a decrease in the firing activity of DRN 5-HT neurons. The dose of OPC used was likely sufficient enough for one to

assume that the pre- and postsynaptic 5-HT_{1A} receptors are activated, and dose was likely not the issue leading to the lack of inhibitory effects of OPC on 5-HT firing activity.

Alternatively, an issue that more likely explains the observed discrepancy between intravenous administration and the microiontophoretic application of OPC is the involvement of input from other brain regions that would occur when OPC is administered intravenously. Due to the reported lower density of sigma receptors in the DRN, brain regions with higher densities of sigma receptors such as the cortex or hippocampus may be mediating the effects of intravenous OPC. For example, sigma ligands, including OPC, can induce acute excitatory effects on NMDA receptor-mediated signaling in the hippocampus (Chapter 5). This excitation of hippocampal CA_3 pyramidal neurons could in turn influence the firing activity in the DRN. Therefore, when OPC is administered intravenously, it could be acting on sigma receptors in forebrain regions, which may in turn be sending afferent inputs to the DRN. The activity of the DRN is dependent on the sum of the inhibitory and excitatory inputs from other brain regions, and through OPC activating excitatory inputs it could be negating the inhibitory effects of 5-HT_{1A} agonism. Conversely, microiontophoresis localizes the application of OPC to the DRN, therefore, any effects observed are mediated only by 5-HT_{1A} and/or sigma receptors situated in the DRN and there are no effects of afferent inputs.

The putative method underlying the mechanism of action of sigma ligands on the DRN is further discussed in the Conclusion of this thesis, where further details are discussed with respect to the various feedback loops what may be involved in the regulation of DRN 5-HT neuronal activity by sigma ligands.

Chapter 8: Conclusion

Sigma receptors, since first identified in 1976 (Martin et al.), have been the focus of many lines of research in order to better understand their role in the CNS. They have since been found to play a modulatory role on neurotransmission, and in the modulation of Ca^{+2} (Brent et al. 1996, 1997, Hayashi et al. 2000, Hayashi and Su 2001) and K^{+} signaling (Soriani et al. 1999, Wilke et al. 1999, Aydar et al. 2002). Years of research on sigma receptors and their ligands have permitted their clinical potential to come to light, particularly in psychiatry, in such disorders as schizophrenia (Weissman et al. 1991, Helmeste et al. 1996, Takahashi et al. 1999, Guitart and Farre 1998) and drug abuse (reviewed by Maurice et al. 2002). Our focus for the purpose of this thesis was to investigate sigma ligands' potential as antidepressants.

To study sigma ligands' potential as antidepressants, we have focused on sigma receptor's modulation of the glutamatergic and 5-HT neurotransmissions, as both of these systems both have been heavily implicated in depression and antidepressant action (see Introduction).With respect to the glutamatergic system, our laboratory previously established a model demonstrating that sigma receptors modulate NMDA-induced neuronal responses in the dorsal hippocampus (Monnet et al. 1990, 1992, Debonnel et al. 1992, Bergeron et al. 1995, Couture and Debonnel 1998).Given this documented modulatory effect of sigma ligands and the fact that sigma receptors are present at a higher density in the hippocampus compared to the DRN (Bouchard and Quirion 1997, Alonso et al. 2000, Inoue et al. 2000), we deemed it relevant to further investigate sigma ligand's modulation of glutamatergic transmission in the hippocampus. This was combined with

investigating sigma ligands' potential modulatory effects on 5-HT neurotransmission in the DRN, as both have implications for the antidepressant potential of sigma ligands.

In the first study, we investigated the effects of treatments with sigma ligands on NMDA receptor-mediated behaviours in the OBX model, an established animal model of depression (Jesberger and Richardson 1988, Lumia et al. 1992, Kelly et al. 1997). A role for NMDA receptors has been established in this model as OBX surgery induces a decrease in NMDA receptor ligand binding (Robichaud et al. 2001).

There were a few key finding from this study. Firstly, treatments with sigma ligands were able to reverse the effects of OBX surgery on NMDA receptor-mediated behaviours. The effects of the sigma ligands tested were dependent on the dose administered and the duration of treatment. The effects of the different doses supports the bell-shaped dose-response curve previously suggested for sigma ligands in electrophysiological studies (Bergeron et al. 1995, Monnet et al. 1996) and behavioural models (Maurice et al. 1994 a,b, Tottori et al. 2002).

Importantly, this study using the OBX model of depression, suggests sigma ligands' potential role as antidepressants in an animal model that is not based solely on behaviour. This study clearly demonstrates that treatments with sigma ligands can reverse OBX-induced behaviours in a dose- and duration-dependent manner. Although the study focused on NMDA-mediated behaviours, the "antidepressant-like" effects observed could be due to a modulation of glutamatergic or serotonergic neurotransmission by sigma receptors.

When examining potential novel antidepressants, one cannot exclude the involvement of the 5-HT system, as 5-HT has been found to play a key role in depression and/or the mechanism of action of antidepressants (See Introduction). Electrophysiological and microdialysis studies have clearly shown that the 5-HT system is altered by short- and long-term treatments with various classes of antidepressants (Chaput et al. 1986, Blier and de Montigny 1994, Kreiss and Lucki 1995). Thus, it begs the question, when examining sigma ligands' potential as antidepressants, whether they can modulate 5-HT neurotransmission.

Therefore, in the second study we examined the effects of short- and long-term administration of various sigma ligands on the basal firing activity of 5-HT neurons of the DRN. Past studies using this electrophyiological model have shown that acute and short-term treatments with antidepressants such as SSRI's and MAOI's induce a decrease in the firing activity of 5-HT neurons of the DRN (Blier et al. 1984, Blier and de Montigny 1985, Chaput et al. 1986). However, after long-term treatments there is a restoration of the firing activity of the 5-HT neurons (Blier et al. 1984, 1988, Chaput et al. 1986, 1991), due to the desensitization of the 5-HT_{1A} autoreceptors (Blier and de Montigny 1994, Kreiss and Lucki 1995, LePoul et al. 2000). This model of recording from 5-HT neurons of the DRN provided an ideal model to study sigma ligands' effect on 5-HT neurotransmission in a manner in which the results can be compared to those seen with classical antidepressants. Based on the different outcomes observed previously in this model by varying the duration of treatments, it was crucial to examine the effects of sigma ligands after both short- and long-term treatments.

Interestingly, in our second study, we found that the sigma ligands 4-IBP and (+)-pentazocine, following 2 and 21-day treatments, induced significant increases in the firing activity of 5-HT neurons of the DRN. First, these findings suggest a clear modulation of 5-HT neurotransmission by sigma ligands *in vivo*, a novel finding with respect to sigma receptor research. This modulation of 5-HT transmission supports a potential role for sigma receptors in depression and/or antidepressant action. Second, this study suggested that these sigma ligands modulate 5-HT neurotransmission by a mechanism different from classical antidepressants, as they induced an increase in the firing activity of 5-HT neurons after only 2 days of treatment. This suggests that sigma ligands may be capable of acting as antidepressants with a fast onset of action, due to the fact that by an unknown mechanism, sigma receptors lead to an increase in the firing activity of 5-HT neurons.

A second line of conclusions from this study is related to the suggestion of subtypes of the $sigma_1$ receptor (Introduction Section 1.1). In this study, the effects of (+)-pentazocine were blocked by the co-administration of the $sigma_1$ antagonist NE-100. However, 4-IBP's effects on 5-HT neurotransmission were blocked by haloperidol, a non-selective sigma antagonist, and not by NE-100. This data suggested that perhaps 4-IBP and (+)-pentazocine were acting through different subtypes of the $sigma_1$ receptors. As discussed in the Introduction, the existence of $sigma_1$ receptor subtypes has been previously suggested by our and other laboratories (Basile et al. 1992, Monnet et al. 1994, Vilner et al. 1995, Debonnel et al. 1996, Tsao and Su 1997, Couture and Debonnel 2001). A new classification of sigma receptor subtypes would represent a key step in fully

understanding sigma receptors' role in the modulation of neurotransmission. As more selective sigma ligands are developed, future studies will be able to further elucidate the role of particular $sigma_1$ receptor subtypes.

Data from the cloning of the $sigma_1$ receptor showed that the $sigma_1$ receptor is composed of a one-transmembrane segment (Kekuda et al. 1996, Seth et al. 1998). A possible explanation is that the sigma receptor is made up of multiple subunits and that the different combinations of these subunits will lead to different actions, likely through different effector systems. Based on this theory, what was cloned was possibly only the ligand binding subunit of the receptor. Thus, the sigma receptor may possess multisubunit structures similar for those previously described for $GABA_A$ and NMDA receptors, such that different subunit compositions yield different functional properties (reviewed by Rudolph et al. 2001, Yamakura and Shimoji 1999, Cull-Candy et al. 2001).

In support of a multiunit receptor concept, sigma receptors have been shown to interact with other proteins and previous studies suggested that protein-protein interactions are the method of signal transduction employed by sigma receptors (Lupardus et al. 2000, Hayashi and Su 2001). In addition, a recent study demonstrated that the cloned $sigma_1$ receptor serves as an auxiliary subunit for voltage-dependent K^+ channels (Aydar et al. 2002). The involvement of different subunit combinations is one way to explain the discrepant data we have seen thus far with respect to sigma receptor research, such as the variation the effector systems involved, and sigma receptor subtypes. Based on this hypothesis, our data suggests that the 5-HT and glutamatergic systems can be modulated by different

sigma receptor subunit combinations, thus, explaining why different ligands work in different ways and are blocked by different antagonists.

The discrepancy between 4-IBP and (+)-pentazocine raises the question as to the mechanism of action of 4-IBP in the model of 5-HT transmission in the DRN. (+)-Pentazocine has previously been shown to be a $sigma_1$ agonist in our model of the modulation of the NMDA response in the dorsal hippocampus (Monnet et al. 1992, Bergeron et al. 1995), however 4-IBP had yet to be tested in this model. Thus, we deemed it necessary to investigate the effects of 4-IBP on glutamatergic neurotransmission in the hippocampus to determine whether it would present agonist or antagonist activity at $sigma_1$ receptors.

In the third study, 4-IBP showed an interesting profile in the model of the modulation of glutamatergic transmission in the dorsal hippocampus. 4-IBP was able to show different responses, such that in half the neurons encountered it induced a potentiation in the NMDA- and QUIS-induced responses. However, in the other half of the neurons encountered it induced an attenuation of the NMDA- and QUIS-induced responses. This response induced by 4-IBP was novel in a few ways. First, 4-IBP appeared to act as an inverse agonist *in vivo* at sigma receptors in this experimental paradigm. This is based on the observations that 4-IBP acted in an opposite manner to sigma agonists and was blocked by the administration of a $sigma_1$ antagonist. Inverse agonism activity has yet to demonstrated at sigma receptors *in vivo*, but *in vitro* inverse agonist activity has been shown by sigma ligands in an *in vitro* model of NMDA-mediated [^{3}H]NE release from hippocampal slices (Monnet et al.1995).

Second, the findings in the third study support that 4-IBP acts differently than other sigma agonists in the hippocampus, which is in accordance with the difference observed in the DRN model and constitutes a further argument for subtypes of the $sigma_1$ receptor, discussed previously. This could to explain the profile we see in the hippocampus with 4-IBP, where a different subtype of the sigma receptor may be involved depending on the initial response of CA_3 pyramidal neurons to excitatory agents. Despite the interesting implications of the potential subtypes toward sigma receptor pharmacology the main focus of this project remains the antidepressant potential of sigma ligands.

A new compound, OPC-14523 was recently developed (Oshiro et al. 2000). OPC-14523 is a sigma ligand that also has high affinity for the $5\text{-}HT_{1A}$ receptor and moderate 5-HT reuptake blocking activity (Oshiro et al. 2000, Tottori et al. 2001). OPC-14523 was clearly an interesting compound for us study based on its combination of properties, as OPC-14523's affinity at $5\text{-}HT_{1A}$ receptors has implications towards the modulation of the 5-HT system. Both somatodendritic and postsynaptic $5\text{-}HT_{1A}$ receptors have been shown to play a role in the control of 5-HT firing activity in the DRN (Aghajanian 1978, Blier and de Montigny 1987, Sprouse and Aghajanian 1988, Romero 1994, Hajos et al. 1999, 2003), and in the mechanisms of action of antidepressants (Blier and de Montigny 1994, Kreiss and Lucki 1995, Haddjeri et al. 1998, LePoul et al. 2000, Hervas et al. 2001). Thus, the forth study investigated the impact of this combination of properties of OPC-14523 in the model of hippocampal glutamatergic neurotransmission and the fifth study examined OPC-14523's effects on DRN 5-HT neurotransmission.

First, the study in the hippocampus demonstrated that OPC-14523 acts as a sigma agonist in the model of the modulation of NMDA-mediated neuronal activity in the dorsal hippocampus. Furthermore, OPC-14523 displayed a bell-shaped dose-response curve, similar to that reported for other sigma ligands (Debonnel et al. 1992, Bergeron et al. 1995), however it was narrower. The narrower curve may be due to activation of postsynaptic 5-HT_{1A} receptors in the hippocampus by OPC-14523. Furthermore, this study showed that OPC-14523 modulated NMDA- and QUIS-induced responses, an effect mediated mainly by sigma receptors. This modulation of glutamatergic activity shown by OPC-14523, has implications towards OPC-14523's potential as an antidepressant, given the role of glutamatergic neurotransmission in depression and the mechanism of action of antidepressants (reviewed by Paul and Skolnick 2003, Palucha and Pilc 2002).

The fifth study on OPC-14523's effects on 5-HT neurotransmission in the DRN also yielded interesting results. OPC-14523, similarly to other sigma ligands tested in the second study, induced a significant increase in the firing activity of 5-HT neurons of the DRN after two days of treatment. This increase in the firing activity was blocked by the co-administration of NE-100, suggesting that it was mediated by $sigma_1$ receptors. In addition, OPC-14523 after a short-term (two days) treatment induced a decrease in the responsiveness of the 5-HT_{1A} autoreceptor. This is particularly significant given that classical antidepressant medications require chronic treatment for this decrease autoreceptor response to occur (Chaput et al. 1986, Kreiss and Lucki 1995, Le Poul et al. 2000). This rapidly decreased responsiveness of the 5-HT_{1A} autoreceptor in addition to the

observed rapid increase in the firing activity of 5-HT neurons after only two days of treatment with OPC-14523 suggests that OPC-14523 has potential to produce a fast onset of its antidepressant action.

OPC-14523's exact effects at 5-HT_{1A} receptors were not known. Thus, the sixth study used microiontophoresis techniques in the DRN and hippocampus to elucidate OPC-14523's effects at 5-HT_{1A} receptors pre- and postsynaptically, respectively. The results from this study showed that OPC-14523 acts as a 5-HT_{1A} agonist, inducing a suppression of the firing activity in both the DRN and hippocampus. Thus, the profile for OPC-14523 is particularly intriguing as both 5-HT_{1A} receptor populations play key roles in antidepressant therapy (Blier and de Montigny 1994, Lucki et al. 1994, Haddjeri et al. 1998).

The question remains as to how OPC-14523 induced such a rapid increase of the firing activity. This may not only involve the rapid decreased autoreceptor response, as other sigma ligands also induced an increase in the firing activity without an effect on the autoreceptor (Bermack and Debonnel 2003). Therefore, the results suggest that two separate mechanisms of action are involved with respect to the effects on the autoreceptor and the increased firing activity. The desensitization of 5-HT_{1A} autoreceptors by OPC-14523 is most likely due to OPC-14523's action as a presynaptic 5-HT_{1A} agonist combined with its action at sigma receptors. However, the increase in the firing rate induced by sigma ligands involves a mechanism common to all sigma ligands.

Numerous results from our laboratory, some included in this thesis, have demonstrated that sigma ligands modulate NMDA receptor-mediated transmission in the dorsal hippocampus (Monnet et al. 1990, 1992, Bergeron et al. 1995). This

ability of sigma ligands is present in other brain regions in addition to the hippocampus, as sigma ligands also modulate NMDA receptor-mediated responses in the ventral tegmentum, nucleus accumbens and striatum (Monnet et al 1990, 1995, Gonzalez-Alvear and Werling 1995, Gronier and Debonnel 1999, Nuwayhid and Werling 2003). Therefore, it is possible that sigma ligands modulate 5-HT neurotransmission in the DRN through modulating NMDA-mediated excitatory inputs to the DRN. However, no acute effects of sigma ligands were seen in the DRN in these studies, whereas sigma ligands acutely modulated NMDA responses in the hippocampus, suggesting that a more indirect mechanism may underlie the effects of sigma ligands in the DRN.

Taking into account the higher concentration of sigma receptors in the hippocampus, the mechanism by which sigma ligands modulate 5-HT neurotransmission in the DRN may be consequent to the modulation of glutamatergic neurotransmission. Specifically, treatments with sigma ligands may rapidly modulate NMDA receptor-mediated transmission in the hippocampus, and potentially other forebrain regions, which in turn leads to a modulation of 5-HT neurotransmission in the DRN via feedback loops to DRN 5-HT neurons. Indeed an afferent connection has been identified that projects from the hippocampus to the DRN via the lateral habenula (Aghajanian and Wang 1977, Kalen et al. 1985, Peyron et al. 1998). This afferent was found to be excitatory as stimulation of the lateral habenula increases the rate of discharge of DRN 5-HT neurons (Ferrero et al. 1996, Varga et al. 2003).

It was also determined that this excitatory connection between the DRN and the lateral habenula involves the activation of NMDA receptors as

ionotophoretic application of NMDA in the DRN significantly increase the firing rate of DRN 5-HT neurons, while iontophoretic applications of 2-amino-5-phosphovaleric acid (2-APV), a competitive NMDA receptor antagonist, suppress the effects of lateral habenular stimulation on 5-HT neurons of the DRN (Ferrero et al. 1996). These findings are in agreement with previous data demonstrating that 5-HT neuronal discharge and 5-HT release in the DRN is increased by glutamate agonists including NMDA (Vandermaelen et al. 1986, Becquet et al. 1993, Tao and Auerbach 1996, 2000). Thus, excitation of the lateral habenula is one way through which activation of dorsal hippocampal pyramidal neurons may lead to excitation of 5-HT neurons of the DRN.

Another feedback loop that may be involved in the results observed is the "long feedback loop" that projects from the DRN to the mPFC and back to the DRN (O'Hearn and Molliver 1984, Ceci et al. 1994, Hajos et al. 1998, Peyron et al. 1998, Celada et al 2001, Varga et al. 2001, Hajos et al. 2003). Stimulation of the mPFC has been shown to excite a subpopulation of DRN 5-HT neurons (Hajos et al. 1998, Celada et al. 2001, Varga et al. 2003). Moreover, NMDA and AMPA-kainate receptors have been shown to be involved in the activation of DRN 5-HT neurons by mPFC afferents as antagonist of these receptors block the excitation produced by mPFC stimulation (Celada et al. 2001). However, this long feedback loop also exerts inhibitory influences on DRN 5-HT neuronal activity, depending on the involvement of GABAergic interneurons, as recent data suggests that the mPFC projections to the DRN preferentially target local GABAergic neurons (Hajos et al. 1998, Varga et al. 2001, 2003). To this end, the activity in the DRN is dependent on the balance between the excitatory input from

various brain regions (e.g. lateral habenula and mPFC) and inhibitory input from GABAergic interneurons in distal areas (e.g. periaqueductal gray area) and local GABAergic interneurons situated in the DRN (Pan and Williams 1989, Wang et al. 1992, Abellan et al. 2000, Varga et al. 2003).

Thus, since the sigma ligands in the experiments of the present thesis have been administered subcutaneously via osmotic minipump it is possible that they are leading to a potentiation of NMDA receptor-mediated responses in the mPFC or other brain regions in addition to the hippocampus. Thus, if the administration of sigma ligands potentiates NMDA receptor-mediated responses in the mPFC this could cause an increase in the excitatory input from the mPFC to the DRN. Therefore, it is possible that the effect of sigma ligands over time leads to a gradual increase in the excitatory feedback to the DRN, which at some point may overcome the GABAergic inhibitory influences and shift the balance controlling the activity of the 5-HT neurons of the DRN leading to an overall net increase in DRN 5-HT neuronal activity. This theory could explain why chronic treatments are needed for any alterations to be observed in the DRN but not in the hippocampus.

Another factor contributing to the time delay is likely based on the density of sigma receptors at the plasma membrane, which has been shown to be altered by the presence of sigma ligands. This was shown in cell lines in which treatments with sigma agonists induce an increase in the sigma receptor density at the plasma membrane, but this requires approximately two days of treatment (Takebayashi et al. 2002). Thus, as treatments with sigma ligands continue, and sigma receptors increase their density at the plasma membrane, they can exert more significant

effects on NMDA receptor-mediated signaling. Based on this theory, sigma receptors may also be increasing in concentration in DRN neuronal plasma membranes so that a stronger modulation of NMDA receptor-mediated signaling occurs.

The molecular mechanism underlying sigma receptors' ability to modulate 5-HT and glutamatergic transmissions may involve sigma receptors' ability to modulate Ca^{+2} (Brent et al. 1997, Morin-Surun et al. 1999, Hayashi et al. 2000, Hayashi and Su 2001) or K^{+} signaling (Soriani et al. 1998, Wilke et al. 1999, Lupardus et al. 2000, Aydar et al. 2002). This modulation of cell excitability may provide a mechanism by which sigma ligands are capable of inducing relatively rapid effects on neurotransmission versus classical antidepressants.

It is likely that sigma ligands' ability to modulate both glutamatergic and 5-HT transmissions contribute to the "antidepressant-like" effects observed in behavioural models. It is possible that sigma receptors act at a downstream target involved with both systems, thus leading to one primary goal or target. The effect of antidepressants on downstream targets has been a recent and important shift in antidepressant research. As reviewed by recent literature, downstream targets are the focus for a mechanism of action for antidepressants and downstream adaptive changes could explain the time lag seen between changes in neurotransmitter systems and therapeutic effects seen in patients (reviewed by Duman 1997, 2003, Young et al. 2002, Chao 2003, Manji et al. 2003). One downstream target of particular interest in antidepressant treatments is neurotrophic factors (reviewed

by Duman 1997, Coyle and Duman 2003, Young et al. 2002, Chao 2003, Manji et al. 2003).

One member of the neurotrophin family, BDNF, has been heavily implicated in the action of antidepressants, as chronic treatments with a variety of antidepressant therapies induce an increase in BDNF expression (Nibuya et al. 1995, Zetterstrom et al. 1998, Rosello-Neustadt et al. 1999, Altar et al. 2003). Moreover, BDNF administration itself has been shown to produce antidepressant effects in behavioural models of depression (Siuciak et al. 1997, Shirayama et al. 2002).

There is some evidence that supports the hypothesis of sigma ligands having effects on neurotrophins, specifically NGF, as Su and colleagues have shown that treatments with sigma agonists induce a potentiation of NGF activity (Takebayashi et al. 2002). Interestingly, classical antidepressant treatments have similar effects as sigma agonists in the potentiation of NGF-induced neurite sprouting, and these effects were found to be dependent on sigma receptor activation (Takebayashi et al. 2002). This effect of sigma ligands on NGF activity could be involved in the effects of sigma ligands observed following short- and long-term treatments in the DRN, similarly to BDNF's putative role in antidepressant action. To this end, sigma ligands could be either activating neurotrophin signaling pathways or activating pathways that act synergistically to neurotrophin pathways.

It would be interesting to study the effects of sigma ligands on BDNF. Thus far this has only been studied with the sigma ligand E-5842, which showed no effects on BDNF or NGF levels following chronic treatments (Ovalle et al.

2002). However, E-5842 presents a sigma antagonist profile, so more studies are necessary to investigate whether sigma agonists may potentiate the effects of BDNF similarly to that observed previously with NGF.

More research is required to elucidate the basis for the observed potentiation of neurotrophin effects by sigma agonists and whether sigma ligands affect neuronal survival and neurogenesis. From there it would be interesting to see whether these effects on neurotrophins are necessary for the observed modulatory effects of sigma receptors on many neurotransmitter systems. This provides many new avenues to explore in the science of sigma receptors, which will offer valuable information for the clinical potential of these ligands.

Overall, the data presented in this thesis further characterize the *in vivo* action of sigma ligands on glutamatergic and 5-HT neurotransmissions. The novel evidence of an interaction between sigma receptors and the 5-HT system provides a mechanism of action for the "antidepressant-like" effects of sigma ligands observed previously in behavioural models. Furthermore, together these data support the further investigation into sigma ligands as potential antidepressants, and open many areas for future research.

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APPENDIX

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ARTICLE

Effects of sigma ligands on NMDA receptor function in the bulbectomy model of depression: a behavioural study in the rat

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Abstract

Sigma (σ) ligands have been shown to modulate NMDA receptor activity. In the present study we used the olfactory bulbectomy (OBX) animal model of depression to assess the effects of the σ_1 ligand igmesine on OBX-induced behaviour. Behavioural experiments demonstrated OBX (saline-treated) rats to have increased dizocilpine-induced behavioural modifications, including locomotor and circling activity as compared to Sham rats (saline-treated). A short-term (7 d) treatment with low doses of igmesine (50–200 μg/kg.d s.c.) had no effect on dizocilpine-induced behaviour while long-term treatments (14 d) with low doses of igmesine reversed the effect of the bulbectomy such that the treated OBX rats' behaviour was not significantly different from Sham-saline rats. Short-term treatments with high doses of igmesine (500–1000 μg/kg.d) also reversed the increased locomotor and circling behaviour seen in OBX rats (saline-treated) while long-term treatments with the same high doses did not. These results provide behavioural evidence for σ ligand's potential to reverse some OBX-induced behaviours. Moreover, they support the notion of a bell-shaped dose–response curve previously reported for σ ligands.

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Key words: Antidepressant, dizocilpine, igmesine, locomotor behaviour.

Introduction

The existence of sigma (σ) receptors was first reported by Martin et al. (1976) who initially classified them as belonging to the opiate receptor family. Sigma receptors were later divided into 2 subtypes σ_1 and σ_2 based on their different ligand affinity, stereoselectivity, and response to various treatments (Itzhak and Stein, 1991; Quirion et al., 1992). In 1996, σ_1 receptors were cloned from guinea-pig liver, human placental cell line, mouse kidney and brain and rat brain (Hanner et al., 1996; Kekuda et al., 1996; Pan et al., 1998). In recent years, using an in vivo electrophysiological paradigm of unitary extracellular recordings from pyramidal neurons of the CA_1 or CA_3 region of the dorsal hippocampus, we have shown that acute intravenous administration of low doses of several high-affinity σ agonists, including igmesine (JO-1784) and (+)-pentazocine, does not affect the spontaneous firing activity of CA_3 pyramidal neurons, but produces a marked and selective dose-dependent potentiation of NMDA-induced firing activity (Bergeron et al., 1993; Monnet et al., 1990, 1992). Sigma ligands such as haloperidol, NE-100 and progesterone act as antagonists, not by modifying NMDA-induced firing activity, but by preventing and reversing the effects of the above-mentioned σ agonists (Monnet et al., 1990, 1992).

The majority of data implicating the σ receptor's role in depression involves the σ_1 subtype. Several σ_1 ligands (e.g. SA-4503, (+)-pentazocine, DTG, igmesine, and OPC-14523) have been shown to have antidepressant abilities in behavioural tests for antidepressants including the forced swimming test and tail suspension test, with NE-100, a selective σ_1 antagonist, blocking this effect (Kinsora et al., 1998; Matsuno et al., 1996; Tottori et al., 1997; Ukai et al., 1998). Moreover, preliminary results in a clinical trial suggest that igmesine might have antidepressant properties (Pande et al., 1998).

Olfactory bulbectomy (OBX) is currently recognized as a valuable animal model of major depression and useful in the study of the mechanisms of action of antidepressant drugs (Jesberger and Richardson, 1988; Kelly et al., 1997; Lumia et al., 1992). OBX in rodents provokes a variety of neurochemical and behavioural alterations, which are not related to anosmia and are reversible by a wide range of antidepressants (Grecksch et al., 1997; Jesberger and

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Richardson, 1985; Kelly et al., 1997; Leonard and Tuite, 1981; Lumia et al., 1992; van Riezen and Leonard, 1990). It is thought to represent the neurochemical actions of antidepressants on depressive substrates relative to their actions on normal substates (reviewed in Jesberger and Richardson, 1986). The behavioural changes induced by OBX appear 2–3 wk after the surgery and are characterized by hyperactivity, irritability, disruption of sexual behaviour (reviewed in Leonard and Tuite, 1981), deficits in learning avoidance responses, spatial memory (Archer et al., 1984) and sleep disturbances (Sakurada and Kisara, 1977; Sakurada et al., 1976). Furthermore, the measurable behavioural and biochemical alterations are normalized by chronic, but not acute administration of clinically efficacious antidepressant drugs from a variety of families (Jesberger and Richardson, 1986).

Indeed, previous studies in our laboratory have shown that OBX induces a down-regulation of NMDA receptors as, following OBX, the hyperactivity induced by the acute administration of the non-competitive NMDA antagonist dizocilpine was markedly decreased (Robichaud et al., 2001). In keeping with this finding, within 1 wk following OBX, [^{125}I]iodo-dizocilpine binding was decreased in the frontal and piriform cortices, in the anteroventral thalamic nucleus and in certain amygdaloid nuclei, whereas, after 3 wk, this binding was also decreased in the posteromedial cortex, the hippocampus and the lateral hypothalamus (Robichaud et al., 2001). The present study investigates the effects of short- and long-term treatments with the σ_1 agonist igmesine on dizocilpine-induced locomotor activity in OBX rats.

Method

Male Sprague–Dawley rats (180–275 g) were used. Animals were housed in temperature- (25 °C) and humidity-controlled rooms with a 12 h light/dark cycle (lights on at 07:00 hours) with food and water ad libitum. Rats were allowed 48 h of adaptation before undergoing surgery. Ethical Committee approval was given by the McGill University Animal Ethical Care Committee and all their rules and regulations were followed.

Surgery

Six groups of animals were studied. Three groups underwent OBX surgery, while three groups were Sham-operated. For OBX surgery (Jesberger and Richardson, 1986), animals were anaesthetized (chloral hydrate, 400 mg/kg i.p.) and fixed in a stereotaxic frame. Bilateral burr holes were made in the skull surface at the following coordinates: A, +5 mm (from Bregma); L, ±2 mm. Olfactory bulbs were sectioned and removed by aspiration; the cavities being filled with haemostatic sponges. For Sham surgery, animals were similarly operated on but the bulbs were left intact. Following surgery animals were given 2 wk to recover and to permit the appearance of the 'OBX syndrome'. Two weeks after lesion, OBX and Sham-operated rats were randomly assigned to one of the five pharmacological treatments (saline, 50, 100, 200, 500 or 1000 μg/kg.d igmesine) for either 7 or 14 d. Drugs and saline were administered via an Alzet osmotic minipump (Alza, CA) inserted subcutaneously under halothane anaesthesia and aseptic conditions.

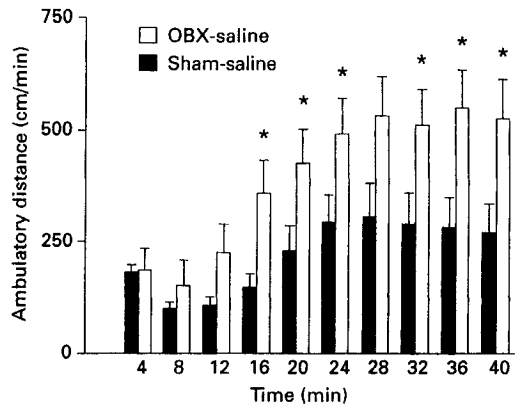

Figure 1. Mean (±S.E.M.) ambulatory distance travelled expressed in cm/min in OBX-saline rats (light bars) vs. Sham-saline rats (dark bars) following the acute injection of dizocilpine (200 μg/kg i.p.) administered at time 0. * $p < 0.05$ Student's t test. Experiments were carried out in 14 OBX rats and 16 Sham rats.

Behavioural experiments

All animals (Sham-operated and OBX rats) received a single injection of dizocilpine (200 μg/kg, i.p.). Animals were then placed in 80 cm diameter, circular activity-measuring wooden boxes with a 45 cm high wooden wall. Floors and walls were painted black and the only lighting was indirect and provided by a 40 W bulb located 2 m from the box. Locomotor activity was recorded with a video-tracking system (Videotrack, France) for 4-min time periods up to 40 min, encompassing the time-course for the maximal behavioural effects of dizocilpine (Löscher and Hönack, 1992). The different other behaviours measured: circling, head weaving and falling over were measured manually. Rectal temperature was also measured at 5 and 45 min. For assessing the effect of the acute administration of dizocilpine on locomotor activity, the mean ambulatory distance per minute was compared for each 4-min period from time 0 to time 40 min. As the pattern of changes in locomotion were similar in Sham and OBX animals (Figure 1), for

assessing the effects of the short- and long-term treatments with igmesine, the mean value of the ambulatory distance per minute during the whole periods of 40 min were compared.

Drugs

Dizocilpine was purchased from Research Biochemicals International (Natick, MA, USA), igmesine was a generous gift from F. Roman (Institut de Recherche Jouveinal, Fresnes, France).

Histological verifications

Following behavioural experiments, the rats were sacrificed and all surgical procedures were verified. If any residual tissue of the main olfactory bulbs remained or if the frontal cortex had been damaged during the surgical procedures, then the behavioural data were not included in the final analysis.

Results

Exploratory behaviour

Following their introduction in the open field, the rats presented the usual exploratory behaviour, which progressively disappeared within 5–10 min. In the Sham-operated animals, the effects of the i.p. administration of dizocilpine (200 μg/kg) appeared within 10–15 min. The first manifestation was a progressive increase in locomotion followed by the appearance of stereotypies, head weaving, and circling behaviour and a marked decrease of rearing behaviour (Table 1, Figures 1, 2). Approximately 30 min following the administration of dizocilpine, the maximal behavioural effects were observed, the increased locomotion was generally reduced after that time since

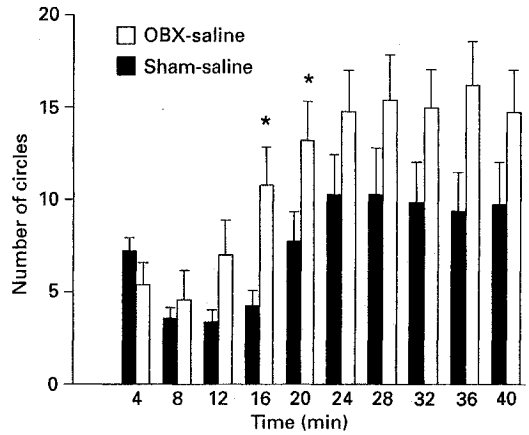

Figure 2. Mean (±S.E.M.) number of circling behaviours observed in OBX-saline rats (light bars) vs. Sham-saline rats (dark bars) following the acute injection of dizocilpine (200 μg/kg i.p.) administered at time 0. * $p < 0.05$ Student's t test. Experiments were carried out in 14 OBX rats and 16 Sham rats.

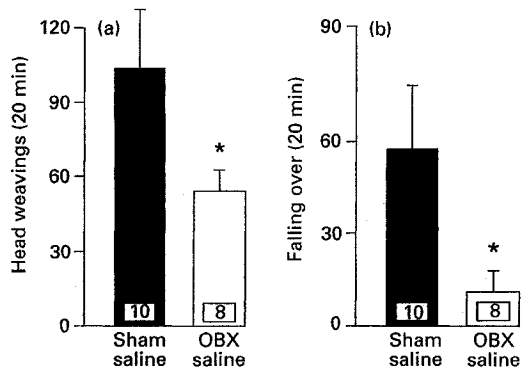

Figure 3. Mean (±S.E.M.) number of (a) head weavings or (b) falling over behaviours recorded in Sham-saline vs. OBX-saline rats observed for a period of 20 min, starting 8 min following the injection of dizocilpine (200 μg/kg). * $p < 0.05$ Student's t test. In this and the following figures the numbers at the foot of the columns indicate the number of rats observed.

Table 1. Behaviours observed before and after the acute administration of MK-801 (200 μg/kg i.p.)

Behaviour	Before MK-801	After MK-801 (200 μg/kg i.p.)	No. of rats	p value
Rearing	6.12±0.93	0.25±0.15	16	0.00001
Head weaving	0±0	5.88±1.38	16	0.0007
Circling	1.94±0.31	6.01±1.49	16	0.01
Falling over	0±0	1.48±0.51	16	0.01

Numbers represent the mean±standard error, p value according to Student's t test. Post-injection observation period lasted from $t+8$ to $t+40$ min.

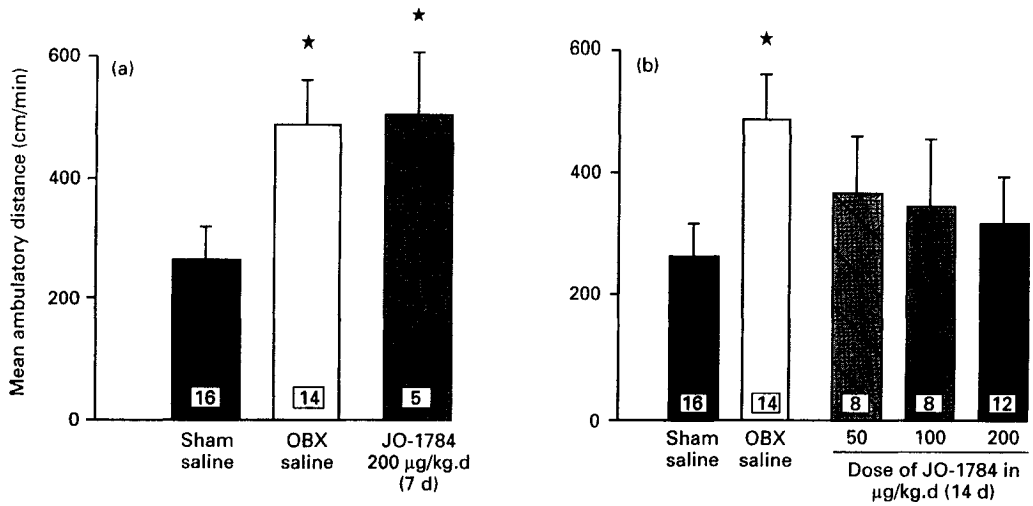

Figure 4. Ambulatory distance travelled by Sham-saline, OBX-saline and OBX rats treated for (a) 7 d or (b) 14 d with igmesine (JO-1784). Values represent the mean (±S.E.M.) distance expressed in cm/min measured during the 40 min duration of the experiment, following dizocilpine injection (200 µg/kg). $^*p < 0.05$ Student's t test vs. Sham-saline.

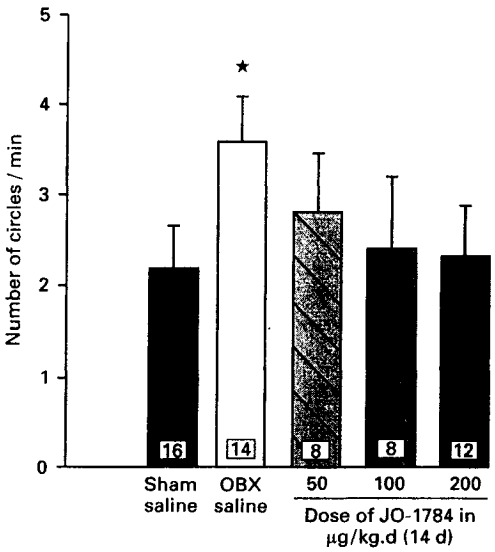

Figure 5. Mean (±S.E.M.) number of circling behaviours observed in Sham-saline, OBX-saline, or OBX rats treated with 50–200 µg/kg.d of igmesine (JO-1784) for 14 d, following the acute administration of dizocilpine (200 µg/kg i.p.) at time 0. $^*p < 0.05$ Student's t test vs. Sham-saline.

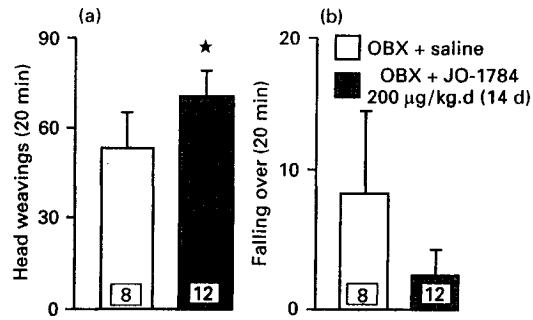

Figure 6. Mean (±S.E.M.) number of (a) head weavings or (b) falling over behaviours recorded in OBX-saline, or OBX rats treated with 200 µg/kg.d of igmesine (JO-1784) for 14 d. Rats were observed for a period of 20 min, starting 8 min following the injection of dizocilpine (200 µg/kg i.p.). $^*p < 0.05$ Student's t test.

most of the animals spent most of their time in stereotypies, or were unable to move without falling over (Figures 1, 2).

Effects of olfactory bulbectomy

OBX rats treated with saline (OBX-saline) had significantly increased locomotor activity vs. Sham rats treated with saline (Sham-saline) in the 4–40 min period following the injection of dizocilpine (Figure 1). In OBX-saline rats, the behavioural effects of dizocilpine measured from 20 to 40 min following its administration, showed a greater than 30% increase in locomotor activity as measured by the distance travelled per minute (Figure 1). The grooming activity was not significantly changed by the injection of dizocilpine nor by the bulbectomy (data not shown). Circling behaviour was increased in OBX-saline vs. Sham-saline rats, even if the difference was statistically significant only during the first 20 min (Figure 2). In contrast, head weaving and falling over (Figure 3) were markedly reduced (between 50 and 80%) following OBX.

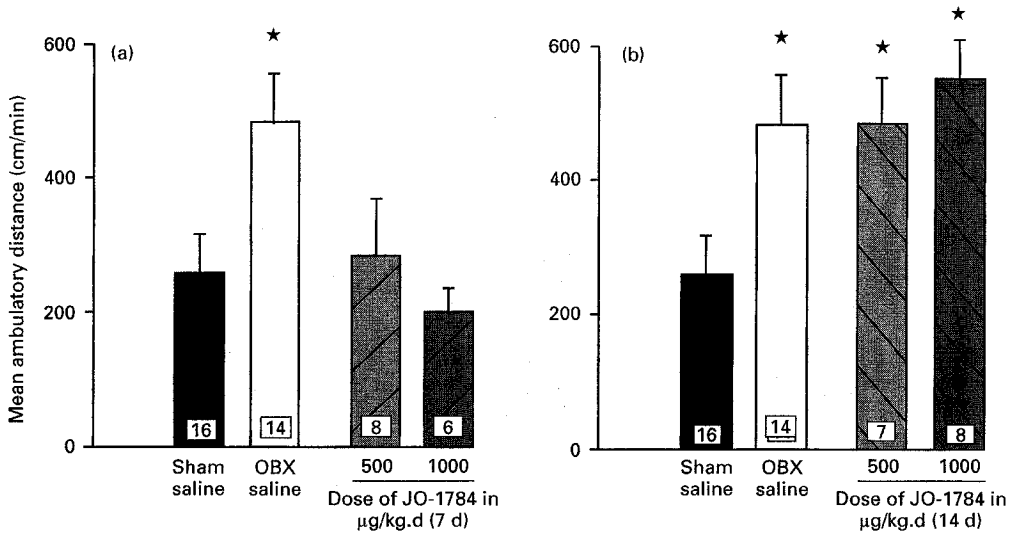

Figure 7. Ambulatory distance travelled by Sham-saline, OBX-saline, or OBX rats treated with 500 or 1000 μg/kg.d of igmesine (JO-1784) for (a) 7 d or (b) 14 d. Values represent the mean (±S.E.M.) distance expressed in cm/min measured during the 40 min duration of the experiments, following injection of dizocilpine (200 μg/kg i.p.). $^*p < 0.05$ Student's t test vs. Sham-saline.

Effects of low doses of igmesine

Short-term (7-d) treatments of OBX rats with a low dose (200 μg/kg.d) of igmesine failed to produce any effect on the dizocilpine-induced locomotor behaviour (Figure 4a). However, in OBX rats, long-term treatments (14 d) with low doses of igmesine (50–200 μg/kg.d) dose-dependently decreased dizocilpine-induced motor effects in OBX rats (Figure 4b). Long-term treatments with low doses of igmesine also markedly decreased the circling behaviour in OBX rats vs. OBX-saline rats (Figure 5). In addition, long-term treatments with low doses of igmesine reversed the decrease in head-weaving behaviour seen in OBX-saline rats (Figure 6a). In contrast, 14-d treatment with igmesine (200 μg/kg.d) did not reverse the decrease in falling over seen in OBX compared to Sham-saline rats (Figure 6b).

Effects of high doses of igmesine

Short-term treatments with igmesine at higher doses (500–1000 μg/kg.d) induced a dose-dependent decrease in locomotor activity after dizocilpine injection in OBX rats, and therefore, a normalized response to dizocilpine vs. OBX-saline rats, comparable to Sham-saline-treated rats (Figure 7a). Long-term treatment with igmesine in high doses (500–1000 μg/kg.d) produced no significant difference between OBX saline- and OBX igmesine-treated rats regarding dizocilpine-induced behavioural modifications (Figure 7b). In agreement with these data, when circling behaviour was assessed, no effect of

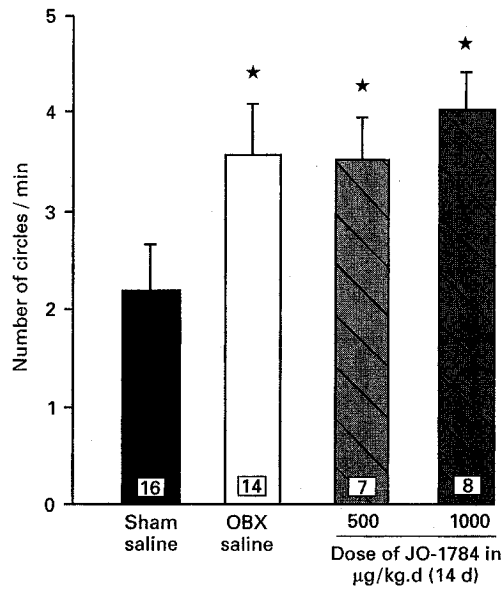

Figure 8. Mean (±S.E.M.) number of circling behaviours recorded in Sham-saline, OBX-saline, or OBX rats treated with 500 or 1000 μg/kg.d of igmesine (JO-1784) for 14 d, following the acute administration of dizocilpine (200 μg/kg i.p.) at time 0. $^*p < 0.05$ Student's t test vs. Sham-saline.

igmesine could be observed with long-term treatments with high doses (500 and 1000 μg/kg.d) (Figure 8). In Sham-operated animals treated for 2 wk with either low or high doses of igmesine, the locomotor and circling behaviour (data not shown) induced by dizocilpine, were

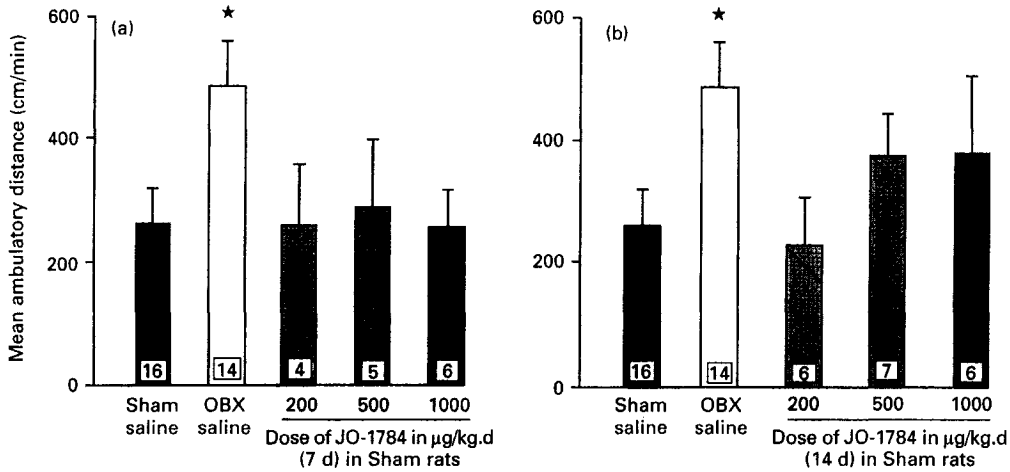

Figure 9. Ambulatory distance travelled by Sham-saline, OBX-saline and Sham rats treated with igmesine (JO-1784) for (a) 7 d or (b) 14 d. Values represent the mean (± S.E.M.) distance expressed in cm/min measured during the 40 min duration of the experiments, following the injection of dizocilpine (200 µg/kg i.p.).

not significantly different from those obtained in Sham-saline rats and thus were decreased vs. OBX-saline rats (Figure 9a, b).

Discussion

Five weeks after a bilateral OBX, and following 3 wk of saline treatment, the behavioural modifications such as ambulatory distance and circling behaviour induced by the acute administration of 200 µg/kg i.p. of dizocilpine were drastically increased in OBX-saline compared to Sham-saline rats (Figures 1, 2). In contrast, head weaving decreased in OBX-saline rats vs. Sham-saline rats. The hyper-locomotion induced by OBX as well as the marked behavioural effects and stereotypies induced by the acute administration of a low dose of dizocilpine are in keeping with previous studies (Deutsch and Hitri, 1993). The potentiation of the behavioural effects induced by the acute administration of dizocilpine is in agreement with the previous results of Redmond et al. (1997) who showed that acute treatment with dizocilpine (300 µg/kg) produced increased home cage locomotor activity while a lesser dose (100 µg/kg) attenuated home cage locomotor activity. Though this is a different setting than the 'open field' used in the present study, these results do correspond to the increase in locomotor activity seen in the present study in the 'open field' as we administered a dose of 200 µg/kg. Furthermore, chronic treatments with dizocilpine decreased the locomotor activity produced by OBX. This does not go against our observation as we administered only acute doses and the chronic dose could have different effects due to changes in receptor function that would not occur in our paradigm with the acute doses. As both the administration of dizocilpine and the OBX surgery alone increase locomotor activity, it is not surprising to find that their combined effect further increases locomotor activity.

The 2-wk treatment of OBX rats with low doses of igmesine (50–200 µg/kg.d), induced a reversal of the behavioural response to the injection of dizocilpine compared to what was observed in OBX-saline rats (Figure 4b). However, the short-term treatment with the same low doses did not lead to any significant difference in the dizocilpine-induced behavioural response compared to OBX-saline (Figure 4a). Conversely, a short-term treatment with the high doses of igmesine reversed the behavioural modifications induced by OBX, whereas a long-term treatment with the same doses was without any effect (Figure 7a, b).

We have previously reported that the dose–response curves of the potentiation of the NMDA response by σ ligands has a bell-shaped aspect (Bergeron et al., 1995). More specifically, following an acute intravenous administration, the maximum potentiation of the NMDA response was observed with 50 µg/kg of (+)-pentazocine and 4 µg/kg of igmesine (Bergeron et al., 1995). When higher doses were administered, the potentiating effect would progressively decrease and finally disappear and at higher doses, σ agonists were acting purely as σ antagonists (Bergeron et al., 1995). Moreover, we have shown that long-term treatments with low doses of σ agonists induces an up-regulation of σ receptors, but following a 3-wk treatment with low doses of DTG, (+)-pentazocine or igmesine, the neuronal activation induced by microiontophoretic applications of NMDA is markedly increased (Bergeron and Debonnel, 1997). Finally, it has been well established that long-term treatments with

antagonists induce a down-regulation of σ receptors (Bergeron and Debonnel, 1997; Itzhak and Alerhand, 1989; Jansen et al., 1992; Riva and Creese, 1990).

Therefore, during a long-term treatment with σ agonists a certain accumulation is needed in order for the ligands to function in the active 'agonist range'. As the concentration of the ligand increases, its agonist effects increase as well as the sensitivity of the σ receptor. This continues until a peak after which the ligand begins to function as an antagonist and the effects of the ligand progressively decrease as the concentration increases and the σ receptor desensitizes. Thus, in the present study it is likely that at the lower dose the longer treatment was necessary in order for the igmesine to reach the 'agonist range'. This explains why a shorter duration of treatment with a higher dose produced the same effects. Furthermore, the long-term treatment at high doses no longer produced any effect most likely due to the concentration of igmesine being in the 'antagonist range'. This dose–response curve thus involves a change in the properties of the σ receptor and modify the NMDA response without affecting the sensitivity of the dizocilpine-binding site.

Furthermore, igmesine is a highly selective σ ligand, which has negligible affinity for other receptor subtypes including PCP, adrenergic, dopaminergic and serotonergic receptors (Roman et al., 1990), therefore, the effects observed in the present study are not likely mediated through activation of another receptor. Indeed, the present results suggest that, following a short-term treatment with a high dose and a long-term treatment with a low dose, igmesine is acting as an agonist and potentiates the response induced by the endogenous ligand for the still functional NMDA receptor. Therefore, in these conditions, it can be postulated that a chronic treatment with low doses of igmesine, by potentiating the effects of the down-regulated NMDA receptors produced by surgery in OBX, will compensate for this down-regulation, thereby normalizing the response to dizocilpine, and thus inducing smaller behavioural effects (Figures 4b, 5, 7a).

Recent studies have demonstrated interactions between several antidepressant drugs and the NMDA receptor complex. For example, the acute administration of desipramine, imipramine and nortriptyline drastically reduces NMDA-induced epileptiform response and long-term potentiation (LTP) in rat hippocampal slices (Watanabe et al., 1993), whereas a 5-wk treatment with desipramine, imipramine and amitryptiline inhibits the binding of [^{3}H]dizocilpine in a concentration-dependent manner (Kitamura et al., 1991). Acute and chronic treatments with imipramine, amitriptyline, citalopram and fluoxetine potentiate the hyperactive behaviour induced by dizocilpine, an effect which is blocked by haloperidol, but not by the D_1 and the D_2 selective antagonists SCH-23390 or sulpiride, respectively (Maj et al., 1991, 1992), suggesting that the effect of haloperidol is likely due to its affinity for σ receptors and not for dopamine receptors. It has also been reported that chronic treatments with fluoxetine or imipramine reduce [^{3}H](+)-pentazocine binding in the rat brain (Shirayama et al., 1993). In addition, several antidepressants have been found to decrease NMDA-activated ion current (Sernagor et al., 1989; White et al., 1990).

Swim stress and exposure to unpredictable mild stress increase the potency of glycine to displace [^{3}H]5,7-dichlorokynurenic acid (DCKA) from the glycine site on the NMDA receptor in an imipramine-reversible manner (Nowak et al., 1995). This was specific to antidepressants after repeated treatments as structurally related non-antidepressant molecules did not produce this effect (Nowak et al., 1995). Similarily, adaptive changes of the NMDA receptor have been observed to occur selectively in the mouse cortex, where chronic SSRI treatments also decreased glycine-displaceable binding (Nowak et al., 1996, 1998). These data further strengthen the hypothesis that NMDA receptor modification could represent the final pathway of antidepressant action as already suggested by several groups (Paul et al., 1994; Skolnick et al., 1996). Combined with our experimental data, these different observations suggest that the NMDA and σ receptors may be involved in the pathophysiology of depression and in the mechanism of action of antidepressant treatments. Thus, a σ ligand that modulates NMDA receptor function could be expected to have some potential interest as an antidepressant treatment.

The inability of igmesine to reverse the dizocilpine-induced head weaving and falling over in OBX rats (Figure 6a, b) is likely due to these behaviours being mediated by pathways different from those involved in ambulation or circling behaviour. We have previously reported that following OBX, the modifications of NMDA-binding parameters differ in several brain regions (Robichaud et al., 2001). It is therefore plausible that some areas are either less enriched in σ receptors or have sustained greater modifications of NMDA-binding parameters which cannot be compensated for. This could also explain why the different responses to OBX surgery, such as head weaving and falling over are decreased in OBX rats (Figure 3a, b). Nonetheless, our results showed OBX to decrease MK-801-induced ataxia (falling over) (Figure 3b). Following treatments with igmesine (200 μg/kg), there was a further decrease in ataxia in addition to an increase in locomotor activity (Figure 6b). Therefore, this increase in locomotor activity could, in part, be due to the decrease in ataxia.

The effects observed in the present studies with igmesine may be due to the σ ligand's modulation of NMDA receptors previously discussed, however, a direct interaction with the serotonergic system cannot be ruled out. Recently, we have demonstrated the σ ligands (+)-pentazocine and 4-IBP to modulate serotonergic neurotransmission, as 2-d treatments (2 μg/kg.d) induced a 35% increase in average basal firing rate of the serotonergic neurons of the dorsal raphe nucleus, while igmesine at the same dose produced no change after short- or long-term treatments, using an electrophysiological paradigm of extracellular recordings in vivo (Bermack and Debonnel, 2001). Akunne et al. (2001) demonstrated that chronic treatment with igmesine (15 mg/kg.d) produced no change in 5-HT_{1A} receptor densities, only minor reductions in tyrosine hydroxylase activity, no effects on 5-HT, norepinephrine reuptake nor 5-HT synthesis. Their study, in agreement with ours, suggested the pharmacological actions of igmesine may be in part due to mechanisms not mediated by the monoaminergic system and may involve NMDA receptors based on their observation of igmesine treatment blocking NMDA-induced increases in cGMP. This is in agreement with the published data mentioned by the reviewer showing 16-d treatment with igmesine (3 mg/kg) produced no change in serotonin turnover (Song et al., 1997).

Effective antidepressant treatments are expected to reverse OBX-induced alterations, thus normalizing NMDA receptor binding levels. Therefore, our results suggest that igmesine could have antidepressant properties in the OBX model. However, this would require further investigation comparing σ ligands to antidepressants with respect to various OBX-induced alterations. These experiments establish the first behavioural model indicating a behavioural effect of long-term treatment with low doses of σ ligands, most likely related to their affinity for σ receptors, reversing OBX-induced alterations.

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Modulation of serotonergic neurotransmission by short- and long-term treatments with sigma ligands

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1 Sigma receptors were first described in 1976 as opiate receptors but were later determined to be a distinct class of receptors with two subtypes, $sigma_1$ and $sigma_2$. Although the endogenous ligand is yet to be elucidated, the $sigma_1$ receptor has recently been cloned.

2 Behavioural models used to test potential antidepressants have shown sigma ligands to produce antidepressant effects but their mechanism of action is unknown.

3 The goal of the present study was to assess the effects of various $sigma_1$ ligands on the firing activity of serotonin (5-HT) neurons of the dorsal raphe nucleus (DRN) using extracellular *in vivo* recordings in anaesthetized rats.

4 The $sigma_1$ ligands (+)-pentazocine and 4-(N-benzylpiperidin-4-yl)-4-iodobenzamide (4-IBP) (2 mg kg^{-1} day^{-1}) increased markedly 5-HT firing activity after 2 days of treatment and maintained the same increased firing rate after long-term (21 days) treatments. Furthermore, the increased firing rate produced by 2 and 21 day treatments with (+)-pentazocine was prevented by the co-administration of N,N-dipropyl-2-(4-methoxy-3-(2-phenylethoxy)phenyl)-thylamine (NE-100) (10 mg kg^{-1} day^{-1}) a selective $sigma_1$ antagonist, confirming the $sigma_1$ receptor's modulation of these effects. In contrast, the $sigma_1$ ligands (+)-N-cyclopropylmethyl-N-methyl-1,4-diphenyl-1-1-ethyl-but-3-en-1-ylamine hydrochloride (JO-1784) and 2-(4-morpholinoethyl 1-phenyl-cyclohexane-1-carboxylate hydrochloride (PRE-084) had no effect.

5 Following a 21-day treatment with (+)-pentazocine there was a marked reduction in the number of neurons found per track. This decrease was not seen after chronic treatment with 4-IBP and may represent a depolarization block.

6 These results suggest a modulation of serotonergic neurotransmission by some sigma receptors and provide a potential mechanism for the 'antidepressant effects' reported and provide evidence toward $sigma_1$ ligands as potential antidepressants with a rapid onset of action.
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Keywords: Antidepressant; electrophysiology; dorsal raphe nucleus; (+)-pentazocine; 4-IBP; PRE-084; JO-1784

Abbreviations: 4-IBP, 4-(N-benzylpiperidin-4-yl)-4-iodobenzamide; 5-HT, serotonin; 8-OH-DPAT, 8-hydroxy-2-(di-n-propylamino)tetralin; AF-DX116, 11-[[2-[(diethylamino)methyl]-1-piperidinyl]acetyl]-5-11-dihydro-6H-pyrido[2,3-6][1,4]benzodiazepine-6-one; BD-737, (+)-cis-N-[2,(3,4-dichlorophenyl)ethyl]-2-(1-pyrrolidinyl-cyclohexlamine; BMY-14802, α-(4-fluorophenyl)-4-(5-fluoro-2-pyrimidinyl)-1-piperazinebutanol; DHSO, dimethylsulphoxide; DRN, dorsal raphe nucleus; DTG, 1.3-di(2-tolyl)guanidine; i.p., intraperitoneal; GABA, γ-aminobutyric acid; JO-1784, (+)-N-cyclopropylmethyl-N-methyl-1,4-diphenyl-1-1-ethyl-but-3-en-l-ylamine hydrochloride; L687-384, 1-benzylspiro[1,2,3,4-tetrahydronaphthalene-1,4-piperidine; MAOI, monoamine oxidase inhibitor; MK-801 (dizocilpine), (+)-5-methyl-10,11-dihydro-5H-dibenzo(a,d)cyclohepten-5-10-imine maleate; NE-100, N,N-dipropyl-2-(4-methoxy-3-(2-phenylethoxy)phenyl)-thylamine; NMDA, N-methyl-D-aspartate; OPC-14523, 1-[3-[4-(3-chlorophenyl)-1-piperazinyl]propyl]-5-methoxy-3,4-dihydro-2-quinolinone monomethanesulphonate; PRE-084, 2-(4-morpholinoethyl 1-phenyl-cyclohexane-1-carboxylate hydrochloride; SA-4503, 1-(3,4-dimethoxyphenethyl)-4-(3-phenylpropyl) piperazine dihydrochloride; SCH-50911, (25)(+)-5,5-dimethyl-2-morpholineacetic acid; SEM, standard error mean; SSRI, selective serotonin reuptake inhibitor; (+)SKF-10,047, (+)-N-allyl-normetazocine

Introduction

Sigma receptors were first described by Martin *et al.* (1976) as a subtype of opiate receptors. They were later distinguished from opiate receptors by the development of selective sigma ligands and classified into $sigma_1$ and $sigma_2$ subtypes (Quirion *et al.*, 1987; 1992). Their endogenous ligand is not known but the endogenous steroid progesterone has high affinity for $sigma_1$ receptors (Su *et al.*, 1988). Many selective $sigma_1$ ligands have been synthesized including (+)-pentazocine, 4-IBP, (+)-N-cyclopropylmethyl-N-methyl-1,4-diphenyl-1-1-ethyl-but-3-en-1-ylamine hydrochloride (JO-1784) and 2-(4-morpholinoethyl 1-phenyl-cyclohexane-1-carboxylate hydrochloride (PRE-084). Recently the $sigma_1$ receptor was cloned and found to be different from all known mammalian receptors (Hanner *et al.*, 1996).

Our laboratory previously used an electrophysiological model to differentiate between sigma agonists and antagonists. Specifically, it was demonstrated that sigma ligands

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modulate NMDA (N-methyl-D-aspartate) receptors such that low doses (0.5–50 μg) of sigma ligands have no effect on the spontaneous firing activity of hippocampal CA_3 neurons but dose-dependently and selectively modulate the response to NMDA (Monnet *et al.*, 1990). In this model, sigma agonists (1,3-di(2-tolyl)guanidine (DTG), (+)-pentazocine, JO-1784) potentiate the NMDA response and $sigma_1$ antagonists (N,N-dipropyl-2-(4-methoxy-3-(2-phenylethoxy)phenyl)-thylamine (NE-100), progesterone, haloperidol) have no effect on their own but block the effects of sigma agonists (Monnet *et al.*, 1990; 1992; Bergeron *et al.*, 1996).

$Sigma_1$ ligands have many potential functions one of which could be a role in the pathophysiology of depression or as antidepressants. Several sigma ligands have been shown to have antidepressant effects in behavioural models of depression such as the tail suspension and forced swimming tests (Matsuno *et al.*, 1996; Tottori *et al.*, 1997; Kinsora *et al.*, 1998; Ukai *et al.*, 1998). In addition, representatives from all classes of antidepressants have been shown to interact with sigma receptors (Bergeron *et al.*, 1993; Narita *et al.*, 1996; Shirayama *et al.*, 1993). An enormous corpus of evidence suggests the involvement of serotonin in the pathophysiology of depression (Delgado, 2000). Just as an example, electrophysiological studies have demonstrated that all long-term treatments with antidepressants, through various mechanisms, increase 5-HT neurotransmission (Chaput *et al.*, 1991; Blier & de Montigny, 1994).

The purpose of this study was to assess the effect of short and long-term treatments with several $sigma_1$ ligands on serotonergic neurotransmission using an electrophysiological model of extracellular recordings of the firing rate of serotonin (5-HT) neurons from the dorsal raphe nucleus (DRN). Previous results using this model, demonstrated that acute and short-term treatments with SSRI's lead to a decreased firing activity of 5-HT DRN neurons, while long-term treatments lead to the restoration of 5-HT firing activity (Chaput *et al.*, 1986; Blier *et al.*, 1984; Blier & de Montigny, 1985). Therefore, we investigated the effects of short-term (2 days) and long-term (21 days) treatments with $sigma_1$ ligands to assess their effects on basal 5-HT firing rate.

Methods

Animals

Experiments were performed in male Sprague-Dawley rats (Charles River, St. Constant, Québec) weighing 250–300 g. They were housed under standard laboratory conditions including a 12–12 h light-dark cycle with free access to food and water.

Treatments

For short-term treatments, rats 250–275 g were anaesthetized with halothane and osmotic minipumps (ALZA Corporation, Palo Alto, CA, U.S.A.) were implanted subcutaneously. Minipumps contained either JO-1784, PRE-84, (+)-pentazocine, 4-IBP (4-(N-benzylpiperidin-4-yl)-4-iodobenzamide) (all 2 mg kg^{-1} day^{-1}) or saline for controls (50% saline, 50% dimethylsulphoxide (DMSO) for 4-IBP controls). Separate series of rats were implanted with two osmotic minipumps simultaneously, one containing (+)-pentazocine or 4-1BP (2 mg kg^{-1} day^{-1}) and the other containing NE-100 (10 mg kg^{-1} day^{-1}) for 2 days.

For long-term treatments, rats 125–150 g were anaesthetized and implanted in a similar fashion to that done for 2-day treatments. Pumps contained either (+)-pentazocine, JO-1784 or 4-IBP (all 2 mg kg^{-1} day^{-1}) or saline for controls (50% saline 50% DMSO for 4-IBP controls). In addition, a separate series of rats were implanted with two osmotic minipumps simultaneously, one containing (+)-pentazocine or 4-1BP (2 mg kg^{-1} day^{-1}) and the other containing NE-100 (10 mg kg^{-1} day^{-1}) for 21 days. The electrophysiology experiments were performed with the minipumps on board.

Electrophysiology

The experiments were performed on rats anaesthetized with chloral hydrate (400 mg kg^{-1} intraperitoneal (i.p.)) and mounted in a stereotaxic apparatus. Supplemental doses of chloral hydrate (100 mg kg^{-1} i.p.) were administered as necessary to prevent any nociceptive reaction to pinching of the hind paw. The rat's body temperature was maintained at approximately 37°C by a thermistor-controlled heating pad.

Extracellular unitary recording of DRN 5-HT neurons was obtained with single-barrelled glass micropipettes pulled in a conventional manner (Haigler & Aghajanian, 1974) with the tips broken back 1–3 μm and filled with 3% fast green solution. Electrode impedance ranged between 2 and 4 MΩ. A burr hole 4 mm in diameter was drilled 1 mm anterior to lambda on the midline. The electrode was then lowered along descents covering the DRN from 300 μm to approximately 1500 μm anterior of lambda. Spontaneously firing DRN 5-HT neurons were identified by their characteristic slow and regular rhythmical firing (Aghajanian & Vandermaelen, 1982). Following the experiments each rat was sacrificed with an intravenous injection of air (1 ml).

Data collection

For each treatment group, the mean DRN 5-HT basal firing rate was determined by averaging the firing rate of all the neurons measured in the population (treatment). Each neuron was recorded for 90 s, and five descents were performed per rat in the DRN of 3–6 rats with the total number of neurons averaged being greater than 40. Student's paired *t*-tests were done comparing treatments to controls using the program Sigmaplot 4.0. A value was considered significant if $P<0.05$.

Drugs

The following substances were used: JO-1784 (a gift from F. Roman, Institut de Recherche Jouveinal, Fresnes, France), (+)-pentazocine, 8-hydroxy-2-(di-n-propylamino)tetralin(8-OH-DPAT), (−)bicuculline methiodide (RBI Pharmaceuticals, Natick, MA, U.S.A.), PRE-084 (a gift from Dr T.-P. Su, NIDA/NIH, Baltimore, MD, U.S.A.). NE-100 (a gift from Taisho Pharmaceutical Co. Ltd. Tokyo, Japan), 4-IBP and R(+)baclofen (Tocris Cookson Inc. Ballwin, MO, U.S.A.).

Results

The doses used in the present series of experiments were chosen according to data obtained previously. We have shown that doses of (+)-pentazocine and JO-1784 between 500–3000 $\mu g\ kg^{-1}$ induced the maximal agonistic effect on the potentiation of the NMDA response (Monnet *et al.*, 1990; 1992). The same doses of PRE-084 and 4-IBP were used since these molecules possess very high affinity for $sigma_1$ receptors, similar to that of (+)-pentazocine and JO-1784 (Su *et al.*, 1991; John *et al.*, 1994; Steinfels *et al.*, 1988; Roman *et al.*, 1990).

In control animals, 5-HT neurons were encountered, starting at a depth of 5033 μm with an average of 2.8 neurons per track and a firing activity of 1.0 Hz. Figure 1 depicts a representative tracing of serotonergic neurons recorded in the DRN along a descent. In this example, the tracing is from a control rat or one treated for 2 days with (+)-pentazocine (2 mg kg^{-1} day^{-1}) showing an increased firing rate.

Treatment with JO-1784

The short-term (2 days) administration of JO-1784 (2 mg kg^{-1} day^{-1}) produced no significant change in the basal firing rate of DRN 5-HT neurons (Figure 2). In addition, a 21-day administration of the same dose of JO-1784 also did not affect the average firing rate of the 5-HT neurons of the DRN (Figure 2).

Treatment with PRE-084

The 2-day administration of PRE-084 (2 mg kg^{-1} day^{-1}) produced no significant change in the basal firing rate of the 5-HT neurons of the DRN (Figure 2).

Treatment with 4-IBP

A 2-day treatment with 4-IBP (2 mg kg^{-1} day^{-1}) produced a 35% increase in the basal firing rate of 5-HT neurons compared to saline-treated animals ($P=0.002$) (Figure 3). Furthermore, a 21-day treatment with 4-IBP maintained a 36% increase in firing rate as seen after 2 days (Figure 3). The co-administration of NE-100 (10 mg kg^{-1} day^{-1}) with 4-IBP (2 mg kg^{-1} day^{-1}) for 2 days did not modify the effect of 4-IBP and a significant increase in the average firing activity of 5-HT neurons was observed (Figure 3).

Treatment with (+)-pentazocine

As illustrated in Figure 1, a 2-day treatment with (+)-pentazocine (2 mg kg^{-1} day^{-1}) produced a 33% increase in the basal firing rate compared to saline-treated rats ($P=0.001$) (Figure 4). Co-administration of NE-100 (10 mg kg^{-1} day^{-1}) with (+)-pentazocine (2 mg kg^{-1} day^{-1}) for 2 days completely prevented the increase of 5-HT firing activity caused by a 2-day eatment with (+)-pentazocine (Figure 4). (+)-Pentazocine eatment (2 mg kg^{-1} day^{-1}) for 21 days maintained a 43% crease in basal firing rate compared to saline-treated rats igure 5). Similarly, co-administration of NE-100 (10 mg kg^{-1} ay^{-1}) with (+)-pentazocine (2 mg kg^{-1} day^{-1}) for 21 days oduced no significant change in average firing rate nor neurons found per track compared to controls (Figures 5 and 6).

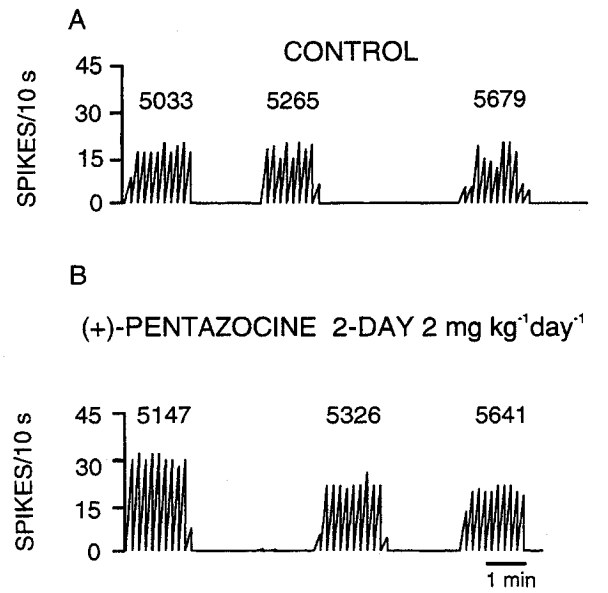

Figure 1 Integrated firing rate histograms of dorsal raphe 5-HT neurons obtained in anaesthetized rats following 2-day treatment with saline (control) (A) or (+)-pentazocine (2 mg kg^{-1} day^{-1}) (B). The numbers above the histogram represent the depth at which the neuron was found.

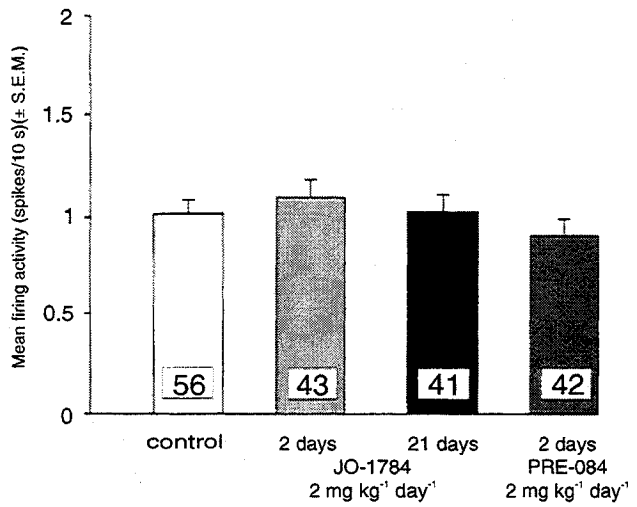

Figure 2 Mean firing activity expressed as spikes/10 s (mean ± s.e.m.) of dorsal raphe nucleus serotonergic neurons measured in anaesthetized rats. Rats were treated with saline (control), JO-1784 (2 mg kg^{-1} day^{-1} for 2 days), PRE-084 (2 mg kg^{-1} day^{-1} for 2 days) or JO-1784 (2 mg kg^{-1} day^{-1} for 21 days). In this and the following figures, numbers in columns indicated the number of neurons tested.

Neurons found per track following long-term treatments

As shown in Figure 6, in rats treated for 21 days with (+)-pentazocine (2 mg kg^{-1} day^{-1}), 94% less neurons were encountered per track. In rats treated for 21 days with 4-IBP there was no significant difference in the amount of neurons found per track compared to saline-treated rats. Various durations of treatment with (+)-pentazocine (2 mg kg^{-1} day^{-1}) did not significantly change the amount of neurons found per track versus controls until day 21. Specifically,

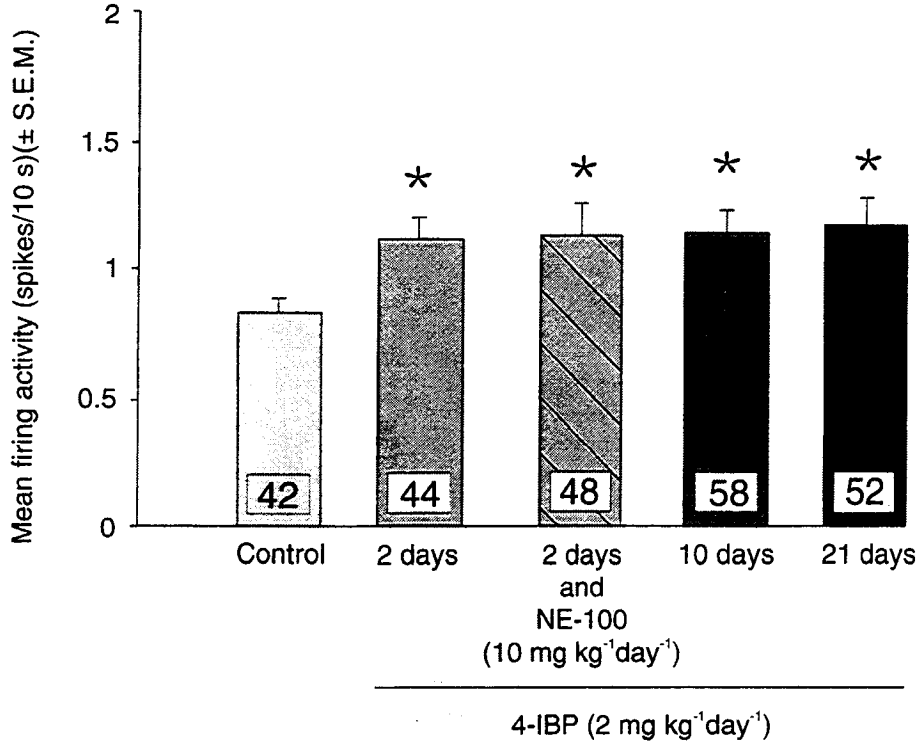

Figure 3 Mean firing activity expressed as spikes/10 s (mean ± s.e.m.) of dorsal raphe nucleus serotonergic neurons measured in anaesthetized rats. Rats were treated with saline (control) for 2 days or 4-IBP (2 mg kg^{-1} day^{-1}) for 2, 10 or 21 days or co-administered 4-IBP (2 mg kg^{-1} day^{-1}) and NE-100 (10 mg kg^{-1} day^{-1}) for 2 days. $*P<0.05$.

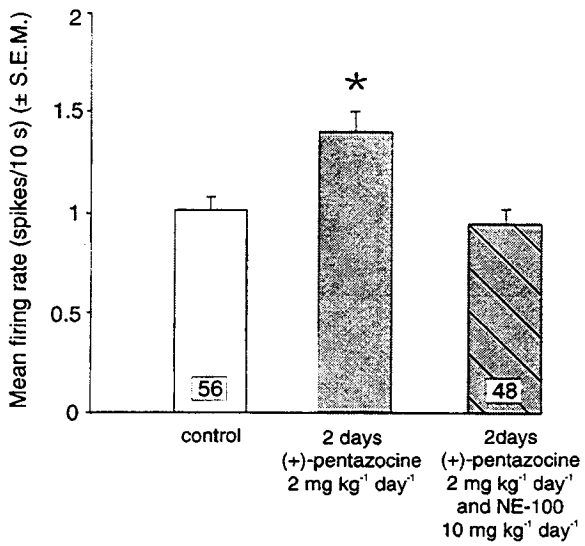

Figure 4 Mean firing activity expressed as spikes/10 s (mean ± s.e.m.) of dorsal raphe nucleus serotonergic neurons measured in rats treated with saline (control), (+)-pentazocine (2 mg kg^{-1} day^{-1}) or co-administered (+)-pentazocine (2 mg kg^{-1} day^{-1}) and NE-100 (10 mg kg^{-1} day^{-1}) for 2 days. $*P<0.05$.

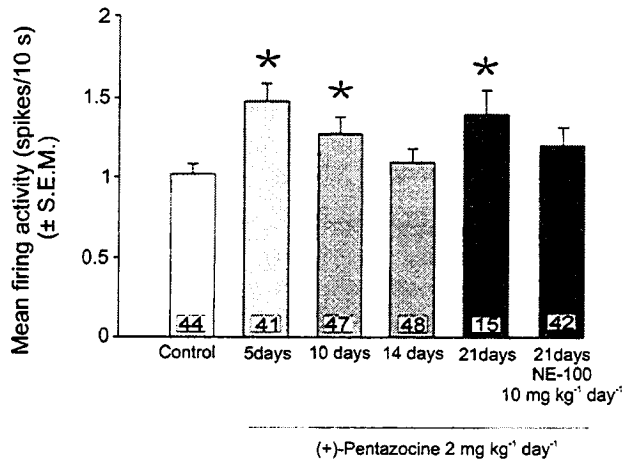

Figure 5 Mean firing activity expressed as spikes/10 s (mean ± s.e.m.) of dorsal raphe nucleus serotonergic neurons in rats treated with saline (control) or with (+)-pentazocine (2 mg kg^{-1} day^{-1}) for 5, 10, 14 or 21 days or co-administered (+)-pentazocine (2 mg kg^{-1} day^{-1}) with NE-100 (10 mg kg^{-1} day^{-1}) for 21 days. $*P<0.05$.

)llowing 5- and 10-day treatments, increased firing rates were ıaintained (40% and 27% respectively) compared to controls, ithout decreasing the number of neurons found per track Figures 5 and 6). In addition, when treated with a lesser dose of .+)-pentazocine (0.5 mg kg^{-1} day^{-1}) no change was seen for neurons yielded per track nor average firing rate compared to controls (Figure 7). To investigate the nature of the finding of decreased number of neurons per track, we injected animals treated with (+)-pentazocine for 21 days (2 mg kg^{-1} day^{-1}) with 8-OH-DPAT (4 μg kg^{-1} i.v.), bicuculline (375 μg kg^{-1} i.v.) or (+)baclofen (5–15 mg kg^{-1} i.v.). These different approaches did not restore the amount of neurons found per track to that of saline-treated rats (8-OH-DPAT 0.0 ± 0, bicuculline 0.2 ± 0.2 and baclofen 0.63 ± 0.24 versus (+)-pentazocine 0.41 ± 0.16, not significant).

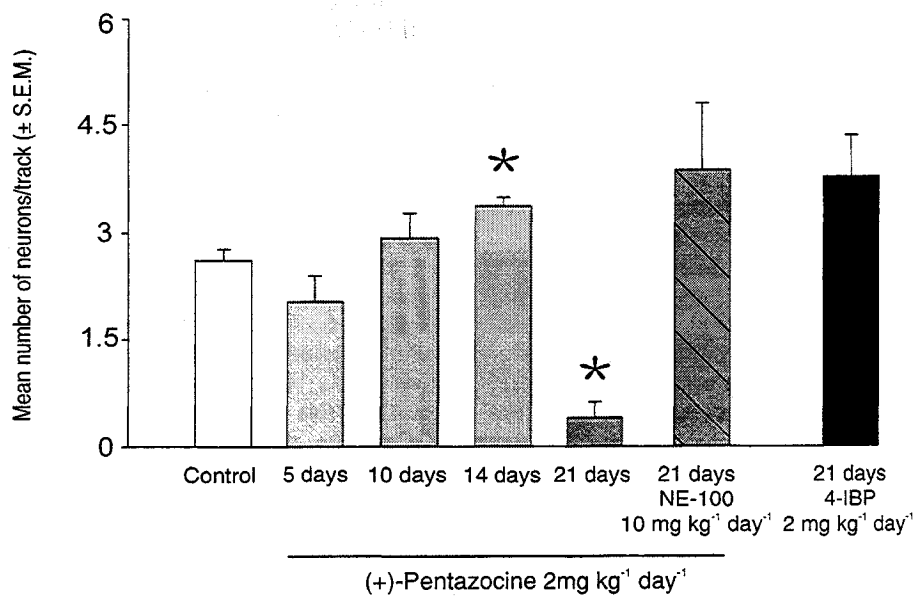

Figure 6 Mean number of neurons per track (±s.e.m.) encountered in rats treated with saline (control) or with (+)-pentazocine (2 mg kg^{-1} day^{-1}) for 5, 10, 14 or 21 days or co-administered (+)-pentazocine (2 mg kg^{-1} day^{-1}) with NE-100 (10 mg kg^{-1} day^{-1}) for 21 days or treated with 4-IBP (2 mg kg^{-1} day^{-1}) for 21 days. $*P<0.05$.

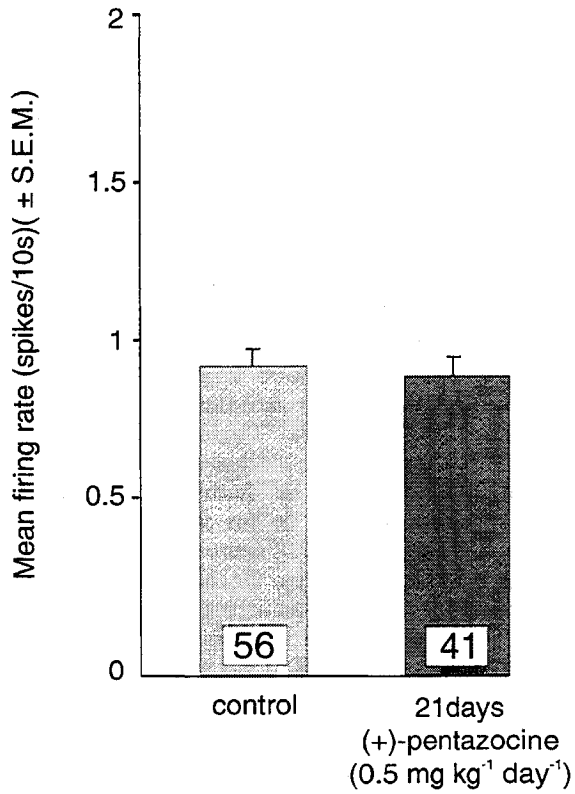

Figure 7 Mean firing activity expressed as spikes/10 s (mean± s.e.m.) of dorsal raphe nucleus serotonergic neurons in rats treated with saline (control) or for 21 days with (+)-pentazocine).5 mg kg^{-1} day^{-1}).

Discussion

-IBP is a selective sigma ligand with a high affinity for the $sigma_1$ receptor (K_i=1.7 nM) and moderate affinity for the $sigma_2$ receptor (K_i=25.2 nM) (John *et al.*, 1994). Short-term treatments with 4-IBP (2 mg kg^{-1} day^{-1}) for 2 days, produced a significant 35% increase in the basal firing rate of DRN 5-HT neurons (Figure 3). Similarly, the selective $sigma_1$ ligand (+)-pentazocine produced a 33% increase in the firing activity of 5-HT neurons of the DRN (Figures 1 and 4). This increase was not seen after treatment with the selective $sigma_1$ ligands PRE-084 and JO-1784 as their firing rates did not differ significantly from controls (Figure 2).

The increased firing rates observed after both short- and long-term treatments with (+)-pentazocine were completely prevented by co-administration with NE-100 (10 mg kg^{-1} day^{-1}), a selective $sigma_1$ antagonist (Figures 4 and 5). This confirms that the modulation of serotonergic firing activity demonstrated here is indeed mediated by $sigma_1$ receptors. However, as shown in Figure 3, when NE-100 (10 mg kg^{-1} day^{-1}) was co-administered with 4-IBP for 2 days, the increase in the firing activity of the 5-HT neurons which was induced by 2 day treatments with 4-IBP was not prevented. Thus, the average firing activity remained significantly increased versus controls.

Various preclinical results for a variety of sigma ligands have already suggested that these compounds could produce antidepressant effects. Specifically, the sigma ligands 1-(3,4-dimethoxyphenethyl)-4-(3-phenylpropyl) piperazine dihydrochloride (SA-4503), (+)-pentazocine, DTG, JO-1784 and 1-[3-[4-(3-chlorophenyl)-1-piperazinyl]propyl-5-methoxy-3,4-dihydro-2-quinolinone monomethanesulphonate (OPC-14523) dose-dependently decreased immobility in mice in the forced swimming test and this decrease was antagonized by pretreatment with the sigma antagonist NE-100 (Matsuno *et al.*, 1996; Tottori *et al.*, 1997; Kinsora *et al.*, 1998). In keeping with these data, the acute administration of SA-4503 and (+)-pentazocine decreased immobility in mice exposed to the tail suspension test, at doses that failed to influence motor activity and these effects were antagonized by NE-100 (Ukai *et al.*, 1998).

A second line of evidence suggesting sigma receptor's potential involvement in the pathophysiology and/or the treatment of depression comes from many antidepressant's interaction with and/or high affinity for sigma receptors. For example, serotonin (5-HT) reuptake inhibitors (SSRI's) and monoamine oxidase inhibitors (MAOI's) prevent [^{3}H](+)3-PPP binding to sigma receptors in rat and mouse brains (Schmidt *et al.*, 1989; Itzhak & Kassim, 1990). Furthermore, sertraline, an SSRI, and clorgyline, an MAOI, potentiate the NMDA response with a bell-shaped dose response curve, potentiations, which are reversed by haloperidol (a sigma$_1$ antagonist). Paroxetine and tranylcypromine, with monoaminergic profiles similar to sertraline and clorgyline except that they are devoid of sigma affinity, did not affect the NMDA response, therefore, indicating that the effects of sertraline and clorgyline were not due to monoaminergic effects (Bergeron *et al.*, 1993). Thirdly, in rats, chronic treatments with imipramine or fluoxetine result in a down regulation of sigma receptors in the striatum, hippocampus and cerebral cortex, brain regions implicated in regulation of emotions. This down regulation involves a decrease in B_{max} and depends on cerebral serotonergic transmission as it was reversed by p-chlorophenylalanine (Shirayama *et al.*, 1993).

The significant increase in the firing activity of 5-HT neurons observed after only 2 days of treatment contrasts what has been seen up to now in electrophysiological studies assessing the effects of antidepressant medications. More specifically, electrophysiological data demonstrate that all antidepressants after chronic treatments, through various mechanisms, increase 5-HT neurotransmission (Chaput *et al.*, 1991; Blier & de Montigny, 1994). For example, acute treatments with MAOI's or SSRI's lead to decreased firing activity of 5-HT neurons in the DRN but as treatment continues the 5-HT neurons regain normal firing activity due to desensitization of the 5-HT$_{1A}$ somatodendritic autoreceptors. This desensitization may be an adaptive change that explains the delayed enhancement of 5-HT mediated neurotransmission, which is consistent with the clinical onset of action of SSRI's (Chaput *et al.*, 1986; Blier *et al.*, 1984; Blier & de Montigny, 1985; 1994). In agreement with electrophysiologial results, microdialysis experiments show that following a 14-day administration of an SSRI, there is a 6 fold increase in extracellular 5-HT concentration in the frontal cortex (Bel & Artigas, 1993). Indeed up to now only one antidepressant (mirtazapine) has been shown to induce an increase in the firing activity of DRN neurons following acute and long-term treatments (Haddjeri *et al.*, 1998; Besson *et al.*, 2000). Interestingly, mirtazapine was recently reported a showing a more rapid onset of action of its antidepressant properties (Benkert *et al.*, 2000). Thus, the present data could suggest that not only sigma agonists might have antidepressant properties, but also that their onset of action might be more rapid than those of classical antidepressants.

Following 21 days of treatment with (+)-pentazocine or 4-IBP (2 mg kg^{-1} day^{-1}) the increase in firing activity seen after 2 days persists, suggesting this is not a transient effect. Interestingly, after 21 days of treatment with (+)-pentazocine but not with 4-IBP there was a drastic decrease in neurons found per track (Figure 6). This did not occur after shorter treatments of 10 or 14 days (Figure 6) nor after 21-day treatment with a lower dose of 0.5 mg kg^{-1} day^{-1} of (+)-pentazocine (Figure 7). Furthermore, the co-administration of NE-100 prevented the decreased neurons per track seen after 21 days of treatment with (+)-pentazocine (Figure 6). Therefore, this phenomenon appears to be selective for (+)-pentazocine and specific to long-term treatments over a certain dosage.

One possible explanation for the decrease in the number of neurons found per track after chronic (+)-pentazocine treatment is a decrease of spontaneously active 5-HT neurons, due to a depolarization blockade, as seen in the dopaminergic neurons of the midbrain following chronic haloperidol administration (Grace & Bunney, 1986; Hollerman *et al.*, 1992). Thus far, we have first investigated the reality of this potential depolarization blockade by testing if it could be reversed by a 5-HT$_{1A}$ agonist. Following the intravenous administration of 8-OH-DPAT, a 5-HT$_{1A}$ agonist at somatodendritic autoreceptors (Peroutka, 1985), the amount of neurons found per track was not changed. One would expect that the activation of the somatodendritic 5-HT$_{1A}$ autoreceptor by 8-OH-DPAT would reverse a depolarization blockade since it repolarizes the neuron, as the depolarization blockade seen in dopaminergic neurons was reversed by apomorphine, a dopamine autoreceptor agonist (Grace & Bunney, 1986; Hollerman *et al.*, 1992). Thus, the lack of effect of 8-OH-DPAT suggests, either that the decreased number of neurons per track was not due to a depolarization blockade, or that higher doses of the 8-OH-DPAT were required. However, the latter appears unlikely as we used the dose previously shown to completely suppress 5-HT firing activity in the DRN (Blier *et al.*, 1998). In a second attempt to repolarize the neurons, rats were injected with (+)baclofen (5–15 mg kg^{-1} i.v.), a γ-aminobutyric acid$_B$ (GABA) agonist, which also, did not restore the number of spontaneously firing neurons suggesting that the silent neurons were not depolarized. However, a lack of repolarizing effect of the GABA$_B$ agonist could not be totally excluded based on recent findings suggesting that, under some circumstances, (+)baclofen might disinhibit DRN 5-HT neurons by preferentially activating GABA$_B$ autoreceptors (Abellan *et al.*, 2000). Therefore, at present the possibility of a depolarization blockade cannot be completely ruled out and we will be further investigating this phenomenon as this would be the first report of such a phenomenon occurring in 5-HT neurons.

A second possible explanation for the decreased number of neurons found per track is an increased endogenous tonic GABA inhibition of the 5-HT neurons of the DRN (Hajos *et al.*, 1999; Abellan *et al.*, 2000). It has been suggested that the inhibitory effect of 8-OH-DPAT on firing activity of DRN neurons involves, in part, the activation of a 5-HT$_{1A}$ receptor-mediated postsynaptic long feedback loop centred on the medial prefrontal cortex (Ceci *et al.*, 1994; Hajos *et al.*, 1999; Casanovas *et al.*, 1999). This inhibition by the prefrontal cortex is thought to involve activation of GABA interneurons by glutaminergic cortical input (Hajos *et al.*, 1999; Haddjeri *et al.*, 2000; Abellan *et al.*, 2000). To test this possibility we injected (−)bicuculline (375 μg kg^{-1} i.v.), a GABA$_A$ antagonist, but this did not restore the number of neurons found per track, suggesting overactive GABA tonic inhibition is not responsible.

It has also been shown that in addition to GABAergic modulation of neurons in the long feedback loop cholinergic

and glutamatergic systems play key roles. This was demonstrated by the finding that the muscarinic antagonist atropine, the M_2 antagonist 11-[[2-[(diethylamino)methyl]-1-piperidinyl]acetyl]-5-11-dihydro-6H-pyrido[2,3-6][1,4]benzodiazepine-6-one (AF-DX116), the NMDA antagonist (+)-5-methyl-10,11-dihydro-5H-dibenzo(a,d)cyclohepten-5-10-imine maleate (MK-801) and $GABA_B$ antagonist (25)(+)-5,5-dimethyl-2-morpholineacetic acid (SCH-50911) all dampened the suppressant effect of 8-OH-DPAT on the firing activity of DRN 5-HT neurons while (−)bicuculline did not (Haddjeri *et al.*, 2000). Therefore, the possible effect(s) of these other systems on the firing activity of neurons of the DRN and thus the neurons found per track should be assessed especially in the light of the known interaction of sigma ligands with NMDA receptors discussed previously. Nevertheless, this dramatic decrease in the number of spontaneously active 5-HT neurons could suggest that the net effect of some sigma ligands will not always be beneficial from an 'antidepressant' perspective.

The effects of (+)-pentazocine changed over the duration of the treatments, as shown in Figures 5 and 6. The magnitude of the increase in the average firing rate of DRN 5-HT neurons progressively reduced in parallel to a progressive increase in the number of neurons found per track. We do not have any definite explanation for this phenomenon, however, if one assumes that the decreased number of spontaneously active neurons is due to a depolarization blockade, one possible explanation could be that, at the beginning of the treatment, spontaneously active neurons and silent neurons will see their firing activity progressively increase and then decrease before reaching the final stage of the depolarization blockade. In such a paradigm, day 14 could represent the time with the maximum number of neurons firing, with some being already on the descending phase of the curve before the depolarization, while the initially silent ones have not yet reached their maximal firing activity. We are currently investigating the potential mechanisms responsible for this phenomenon.

The discrepancy between (+)-pentazocine and 4-IBP producing an increase in firing activity while PRE-084 and JO-1784 did not, is surprising. Firstly, like (+)-pentazocine, JO-1784 was shown to be a sigma agonist in our model of modulation of the NMDA response (Monnet *et al.*, 1992). Secondly, although PRE-084 has not been tested in our model of NMDA modulation, it was found to act as an agonist in several behavioural models of learning and memory deficits. Specifically, PRE-084 attenuated MK-801-induce learning impairments in mice similar to $sigma_1$ agonists (+)-pentazocine and (+)-N-allyl-normetazocine ((+)SKF-10,047) and was antagonized by the $sigma_1$ antagonist α-(4-fluorophenyl)-4-(5-fluoro-2-pyrimidinyl)-1-piperazinebutanol (BMY-14802) (Maurice *et al.*, 1994a, b). Similarly, treatments with JO-1784 and PRE-084 (0.1–3 mg kg^{-1}) improved learning impairments in a BMY-14802 sensitive manner in senescense accelerated mice (Maurice *et al.*, 1996).

This lack of effect of JO-1784 and PRE-084 on the firing activity of serotonergic neurons of the DRN may be explained by the existence of subtypes of $sigma_1$ receptors, which has been previously suggested by results from our laboratory. Specifically, potentiation of the NMDA response by DTG and JO-1784 is mediated by a subtype of $sigma_1$ receptor linked to a $G_{i/o}$ protein, whereas potentiation induced by (+)-pentazocine is mediated by another subtype of the $sigma_1$ receptor not linked to a $G_{i/o}$ protein, as only this response is pertussis toxic insensitive (Monnet *et al.*, 1994). Furthermore, following colchicine pretreatment, which destroys the mossy fibre system, the neuronal response induced by DTG and JO-1784 was abolished while (+)-pentazocine's effect persisted, indicating the $sigma_1$ receptor subtype mediating (+)-pentazocine's effect is located post-synaptically on pyramidal neurons while the $sigma_1$ receptor subtype mediating DTG and JO-1784's effects is located presynaptically (Debonnel *et al.*, 1996). Further evidence in support of the existence of subtypes of $sigma_1$ receptors was demonstrated recently as the potentiation of the NMDA response by (+)-pentazocine is reversed by naloxone, an opiate antagonist, while the potentiating effects of JO-1784, (+)-cis-N-[2,(3,4-dichlorophenyl)ethyl]-2-(1-pyrrolidinyl-cyclohexlamine (BD 737) and 1-benzylspiro[1,2,3,4-tetrahydronaphthalene-1,4-piperidine (L 687-384) were not (Couture & Debonnel, 2001). Thus, the modulation of serotonergic firing activity seen after a 2 day treatment with (+)-pentazocine and 4-IBP may be mediated by a specific subtype of $sigma_1$ receptor to which (+)-pentazocine and 4-IBP possess high affinity, while JO-1784 and PRE-084 may not.

(+)-Pentazocine and 4-IBP are probably not acting *via* the same $sigma_1$ receptors. Evidence for this includes the fact that (+)-pentazocine after chronic treatments induced a decrease in the number of neurons encountered per track while chronic treatment with 4-IBP did not. In addition, (+)-pentazocine's effect of increasing the 5-HT firing activity was reversed by the co-administration of NE-100 while 4-IBP's effect was not. These differences are likely due to effects mediated by different subtypes of the $sigma_1$ receptor. There has been previous evidence of multiple binding sites for (+)-pentazocine in addition to the aforementioned results by Couture & Debonnel (2001), for example, saturation studies, in the presence of ions including Zn^{2+}, Ca^{2+}, Mg^{2+} and in Krebs-Ringer buffer have demonstrated multiple (+)-[^{3}H]-pentazocine binding sites *in vivo* (Basile *et al.*, 1992). Further evidence showed [^{3}H]-pentazocine to label three different sites with different K_d values when various cell lines were tested (Vilner *et al.*, 1995).

It is important to mention that JO-1784 or PRE-084's ability to modulate serotonergic neurotransmission cannot be completely ruled out. Maurice *et al.* (1994b), has shown that PRE-084 follows a bell-shaped dose-response curve, which has been previously described in the modulation of the NMDA response by sigma ligands, including JO-1784, in the hippocampus (Bergeron *et al.*, 1995). Our doses were chosen based on those shown to produce an optimal response in the modulation of the NMDA response previously tested in our laboratory (Monnet *et al.*, 1990; 1992). Thus, it is indeed possible that the dose of PRE-084 or JO-1784 tested may be too low to reach the agonist range, or conversely, it may be too high and functioning as an antagonist. After chronic treatments with sigma ligands in the NMDA model, our laboratory has shown that low doses of JO-1784 or DTG potentiate the response to NMDA, however, at high doses they function as antagonists having no effect on their own but blocking the effect of sigma agonists (Bergeron *et al.*, 1997). Thus, the effect of these two ligands on serotonergic neurotrans-

mission cannot be completely ruled out until a range of doses is tested.

Even if this was not the case, it is also possible that PRE-084 and JO-1784 could possess some antidepressant properties but act *via* another mechanism. This may involve the modulation of NMDA receptors as other compounds that antagonize NMDA receptors have been shown to produce antidepressant effects in behavioural models of depression (Trullas & Skolnick, 1990; Maj *et al.*, 1992; Papp & Moryl, 1994). In addition, an alternative theory is that these sigma ligands could be modulating noradrenergic activity.

The precise mechanisms underlying the modulation of serotonergic neurotransmission evidenced in the present study remain to be elucidated and are the focus of current investigations in our laboratory.

In conclusion, this series of experiments provides the firs evidence of sigma receptor interaction with the 5-HT system. Thus, it strengthens the argument for sigma receptor's role in depression and provides a plausible mechanism of action to explain the antidepressant-like effects observed with some sigma ligands in behavioural models of depression. Importantly, these experiments show sigma ligands to produce an increase in 5-HT firing activity in just 2 days, a more rapid and robust effect than the vast majority of known antidepressant medications, thus, indicating its potential as an antidepressant with a rapid onset of action.

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