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ELECTROPHYSIOLOGICAL STUDIES IN RAT DORSAL HIPPOCAMPUS: MODULATION OF THE NEURONAL RESPONSE TO N-METHYL-D-ASPARTATE BY SELECTIVE SIGMA$_1$ AND SIGMA$_2$ LIGANDS

BY

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July 1998

A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfilment of the degree of Master in Science

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ACKNOWLEDGEMENTS

These experiments were carried out in the Neurobiological Psychiatric Unit at McGill under the supervision of Dr. Guy Debonnel. The research was conducted to fulfil the requirements of a Master in Science. I wish to thank Dr. Debonnel for his expert advice and his support and guidance throughout my studies.

I would also like to thank the members of the selection committee, namely Dr. de Montigny, Dr. Debonnel and Dr. Blier for accepting my candidacy as a graduate student and, providing me with such a stimulating environment. I am also grateful to Claude Bouchard, Normand Lavoie and Yves Simard for always being available for technical assistance and advice. A special "thanks" to all staff members and fellow graduate students for their continuous support and encouragement during my studies at McGill University.

Finally, I would like to covey a very special thank you to my friend and fiance, Patrice for his encouragement and patients and, my parents for their understanding and continuous support.

Manuscripts and authorship

Guidelines for Thesis Preparation

Faculty of Graduate Studies and Research, McGill University

The review of the literature and the studies presented in this Master thesis concern the modulation of the neuronal response by selective sigma ligands.

Two experimental series are presented in this thesis. One article has been published, the second one has been submitted. I have conducted all the experimental work, analyzed the data, drew the figures and written the papers. In regards to the second article, the technician Normand Lavoie also contributed to a minor extent in the obtention of experimental datas.

It is the policy of the Faculty at McGill University to allow the students to include as chapters "original publications" concerning the thesis research project. The faculty regulations can be summarized as follows:

Candidates have the option, subject to the approval of their Department, of including, as part of their thesis, copies of the text of a paper(s) submitted for publication, or the clearly-duplicated text of published paper(s), provided that these copies are bound as an integral part of the thesis. If this option is chosen, connecting texts, providing logical bridges between the different papers, are mandatory.

The thesis must still conform to all other requirements of the "Guidelines Concerning Thesis Preparation" and should be in a literary form that is more than a mere collection of manuscripts published or to be published.

The thesis must include, as separate chapters or sections:

(1) a table of contents,

(2) a general abstract in English and French,

(3) an introduction which clearly states the rationale and objectives of the study,

(4) a comprehensive review of the background literature to the subject of the thesis, when this review is appropriate,

(5) a final overall conclusion and/or summary.

Additional material (procedural and design data, as well as descriptions of equipments used) must be provided where appropriate and in sufficient detail (e.g. in appendices) to allow a clear and precise judgement to be made of the importance and originality of the research reported in the thesis.

LIST OF PUBLICATIONS

Published Abstracts

1. Debonnel G, and Couture S (1997) Modulation of the neuronal response to *N*-Methyl-*D*-Aspartate by selective σ_2 ligands, Neuroscience Meeting, New Orleans.

2. Couture S, and Debonnel G (1997) Sensitivity of selective σ ligands to the opiate antagonist naloxone, Neuroscience Meeting, New Orleans.

3. Couture S, and Debonnel G (1996) Modulation of the neuronal response to *N*-Methyl-*D*-Aspartate by selective σ_2 Ligands, ACNP abstract.

4. Couture S, and Debonnel G (1996) Modulation of the neuronal response to *N*-Methyl-*D*-Aspartate by selective σ_2 Ligands, Research Day, Dept. of Psychiatry, Mcgill University.

5. Couture S, and Debonnel G (1997) Sensitivity of selective σ ligands to the opiate antagonist naloxone, Research Day, Dept.of Neurology, McGill University.

6. Couture S, and Debonnel G (1997) Sensitivity of selective σ ligands to the opiate antagonist naloxone, Research Day, Dept. of Psychiatry.

7. Couture S, and Debonnel G (1997) Implication of σ ligands as potential anticonvulsants or proconvulsants, Symposium on Epilepsy, Laurentides.

Published Articles

1. Couture S, and Debonnel G (1998) Modulation of the neuronal response to *N*-Methyl-*D*-aspartate by selective σ_2 ligands, SYNAPSE, 29:62-71.

2. Couture S, Lavoie N, and Debonnel G (1998) Suppression of (+)-pentazocine effects by naloxone (submitted, SYNAPSE).

ABSTRACT

It has now been accepted for several years, that σ receptors exist as at least two distinct entities denoted σ_1 and σ_2. We have previously shown in our laboratory that several selective σ_1 ligands potentiate the neuronal response to NMDA in the dorsal hippocampus of rat. The non selective σ_1/σ_2 ligand, DTG, also potentiates the neuronal response. However, when it is administered at doses between 3 and 40 μg/kg, the increase of NMDA-induced activation turns in to an epileptoid activity. Data presented in this thesis suggest that similarly to σ_1 ligands, selective σ_2 ligands such as Lu 28-179, Lu 29-252 and BD 1008 dose-dependently potentiate the NMDA response. Interestingly, the effects of these drugs are not reversed by the non selective σ_1/σ_2 antagonist haloperidol, by the neurosteroid progesterone nor by the selective σ_1 antagonist NE-100. The σ_2 ligand CB-64D also potentiates, dose-dependently, the NMDA response, its effect, however is reversed by haloperidol and by the neurosteroid progesterone. All σ_2 ligands failed to generate any epileptoid activity on their own but, with the subsequent administration of a σ_1 agonist (JO-1784), an epileptoid activity was induced. This epileptoid activity was not observed following the subsequent administration of the σ_1 agonist (+)-pentazocine.

The σ receptor is believed to be a non opioid receptor insensitive to naloxone. However, recently, a protein has been identified that resembles the σ opioid receptor originally proposed by Martin and colleagues in 1976. Therefore to answer this discrepancy, we verified the spectrum of σ ligands for which the potentiation of the NMDA response is mediated by the naloxone-sensitive σ receptor. The potentiation of the NMDA response induced by (+)-pentazocine, but not JO-1784, BD-

737 and L, 687-384, was suppressed by naloxone.

The data presented in this thesis suggest that: (1) similarly to σ_1 ligands, σ_2 agonists potentiate the NMDA response; (2) the coactivation of one type of σ_1 and σ_2 receptors appears to be necessary to induce epileptoid activity; (3) haloperidol may not act as a σ_2 antagonist; (4) and (+)-pentazocine might act on two types of σ_1 receptor: one which is haloperidol sensitive and an other, which is naloxone sensitive but different than the opiate receptors.

ABRÉGÉ

On reconnaît maintenant l'existence d'au moins deux sous types de récepteurs σ: les récepteurs σ_1 et σ_2. Plusieurs études de notre laboratoire ont démontré que de nombreux ligands σ_1 potentialisent la réponse neuronale au NMDA dans l'hippocampe dorsal du rat. Il a également été démontré que le DTG, un ligand non sélectif σ_1 /σ_2 , potentialise aussi la réponse neuronale au NMDA mais que, lorsque il est administré à des doses comprises entre 3 et 40 µg/kg, il induit une activité épileptoïde. Les données présentées dans cette thèse suggèrent que les ligands σ_2 sélectifs tels que le BD 1008, le Lu 28-179 et le Lu 29-252 potentialisent aussi la réponse neuronale au NMDA, mais les effets induits par ces ligands ne sont pas renversés par l'halopéridol, un antagoniste sigma non sélectif σ_1/σ_2, par le neurosteroide progestérone ou par l'antagoniste sélectif au récepteur σ_1, le NE-100. Cependant, le ligand σ_2 CB-64D potentialise aussi la réponse neuronale au NMDA mais ses effects sont renversés par l'haloperidol ou par le neurostéroide progesterone. Ces différents ligands σ_2 ne génèrent aucune activité épileptoïde par eux-mêmes. Par contre, en présence d'un autre ligand sélectif σ_1 tel que le JO-1784, une activité épileptoïde est observée alors que ceci n'est pas le cas après l'administration d'un autre ligand σ_1 tel que la (+)-pentazocine.

Les récepteurs σ sont maintenant reconnus comme étant des récepteurs non opiacés et insensible à la naloxone. Cependant, récemment, une protéine a été purifiée qui ressemble au récepteur opiacé et pour lequel la naloxone a une haute affinité. Nous avons vérifié si la potentialisation de la réponse au NMDA induite par certains ligands σ pourrait être renversée par la naloxone. La potentialisation induite par la (+)-pentazocine mais non celles induites par le JO-1784, le BD-737 ou le L, 687-384 est effectivement renversée par la naloxone.

Les données présentées dans cette thèse suggèrent que: (1) les ligands σ_2 potentialisent la reponse neuronale au NMDA; (2) l'administration concommitante de ligands σ_1 et σ_2 seraient necessaire pour induire une activité epileptoide; (3) l'haloperidol n'agirait peut-être pas comme antagoniste σ_2; (4) et le ligand (+)-pentazocine agirait possiblement sur deux sous-types de récepteurs σ_1: un récepteur sensible à l'halopéridol et un autre récepteur sensible à la naloxone tout en étant différent des récepteurs opiacés.

TABLE OF CONTENTS

6. **PHYSICAL CHARACTERISTICS OF SIGMA RECEPTORS**

6.1 G-protein coupling and second messenger system
6.2 Endogenous ligands

7. **SIGMA RECEPTORS IN PSYCHIATRY**

CHAPTER II **Modulation of the Neuronal Response to N-methyl-D-aspartate by selective σ_2 ligands**

CHAPTER III **The suppression of (+)-pentazocine effects by naloxone**

CHAPTER IV **GENERAL DISCUSSION**

À la mémoire de Geneviève

CHAPTER 1

1. INTRODUCTION

Excitatory amino acids and glutamate receptors are important in regards to sigma receptors (σ) since *in vivo* electrophysiological studies have shown that σ receptors play an important role in modulating *N*-methyl-*D*-aspartate (NMDA).

1.1 Excitatory amino acids

L-Glutamate, l-aspartate, cysteic acid and possibly homocysteate are the principal excitatory amino acids in the central nervous system and their role consists of mediating fast excitatory synaptic responses. Excitatory amino acids are fairly uniformly distributed in the central nervous system and are found in virtually all cells. In the brain, l-glutamate is synthesized in nerve terminals from glucose, via the Krebs cycle, by transamination of α-oxoglutarate and by synthesis of glutamine in glial cells. It is transported into nerve terminals where the enzyme glutaminase converts it into glutamate. It is stored in presynaptic terminals by a Mg^{2+}-ATP dependent process and released from vesicles by a common calcium dependent mechanism. Once released, it acts as a synaptic transmitter, both on presynaptic or postsynaptic receptors. It is removed from the extracellular space by high-affinity uptake site via the plasma membrane glutamate transporter on the presynaptic nerve and on glial cells (for review, see Fykse and Fonnum, 1996).

The main pathways in the nervous system that use excitatory amino acids as neurotransmitters are the projections from the cerebral cortex to the striatum (Herrling et al., 1983) and the

hippocampus (Crunezzi et al., 1983; Ganong et al., 1983; Koerner and Cotman, 1982); the intrahippocampal mossy fibber system (Ganong et al., 1983; Lanthorn et al., 1984); the Schaffer collaterals (Ganong et al., 1983; Koerner and Cotman., 1981); the dorsal root evoked-monosynaptic response (Davies et al., 1982, 1984); the lateral olfactory tract (Collins, 1982) and the primary afferents to the trigeminal nucleus (Salt and Hill., 1981).

1.2. Receptors

Five categories of glutamate receptors mediating the excitatory synaptic actions of glutamate in the CNS have been identified on the basis of their selective agonists (Biscoe et al., 1978; Cotman et al., 1981; Foster et al., 1981; Haldeman et al., 1972; McLennan and Hendry, 1981; Watkins, 1984). The three main classes of ligand-gated glutamate receptors located on postsynaptic sites are the *N*-Methyl-*D*-aspartate (NMDA) receptor, the α-amino-3-hydroxy-5-methyl-isoxazole (AMPA) receptor and the kainate receptors (KA). The fourth one is AP4 receptor (1-2-amino-4-phosphonobutyrate) and represents an inhibitory autoreceptor while the last one is the metabotropic receptor for ACPD (trans-1-aminocyclopentane-1-3-dicarboxylic acid).

1.2.1 Metabotropic receptor

This receptor has been proposed to be implicated in synaptic plasticity and in excitotoxicity. The metabotropic receptor have been subdivided into three subgroups based on their amino acid sequences (Pin and Duvoisin, 1995). These receptors fall in the class of G-protein coupled receptors. They contain three structural domains: a hydrophillic NH2 terminal sequence, seven membrane

spanning domain and an intracellular COOH terminal sequence. They act mainly by releasing inositol phosphate and diacylglycerol as second messengers. Molecular biology studies have revealed in rats the presence of 6 cDNAs for this type of receptor (Nakanishi et al., 1992).

1.2.2 AMPA and Kainate receptor

These receptors bind the glutamate agonist AMPA (kainate and quisqualate) but neither binds the glutamate agonist NMDA. They gate a low-conductance cation channel that is permeable to both sodium and potassium. They are responsible for fast excitatory transmission and operate in most CNS synapses. AMPA receptor channels are dimeric molecules formed by the combination of any one or two of four subunits (GluR1-GluR4) and are 900 amino acids long while the KA subclass includes GLUR5-GLUR7 and KA1-KA2 subunits (Sommer et al.,1990). Autoradiographical studies have revealed that these receptors are mainly located in telecenphalic regions; however, both the kainate and AMPA receptors have other distributions (Cotman et al, 1981). AMPA depolarization is antagonized by glutamate diethylester (GDEE) while KA-induced depolarization is unaffected by GDEE (Foster et al., 1984; Krogsgaard-Larsen et al., 1980; McLennan and Hendry, 1981).

1.2.3 NMDA receptor

The NMDA receptor can be differentiated from the other AMPA receptors because it is blocked selectively by the glutamate antagonist APV and is involved in slower excitatory synaptic responses. The NMDA receptor is a ligand-gated channel associated with several binding sites (i.e. that for NMDA, PCP, glycine, polyamine and zinc). The NMDA receptor is the only ligand-gated

channel that is voltage dependent and permeable to calcium (Daw et al., 1993). It controls a cation channel of high conductance that is also permeable to sodium and potassium

1.2.3.1 PCP

Phencyclidine (PCP) also known as "Angel dust " induces symptoms such as delusions, hallucinations, depersonalization and dysphoria resembling those seen in schizophrenia (Haertzen et al.,1970; Brady and Balster, 1982; Shannon, 1982; Shearman and Hertz, 1982). The PCP site is located inside the channel of the NMDA complex. By blocking the cationic channel, PCP antagonizes the response to NMDA in a non competitive fashion (Anis et al., 1983; Snell et al., 1987) (see below).

1.2.3.2 Magnesium

Similarly to PCP, the magnesium (Mg^{2+}) binding site is located inside the ion channel of the NMDA receptor (Nowak et al., 1984; Mayer et al., 1984). Mg^{2+} can readily block the NMDA receptor in a voltage dependent fashion. For example, when the membrane potential of the NMDA receptor is negative (cell is polarized), the Mg^{2+} has positive charge and is attracted to the inside of the cell preventing the NMDA channels from opening. However, if the membrane potential is positive (cell is depolarized), there is no electrical gradient to incite the Mg^{2+} to bind to and block the NMDA channel.

1.2.3.3 Glycine

In 1987, Johnson and Asher discovered that glycine causes a facilitation of the NMDA

response in dissociated cell cultures via strychnine insensitive binding sites. It is now known that glycine is an obligatory coagonist required for the activation of the NMDA receptor (Kleckner and Dingledine, 1982; Laube et al., 1997). The binding of two glycine and two glutamate molecules is required for NMDA to stimulate channel opening (Kemp and Leeson, 1993).

1.2.3.4 Zinc

The zinc binding site is located on the extracellular side of the membrane and blocks the NMDA response (Mayer and Westbrook, 1987; Monaghan et al., 1989; Yoneda and Ogita., 1991). The blockade of NMDA by zinc acts in a voltage-independent matter (Westbrook and Mayer, 1987) and its role consists of reducing the open time and the frequency at which NMDA receptors are open.

1.2.3.5 Polyamine

Polyamine is an allosteric co-activator of the NMDA receptor (Ransom and Stec, 1988). Polyamines such as spermine and spermidine function as allosteric modulators of the NMDA receptors and potentiate NMDA currents in the presence of saturating concentrations of glutamate and glycine. In contrast to glycine, their presence is not a requirement for NMDA activation. It has been proposed that polyamine may potentiate or mediate the excitotoxic mechanisms responsible for neuronal damage (Ranson and Stec, 1988).

1.2.3.6 Molecular characterisations of the NMDA receptor

The NMDA receptor has been cloned and is subdivided in two main subunits. The first subunit

is NMDA1R (or NR1) and the second subunit is NMDA2R (or NR2). The NMDA1R forms homomeric or heteromeric channels and are expressed at high levels in most neurons (Moriyoshi et al., 1991). Seven splice variants of NMDA1R have been identified. The NMDA2R acts as a modulatory subunit and forms heteromeric channels. Four different genes encoding variants of NMDA2R have been identified (Moriyoshi et al., 1991).

1.2.3.7 LTP

Long term potentiation (LTP) was first discovered in the hippocampus (Bliss and Lomo, 1970; Lomo, 1986) and is defined as an increase in the amplitude of excitatory postsynaptic potentials at various CNS synapses following a short burst of presynaptic stimulation (11 Hz for 1 second). LTP has been proposed to underlie the synaptic plastic changes that are thought to occur during learning and memory. The voltage-dependent channel block by magnesium and the high calcium permeability of the NMDA receptor seem to underlie the involvement of this receptor in long term potentiation. Short-term potentiation and long term depression (LTD) are distinct processes but might also involve excitatory amino acids (Malenka et Nicoll, 1993). However, some recent studies *in vivo* and *in vitro* have demonstrated that neither LTP nor LTD are reliable models of learning abilities in rats and mice (for review, see Holscher, 1997). These recent results have to be interpreted carefully as they inform us that LTP and LTD might not be as important in certain models of learning as previously assumed.

1.2.3.8 Excitotoxicity

Olney (1969) was one of the first to observe excitotoxicity, when the systemic administration

of glutamate produced neurotoxic lesions in certain brain regions of infant mice. Excitatory amino acids are able to cause neuronal death in murine cortical cell cultures (Choi, 1988). Excitotoxicity is believed to be caused by an increase in extracellular glutamate and has been proposed as a mechanism of many pathologies, including epilepsy, ischemia and possibly Huntington and Alzheimer's diseases (Choi et al., 1992). However, a recent study by Obrenovitch and Urenjak (1997) showed that the glutamate level does not necessarily correlate with neuronal dysfunction and death *in vivo*. These authors suggest other hypotheses to explain excitotoxicity. For example, an increase in glutamate receptors, an altered ionic selectivity of inotropic glutamate receptors abnormalities in sensitivity or an enhancement of glutamate-mediated synaptic efficacy may account for these effects. The conclusions proposed by these authors do not exclude the fact that most studies have found that glutamate is implicated in excitotoxicity.

2. HISTORICAL PERSPECTIVE ON SIGMA RECEPTORS

Sigma receptors (σ) were discovered in 1976 by Martin and colleagues in the nondependent chronic spinal dogs. They were first classified as opiate receptors. Three subtypes of opiate receptors were denoted from their prototypal agonists: μ for morphine, κ for ketocyclazocine and σ for SKF-10047. A fourth opioid receptor, δ, was later identified (Chang and Cuatracasas, 1979). The three opiate receptors, μ, κ and δ mediate the effects of opioid drugs and peptides. Specifically, the μ receptor mediates analgesia (Chang et al., 1980; Pert and Snyder, 1974), the κ receptor mediates analgesia and sedation (James et al., 1982); while δ receptor mediates satisfaction, reward and seizure. The σ receptors were believed to mediate the psychomimetic effects produced by some

opiates and were shown to cause canine delirium in dogs (Keats and Yelford, 1964; Haertzen et al., 1970). However, the opiate antagonist naloxone, which is known to block opioid receptors, failed in both *in vivo* and *in vivo* models to reverse the effects of these σ ligands suggesting that σ receptors do not belong to the opiate family (Su, 1993). The σ receptors are now recognized as non-opioid receptors, insensitive to naloxone (Quirion et al., 1987, 1992).

The racemic ligand SKF-10047 binds both to a low and a high affinity site, the PCP and the σ site, respectively. It is noteworthy that, for several years, the PCP site and the σ receptor were confused, since PCP and σ sites share moderate to high affinities for different chemical classes of drugs, such as psychotomimetic benzomorphans. It is now well established that PCP and σ binding sites are different receptors and are therefore differentially distributed throughout the brain (Largent et al., 1984).

3.SUBTYPES OF SIGMA RECEPTORS

The existence of at least 2 subtypes of σ receptors denoted σ_1 and σ_2 is now widely accepted (Quirion et al., 1992). The σ_1 and σ_2 sites are distinct entities and do not represent 2 different affinity states of a single type of binding site. They do not appear to be located on common macromolecules and their respective concentrations in the CNS vary in different areas. These two subtypes, σ_1 and σ_2 receptors, can be differentiated by their (1) anatomical and tissue distribution, (2) their physical characteristics, (3) the mechanism underlying G-protein coupling, (4) their specific role and (5) their drug selectivity (McCann et al., 1992).

Only the σ_1 receptor is believed to be linked to a G protein (Quirion et al., 1992). Studies using photoaffinity labelling revealed a molecular weight of 25 kDa for the σ_1 receptor in guinea-pig brains and of 18-21 kDA for the σ_2 receptors in PC12 cells (Hellewell and Bowen, 1990). The σ subtypes also differ in their affinity for (+)-benzomorphans and (Hellewell and Bowen, 1990; Walker et al., 1990; Quirion et al., 1992) the σ_1 site, unlike the σ_2, displays restricted stereospecificity for (+)-isomers of the benzomorphans, morphinans and other opiates (Hellewell and Bowen, 1990). Relatively to σ_1 receptors, σ_2 receptors are more resistant to solubilizations (Torrence-Campbell and Bowen, 1996). The σ_1-binding site was purified as a 30-kDa protein in guinea pigs with only one transmembrane segment. The deduced amino acid sequences shares homology with fungal proteins implicated in sterol synthesis but does not resemble any type of known receptor (Hanner and al, 1996). In humans and rat brains, the σ_1 binding site was purified as a 223 amino acid protein with one transmembrane domain. It exhibits 93% homology with the guinea pig σ receptor (Kekuda et al., 1996; Seth et al., 1998). Additionally, the σ_1 receptor was purified in the mouse as a 28-kDa protein sharing 88% homology with guinea pigs and 90% homology with humans (Pan et al.,1998).

Recently, σ_3 and σ_4 have been proposed, with properties distinct from σ_1 and σ_2 sites (Booth et al., 1993; Bowen et al., 1995B).

4. ANATOMY OF SIGMA RECEPTORS

4.1 Anatomical, tissue distribution and cellular localisation

σ receptors are particularly concentrated in the limbic system and in the motor areas. High densities of σ receptors are present in the pars compacta of the substantia nigra, the caudate nucleus, the cerebellum, the red nucleus as well as some cranial nerve nuclei. In the limbic system, there is a high concentration of σ receptors in the hippocampus, the amygdala, the cingulate, the lateral septum and the bed nucleus of the stria terminalis (Gundlack et al., 1986; Contreras et al., 1987; Mcclean and Webber, 1988). Some autoradiographical studies have also shown that σ receptors are enriched in the locus coeruleus, as well as in the Purkinje molecular and granular layers and in the interpositus nucleus of the cerebellum (Gonzalez-Alvear et al., 1995). In the hindbrain, there is a 10 fold greater density of σ_1 receptor as compared to the cortex and the σ_1 binding sites are more abundant in the dentate gyrus of the hippocampal formation, facial nucleus and various thalamic and hypothalamic nuclei (Bouchard and Quirion, 1997). The highest concentrations of σ_2 receptors occurs in the cerebellum and in the cortex, where they exceed σ_1 by 300 folds and the σ_2 binding sites are found in structures such as the nucleus accumbens, substantia nigra pars reticulata, central grey matter and oculomotor nucleus (Bouchard and Quirion, 1997).

The σ receptors are also distributed outside the central nervous system in various peripheral tissues: rat pituitary gland, adrenal gland, testis, ovary (Wolfe et al., 1988), in tissues of the immune system (Wolfe et al., 1988), kidney (Musacchio et al.. 1988) and in the liver (Musacchio et al., 1988; Ross, 1991). In rat liver and kidney, both σ_1 and σ_2 are found in high densities (Hellewell and Bowen, 1990; Hellewell et al., 1994) and finally, σ receptors are also present in various cell lines (Vilner and Bowen, 1993; Vilner et al., 1995).

The synaptosomal marker choline acetyltransferase (McCann and Su, 1990) and mitochondrial markers such as succinic dehydrogenase (Whitaker et al., 1962) differ from the distribution of both σ_1 and σ_2 bindings. Thus, it is unlikely that σ sites are located in either synaptic or mitochondrial membrane. It has been suggested that microsomal σ binding sites may represent synthesized receptors or internalized receptors as a results of endocytosis (Itzhak, 1994).

5. PHYSIOLOGY OF SIGMA RECEPTORS

5.1 Role σ_1 receptor

Many roles have been proposed for the σ_1 receptors. They have been investigated as potential antitussive and anticonvulsant (Roth and al., 1993), it has also been suggested that σ ligands possess proconvulsant or anticonvulsant effects. Indeed, following a pretreatment with fluorothyl or supramaximal electroshocks, the σ ligand (+)-3-(PPP) acted as a proconvulsant while the σ ligand SKF-10047 and (±) cyclazocine both demonstrated anticonvulsant effects (Tortella et al., 1990).

More recently, the σ_1 receptor has been reported to be associated with memory processing in the hippocampus (Leonard and Nicholson, 1994) Matsuno and colleagues (1997) have proposed that σ_1 ligands are implicated in learning and memory processes through the interaction of cholinergic and glutamatergic systems. SA 4503, a selective σ_1 ligand, is known to reverse the amnesia induced by cholinergic antagonists in passive avoidance tasks. This effect is prevented by σ antagonists haloperidol and NE-100 suggesting that the attenuation of amnesia is mediated through σ_1 sites.

The σ ligands could possibly exert some neuroprotective effects by inhibiting the ischemia-induced presynaptic release of excitatory amino acids (Maurice and Lockart, 1996). For example, 4-phenyl-1-(4-phenylbutyl)piperidine afforded protection when administered at the end of ischemia and during reperfusion in the cat hippocampus (Takahashi et al., 1995). Additionally, the ligands SL 82-075 and ifenprodil have proven to exert antiischemic effects, however it remains unclear wether these effects are mediated via σ receptors or via the polyamine site of the NMDA receptor (Contreras et al., 1990).

5.2 Role of σ_2 receptors

Due to the absence of selective σ_2 ligands, the role of this subtype of receptors has been less studied. Recent studies have suggested that σ_2 receptors are involved in motor function but does not appear to display any neuroprotective function (DeCoster et al., 1995). Walker and colleagues (1992) pointed to the possibility that σ_2 receptors are involved in motor control, after showing that the non selective σ ligand DTG, locally injected in the rat red nucleus, induces torticollis. In behavioral studies, BD 1047 and BD 1063 had no effects on their own when unilaterally microinjected into the rat red nucleus, but both compounds attenuated the dystonia produced by DTG and haloperidol (Matsumoto et al., 1995). Moreover, in rats, a potent anxiolytic activity in the black\white box exploration was found with Lu 28-179 (Perregaard et al., 1995).

5.3 Interaction with the glutamatergic system

Electrophysiological and neurochemical studies have shown that σ receptors modulate the

NMDA/PCP receptor complex. For example, the σ ligands (+)-SKF-10047 and (+)-pentazocine increase dopamine metabolism in the rat brain and their effects are suppressed by the NMDA antagonist PCP (Iyengar et al., 1990). Moreover, σ ligands antagonize the level of cGMP in mouse cerebellum in the presence of an agonist of the NMDA/PCP complex (Rao et al., 1990; Ferris et al., 1991). In our electrophysiological model, σ ligands either acted as agonists by potentiating the NMDA response or as antagonists by suppressing the effects induced by σ agonists. Previous studies have demonstrated that low doses (0.5-3 μg/kg, i.v.) of DTG potentiate the neuronal response to NMDA in the CA_3 region of the rat dorsal hippocampus but do not modify KA- and QUIS-induced activations (Monnet et al., 1990). This potentiation of the NMDA response was observed with several σ ligands including (+)pentazocine, BD737, L687-384, APDQ, AdipG, DnBG, sertraline, clorgyline, JO-1784 and JO-1783 (Monnet et al., 1992a; Bergeron et al., 1993; Bergeron et al., 1995). Only four σ ligands were acting as "antagonists": haloperidol, BMY-14802, NE 100 and (+)3-PPP, as they failed to potentiate the neuronal response to NMDA but instead suppressed the potentiation of the NMDA response induced by σ "agonists" (Monnet et al., 1992a). Spiperone, another butyrophenone with a binding profile similar to that of haloperidol but with a low affinity for σ receptors (Taylor and Dekleva, 1988; Weber et al., 1986; Tam et al., 1988), failed to reverse the potentiation of NMDA-induced activation by the σ agonists, suggesting that the antagonistic effect of haloperidol is due to its affinity for σ receptors.

The majority of σ ligands generate bell shaped dose-response curves. The degree of potentiation of the NMDA response increases dose-dependently with low doses, then decreases

progressively with higher doses and disappears completely with doses higher than 1 mg/kg. The disappearance of the potentiation of the NMDA response with high doses of σ ligands is not associated with a desensitization of the σ receptors, but could be due to the activation of another subtype of σ receptor. Bell-shaped dose-response curves have also been observed in the effects of σ ligands in different behaviour or biochemical models. For example, Nabeshima's group showed that doses of DTG or (+)-pentazocine between 1 and 1000 µg/kg had no behavioral effect in naive rats, but dose-dependently reversed the effect of MK-801. The same type of bell-shape dose-response curve was obtained with maximal effects at doses of 100 µg/kg of DTG and of (+)-pentazocine.

5.4 Interaction with the dopaminergic system

It has been proposed that σ receptors in the central nervous system can modulate the dopaminergic system. For example, the microinjection of DTG in the rat substantia nigra produces contralateral turning (Walker et al., 1988, 1992). This turning, induced by σ ligands, is prevented when the nigrostriatal dopaminergic neurons are destroyed by 6-hydroxydopamine (Goldstein et al., 1989). Microdialysis experiments have also shown an interaction of σ ligands with the dopaminergic system such as an increase in extracellular dopamine in the striatum following the injection of the σ ligands DTG and (+)-pentazocine (Patrick et al., 1993; Gudelsky et al., 1992). Weatherspoon and colleagues (1996) found that in the region of the nucleus accumbens, dopamine release induced by (+)pentazocine is primarily mediated by σ_1 receptors, while in the region of the prefrontal cortex, its action is mainly via the σ_2 receptors. Some electrophysiological studies have demonstrated that the acute administration of high doses of σ ligands such as (+)-pentazocine, SR 31742A, BMY-14802

and (+)SKF 10,047 increases, whereas DTG and (+)3-PPP decreases the firing activity of dopaminergic neurons (Clark et al., 1995; Freeman and Zhang, 1992). In contrast, other studies have shown that DTG, BMY-14802, JO-1784, SKF 10,047 and (+)-pentazocine had no effect on A_9 and A_{10} dopaminergic neurons (Zhang et al., 1994). Furthermore, SR 31, 742A increases the firing rate of dopaminergic neurons in A_9 and A_{10} (Poncelet et al., 1993) while BMY 1482 decreases the dopaminergic neuron activity in A_{10} but not in A_9 and, SKF-10047 applied by microiontophoresis is devoid of effects (Freeman and Zhang, 1992). Finally, microiontophoretic applications of the σ ligand S212378 had a slight effect on both A_9 and A_{10} neurons, the σ_2 ligand S21272 increased the firing activity of both A_9 and A_{10} neurons while JO-1784 did not induce any change on the dopaminergic system (Gronier and Debonnel, 1996).

As mentioned earlier, the σ_2 receptor is believed to play an important role in motor function through its interaction with the dopaminergic system. It is not yet fully understood if σ_2 ligands alone are responsible for the interaction of σ receptors with the dopaminergic system. Some σ_1 ligands have also been proposed to interact with the dopaminergic system.

5.5 Interaction with other drugs

σ receptors are implicated in many pharmacological functions through their interaction with a wide variety of drugs. Indeed, they suppress the hyperlocomation and supersensibility induced by cocaine and amphetamine (Ujike et al., 1992). It was suggested that they mediate the effects of some atypical antipsychotic agents, however, all clinical studies failed to demonstrate their antipsychotic

properties. (Taylor and Dekleva, 1987; Den Boer et al., 1990; Taylor et al., 1991; Cook et al., 1992). In addition, they block potassium channels (Wu et al., 1991), inhibit [^{3}H] norepinephrine uptake (Roger and Lemaire, 1991) and induce [^{3}H] norepinephrine (Roman et al., 1991) and [^{3}H] acetylcholine (Junien et al., 1991) release from hippocampal slices. Moreover, they interact with antidepressant, anticonvulsant, antihistamine, antimuscarinic, antitussive drugs and steroids. For example, competition studies using [^{3}H](+)-pentazocine have revealed that some serotonin reuptake inhibitors and tricyclic antidepressants interact with the σ_1 sites in the following order:fluvoxamine>sertraline>(+)fluoxetine...(Narita et al., 1996).

6. PHYSICAL CHARACTERISTICS OF SIGMA RECEPTOR

6.1 G-protein coupling and second messenger

It has been proposed that σ receptors interact with guanine nucleotide binding protein and may be part of the G-protein coupled family of receptors (Itzhak and Khouri, 1988; Itzhak, 1989; Itzhak and Stein, 1991; Connick et al., 1992). The σ receptor binding site is sensitive to both GTP and its analog Gpp (NH)p (Itzhak and Khouri, 1988). Numerous studies have demonstrated an interaction between Gpp (NH)p, GTP and σ ligands (such as [^{3}H]+ PPP), while a resistance of DTG binding site to these nucleotides was observed, suggesting that σ_1 and not σ_2 receptors are linked to G-proteins. (Itzhak and Stein, 1991). Indeed, Quirion and colleagues proposed in 1992 that only the σ_1 receptor is linked to a G-protein and not the σ_2 receptor. It has been proposed that the σ_1 site is modulated through $G_{i/o}$ proteins which in turn, regulate secondary messenger systems including protein kinase C translocation, phosphatidyl inositol turnover and the arachidonic acid cascade

(Itzhak, 1989). Moreover, Bowen and co-workers have shown that σ_1 ligands and not σ_2 ligands inhibit acetylcholine-and norepinephrine-induced phospoinositide response in rat brain synaptoneurosome preparations (Bowen et al., 1988). The σ_2 receptor is not linked to a G-protein nor is it involved in secondary messenger systems. However, some studies have suggested that σ_2 receptors play a role in the modulation of cellular Ca^{++} level. For example, it has been proposed that σ_2 ligands produce a rise in intracellular calcium by releasing it from intracellular stores thereby leading to a diminution in depolarization-induced calcium influx through voltage-dependant calcium channels (Rothman, 1994).

At this moment, the biochemical nature of the G-protein coupled to σ receptors is unknown and many authors argue against G protein coupling because of the molecular size of the σ_1 receptor as compared to the molecular size of G-protein (Hellewell et al., 1990, 1994). They suggest instead that σ sites are multi-subunit motifs of the G protein-coupled receptor (De Costa et al., 1994). It is noteworthy that even if the σ_1 receptor has been cloned as a one transmembrane domain protein and that G-proteins are known to have 7 transmembrane domains, the σ ligand used to label the σ receptor for its cloning was (+)-pentazocine and numerous studies have shown that (+)-pentazocine is not linked to a G protein. For example, a pretreatment with pertussis toxin suppresses the potentiation of the NMDA response induced by low doses of the σ_1 ligand JO-1784 but not that induced by (+)pentazocine suggesting that (+)-pentazocine (Monnet et al., 1994).

6.2 Endogenous ligands and neuropeptides

The endogenous ligands for σ receptors have not yet been identified, it has been proposed that several neuropeptides interacting with σ receptors might be endogenous ligands for σ binding sites, for which they have a high affinity. For example, the neuropeptide Y (NPY) has a high affinity *in vivo* for the σ receptor (Quirion et al., 1987). It selectively potentiates the NMDA response of CA_3 pyramidal neurons and the neuropeptide YY (PYY) suppresses the effect of NPY. The effect of NPY is also suppressed by haloperidol and by BMY-14802 but not by spiperone, suggesting that NPY suppression is mediated via σ receptors (Monnet et al., 1992b). Also, the neuropeptide calcitonin gene related protein (CGRP) potentiates the neuronal activation induced by NMDA (Bouchard and al., 1993) in the CA_3 region of the dorsal hippocampus.

It has also been suggested that several neurosteroids interact with σ receptors: Su et al., in 1988 demonstrated that progesterone and testosterone competitively displace, with a high potency, haloperidol and (+) SKF 10,047. Finally, it has been proposed that zinc may act as an endogenous ligand at σ_2 sites in the hippocampus because zinc is present in synaptic vesicles in the brain and may function to regulate binding at the σ_2 site.

Neuropeptides, such as CCK may also be involved in the modulation of the NMDA response by σ ligands. Indeed, the excitatory effect of CCK-8S in CA_3 pyramidal neurons is mediated via CCK-B receptors and the potentiation of the NMDA response induced by σ ligands is selectively suppressed by CCK-A antagonists (Gronier and Debonnel, 1995). Moreover, *in vivo*

electrophysiological studies have demonstrated that the neurosteroid dehydroepiandrosterone (DHEA) potentiates the neuronal response to NMDA in CA_3 hippocampal pyramidal neurons. However, instead of generating a bell-shape curve with high doses, a plateau is reached. The effects of DHEA are suppressed by progesterone, haloperidol and NE-100, whereas progesterone suppresses the potentiation of the NMDA response induced by (+) pentazocine and DTG (Bergeron et al., 1996).

7. SIGMA RECEPTOR IN PSYCHIATRY

The psychotomimetic effects of some σ ligands, the high affinity of several antipsychotic medications for σ receptors, and the fact that post mortem studies have demonstrated a reduction in the density of σ ligand binding in the cortex and cerebellum of schizophrenic patients (Simpson et al., 1991) and the fact that chronic treatments with haloperidol (a non selective σ_1/σ_2 ligand) result in a down-regulation of σ receptor density (Itzhak, 1989) all suggest that σ receptors could be implicated in schizophrenia. Several studies have proposed that σ ligands regulate the activity of the glutamatergic system via the NMDA receptor. Thus, a modulation of the glutamatergic system which could interfere with dopaminergic neurotransmission or a direct interaction of σ ligands with dopaminergic neurons (Halberstadt, 1995) could account for the implication of σ receptors in the pathophysiology of schizophrenia (Debonnel, 1993). This may further be supported by the fact that dysfunctional glutamatergic pathways are involved in psychotic pathology. However, all clinical trials using σ ligands such as BMY-14802 and Dup 734 failed to detect any significant results in treating schizophrenic patients (Borisson et al., 1992; Gewirtz et al., 1994). There is still hope that σ ligands could be useful tools in the treatment of schizophrenia. For example, in a clinical study where 10

chronic schizophrenic patients with negative symptomology received the σ antagonist SL 82.0715, four patients showed improvement of negative symptoms, two patients deteriorated and the others showed no visible difference in their symptoms (Modell et al., 1996).

The implication of σ receptors in affective disorders has also been supported by the finding that some antidepressant drugs like sertraline or clorgyline have high affinity for σ receptors and both of these antidepressants modulate selectively the NMDA response (Bergeron et al., 1993). More recently, a double blind clinical study provided evidence that the σ_1 ligand JO-1784 has therapeutic potential for treating depression (Pande et al., 1997).

Sigma receptors may also play a role in dementia. For example, the role of the σ receptor in Alzheimer has been investigated and it has been shown in post-mortem study of brains of Alzheimer's patients that the binding of [^{3}H] DTG to σ binding sites in the CA_1 region is reduced by 26% and that there is an average lost of 29% of pyramidal neurons (Jansen et al., 1993). Finally, it has also been proposed that the interaction of NMDA and σ_1 binding sites may also contribute to the antiparkinsonian effects of budipine (Kornhuber et al., 1995).

The role of σ receptors in psychiatry is not clearly elucidated although it is clear that these receptors interact with many neuronal systems and may be implicated in many psychiatric diseases. Further clinical studies would be warranted in order to confirm the efficacy of selective σ ligands in treating depression and assess their efficacy as potential antipsychotic.

CHAPTER II

Modulation of the neuronal response by *N*-methyl-D-aspartate by selective σ_2 ligands

These σ_2 receptors has been less studied than σ_2 due to the absence of selective σ_2 ligands. In our laboratory, it has previously been shown that when the non selective $\sigma_1\backslash\sigma_2$ ligand, DTG, was administered intravenously in doses ranging from 3-40 µg/kg, an epileptoid activity was observed upon the microiontophoretic application of NMDA, whereas this type of activity has never been observed following the administration of a σ_1 ligands thus suggesting that this effect was related to the affinity of DTG for the σ_2 receptor. Recently, some selective σ_2 ligands such as Lu 28-179, Lu 29-152 (Perregard et al., 1995,; Moltzen et al., 1995), BD 1008, ibogaine, CB-64D, CB-164 and BD 10047 (Bowen and al., 1995A) have become available.

The first goal of the present in vivo electrophysiological studies was to use these new tools in our model to obtain a better understanding of the physiological role of σ_2 receptors and to determine if, similarly to DTG, these σ_2 ligands would be capable of generating an epileptoid activity on their own or if the coactivation of σ_1 and σ_2 receptors was necessary to induce this type of effect.

Modulation of the neuronal response to *N*-Methyl-D-Aspartate by selective sigma $_2$ ligands

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Published : Synapse 29:62-71, 1998

ABSTRACT

It has now been accepted for several years that sigma (σ) receptors exist in, at least, two distinct entities denoted σ_1 and σ_2. Previous electrophysiological studies from our laboratory have demonstrated that several selective σ_1 ligands potentiate the neuronal response to NMDA. The non selective σ_1/σ_2 ligand DTG also potentiates the NMDA response. However, when DTG is administered at doses between 3 and 40 µg/kg, the increase of NMDA-induced activation turns to an epileptoid activity. Until recently, the physiological role of σ_2 receptors had been less studied due to the lack of selective σ_2 ligands. The goal of the present electrophysiological studies was to assess the effect of the intravenous administration of new selective σ_2 ligands on the neuronal response to NMDA in the CA_3 region of the rat dorsal hippocampus. Lu 28-179 and BD 1008 potentiated dose-dependently the NMDA response and generated bell-shape dose response curves. These ligands failed to generate any epileptoid activity on their own but, the subsequent administration of a low dose of a σ_1 agonist (JO-1784), induced an epileptoid activity. Interestingly, the potentiations of the NMDA response induced by Lu 28-179 or BD 1008 were not reversed by haloperidol, by the neurosteroid progesterone nor by the selective σ_1 antagonist NE-100. Ibogaine, a high affinity σ_2 ligand slightly increases the NMDA response which was reversed by progesterone. These data suggest that, similarly to σ_1 ligands, σ_2 agonists potentiate the NMDA response, that the coactivation of σ_1 and σ_2 receptors could be necessary to induce an epileptoid activity. They also suggest that haloperidol may not act as a σ_2 antagonist and that several subtypes of σ_2 receptors could exist.

Key words: NMDA, hippocampus, BD.1008, LU 28-179

INTRODUCTION

The existence of at least 2 different subtypes of σ receptors denoted σ_1 and σ_2 is now widely accepted (Quirion et al., 1992). The σ_1 and the σ_2 sites are distinct entities and do not represent 2 different affinity states of a single type of binding site. These two receptors do not appear to be located on a common macromolecule and their respective concentrations vary in different areas on the CNS .

Previous studies using an electrophysiological model of extracellular recordings have demonstrated that low doses of several σ_1 ligands including (+)-pentazocine, BD 737, L 687-384, sertraline, clorgyline and JO-1784 (Bergeron et al., 1993; Bergeron et al., 1995; Debonnel et al., 1992; Monnet et al., 1990) potentiate the neuronal response to *N*-methyl-D-aspartate (NMDA) in the CA_3 region of the rat dorsal hippocampus but do not modify Kainate-(KA) nor Quisqualate-(QUIS) induced activations (Monnet et al., 1990). Interestingly, the majority of σ ligands tested thus far generates bell shaped dose-response curves with respect to the observed potentiations of the NMDA response. Indeed, the degree of potentiation increases dose-dependently with low doses, then decreases progressively as the doses increase and disappears completely with doses higher than 1 mg/kg. Such a modulation of the NMDA response has also been shown with neuropeptides such as NPY or neuroactive steroids such as dehydroepiandrosterone (DHEA), progesterone or testosterone which present a high affinity for σ receptors (Monnet et al., 1990; Roman et al., 1989). The non selective σ_1/σ_2 ligand DTG also potentiates the neuronal response to NMDA. However, it has also been shown that DTG, when administered intravenously at doses ranging from 3 to 40 µg/kg, induces

an epileptoid activity upon microiontophoretic applications of NMDA (Bergeron et al., 1995). This is most likely due to the simultaneous activation of numerous neurons in the hippocampus. The resultant epileptoid activity makes it impossible to record a single neuron, this activity being replaced by "field potential". This type of epileptoid activity has never been observed following the administration of any other σ_1 ligand.

Due to the absence of selective σ_2 ligand, the role of this subtype of receptors has been much less studied but recently, some selective σ_2 ligand became available. Lu 28-179 (1'-(4-(1-(4-flurophenyl)-1H-indol-3-yl)-1-butyl) spiro (isobenzofuran-1(3H),4'piperidine) has been shown to be a potent σ_2 ligand with an affinity in the subnanomolar range (Moltzen et al., 1995). The compound has also a relatively low affinity for serotonin $5HT_{1A}$ (K_i = 21000 nM), $5HT_{2A}$ (K_i = 2000 nM), dopaminergic D_2 (K_i = 800 nM) and α_1 adrenergic receptors (K_i = 330 nM) (Perregaard et al., 1995). BD 1008 is a novel non selective diamine with high affinity for both the σ_2 receptor (K_i= 13.8 nM) and for the σ_1 receptor (K_i = 0.6 nM) (Hellewell et al., 1994). Finally, ibogaine is an indole alkaloid exhibiting a high affinity for σ_2 receptors (K_i= 201nM) and low affinity for the σ_1 site (K_i= 8554 nM), thus showing a 43-fold selectivity for σ_2 receptors (Bowen et al., 1995). It also possesses a low affinity (> 100 nM) for several neurotransmitters: noradrenergic (α_1, α_2 and β), dopaminergic (D_1 and D_2), cholinergic (both nicotinic and muscarinic) and serotonergic ($5HT_{1A/D}$, $5HT_2$, $5HT_3$) (Sershen et al., 1996).

The goal of the present *in vivo* electrophysiological studies was to assess the effects of these

σ_2 ligands in our electrophysiological model, to clarify the role of σ_2 receptors and to determine if, similarly to DTG, these σ_2 ligands would generate an epileptoid activity on their own or if a coactivation of σ_1 and σ_2 receptors would be necessary to induce this type of effect.

MATERIALS AND METHODS

Male Sprague-Dawley rats weighing 225-250 g were obtained from Charles Rivers (Saint-Constant, Québec, Canada). They were maintained at constant temperature (25°C) under a 12 h-12 h light-dark cycle with free access to food and water, for two to three days before the electrophysiological experiments.

In vivo electrophysiological experiments

Animals were anesthetized with urethane (1.25 g/kg, i.p.) and mounted in a stereotaxic apparatus. Body temperature was maintained at 37° C throughout the experiment and a catheter was installed, prior to recording, in a lateral tail vein for the intravenous administration of drugs.

Extracellular unitary recordings of CA_3 dorsal hippocampus pyramidal neurons

Five-barrelled glass micropipettes were pulled in a conventional manner and their tips were broken to a diameter of 8-12 µm under microscopic control (Haigler and Aghajanian, 1974). The central barrel, used for extracellular recordings, was filled with a 2M NaCl solution containing fast green FCF. Two side barrels, used for microiontophoresis were filled with QUIS (1.5 mM in 400 mM NaCl, pH 8) and with NMDA (5 mM in 200 mM NaCl, pH 8). A third barrel was used for automatic current balancing and contained a 2M NaCl solution. The extracellular recordings were obtained in the CA_3 region at a depth of 3.5 to 4.5 mm below the cortical surface in a area defined stereotaxically as 4.2$\pm$ 0.2 mm anterior and 4.2 $\pm$ 0.2 mm laterally (Paxinos and Watson, 1986). Pyramidal neurons of the CA_3 region were identified by their characteristic large-amplitude (0.5-1.2mV) and long-

duration (0.8-1.2 ms) single action potentials, alternating with complex spike discharges (Kandel and Spencer, 1961). The currents used for ejecting the NMDA ranged from -8 to -15 nA and from 2 to -3 for QUIS. The duration of the microiontophoretic ejections was kept constant at 50 seconds. The σ ligands were administered intravenously, at doses between 1-2000 μg/kg. Only one dose of each drug was administered to one rat while recording from one neuron.

Calculations

The computer calculated the effect of each microiontophoretic application of NMDA and QUIS as the total number of spikes/nanocoulomb (nC). The effect of the intravenous administration of the sigma ligands was measured as the ratio (N_2/N_1) obtained by averaging the number of spikes generated by nanocoulomb of three successive applications of each excitatory substance before (N_1) and after (N_2) the intravenous administration of the drug tested. For the epileptoid activity, as it becomes impossible to record any single cell activity, the calculations were based on the number of cells for which such activity was appearing following the administration of a σ ligand. The dose-response curves of the effects of the intravenous administration of σ ligands were obtained by fitting experimental data to general logistic equations obtained with the software tablecurve (version 3.0, Jandel Scientific, 1991). Each data point represents the mean value of the results obtained with 4 neurons.

Drugs

The following substances were used: BD 1008 was a generous gift from W. Bowen (Laboratory of Medicinal Chemistry, NIDDK, NIH, Bethesda, USA); Lu 28-179 a gift from T. Skärsfeldt (Lundbeck A/S, Copenhagen, Denmark); JO-1784 a gift from J.L. Junien (Institut de Recherche Jouveinal, Fresnes, France); NE-100 was a gift from S. Okuyama (Taisho, Pharmaceutical Ltd, Tokyo, Japan); The following compounds were purshased: (+)-pentazocine and progesterone from RBI (Natick, Mass, USA); NMDA and Ibogaine from Sigma (St-louis, Mo, USA); haloperidol from McNeil laboratories (Stoufille, Ontario, Canada) and QUIS from Tocris Cookson (St-louis, USA); Lu 28-179 was solubilized in propylene glycol and methanesulfonic acid. All other drugs were dissolved in saline.

Statistical analysis

Results are expressed as means $\pm$ SE.M. The means were compared using a one factor ANOVA with repeated measures followed by Tukey's pairwise comparison test. Probability values smaller than 0.05 were considered as significant. To compare the number of neurons demonstrating an epileptoid activity after the intravenous administration of σ ligands , Fisher Exact tests were used.

RESULTS

Effect of the acute intravenous administration of Lu 28-179 on the firing activity of CA_3 pyramidal neurons of the dorsal hippocampus

Administered intravenously at doses of 50, 100, 200, 250, 500, 750 and 1000 µg/kg, Lu 28-179 potentiated selectively the NMDA response at low doses ranging from 100-2000 µg/kg, i.v., the response to QUIS remaining unchanged. The degree of this potentiation of the neuronal response induced by NMDA was dose-dependent and generated a bell-shape dose-response curve (Fig. 1A). The dose response curve of Lu 28-179 was fitting the table curve equation: y = a + b exp {-exp(-(x -c)/d)} -{(x -c) /d) + 1} [Extr.Val] and the r-value was of 0.97. The potentiation of the NMDA response increased dose-dependently with low doses to reach an approximate maximum peak with a dose of 500 µg/kg, i.v. which induced a 75% increase of the NMDA response (Fig. 1B) then decreased and disappeared with i.v. doses higher than 2000 µg/kg. Lu 28-179 did not induce any epileptoid activity at any of the doses tested. However, when a dose of 40 µg/kg, i.v. of the σ_1 ligand JO-1784 was administered following the administration of Lu 28-179, an epileptoid activity was observed upon applications of NMDA (Fig. 2A) (Tab. 1). This epileptoid activity was not observed when a dose of (+)-pentazocine (100 µg/kg,i.v.) (Tab. 1) was administered following the injection of Lu 28-179 but (+)-pentazocine further increased slightly but not significantly the potentiation of the NMDA response(Fig. 2B). Administered at doses of 1000 µg/kg,i.v., haloperidol did not reverse the potentiation of the NMDA response induced by Lu 28-179 nor did it prevent the effect of Lu 28-179 on the NMDA response (Fig. 3A). Conversely, haloperidol administered after Lu 28-179

increased slightly the response to NMDA but this increase did not reach any statistical significance. The potentiation of the NMDA response induced by 500 µg/kg, i.v. of Lu 28-179 was neither reversed by 50 µg/kg, i.v. of progesterone, a neuroactive steroid with a high affinity for σ receptors (Fig. 3B) nor by 200 µg/kg, i.v. of the σ_1 antagonist NE-100 (Fig. 3C). Higher doses of progesterone (up to 1000 µg/kg, i.v.) or NE-100 (up to 1000 µg/kg, i.v.) also failed to suppress the potentiation induced by Lu 28-179 (data not shown). As illustrated for BMY-7378 in Fig. 3D, the $5HT_{2A}$ antagonist nefazodone and the $5HT_{1A}$ and α_1 antagonist BMY-7378 did not modify the potentiation of the NMDA response nor did they prevent the potentiation of the NMDA response induced bu Lu 28-179.

Effect of the acute intravenous administration of BD 1008 on the firing activity of CA_3 pyramidal neurons of the dorsal hippocampus

BD 1008 administered at doses of 1, 10, 25, 50, 100, 200, 250 and 500 µg/kg potentiated dose-dependently the NMDA response between the doses of 25-250 µg/kg, i.v, the maximal approximate peak was obtained with a dose of 100 µg/kg, i.v. and disappeared with a i.v. dose of 500 µg/kg. Interestingly, this ligand generated a bell-shape dose-response curve for which the best fit equation was the same as for Lu 28-179 with a r value of 0.917. This ligand proved to be non selective as it also potentiated the QUIS response with doses between 10-250 µg/kg, i.v. and generated also a bell-shape dose-response curve fitting the same equation as mention above and with a r value of 0.948 (Fig. 4B). Similarly to what was observed with Lu 28-179, BD 1008 did not induce an epileptoid activity by itself. However, when a dose of 40 µg/kg, i.v. of JO-1784 was administered

subsequently to that of BD 1008, an epileptoid activity was observed during microiontophoretic applications of NMDA (Fig. 4C) (Tab. 2). (+)-Pentazocine (100 µg/kg, i.v.) again failed to induce any epileptoid activity when administered after BD 1008 (Fig. 5B) (Tab. 2). Moreover, the potentiation of the NMDA response induced by BD-1008 was not reversed by low nor by high doses of haloperidol (Fig. 5A). Progesterone (50 µg/kg, i.v) (Fig. 5C) and NE-100 (200 µg/kg,i.v.) (Fig. 5D) also failed to reverse the potentiation induced by BD 1008.

Effect of the acute intravenous administration of ibogaine on firing activity of CA_3 pyramidal neurons of the dorsal hippocampus

The effects of ibogaine (200 µg/kg, i.v) were assessed in four experimental series where its administration was followed by that of haloperidol, progesterone, NE-100 and JO-1784. Ibogaine potentiated only slightly the NMDA response (Fig. 6A), and its effect was only significant in two series or when all the data were pooled together. Interestingly, this slight increase of the NMDA response was reversed by 50 µg/kg, i.v. of the neurosteroid progesterone (Fig. 6C) but not by 100 µg/kg, i.v. of haloperidol (Fig. 6B) nor by 200 µg/kg, i.v. of NE 100 (Fig. 6D). Ibogaine administered at higher or lower doses (50, 100, 250, 500 and 1000 µg/kg) did not potentiate significantly the NMDA response and therefore a dose-response curve could not be obtained. Moreover, the intravenous adminstration of 200 µg/kg, i.v. of ibogaine did not supress the potentiation of the NMDA response induced by Lu 28-179 (500 µg/kg, i.v.) (Fig. 7A) or of JO-1784 (40 µg/kg) (Fig. 7B). When, JO-1784 (40 µg/kg) was administered, following a potentiation of the NMDA response by a prior adminstration of ibogaine, no further significant potentiation of the NMDA response could

be obtained (Fig. 7C).

DISCUSSION

Numerous studies have been conducted with σ_1 ligands but, relatively few data are available concerning the role of σ_2 receptors. Walker and colleagues (1993) pointed out the possibility that σ_2 might be involved in motor function, as suggested by their finding that the non selective σ_1/σ_2 DTG, injected locally in the rat red nucleus, induced torticollis (Walker et al., 1993). In behavorial studies, BD 1047 and BD 1063, two high affinity σ_2 ligands had no effects on their own when unilaterally microinjected into the red nucleus of rats, but both compounds attenuated the dystonia produced by DTG and haloperidol (Matsumoto et al., 1995). Moreover, in rats, a potent anxiolytic activity in the black\white box exploration was found with Lu 28-179 (Perregaard et al., 1995).

The data obtained in the present studies suggest that the σ_2 ligands Lu 28-179 and BD 1008 administered intravenously potentiate dose-dependently the neuronal response to NMDA in the CA_3 region of the rat dorsal hippocampus. The degree of potentiation of the NMDA response increased dose-dependently with low doses, then decreased and disappeared at higher doses. This type of bell shape dose-response curve had already been observed previously in the same electrophysiological model with several high-affinity σ_1 ligands including (+)-pentazocine, L 687,384, sertraline, clorgyline and JO-1784 (Bergeron et al., 1993; Bergeron et al., 1995) as well as in other experimental models. For exemple, Nabeshima's group showed that doses of DTG or (+)-pentazocine between 1 and 1000 µg/kg had no behavioral effect in naive rats, but dose-dependently reversed the effect of MK-801. The same type of bell-shaped dose-response curves was obtained with maximal effects at the dose of 100 µg/kg of DTG and of (+)-pentazocine (Maurice et al., 1994).

In our model, BD 1008 also potentiated the QUIS response. Such a potentiation of the QUIS response has already been observed with the σ_1 ligand BD 737 whereas an inhibition of QUIS-induced response has already been observed with NPY-COOH (Monnet et al., 1992). Morever, the potentiation of the QUIS response is not general to σ_2 ligands since Lu 28-179 failed to potentiate the quisqualate response. Thus, this effect of BD 1008 appears to be a non specific effect.

Despite a very high affinity for σ_2 receptors, the doses of the σ_2 ligands for Lu 28-179 and BD 1008 required to induce a potentiation of the NMDA response were generally 5 to 10 times higher than those required with the σ_1 ligands and the degree of potentiation was less impressive than that observed with σ_1 ligands. The fact that Lu 28-179 and BD 1008 have a lower potency could be attributed to an effect of these ligands on σ_1 receptors. However, this is unlikely since Lu 28-179 has a much lower affinity for the σ_1 receptor compared to that of BD 1008 and the degree of potentiation of the NMDA response induced by the same dose of these two ligands is nearly identical. Finally, the lack of effect of the $5HT_{1A}$ and α_1 antagonist BMY 7378, of the $5HT_{2a}$ antagonist nefazodone and of the σ_1 antagonist NE-100 on the potentiation of the NMDA response induced by Lu 28-179 further suggest that this potentiation is not mediated via the σ_1, $5HT_{1a}$, $5HT_{2a}$ or α_1 receptors, but rather by the σ_2 receptors. It has been shown that DTG, a non selective σ_1/σ_2 ligand, induces an epileptoid activity upon microiontophoretic applications of NMDA when administered intravenously at doses ranging from 3 to 40 µg/kg (Bergeron et al., 1995). In the present series, BD 1008 and Lu 28-179 did not induce any epileptoid activity by themselves. However, following the subsequent administration of the σ_1 ligand JO-1784 but not following the subsequent administration of the σ_1

ligand (+)-pentazocine, an epileptoid activity was observed. These results suggest that the coactivation of both σ_1 and σ_2 receptors could be required to induce an epileptoid activity. BD 1008 has a high affinity for σ_2 receptors and a high affinity for σ_1 receptors as shown by its capacity to displace [^{3}H](+)-pentazocine. Therefore, one might have expected BD 1008 to induce an epileptoid activity of its own. However, previous studies have demonstrated the existence of at least two types of σ_1 receptors. One on which JO-1784 would act and a second one on which (+)-pentazocine is acting (Debonnel et al., 1996; Monnet et al., 1994). Since, in the present series of experiments, the addition of (+)-pentazocine to Lu 28-179 and BD 1008 was unable to induce any epileptoid activity, it is plausible that only σ_1 ligands acting on the subtype for which JO-1784 has high affinity would generate an epileptoid activity in the presence of σ_2 agonists.

Haloperidol is known as having a high affinity for both σ_1 and σ_2 receptors and is generally presented as a σ_1/σ_2 antagonist in almost all models. It prevents and reverses the potentiations induced by (+)-pentazocine, DTG, JO-1784 and DHEA in our model (Bergeron et al., 1996). However, in the present experimental series, haloperidol did not suppress the effects induced by BD 1008 or by Lu 28-179; it even enhanced non-significantly the NMDA response. These data suggest that haloperidol is not a σ_2 antagonist and that it may even act as weak σ_2 agonist.

The neurosteroid progesterone also failed to suppress the effect of Lu 28-179 and of BD 1008. The first indication of the interaction of neurosteroids with σ receptors was provided by Su et al in 1988 who showed that progesterone competitively displace [^{3}H] SKF 10,047 and [^{3}H]-

haloperidol in rat CNS membranes (Su et al., 1988). This observation was latter confirmed in the CNS (Yamada et al., 1994; McCann et al., 1994) and in placental membranes (Ramamoorthy et al., 1995). It has been shown previously that the neuroactive steroid DHEA also potentiate selectively the NMDA response and that the potentiation is suppressed by progesterone, haloperidol and NE-100 (Bergeron et al., 1996). Progesterone also suppresses the potentiations of the NMDA response induced by non steroid σ ligands such as (+)-pentazocine and DTG (Bergeron et al., 1996). The fact that progesterone reverses the effects of the non selective σ_1/σ_2 DTG but not those of Lu 28-179 could be explained by its higher affinity for the σ_1 receptor since progesterone is known to be also a potent σ_1 antagonist. However, BD 1008 has also high affinity for the σ_1 receptor and its effects are not reversed by progesterone. This constitute another argument suggesting that the effect of BD 1008 was mediated via σ_2 receptors on which progesterone is inactive.

The σ ligand ibogaine increased slightly but significantly the NMDA response at the dose of 200 μg/kg but no dose-response curve could be obatined. This ligand did not act either as a σ_2 antagonist in our model since it failed to reverse the potentiation of the NMDA response induced by Lu-28-179, or as a σ_1 antagonist since it did not reverse the effect induced by JO-1784. The lack of significant effect of JO-1784 when administered after ibogaine , should not be interpreted as a suggestion that ibogaine is acting as a σ_1 antagonist but rather as an indication that a plateau effect was reached. Thus, this ligand had only a small agonistic effect in our electrophysiological model, which could suggest it might be acting as a partial σ agonist.

In conclusion, the effects on the NMDA response induced by BD 1008 and Lu 28-179 are not suppressed by haloperidol nor progesterone while those induced by ibogaine are suppressed by progesterone. The most likely explanation for these results is that several subtypes of σ_2 receptors may exist as it has already been demonstrated for σ_1 subtypes of receptors. BD 1008 and Lu 28-179 could be acting on one subtype of σ_2 receptor while ibogaine would act on another subtype. Further studies with others σ_2 ligands would be necessary to fully elucidate the role of haloperidol and progesterone on σ_2 receptors.

ACKNOWLEDGEMENTS

The author wish to thank Claude Bouchard, Normand Lavoie and Yves Simard for computer programming and for illustrations, and Martine Le Dû for her secretarial assistance. This work was supported by the Fonds de la Recherche en Santé du Québec (FRSQ) and by the Royal Victoria Hospital Research Institute. G.D. is a receipt of a scholarship from the F.R.S.Q. and S.C. is a fellow from the Royal Victoria Hospital Research Institute.

LEGENDS TO FIGURES

Figure 1

(A) Dose-response curve of the effect of intravenous administrations of Lu 28-179 on the neuronal activation of CA_3 dorsal hippocampus pyramidal neurons induced by microiontophoretic applications of NMDA. Each dot represents the effect of one dose of the drug administered to one rat while recording for one neuron. The effect was assessed by determining the ratio (N_1/N_2) of the number of spikes generated/nC of NMDA before (N_1) and after (N_2) the injection of the drug.

(B) Integrated firing rate histogram of a CA_3 dorsal hippocampus pyramidal neuron illustrating the effects of microiontophoretic applications of NMDA and QUIS before and after the intravenous administration of Lu 28-179, and after the subsequent administration of haloperidol. In this and the subsequent continuous integrated firing histograms, bars indicate the duration of applications for which currents are given in nA. Open circles (oo) represent an interruption of the illustration of the continuous recording.

Figure 2

(A) Integrated firing rate histogram of a CA_3 dorsal hippocampus pyramidal neuron illustrating the effects of microiontophoretic applications of NMDA and QUIS before and after the injection of Lu 28-179 and after the subsequent intravenous administration of JO-1284. Dotted picks represent epileptoid activity, in this and the subsequent figures.

(B) Responsiveness, expressed as the number of spikes generated/nc (mean ± SEM), of CA_3 dorsal hippocampus neurons to microiontophoretic applications of NMDA before (open column), after (grey column) the administration of Lu 28-179 and after the subsequent administration of Lu 28-179 and (+)-pentazocine (dark grey colum). *p<0.05, using 1D RMANOVA.

Figure 3

(A) Responsiveness, expressed as the number of spikes generated/nc (mean ± SEM), of CA_3 dorsal hippocampus neurons to microiontophoretic applications of NMDA before (open columns), after (hatched columns) the administration of haloperidol, after the subsequent administration of Lu 28-179 (grey column) and after the subsequent administration of Lu 28-179 and haloperidol (grey hatched column). *p<0.05, using 1D RMANOVA.

(B. C. D) Responsiveness of CA_3 dorsal hippocampus neurons to microiontophoretic applications of NMDA before (open columns) and after (grey column) the administration of Lu 28-179 and after the subsequent administration (grey hatched column) of progesterone (B) NE-100 (C) BMY 7378 (D). *p<0.05, using 1D RMANOVA.

Figure 4

(A) Dose-response curve of the effect of the intravenous administration of BD-1008 on the neuronal activation of CA_3 dorsal hippocampus pyramidal neurons induced by microiontophoretic applications of NMDA (A)QUIS (B).

(C) Integrated firing rate histogram of a CA_3 dorsal hippocampus pyramidal neuron illustrating the effects of microiontophoretic applications of NMDA and QUIS before and after the injection of BD 1008 and after the subsequent intravenous administration of JO-1784.

Figure 5

(A) Responsiveness of CA_3 dorsal hippocampus neurons to microiontophoretic applications of NMDA before (open column), after (hatched column) the administration of haloperidol, after the subsequent administration of BD 1008 and haloperidol (grey hatched column). *$p<0.05$, using 1D RMANOVA.

(B. C. D) Responsiveness of CA_3 dorsal hippocampus neurons to microiontophoretic applications of NMDA before (open column) and after (grey column) the administration of BD-1008 and after the subsequent administration (grey hatched column) of (+)-pentazocine (B) progesterone (C) NE-100 (D). *$p<0.05$, using 1D RMANOVA.

Figure 6

(A) Integrated firing rate histogram of a CA_3 dorsal hippocampus pyramidal neuron illustrating the effects of microiontophoretic applications of NMDA and QUIS before and after the injection of ibogaine and after the subsequent intravenous administration of haloperidol.

(B. C. D) Responsiveness of CA_3 dorsal hippocampus neurons to microiontophoretic applications of

NMDA before (open columns) and after (grey column) the administration of ibogaine and after the subsequent administration (grey hatched column) of haloperidol (B) progesterone (C) and NE-100 (D). *p<0.05, using 1D RMANOVA.

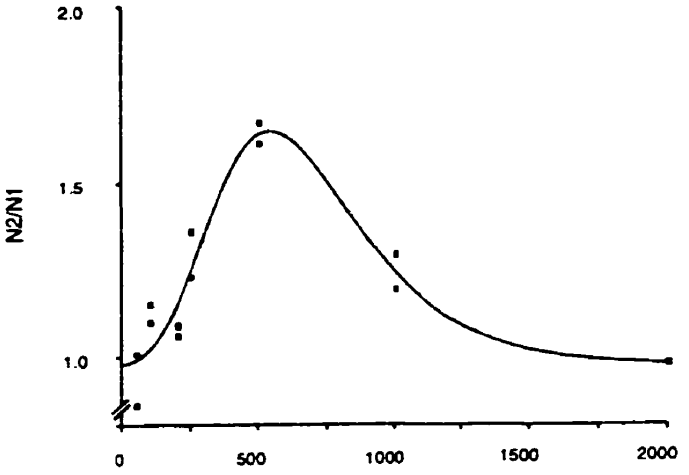
Figure 7

(A) Responsiveness of CA_3 dorsal hippocampus neurons to microiontophoretic applications to NMDA before (open column) and after (grey column) the administration of Lu 29-179 and after the subsequent administration of ibogaine (grey hatched column). *p<0.05, using 1D RMANOVA.

(B) Responsiveness of CA_3 dorsal hippocampus neurons to microiontophoretic applications to NMDA before (open column) and after (grey column) the administration of ibogaine and after the subsequent administration of JO1784 (grey hatched column). *p<0.05, using 1D RMANOVA.

(C) Responsiveness of CA_3 dorsal hippocampus neurons to microiontophoretic applications to NMDA before (open column) and after (grey column) the administration of JO-1784 and after the subsequent administration of ibogaine (dark grey column). *p< 0.05, using 1D RMANOVA.

A.


2.0
1.5
1.0
N2/N1
0
500
1000
1500
2000
Dose of Lu 28-179 (µg/kg, i.v.)

B.

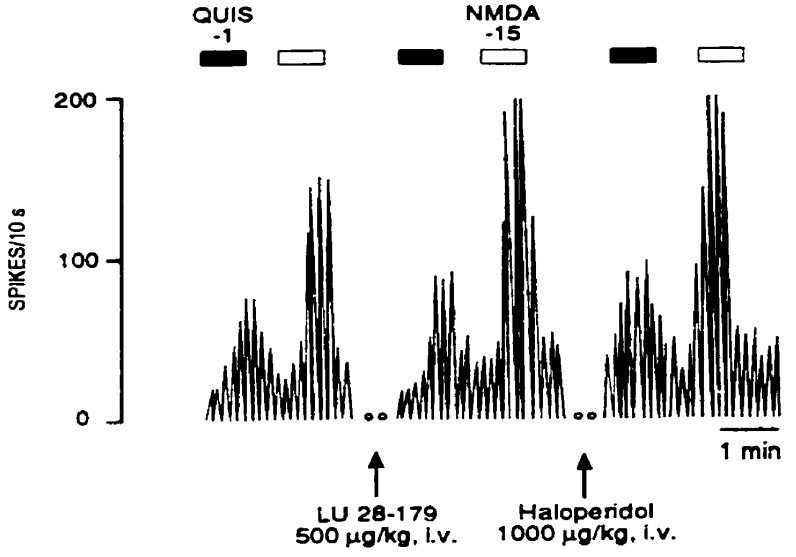
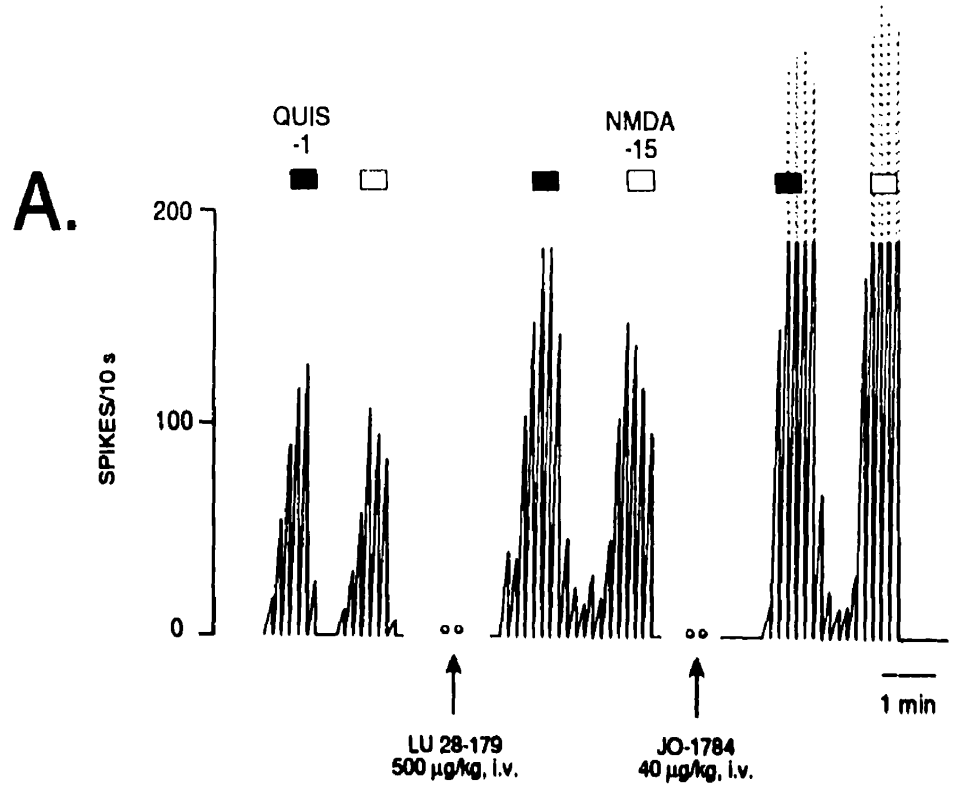

QUIS
-1
NMDA
-15
200
100
0
SPIKES/10 s
1 min
LU 28-179
500 µg/kg, i.v.
Haloperidol
1000 µg/kg, i.v.

Fig. 1


A.
QUIS
-1
NMDA
-15
200
100
0
SPIKES/10 s
1 min
LU 28-179
500 µg/kg, i.v.
JO-1784
40 µg/kg, i.v.

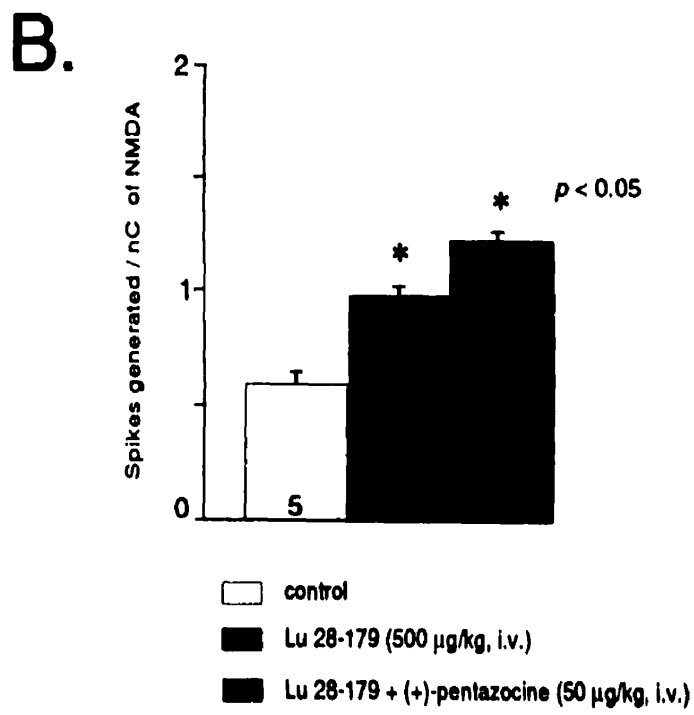

B.
2
1
0
Spikes generated / nC of NMDA
p < 0.05
*
*
5
control
Lu 28-179 (500 µg/kg, i.v.)
Lu 28-179 + (+)-pentazocine (50 µg/kg, i.v.)

Fig. 2

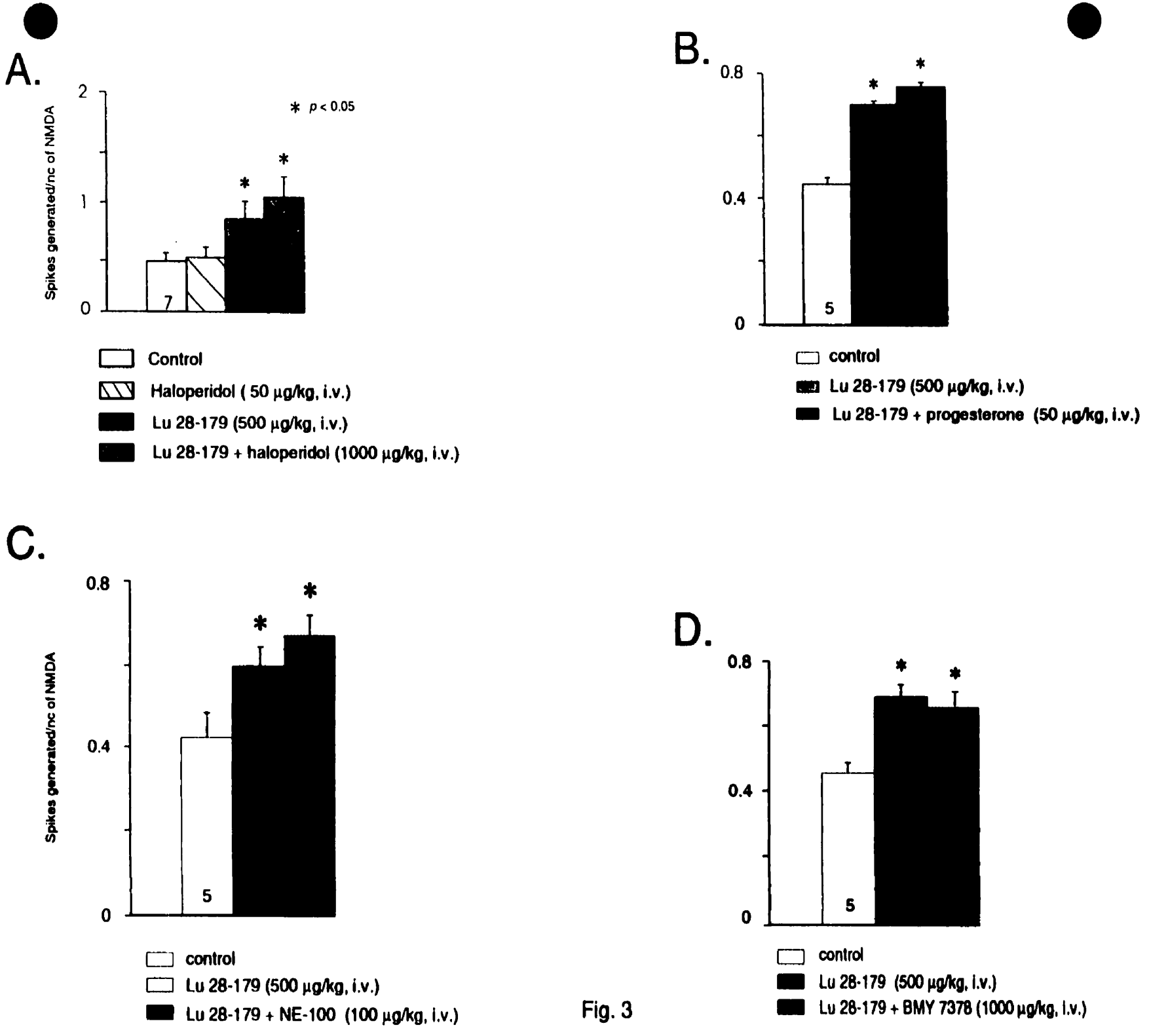
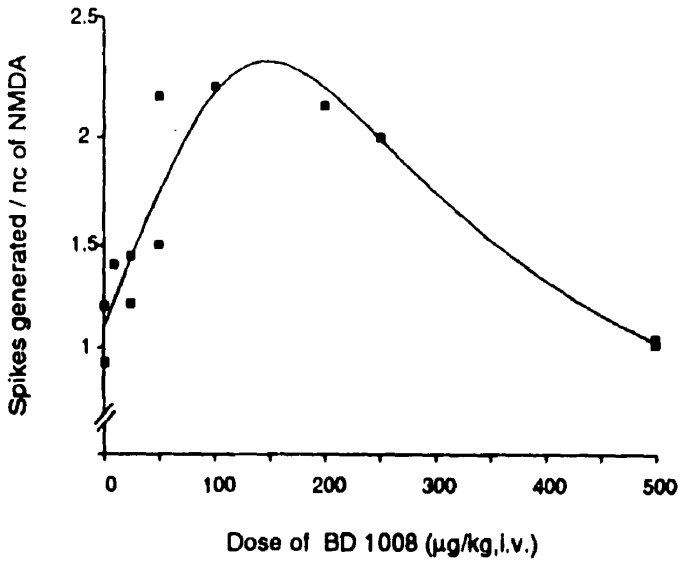
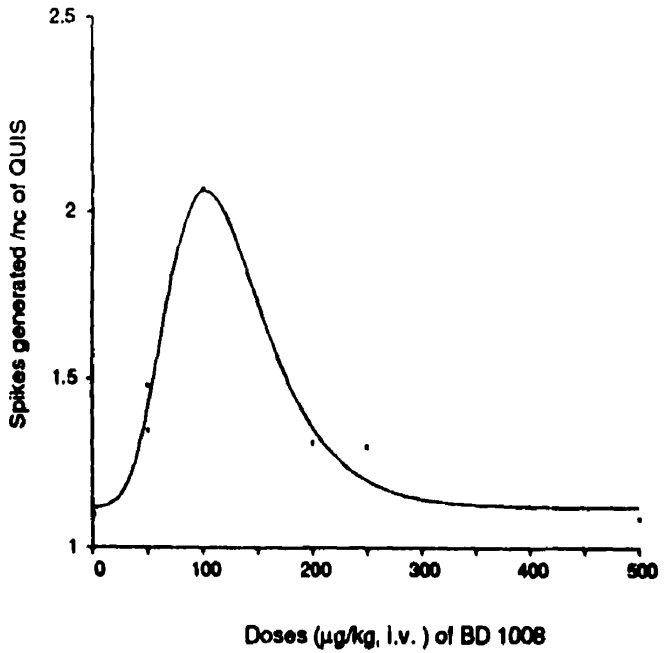

A.
Spikes generated/nc of NMDA
2
1
0
* p < 0.05
*
*
7
Control
Haloperidol (50 µg/kg, i.v.)
Lu 28-179 (500 µg/kg, i.v.)
Lu 28-179 + haloperidol (1000 µg/kg, i.v.)
B.
0.8
0.4
0
*
*
5
control
Lu 28-179 (500 µg/kg, i.v.)
Lu 28-179 + progesterone (50 µg/kg, i.v.)
C.
Spikes generated/nc of NMDA
0.8
0.4
0
*
*
5
control
Lu 28-179 (500 µg/kg, i.v.)
Lu 28-179 + NE-100 (100 µg/kg, i.v.)
D.
0.8
0.4
0
*
*
5
control
Lu 28-179 (500 µg/kg, i.v.)
Lu 28-179 + BMY 7378 (1000 µg/kg, i.v.)

Fig. 3

A.


Spikes generated / nc of NMDA
2.5
2
1.5
1
0
100
200
300
400
500
Dose of BD 1008 (µg/kg,i.v.)

B.


Spikes generated /nc of QUIS
2.5
2
1.5
1
0
100
200
300
400
500
Doses (µg/kg, i.v.) of BD 1008

C.

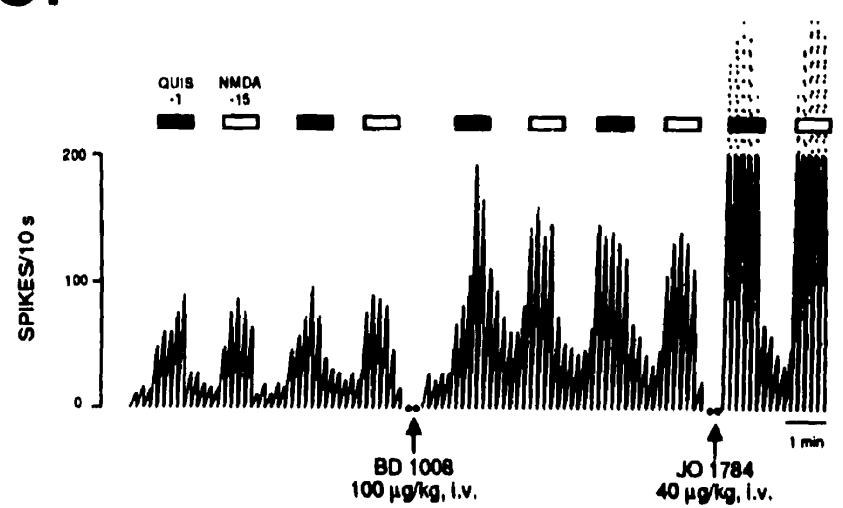

QUIS
-1
NMDA
-15
SPIKES/10 s
200
100
0
1 min
BD 1008
100 µg/kg, i.v.
JO 1784
40 µg/kg, i.v.

Fig. 4

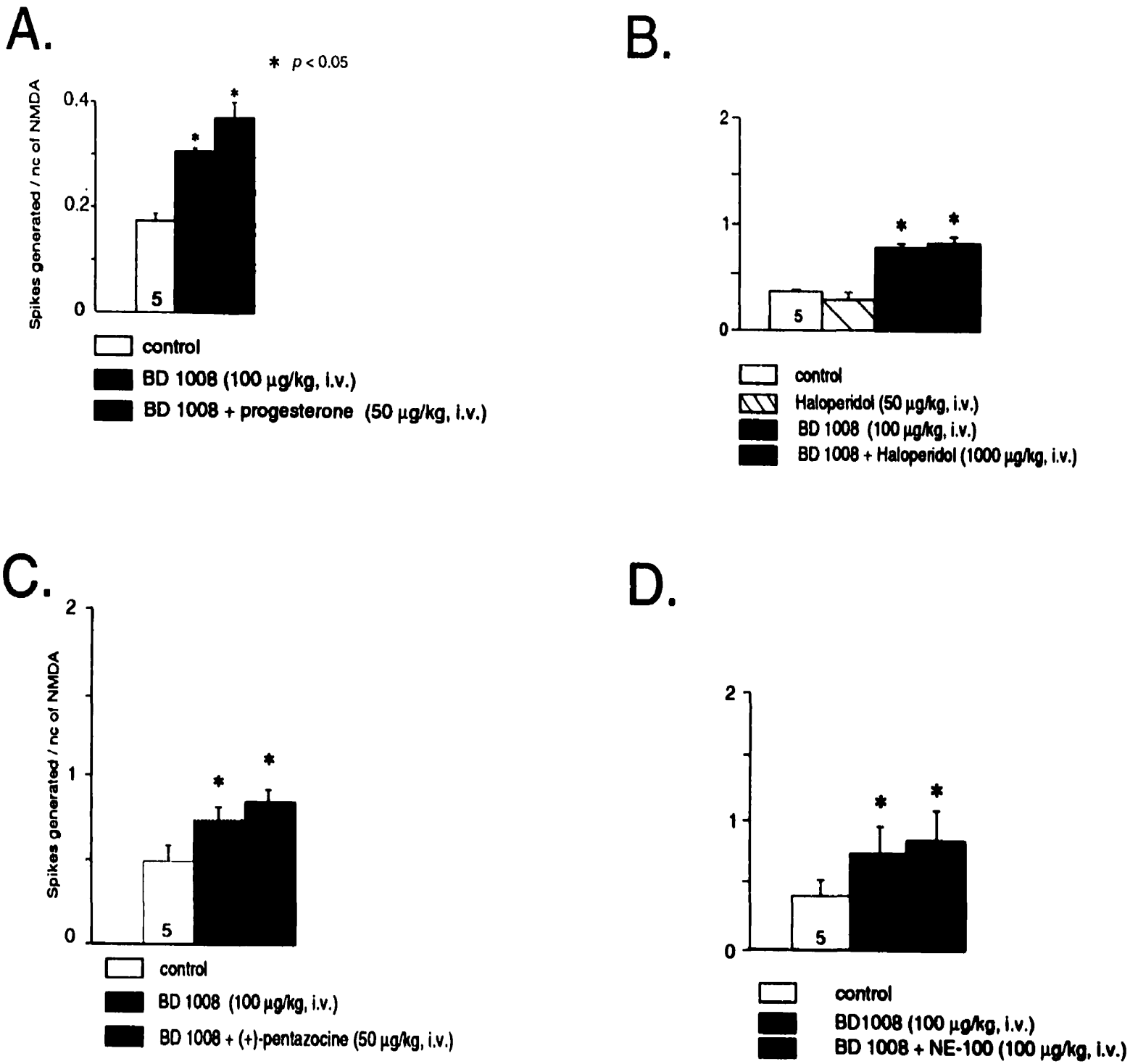
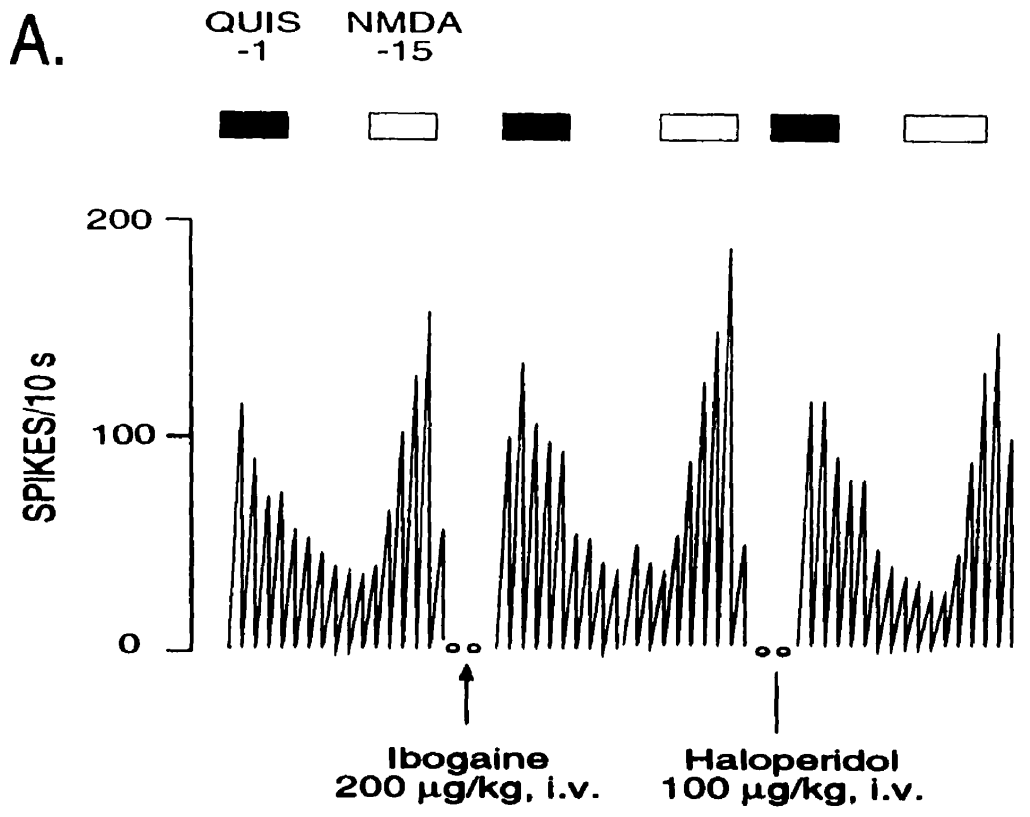

A.
* p < 0.05
Spikes generated / nc of NMDA
0.4
0.2
0
5
control
BD 1008 (100 µg/kg, i.v.)
BD 1008 + progesterone (50 µg/kg, i.v.)
B.
2
1
0
5
control
Haloperidol (50 µg/kg, i.v.)
BD 1008 (100 µg/kg, i.v.)
BD 1008 + Haloperidol (1000 µg/kg, i.v.)
C.
Spikes generated / nc of NMDA
2
1
0
5
control
BD 1008 (100 µg/kg, i.v.)
BD 1008 + (+)-pentazocine (50 µg/kg, i.v.)
D.
2
1
0
5
control
BD1008 (100 µg/kg, i.v.)
BD 1008 + NE-100 (100 µg/kg, i.v.)

Fig. 5


A.
QUIS
-1
NMDA
-15
200
100
0
SPIKES/10 s
Ibogaine
200 µg/kg, i.v.
Haloperidol
100 µg/kg, i.v.

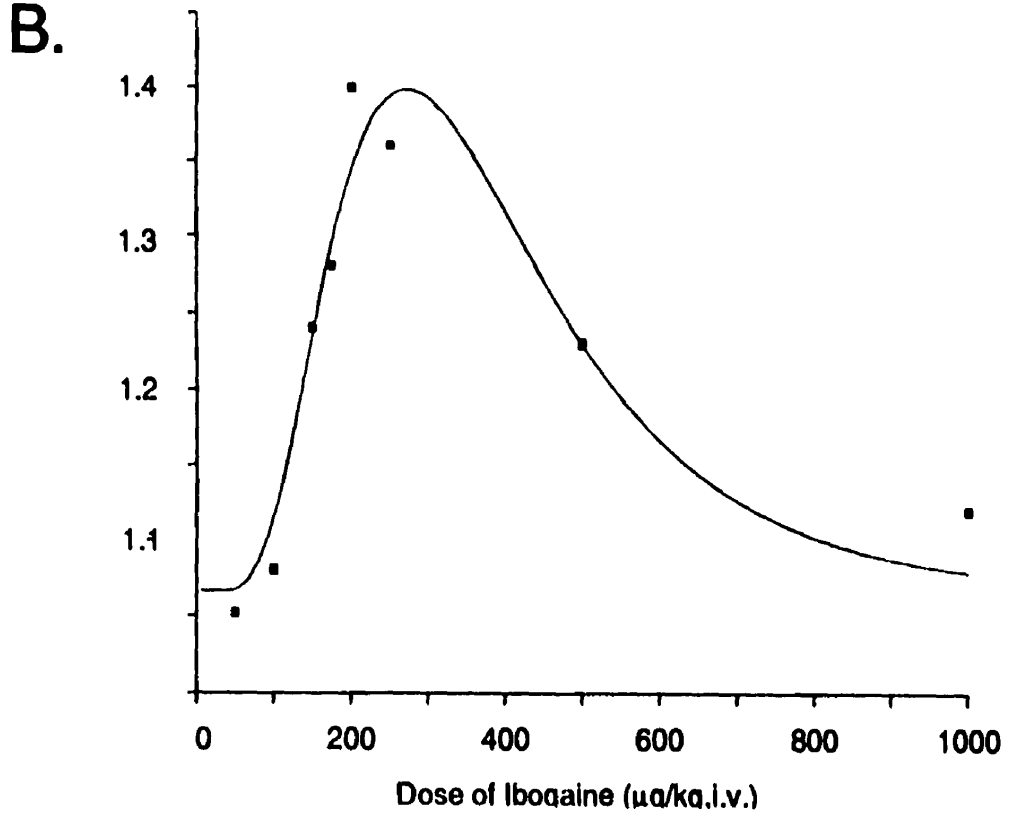
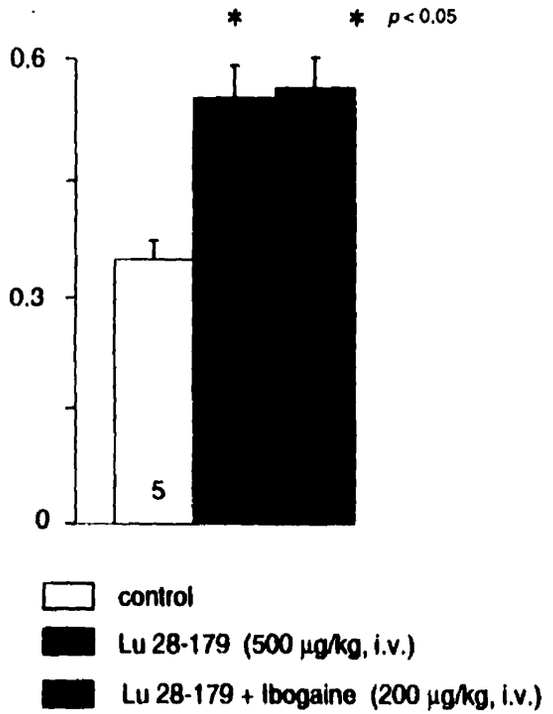

B.
1.4
1.3
1.2
1.1
0
200
400
600
800
1000
Dose of Ibogaine (µg/kg,i.v.)

Fig. 6

A.


* * p < 0.05
0.6
0.3
0
5
control
Lu 28-179 (500 µg/kg, i.v.)
Lu 28-179 + Ibogaine (200 µg/kg, i.v.)

B.

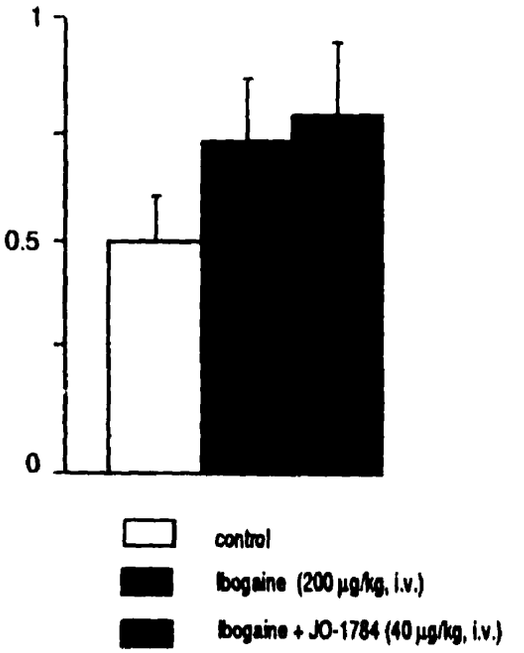

1
0.5
0
control
Ibogaine (200 µg/kg, i.v.)
Ibogaine + JO-1784 (40 µg/kg, i.v.)

Fig. 7

LEGENDS TO TABLES

Table 1

(A, B) Number of neurons inducing an epileptoid activity or not inducing an epileptoid activity following the intravenous administration of Lu 28-179 and after the subsequent administration of Lu 28-179 + JO-1784 (A) and of Lu 28-179 + (+)-pentazocine (B). *$p<0.05$, using Fisher Exact test

Table 2

(A, B) Number of neurons inducing an epileptoid activity or not inducing an epileptoid activity following the intravenous administration of BD 1008 and after the subsequent administration of BD 1008 + JO-1784 (A) and of BD 1008 + (+)-pentazocine (B). *$p<0.05$, using Fisher Exact test

TABLE 1

Number of neurons generating an epileptoid activity after the administration of σ ligands

Number of neurons		
Following the administration of	No epileptoid activity	Epileptoid activity
(A) Lu 28-179 (500 µg/kg)	13	0
(A) Lu 28-179 + JO-1784 (40 µg/kg)	4*	9*
(B) Lu 28-179 (500 µg/kg)	7	0
(B) Lu 28-179 + (+)-pentazocine (100 µg/kg)	6	1

*$p<0.05$, Fisher Exact test

TABLE 2

Number of neurons generating an epileptoid activity after the administration of σ ligands

	Number of neurons	
Following the administration of	No epileptoid activity	Epileptoid activity
(A) BD 1008 (100 µg/kg)	9	1
(A) BD 1008 + JO-1784 (40 µg/kg)	4*	6*
(B) BD 1008 (100 µg/kg)	5	1
(B) BD 1008 + (+)-pentazocine (100 µg/kg)	4	2

*$p<0.05$, Fisher Exact test

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CHAPTER III

Suppression of (+)-pentazocine effects by naloxone

The σ_1 receptor was purified as a 30 kDa protein. Meanwhile, Su and colleagues, trying to clone this receptor using affinity chromatography purified a protein from rat liver and rat brain that appeared to resemble the σ opioid receptor proposed by Martin in 1976. In addition, it was observed that (+)-pentazocine, (±)-cyclazocine and naloxone had high affinity for this purified protein. This created some confusion since that σ receptors had been believed, since 1983, to be distinct from the opiate family. Electrophysiological studies were performed to assess the effects of naloxone and (±)-cyclazocine on the potentiation of the NMDA response induced by σ ligands already tested in our model.

SUPPRESSION OF NALOXONE BY (+)-PENTAZOCINE

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Submitted to: Synapse

Date: July 6, 1998

ABSTRACT

Recently, in an attempt to purify the non opioid σ receptor, Su and colleagues identified a protein from rat liver and brain which appeared to resemble the σ opioid receptor as proposed by Martin in 1976, and for which the non-opiate σ_1 ligand (+)-pentazocine presents a high affinity. Previous electrophysiological studies from our laboratory have demonstrated that several selective σ_1 ligands potentiate the neuronal response to NMDA. The goal of the present electrophysiological studies was to assess if the effects of these selective σ_1 ligands on the potentiation of the NMDA response was mediated by the naloxone-sensitive σ receptor.

Extracellular unitary recordings were obtained from pyramidal neurons of the CA_3 region of the rat dorsal hippocampus. The σ_1 ligands BD 737, L, 687-384, JO-1784 and (+)-pentazocine administered intravenously at low doses, potentiated selectively the NMDA response. The opiate antagonist naloxone failed to reverse the potentiation induced by BD 737, L,687-384 and JO-1784, however, it did suppress the potentiation of the NMDA response induced by the σ_1 ligand (+)-pentazocine. The potentiation of the NMDA response was not suppressed by the μ, κ nor by the δ antagonists.

(±) Cyclazocine, another σ ligand with a high affinity for the above mentioned protein purified by Su and colleagues acted as an antagonist by suppressing the potentiation of the NMDA response induced by both JO-1784 and (+)-pentazocine. These results suggest that the effects induced by some ligands may be in fact sensitive to naloxone while others are not, which constitute another argument in favour of the heterogeneity of σ receptors. *KEY WORDS: opiate, NMDA, hippocampus, sigma*

INTRODUCTION

Sigma (σ) receptors were discovered in 1976 by Martin and colleagues who first classified them as opiate receptors. Three subtypes of opiate receptors were described based on their protypal agonists: μ for morphine, κ for ketocyclazocine and σ for SKF-10047. The racemic ligand SKF-10047 binds both to a low and a high affinity site which represent, the PCP and the sigma sites, respectively. These σ receptors were initially believed to mediate the psychomimetic effects produced by some opiates but it was soon shown that the opiate antagonist naloxone failed to reverse these psychomimetic effects, and it was thus concluded that σ receptors do not belong to the opiate family (Su, 1993). Sigma receptors are now recognized as non opiate receptors (Quirion et al., 1987, 1992). Recently, the σ_1 receptor was purified as a 30-kDa protein in guinea pigs with only one transmembrane segment. The deduced amino sequence shares homology with fungal proteins implicated in sterol synthesis and does not resembles any type of known receptor (Hanner and al, 1996). In humans and rats, the σ_1 binding site was purified as a 223 amino acid protein with one transmembrane domain (Kekuda et al., 1996; Seth et al., 1998). Meanwhile, the group of Su was also attempting to purify this receptor. Using high affinity chromatography, they isolated a protein from rat liver and rat brain (Tsao and Su, 1996). This protein appears to resemble the original σ opioid receptor as proposed by Martin in 1976, since not only (+)-pentazocine, but also naloxone and (±)cyclazocine bind it with a high affinity.

Different classifications of σ receptors have been proposed but it is generally accepted that at least 2 different types of non-opioid σ receptors denoted σ_1 and σ_2 exist (Quirion, 1992).

Moreover, it has been shown in our laboratory that within one category of σ receptors, several subtypes exist (Debonnel et al., 1992; Monnet et al., 1994; Couture and Debonnel, 1997). Previous studies have demonstrated that low doses of several σ ligands, including (+)-pentazocine, BD 737, L-687,384, JO-1784 potentiate the neuronal response to *N*-methyl-*D*-aspartate (NMDA) in the CA_3 region of the rat dorsal hippocampus but do not modify kainate-(KA) nor quisqualate-(QUIS) induced activations (Monnet et al., 1990; Bergeron et al, 1993; Bergeron et al., 1994).

The purpose of the present electrophysiological studies, carried out *in vivo*, in the CA_3 region of the rat dorsal hippocampus, was to assess if the potentiation of the NMDA response induced by these σ_1 ligands could be altered by the opiate antagonist naloxone.

MATERIALS AND METHODS

Male Sprague-Dawley rats weighing 225-250 g were obtained from Charles Rivers (Saint-Constant, Québec, Canada). They were maintained at constant temperature (25°C) under a 12 h-12 h light-dark cycle with free access to food and water, for two to three days before the electrophysiological experiments.

In vivo electrophysiological experiments

Animals were anesthetized with urethane (1.25 g/kg, i.p.) and mounted in a stereotaxic apparatus. Body temperature was maintained at 37° C throughout the experiment and a catheter was installed, prior to recording, in a lateral tail vein for the intravenous administration of drugs.

Extracellular unitary recordings of CA_3 dorsal hippocampus pyramidal neurons

Five-barrelled glass micropipettes were pulled in a conventional manner and their tips were broken to a diameter of 8-12 µm under microscopic control (Haigler and Aghajanian, 1974). The central barrel, used for extracellular recordings, was filled with a 2M NaCl solution containing fast green FCF. Two side barrels, used for microiontophoresis were filled with QUIS (1.5 mM in 400 mM NaCl, pH 8) and with NMDA (5 mM in 200 mM NaCl, pH 8). A third barrel was used for automatic current balancing and contained a 2M NaCl solution. The extracellular recordings were obtained in the CA_3 region at a depth of 3.5 to 4.5 mm below the cortical surface in a area defined stereotaxically as 4.2 ± 0.2 mm anterior to lambda and 4.2 ± 0.2 mm lateral to midline (Paxinos and Watson, 1986). Pyramidal neurons of the CA_3 region were identified by their characteristic large-amplitude (0.5-

1.2mV) and long-duration (0.8-1.2 ms) single action potentials, alternating with complex spike discharges (Kandel and Spencer, 1961). The currents used for ejecting the NMDA ranged from -8 to -15 nA and from 2 to -3 for QUIS. The duration of the microiontophoretic ejections was kept constant at 50 seconds. The σ ligands were administered intravenously. Only one dose of each σ ligand was administered to one rat while recording from one neuron.

Calculations

The computer calculated the effect of each microiontophoretic application of NMDA and QUIS as the total number of spikes/nanocoulomb (nC). The effect of the intravenous administration of the σ ligands was measured by averaging the number of spikes generated by nanocoulomb of three successive applications of each excitatory substance tested for a 50 seconds period. The maximal effect generally occurred within 10 minutes following the intravenous administration of the σ ligand. The potential "antagonistic" effect of naloxone and (±)cyclazocine was assessed by comparing the number of spikes generated/nC of each excitatory substance following the administration of a σ agonist and following that of naloxone or of (±) cyclazocine.

Drugs

The following substances were used: JO-1784 a gift from J.F. Roman (Institut de Recherche Jouveinal, Fresnes, France); BD-737, a gift from W.D. Bowen (laboratory of Medicinal Chemistry, NIDDK, NIH, USA); L-687,384, a gift from L.L. Iversen (Merck-Sharp and Dome, Tyler park, UK); (±)cyclazocine, a gift from T. P. Su (NIDA/NIH, Baltimore, MD).The following compounds were

purchased: (+)-pentazocine, cyprodime hydrobomide, naltrindole and 3-isothyocyanate 3 (DIPPA) from (RBI, Natick, MA, USA); NMDA from (Sigma St-louis, MO, USA); and QUIS from (Tocris Cookson, St-louis, MO, USA).

Statistical analysis

Results are expressed as means ± S.E.M. The means were compared using a one factor ANOVA with repeated measures followed by Tukey's post-hoc comparison tests. Probability values smaller than 0.05 were considered as significant.

RESULTS

Effect of the acute intravenous administration of sigma agonists on the firing activity of CA_3 pyramidal neurons of the dorsal hippocampus

As previously observed, in 15 rats, the intravenous administration of a dose of 50 µg/kg of the σ_1 ligand (+)-pentazocine, significantly potentiated the NMDA response, which increased by 50% (Fig. 1A). This potentiation was nearly completely reversed by the subsequent administration of naloxone, 200 µg/kg, i.v. (Fig. 1B); higher doses did not induce further suppression (data not shown). The suppression of the potentiating effect of (+)-pentazocine occurred within 3 to 4 minutes after the administration of naloxone, similar to what has previously been seen with σ antagonists such as haloperidol and NE-100. In order to determine which type of opiate receptor was mediating this suppressant effect of naloxone, selective antagonists for the three subtypes were tested. The µ antagonist cyprodime hydrobomide (Chen, 1993), the κ antagonist DIPPA (Chang, 1994) and the δ antagonist naltrindole (Portocheze, 1988) administered at the same dose of 200 µg/kg all failed to reverse the potentiation induced by (+)-pentazocine and nor did they modify the NMDA response (Fig. 2A, 2B, 2C).

The σ_1 ligands JO-1784 (40 µg/kg), L-687,384 (1 µg/kg) and BD 737 (200 µg/kg) also selectively potentiated the NMDA response. However, the potentiation induced by these σ agonists was not modified by naloxone administered at a dose of 200 µg/kg (Fig. 2A, 2B, 3A, 3B). When administered at doses up to 1000 µg/kg, i.v., naloxone still failed to modify the potentiation of the

NMDA response (Fig. 3C).

Effect of the acute intravenous administration of (±) cyclazocine on the firing rate activity of CA_3 pyramidal neurons of the dorsal hippocampus

(±) Cyclazocine, administered at doses up to 1000 μg/kg, i.v., had no effect on the NMDA response. However, when the σ_1 ligands JO-1784 or (+)-pentazocine were administered following the administration of 1000 μg/kg of (±)cyclazocine, no potentiation of the NMDA response was obtained suggesting that (±)cyclazocine was acting as a σ antagonist (Fig. 4A, 4B). Indeed, if (±)cyclazocine was administered during a potentiation of the NMDA response induced by a prior administration of JO-1784 or (+)-pentazocine, it completely suppressed the effects induced by both of these σ_1 ligands. (Fig. 4C, 4D).

DISCUSSION

The data obtained in the present studies confirm that low doses of the σ_1 ligands (+)-pentazocine, BD 737, L-687,384 and JO-1784 administered intravenously, potentiate the neuronal response to NMDA in the CA_3 rat dorsal hippocampus as observed previously in the same electrophysiological model (Monnet et al., 1990; Bergeron et al., 1994). Interestingly, the potentiation of the NMDA response induced by (+)-pentazocine was reversed by the opiate antagonist naloxone but not by the opiate antagonists μ, κ or δ, while the potentiating effects induced by JO-1784, BD 737 and L, 687-384 were not altered.

Sigma receptors were originally classified as opiate receptors. They were believed to account for the psychomimetic effects such as delirium in the dog and manic, delusional and hallucinatory effects in humans induced by the acute or the chronic administration of the σ ligand ($\pm$)SKF-10047 (Martin et al., 1976). The psychomimetic symptoms induced by long term SKF-10047 such as delirium were reversed by the opiate antagonist naloxone in the chronic spinal dog (Martin et al., 1976, 1980). Additionally, Vaupel later found that the psychomimetic symptoms mentioned above, induced by the intravenous, acute administration of ($\pm$)SKF -10047 were in fact insensitive to naloxone in the chronic spinal dog (Vaupel et al., 1983).

Following these results, it was suggested that σ receptors differ from the opiate family for two reasons. First, naloxone failed in *in vivo* models to reverse the effect of these σ ligands and second, the opiate family of receptors has higher affinity for the (-)-enantiomers, while σ receptors has higher

affinity for (+)-enantiomers (Su, 1993). This proposition was finally accepted in 1987 (Quirion et al., 1987).

Recently, Tsao and Su purified a naloxone-sensitive, haloperidol-sensitive [^{3}H](+)SKF-10047-binding protein from rat liver and rat brain membranes. The [^{3}H](+)SKF-10047 binding to the protein was inhibited by the following compounds listed in decreasing order of potency:(+)pentazocine>(-)pentazocine>(±)cyclazocine>(-)morphine>(-)naloxone>haloperidol>(+)SKF-10047>DADLE>(-)SKF-10047. In the present studies, we show that the effects of 50 µg/kg of (+)-pentazocine were consistent with the data of Su and colleagues.

The racemic form of pentazocine was initially used to generate psychomimetic symptoms. Further studies have focussed on discovering which isomer mediates these adverse side effects (Forrest et al, 1969). It was shown that the psychomimetic effects are produced by the l-isomer, although d-pentazocine produces, to a lesser extent, some degree of anxiety (Bellville and Forrest, 1968). In our studies, we used the d-isomer which is known to have poor affinity for the classical opiate receptors unlike (-)pentazocine (Pasternak, 1988; Clark et al. 1989; Su, 1986). (+)-Pentazocine is inactive in the opioid models of analgesia and has always been presented as the prototypal σ_1 ligand (Quirion, 1987). Itzhak (1989) found that the binding of (+)-[^{3}H]pentazocine to the σ site labelled with [^{3}H]-3PPP is coupled to a G protein. However, numerous evidence suggest that (+)-pentazocine is also acting on a σ receptor different than the one on which are acting other σ_1 ligands. For example, following a pretreatment with pertussis toxin, the potentiation of the NMDA response induced by low doses of

the σ_1 ligand JO-1784 is suppressed but not that induced by (+)-pentazocine suggesting that the effect of (+)-pentazocine is not mediated by a receptor linked to a $G_{i/o}$ protein and thus that these two σ_1 ligands act on two different receptors (Monnet et al., 1994). This is in keeping with the data of de Haven Hudkins et al (1992) who have reported that the binding of (+)-[^{3}H]pentazocine is insensitive to GTP and Gpp(NH)p, suggesting that this binding of (+)-pentazocine was on a receptor not linked to a $G_{i/o}$ protein. Finally, saturation binding studies in the presence of ions such as Zn2+, Ca2+ and Mg2+ and studies in Krebs-Ringer buffer have demonstrated that multiple (+)-[^{3}H]pentazocine binding sites exist (Basile et al, 1992). These monovalent cations decreased the k_d for (+)-[^{3}H] pentazocine binding while divalent cations split (+)-[^{3}H]pentazocine into low and high affinity components.

The classification of σ receptors as non-opiate is now widely accepted. However, the controversy as to whether the effects of some σ ligands were reversed by naloxone still persisted. Indeed, studies shown that some effects of certain σ ligands are reversed by naloxone. For example, (+)-pentazocine is known to induce slight respiratory depression in humans (Bellville and Forrest, 1968) and this effect is blocked by naloxone (Martin, 1983). Additionally, in a model of inward potassium currents responses in xenopus oocytes, Kobayashi et al., (1996) found that the currents induced by several σ ligands were reduced by naloxone.

The results obtained in the present studies demonstrate that the effects induced by (+)-pentazocine are reversed by naloxone while the other σ_1 ligands are insensitive to naloxone. Naloxone is a non selective opiate antagonist which is more potent on the μ receptor that for the other opiate

receptors. The fact that naloxone reverse the effects of (+)-pentazocine might not be attributed to the affinity of (+)-pentazocine for the μ receptor because primarily, it has only a very low affinity for this site and secondly, the μ antagonist cyprodime hydromide failed to reverse the potentiation of the NMDA response induced by (+)-pentazocine. The antagonistic effect of naloxone on (+)-pentazocine-induced effects attributed to the affinity of this σ ligand for κ or δ receptor because the κ and δ antagonists failed to reverse the potentiation of (+)-pentazocine. With regards of those results, we can therefore suggest that the sensitivity of (+)-pentazocine to naloxone is not mediated via the μ, κ or δ receptors, but most probably on a different subtype of σ receptor, which is naloxone sensitive but different from the one described by Kobayashi et al (1996) for which μ and δ ligands are potent agonists and antagonists.

(±)Cyclazocine is a benzomorphan derivative which was first synthesized in 1962. This compound has high affinity for σ receptor and low affinity for μ and κ opioid receptor. (For review, see Gavend et al, 1995). It is known that (±)cyclazocine produces moderate dysphoric and psychomimetic side effects (Keats and Telford, 1964) and that the effects are reversed by naloxone (Jasinski et al, 1968). In our model, (±)cyclazocine acted as an antagonist by suppressing the effects induced by both JO-1784 and (+)-pentazocine. It is thus probable that (±)cyclazocine is a non selective σ ligand which acts on the opiate σ receptor as does (+)-pentazocine and on the non-opiate σ receptor as does JO-1784.

In conclusions, our data further support the notion that the effects of at least the σ ligand (+)-

pentazocine are in part sensitive to naloxone, while the effects mediated by others σ_1 are not. It was proposed by Martin and colleagues in 1980 that there may exist two types of σ receptor, one which is naloxone sensitive (defined as a high-affinity opiate receptor), and one which is naloxone insensitive. Our results suggest that at least three types of σ receptor exist. Within the σ_1 receptors, two distinct entities exist: one corresponding to the protein isolated by Su and colleagues and for which, (+)-pentazocine has a high affinity and which is naloxone sensitive and, an other on which most of the σ_1 ligands are acting and coupled to a $G_{i/o}$ protein and for which (+)-pentazocine has a low affinity. (+)-Pentazocine would act on two types of receptors: one which is naloxone sensitive and an other which is haloperidol sensitive. Moreover, these data provide additional evidence that (+)-[^{3}H]pentazocine is acting on a different subtype of σ_1 receptor than JO-1784, L-687, 384 or BD 737.

ACKNOWLEDGEMENTS

The author wish to thank Claude Bouchard and Yves Simard for computer programming and for illustrations, and Martine Le Dû for her secretarial assistance. This work was supported by the Fonds de la Recherche en Santé du Québec (FRSQ) and by the Royal Victoria Hospital Research Institute. G.D. is a receipt of a scholarship from the F.R.S.Q. and S.C. is a fellow from the Royal Victoria Hospital Research Institute.

LEGENDS TO FIGURES

Figure 1A

Integrated firing rate histogram of CA_3 dorsal hippocampus pyramidal neuron illustrating the effects of microiontophoretic applications of NMDA and QUIS before and after the intravenous administration of (+)-pentazocine, and after the subsequent administration of naloxone. In this and subsequent continuous integrated firing histograms, bars indicate the duration of applications for which currents are given in nA. Open circles (oo) represent an interruption of the illustration of the continuous recording.

Figure 1B

Responsiveness, expressed as the number of spikes generated/nC (mean $\pm$ S.E.M), of CA_3 dorsal hippocampus neurons to microiontophoretic applications of NMDA before (open column), after (grey column) the administration of (+)-pentazocine and after the subsequent administration of naloxone (grey hatched column) (B) *$p<0.05$, using one-way RMANOVA .

Figure 2

Responsiveness, expressed as the number of spikes generated/nC (mean $\pm$ S.E.M), of CA_3 dorsal hippocampus neurons to microiontophoretic applications of NMDA before (open column), after (grey column) the administration of cyprodime hydromide and after the subsequent administration of naloxone (grey hatched column) (A) and after the subsequent administration of DIPPA (grey hatched

column) (C) and after the subsequent administration of naltrindole (grey hatched column) (D) *p<0.05, using one-way RMANOVA .

Figure 3A

Integrated firing rate of CA_3 dorsal hippocampus pyramidal neuron illustrating the effects of microiontophoretic applications of NMDA and QUIS before and after the intravenous administration of JO-1784, and after the subsequent administration of naloxone.

Figure 3B

Responsiveness, expressed as the number of spikes generated/nc (mean ± S.E.M), of CA_3 dorsal hippocampus neurons to microiontophoretic applications of NMDA before (open columns), after (grey column) the administration of JO-1874 and after the subsequent administration of naloxone (Grey hatched column).*p<0.05,using one-way RMANOVA.

Figure 4A

Integrated firing rate histogram of CA_3 dorsal hippocampus pyramidal neuron illustrating the effects of microiontophoretic applications of NMDA and QUIS before and after the intravenous administration of L, 687-384, and after the subsequent administration of naloxone.

Figure 4

Responsiveness of CA_3 dorsal hippocampus neurons to microiontophoretic applications of NMDA

before (open columns) and after (grey column) the administration of L, 687-384 (A), BD-737 (B) and after the subsequent administration of naloxone (grey hatched column).

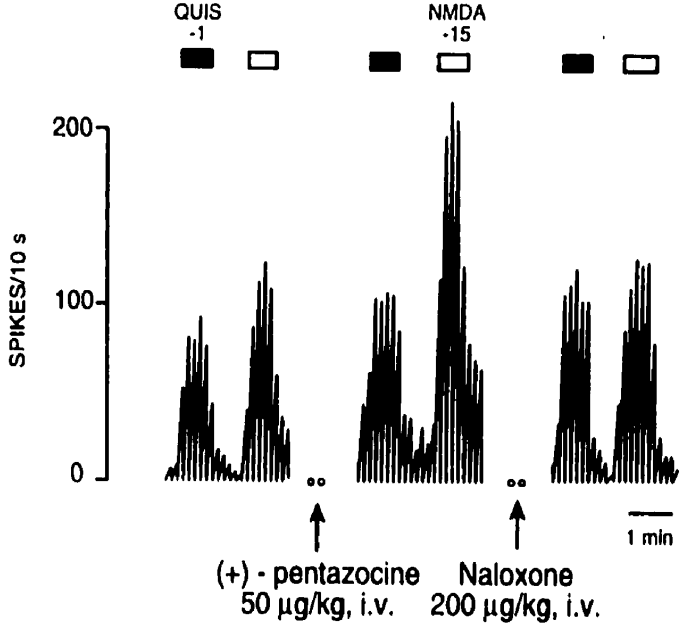
*p< 0.05, using one-way RMANOVA.

Figure 5

Responsiveness of CA_3 dorsal hippocampus neurons to microiontophoretic applications of NMDA before (open column) and (in A and B) after (grey column) the administration of (A) (+)-pentazocine (B) JO-1784, and after the subsequent administration of (+)-cyclazocine (Grey hatched column) or (in C and D) after (grey hatched column) the administration of (+)-cyclazocine and after the subsequent administration of (C) (+)-pentazocine or (D)JO-1784 (grey columns).

*p<0.05, using one-way RMANOVA

A.


QUIS
-1
NMDA
-15
200
100
0
SPIKES/10 s
1 min
(+) - pentazocine
50 µg/kg, i.v.
Naloxone
200 µg/kg, i.v.

B.

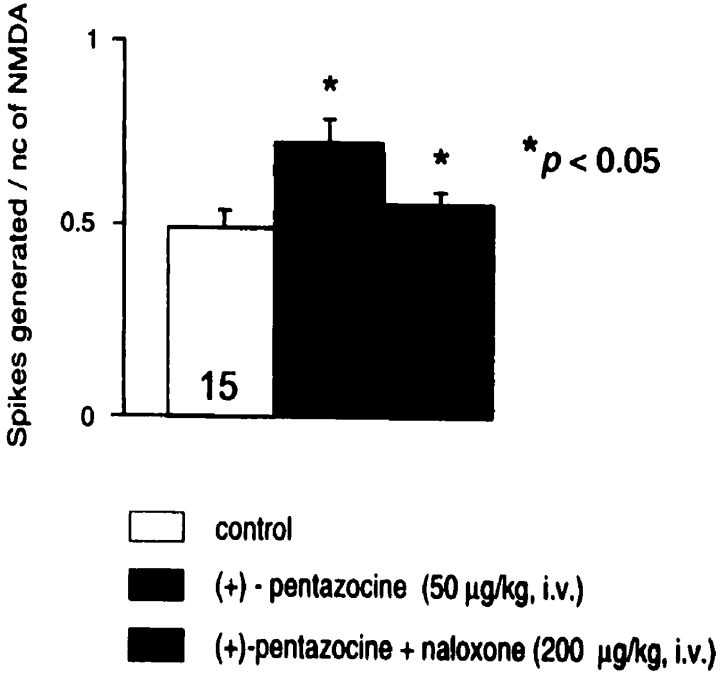
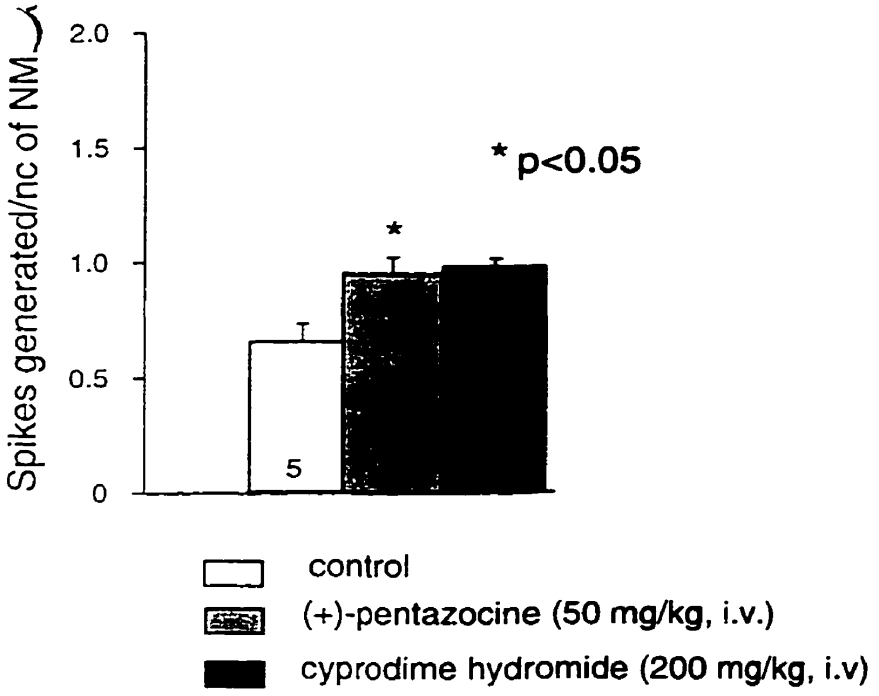
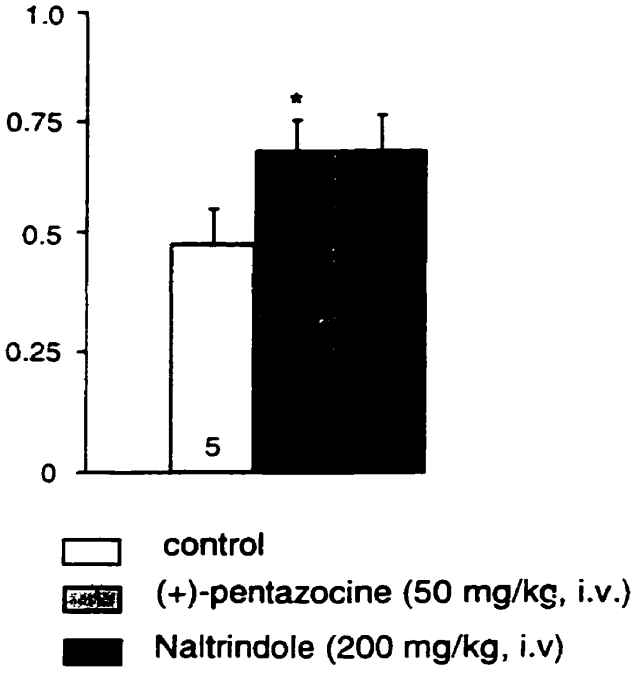

Spikes generated / nc of NMDA
1
0.5
0
15
*
*
*p < 0.05
control
(+) - pentazocine (50 µg/kg, i.v.)
(+)-pentazocine + naloxone (200 µg/kg, i.v.)

Fig. 1

C.


Spikes generated/nc of NM
2.0
1.5
1.0
0.5
0
* p<0.05
*
5
control
(+)-pentazocine (50 mg/kg, i.v.)
cyprodime hydromide (200 mg/kg, i.v)

D.


1.0
0.75
0.5
0.25
0
*
5
control
(+)-pentazocine (50 mg/kg, i.v.)
Naltrindole (200 mg/kg, i.v)

E.

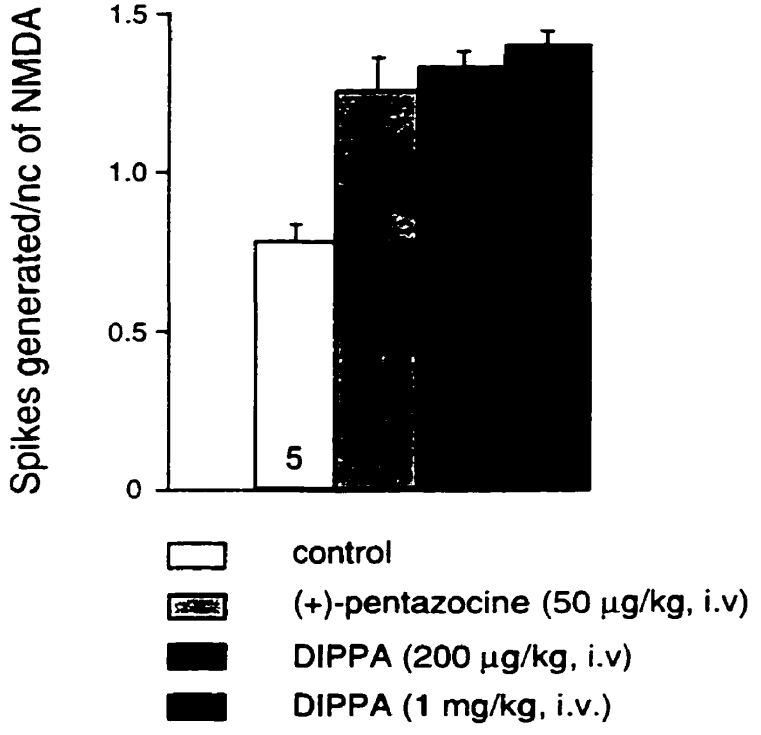
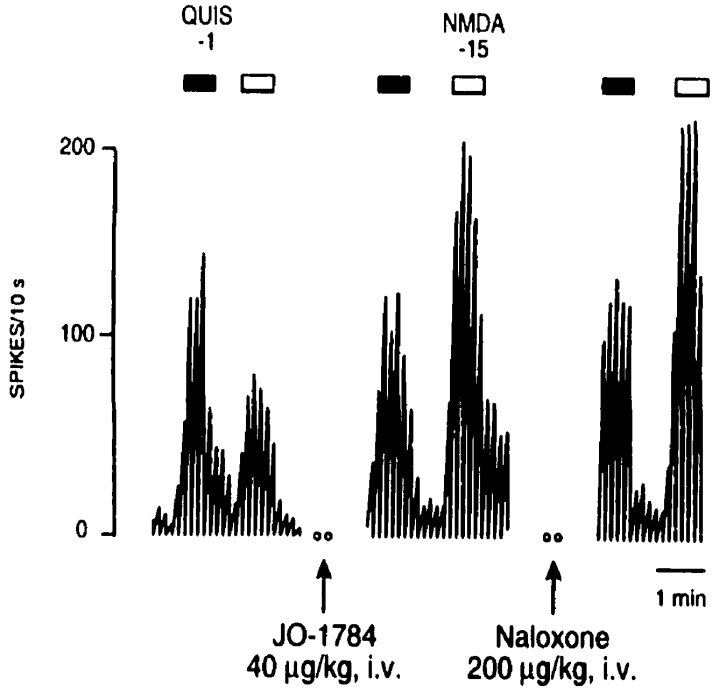

Spikes generated/nc of NMDA
1.5
1.0
0.5
0
5
control
(+)-pentazocine (50 µg/kg, i.v)
DIPPA (200 µg/kg, i.v)
DIPPA (1 mg/kg, i.v.)

Fig.2

A.


QUIS
-1
NMDA
-15
200
100
0
SPIKES/10 s
1 min
JO-1784
40 μg/kg, i.v.
Naloxone
200 μg/kg, i.v.

B.

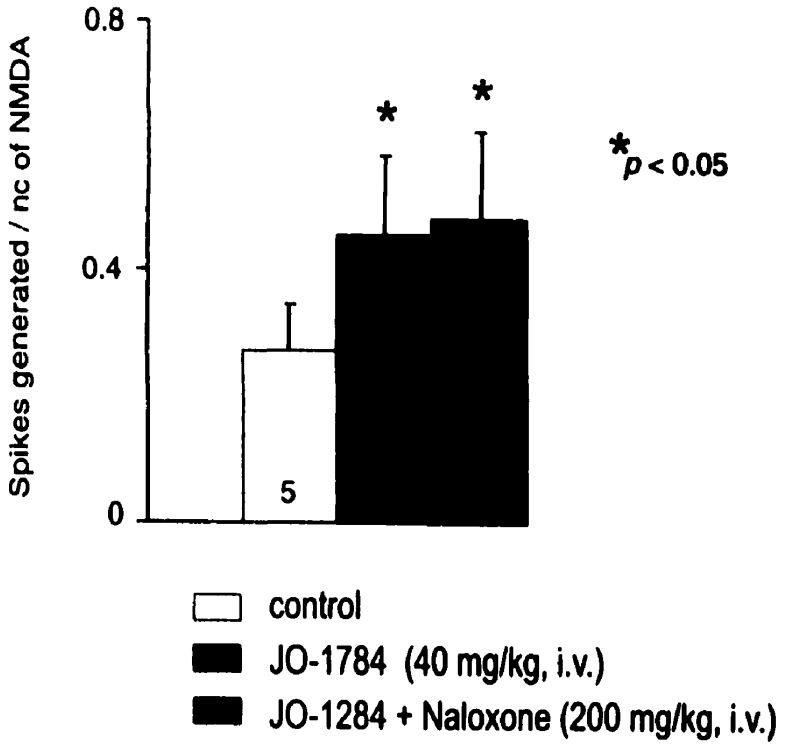

0.8
0.4
0
Spikes generated / nc of NMDA
5
*
*
*p < 0.05
control
JO-1784 (40 mg/kg, i.v.)
JO-1284 + Naloxone (200 mg/kg, i.v.)

Fig.3

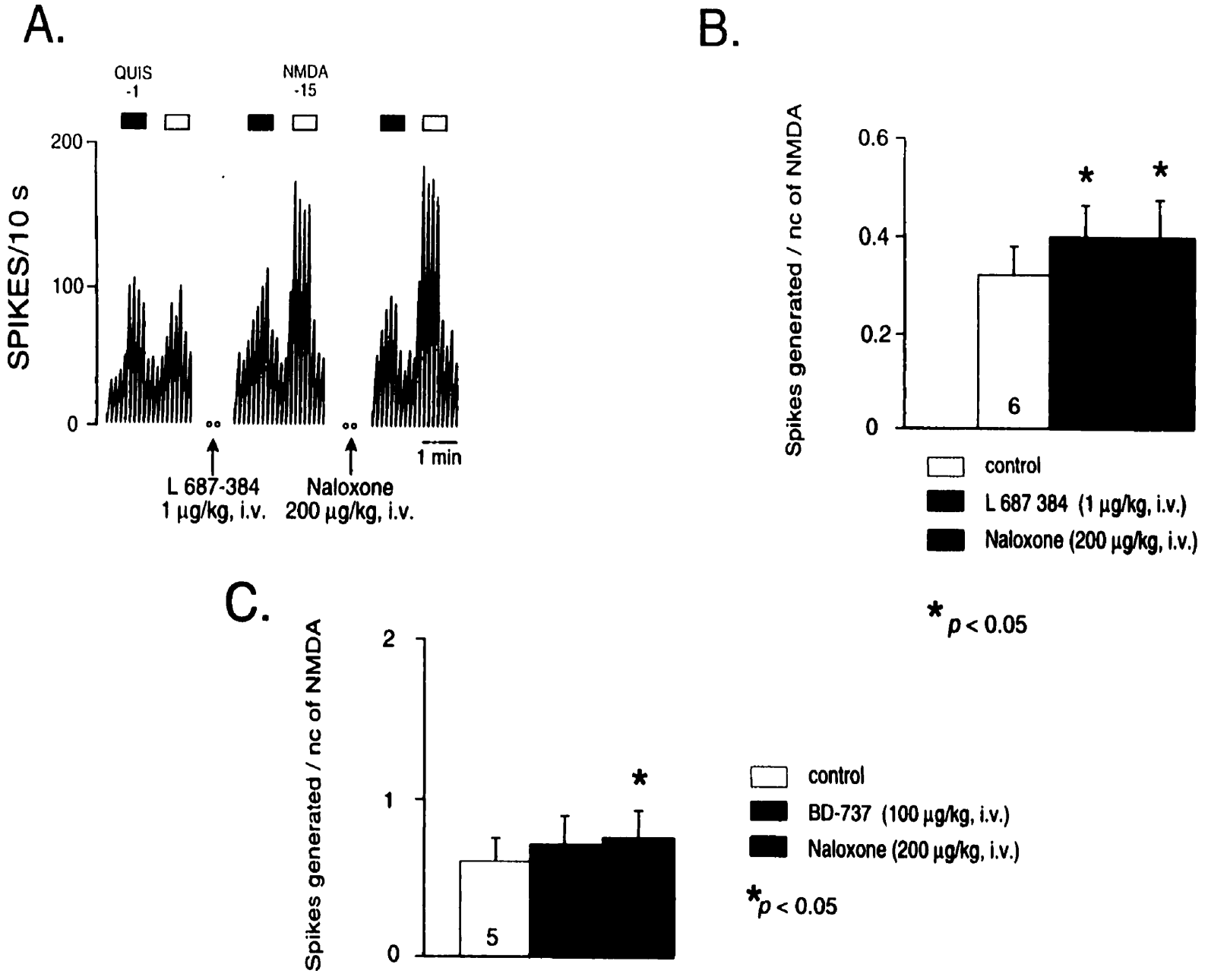

A.
QUIS
-1
NMDA
-15
200
100
0
SPIKES/10 s
1 min
L 687-384
1 µg/kg, i.v.
Naloxone
200 µg/kg, i.v.
B.
Spikes generated / nc of NMDA
0.6
0.4
0.2
0
6
control
L 687 384 (1 µg/kg, i.v.)
Naloxone (200 µg/kg, i.v.)
* p < 0.05
C.
Spikes generated / nc of NMDA
2
1
0
5
control
BD-737 (100 µg/kg, i.v.)
Naloxone (200 µg/kg, i.v.)
* p < 0.05

Fig.4

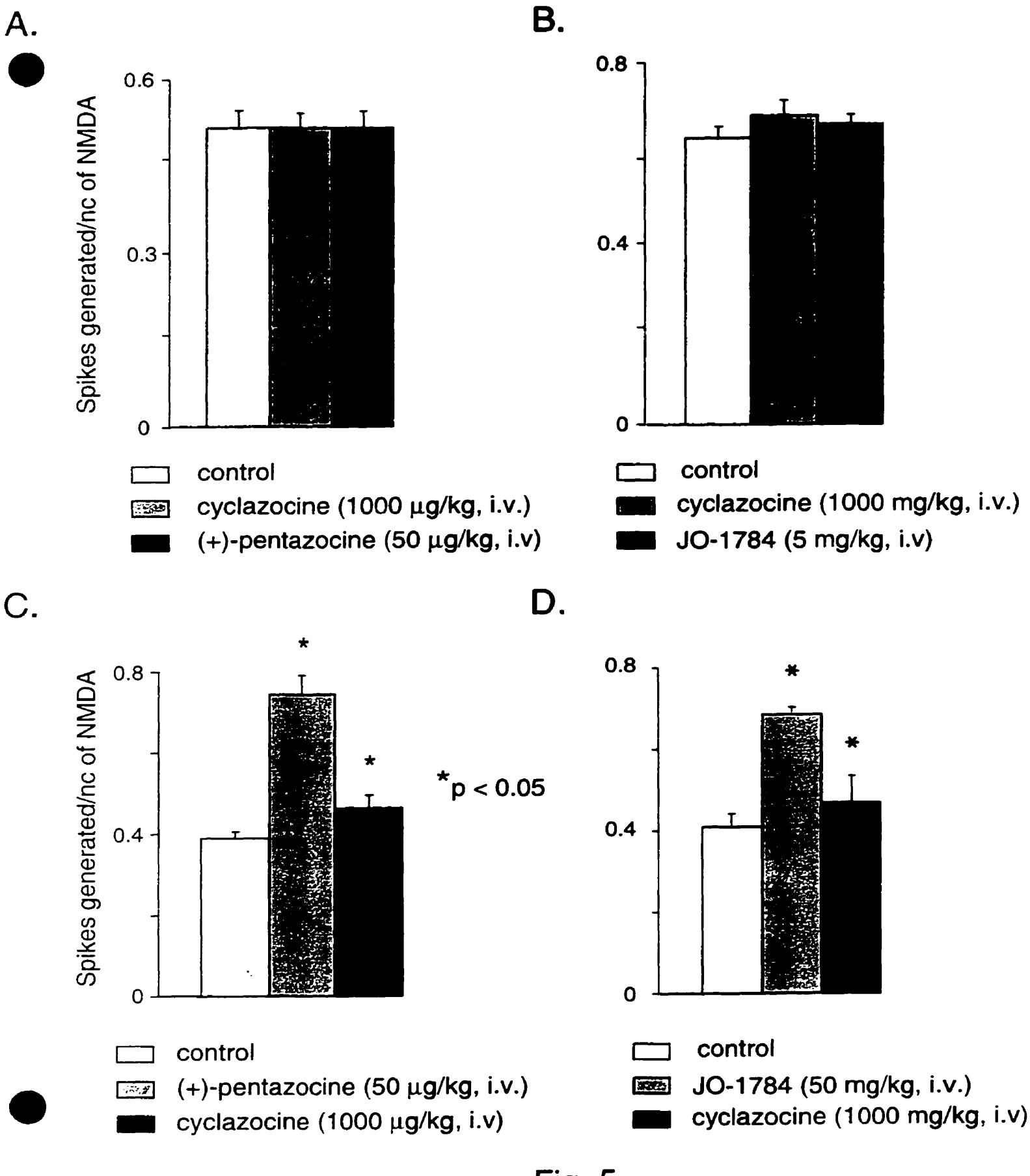

A.
B.
C.
D.
Spikes generated/nc of NMDA
0.6
0.3
0
0.8
0.4
control
cyclazocine (1000 µg/kg, i.v.)
(+)-pentazocine (50 µg/kg, i.v)
control
cyclazocine (1000 mg/kg, i.v.)
JO-1784 (5 mg/kg, i.v)
*
*p < 0.05
control
(+)-pentazocine (50 µg/kg, i.v.)
cyclazocine (1000 µg/kg, i.v)
control
JO-1784 (50 mg/kg, i.v.)
cyclazocine (1000 mg/kg, i.v)

Fig. 5

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GENERAL DISCUSSION

The results presented in this thesis provide a better understanding of the physiological role and of the pharmacological profile of σ_1 and σ_2 receptors.

In the first study, the σ_2 ligands Lu 28-179, CB-64D and BD 1008, when administered intravenously, potentiated dose-dependently the NMDA response in the CA_3 region of the rat dorsal hippocampus. These ligands generated bell-shape dose-response curves as observed previously in the same electrophysiological models with several high affinity σ_1 ligands including (+)-pentazocine, L,687-384, sertraline, clorgyline and JO-1784 (Bergeron et al., 1995). Ibogaine, a ligand with high affinity for the σ_2 receptor, increased slightly but significantly the NMDA response at a dose of 200 µg\kg.

In the second study, the effect of (+)-pentazocine on the NMDA response was reversed by naloxone and not by selective antagonists of the opiate family suggesting that σ receptors are distinct from the opiate receptors µ, δ and κ. As mentioned earlier, Su and colleagues had found that (+)-pentazocine and naloxone had high affinity for their purified protein. Our results are consistent with those obtained by these investigators. Musacchio and colleagues also provides evidence that at least three binding sites for σ ligands (Klein and Musacchio, 1989; Musacchio et al., 1988, 1989). It is therefore plausible to think that (+)-pentazocine have multiple binding sites as proposed by many groups (Klein and Musacchio, 1989; Musacchio et al., 1988, 1989).

The results obtained in the first study suggest two series of conclusion:

1) Similarly to σ_1 ligands, σ_2 ligands also induce a modulation of the NMDA response. However, the doses of σ_2 ligands required to induce a potentiation of the NMDA response are generally 5 to 20 times higher than those required for the σ_1 ligands and the degree of potentiation was less impressive than that observed with σ_1 ligands

2) They may exist at least two subtypes of σ_2 receptors: the σ_{2a} and σ_{2b} receptors. The σ_{2a} receptor, on which Lu 28-179, Lu 29-252 and BD 1008 act and on which haloperidol and progesterone would not act as antagonists. The σ_{2a} might be located postsynaptically and release an endogenous ligand that would triggered the intracellular release of glutamate and act on the NMDA channel. In fact, preliminary studies have shown that after lesioning the mossy fibbers of the hippocampus of rats with colchicine, Lu 28-179, Lu 29-252 and BD 1008 continue to potentiate the NMDA response, suggesting that they are located postsynaptically (unpublished data).

The σ_{2b} receptor on which CB-64D and DTG would act and on which haloperidol and progesterone act as antagonists. The σ_{2b} might be located presynaptically and modulate the NMDA response via a different endogenous ligand than Lu 28-179, Lu 29-252 and BD 1008. Indeed, in pertussis toxin pretreated rats, the intravenous administration of CB-64D, no potentiation of the NMDA response was observed suggesting that the receptor for this ligand is located presynaptically (unpublished data). The results obtained with CB-64D were similar to those observed with DTG

(Bergeron et al, 1995).

The results obtained in the second series of experiments allow to propose a new classification for what was formally identified as σ_1 receptor, which could comprise two distinct entities: the σ_p and the σ_j. The ligand (+)-pentazocine would act as a selective agonist of the σ_p receptor, while the ligand JO-1784 would act as a selective agonist of the σ_j receptor. The main distinction between these two receptors are:

σ_p	σ_j
The σp is located postsynaptically (Debonnel et al., 1996);	σ_j is located presynaptically (Debonnel et al., 1996);
The activation of σp does not induced an epileptoid activity by its own, nor when it is combined with the activation of σ_2 (Couture and Debonnel, 1998);	The activation of σ_j combined with that of the σ_2 receptor induced an epileptoid activity (Couture and Debonnel, 1998);
The σ_p receptor as been cloned as a one transmembrane domain protein, and is not linked to a G protein (Kekuda et al., 1996; Seth et al., 1998.);	σ_j is linked to a $G_{i/o}$ protein (Itzhak, 1989; Quirion et al., 1992; Monnet et al., 1994)
σ_p has a high affinity for naloxone which acts as a σ_p antagonist (Couture et al., submitted to synapse, 1998).	σ_j is naloxone insensitive (Couture et al., submitted to synapse, 1998).

In order to verify the validity of this new classification, the cloning of the former σ_j receptor would be warranted.

Globally, the results obtained are suggesting that the σ receptor family is larger and more

heterogenous than formally expected and that at least four σ receptor exist (σ_j , σ_p, σ_{2a} and σ_{2b}). The σ ligands (JO-1784, (+)-pentazocine, Lu 29-252, Lu 28-179, DTG) interacting with one of the four σ receptor mentioned above all act in a similar fashion by potentiating the NMDA response in our *in vivo* electrophysiological model. The main distinction between the σ_1 receptors (σ_j and σ_p) and the σ_2 receptors (σ_{2a} and σ_{2b}) is that they are implicated in different roles. Indeed, it has been shown that σ_1 ligands are implicated in learning and memory processes through their interaction with the glutamatergic system (Matsuno et al., 1997) while the σ_2 ligands are implicated in motor function through their interaction with the glutamatergic system who consequently interact with the dopaminergic system.

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