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Effect of short-term and long-term treatments with σ ligands on the *N*-methyl-D-aspartate response in the CA_3 region of the rat dorsal hippocampus

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1 Long-term treatments with the σ ligand haloperidol decrease the density of σ receptors in mammalian CNS. We have shown that σ ligands, such as di(2-tolyl)guanidin (DTG), potentiate dose-dependently, with bell-shaped dose-response curves, the neuronal response of pyramidal neurones to *N*-methyl-D-aspartate (NMDA) in the CA_3 region of the rat dorsal hippocampus. σ Ligands producing such a potentiation were denoted 'agonists'. This potentiation was suppressed by low doses of other σ ligands denoted 'antagonists'. High doses of DTG and JO-1784 did not modify the NMDA response but acted as 'antagonists' by suppressing the potentiation induced by σ 'agonists'.

2 Following a 21-day treatment with haloperidol as well as with high doses of DTG or JO-1784, after a 48 h washout, the acute administration of σ 'agonists' failed to induce any potentiation of the NMDA response. Following a 21 day treatment with a low dose of DTG or JO-1784, after a 48 h washout, the neuronal response to microiontophoretic applications of NMDA was markedly increased. A 21 day treatment with low or high doses of (+)-pentazocine, after a 48 h washout, did not produce any change.

3 Following a two day treatment with a high dose of haloperidol, DTG, JO-1784 and (+)-pentazocine, after a 24 h washout, the potentiation of the NMDA response induced by the acute administration of the σ 'agonists' was unchanged.

4 With the minipumps on board, with DTG and JO-1784, a dose-dependent enhancement of the NMDA response was seen but no effect was observed in the groups of rats treated at the same doses with haloperidol or (+)-pentazocine.

5 The present data suggest that long-term treatments with σ 'antagonists' induce a desensitization of the σ receptors, whereas long-term treatments with σ 'agonists' induce a supersensitivity of the σ receptors.

Keywords: Sigma (σ) Receptors; haloperidol; di(2-tolyl)guanidin (DTG); JO-1784; pentazocine; hippocampus; electrophysiology; long-term treatment

Introduction

Many neuroleptics, several antidepressants and some neuroactive steroids as well as psychotomimetic drugs, such as pentazocine and cocaine, exhibit high to moderate affinity for σ receptors, suggesting that these receptors might be involved in the control of behavioural and emotional states (for review see: Walker *et al*., 1990; Su, 1991; Debonnel, 1993; Debonnel & de Montigny, 1996). Moreover, some recently developed neuroleptic drugs, having a low affinity for dopamine receptors and high to moderate affinities for σ receptors, were shown to exert a therapeutic effect in schizophrenia (Snyder & Largent, 1989; Munetz *et al*., 1989; Ashwood *et al*., 1992). Post-mortem studies have shown a decrease of the densities of σ binding sites in several regions of the brain of schizophrenic patients (Weissman *et al*., 1988). Moreover, a down-regulation of σ binding sites has been observed following long-term haloperidol administration in animals (Itzhak & Alerhand, 1989; Matsumoto *et al*., 1990; Reynolds *et al*., 1991). Binding studies with selective σ radioligands have provided evidence for the existence of at least two subtypes of σ receptors (for review see: Hellewell & Bowen, 1990; Quirion *et al*., 1992). The most commonly used σ ligands, including DTG (di(2-tolyl)guanidin) (Weber *et al*., 1986) and haloperidol (Tam & Cook, 1984) do not discriminate between σ_1 and σ_2. In contrast, (+)-pentazocine (Steinfels *et al*., 1988) and JO-1784 ((+)N-cyclopropylmethyl-N-methyl-1,4-diphenyl-1-ethyl-but-3-en-1-ylamine hydrochloride) (Roman *et al*., 1990) are more selective for the σ_1 subtype (Bowen *et al*., 1989; Hellewell & Bowen, 1990; Rothman *et al*., 1991).

Hanner *et al*. 1996), have recently cloned the σ receptor but its physiological role is not yet completely understood. We have previously shown that the acute administration of low doses of selective σ ligands, such as DTG, JO-1784, (+)-pentazocine, BD-737 (Contreras *et al*., 1991) and L-687,384 (1-benzyl-spiro[1,2,3,4-tetrahydronaphthalene-1,4-piperidine) (Middlemiss *et al*., 1991; Barnes *et al*., 1992), potentiate selectively the neuronal response of CA_3 dorsal hippocampus pyramidal neurones to microiontophoretic application of *N*-methyl-D-aspartate (NMDA) (Monnet *et al*., 1990; 1992; Bergeron *et al*., 1993; 1995). These ligands were denoted σ 'agonists' as an operational definition. This potentiation can be suppressed by other σ ligands such as haloperidol, BMY-14802 (α-(4-fluorophenyl)-4-(5-fluoro-2-pyrimidinyl)-1 piperazine butanol) (Taylor & Dekleva, 1987) and (+)-3-(3-hydroxyphenyl)-N-(1-propyl) piperidine (+)-3-PPP; Largent *et al*., 1984) which were thus denoted σ 'antagonists'. The degree of the potentiation obtained with σ 'agonists' is dose-dependent and presents a bell-shaped dose-response curve (Bergeron *et al*., 1995). For most of the σ 'agonists', the maximal potentiation is observed at doses in between 100–200 $\mu g\ kg^{-1}$, i.v. At higher doses, the degree of the potentiation progressively decreases to finally vanish at doses higher than 500 $\mu g\ kg^{-1}$, i.v. At the dose of 1000 $\mu g\ kg^{-1}$, i.v., σ ligands such as DTG and JO-1784 do not modify the NMDA response but then act as 'antagonists' by preventing and suppressing the potentiation induced by σ agonists (Debonnel *et al*., 1992; Bergeron *et al*., 1995). It is noteworthy that these selective σ ligands have no significant affinity for any of the binding sites of the NMDA receptor complex, given the doses administered (Walker *et al*., 1990).

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The purpose of the present study was to determine the effects of acute administration of low doses of ligands DTG, JO-1784, (+)-pentazocine and L-687,384 on NMDA-induced activation, following long- and short-term treatments with low or high doses of several σ ligands. These experiments were carried out in the CA_3 region of the rat dorsal hippocampus, a region with high densities of σ and NMDA receptors (Cotman & Monaghan, 1988), by use of an *in vivo* electrophysiological paradigm whereby the neuronal responsiveness to microiontophoretic applications of NMDA, quisqualate (Quis) and acetylcholine (ACh) can be quantified by extracellular unitary recording.

Methods

Long- and short-term treatments

Male Sprague-Dawley rats (125–150 g) were anaesthetized with halothane for the subcutaneous implantation of osmotic minipumps. For the long-term treatments, saline or the following σ ligands were delivered at the doses of 200 μg kg^{-1} day^{-1} or 2000 μg kg^{-1} day^{-1} for haloperidol, JO-1784 and (+)-pentazocine and of 100 μg kg^{-1} day^{-1} or 1000 μg kg^{-1} day^{-1} for DTG. In a first study, rats were treated for 21 days followed by a washout period of two days before the electrophysiological experiments. In a second study, animals received one of the following σ ligands: haloperidol, JO-1784 of (+)-pentazocine at the dose of 2000 μg kg^{-1} day^{-1} or DTG at the dose of 1000 μg kg^{-1} day^{-1} for two days, followed by a washout period of one day before the experiments. In a third study, animals were treated for two days and the electrophysiological experiments were carried out while the minipumps were still on board. For this last series, DTG, JO-1784 and haloperidol were administered to the rats, at the doses of 1, 10, 100 or 1000 μg kg^{-1} day^{-1}.

Recording from CA_3 dorsal hippocampus pyramidal neurones

Rats were anaesthetized with urethane (1.25 g kg^{-1}, i.p.) and mounted in a stereotaxic apparatus. Body temperature was maintained at 37°C throughout the experiments. Five-barrelled glass micropipettes, preloaded with fibreglass strands in order to promote capillary filling, were pulled in a conventional manner (Haigler & Aghajanian, 1974) and their tips broken back to 8 to 12 μm under microscopic control. The central barrel, used for extracellular unitary recordings of the activity of CA_3 dorsal hippocampus pyramidal neurones, was filled with a 2 M NaCl solution as well. The impedance of the central barrel was typically between 2 and 5 MΩ. One side barrel, filled with 2 M NaCl, was used for current balancing. The other side barrels, used for microiontophoresis, were filled with NMDA (10 mM in 200 mM NaCl, pH 8), Quis (1.5 mM in 400 mM NaCl, pH 8) and ACh (29 mM in 200 mM NaCl, pH 4). After removal of the dura mater, the micropipette was lowered into the CA_3 region of the dorsal hippocampus (lateral: 4.2 mm and anterior: 4.2 mm) at a depth of 3.5 to 4.5 mm from the cortical surface (Paxinos & Watson, 1986). Action potentials were detected by a differential amplitude discriminator generating square pulses which were fed to a computer and to a counter from which integrated firing rate histograms were generated and displayed on a Gould paper chart recorder (model RS 3200). Pyramidal neurones were identified according to their long duration (0.8–1.5 ms) and large amplitude (0.5–2 mV) action potentials, and by the presence of characteristic 'complex spike' discharges alternating with simple spike activity (Kandel & Spencer, 1961). The duration of the microiontophoretic applications and the intensity of the current used were also stored in the computer. The effect of the microiontophoretic applications of either NMDA, Quis or ACh on pyramidal neurone firing activity was expressed as the number of spikes generated per nC (1 nC being the charge generated by 1 nA applied for 1 s). The duration of microiontophoretic ejections of these excitatory substances was kept constant at 50 s. The currents used for ejecting NMDA ranged from −8 to −20 nA, from −2 to −6 nA for Quis and 5 to 10 for ACh. For a given neurone, the current was adjusted to obtain a firing frequency in between 7 and 15 Hz and was thereafter maintained constant for the remainder of the experiment.

The effects of long- and short-term treatments were measured by comparing the neuronal response to NMDA, Quis and ACh in rats treated with σ ligands or saline. The effects of these treatments were also assessed by comparing the degree of potentiation of the NMDA response following the acute intravenous administration of a low dose of a σ 'agonist' or during the microiontophoretic application of a σ 'agonist', in control and treated rats.

Calculations

The computer calculated the effect of each 50 s microiontophoretic application of an excitatory substance, as the total number of spikes generated nC^{-1}. Each value was calculated by the computer as the mean of the effect of three consecutive applications of the same excitatory substance. The effects of the intravenous administration of σ ligands were assessed by determining the ratio (N_2/N_1) of the number of spikes generated nC^{-1} of each of the three excitatory sub-

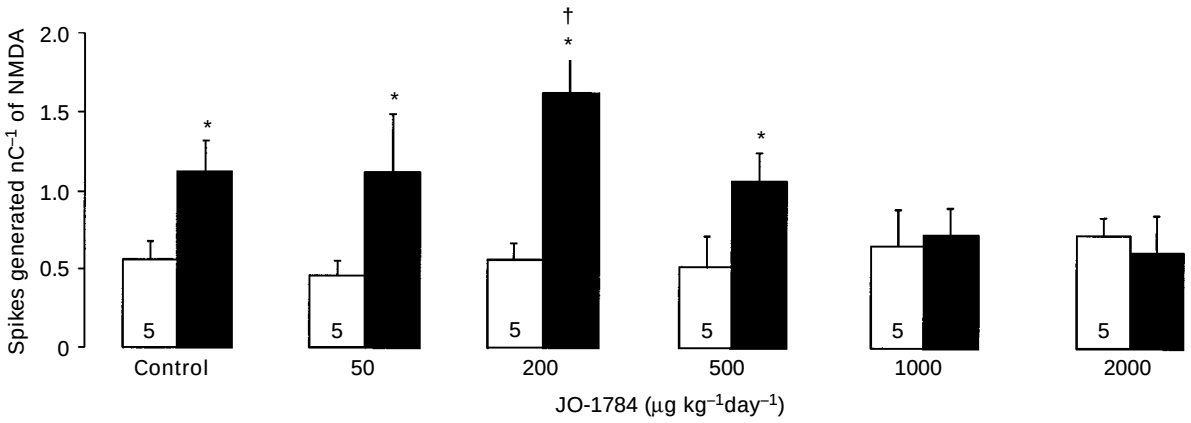

Figure 1 Effects of 21 day treatments with various doses of JO-1784 on the neuronal response induced by NMDA (open columns) or NMDA during concomittent application of JO-1784 (20 nA) (solid columns). The responsiveness of CA_3 dorsal hippocampus pyramidal neurones to microiontophoretic application of NMDA expressed as the number of spikes generated per nanocoulomb (nC: mean ± s.e.mean). In this and the following figures, the numbers at the bottom of the first column indicate the number of neurones tested. $^*P<0.05$, by Student's t test.

stances NMDA, Quis or ACh before (N_1) and after (N_2) the injection of the σ ligand.

Statistical analyses

All results are expressed as the mean ± s.e.mean of the number of spikes generated/nC of NMDA, Quis and ACh. Statistical significance was assessed by Student's *t* test with the Dunnett's correction for multiple comparisons. Probability values smaller than 0.05 were considered significant. Covariance analyses were used to compare the degree of the potentiation of the NMDA response in the groups of rats treated with different doses of DTG and JO-1784 while keeping the minipumps on board as well as the series of experiments of long-term treatments with different doses of JO-1784.

Results

The short- and long-term treatments with various doses of the σ ligands studied did not change the spontaneous firing rate of CA_3 pyramidal neurones nor their response to Quis and ACh nor did they modify the baseline neuronal response to NMDA with the exception of the series of experiments carried out with the minipumps on board.

Effects of 21 day treatments with different doses of JO-1784 followed by a 48 h washout

Several series of long-term treatments with JO-1784 (50, 200, 500, 1000, 2000 μg kg^{-1} day^{-1}) were performed. Following 21 day treatments and after a 48 h washout, no significant differences were found between the degrees of potentiation of the NMDA response induced by microiontophoretic applications of JO-1784 (20 nA) in the control animals and the rats treated with 50 and 500 μg kg^{-1} day^{-1} (Figure 1). However, in the group of rats treated with the dose of 200 μg kg^{-1} day^{-1}, a significant enhancement of the potentiation of the NMDA response was observed (Figure 1). In the group of rats treated at the dose of 1000 and 2000 μg kg^{-1} day^{-1}, no potentiation of the NMDA response was found following the administration of JO-1784 (Figure 1). This type of bell-shaped dose-response curve is very similar to those already found following acute treatments with σ 'agonists' (Bergeron *et al*., 1995). Therefore, in the following series of experiments, the dose inducing the maximal degree of potentiation and a high dose were chosen.

Effects of 21 day treatments with high doses of DTG, JO-1784 and (+)-pentazocine followed by a 48 h washout

Following 21 day treatments with 1000 μg kg^{-1} day^{-1} of DTG or 2000 μg kg^{-1} day^{-1} of JO-1784, after a 48 h washout, neither the intravenous administration of low doses of DTG, JO-1784 or (+)-pentazocine nor the microiontophoretic application of these σ ligands induced any potentiation of the NMDA response (Figure 2a and b). However, following a 48 h washout, the 21 day treatment with the high dose of 2000 μg kg^{-1} day^{-1} of (+)-pentazocine, the degree of potentiation of the NMDA response induced by low doses of DTG, JO-1784 and (+)-pentazocine was similar in treated and in control rats as illustrated for (+)-pentazocine in Figure 2c.

Effects of 21-day treatments with low doses of DTG, JO-1784 and (+)-pentazocine followed by a 48 h washout

Following a 21 day treatment with DTG at the dose of 100 μg kg^{-1} day^{-1}, after a 48 h washout, the effects of the acute administration of DTG was significantly increased. In control rats, the potentiation of the NMDA response induced by microiontophoretic application of DTG (20 nA), was a 2

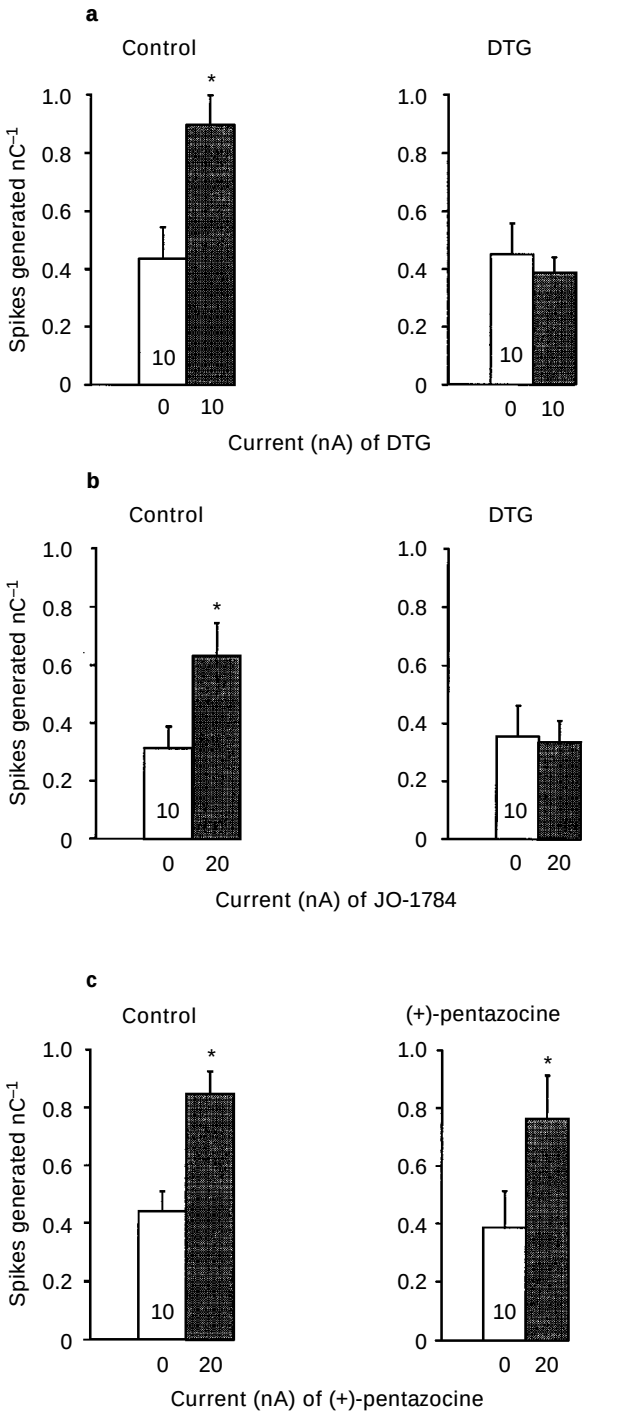

Figure 2 Responsiveness expressed as the number of spikes generated per nanocoulomb (mean ± s.e.mean) (open columns) of CA_3 dorsal hippocampus pyramidal neurones to microiontophoretic applications of NMDA before and during microiontophoretic applications (stippled columns) of DTG (a), JO-1784 (b) and (+)-pentazocine (c) at current of 10 nA in control and rats treated with a high dose of DTG (1000 μg kg^{-1} day^{-1}, s.c., a and b) or in control and rats treated with high dose of (+)-pentazocine (2000 μg kg^{-1} day^{-1}, s.c., c) for 21 days following a washout period of two days. *$P<0.05$, by Student's *t* test.

fold (Figure 3a), whereas, in rats treated with DTG, microiontophoretic application of this ligand with the same current induced a 4 fold increase of the NMDA response (Figure 3a). In these DTG-treated rats, the intravenous administration of DTG (1 μg kg^{-1}) induced an epileptoid activity upon NMDA application (data not shown), a phenomenon observed only with doses higher than 3 μg kg^{-1}, i.v. in naive rats (Monnet *et al*., 1992; Bergeron *et al*., 1995). The acute administration of

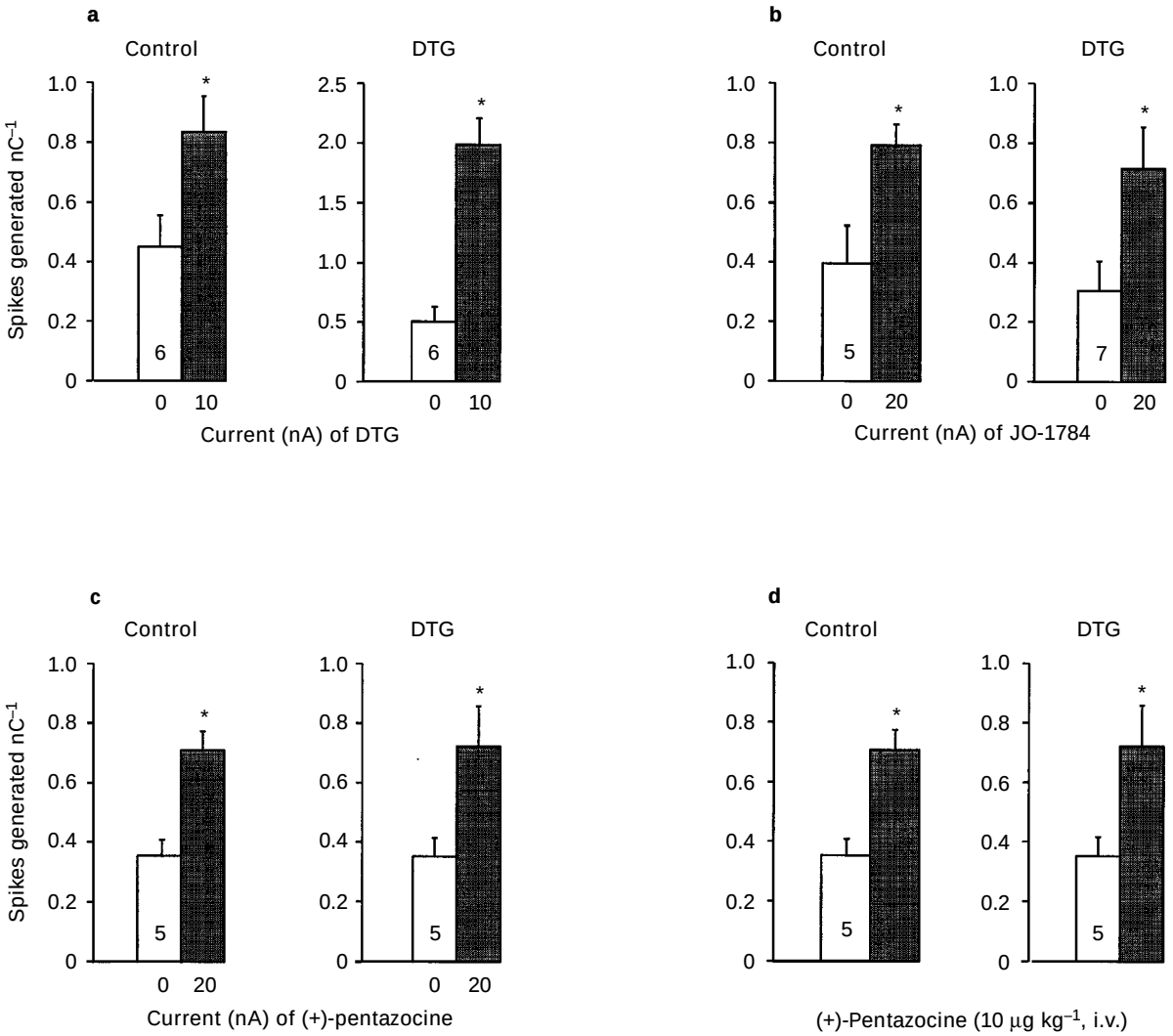

Figure 3 Responsiveness expressed as the number of spikes generated per nanocoulomb (mean ± s.e.mean) of CA_3 dorsal hippocampus pyramidal neurones to microiontophoretic applications of NMDA before (open columns) and during the microiontophoretic applications (stippled columns) of (a) DTG, (b) JO-1784 or (c) (+)-pentazocine (c) before and (d) after the intravenous administration of (+)-pentazocine in control rats and rats treated with a low dose of DTG (100 μg kg^{-1} day^{-1}, s.c.) for 21 days following a two day washout.

JO-1784 (4 μg kg^{-1}, i.v. or 20 nA) (Figure 3c) and that of (+)-pentazocine (10 μg kg^{-1}, i.v. or 20 nA) (Figure 3c, d) induced a potentiation of the NMDA response similar to that produced in naive rats.

Following long-term treatment with JO-1784 at the dose of 200 μg kg^{-1} day^{-1} for 21 days, after a 48 h washout, the intravenous administration of 1 μg kg^{-1} JO-1784 (a dose which did not induce any modification of the neuronal response to NMDA in the control rats), produced a 2 fold increase of the NMDA response (Figure 4a). However, the subsequent administration of 4 μg kg^{-1}, i.v., of JO-1784 did not induce any further enhancement of the NMDA response (Figure 4b). In contrast, the injection of 10 μg kg^{-1}, i.v., of (+)-pentazocine induced a potentiation of the NMDA response similar to that obtained in control rats (Figure 4c). Moreover, as was the case for rats treated with a low dose of DTG, in JO-1748-treated rats, the acute administration of DTG (1 μg kg^{-1}, i.v.) induced epileptoid activity upon microiontophoretic application of NMDA (data not shown).

Following long-term treatment with the dose of 200 μg kg^{-1} day^{-1} of (+)-pentazocine, after a 48 h washout, no significant difference was found between the degrees of potentiation of the NMDA response induced by microiontophoretic applications or by intravenous administrations of low doses of DTG, (+)-pentazocine or JO-1784 in the treated and control rats.

Effects of 21-day treatments with a low or a high dose of haloperidol followed by a 48 h washout

In a first series of experiments, rats were treated for 21 days with haloperidol (200 or 2000 μg kg^{-1} day^{-1}, s.c.), or with saline, delivered subcutaneously by osmotic minipumps. After a 48 h washout (removal of the minipump), the effects of the intravenous administration and of the microiontophoretic applications of the three selective and high affinity σ ligands DTG (1 μg kg^{-1}, i.v., or 20 nA), JO-1784, (4 μg kg^{-1}, i.v., or 20 nA) or (+)-pentazocine (10 μg kg^{-1}, i.v., or 20 nA) were assessed.

As illustrated for DTG in Figure 5, no potentiation of the NMDA response was induced by the intravenous administration of a low dose of DTG (Figure 5a) or by microiontophoretic applications of DTG (Figure 5b) in the rats treated with haloperidol for 21 days at the dose of 200 μg kg^{-1} day^{-1} and at the dose of 2000 μg kg^{-1} day^{-1}. Similarly, the intravenous administration of a low dose of JO-1784 or (+)-pentazocine or the microiontophoretic application of JO-1784 and (+)-pentazocine failed to potentiate the NMDA response in the rats treated for 21 days with 200 or 2000 μg kg^{-1} day^{-1} of haloperidol (data not shown).

To rule out the possibility that the absence of potentiation of the NMDA response was due to the residual haloperidol despite the 48 h washout, a group of rats was treated with the

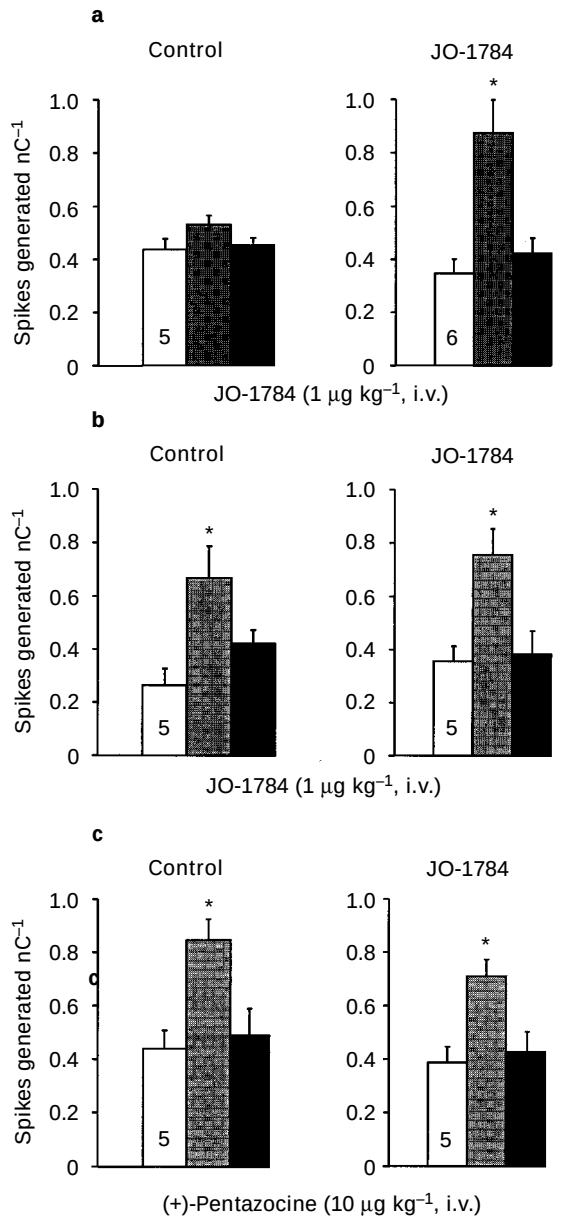

Figure 4 Responsiveness expressed as the number of spikes generated per nanocoulomb (mean±s.e.mean) of CA_3 dorsal hippocampus pyramidal neurones to microiontophoretic applications of NMDA before (open columns) and after the intravenous administration (stippled columns) of a low dose of JO-1784 (a and b) and (+)-pentazocine (c) in control rat and rats treated with a low dose of JO-1784 (200 μg kg^{-1} day^{-1}, s.c.) for 21 days following a two day washout. The potentiation obtained following the intravenous administration of a σ ligand was reversed by the intravenous administration of 10 μg kg^{-1} haloperidol (solid columns). *$P<0.05$, by Student's t test.

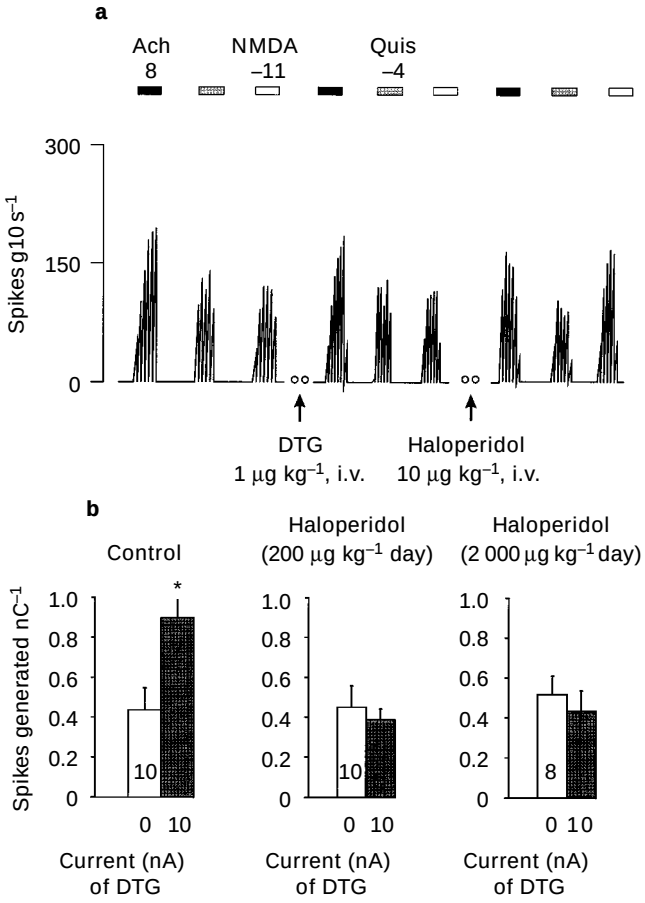

Figure 5 (a) Integrated firing rate histogram of a CA_3 dorsal hippocampus pyramidal neurone showing the effects of microiontophoretic applications of ACh, quisqualate (Quis) and NMDA before and after the intravenous administration of a low dose of DTG in a rat treated with haloperidol 200 μg kg^{-1} day^{-1} for 21 days following a washout period of two days. Bars indicate the duration of applications for which the currents are given in nA. Each neuronal response to each excitatory agent represents the computed-generated mean of the effects of three successive applications. The open circles (○○) represent an interruption of the illustration of the continuous recording. (b) Responsiveness expressed as the number of spikes generated per nanocoulomb (mean±s.e.mean) of CA_3 dorsal hippocampus pyramidal neurones to microiontophoretic applications of DTG at a current of 10 nA in control rats and rats treated with a low or high dose of haloperidol (s.c.) for 21 days. *$P<0.05$, by Student's t test.

same doses of haloperidol (200 or 2000 μg kg^{-1} day^{-1}) for 21 days but the electrophysiological experiments were carried out following a 7 day washout. In this series, DTG injected intravenously (1 μg kg^{-1}, i.v.; Figure 6a) or applied by microiontophoresis (20 nA) and (+)-pentazocine (10 μg kg^{-1}, i.v.; Figure 6b) or applied by microiontophoresis (20 nA), failed to induce any potentiation of the NMDA response.

Effects of 2 day treatments with DTG, JO-1784, (+)-pentazocine and haloperidol followed by a 24 h washout

In this series of experiments, rats were treated for 48 h with DTG, JO-1784, (+)-pentazocine and haloperidol at the high dose of 2000 μg kg^{-1} day^{-1} followed by a 24 h washout. In rats treated with 2000 μg kg^{-1} day^{-1} of haloperidol, the intravenous administration of DTG (1 μg kg^{-1}, i.v.) and JO-1784 (4 μg kg^{-1}, i.v.) or the microiontophoretic application of DTG or JO-1784 with a current of 20 nA, induced a potentiation of the NMDA response similar to that obtained in the control rats (Figure 7a). Similarly, the 48 h treatments with DTG (2000 μg kg^{-1} day^{-1}, s.c.), with JO-1784 (2000 μg kg^{-1} day^{-1}, s.c.) or with (+)-pentazocine (2000 μg kg^{-1} day^{-1}, s.c.) did not modify the degree of the potentiation of the NMDA response induced by the intravenous administration of low doses or by microiontophoretic applications of DTG or JO-1784 (Figure 7b, c).

Effects of 2-day treatments with DTG, JO-1784, (+)-pentazocine and haloperidol with minipumps on board

In a last series of experiments, doses of 1, 10 100 and 1000 μg kg^{-1} day^{-1}, s.c. of each of the four σ ligands studied (DTG, JO-1784, (+)-pentazocine and haloperidol) were administered for 48 h. Electrophysiological experiments were carried out while the minipumps were still on board.

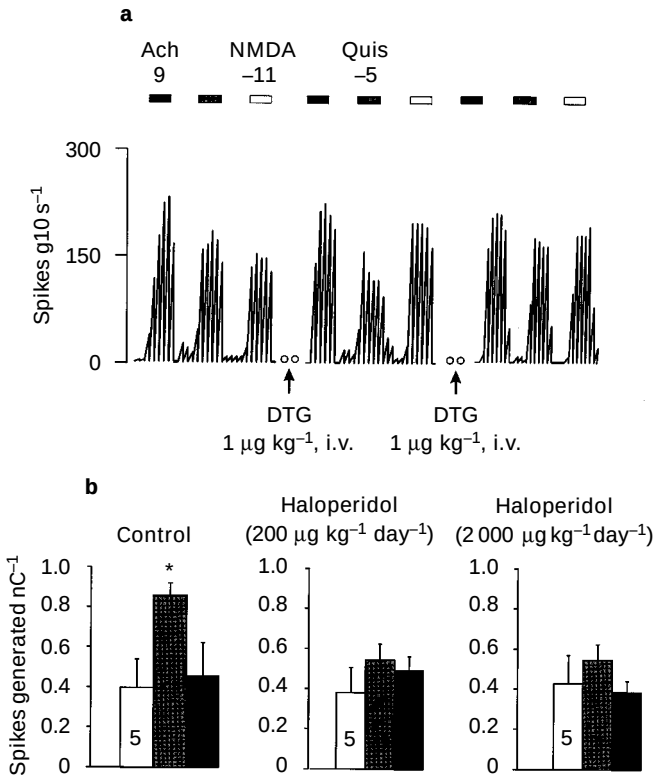

Figure 6 Integrated firing rate histogram of CA_3 dorsal hippocampus pyramidal neurones showing the effects of microiontophoretic applications of ACh, quisqualate (Quis) and NMDA before and after the intravenous administration of two consecutive low doses of DTG in rats treated with haloperidol 200 μg kg^{-1} day^{-1} for 21 days following a washout period of seven days. (b) Responsiveness expressed as the number of spikes generated per nanocoulomb (mean $\pm$ s.e.mean) of CA_3 dorsal hippocampus pyramidal neurones to microiontophoretic applications of NMDA before (open columns) and after (stippled columns) the intravenous administration of (+)-pentazocine (10 μg kg^{-1}, i.v.) and following the acute administration of haloperidol (10 μg kg^{-1}, i.v.) (solid columns) in rats treated with low or high dose of haloperidol for 21 days, following a seven day washout. *$P<0.05$, by Student's t test.

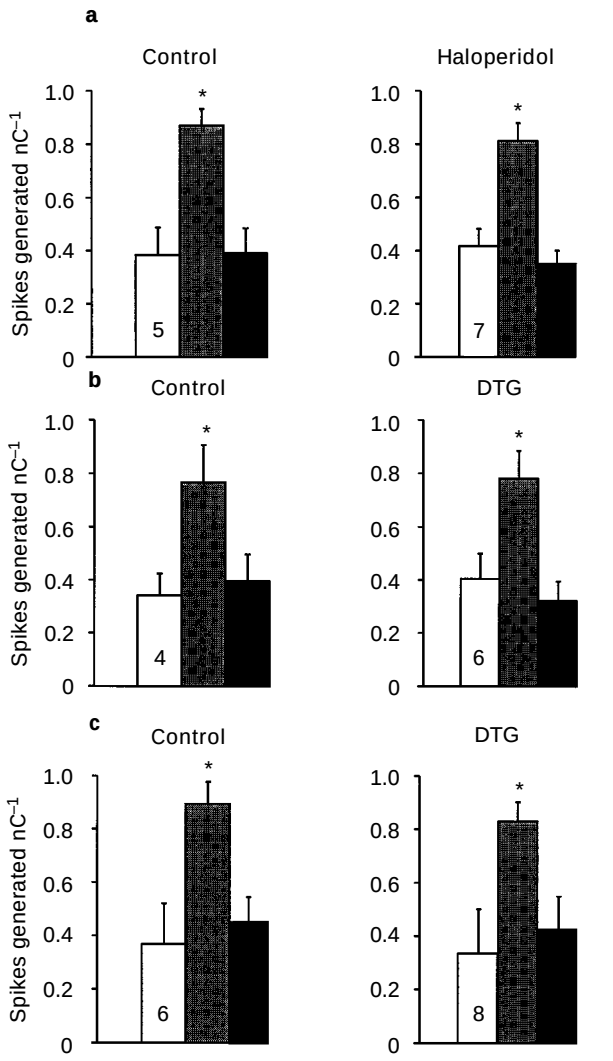

Figure 7 Responsiveness expressed as the number of spikes generated per nanocoulomb (mean $\pm$ s.e.mean) of CA_3 dorsal hippocampus pyramidal neurones to microiontophoretic applications of NMDA before (open columns) and after the intravenous administration of a low dose (stippled columns) of DTG (1 μg kg^{-1}, i.v.,) a and b or JO-1784 (4 μg kg^{-1}, i.v., c) in control rat and rats treated with a high dose of haloperidol (2000 μg kg^{-1} day^{-1}, s.c.) for 2 days (a) or with a high dose of DTG (2000 μg kg^{-1} day^{-1}, s.c., b and c) following a 24 h washout. The potentiation obtained following the intravenous administration of a σ ligand was reversed by the intravenous administration of haloperidol 10 μg kg^{-1} (solid columns). *$P<0.05$, by Student's t test.

In rats treated with DTG or JO-1784, the neuronal response to microiontophoretic application of NMDA was increased. As shown in Figure 8, the degree of activation of CA_3 pyramidal neurones induced by microiontophoretic applications of NMDA, with the same ejecting current, was related to the dose of the σ ligands administered during the 48 h.

The degree of activation by NMDA in the group of rats treated with DTG (10 μg kg^{-1} day^{-1}) could not be quantified because the microiontophoretic applications of NMDA induced an epileptoid activity. This epileptoid activity was not affected by diazepam (5 mg kg^{-1}, i.v.), but was completely reversed by a very low dose of haloperidol (10 μg kg^{-1} day^{-1}, i.v.).

In rats treated with (+)-pentazocine, the neuronal response to microiontophoretic applications of NMDA was not increased (data not shown).

In rats treated for 48 h with low doses of haloperidol (1 and 10 μg kg^{-1} day^{-1}), the neuronal response to microiontophoretic applications of NMDA was similar to that of control rats. In rats treated with higher doses of haloperidol (100 and 1000 μg kg^{-1} day^{-1}), the microiontophoretic applications of DTG or (+)-pentazocine failed to induce a potentiation of the NMDA-induced activation.

Discussion

Twenty-one day treatments with various doses of the σ 'agonist' JO-1784 modified the effects of the acute administration of the same σ ligand. The dose-response curve of this effect presented a bell-shaped aspect similar to that observed following an acute treatment (Bergeron *et al*., 1997).

Twenty-one day treatments, followed by a 48 h washout, with a low or a high dose of the high affinity σ ligand haloperidol, as well as with high doses of DTG or JO-1784, prevented the potentiation of the NMDA response by an acute administration of low doses of σ 'agonists'. In our electrophysiological paradigm, we have previously shown that haloperidol is acting as a σ 'antagonist' since it does not have any effect on NMDA-induced activation by itself, but prevents and reverses the potentiation of the NMDA response induced by the acute administration of low doses of σ 'agonists'. The possibility that the lack of potentiation by the acute administration of low doses of σ 'agonists' in rats treated with a low or a high dose of haloperidol, could have been due to residual haloperidol following the 48 h washout is ruled out by the observation of the same phenomenon following a seven day washout. A more plausible explanation could be that long-term treatment with haloperidol induces a down-regulation of

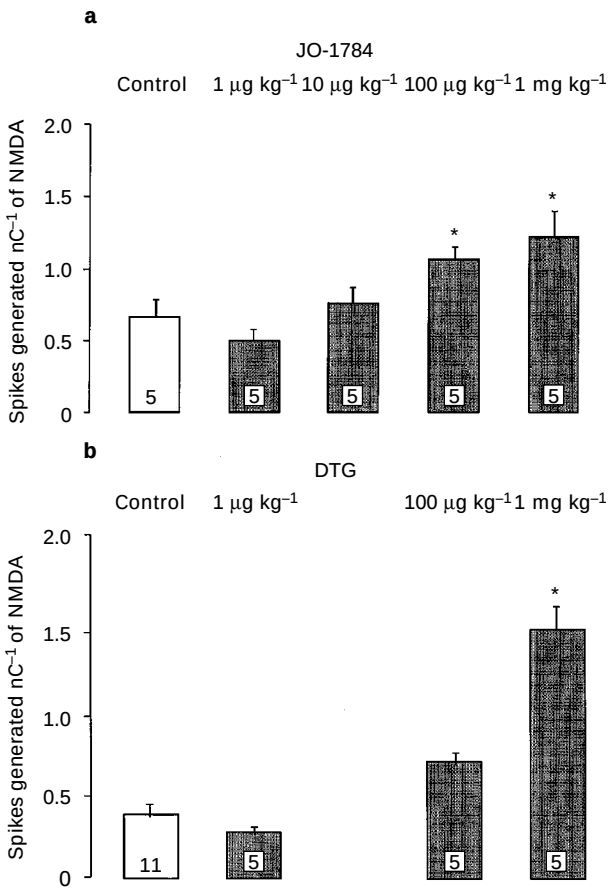

Figure 8 Responsiveness expressed as the number of spikes generated per nanocoulomb (nC: mean ± s.e.mean) of CA_3 dorsal hippocampus pyramidal neurones to microiontophoretic applications of NMDA in control rats and rats treated for two days with (a) JO-1784 or (b) DTG 1, 10, 100 and 1000 μg kg^{-1} day^{-1} by osmotic minipumps. The effect of microiontophoretic applications of NMDA in the group of rats treated with DTG 10 μg kg^{-1} day^{-1} could not be determined as epileptoid activity was induced. Experiments were carried out with the minipumps on board. *$P<0.05$, by Student's t test.

the σ receptors, sufficient to hinder the potentiating effects of σ 'agonists' on the NMDA response. Indeed, with [^{3}H]-(+)-SKF-10,047 or [^{3}H]-(+)-3-PPP, several groups have observed in rodents, that long-term haloperidol induces a decrease in the affinity and in the number of σ sites (Itzhak & Alerhand, 1989; Riva & Creese, 1990; Itzhak & Stein, 1991; Karbon *et al*., 1991; Jansen *et al*., 1992; Bailey & Karbon, 1993).

Long-term treatments with other neuroleptics with low affinity for the σ receptors, such as clozapine and raclopride (Brent & Chahl, 1993), do not modify σ binding parameters (Riva & Creese, 1990). Moreover, in brains of schizophrenic patients treated with haloperidol, there is a clear deficit of binding of [^{3}H]-DTG (Reynolds *et al*., 1991). In contrast, in schizophrenic patients treated with phenothiazines, neuroleptics with low or moderate affinity for σ binding sites (Walker *et al*., 1990), no significant alteration of [^{3}H]-DTG binding parameters could be detected (Reynolds *et al*., 1991). A down-regulation of σ receptors following long-term treatment with a σ 'antagonist' might appear surprising. However, although down-regulations are most commonly observed with agonists (Burt *et al*., 1977; Blackshear & Sanders-Bush, 1982; Fleminger *et al*., 1983), it has been shown that β-adrenoceptor and 5-HT_2 receptor antagonists can produce homologous receptor down-regulation (Conn & Sanders-Bush, 1987; De Blasi *et al*., 1988).

Twenty-one day treatments with high doses of DTG or JO-1784, after a 48 h washout, also prevented the potentiation of the NMDA response by the acute administration of σ 'agonists'. By use of an *in vivo* electrophysiological paradigm, we have previously shown that, when administered acutely, high doses of DTG and JO-1784 (1000 μg kg^{-1}, i.v.) act as σ 'antagonists' by preventing and suppressing the potentiation induced by low doses of σ 'agonists' (Debonnel *et al*., 1992). The results obtained in the present study are thus in keeping with these data, suggesting that, similar to 21 day treatment with haloperidol, 21 day treatments with high doses of DTG or JO-1784 may also induce a down-regulation of σ receptors. This possibility is consistent with the data of Beart *et al*. (1989) showing that a 5 day treatment with a high dose of DTG, 5 mg kg^{-1} day^{-1}, reduces [^{3}H]-(+)-3-PPP binding sites in the rat brain (Beart *et al*., 1989).

Twenty-one day treatments with low doses of DTG (100 μg kg^{-1} day^{-1}) and JO-1784 (200 μg kg^{-1} day^{-1}), after a 48 h washout, enhanced markedly the degree of potentiation of the NMDA response induced by the acute administration of low doses of these σ 'agonists'. In some rats, plasma levels of JO-1784 were assessed following a 21 day treatment or an acute administration following a 21 day treatment with a dose of 200 μg kg^{-1} day^{-1}, when the measure was carried out with the minipump still 'on board' a plasma level of 1.07 μg ml^{-1} was obtained. However, when the dosage was made 15 min following the acute administration of a dose of 10 μg kg^{-1}, at the time when the peak of electrophysiological effect is observed, the serum level of JO-1784 was 3.2 μg ml^{-1} ($n=3$) (G. Debonnel and J.L. Junien, unpublished observations). This indicates that a 21 day treatment with 200 μg kg^{-1} day^{-1} JO-1784 induces plasma levels in the range of those obtained with an acute 'agonistic' dose.

The phenomenon of an epileptoid activity induced by the microiontophoretic applications of NMDA, following the acute administration of doses of DTG higher than 3 μg kg^{-1} in naive rats, has been described previously (Monnet *et al*, 1992; Bergeron *et al*., 1995). In these experimental series, epileptoid activity appeared following the administration of doses of DTG between 3–40 μg kg^{-1}, i.v. Importantly, this activity has never been observed with any of the σ_1 ligands tested (Bergeron *et al*., 1995). In the present experiments, in the group of rats treated for 21 days with a low dose of DTG, the same phenomenon occurred following the intravenous administration of only 1 μg kg^{-1} DTG, but not following the intravenous administration of 4 μg kg^{-1} JO-1784. Similarly, following a 21 day treatment with 200 μg kg^{-1} day^{-1} of JO-1784, the intravenous administration of 1 μg kg^{-1} DTG also induced epileptoid activity whereas the intravenous administration of JO-1784 at doses of 1 and 4 μg kg^{-1} did not produce such a phenomenon. One can thus assume that long-term treatment with a low dose of DTG increases the sensitivity of both σ_1 and σ_2 receptors, whereas long-term treatment with JO-1784 increases the sensitivity of σ_1 receptors. The finding that a dose as low as 1 μg kg^{-1}, i.v. of DTG, but not a low dose of JO-1784, induced epileptoid activity suggests that the activation of σ_2 receptors is required to induce this effect. The validity of this hypothesis will be easily verified when selective σ_2 ligands become available.

Long-term treatments with either a low or a high dose of (+)-pentazocine did not modify the effects of the acute administration of a low dose of σ 'agonists' on the NMDA-response. Radioligand binding studies of the effect of long-term treatments with the racemic form of pentazocine have yielded conflicting results. Kizu *et al*. (1991) have shown that a 14 day administration of a high dose of (+)-pentazocine (7.5 mg kg^{-1} day^{-1}) reduced the affinity, but not the density of σ sites labelled with [^{3}H]-haloperidol in the rat brain, whereas Weissman & De Souza (1991) found that a 28 day treatment with (+)-pentazocine (10 mg kg^{-1} day^{-1}) modified neither the K_D nor the B_{max} values of [^{3}H]-haloperidol binding in the same species. The lack of effect, in the present study, of low or high doses of (+)-pentazocine could be due to the fact that a two-day washout period was sufficient to cancel out the effects that the long-term treatment might have induced. However, this is very unlikely since, contrary to the effect of a two day treatment with the other σ_1 'agonist' JO-1784, a two-day treatment

with a low dose of (+)-pentazocine did not induce any change of the NMDA response when the experiments were carried out with the minipumps on board. The present results rather suggest that long-term treatments with a low or a high dose of (+)-pentazocine do not alter the sensitivity of σ_1 receptors.

When the minipumps were removed 24 h before the electrophysiological experiments, none of the two-day treatments with either of the four σ ligands tested (haloperidol, DTG, JO-1784 and (+)-pentazocine) modified the potentiation of the NMDA response induced by the acute administration of the σ 'agonists'. These results suggest that modification of the σ receptors by the σ ligands cannot be achieved over a short period.

In contrast, in the experimental series carried out with the minipumps on board, DTG and JO-1784 (administered for 48 h at each of the following four doses: 1, 10, 100 or 1000 μg kg^{-1} day^{-1}, s.c.) enhanced the NMDA response in a dose-dependent manner, whereas in the group of rats treated with haloperidol with the same four doses, no potentiation of the NMDA response was observed. Moreover, 48 h treatments with 1 and 10 μg kg^{-1} day^{-1}, s.c., of haloperidol, did not prevent the potentiation induced by microiontophoretic applications of DTG and JO-1748 with a current of 20 nA, whereas in the group of rats treated with 100 and 1000 μg kg^{-1} day^{-1}, s.c., of haloperidol, no potentiation of the NMDA response was observed upon microiontophoretic applications of the same two ligands with the same current. This suggests that when the experiments were performed with the minipumps on board delivering haloperidol at doses of 100 and 1000 μg kg^{-1} day^{-1}, s.c., the amount of haloperidol in the brain was sufficient to occupy the σ receptors and consequently prevent the action of the σ 'agonists' DTG and JO-1784.

In rats treated with (+)-pentazocine (at any of the four doses administered), the microiontophoretic applications of NMDA induced the same degree of neuronal activation. Over the last few years, we have observed that (+)-pentazocine presents a pharmacological profile distinct from that of other σ ligands: (1) pertussis toxin pretreatment abolishes the potentiation induced by JO-1784, L-687,384 and neuropeptide Y (NPY), but not that induced by (+)-pentazocine (Monnet *et al*., 1994); (2) following a unilateral lesion of the mossy fibre system by a local injection of colchicine, the potentiating effect of JO-1784 is abolished whereas the effect of (+)-pentazocine on the NMDA response is still present (Debonnel *et al*., 1996); (3) high doses of the σ_1 ligands, JO-1784 BD-737 and L-687,384 (which are acting as 'agonists' at low doses) prevent and suppress the potentiation of the NMDA response induced by low doses of the σ 'agonists' whereas high doses of (+)-pentazocine fail to produce such an effect. These observations, in keeping with the present results, suggest that (+)-pentazocine, a selective σ_1 receptor 'agonist' (Quirion *et al*., 1992), acts on a distinct subtype of σ_1 receptor. However, the present electrophysiological data do not allow us to ascertain that the metabolism of the drugs used may not contribute to the changes observed following long-term treatments.

In conclusion, the results of the present study suggest that long-term treatments with σ 'agonists' induce a supersensitivity of σ receptors whereas long-term treatments with σ 'antagonists' induce a desensitization of σ receptors.

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