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Involvement of σ receptors in the modulation of the glutamatergic/NMDA neurotransmission in the dopaminergic systems

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Abstract

Extracellular single-unit recordings and iontophoresis were used to examine the effects of different selective σ receptor ligands on dopaminergic and glutamatergic *N*-methyl-D-aspartate (NMDA) neurotransmissions both in origin (A10 and A9 areas) and terminal (nucleus accumbens and caudate nucleus) regions of the rat mesolimbic and nigrostriatal dopaminergic systems. The selective σ_1 receptor ligands 2-[4-(4-methoxy-benzyl)piperazin-1-yl-methyl]4-oxo[4*H*]-benzo-thiazolin-2-one (S-21377), systemically administered (1.2 mg/kg, i.v., cumulative dose), and 2[(4-benzyl piperazin-1-yl) mothyl] naphthalene, dichiorydrate (S-21378), iontophoretically applied, slightly increased the spontaneous firing rate and potentiated the NMDA-induced neuronal activation of dopaminergic neurons in the A9 and A10 regions. (+)*N*-cyclopropylmethyl-*N*-methyl-1,4-diphenyl-1-ethyl-butyl-2-*N* (JO-1784), another selective σ_1 receptor ligand produced no or little effect in these areas. The systemic administration of the selective σ_2 receptor ligand 1,4-bis-spiro[isobenzofuran-1(3*H*), 4′-piperidin-1′yl]butane (Lu 29–252) (2 mg/kg, i.v., cumulative dose) did not modify the firing activity of A9 and A10 dopaminergic neurons, but significantly potentiated the NMDA-induced increase in firing activity of A10 dopaminergic neurons. None of the σ receptor ligands tested had any effects on the dopamine-induced suppression of firing. In the nucleus accumbens, the systemic administration of (JO-1784), (40 μg/kg, i.v.), (+)-pentazocine (30 μg/kg, i.v.), another selective σ_1 receptor ligand, and of the non selective σ_1 receptor ligand di-tolyl-guanidine (DTG) (20 μg/i.v.) produced a significant increase of NMDA-induced neuronal activation. Microiontophoretic applications of JO-1784 also potentiated the NMDA response. They also increased significantly the suppressant effect of dopamine on NMDA and kainate-induced activations of accumbens neurons. In the caudate nucleus, (+)-pentazocine, but not JO-1784, potentiated slightly the neuronal response to NMDA. None of the σ receptor ligands tested did modify significantly the responses of caudate and accumbens neurons to kainate. These findings suggest that at least two subtypes of σ_1 receptors may affect differentially the glutamate NMDA neurotransmission in the terminal and origin regions of the mesolimbic and nigrostriatal dopaminergic systems. These results also demonstrate the existence of a functional interaction between σ_2 and NMDA receptors in the A10 region.

Keywords: Dopamine neuron; Extracellular recording; Ventral tegmental area; Substantia nigra compacta; Nucleus accumbens; Caudate nucleus; Microiontophoresis

1. Introduction

Since early observations had reported that several neuroleptics, including haloperidol, have high affinity for σ receptors, the possibility that these receptors may be a potential target for antipsychotic drugs has been intensively considered (Debonnel and de Montigny, 1996). Many investigations have focused on potential interactions of σ receptors with the central dopaminergic activity. Radioligand binding studies have demonstrated that σ receptors are abundant in brain dopamine-rich regions. In particular, in the *substantia nigra compacta* their density has been found to decrease markedly after a selective lesion of dopaminergic neurons (Gundlach et al., 1986; Graybiel et al., 1989). Despite this neuroanatomical relation, neurochemical and electrophysiological studies have failed to elucidate the precise interaction between σ receptors and dopaminergic neurotransmission (Debonnel and de Montigny, 1996). Many studies, however, have been

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hampered by the fact that most of the σ receptor ligands tested were not completely selective, displaying, beside their affinity for σ receptors, a moderate to high affinity for phencyclidine sites and/or monoamine receptors. Some newer drugs, such as (+)-pentazocine, di-tolyl-guanidine (DTG) and (+)*N*-cyclopropylmethyl-*N*-methyl-1,4-diphenyl-1-ethyl-butyl-2-*N*-1-ylamine-hydrochloride (JO-1784) exhibit a higher selectivity for σ receptors. In previous electrophysiological studies, they were found to have little or no effect on dopaminergic cell firing activity when administered chronically or acutely at high doses (Zhang et al., 1992, 1993a,b). However, several σ receptor ligands have been shown to exert complex effects, with biphasic dose–response curves in other experimental paradigms (Earley et al., 1991; Maurice et al., 1994a,b; Bergeron et al., 1995), indicating that a more thorough evaluation of the effects of low doses was required.

In the hippocampus, where they are also abundant (Gundlach et al., 1986), σ receptors are thought to play a role in the modulation of the glutamatergic neurotransmission, via an indirect modulation of the *N*-methyl-D-aspartate (NMDA) receptor ion channel complex (Debonnel et al., 1996). Previous results from our laboratory, using extracellular recordings from pyramidal neurons of the CA_3 region of the dorsal hippocampus of anaesthetized rats have demonstrated that several selective σ ligands, when applied by microiontophoresis or administered intravenously at low doses, exert a selective potentiating effect on NMDA-induced neuronal activation (Debonnel et al., 1990; Monnet et al., 1992; Gronier and Debonnel, 1996). Interestingly, the dose–response curves obtained with most of the agonists tested thus far in this model have a bell-shaped aspect (Couture and Debonnel, 1998).

Like the hippocampus, both the origin and projection areas of the meso(cortico)limbic and nigrotriatal dopaminergic systems receive large glutamatergic inputs (Beckstead et al., 1979; Phillipson, 1979). Recent electrophysiological studies have indicated that the glutamate innervation of midbrain dopaminergic nuclei exerts a tonic influence on the firing activity of dopaminergic neurones, via NMDA receptor activation (Overton and Clark, 1992; Chergui et al., 1993). There is also strong electrophysiological evidence for a close functional interaction between the glutamate and dopamine inputs in dopaminergic terminal regions, such as the nucleus accumbens (Debonnel and de Montigny, 1988; Mulder et al., 1996) and the striatum (Calabresi et al., 1993).

Therefore, for a better understanding of the putative functional role of the σ receptors on dopaminergic activity, it became important to examine whether these sites could influence the glutamate neurotransmission in the brain dopaminergic systems. In the present electrophysiological studies, we assessed the effects of σ ligands, applied iontophoretically or administered systemically at low doses, on glutamate- and dopamine-mediated responses. Those experiments were carried out both in the origin areas (ventral tegmental area or A10 and substantia nigra compacta or A9) and in the main projection areas (nucleus accumbens and the adjacent part of the caudate nucleus) of the nigrostriatal and mesolimbic dopaminergic systems.

2. Material and methods

Male Sprague–Dawley rats were anestethized and mounted in a stereotaxic apparatus. Recordings were obtained with either single barrel or 5-barrel microiontophoretic pipettes which comprises a central recording barrel, 3 surrounding barrels for drug ejection and a balance channel filled with 2 M NaCl for current neutralization. The recording barrel was filled with a 2 M NaCl solution saturated with fast green FCF. Action potentials were screened via a differential amplitude discriminator which generated square pulses. These pulses were fed to a computer, from which integrated firing rate histograms were obtained.

2.1. Recording in the nucleus accumbens and the caudate nucleus

In this series of experiments, rats were anesthetized with urethane (1.25 g/kg, i.p.). This anaesthetic was chosen to obtain data comparable with those previously obtained with our studies in the hippocampus. The tips of the recording electrodes were broken back under microscope control to an 8–12 μm diameter. The side barrels used for drug ejection were filled with the following solutions: DA 500 mM, NaCl 50 mM, pH 4; kainate 1 mM, NaCl 200 mM, pH 8; NMDA 5 mM, NaCl 200 mM, pH 5; JO-1784 5 mM, NaCl 150 mM, pH 4. In theses conditions, the impedance of the central barrel was typically between 1 and 3 MΩ and those of the other side barrels were typically between 25 and 50 MΩ. Caudate neurons were recorded in a region defined stereotaxically as 9.5–11 mm anterior to lambda, 0.8–1.5 mm lateral to the midline and 4.0–5.5 mm below the cortical surface. Accumbens neurons were recorded in a region defined stereotaxically as 9.5–11 mm anterior to lambda, 0.8–1.5 mm lateral to the midline and 6.0–7.9 mm below the cortical surface. The lower limit of the nucleus accumbens was recognized by the appearance of the fast firing (> 25 spikes/s) neurons of the olfactory tubercles. A total of one to five cells was recorded from each animal. The location of the last cell was marked by ejecting a spot of Fast Green dye to allow subsequent histological verification of electrode placement.

2.2. Recording in the substantia nigra compacta and in the ventral tegmental area

In this series of experiments, rats were anesthetized with chloral hydrate (400 mg/kg). This anaesthetic was

chosen because the majority of studies concerning the effects of σ receptor ligands on dopamine cells firing activity have been carried out using chloral hydrate (Zhang et al., 1992, 1993a,b). In our experimental conditions, dopamine neurons could only be studied with recording electrodes of higher impedance than those used for accumbens neurons (typically between 3 and 10 MΩ). Consequently, the tips of the electrodes were broken back to a lower diameter (3–8 μm diameter). In these conditions, we found that it was necessary to fill the side barrel with higher concentration solutions in order to insure the ejection of a reasonable amount of drug (NMDA 50 mM, NaCl 100 mM, pH 4.5; 2[(4-Benzyl piperazin-1-yl) mothyl] naphthalene, dichiorydrate (S-21378) 5 mM in NaCl 100 mM, pH 5.5). Each barrel, including the recording barrel, was supplemented with a glass wire (100 μm). Impedances of the side barrels were typically between 60 and 90 MΩ. The dopaminergic neurons were identified according to their location within the ventral tegmental area region (2.7–3.9 mm anterior to lambda and 0.3–1.1 mm lateral to the midline, and 6.5 to 9 mm below the cortical surface) and the substantia nigra compacta (2.7–3.8 mm anterior to lambda and 2 to 2.4 mm lateral to the midline, and 6 to 8 mm below the cortical surface), and well established electrophysiological criteria (Grace and Bunney, 1983a,b), including spontaneous firing rate between 0.5 and 10 spikes/s (occurring sometimes in burst), triphasic waveforms (usually with a notched initial positive deflection), and long action potentials (duration 2–4 ms).

2.3. Drugs

The following substances were used: dopamine and DTG (Aldrich Chemical, Milwaukee, WI, USA); NMDA and kainate (Sigma, St. Louis, MO, USA); (+)-pentazocine (RBI, Natick, MA, USA); JO-1784 and JO-5220 were generous gifts from Dr. J.L. Junien (Jouveinal Research Institute, Fresnes, France), 1,4-bis-spiro[isobenzofuran-1 (3*H*), 4′-piperidin-1′yl]butane (Lu 29–252) a generous gift from Dr. T. Skärsfeldt (Lundbeck, Copenhagen, Denmark), 2-[4-(4-methoxy-benzyl) piperazin-1-yl-methyl]4-oxo[4*H*]-benzo-thiazolin-2-one (S-21377) and S-21378 were generous gifts from Dr. M.C. Rettori (Servier Research Institute, Paris, France).

2.4. Administration of σ receptor ligands

Intravenous doses of DTG, JO-1784, (+)-pentazocine, S-21377, Lu 29–252 (solubilized in saline pH 4.5) were administered via a lateral vein of the tail. JO-1784 and S-21378 were iontophoretically ejected at currents ranging from +10 to +20 nA and −5 to −15 nA, respectively. S-21377, Lu 29–252 and JO-5220 were very poorly soluble and therefore could not be applied by microiontophoresis.

2.5. Activation by excitatory substances

Alternate microiontophoretic applications of the excitatory substances (kainate and NMDA) were carried out, adjusting the ejecting currents to induce a similar degree of neuronal activation with each of them. For studies of A9 and A10 dopaminergic neurons, we used iontophoretic currents of NMDA ranging from −1 to −10 nA, ensuring an increase in the firing activity of 20% to 60%. For studies of accumbens and caudate neurons, the current used for NMDA ranged from −10 to −60 nA, and from −5 to −30 nA for kainate. Care was taken to keep the activations induced by kainate and NMDA in a physiological range (10–20 spikes/s). All applications of the excitatory amino acids were of 50–100 s. A small current (from 3 to 15 nA), of opposite polarity to the ejecting current, was used for retaining each compound between microiontophoretic applications. The degree of activation induced by each excitatory substance was calculated by determining the total number of supplementary spikes generated per nC (1 nC being the charge generated by 1 nA applied for 1 s). The effects of σ receptor ligands were assessed by determining the degree of activation induced by NMDA or kainate, before and after the i.v. administration of the σ receptor ligands, or before and during the long lasting microiontophoretic application of JO-1784 or S-21378.

2.6. Suppression by dopamine

In A9 and A10 regions, serial 60–80 s applications of dopamine at fixed intervals with intensities of current (1 to 15 nA), adjusted to ensure a degree of suppression of the firing activity of about 30%, were carried out. The degree of suppression of firing induced by dopamine was determined by measuring the number of spikes suppressed per nC of dopamine. In the accumbens and caudate nuclei, long term applications of dopamine were carried out during serial applications of kainate and NMDA. The effects of dopamine were evaluated by comparing the degree of activation of NMDA and kainate before and during the microiontophoretic applications of dopamine. The effect of σ receptor ligands on the responsiveness to dopamine was assessed by comparing the suppressing effects of dopamine before and during the microiontophoretic application of the σ receptor ligand.

2.7. Statistical analysis

All results are expressed as the mean ± standard error of the mean (S.E.M.) of the number of spikes generated/nC. Statistical significance was assessed using the two-tailed Student's *t*-test and paired Student's *t*-test,

when appropriate. Probability values smaller than 0.05 were considered as significant.

3. Results

3.1. Effects of σ receptor ligands on dopaminergic neurons (substantia nigra compacta and ventral tegmental area)

A total of 116 cells were studied. Fifty-five neurons were recorded from the substantia nigra compacta and 61 neurons were recorded from the ventral tegmental area. All filled electrophysiological criteria previously described for midbrain dopaminergic cells (Grace and Bunney, 1983a,b). Given their potential biphasic effects, the σ receptor ligands were first tested at doses (ranging from 30 to 200 μg/kg, i.v.) lower than those used in previous electrophysiological studies, bearing on the effects of σ receptor ligands on dopaminergic activity. These doses have previously been shown to produce a marked potentiating effect on the neuronal response to NMDA in the dorsal hippocampus (Debonnel et al., 1990; Monnet et al., 1990; Monnet et al., 1992; Debonnel et al., 1995; Gronier and Debonnel, 1996; Couture and Debonnel, 1998). None of the four σ receptor ligands tested (DTG, JO-1784, S-21377 and Lu 29–252; $n = 5–12$ cells/group) did produce any

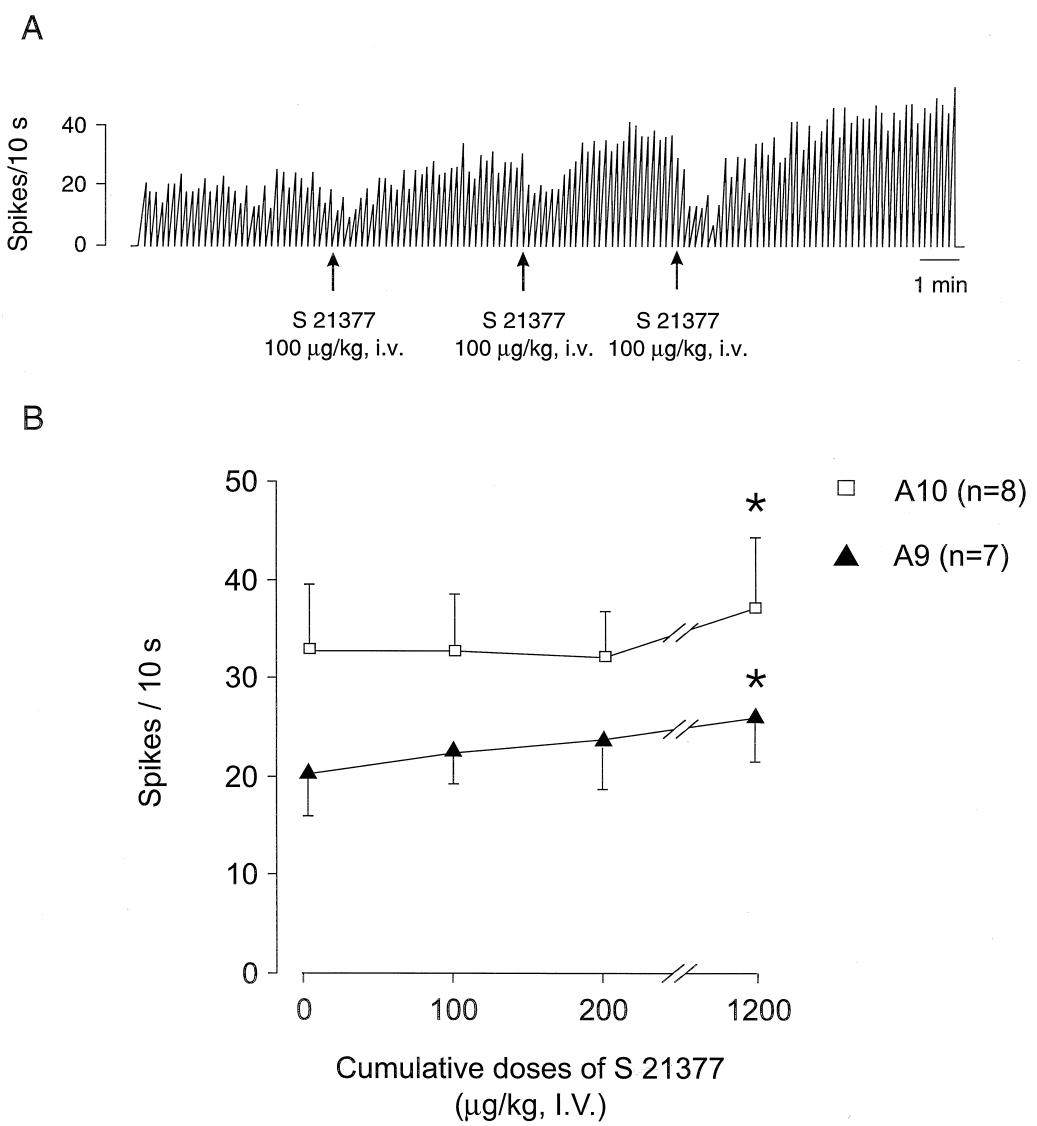

Fig. 1. (A) Firing rate histogram showing the response of one representative A9 midbrain dopaminergic neuron to cumulative i.v. administration of the selective σ_1 agonist S-21377. (B) Effect of a cumulative i.v. administration of the selective σ1 agonist S-21377 on the mean basal firing rate (±S.E.M.) of A9 and A10 midbrain dopaminergic neurons. * $P < 0.02$, compared to the corresponding values before the administration of S-21377; paired Student's *t*-test.

significant effect on the basal firing activity of the A9 and A10 dopaminergic neurons, when administered at low doses (30 μg/kg and 200 μg/kg).

Given that the low doses were ineffective in modifying the firing rate of dopaminergic neurons, the effects of cumulative doses were investigated using the two ligands

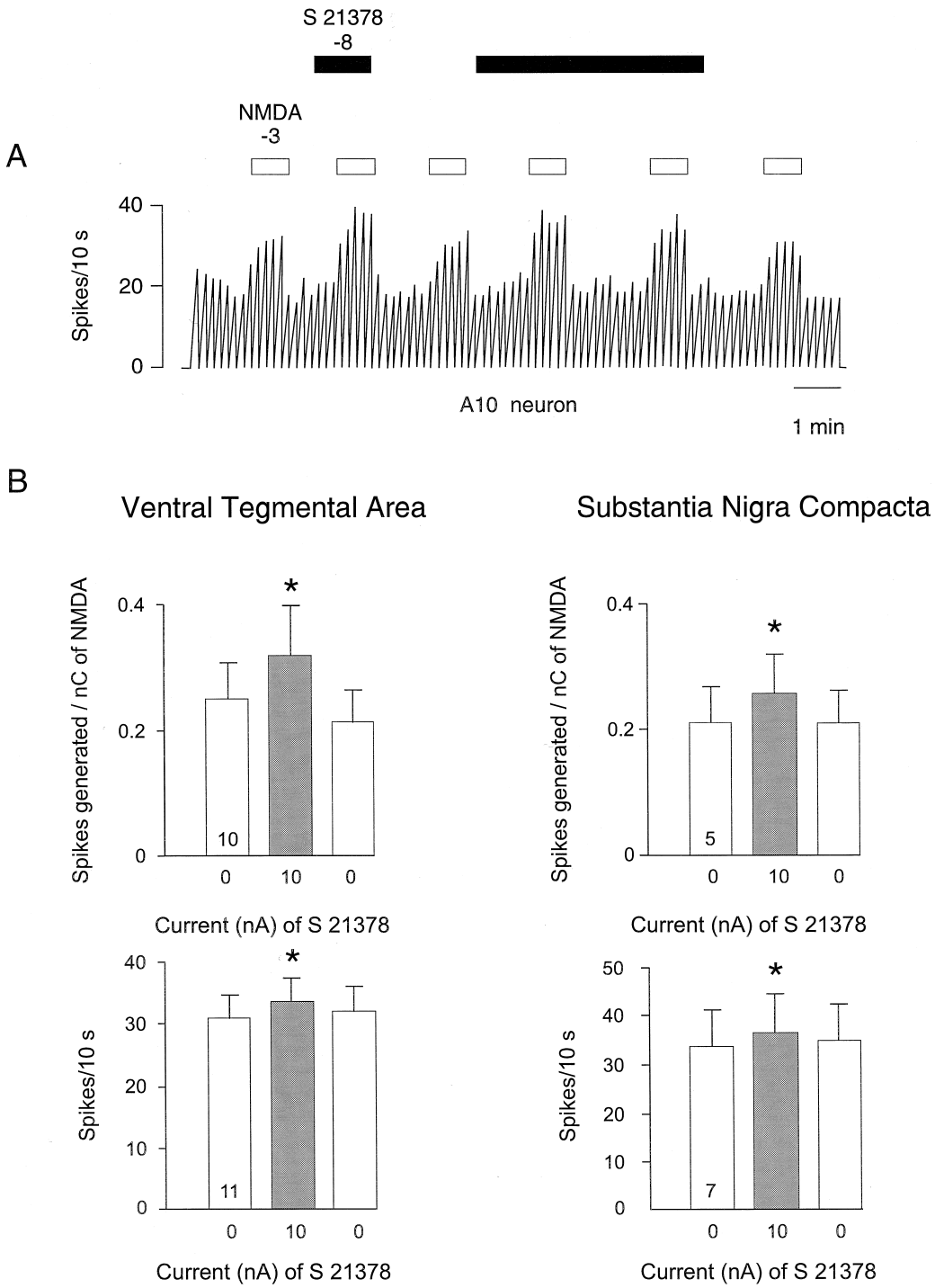

Fig. 2. (A) Firing rate histogram showing the response of one representative A10 midbrain dopaminergic neuron to iontophoretic application of low currents of NMDA before, during and after the iontophoretic application of the selective σ_1 agonist S-21378. In this and the following histograms, bars indicate the duration of the application of a compound for which currents are given in nA. The microiontophoretic application of S-21378 only very slightly increase the basal firing activity of this particular dopamine neuron while it increased the NMDA response by more than 25%. (B) Mean firing activity (mean ± S.E.M.) and responsiveness (expressed as the number of spikes generated per nC ± S.E.M.) to applications of NMDA of A9 and A10 dopaminergic neurons before, during, and after the iontophoretic application of the selective σ ligand S-21378. In this series of experiments, the same neurons were recorded during the complete sequence of drug administration. $* P < 0.02$, compared to the corresponding values before the application of S-21378; paired Student's *t*-test.

S-21377 and Lu 29–252, which display high affinity and a very high selectivity for σ_1 and σ_2 receptors, respectively (Debonnel et al., 1995; Perregaard et al., 1995). Administration of increasing doses of the selective drug S-21377 (100–1200 μg/kg), accelerated the firing activity of A9 neurons (Fig. 1A) in a dose-dependent manner, up to 25% above the baseline, whereas only the highest dose of S-21377 effectively stimulated A10 dopaminergic cells

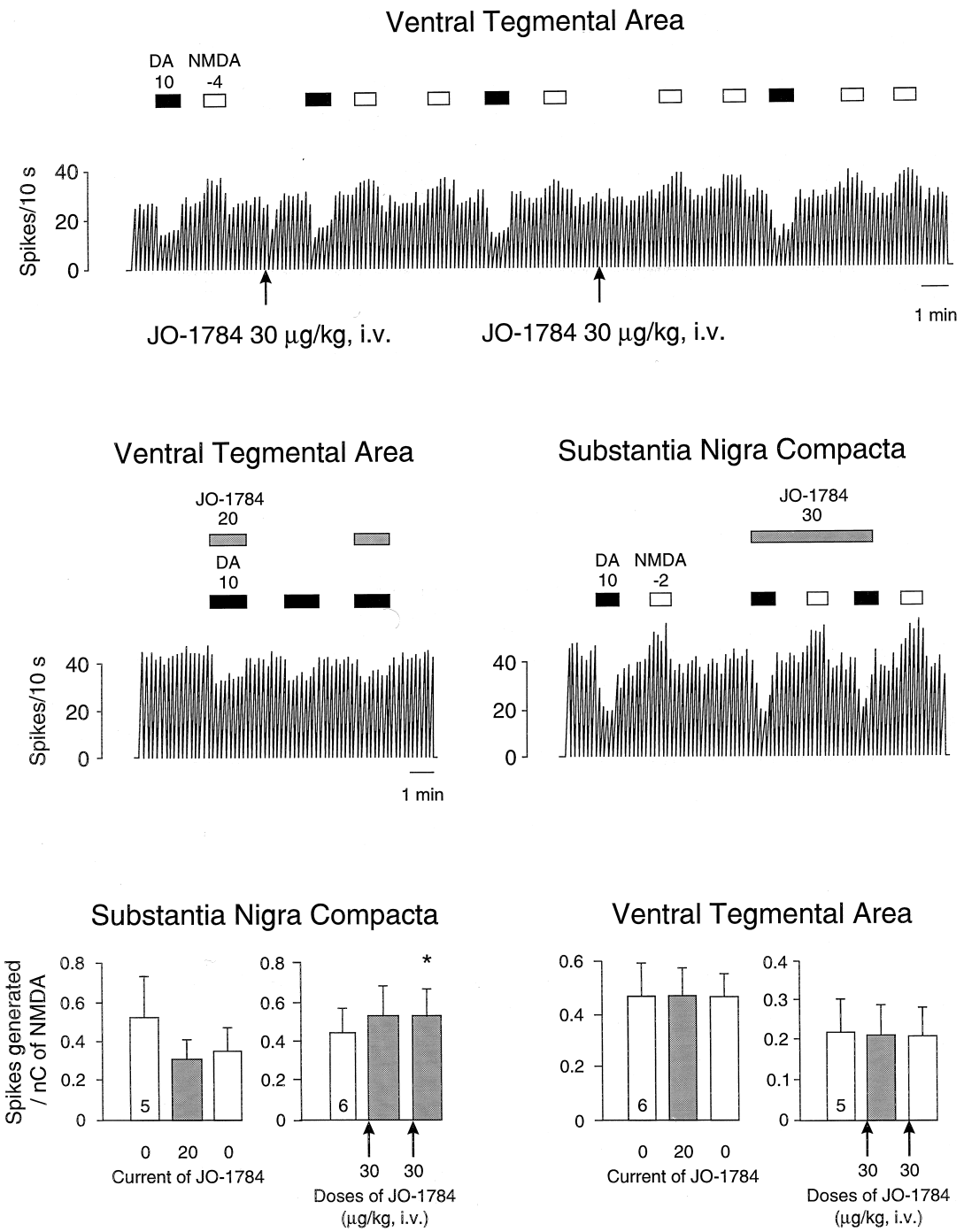

Fig. 3. Top: Firing rate histograms showing the responses of three midbrain A9 and A10 dopaminergic neurons to iontophoretic applications of NMDA and dopamine before, during and after the iontophoretic application of the selective σ_1 ligand JO-1784, or before and after the i.v. administration of JO-1784. Bottom: Responsiveness (expressed as the number of spikes generated per nC ± S.E.M.) to applications of NMDA of A9 and A10 dopaminergic neurons before, during and after the iontophoretic application of the selective σ_1 ligand JO-1784, or before and after the i.v. administration of JO-1784. In this series of experiments, the same neurons were recorded during the complete sequence of drug administration. $^{*}P < 0.05$, compared to the corresponding values before the administration of JO-1784; paired Student's *t*-test.

(Fig. 1B). This acceleration was sometimes preceded by an initial short-lasting depression of the firing activity (Fig. 1A). Such effect occurs immediately after the drug administration and was probably not related to a central effect of the σ agonist. The basal firing activity of both A10 ($n = 16$), and A9 ($n = 12$), dopaminergic neurons remained unchanged during the administration of the selective σ_2 receptor ligand Lu 29–252 at doses up to 1.5 mg/kg (data not shown). Iontophoretic applications of the ligand JO-1784 had no effect on the basal firing activity of A9 and A10 dopaminergic neurons, whereas S-21378, another selective σ_1 receptor ligand and a close analog of S-21377, very slightly, but significantly increased the basal firing activity during its local application (Fig. 2).

All the dopaminergic cells in the A9 and A10 regions responded to the microiontophoretic application of NMDA by an increase of their firing activity. As previously described, this increase in firing rate was dose-dependent and was accompanied by an augmentation in burst-firing (Overton and Clark, 1992; Chergui et al., 1993). The ejection currents of NMDA required to induce a cell activation were low. In general, currents between −1 and −7 nA were adequate to insure an increase in the firing activity of more than 20%. Systemic (60 μg/kg i.v., cumulative doses) or iontophoretic administrations of JO-1784 had no significant effect on the neuronal response to NMDA of A10 dopaminergic neurons (Fig. 3). In the A9 region, the NMDA response of dopaminergic neurons

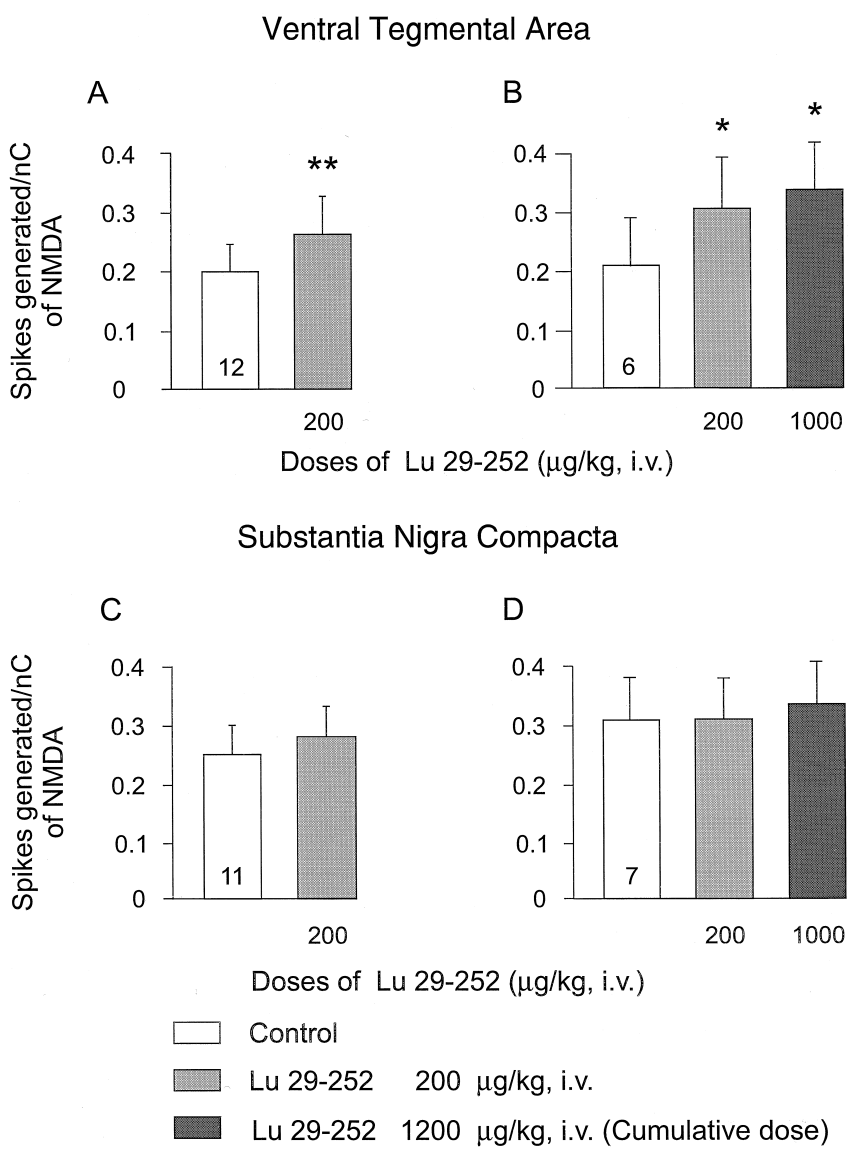

Fig. 4. Responsiveness (expressed as the number of spikes generated per nC + S.E.M.) to applications of NMDA of A10 (A, B) and A9 (C, D) dopaminergic neurons before and after the i.v. administrations of one dose (200 μg/kg, A, C), or two subsequent doses (200 and 1000 μg/kg, B, D) of the selective σ_2 agonist Lu 29–252. In this series of experiments, the same neurons were recorded during the complete sequence of drug administration. $^{*}P < 0.05$, $^{**}P < 0.02$, compared to the corresponding values before the administration of Lu 29–252; paired Student's *t*-test.

remained unchanged during the local application of JO-1784, but was slightly increased after the subsequent i.v. administration of two cumulative doses of JO-1784 (60 μg/kg). This increase, however, was short-lasting, occurring generally during the first 15 min after the second i.v. administration of JO-1784. Contrary to JO-1784, the long-lasting microiontophoretic applications of the σ_1 selective ligand S-21378 potentiated the neuronal response induced by NMDA of both A9 and A10 dopaminergic neurons (Fig. 2). S-21377, at the same dose which accelerated the activity of A10 neurons (cumulative dose of 1.1 mg/kg, i.v.), also exerted a slight potentiating effect of the neuronal response to NMDA of A10 dopaminergic neurons (0.132 ± 0.018 vs. 0.110 ± 0.02, spikes generated/nC of NMDA, $n = 6, P < 0.05$). The i.v. administration of 200 μg/kg of the selective σ_2 receptor ligand Lu 29–252 induced a significant potentiation of the NMDA response of dopaminergic neurons in the A10 region (Fig. 4). An additional dose of 1 mg/kg did not produce a greater effect than the initial dose of 200 μg/kg (Fig. 4). There was a large variation in the degree of potentiation from one neuron to another, from 0% to 120%. In the A9 region, the global effect of Lu 29–652 on the NMDA response of dopaminergic neurons was not significant ($p < 0.11$, $n = 11$; Fig. 4). Four A9 dopaminergic neurons out of the 12 studied, however, had their NMDA response increased by more than 30%.

The microiontophoretic application of dopamine (1–10 nA) resulted in a current-dependent suppression of the firing activity of A9 and A10 dopaminergic neurons. The suppressant effect of dopamine was variable from one neuron to another. Currents of dopamine (3–10 nA) were adjusted to ensure a degree of suppression of firing of 20–30%. Neither the σ_1 selective ligands JO-1784 and S-21378 (iontophoretically or intravenously administered, 60 μg/kg), nor the σ_2 selective drug Lu 29–252 (200 and 1000 μg/kg, i.v.) did produce any effect on the dopamine-induced suppression of firing in both the A9 and A10 regions ($n = 5$–10 cells/group, Fig. 3).

3.2. Nucleus accumbens and caudate nucleus

A total of 99 neurons were studied, 63 neurons were recorded from the nucleus accumbens and 36 neurons from the adjacent part of the caudate nucleus. As previously described, in anesthetized animals, nucleus accumbens and caudate nucleus contain both quiescent and spontaneously active neurons with low spontaneous firing rates (0–5 Hz). Microiontophoretic applications of kainate and NMDA activated all the neurons recorded. There was no difference between the two regions studied with respect to their neuronal responses to kainate and NMDA. In general, both kainate and NMDA promoted a neuronal activation characterized by periodic bursts separated by long silent periods. In some neurons, the amplitude of the neuronal response to kainate and NMDA decreased markedly upon repeated applications. This phenomenon may be due to a progressive desensitization of the cells during excitatory amino acid receptor activation, since we observed more stable responses after increasing the time interval between each application. Only the data obtained from neurons showing a stable response to repeated applications of excitatory substances were included in the present studies. The three ligands tested (JO-1784, (+)-pentazocine and DTG) were used at the same low doses that had previously been used in the rat dorsal hippocampus.

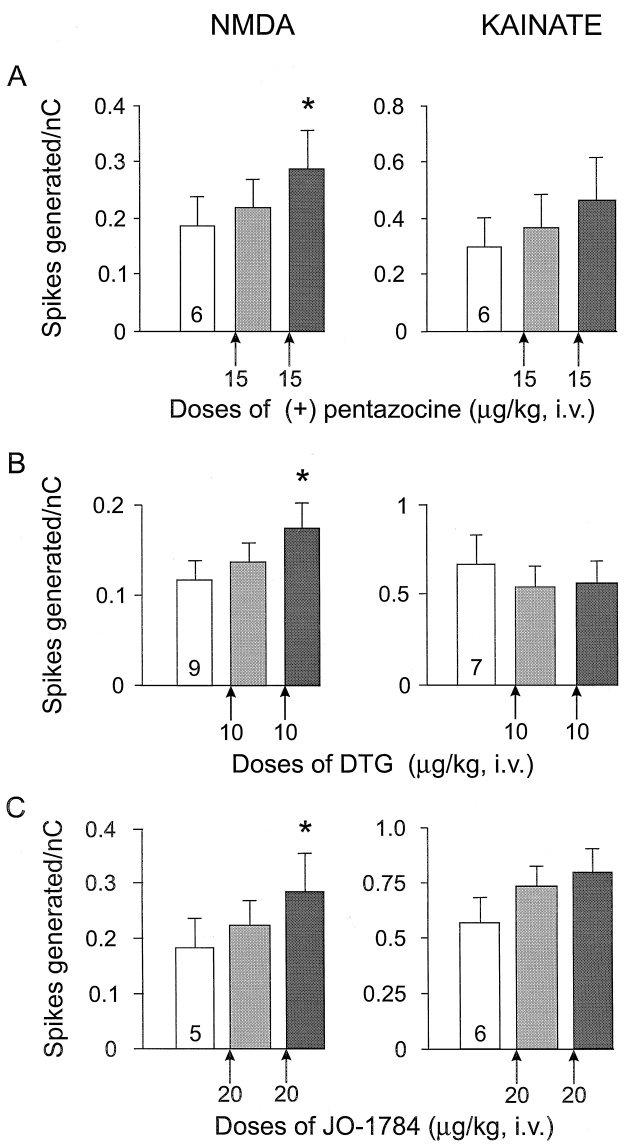

Fig. 5. Responsiveness (expressed as the number of spikes generated per nC ± S.E.M.) to applications of NMDA (left) and kainate (right) of accumbens neurons before and after two subsequent i.v. administrations of the selective σ receptor ligands (+)-pentazocine (A), DTG (B) and JO-1784 (C). In this series of experiments, the same neurons were recorded during the complete sequence of drug administration. $^{*}P < 0.03$, compared to the corresponding values before the administration of the σ agents; paired Student's *t*-test.

In the nucleus accumbens, two subsequent i.v. administrations of (+)-pentazocine (15 μg/kg), DTG (10 μg/kg) and JO-1784 (30 μg/kg) exerted a potentiating effect on the neuronal response to NMDA (Fig. 5). In one third of the neurons tested with DTG and JO-1784, the increase in NMDA neuronal response was followed by an enhancement of the basal firing activity. The neuronal response to kainate was not significantly affected by the i.v. administration of each of these σ receptor ligands, though some neurons treated with JO-1784 (2 out of 6) and (+)-pentazocine (4 out of 12) showed a concomitant increase in the neuronal responses to kainate and NMDA. In the caudate nucleus, (+)-pentazocine, but not JO-1784, increased slightly but significantly the NMDA-induced neuronal activation (Fig. 6). Fig. 7 shows that the neuronal response to NMDA of accumbens neurons was markedly enhanced upon long lasting applications of JO-1784. This enhancing effects of the NMDA response of accumbens neurons was reversible, the neurons returning to their initial level of NMDA activation after the cessation of JO-1784 application (data not shown). Kainate-induced activations were also substantially increased in 9 out of 16 accumbens neurons tested during the JO-1784 applications. However, this increase persisted after the cessation of JO-1784 applications (data not shown). The potentiation of the NMDA response upon JO-1784 application was completely suppressed following the i.v. administration (100 μg/kg) of the new putative σ antagonist JO-5220 (Fig. 7). In contrast with the high affinity σ antagonist haloperidol, this compound is devoid of affinity for dopaminergic receptors (Junien and Roman, personal communication). As observed in Fig. 7, both kainate and NMDA-induced activations appear to decrease below their initial levels following the administration of JO-5220. This phenomenon had already been sometimes observed in the hippocampus with the σ antagonist haloperidol, which not only reversed the potentiation of the NMDA response induced by σ agonist, but also render the NMDA and quisqualate responses

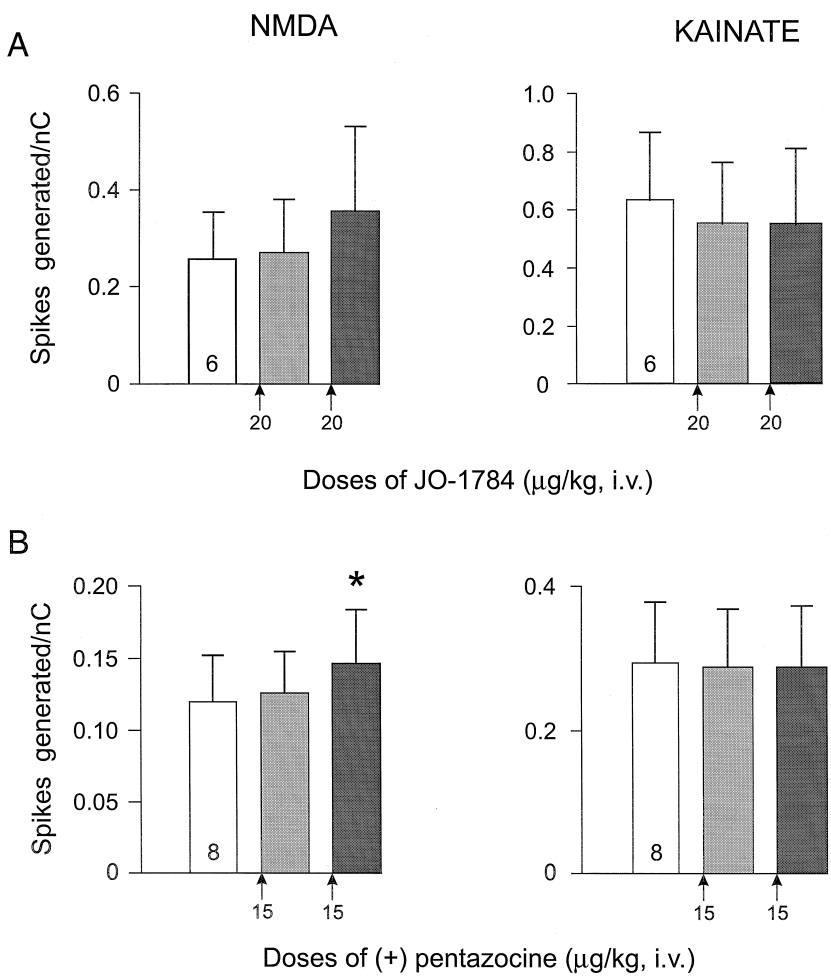

Fig. 6. Responsiveness (expressed as the number of spikes generated per nC ± S.E.M.) to applications of NMDA (left) and kainate (right) of caudate neurons before and after two subsequent i.v. administrations of the selective σ receptor ligands JO-1784 (A) and (+)-pentazocine (B). In this series of experiments, the same neurons were recorded during the complete sequence of drug administration. * $P < 0.05$, compared to the corresponding values before the i.v. administration the drugs; paired Student's *t*-test. * $P < 0.05$, compared to the corresponding values before the application of JO-1784; paired Student's *t*-test.

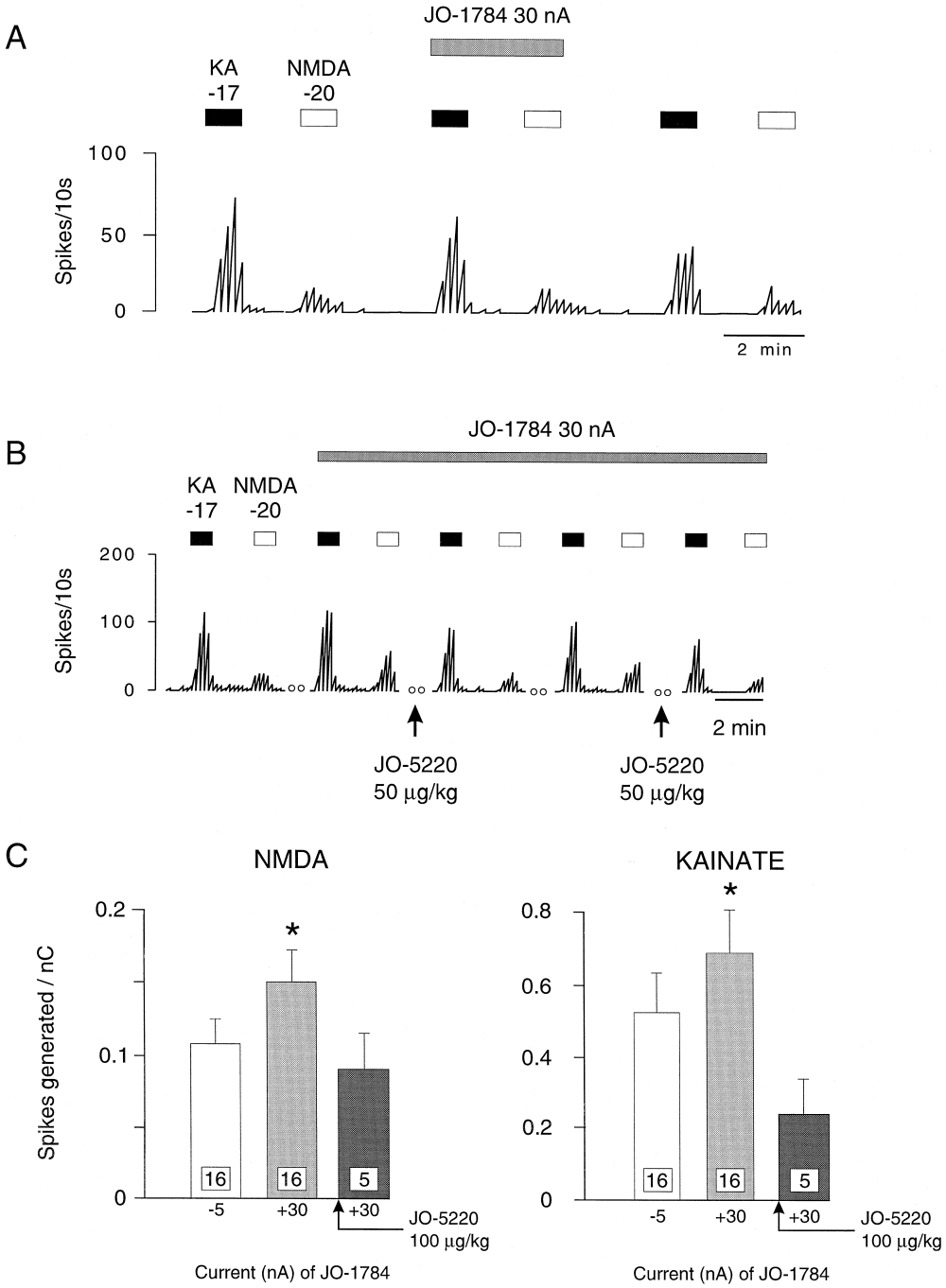

Fig. 7. (Top) Firing rate histogram showing the responses of a caudate neuron to iontophoretic applications of NMDA and kainate before, during, and after the iontophoretic application of the selective σ agonist JO-1784. (Bottom) Firing rate histogram showing the response of an accumbens neuron to iontophoretic application of NMDA and kainate before and during the iontophoretic application of the selective σ_1 receptor ligand JO-1784, and after two subsequent i.v. administrations of the putative σ antagonist JO-5220. (C) Responsiveness (expressed as the number of spikes generated per nC ± S.E.M.) to applications of NMDA and kainate before and during the iontophoretic application of the selective σ_1 agonist JO-1784, and after the i.v. administration of the putative σ antagonist JO-5220. $^{*}P < 0.05$, compared to the corresponding values before the application of JO-1784; paired Student's *t*-test.

sometimes lower than their initial levels. However, when JO-5220 was administered in another group of accumbens neurons or in hippocampal pyramidal cells which were not previously treated with JO-1784, there was no evidence that JO-5220 induced a decrease in the NMDA and kainate activation (data not shown). Contrarily to accumbens neurons, the response of caudate neurons to NMDA and kainate application remained unchanged during the iontophoretic application of JO-1784 (NMDA 0.19 ± 0.07 vs. 0.2 ± 0.07 spikes generated per nC ($n = 12$) and kainate 0.36 ± 0.07 vs. 0.42 ± 0.12 spikes generated per nC ($n = 10$), before and during JO-1784 applications, respectively).

Long term applications of 20 or 30 nA of dopamine decreased both NMDA and kainate-induced firing activities of accumbens neurons. The degree of inhibition induced by dopamine was not significantly different whether when applied concomitantly with NMDA or with kainate. When JO-1784 was applied concomitantly with dopamine, its suppression of the NMDA- and kainate-induced firing activities was significantly enhanced (Fig. 8).

4. Discussion

4.1. A9 and A10 regions

Some of the drugs used in the present study have been tested previously on the basal firing activity of dopaminergic cells (Zhang et al., 1992, 1993b,a), but their effects had been investigated at very high doses compared to those

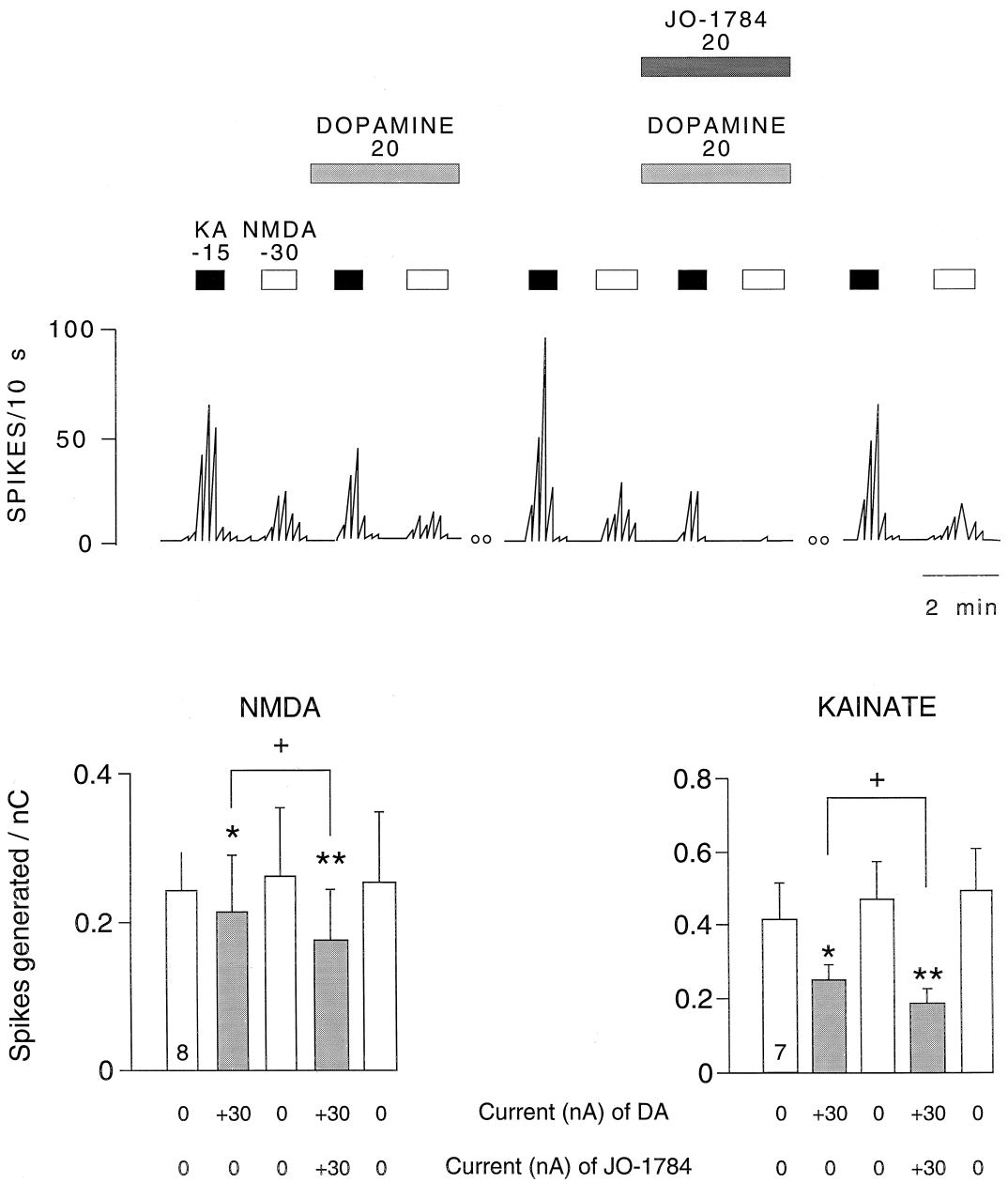

Fig. 8. (Top) Firing rate histogram of an accumbens neuron illustrating the inhibitory effect of iontophoretic applications of dopamine on NMDA and kainate-induced activation, before and during the iontophoretic application of the selective σ_1 agonist JO-1784. (Bottom) Responsiveness (expressed as the number of spikes generated per nC ± S.E.M.) of accumbens neurons to applications of NMDA and kainate before and during the iontophoretic application of dopamine, and before and during the concomitant application of dopamine with JO-1784. In this series of experiments, the same neurons were recorded during the complete sequence. $^{*}P < 0.05$, $^{**}P < 0.03$ compared to the corresponding values before the application of dopamine; $^{+}P < 0.05$, compared to the corresponding values before the application of JO-1784, paired Student's *t*-test.

required to exert an effect in the dorsal hippocampus. Given that these drugs may have biphasic action (Earley et al., 1991; Maurice et al., 1994a,b; Bergeron et al., 1995), it was of interest to undertake a more detailed evaluation on the effect of low doses, on the firing activity. It appears clearly in our study that none of the σ receptor ligands tested, when administered at low doses, significantly affected the firing activity. Only the σ_1 receptor ligand S-21377, when administered at doses above 1 mg/kg had a slight excitatory effect on both A9 and A10 dopaminergic neurons. Previous ligand binding studies have shown that S-21377 displays high and selective affinity for σ_1 vs. σ_2 receptors and is apparently devoid of affinity for other receptors including the phencyclidine receptor (Debonnel et al., 1995), suggesting that σ receptors may contribute directly to this excitatory effects. However, it appears that S-21377 exerts its effect at relatively high doses, suggesting that a large occupancy of receptors would be necessary to promote a small activatory effect. In this regard, S-21377 produces the same weak accelerating effects on dopaminergic neurons as the other selective σ receptor ligands (*Z*)-3(hexahydroazepin-1-yl)-1-(3-chloro-4-cyclohexylphenyl)propene-1, hydrochloride (SR31742A) and 6-[6-(4-hydroxypiperidinyl)hexyloxy]-3-methylflavone HCl (NPC16-37) (Poncelet et al., 1993; Shepard et al., 1994).

On the other hand, σ_2 receptors do not appear to be involved in the regulation of the basal firing activity of dopaminergic neurons. The σ_2 receptor ligand Lu 29–252 was ineffective in modifying the spontaneous activity, at a dose which provides a large occupancy of brain σ_2 receptors, since five-time lower doses were adequate in the present study to potentiate the NMDA neuronal response in the ventral tegmental area.

The modest electrophysiological changes induced by S-21377 differ from those obtained with classical neuroleptic-like drugs such as haloperidol or chlorpromazine which increase the firing rate by as much as 100% at lower doses (Bunney et al., 1973). In addition, unlike typical antipsychotics and like all other σ receptor ligands tested in this study, S-21377 failed to reverse the inhibitory effect induced by dopamine-autoreceptor stimulation.

JO-1784, another selective σ receptor ligand, administered at low (present study) or high doses (Zhang et al., 1992) has been found to be ineffective in modifying the firing rate of A9 and A10 dopaminergic neurons. This may suggest that JO-1784 and S-21377 bind to distinct subtypes of receptors. This is in keeping with previous electrophysiological studies carried out in our laboratory that have demonstrated the existence of at least two classes of σ_1 receptors, with distinct neuronal localization (Debonnel et al., 1996) and associated with different second messenger systems (Monnet et al., 1994), in the rat dorsal hippocampus.

Our findings obtained with S-21377 could be consistent with those of some neurochemical in vivo studies, showing that some σ agonists like DTG or (+)-pentazocine increase the basal dopamine release and/or turnover from dopaminergic cell terminals in the striatum (Patrick et al., 1993; Gudelsky, 1995), the olfactory tubercles (Iyengar et al., 1990), and the frontal cortex (Gudelsky, 1995), indicating that they possibly promote an activation of mesocorticolimbic and nigrostriatal dopaminergic neurons (Gudelsky, 1995). However, these stimulatory effects were often observed at very high doses, indicating that they may be associated to non-σ properties. On the other hand, SR31742A and NPC16377, two other more selective σ receptor ligands which increased slightly the firing activity of midbrain dopaminergic neurons were found to have no detectable effect on the basal dopaminergic metabolism in dopaminergic terminal regions (Karbon et al., 1993; Poncelet et al., 1993).

Our present data confirm previous observations that microiontophoretic applications of NMDA elicit a profound excitatory effect on midbrain dopaminergic neurons (Overton and Clark, 1992; Chergui et al., 1993). Contrarily to what was observed in the hippocampus, JO-1784 failed to induce any change of the NMDA neuronal response in A10 dopaminergic neurons. On the other hand, we found that iontophoretic applications of S-21378, another selective σ receptor ligand, potentiate the neuronal response to NMDA as it did in the hippocampus (Debonnel et al., 1995, 1996). These results constitute another argument for the existence of two subtypes of σ_1 receptors, non uniformly distributed in the rat central nervous system. The other σ_1 receptor ligand S-21377 was also found to exert a potentiating effect on the NMDA response of A10 neurons when administered at high doses, suggesting that probably S-21377 and S-21378, which have a very similar chemical structure, bind to the same subtype of σ receptor.

The administration of moderate doses of the selective σ_2 receptor ligand Lu 29–252 markedly potentiates NMDA-induced neuronal response to dopaminergic neurons in the Ventral Tegmental Area and of some A9 neurons. Other σ_2 selective ligands have previously been found to potentiate effectively the NMDA neuronal response in the rat dorsal hippocampus at similar doses (Couture and Debonnel, 1998). It is noteworthy that, in this area, and contrarily to what was found in the hippocampus, σ_2 receptors, rather than σ_1 receptors, appear to play a more pivotal role in the control of the glutamate/NMDA neurotransmission. As a matter of fact, Lu 29–252 was found to stimulate the neuronal response to NMDA by more than 100% in more than one third of the A10 dopaminergic neurons tested. Such a level was never reached with σ_1 agonists. The situation in the A9 region is different since only a minority of dopaminergic neurons were sensitive to the σ_2 agonist.

4.2. Nucleus accumbens and caudate nucleus

Our observations, obtained from accumbens neurons, are qualitatively similar with previous results obtained

from our laboratory in the rat hippocampus (Debonnel et al., 1990; Gronier and Debonnel, 1996), since all the ligands tested potentiate selectively the neuronal response to NMDA at low to moderate doses. Indeed, much less consistent effects of σ receptor ligands were observed on the kainate response. Even if some accumbens neurons tested had their response to kainate increased upon the application of JO-1784, it is noteworthy that none of the σ receptor ligands which were systemically administered had any significant effect on this response. Our results raise the possibility that the regulation of the level of activation of the NMDA receptor may be an important function of σ receptors in the limbic system in general. However, in the nucleus accumbens, the sensitivity of NMDA receptors to σ agonists appears to be lower than in the hippocampus (Debonnel et al., 1990; Gronier and Debonnel, 1996). Indeed, JO-1784 and DTG were found to enhance the neuronal responses of hippocampal neurons to NMDA by 100–200%, whereas more modest activations, of only 20–50% were found in the nucleus accumbens. This discrepancy may in part be due to a more rapid desensitization of accumbens neurons to NMDA receptors activation.

In the caudate nucleus, there was no change in the responsiveness of neurons to NMDA following JO-1784 administration, whereas (+)-pentazocine, another selective ligand, exerted a weak potentiating effect. The difference of sensitivity of caudate neurons to JO-1784 and (+)-pentazocine is in keeping with earlier observations showing that, in the hippocampus, JO-1784 interacts with a subtype of receptors different from that activated by (+)-pentazocine (Monnet et al., 1994), suggesting again the existence of at least two subtypes of σ_1 receptors.

The fact that JO-1784 seems to modulate the inhibitory action of dopamine in the accumbens suggests the existence of a functional interaction between σ and dopaminergic receptors in this terminal dopaminergic region. These results may be considered in light of recent observations in the rat central nervous system which suggest that different neuropeptides including cholecystokinin (CCK) may be implicated in the mediation of the effect of σ receptors (Gué et al., 1992; Bouchard et al., 1995; Gronier and Debonnel, 1996). In the nucleus accumbens CCK coexists and interacts closely with dopamine (Debonnel and de Montigny, 1988; Crawley, 1991). As a matter of fact, this neuropeptide has been shown to potentiate the inhibitory effect of dopamine in a population of accumbens neurons (White and Wang, 1984). According to different experimental evidence, σ receptor activation might promote the central release of CCK (Gué et al., 1992; Gronier and Debonnel, 1996). Therefore, it is possible that the increase in dopaminergic sensitivity of accumbens neurons induced by JO-1784 is mediated by an indirect mechanism involving the activation of CCK neuronal pathway.

In conclusion, our study indicates that one role of the σ receptors located in the mesolimbic and nigrostriatal dopaminergic systems could be the modulation of the sensitivity of neurons to glutamate, via an interaction with NMDA receptors. These effects are variable, depending on the regional localization of the different subtypes of σ receptors involved. Our results also indicate that a functional interaction between σ and dopaminergic receptors may exist in the nucleus accumbens, but is not present in the midbrain dopaminergic nuclei.

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