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Competitive NMDA receptor antagonists do not produce locomotor hyperactivity by a dopamine-dependent mechanism

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Abstract

The involvement of dopaminergic activity in the mediation of the behavioural effects produced by blockade of NMDA receptors in the nucleus accumbens was investigated. Intra-accumbens infusion of the competitive NMDA receptor antagonist, DL-2-amino-5-phosphonovaleric acid (AP-5) (2, 4 and 10 μg/0.5 μl) induced a dose-dependent increase in locomotor activity in rats. Pharmacological blockade of dopamine receptors locally in the nucleus accumbens with haloperidol (5 μg/μl) failed to reduce the locomotor effects of AP-5 (10 μg), but antagonized the effects induced by the non-competitive NMDA receptor antagonist, MK-801 ((+)-5-methyl-10,11-dihydro(*a*,*d*)-cyclohepten-5,10-imine hydrogen maleate salt) (10 μg). The effects of dopamine co-administered with AP-5 at various doses in the nucleus accumbens were also examined. When the level of locomotor activity induced by AP-5 (10 μg) was similar to that produced by dopamine (10 μg), the simultaneous infusion of both compounds at this dose did not increase or decrease the locomotor response. When the level of locomotor activity induced by AP-5 (10 or 4 μg) was lower than that produced by a higher dose of dopamine (20 μg), the combined infusion of both compounds resulted in a locomotor response similar to that induced by AP-5 alone, indicating a reduction of dopamine locomotor effects. These results show that the locomotor hyperactivity induced by AP-5 was not modified when the dopaminergic activity in the nucleus accumbens was either reduced or enhanced, suggesting that the behavioural effects resulting from the blockade of NMDA receptors with the competitive NMDA receptor antagonist, AP-5, is not mediated by endogenous dopamine in this brain area.

Keywords: Excitatory amino acid; NMDA receptor antagonist; AP-5 (DL-2-amino-5-phosphonovaleric acid); MK-801 ((+)-5-methyl-10,11-dihydro(*a*,*d*)-cyclohepten-5,10-imine hydrogen maleate salt); Dopamine; Locomotor activity

1. Introduction

Excitatory amino acids acting through NMDA receptor subtypes appear to affect a variety of functions in the central nervous system. They have been involved in the coordination and the regulation of motor activity, sensory-motor integration and memory functions (Fagg, 1985; Willets et al., 1990). Behavioural studies in rodents showed that a systemic injection of non-competitive NMDA receptor antagonists such as phencyclidine or MK-801 ((+)-5-methyl-10,11-dihydro(*a*,*d*)-cyclohepten-5,10-imine hydrogen maleate salt), which are known to act directly at the phencyclidine binding site located within the ion channel of the NMDA receptor complex, induced a behavioural activation (locomotor hyperactivity and stereotypies) resembling that observed after administration of drugs enhancing dopaminergic neurotransmission (Schmidt et al., 1992). The behavioural stimulant effects of non-competitive NMDA receptor antagonists were reduced after pharmacological blockade of dopamine receptors or dopamine depletion (Clineschmidt et al., 1982; Dall'Olio et al., 1992; Ouagazzal et al., 1993; Gattaz et al., 1994; Martin et al., 1994) suggesting that these compounds produce their effect through dopaminergic-dependent mechanisms. Consistent with this suggestion, electrophysiological and neurochemical studies showed that the administration of non-competitive NMDA receptor antagonists increased the firing rate of midbrain dopamine neurons and dopamine turnover in areas of dopaminergic nerve terminals (Im-

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perato et al., 1990; French et al., 1991, 1993; Löscher et al., 1991; Svensson et al., 1991; Bubser et al., 1992; Zhang et al., 1992).

The blockade of NMDA receptors directly at the receptor site with competitive NMDA receptor antagonists injected systemically at high doses or in central nervous structures such as the nucleus accumbens was also found to induce locomotor activation and stereotyped behaviours (for review see Schmidt et al., 1992; Carlsson, 1993). Interestingly, prior administration of the mixed dopamine D_1/D_2 receptor antagonist, haloperidol, was found to reduce the locomotor activity induced after systemic administration of the competitive NMDA receptor antagonist, CPP (3-(-2-carboxy-piperazin-4-yl)-propyl-1-phosphonic acid) (Svensson et al., 1991). However, the prevalent hypothesis that a dopaminergic mechanism might be involved in the behavioural effects of competitive NMDA receptor antagonists has been questioned. Electrophysiological and neurochemical studies have indeed shown that competitive NMDA receptor antagonists injected at doses producing behavioural activation did not modify central dopaminergic neurotransmission (French et al., 1991; Svensson et al., 1991). Furthermore, in monoamine-depleted mice, systemic administration of competitive NMDA receptor antagonists is still able to produce locomotor activation (Carlsson, 1993). Thus, the involvement of dopaminergic mechanisms in the mediation of the behavioural stimulant effects produced by competitive NMDA receptor antagonists is still a matter of debate.

The present study was thus conducted to further elucidate the role of the mesolimbic dopaminergic system in the mediation of the locomotor activity observed after the blockade of NMDA receptors with competitive NMDA antagonists. The mesolimbic dopaminergic nerve terminals in the nucleus accumbens originating from cell bodies in the ventral tegmental area are known to play an important role in the expression of locomotor activity since local infusion of dopamine or dopaminergic agonists in the nucleus accumbens produces robust locomotor stimulation in rats (Pijnenburg et al., 1976; Makanjuola et al., 1980; Boss et al., 1988; Plaznik et al., 1989). Furthermore, either pharmacological blockade of dopamine receptors or selective destruction of dopamine terminals in this brain area inhibited the locomotor stimulation induced by systemic administration of psychostimulants, such as amphetamine (Pijnenburg et al., 1975; Kelly et al., 1976; Boss et al., 1988; Plaznik et al., 1989). The nucleus accumbens, in addition to its dopaminergic input, also receives prominent excitatory (probably glutamatergic) projections from various cortical structures such as the prefrontal cortex, amygdala and hippocampus (Fonnum, 1984). Numerous morphological and biochemical studies have shown that reciprocal cellular interactions linking the activity of the two afferent systems may contribute to the regulation of the functions mediated by the nucleus accumbens (Mogenson and Yang, 1991). The microinfusion of either competitive or non-competitive NMDA receptor antagonists in the nucleus accumbens also induces pronounced locomotor activation in rodents, suggesting that this brain structure may be a critical site of action for NMDA receptor antagonists injected systemically (Donzanti and Uretsky, 1984; Raffa et al., 1989; Carlsson, 1993; Amalric et al., 1994). Given that the activation of dopamine receptors within the nucleus accumbens seems to be critical for the expression of the locomotor activity induced by non-competitive NMDA receptor antagonists (Wallace et al., 1992; Ouagazzal et al., 1994), we further examined whether the blockade or activation of dopamine neurotransmission in the nucleus accumbens affects the locomotor activity induced by the competitive NMDA receptor antagonist, DL-2-amino-5-phosphonovaleric acid (AP-5), injected locally into this brain area.

2. Materials and methods

2.1. Animals

Male albino Wistar rats (Iffa-Credo, France) weighing 280–300 g at the start of the experiment were housed in groups of 3 per cage with free access to food and water. They were maintained under temperature-controlled conditions with an alternating 12-h light-dark cycle.

2.2. Stereotaxic surgery and infusion procedure

Stereotaxic surgery was performed under xylazine (15 mg/kg intra-muscular, i.m.) and ketamine (100 mg/kg i.m.) anaesthesia. The rats were implanted with bilateral stainless-steel guide cannulae (23-gauge) positioned 3 mm above the nucleus accumbens at coordinates: AP +3.2 mm, L ±1.7 mm, from the bregma, DV −4.8 mm from the skull surface with the incisor bar set at +5.0 mm, according to the atlas of Pellegrino et al. (1979). The cannulae were fixed to the skull with dental cement and stainless-steel screws. Wire stylets were inserted into the cannulae to prevent occlusion. One week after implantation, a bilateral 30-gauge injection needle was inserted through the guide to 3 mm beyond its tip and a volume of 0.5 μl was infused at a rate of 0.16 μl/min with a Hamilton syringe mounted on a microdrive pump. Following the injection, the injection needle remained in place for another 2 min to allow the drug to diffuse away from the injection site.

2.3. Drugs

AP-5 (DL-2-amino-5-phosphonovaleric acid; Tocris Neuramin, UK) and MK-801 ((+)-5-methyl-10,11-dihydro(*a*,*d*)-cyclohepten-5,10-imine hydrogen maleate salt; Merck Sharp and Dohme Research Laboratories, UK) were dissolved in a physiological saline solution, and the pH was adjusted to 6.5 with a minimum quantity of NaOH (0.1 N). Haloperidol (Haldol, injectable solution, Janssen) was injected at a dose of 5 μg/μl and control injections using the vehicle (a solvent of the commercial preparation for haloperidol) were tested. Dopamine (Sigma, France) was dissolved in a 0.9% saline solution containing ascorbic acid (0.1 mg/ml) as an antioxidant.

2.4. Behavioural testing

Measurements of locomotor activity were conducted in a bank of 16 individual wire (top, floor and front door) and Plexiglas photocell cages (side walls) 40 × 25 × 23 cm. Each cage was fitted with 2 parallel horizontal infrared beams, 1 cm above the floor, located across the long axis of the cage (Imetronic, France). Interruptions of either beam were accumulated over 1-min intervals and recorded in minute bins by an on-line input to a microcomputer (Tandon PCA 12 SL).

The animals were familiarised with the experimental cages during a 3-h session, one day before the test session. On the test day, spontaneous locomotor activity was monitored for 90 min prior to any drug treatment. Following this period, the compounds were administered to the animals and their locomotor activity was immediately monitored for a 60-min or 80-min period depending on drug treatments. All drugs were administered bilaterally in a volume of 0.5 μl/side, except for haloperidol which was injected in a volume of 1 μl/side. Each subject was used only once.

2.5. Experimental design

Experiment I

One group of animals ($n = 41$) was given a bilateral infusion of AP-5 (2, 4 and 10 μg/0.5 μl by side, $n = 9$–14 per dose) in the nucleus accumbens. The control group received an intra-accumbens infusion of NaCl (0.9%) ($n = 9$).

Experiment II

A first group of animals received a bilateral infusion of the haloperidol (5 μg/μl, $n = 10$) or vehicle solution ($n = 9$), 15 min prior to the AP-5 solution (10 μg/0.5 μl) in the nucleus accumbens. A second group of animals received a bilateral infusion of the haloperidol (5 μg/μl, $n = 10$) or vehicle solution ($n = 10$), 15 min prior to the MK-801 solution (10 μg/0.5 μl) in the nucleus accumbens. A control group ($n = 6$) received a bilateral infusion of the vehicle solution in the nucleus accumbens. The dose and time effects of haloperidol administration were chosen on the basis of previous experiments (Ouagazzal et al., 1994).

Experiment III

The animals ($n = 69$) receiving local co-administration of AP-5 and dopamine in the nucleus accumbens were subdivided in four different experimental groups. Each group received the simultaneous treatment in two consecutive infusions in a volume of 0.5 μl/side each. The first group of animals ($n = 8$) received a bilateral infusion of AP-5 (10 μg) in the nucleus accumbens immediately prior to the microinfusion of dopamine (10 μg). The second group received an infusion of AP-5, 10 ($n = 10$) or 4 μg ($n = 12$), immediately prior to the microinfusion of dopamine (20 μg). To test for potential non-specific effects of consecutive local microinfusions of the drug solutions, two additional control groups were tested: one group received a bilateral infusion of AP-5, 10 ($n = 9$) or 4 μg ($n = 12$), in the nucleus accumbens prior to microinfusion of the dopamine vehicle solution (i.e. 0.1 mg/ml ascorbic acid solution). Another control group received an intra-accumbens infusion of the vehicle solution of AP-5 (i.e. NaCl 0.9%) immediately prior to the microinfusion of dopamine 10 ($n = 9$) or 20 μg ($n = 9$). Finally, a control group ($n = 6$) received a bilateral infusion of a physiological saline solution in the nucleus accumbens.

2.6. Histology

After completion of behavioural testing, the rats were anaesthetized with an intraperitoneal injection of chloral hydrate (400 mg/kg) and perfused with a 10% formalin solution through the left cardiac ventricle. The brains were removed and post-fixed in the same solution. Brain sections were cut at a thickness of 60 μm, and stained with cresyl violet to check the accuracy of the injection sites. Only rats from data analysis showing the appropriate injection sites were used.

2.7. Statistical analysis

The data were evaluated using a two-factor analysis of variance (ANOVA). The different drug treatments were the independent factor, and time was considered the repeated measure. Individual post-hoc comparisons between the different drug treatments were carried out using the Duncan multiple range test. The significance level was taken to be $P < 0.05$.

3. Results

3.1. Histology

Examples of cannula track placements, located within the nucleus accumbens for the animals of experiment I, are shown in Fig. 1. Injection sites for subjects of experiments II and III are not illustrated. However, most placements fell within the nucleus accumbens in the anterior planes from +4.0 to +3.0 from the bregma according to the atlas of Pellegrino et al. (1979). Subjects in which the injection site was located outside the nucleus accumbens were excluded from the statistical analysis.

3.2. Locomotor response to the infusion of AP-5 in the nucleus accumbens

As can be seen in Fig. 2, the infusion of the competitive NMDA receptor antagonist, AP-5 (2, 4 and 10 μg/0.5 μl), in the nucleus accumbens induced a dose-dependent enhancement of locomotor activity in rats. The effect was maximal during the first 5–15 min following the infusion, depending on the dose. A gradual return to control values was then seen around 60 min after the injection. The two-factor analysis of variance (ANOVA) revealed a significant main effect of dose ($F(3,33) = 14.73$, $P < 0.05$), time ($F(11,363) = 24.83$, $P < 0.05$) and dose × time interaction ($F(33,363) = 3.79$, $P < 0.05$). Post-hoc comparisons by means of Duncan's test showed that the locomotor scores following the three doses of AP-5 tested (2, 4 and 10 μg) differed significantly from those following vehicle infusion ($P < 0.05$). Intra-accumbens infusion of AP-5 was also found to induce intensive sniffing and rearing, stereotyped behaviour, at each dose tested.

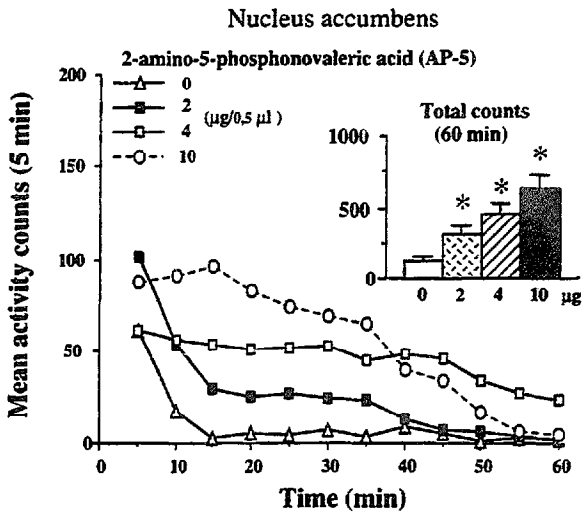

Fig. 2. Effects of AP-5 infusion in the nucleus accumbens on locomotor activity. Immediately after the infusion of AP-5 in the nucleus accumbens, the rats were placed in the photocell activity cages and locomotor activity was recorded for 60 min. Various doses of AP-5 were tested in different groups of animals (0, $n = 9$; 2, $n = 7$; 4, $n = 8$ and 10 μg/0.5 μl, $n = 12$). The ordinate gives the mean number of photocell counts in 5-min periods. Insert: Mean locomotor activity counts (±S.E.M.) during the entire 60-min period. * Significantly different from controls, $P < 0.05$, Duncan's test after significant ANOVA.

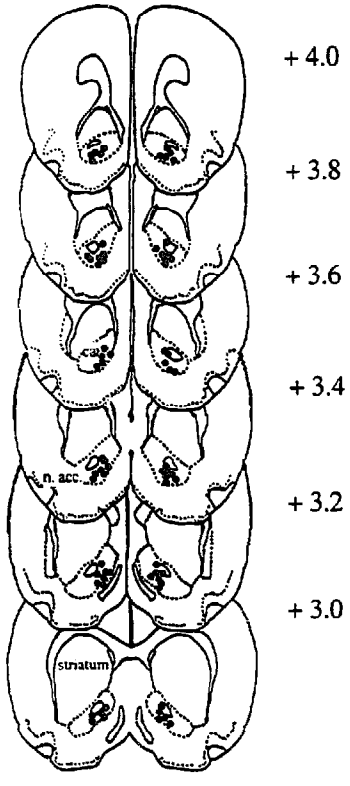

Fig. 1. Frontal sections of rat brain showing histological reconstruction of the injection sites in the nucleus accumbens (n. acc.) of the subjects in experiment I. Black dots indicate the correct location of the tips of needle injections in the nucleus accumbens. Subjects in which the site of injection fell outside the nucleus accumbens were discarded. Values give the distance in mm from bregma, according to the atlas of Pellegrino et al. (1979). ac., anterior commissure.

3.3. Effect of dopamine receptor blockade on locomotor activity induced by AP-5 or MK-801

As illustrated in Fig. 3A, the animals given a bilateral combined infusion in the nucleus accumbens of a vehicle solution, followed by the AP-5 (10 μg) solution, showed a significant enhancement of locomotor activity in comparison to control animals injected with the physiological saline solution. Prior blockade of dopamine receptors with a microinfusion of haloperidol (5 μg) in the nucleus accumbens failed to alter the locomotor stimulation induced by 10 μg of AP-5. The overall ANOVA revealed a significant main effect of the different drug treatments ($F(2,18) = 10.6$, $P < 0.05$) and a significant main effect of time ($F(11,198) = 5.7$, $P < 0.01$). The drug × time interaction was also found to be significant ($F(22,198) = 3.1$, $P < 0.05$). Post-hoc

comparisons showed no significant difference between the locomotor response to AP-5 in the animals pretreated with the haloperidol or the vehicle solution ($P > 0.05$, N.S., Duncan test).

The animals given a combined bilateral infusion of the vehicle solution in the nucleus accumbens, followed by the non-competitive NMDA receptor antagonist, MK-801 (10 μg) solution, showed a significant increase in locomotor activity in comparison to control animals injected with the physiological saline solution (Fig. 3B). In keeping with this trend, the overall ANOVA revealed a significant main effect of drug treatment ($F(2,18) = 9.1$, $P < 0.01$), time ($F(15,270) = 5.9$, $P < 0.01$) and a significant drug × time interaction ($F(30,270) = 3.9$, $P < 0.001$). A pattern of stereotyped behaviour similar to that previously found with AP-5 was observed after MK-801 infusion (data not shown). In contrast to the effects observed in the animals

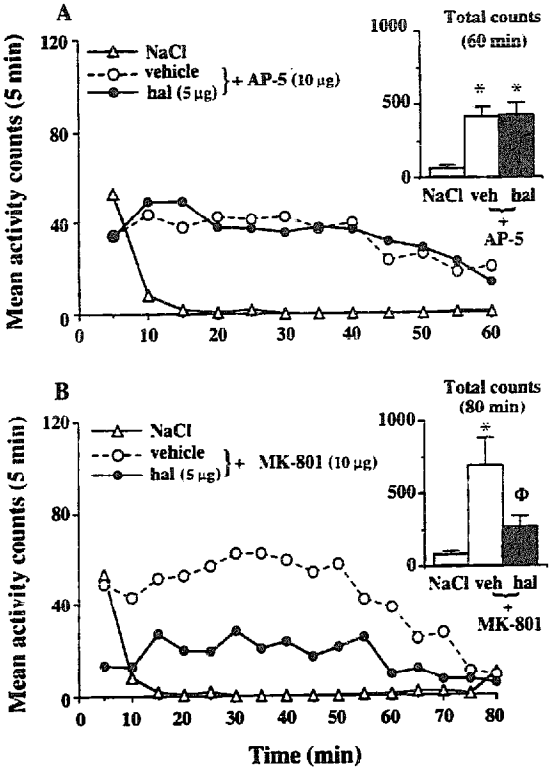

Fig. 3. Effects of haloperidol on the locomotor activation induced by AP-5 (A) or MK-801 (B) infused bilaterally in the nucleus accumbens. A: The animals received a bilateral infusion of the haloperidol (5 μg/μl, $n = 8$) or vehicle solution ($n = 8$) 15 min before AP-5 infusion (10 μg/0.5 μl) in the nucleus accumbens. A control group ($n = 6$) received a physiological saline solution (NaCl 0.9%) bilaterally in the nucleus accumbens. B: The animals received a bilateral infusion of the haloperidol (5 μg/μl, $n = 8$) or vehicle solution ($n = 8$) 15 min before MK-801 infusion (10 μg/0.5 μl) in the nucleus accumbens. Control group, same as in (A). Coordinates, as in Fig. 1. Insert refers to the mean total photocell counts (±S.E.M.) during the 60-or 80-min test. * Significantly different from control group injected with the physiological saline solution, $P < 0.05$, ANOVA. $^{\Phi}$ Significantly different from control group injected with haloperidol vehicle solution, $P < 0.05$. Duncan's test after significant ANOVA.

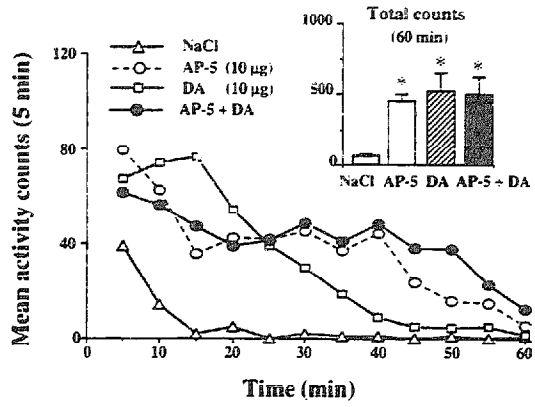

Fig. 4. Locomotor response to a separate ($n = 8$ and 9, respectively) or simultaneous injection ($n = 6$) of AP-5 (10 μg/0.5 μl) and dopamine (10 μg/0.5 μl) bilaterally in the nucleus accumbens. A control group ($n = 6$) received a bilateral infusion of a physiological saline solution (NaCl 0.9%) in the nucleus accumbens. Coordinates as in Fig. 1. Insert refers to mean total photocell counts ± S.E.M. during the 60-min test. * Significantly different from control animals injected with the physiological saline solution. $P < 0.05$, ANOVA.

injected with AP-5, the pretreatment with haloperidol was shown to markedly reduce the locomotor activity induced by MK-801 in the nucleus accumbens ($P < 0.01$, Duncan test, Fig. 3B).

3.4. Locomotor response to the co-administration of AP-5 and dopamine in the nucleus accumbens

As illustrated in Fig. 4, the bilateral dopamine infusion, at a dose of 10 μg, in the nucleus accumbens resulted in a locomotor activation which was comparable in intensity to that observed after AP-5 (10 μg) infusion in the same region. The overall ANOVA revealed a significant main effect of drug treatment ($F(3,25) = 5.45$, $P < 0.01$), a significant main effect of time ($F(11,275) = 14.14$, $P < 0.01$) and a significant drug × time interaction ($F(33,275) = 2.02$, $P < 0.01$). Furthermore, the locomotor hyperactivity induced by both treatments was found to be significantly different from the basal level of activity observed in control animals ($P < 0.01$, Duncan test). The concomitant infusion of AP-5 and dopamine did not result in a cumulative locomotor stimulation. In fact, the mean activity counts, following the separate or simultaneous administration of AP-5 or dopamine, approximated an average of 500 counts in each experimental situation for the total 60-min period of testing (graph inset, Fig. 4). A separate analysis measuring the effects of each drug injected alone or in combination showed no significant difference between the three treatments (ANOVA: $F(2,20) = 0.3$, N.S.).

In order to dissociate the locomotor effects induced by AP-5 or dopamine administration, a higher dose of dopamine (20 μg) was injected in the nucleus accumbens. The level of locomotor activity was found to be

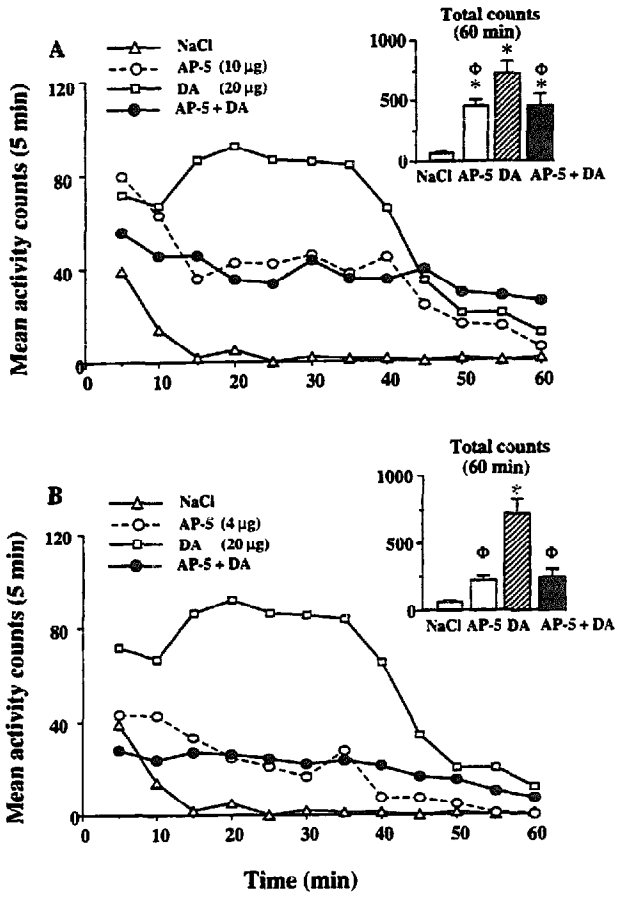

Fig. 5. A: Locomotor response to a separate ($n = 8$ and 9, respectively) or simultaneous injection ($n = 9$) of AP-5 (10 μg/0.5 μl) and dopamine (20 μg/0.5 μl) bilaterally in the nucleus accumbens. Control group ($n = 6$) same as in Fig. 4. B: Locomotor response to a separate ($n = 11$ and 9, respectively) or simultaneous injection ($n = 10$) of AP-5 (4 μg/0.5 μl) and dopamine (20 μg/0.5 μl) bilaterally in the nucleus accumbens. Control group ($n = 6$) same as in Fig. 4. Coordinates as in Fig. 1. Insert refers to mean total photocell counts ± S.E.M. during the 60-min test. * Significantly different from control animals injected with saline solution, $P < 0.05$, ANOVA. $^{\Phi}$ Significantly different from control animals injected with dopamine, $P < 0.05$, Duncan's test after significant ANOVA.

higher than that produced either with dopamine or AP-5, injected at a dose of 10 μg (Figs. 4 and 5A). The dopamine-induced locomotor activation was markedly reduced when dopamine (20 μg) and AP-5 (10 μg) were injected concomitantly (Fig. 5A). Furthermore, the combined infusion of AP-5 and dopamine was found to produce a locomotor stimulation which appeared to be very similar to that observed after the infusion of AP-5 (10 μg) alone and significantly different from the basal level of activity observed in control animals. The overall ANOVA showed a significant main effect of drug treatment ($F(3,28) = 11.7$, $P < 0.01$), main effect of time ($F(11,308) = 11.9$, $P < 0.01$) and a significant drug × time interaction ($F(33,308) = 2.88$, $P < 0.01$). Post-hoc individual comparisons showed that the groups, receiving either a combined injection of AP-5 and dopamine or AP-5 alone, showed a significantly lower locomotor activity level than the group injected with dopamine alone ($P < 0.05$, Duncan's test; graph inset). No significant difference in the locomotor activity produced by both treatment (i.e. AP-5 alone or with dopamine) could be observed.

To see whether the locomotor effect produced by combined treatment with dopamine and AP-5 was always comparable to that produced by AP-5 infusion in the nucleus accumbens, a lower dose of 4 μg of AP-5 was tested further in a new group of animals either alone or as a simultaneous treatment. The overall ANOVA comparing the various drug treatments revealed a significant main drug effect ($F(3,32) = 18.36$, $P < 0.01$), a significant main effect of time ($F(11,33) = 11.99$, $P < 0.01$) and a significant drug × time interaction ($F(33,352) = 3.11$, $P < 0.01$). Post-hoc group comparisons showed that the combined treatment with AP-5 (4 μg) and dopamine (20 μg) significantly reduced the locomotor activity of the animals as compared to those injected with dopamine alone ($P < 0.01$, Duncan's test) (Fig. 5B). Furthermore, the combination of AP-5 and dopamine treatment enhanced locomotor activity to a level comparable to that reached by animals pretreated with AP-5 alone ($P > 0.05$, N.S., Duncan test).

4. Discussion

4.1. Effect of dopamine receptor blockade in the nucleus accumbens on locomotor activity induced by AP-5

The present study showed that, in line with previous findings (Amalric et al., 1994; Donzanti and Uretsky, 1984; Carlsson, 1993; Pulvirenti et al., 1993), the competitive NMDA receptor antagonist, AP-5, infused into the nucleus accumbens produces a robust enhancement of locomotor activity. The local injection into the nucleus accumbens of another competitive antagonist, CPP, produced a similar behavioural activation (O'Neill et al., 1989), emphasizing the involvement of NMDA receptor blockade in mediating the locomotor hyperactivity. Furthermore, a specific action of AP-5 on NMDA receptors was suggested by the dose-dependent locomotor response and the lack of effect on locomotion of other excitatory amino acid receptor antagonists acting at the AMPA receptor subtype (unpublished observations). The combined treatment of AP-5 and a control solution (vehicle for dopamine or haloperidol solution) was, however, found to produce a locomotor response smaller than that induced by AP-5, injected as a single treatment. This effect, presumably due to dilution of the drug solution in a larger volume, led us to investi-

gate the effects of the highest dose of AP-5, 10 μg/0.5 μl, in combination with the local injection of the dopamine or haloperidol solution. The specificity of AP-5 effects on basal locomotor activity after microinfusion in different brain regions was demonstrated in a recent investigation (Amalric et al., 1994). Microinfusion of AP-5 (10 μg) in the nucleus accumbens induced locomotor hyperactivity, whereas the same dose of AP-5 infused in the striatum did not modify basal locomotor activity, but produced stereotyped behaviours (sniffing and rearing), as previously observed by others (Schmidt, 1986; Schmidt and Bury, 1988). Together, these data suggest that the excitatory amino acid system, probably originating from cortical inputs to the nucleus accumbens, exert an inhibitory influence via the NMDA receptors on psychomotor functions. In agreement with this suggestion, the interruption of excitatory amino acid transmission following destruction of the prefrontal cortex was found to enhance spontaneous locomotion in rats (Jaskiw et al., 1990; Yoshida et al., 1991; Whishaw et al., 1992; Burns et al., 1993).

The blockade of dopamine receptors in the nucleus accumbens using haloperidol (5 μg) failed to reduce the locomotor effects induced by AP-5, whereas a lower dose of haloperidol (2.5 μg) was previously shown to reduce amphetamine-induced locomotor activation (Ouagazzal et al., 1994; Pijnenburg et al., 1975). Furthermore, in the present study, although the locomotor stimulation induced by MK-801 was greater than that induced by AP-5 administered at the same dose, haloperidol (5 μg) pretreatment could reduce MK-801 effects on locomotion. These results are in line with previous findings showing that the injection of either haloperidol or selective dopamine D_1 or D_2 receptor antagonists, SCH 23390 and eticlopride respectively, could reduce the locomotor stimulation induced by a systemic injection of MK-801 (Willins et al., 1993; Ouagazzal et al., 1994). Together these results suggest that activation of dopamine receptors within the nucleus accumbens is necessary for the expression of MK-801 effects. In contrast, the locomotor activation produced by AP-5 did not appear to be mediated through a dopaminergic mechanism in the nucleus accumbens. Consistent with this suggestion, the intra-accumbens infusion of AP-5 was found to induce a pronounced locomotor stimulation in monoamine-depleted mice (Svensson and Carlsson, 1992; Carlsson, 1993).

Paradoxically, if injected systemically, dopamine receptor antagonists may attenuate the locomotor hyperactivity induced by a competitive NMDA receptor antagonist administered locally within the nucleus accumbens (Imperato et al., 1990; Amalric et al., 1994). It is therefore possible that with this route of administration, dopamine antagonists may act in other structures, located beyond the nucleus accumbens, to reduce the locomotor hyperactivity response. Anatomical studies have indeed shown that the mesencephalic dopaminergic neurons project, in addition to the striatum, to a number of structures involved in motor functions, such as the subthalamic nucleus and the globus pallidus (Lindvall and Björklund, 1979; Meibach and Katzman, 1979; Klitenick et al., 1992). Hence, the local infusion of dopamine directly in the ventral pallidum, a primary target of the nucleus accumbens output neurons, was recently shown to induce marked locomotor stimulation in rats (Klitenick et al., 1992).

The present results, revealing a differential effect of haloperidol on MK-801- and AP-5-induced locomotor activity, are in agreement with results of electrophysiological and neurochemical studies showing that the administration of non-competitive NMDA receptor antagonists increased the firing rate of midbrain dopamine neurons and dopamine turnover in dopamine nerve terminal areas such as the nucleus accumbens and the striatum, whereas the competitive NMDA receptor antagonists do not (French et al., 1991; Svensson et al., 1991; Bubser et al., 1992). It therefore seems unlikely that the blockade of NMDA receptors is the single mechanism through which competitive and non-competitive NMDA receptor antagonists could induce their behavioural effect. Interestingly, Quirion et al. (1987) have proposed the existence of two phencyclidine (PCP) binding sites where the non-competitive NMDA receptor antagonists act. The PCP_1 site is associated with the NMDA receptor complex and the PCP_2 site may not be linked to the NMDA receptor complex (Quirion et al., 1987). In favour of this hypothesis, subsequent studies have demonstrated the existence of PCP binding sites not coupled to NMDA receptors either in the central nervous system (Maragos et al., 1988; Rao et al., 1990,1991; Kovask and Larsson, 1994) or outside the central nervous system (Sun and Larsson, 1993; Kovask and Larsson, 1994). It may thus be proposed that the behavioural effects produced by the non-competitive NMDA receptor antagonists result from their action on phencyclidine binding sites, coupled or not coupled to NMDA receptors, which may be located within the nucleus accumbens.

4.2. Effect of dopamine receptor activation in the nucleus accumbens on the locomotor activity induced by AP-5

The hypothesis suggesting that the locomotor stimulation induced by AP-5 might not be related to the activation of dopaminergic neurotransmission in the nucleus accumbens, is also supported by the lack of additive locomotor response observed after combined local infusions of various doses of AP-5 and dopamine. The local application of dopamine, in a similar dose

range, in the nucleus accumbens or the striatum is known to produce specific behaviours mediated by these nervous structures, such as locomotor hyperactivity, rotation or motor conditioned responses (Costall and Naylor, 1976; Joyce and Van Hartesveldt, 1984; Baunez et al., 1994). A similar lack of synergism between dopamine and AP-5 injected simultaneously in the striatum in the performance of a conditioned motor response has also been reported (Amalric et al., 1994; Baunez et al., 1994). Interestingly, in the current study, a negative interaction between dopamine and AP-5 treatment to produce their locomotor effect, was revealed when both compounds induced a different level of locomotor activity. Whatever the doses of AP-5 tested (10 and 4 μg), a combined infusion of AP-5 with dopamine, at a dose known to produce a higher locomotor activation than AP-5, increased locomotor activity in a way similar to that observed after infusion of AP-5 alone. These results suggest that, following a combined infusion of AP-5 and dopamine, only the effects of AP-5 might be expressed, thus reducing the locomotor hyperactivity induced by dopamine. Consistent with this effect, it has been shown that intra-accumbens infusion of a dose of AP-5 which did not modify basal locomotor activity, reduced the locomotor stimulation induced by dopamine or amphetamine injected locally in the nucleus accumbens (Hamilton et al., 1986; Pulvirenti et al., 1991; Kelley and Throne, 1992). Hence, similar results were obtained after intra-striatal infusion of AP-5 at subthreshold doses on dopamine-induced stereotypies (Kelley and Delfs, 1994) and motor deficits in a reaction time task (Baunez et al., 1994). Together, these findings indicate that the activation of NMDA receptors in the nucleus accumbens, and more generally within the neostriatum, is required for the expression of the behavioural effects induced by enhancement of dopamine neurotransmission.

Whilst several studies showed that the blockade of NMDA receptors in the nucleus accumbens induced locomotor activation, other reports showed, paradoxically, that NMDA receptor agonists (glutamate or NMDA) injected directly in the same brain area could also induce locomotor stimulation in rodents (Donzanti and Uretsky, 1984; Hamilton et al., 1986; Boldry and Uretsky, 1988; Mogenson and Yang, 1991; Svensson et al., 1994). It thus emerges that the cortical projection to the nucleus accumbens may exert, via the NMDA receptors, either an inhibitory or a stimulatory influence on psychomotor functions. The mechanisms underlying this dual influence are unclear, however. These effects could involve two different subpopulations of functionally distinct NMDA receptors in the nucleus accumbens (likely the same NMDA receptor subtypes located on distinct neuronal systems exerting a specifically stimulatory or inhibitory influence on psychomotor functions). In agreement with this hypothesis, it has recently been shown that the nucleus accumbens, divided in a 'core' and the 'shell' subregion, might be functionally heterogenous (Pulvirenti et al., 1993; Maldonado-Irizarry and Kelley, 1994). Indeed, whereas AP-5 application in the 'shell' induced marked locomotor activation (Maldonado-Irizarry and Kelley, 1994) without affecting cocaine-induced locomotor hyperactivity (Pulvirenti et al., 1993), the same dose injected in the 'core' decreased the spontaneous locomotion and reduced cocaine-induced locomotor hyperactivity (Pulvirenti et al., 1993; Maldonado-Irizarry and Kelley, 1994). It is thus possible that in the present study the blockade of the NMDA receptors located within the 'core' suppressed the dopamine-induced locomotor activation, whereas inactivating receptors located in the 'shell' region may have induced a locomotor stimulation. The volume of diffusion of the AP-5 solution (0.5 μl) probably reaching both regions might thus have affected the two populations of NMDA receptors. Further studies using a smaller injection volume in the two specific subregions should help clarify this issue.

In conclusion, the present study showed that neither pharmacological blockade nor activation of dopamine receptors affected the locomotor response to intra-accumbens blockade of NMDA receptors. These results suggest that the behavioural effects of the competitive NMDA receptor antagonist, AP-5, may not be related to the activation of dopamine neurotransmission in the nucleus accumbens. It thus emerges that the excitatory amino acid systems innervating the nucleus accumbens may have an inhibitory influence on motor function which is likely to be beyond the modulation of dopaminergic output. Alternatively, the pharmacological blockade of dopamine receptors in the nucleus accumbens was found to reduce the locomotor effects of local infusion of glutamate and NMDA in the nucleus accumbens (Donzanti and Uretsky, 1984; Hamilton et al., 1986; Boldry and Uretsky, 1988; Mogenson and Yang, 1991). The functional interaction occurring between the excitatory amino acid and dopamine systems in the nucleus accumbens thus seem to vary considerably depending on the nature of the excitatory amino acid influence on motor function.

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