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Effect of *N*-methyl-D-aspartate on motor activity and in vivo adenosine striatal outflow in the rat

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Abstract

It has been previously found that the systemic administration of low doses of *N*-methyl-D-aspartate (NMDA) in mice induces motor depression. The effects of the systemic administration of different doses of NMDA (10, 30 and 60 mg/kg s.c.) on the motor activity and on the in vivo extracellular levels of adenosine in the striatum was studied in Sprague–Dawley rats. The adenosine concentration in samples of perfusate was determined 24 h after implantation of a transverse microdialysis probe. At 30 and 60 mg/kg, but not 10 mg/kg, NMDA induced both a significant motor depression (motility and rearing) and a significant increase in the striatal extracellular levels of adenosine. Both the motor depression and the changes in the extracellular levels of adenosine were only evident during the first 30 min after NMDA administration. The non-competitive NMDA receptor antagonist MK-801 (0.1 mg/kg s.c.) completely counteracted the effects of NMDA (30 mg/kg s.c.) on motor activity (motility) and on the striatal extracellular levels of adenosine. The correlation between the behavioural and the biochemical data strongly support the hypothesis that adenosine release in the striatum is a main mechanism responsible for the motor depressant effects produced by the systemic administration of NMDA. © 1999 Elsevier Science B.V. All rights reserved.

Keywords: NMDA (*N*-methyl-D-aspartate); Adenosine; Striatum; Microdialysis; Motor activity; (Rat)

1. Introduction

We have previously shown that the systemic administration of low non-convulsant doses of *N*-methyl-D-aspartate (NMDA) in mice induces an initial motor depression, followed by motor activation. These effects seem to be mediated by different mechanisms and, most probably, they originate in different brain structures (Ferré et al., 1994; Giménez-Llort et al., 1995, 1997). The motor activation is partially dopamine-dependent, since it is counteracted by dopamine antagonists (Giménez-Llort et al., 1997). However, a dopamine-independent mechanism seems also to be involved, since NMDA induces motor activation in reserpinized mice (Ferré et al., 1994; Giménez-Llort et al., 1995, 1997). The hippocampus and/or the extended amygdala were suggested as target brain structures responsible for the NMDA-induced motor activation, since this activation was associated with an increase in c-*fos* expression in these areas (Ferré et al., 1996).

Several pieces of evidence suggest that the motor depression induced by NMDA is adenosine-mediated. First, it was antagonized by a low dose of the non-selective adenosine antagonist theophylline, which by itself did not induce motor activation (Giménez-Llort et al., 1995). Second, theophylline exerted a dose-dependent counteraction of the NMDA-induced motor depression (Giménez-Llort et al., 1995). Third, the selective non-competitive NMDA antagonist MK-801 and theophylline, but not amphetamine, were able to counteract the motor depressant effect of NMDA (Giménez-Llort et al., 1996). Further evidence suggests that NMDA receptor-induced striatal adenosine release is the mechanism responsible for the NMDA-mediated motor depression. The striatum seems to

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be the main target area responsible for the motor depressant effects of adenosine agonists (for review, see Ferré et al., 1997) and stimulation of central NMDA receptors has been shown to increase the extracellular concentrations of adenosine in the brain, including the striatum (Hoehn and White, 1990; Chen et al., 1992; Pazzagli et al., 1993, 1994, 1995). This hypothesis was tested in the present work by checking whether the motor depression induced by NMDA in the rat is temporally related with striatal adenosine release measured in vivo by the microdialysis technique.

2. Material and methods

2.1. Animals

Male Sprague–Dawley rats weighing 200–300 g were used. The animals were allowed to adjust to a room with a 12-h light/dark cycle and 22 ± 2°C. They had free access to food and water up to the time of measurement of motor activity or microdialysis experiment.

2.2. Motor activity recording

At least a 5-day adaptation period was allowed prior to any treatment. The rats were randomly assigned to the different groups and housed four per cage. A computerized motion detection system using beams of infrared and red lights in combination with vertical and horizontal photocell arrays was used to detect movement (Ögren et al., 1979). The rats were not acclimatized to the experimental chamber. The experimental chamber contained two horizontal and one vertical array of photocell detectors per cage. Three parameters of movement were measured: motility, locomotion and rearing. Motility represents a measurement of general activity and was measured by counting all movements beyond a distance of 4 cm from the point of origin. Locomotion was measured by counting the number of times the animal moved from one side to other side of the box (32 cm). Rearing was measured by counting the number of times the rat stood on its hindlegs and interrupted the infrared beams situated 25 cm above the cage floor. NMDA (Sigma, Sweden) was dissolved in saline and adjusted to pH 7.4 with NaOH. MK-801 (Sigma) was dissolved in saline. Both drugs were injected s.c. in a volume of of 2 ml/kg. MK-801 and NMDA were administered 60 min and immediately before motor activity recordings, respectively. All values (counts) recorded per 30 min were transformed (squared root of (counts + 0.5)) and analyzed by One-way ANOVA with post-hoc Fisher's PLSD test.

2.3. In vivo microdialysis

The rats were anaesthetized with chloral hydrate (400 mg/kg i.p.) and placed in a stereotaxic frame (Stoelting Stellar). Microdialysis probes were inserted transversaly in both striata (Giovannini et al., 1987). The microdialysis probe (AN 69 membrane, Dasco, Italy; 220 μm internal diameter and 310 μm external diameter, molecular weight cut-off > 15,000 Da) was covered with Super-Epoxy glue along the whole length except for a region corresponding to the striata (two sections of 3.5 mm separated by a glued central zone of 2.5 mm). The coordinates used for the implantation of the microdialysis probe were AP 0.0 mm and DV −5.0 mm from bregma (Paxinos and Watson, 1982). Perfusion was started 24 h after implantation. Each rat was placed in a Plexiglass cage and the inlet of the microdialysis probe was connected to a microperfusion pump (Carnegie Medicine, Sweden) while the outlet was inserted into a 200-μl test tube. The microdialysis fibers were perfused at a constant flow rate of 2 μl/min with Ringer solution (composition in mM: NaCl 147, $CaCl_2$ 2.3, KCl 4.0, pH 7.0). After a 1-h stabilization period,

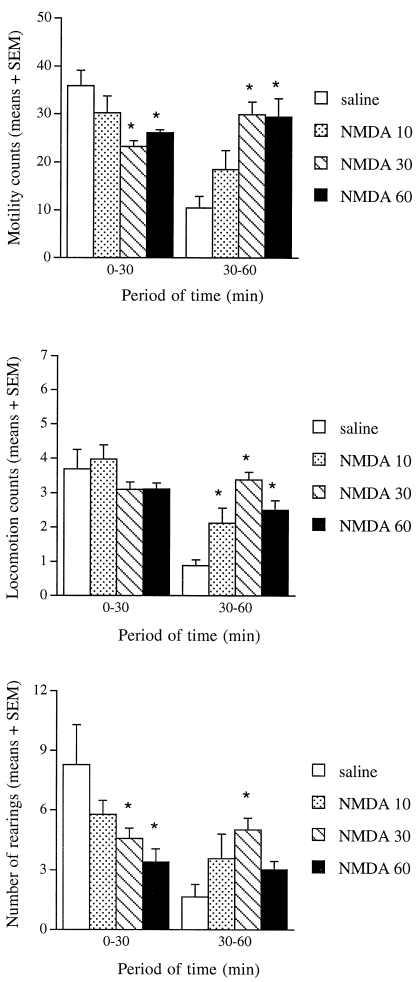

Fig. 1. Effect of the systemic administration of saline or 10, 30 and 60 mg/kg of NMDA (NMDA 10, NMDA 30 and NMDA 60, respectively) on motility, locomotion and rearing. Results represent means + S.E.M. of the transformed data obtained during the first and second 30-min periods after drug or saline administration ($n = 6$/group). * $p < 0.05$ compared with saline.

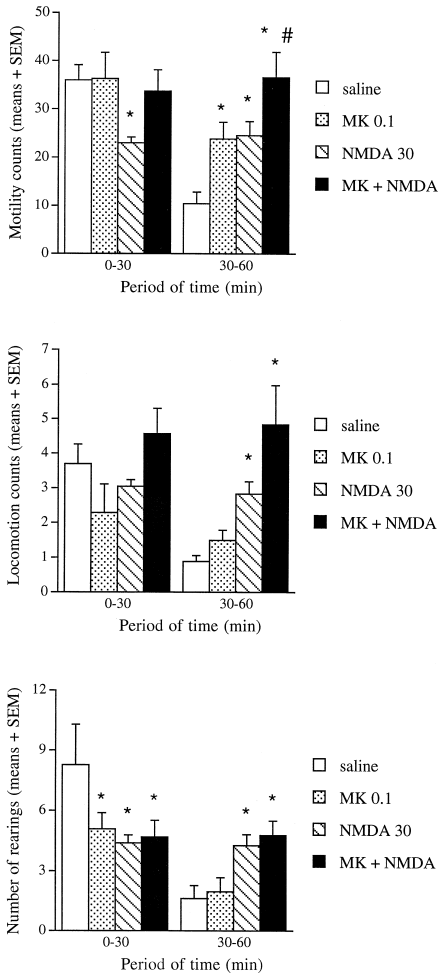

Fig. 2. Effect of the systemic administration of saline, 30 mg/kg of NMDA (NMDA 30), 0.1 mg/kg of MK-801 (MK 0.1) or NMDA plus MK-801 (the same doses; MK + NMDA) on motility, locomotion and rearing. Results represent means + S.E.M. of the transformed data obtained during the first and second 30-min periods after NMDA or saline administration. MK-801 was administered 60 min before NMDA ($n = 6$/group). $^{*}p < 0.05$ compared with saline. $\#p < 0.05$ compared with NMDA 30.

30-min samples were collected. NMDA and MK-801 were dissolved and administered as described above. Control rats received at the same time saline and 30-min samples were collected. The samples were frozen until assay. In vitro adenosine recovery through the probe was 23 ± 2.4% (mean ± S.E.M; $n = 4$) irrespective of the adenosine concentration in the external solution, as previously described (Pazzagli et al., 1995). Adenosine was assayed by means of reverse-phase HPLC with a fluorometric detector, with an excitation wavelength set at 270 nm and emission wavelength set at 394 nm, after derivatization of adenosine to obtain 1, N^6-ethenoadenosine (Wojcik and Neff, 1982). A nucleosil C-18 column (i.d.: 4.6 mm; length: 150 mm; Waters, MA, USA) with a particle size of 3.5 μm was used. The mobile phase was a 50 mM acetate buffer (pH = 5) with 5% acetonitrile (v/v) and 1 mM L-octanesulfonic acid sodium salt (Eastman Kodak, Rochester, NY), which was pumped at a flow rate of 0.8 ml/min. The minimal detectable amount of adenosine was 0.1 pmol. At the end of the experiment, the rats were anaesthetized with chloral hydrate (400 mg/kg i.p.) and killed by decapitation. The brain was rapidly removed and placed in a vial containing 10 ml of 9% phosphate-buffered formaldehyde solution. Coronal slices (50 μm) were examined under a light microscope to verify the position of the probe. Statistical significance of differences between groups was evaluated by Student's two-tailed non-paired *t*-test.

3. Results

3.1. Effects of the systemic administration of NMDA and MK-801 on motor activity

When introduced in the experimental chamber, control rats (injected with saline) showed a pronounced motor activation during the first 30-min period (exploratory period), followed by hypoactivity during the second 30-min period (habituation period). The systemic administration of NMDA (30 and 60 mg/kg) decreased the initial motor activation, which was significant for motility and rearing, but not for locomotion (compared to the control group) (Fig. 1). NMDA (30 and 60 mg/kg) induced motor activation during the second 30-min period, which was significant for motility and locomotion (compared to control) (Fig. 1). NMDA (30 mg/kg) significantly increased rearing and a lower dose of NMDA (10 mg/kg) was also found to induce a significant increase in locomotion (Fig. 1). A low dose of the non-competitive NMDA antagonist MK-801 (0.1 mg/kg) significantly reduced rearing but did not change motility and locomotion during the first 30-min

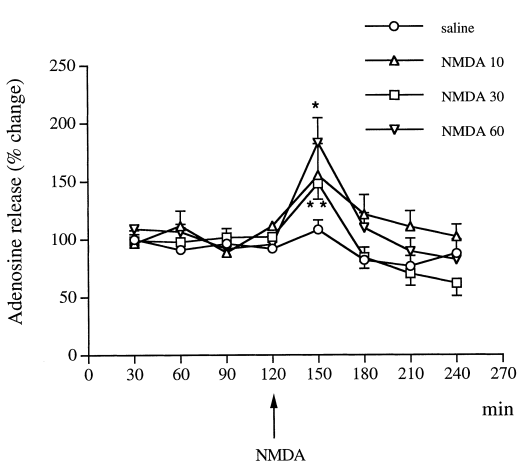

Fig. 3. Effect of the systemic administration of saline or 10, 30 and 60 mg/kg of NMDA (NMDA 10, NMDA 30 and NMDA 60, respectively) on the striatal extracellular levels of adenosine. Results, expressed as means ± S.E.M., represent the percentage variation of the basal value, calculated as the mean of the three first samples ($n = 5–6$/group). The arrow indicates the time of saline or NMDA administration. * and **: $p < 0.01$ and $p < 0.05$ compared with saline, respectively.

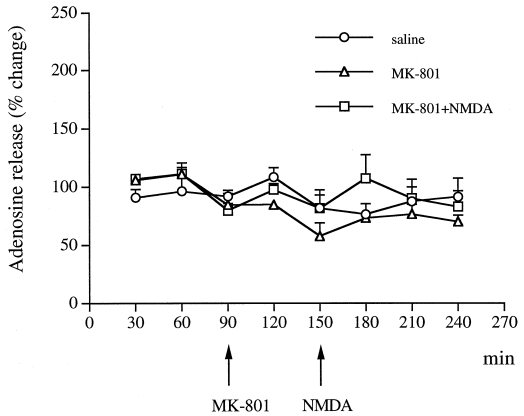

Fig. 4. Effect of the systemic administration of saline, 0.1 mg/kg of MK-801 (MK-801) or 30 mg/kg of NMDA plus 0.1 mg/kg of MK-801 (MK-801 + NMDA) on the striatal extracellular levels of adenosine. Results, expressed as means + S.E.M., represent the percentage variation of the basal value, calculated as the mean of the three first samples ($n = 5–6$/group). The arrow indicates the time of saline, NMDA or MK-801 administration.

period of observation (Fig. 2). During the second 30-min period, MK-801 (0.1 mg/kg) significantly increased motility, but not locomotion or rearing compared to controls (Fig. 2). MK-801 (0.1 mg/kg) counteracted the decrease in motility, but not in rearing, induced by NMDA (30 mg/kg) (Fig. 2). During the second 30-min period of observation, the combined administration of NMDA and MK-801 induced an increase in motility, which was significantly different from that induced by NMDA alone (Fig. 2).

3.2. Effects of the systemic administration of NMDA and MK-801 on the striatal extracellular levels of adenosine

The basal striatal extracellular levels of adenosine were 0.025 ± 0.002 μM (in mean ± S.E.M.), calculated as the mean of the first three samples of all experimental groups (value not corrected for recovery). A statistically significant increase, compared to the control group, in the striatal extracellular levels of adenosine of about 50% and 80% was obtained after the systemic administration of NMDA 30 and 60 mg/kg, respectively. The change was only observed in the first 30 min after drug administration. A non-significant increase was obtained with the lower dose of NMDA (10 mg/kg) (Fig. 3). The systemic administration of MK-801 (0.1 mg/kg) did not induce any significant change in the striatal extracellular levels of adenosine and counteracted the effect of NMDA (30 mg/kg) (Fig. 4).

4. Discussion

The spontaneous motor activity of rodents exposed to a new environment is characterized by an initial period of hyperactivity (exploratory period), followed by hypoactivity (habituation period). We have previously reported that the systemic administration of low, subconvulsant doses of NMDA in mice produces a decrease in motor activity during the exploratory period and an increase in motor activity during the habituation period (Ferré et al., 1994; Giménez-Llort et al., 1995, 1996, 1997). In the rat, a shorter duration of the spontaneous exploratory period (about 30 min) is observed compared to that observed in mice (about 60 min). Although this might reflect differences between species, we believe that it may well be explained by differences in the models used. For instance, in our previous studies, three mice were simultaneously introduced in the experimental chamber, which is a method classically used to decrease the variability of the results (Andén and Grawoska-Andén, 1988). Obviously, the interaction with other subjects adds more stimuli to those associated with the new environment. In the present work, the rat was used instead of the mouse for several reasons. First, a more precise qualitative analysis of motor activity can be carried out. Second, the in vivo microdialysis technique is easier to perform. Third, our previous studies on the extracellular levels of adenosine had been performed in the rat (Pazzagli et al., 1993, 1994, 1995).

The analysis of motor activity showed that NMDA significantly decreased both motility and rearing during the exploratory period, but it did not significantly affect locomotion. On the contrary, the three parameters were increased during the habituation period. A low dose (0.1 mg/kg) of the non-competitive NMDA receptor antagonist MK-801 did not significantly modify motility or locomotion during the exploratory period, but significantly decreased the number of rearings. During the habituation period, MK-801 significantly increased motility, in agreement with results obtained in mice (Giménez-Llort et al., 1995, 1996), and did not antagonize the motor activation induced by NMDA. On the contrary, when MK-801 was injected before NMDA, the increase in motility and locomotion was greater than that induced by NMDA administered alone. These results suggest that the motor activation induced by NMDA and by MK-801 involves different mechanisms.

We previously found in mice that using equipotent motor activating doses of the psychostimulants amphetamine, MK-801, and theophylline, only MK-801 and theophylline were able to counteract the motor depressant effects of NMDA (Giménez-Llort et al., 1995, 1996). These results suggested that the depressant effects of systemically administered NMDA are NMDA receptor-mediated. Furthermore, since theophylline is an adenosine antagonist, the results suggest that adenosine is involved in the NMDA-mediated motor depression. In the present work, we confirmed in the rat that a low dose of MK-801 counteracts the NMDA-induced decrease in motility. It was not surprising that the decrease in rearing induced by NMDA was not counteracted by MK-801, since MK-801

induced a decrease in rearing behaviour by itself. This selective depressive effect of MK-801 on rearing has been previously described as being specific for non-competitive NMDA antagonists, when compared with other psychostimulants (Ögren and Goldstein, 1994).

A dose-dependent increase in the striatal extracellular levels of adenosine was found after the systemic administration of NMDA. The doses of NMDA which were effective in modifying adenosine levels were the same as those that were effective in inducing motor depression (30 and 60, but not 10, mg/kg). Furthermore, both motor depression and the increase in striatal extracellular levels of adenosine were observed during the first 30 min after NMDA administration. Finally, the systemic administration of MK-801 counteracted both the behavioural effects, i.e., the NMDA-induced depressant effect on motor activity, and the increase in the striatal extracellular levels of adenosine. The correlation between the behavioural and the biochemical data strongly support the hypothesis that adenosine release in the striatum is a main mechanism responsible for the motor depressant effects produced by the systemic administration of NMDA.

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