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# Motor depressant effects of systemically administered polyamines in mice: involvement of central NMDA receptors

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**Abstract**

The systemic administration of polyamines (s.c.) produced a dose-dependent motor depression. With high doses the depressant effect was long-lasting and the animals showed signs of toxicity. $ED_{50}$ values for spermine, spermidine and putrescine were 38, 90 and 251 mg/kg respectively. The motor depression induced by the systemic administration of *N*-methyl-D-aspartate (NMDA; 25 mg/kg i.p.) was used as a model for studying the interactions between polyamines and the NMDA receptor. Results indicate that (1) the motor effects elicited by NMDA are very similar to those induced by polyamines at $ED_{50}$ doses; (2) polyamines, even at non-active doses, potentiate the motor depressant effect induced by NMDA; (3) the NMDA receptor antagonist, (5*R*,10*S*)-(+)-5-methyl-10,11-dihydro-5*H*-dibenzo[*a*,*d*]cyclohepten-5,10-imine (MK-801; 0.5 mg/kg i.p.), abolishes the depressant effect elicited by NMDA and by polyamines, even at toxic doses; (4) amphetamine (1.5 mg/kg i.p.) does not counteract the motor depressant effects of NMDA or polyamines. On the other hand, the adenosine receptor antagonist, theophylline (30 mg/kg i.p.), counteracts NMDA- but not polyamine-induced motor depression. The concentration of polyamines in the brain is modified after their systemic administration at high doses and at the $ED_{50}$ dose of putrescine. In conclusion, the data suggest that the NMDA receptor could be a target mediating the motor effect elicited by polyamines. They also show that the quantitative analysis of the motor effects elicited by non-convulsant doses of NMDA might be a powerful tool for studying in vivo the interaction between neurotransmission systems involved in the regulation of motor activity.

*Keywords:* Polyamine; Putrescine; Spermidine; Spermine; NMDA (*N*-methyl-D-aspartate); Motor activity; MK-801; Amphetamine; Theophylline; (Mouse)

## 1. Introduction

Glutamate is considered to be the main excitatory neurotransmitter in the central nervous system of mammals and various observations support its involvement in certain physiological and pathological processes. According to pharmacological (Lodge and Collingridge, 1990) and molecular studies (Hollmann and Hienemann, 1994), glutamatergic neurotransmission is regulated through different ionotropic and metabotropic receptors. The NMDA receptor subfamily (Moriyoshi et al., 1991; Westbrook, 1994) of ionotropic glutamate receptors plays an important role in the central nervous system. It has been associated with neuronal development and plasticity and with pathological conditions such as stroke and epilepsy (Lodge and Collingridge, 1990). Several studies using antagonists of the NMDA receptor (Svensson et al., 1994; Starr and Starr, 1994; Irifune et al., 1995) have identified strong interactions between the glutamatergic and dopaminergic system in the regulation of motor activity in mammals. The NMDA receptor is a complex molecular entity with multiple modulatory sites (Monagham et al., 1989). At present, it is accepted that, in addition to being activated by glutamate and aspartate, NMDA receptors are positively modulated by glycine and polyamines, acting at distinct sites of the same receptor (Rock and Macdonald, 1995).

Polyamines are active compounds which play significant roles in physiological and pathological conditions (Seiler, 1991; Paschen, 1992). However, there is no agreement regarding the cellular mechanisms involved in the action of polyamines in the central nervous system (Carter, 1994) and toxic effects of polyamines have also been reported by several authors (Teradaira et al., 1983; Sakurada et al., 1983; De Vera et al., 1992; Camón et al., 1994). In spite of the poor transport of polyamines between blood and brain (Shin et al., 1985), systemically

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administered polyamines have centrally mediated effects (Sakurada et al., 1983; Seiler, 1991). The positive interaction between polyamines and the NMDA receptor has been demonstrated by using both in vitro (Ransom and Stec, 1988; Williams et al., 1989; Sacaan and Johnson, 1990) and in vivo approaches (Singh et al., 1990; Crawley et al., 1992; Munir et al., 1993; Chu et al., 1994). In the in vivo studies both the NMDA-induced convulsant effects and the NMDA-related excitotoxic damage were shown to increase in the presence of polyamines.

In recent studies we have demonstrated that the systemic administration of non-convulsant doses of *N*-methyl-D-aspartate (NMDA) in rodents exposed to a new environment induces a characteristic pattern of motor activity: an initial motor depression during the initial exploratory period followed by motor activation during the habituation period (Ferré et al., 1994a; Giménez-Llort et al., 1995a). We have postulated that those motor effects induced by NMDA are centrally mediated (Ferré et al., 1994a; Giménez-Llort et al., 1995a). The aims of the present work were, first, to characterise the effects of polyamines on motor activity after their systemic administration to mice and, second, to explore the usefulness of quantitative analysis of the motor activity in mice in order to provide an in vivo model for studying the action of polyamines in the central nervous system.

## 2. Materials and methods

### 2.1. Animals

Male mice of the OF1 strain (24–32 g) were used. The animals were randomly assigned to different groups and maintained under standard laboratory conditions (22 ± 2°C and 12 : 12 h light/dark cycles beginning at 07:00 h). They had free access to food and water up to the time at which motor activity was measured. The mice were used only once.

### 2.2. Motor activity recording

The motor activity of groups of three mice was recorded with a video-computerized system (Videotrack 512, View Point, Lyon, France) using subtraction image analysis (Ferré et al., 1994a; Giménez-Llort et al., 1995b). Subtraction of an image from the previous one generates a shadow which is computed and analyzed in terms of quantity of movement. The system was set to measure any kind of motor activity (locomotion, rearing, intense grooming) and to avoid the monitoring of very small movements (breathing, non-intense grooming, tremors). Four open-field cages (35.5 × 35.5 × 35.5 cm) were recorded simultaneously in a soundproof, temperature-controlled (22 ± 2°C) experimental room, which was uniformly illuminated with two incandescent lamps (100 W) located 1.5 m above the floor. Motor activity was recorded immediately after the animals were treated and placed in the open-field cages for 2 h without any acclimatisation period.

### 2.3. Drugs

The following drugs were used: NMDA (Sigma, St. Louis, MO, USA), (5*R*,10*S*)-(+)-5-methyl-10,11-dihydro-5*H*-dibenzo[*a*,*d*]cyclohepten-5,10-imine (MK-801, RBI, Natick, MA, USA), amphetamine sulphate (generously supplied by the Ministry of Health, Spain), theophylline (Sigma) and the polyamines (Sigma) putrescine dihydrochloride, spermidine trihydrochloride and spermine tetrahydrochloride. All the concentrations refer to free drug. Drugs were dissolved in 5.5% glucose and NMDA solutions were adjusted to pH 7.4 with 0.25 M NaOH. All drugs were administered i.p. except polyamines which were administered s.c. The volume of injection was 10 ml/kg in all cases. Drugs were administered as follows: MK-801, theophylline and polyamines were administered 60, 30, and 15 min before the beginning of the test, respectively. NMDA and amphetamine were administered just before the beginning of the test. Groups not given NMDA or amphetamine received a glucose solution injection just before the beginning of the test. Doses of NMDA and MK-801 were adjusted in accordance with results of previous studies (Ferré et al., 1994a; Giménez-Llort et al., 1995b). Amphetamine and theophylline dosages were adjusted in order to obtain a motor activation equivalent to that reached after MK-801 (0.5 mg/kg). No mortality was observed during the experimentation period.

### 2.4. Liquid chromatographic analysis of polyamines

At various times after the administration of polyamines, mice were decapitated and the brains were quickly removed from the skull. The left hemisphere was dissected, weighed and frozen. Samples were stored at −20°C until biochemical analysis. The determination of polyamines in the brain was performed using a previously described method (Martínez et al., 1991). Briefly, samples were ultrasonically homogenised and deproteinised using a 0.4 M $HClO_4$ solution and centrifuged (10000 × *g* for 15 min). Then, 300 μl of $Na_2CO_3$ (2.5 M) and 500 μl of dansyl chloride (5 mg/ml in acetone) were added to the supernatant, and the mixture was kept for 1 h at 50°C. The samples were then centrifuged for 10 min at 10000 × *g* and the supernatant was carefully separated and extracted with benzene, according to previously described procedures. 1,6-Diaminohexane was used as an internal standard. An aliquot of 100 μl of benzene extract was evaporated under helium flow and redissolved in 200 μl of methanol. Before the injection, the samples were filtered through 0.45 μm membrane filters (Versapor, Gelman Sciences). The liquid chromatographic equipment consisted of a gradient solvent-delivery Waters 600 E (Mil-

ford, MA, USA) pump, a WISP 712 automatic injector, a Waters 470 fluorescence detector (350 and 520 nm for excitation and emission wavelengths, respectively). A SupelcoSil LC-18 column (150 × 4.6 mm, particle size 5 μm) was used. Elution was performed with a gradient consisting of solvent A: 1.2 mM $Na_2HPO_4$ and 12 mM NaCl, and solvent B: methanol. Initial conditions (60% B) were maintained for 3.5 min, and an 18-min linear gradient from 60% to 90% of solvent B was then run. Final conditions were maintained for 4.5 min. A short (2 min) reverse program was run to return to initial conditions. The flow rate was adjusted to 1.2 ml/min. Standard compounds were obtained from Sigma. Other reagents and solvents – chromatographic grade – were purchased from various commercial sources.

### 2.5. Statistical analysis

Dose-response curves of non-transformed motor activity values determined simultaneously in the groups of three mice ($n = 1$), corresponding to intervals of 10 min, were adjusted and analyzed using the program GraphPad INPLOT (Version 4.03) (GraphPad Software, San Diego, CA, USA) for personal computers.

All values recorded for each group of three mice, at 10 min intervals, were transformed (square root of (activity value + 0.5)) (Andén and Grabowska-Andén, 1988) and analysed in different time periods. Analyses of the influence of the treatments on the mean motor activity in the initial period (0–20 min) and on the total motor activity during the entire session were performed using a one-way analysis of variance (one-way ANOVA; SPSS/PC + (Version 5.0.1, SPSS, Chicago, IL, USA) program for personal computer. One-way ANOVA and post-hoc Duncan's test ($P < 0.05$) were also used for analysing the concentration of polyamines in the brain.

## 3. Results

### 3.1. Effect of NMDA, MK-801, amphetamine and theophylline on the motor activity of mice

The motor activity of mice in a 120 min test under different experimental conditions is shown in Fig. 1a. Animals receiving glucose displayed active behaviour during the first 30 min. This activity declined gradually until the mice entered a period of repose. Mice receiving the agonist of the NMDA receptor, NMDA (25 mg/kg) (Fig. 1b), showed the characteristic decrease in motor activity during the first 20 min of the session, followed by a moderate increase in motor activity. Thus, the accumulated motor activity was equivalent to that of the control group by the end of the session. Taking into account the pattern shown with NMDA, the period 0–20 min was chosen for analysis in all treatments. The data in Table 1(A–D) show the analysis of the accumulated motor activity and the mean motor activity displayed during this period after various treatments.

The NMDA receptor antagonist, MK-801 (0.5 mg/kg), amphetamine (1.5 mg/kg) and the non-selective adenosine receptor antagonist, theophylline (30 mg/kg), produced a significant increase in motor activity, though this remained unmodified during the exploratory period (Table 1A and Fig. 1a).

### 3.2. Effect of the combined administration of NMDA with MK-801, amphetamine or theophylline on the motor activity of mice

Fig. 1b and Table 1A show the combined effects of MK-801, amphetamine and theophylline with NMDA (25 mg/kg). MK-801 (0.5 mg/kg) eliminated the initial effect of NMDA, and the hyperactivity induced by this dose of MK-801 was not modified by the combined treatment. Amphetamine (1.5 mg/kg) did not suppress the motor depressant effect of NMDA and the hyperactivity induced by amphetamine was reduced by 25%. Theophylline (30 mg/kg) almost totally counteracted (75%) the decrease in the motor activity induced by NMDA (25 mg/kg) during the exploratory period, and the hyperactivity induced by this dose of theophylline was not modified by the combined treatment.

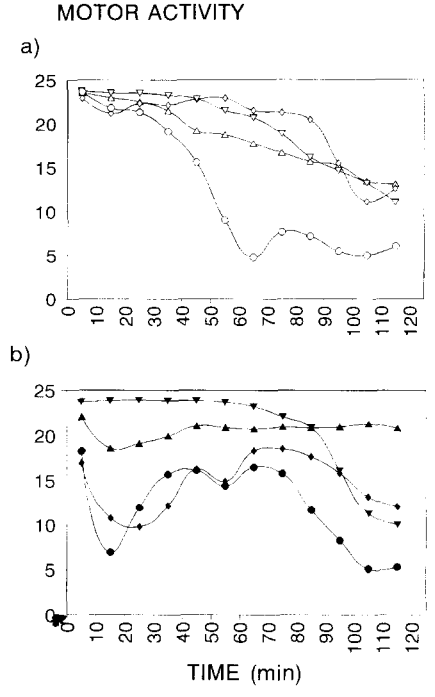


Fig. 1. (a) Time-course of the motor activity of mice after treatment with glucose (○) or motor stimulants, amphetamine 1.5 mg/kg (◇), MK-801 0.5 mg/kg (▽) and theophylline 30 mg/kg (△). (b) Effect of NMDA 25 mg/kg (●) and its combination with amphetamine 1.5 mg/kg (◆), MK-801 0.5 mg/kg (▼) and theophylline 30 mg/kg (▲) on the motor activity of mice. Each point represents the mean value of transformed data accumulated in 10 min intervals.

Table 1
Motor activity during the exploratory period (0, 20 min) and during the total test activity session (0, 120 min)

| Treatment | *n* | Exploratory activity | Total activity |
|---|---|---|---|
| *(A) NMDA and motor stimulants* | | | |
| Control | 10 | 22.7 ± 0.4 | 146.7 ± 6.9 |
| NMDA/25 | 10 | 12.6 ± 0.7 [c] | 145.9 ± 4.9 |
| MK-801/0.5 | 6 | 23.7 ± 0.4 | 233.8 ± 10.2 [c] |
| MK-801/0.5 + NMDA/25 | 6 | 23.7 ± 0.2 [n] | 240.5 ± 6.9 [c,n] |
| Amphetamine/1.5 | 6 | 22.1 ± 0.5 | 236.7 ± 7.5 [c] |
| Amphetamine/1.5 + NMDA/25 | 8 | 13.8 ± 0.4 [c,a] | 176.3 ± 7.5 [a] |
| Theophylline/30 | 5 | 23.3 ± 0.2 | 220.4 ± 6.8 [c] |
| Theophylline/30 + NMDA/25 | 5 | 20.4 ± 0.8 [t,n] | 247.3 ± 7.7 [c,n] |
| *(B) The polyamines* | | | |
| Putrescine/30 | 5 | 22.1 ± 0.2 | 150.1 ± 6.9 |
| Putrescine/60 | 6 | 21.9 ± 0.5 | 140.8 ± 8.5 |
| Putrescine/120 | 6 | 21.5 ± 0.7 | 154.9 ± 11.8 |
| Putrescine/240 | 6 | 16.3 ± 1.3 [c] | 137.7 ± 13.6 |
| Putrescine/480 | 5 | 10.4 ± 0.3 [c] | 56.8 ± 4.2 [c] |
| Putrescine/960 | 5 | 11.3 ± 0.7 [c] | 64.3 ± 2.9 [c] |
| Spermidine/40 | 6 | 22.7 ± 0.3 | 167.2 ± 9.5 |
| Spermidine/80 | 6 | 18.6 ± 0.9 [c] | 138.4 ± 12.7 |
| Spermidine/160 | 6 | 13.2 ± 0.8 [c] | 74.9 ± 10.3 [c] |
| Spermidine/320 | 5 | 10.1 ± 0.4 [c] | 71.3 ± 4.7 [c] |
| Spermine/20 | 6 | 22.3 ± 0.4 | 155.1 ± 12.6 |
| Spermine/40 | 6 | 16.4 ± 1.7 [c] | 114.6 ± 9.0 [c] |
| Spermine/80 | 6 | 9.5 ± 0.4 [c] | 58.8 ± 3.5 [c] |
| Spermine/160 | 5 | 7.7 ± 0.7 [c] | 62.1 ± 7.1 [c] |
| *(C) Polyamines and NMDA* | | | |
| Putrescine/120 + NMDA/25 | 6 | 9.5 ± 0.4 [c,o,n] | 142.6 ± 10.3 |
| Putrescine/240 + NMDA/25 | 5 | 10.0 ± 0.7 [c,o,n] | 117.4 ± 8.9 |
| Putrescine/480 + NMDA/25 | 5 | 9.6 ± 0.6 [c,n] | 79.2 ± 3.2 [c,n] |
| Spermidine/40 + NMDA/25 | 6 | 10.6 ± 0.9 [c,o] | 111.3 ± 8.8 [c,o,n] |
| Spermidine/80 + NMDA/25 | 6 | 10.2 ± 0.6 [c,o] | 128.9 ± 7.4 |
| Spermidine/160 + NMDA/25 | 5 | 8.6 ± 0.6 [c,o,n] | 57.1 ± 4.3 [c,n] |
| Spermine/20 + NMDA/25 | 6 | 12.2 ± 0.6 [c,o] | 142.2 ± 5.6 |
| Spermine/40 + NMDA/25 | 6 | 9.6 ± 0.7 [c,o,n] | 88.4 ± 6.0 [c,n] |
| Spermine/80 + NMDA/25 | 5 | 8.2 ± 0.8 [c,n] | 66.0 ± 4.4 [c,n] |
| *(D) Polyamines and motor stimulants* | | | |
| MK-801/0.5 + putrescine/240 | 6 | 23.2 ± 0.4 [o] | 220.5 ± 12.1 [c,o] |
| MK-801/0.5 + putrescine/480 | 4 | 21.5 ± 0.7 [o] | 170.4 ± 5.5 [o,m] |
| MK-801/0.5 + spermidine/80 | 6 | 22.8 ± 0.4 [o] | 258.4 ± 6.2 [c,o] |
| MK-801/0.5 + spermidine/160 | 4 | 22.1 ± 0.8 [o] | 223.3 ± 6.5 [c,o] |
| MK-801/0.5 + spermine/40 | 6 | 22.7 ± 0.6 [o] | 191.0 ± 8.9 [c,o,m] |
| MK-801/0.5 + spermine/80 | 4 | 18.3 ± 1.4 [c,o,m] | 133.6 ± 14.5 [o,m] |
| Putrescine/240 + amphetamine/1.5 | 6 | 11.5 ± 0.5 [c,o,a] | 131.8 ± 5.4 [a] |
| Spermidine/80 + amphetamine/1.5 | 6 | 13.8 ± 0.9 [c,o,a] | 164.0 ± 14.7 [a] |
| Spermine/40 + amphetamine/1.5 | 6 | 9.6 ± 0.4 [c,o,a] | 86.1 ± 9.6 [c,a] |
| Theophylline/30 + putrescine/240 | 6 | 14.7 ± 1.3 [c,t] | 158.1 ± 12.9 [t] |
| Theophylline/30 + spermidine/80 | 5 | 19.0 ± 1.5 [c,t] | 198.0 ± 12.4 [c,o] |
| Theophylline/30 + spermine/40 | 5 | 16.6 ± 1.9 [c,t] | 167.7 ± 15.4 [o,t] |

Results are expressed as means ± S.E.M. of all 10-min transformed data per three mice ($n = 1$) during the exploratory period (0–20 min) and during the total session (0–120 min). The dose of each treatment (mg/kg) is indicated after the corresponding drug. Statistically significant differences obtained with one-way ANOVA ($P < 0.0001$) followed by post-hoc Duncan's test ($P < 0.05$) are indicated according to the following keys: c, vs. glucose-treated group; n, vs. NMDA/25 group; m, vs. corresponding MK-801/0.5-treated group; a: vs. amphetamine/1.5-treated group; t, vs. theophylline/30-treated group treated group; o, vs. the corresponding polyamine-treated group.

### 3.3. Effect of polyamines on the spontaneous motor activity of mice

Polyamines induced a decrease in the spontaneous motor activity of mice. The data plotted in Fig. 2 correspond to the mean motor activity values during a 120 min session after treatment with polyamines. This shows the existence of a dose-response relationship characterised by an $ED_{50}$ (in mg/kg) of 38, 90 and 251 for spermine, spermidine and putrescine, respectively. Motor activity values ob-

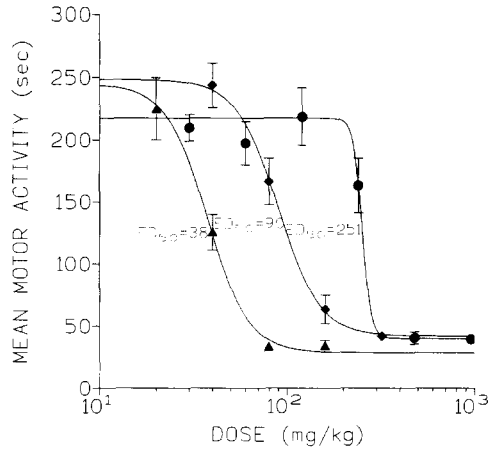


Fig. 2. Dose-response plot of the motor depressant effect induced by polyamines in mice. Data correspond to means ± S.E.M. of non-transformed values of motor activity (s), during a 120-min session accumulated in intervals of 10 min. $ED_{50}$ for putrescine (●), spermidine (♦) and spermine (▲) are indicated. Motor activity for the glucosate group was (mean ± S.E.M) 214 ± 14 s.

tained with low doses of polyamines were equivalent to those obtained in glucose-treated animals and no modification in the pattern of activity was observed. Doses close to the $ED_{50}$ values did not induce toxicity signs and only an initial alteration in motor activity was observed. High doses of polyamines induced severe signs of toxicity with losses of postural tone and ataxia.

The analysis of the results obtained with different doses of putrescine, spermidine and spermine is shown in Table 1B. The non-active doses had a response equivalent to that of the glucose-treated animals. The response to polyamines at the $ED_{50}$ doses was characterised by an initial hypoactivity followed by a moderate increase of activity, delaying the onset of the habituation period. The toxic effect of high doses was reflected by the decrease of motor activity (> 50%) in the exploratory period with no recovery during the session. Data plotted in Fig. 3a illustrate the temporal pattern of the motor activity response of mice to the different doses of putrescine.

### *3.4. Effect of the combined administration of NMDA (25 mg/kg) and polyamines on the motor activity of mice*

Table 1C shows the effects on motor activity of the interaction between NMDA (25 mg/kg) and different doses of polyamines. After the combined treatment with a non-active dose of polyamine (putrescine (120 mg/kg), spermidine (40 mg/kg) and spermine (20 mg/kg)) there was a potentiation of the effect of NMDA and/or of the corresponding polyamine in the initial period, and the accumulated motor activity of the mice was totally (putrescine (120 mg/kg) and spermine (20 mg/kg)) or partially (> 75%) restored (spermidine (40 mg/kg)). A similar result was obtained when NMDA (25 mg/kg) was combined with polyamines at $ED_{50}$ doses (Fig. 3c). The decrease in motor activity was intensified during the exploratory period, and the total activity was totally (spermidine (80 mg/kg)) or partially (> 60%) restored (putrescine (240 mg/kg) and spermine (40 mg/kg)). With higher doses, the data indicated that the enhancement in the motor depressant effect was also detectable during the first period, but in these cases there was a great reduction (> 50%) of motor activity throughout the session. In general, the combined administration of polyamines and

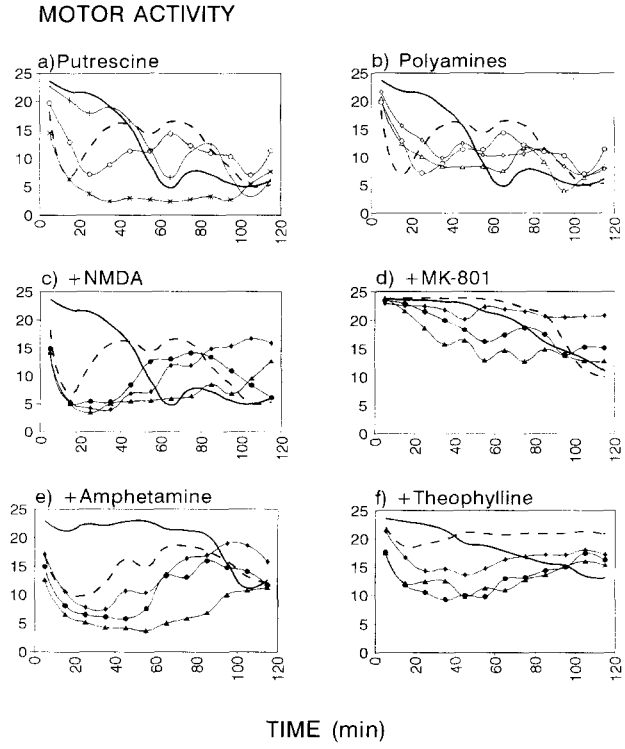


Fig. 3. Time-course of motor activity changes in mice under the following conditions: (a) Effect of the dose after putrescine treatment: 120 mg/kg (+); 240 mg/kg (○) and 480 mg/kg (*). The response of animals treated with glucosate (thick line) and NMDA (25 mg/kg) (dashed line) groups is also indicated. (b) Effect of the $ED_{50}$ dose of putrescine (○), spermidine (◇) and spermine (△). The response of animals treated with glucosate (thick line) and NMDA (25 mg/kg) (dashed line) is also indicated. (c) Effect of the interaction of polyamines at the $ED_{50}$ dose, putrescine (●), spermidine (♦) and spermine (▲) with NMDA (25 mg/kg). The response of animals treated with glucosate (thick line) and NMDA (25 mg/kg) (dashed line) is also indicated. (d) Effect of the interaction of polyamines at the $ED_{50}$ dose, putrescine (●), spermidine (♦) and spermine (▲), with MK-801 (0.5 mg/kg). The response of animals treated with MK-801 (0.5 mg/kg) (thick line) and MK-801 (0.5 mg/kg)+NMDA (25 mg/kg) (dashed line) is also indicated. (e) Effect of the interaction of polyamines at the $ED_{50}$ dose, putrescine (●), spermidine (♦) and spermine (▲), with amphetamine (1.5 mg/kg). The response of animals treated with amphetamine (1.5 mg/kg) (thick line) and amphetamine (1.5 mg/kg)+NMDA (25 mg/kg) (dashed line) is also indicated. (f) Effect of the interaction of polyamines at the $ED_{50}$ dose, putrescine (●), spermidine (♦) and spermine (▲), with theophylline (30 mg/kg). The response of animals treated with theophylline (30 mg/kg) (thick line) and theophylline (30 mg/kg)+NMDA (25 mg/kg) (dashed line) is also indicated. Each point represents the mean value of transformed data accumulated in 10-min intervals.

NMDA potentiated the initial motor depressant effect of both compounds, with a trend to a decrease of the total motor activity.

### 3.5. Effect of the combined administration of polyamines with MK-801, amphetamine and theophylline on the motor activity of mice

The combined administration of polyamines and MK-801 (0.5 mg/kg) (Table 1D) reversed the motor modifications induced by the polyamines at the doses tested ($ED_{50}$ doses (Fig. 3d) and higher) in the initial period. It is noteworthy that spermidine (80 mg/kg) did not modify the overactivation induced by MK-801 (0.5 mg/kg), while putrescine (480 mg/kg), spermine (40 mg/kg and 80 mg/kg) antagonised it partially. In summary, MK-801 (0.5 mg/kg) abolished the hypoactivity induced by polyamines in the exploratory period, and the hyperactivity induced by MK-801 seemed to be regulated in a different way by spermidine or putrescine and spermine.

The combined administration of amphetamine (1.5 mg/kg) and polyamines at $ED_{50}$ doses (Fig. 3e and Table 1D) did not attenuate the effect of polyamines during the exploratory period, but enhanced it. The data for total motor activity indicate that polyamines antagonised the overactivation induced by amphetamine (1.5 mg/kg), with a potency ranging as follows: spermine (40 mg/kg) > putrescine (240 mg/kg) > spermidine (80 mg/kg).

The combined administration of theophylline (30 mg/kg) and polyamines at $ED_{50}$ doses (Fig. 3f and Table 1D) did not modify the effect of polyamines in the initial period. Data for total motor activity indicate that putrescine (240 mg/kg) and spermine (40 mg/kg) partially antagonised (25%) the hyperactivity induced by theophylline (30 mg/kg).

### 3.6. Concentration of polyamines in the brain

Table 2 gives the concentrations of putrescine, spermidine and spermine in the mouse brain 135 min after toxic doses of the polyamines (putrescine (960 mg/kg), spermidine (320 mg/kg) and spermine (160 mg/kg) at min 120 of the motor activity test). The concentrations of brain polyamines after 240, 80 and 40 mg/kg of putrescine, spermidine and spermine respectively, were determined 35 min after polyamine administration (min 20 of the motor activity test). The brain polyamine concentration 75 min after the administration of putrescine 240 mg/kg (min 60 of the motor activity test) is also included.

## 4. Discussion

In agreement with results of previous studies (Sakurada et al., 1983; Hirsch et al., 1987), the systemic administration of polyamines produced a dose-dependent motor depression. With high doses, the depressant effect was long-lasting and the animals showed signs of toxicity. The $ED_{50}$ values of the motor depression induced by the polyamines putrescine, spermidine and spermine were similar to those obtained in acute toxicity studies (Sakurada et al., 1983; Teradaira et al., 1983), suggesting that a common mechanism of action is responsible for both non-toxic and toxic effects of polyamines. Furthermore, the $ED_{50}$ values obtained in the present work were very similar to the $EC_{50}$ values of polyamines reported for displacement of [$^3$H]spermine in rat brain synaptosomes (London et al., 1991). The results obtained from the determination of polyamine concentrations in the brain after their systemic administration suggest that, at least for putrescine, their motor depressant effects could be mediated at a central level. An $ED_{50}$ dose of putrescine induced a significant increase in both putrescine and spermine brain levels 35 min after its administration, the time of a maximal motor depressant effect (20 min after the beginning of motor recording). $ED_{50}$ doses of spermine and spermidine were associated with a non-significant increase in their brain concentrations and higher, toxic, doses were necessary to reach significance. This result might have been due to aspects of the method used: first, the basal values for putrescine were much lower than those for spermidine and spermine; second, the molar concentration of putrescine

Table 2
Brain concentration of polyamines after their systemic administration

| Influence of dose and time | $n$ | Putrescine | Spermidine | Spermine |
|---|---|---|---|---|
| Control | 10 | 11.18 ± 0.29 | 256.52 ± 3.24 | 148.51 ± 5.17 |
| Putrescine/960 (10.91 nmol, 135 min) | 6 | 191.43 ± 31.53 [a] | 272.67 ± 10.19 | 205.16 ± 9.52 [a] |
| Spermidine/320 (2.22 nmol, 135 min) | 6 | 12.29 ± 0.87 | 325.73 ± 20.18 [a] | 262.24 ± 15.80 [a] |
| Spermine/160 (0.79 nmol, 135 min) | 6 | 15.80 ± 0.93 | 319.30 ± 9.62 [a] | 356.08 ± 20.97 [a] |
| Putrescine/240 (2.73 nmol, 35 min) | 8 | 51.19 ± 3.94 [a] | 248.00 ± 3.82 | 185.80 ± 10.78 [a] |
| Putrescine/240 (2.73 nmol, 75 min) | 4 | 35.07 ± 2.48 | 244.43 ± 5.83 | 160.48 ± 3.44 |
| Spermidine/80 (0.56 nmol, 35 min) | 5 | 11.67 ± 0.67 | 265.23 ± 10.86 | 171.16 ± 7.27 |
| Spermine/40 (0.20 nmol, 35 min) | 4 | 12.31 ± 0.71 | 254.07 ± 14.63 | 158.39 ± 13.75 |

Results in nmol/g fresh tissue are expressed as means ± S.E.M. The dose of each treatment (mg/kg) is indicated after the corresponding drug. One-way ANOVA ($P < 0.05$) and post-hoc Duncan's test were used for analyzing the putrescine, spermidine and spermine modifications in brain after the different treatments. [a] $P < 0.05$ vs. control.

administered was much higher than that of the other polyamines. If we consider both these points, it might be assumed that a lower amount of spermidine and spermine penetrates in the mice brain where there is a high endogenous content. Under these conditions only a trend can be detected.

The spontaneous motor activity of mice exposed to a new environment is characterised by an initial hyperactivity (exploratory period) followed by low levels of motor activity (habituation period). We have recently found that dopamine neurotransmission is especially involved in the initial motor hyperactivity, which is inhibited by blocking dopamine receptors (Giménez-Llort et al., submitted). The systemic administration of subconvulsant doses of NMDA in rodents induces motor depression during the exploratory period followed by motor activation during the habituation period (Ferré et al., 1994a; Giménez-Llort et al., 1995a,b). The NMDA-induced motor depression was counteracted by the prior administration of an adenosine receptor antagonist (Giménez-Llort et al., 1995a). Stimulation of central NMDA receptors induces adenosine release (Pazzagli et al., 1993, 1994) and, through specific antagonistic interactions between adenosine and dopamine receptors, adenosine inhibits dopamine neurotransmision (Ferré et al., 1992, 1994b). We have then postulated that, in addition to the antagonistic interaction between the glutamatergic and dopaminergic systems (Svensson et al., 1994; Irifune et al., 1995), NMDA produces motor depression by stimulating central NMDA receptors, which induces adenosine release and, therefore, blocks dopamine neurotransmission (Giménez-Llort et al., 1995a). In agreement with this interpretation, in the present work, the non-competitive NMDA antagonist, MK-801, and the adenosine receptor antagonist, theophylline, but not amphetamine, counteracted the NMDA-induced motor depression. In fact the doses of MK-801, theophylline and amphetamine used induced similar motor activation in animals not receiving NMDA.

It has been suggested that the NMDA receptor is the target site for the pharmacological and toxic effects of polyamines (for review, see Carter, 1994). Various data indicate complex and dose-dependent effects for polyamines in this receptor (see Section Section 1). At present it is accepted that polyamines are able to interact directly with the NMDA receptor (polyamine binding site), enhancing the effects induced by NMDA receptor stimulation. The i.c.v. administration of spermine and spermidine to rodents has been reported to potentiate the convulsant properties of NMDA (Singh et al., 1990; Munir et al., 1993). The motor-depressant effect of polyamines could then also be related to their action as positive modulators of NMDA receptors. In fact, polyamines were found to strengthen the NMDA-induced motor depression even at doses which did not induce motor depression when administered alone (for putrescine and spermidine). Furthermore, MK-801 counteracted the polyamine-mediated motor depression, even when combined with toxic doses of polyamines. The antagonism between polyamines and NMDA receptor blockers, when a behavioural approach was followed, has previously been reported in a model using intracerebral administration in rats (Crawley et al., 1992). Under these conditions, putrescine and spermine were able to antagonise the 'darting' behaviour induced by the NMDA receptor antagonist, 3-(2-carboxypiperazin-4-yl)-propyl-1-phosphonic acid (CPP).

As with the results obtained with NMDA, amphetamine did not counteract the polyamine-induced motor depressant effect. The complete lack of effect shown by amphetamine when coadministered with polyamines is consistent with the results of Hirsch et al. (1987), who found that the intracerebral injection (bilaterally in the nucleus accumbens) of spermine and spermidine inhibited the hyperactivity caused by amphetamine injected into the same nucleus. Unlike the result obtained with NMDA, theophylline did not counteract the motor depressant effects of polyamines. Therefore, from the present results we hypothesise that the differential effect of theophylline on the motor depressant effects induced by NMDA and polyamines may suggest the existence of specific interactions between polyamines and the neuromodulation induced by adenosine. In fact, potentiation of the adenosine-mediated effects by spermine has been described (Wasserkort et al., 1991). Other factors such as the varying sensitivity to polyamines of the subtypes of NMDA receptors (Rock and Macdonald, 1995) could be involved.

Interestingly, the analysis of the results obtained in the study of the interaction between NMDA or polyamines and the different motor-stimulant drugs assayed reveals that spermidine is the polyamine with the pharmacological profile closest to that elicited by NMDA. On the contrary, spermine displays a profile antagonistic to the NMDA-mediated effects. Further work is needed to evaluate the possible significance of these differential interactions between NMDA and polyamines and the complex dose-related interactions of these polyamines in the regulation of the NMDA receptor (Rock and Macdonald, 1995; Marvizón and Baudry, 1994).

To our knowledge, this is the first in vivo agonist-based model reported to study the interaction between polyamines and the NMDA receptor under non-convulsant conditions. The model would appear to be suitable for studying the neurochemical interaction of NMDA/polyamines with other neurotransmission systems involved in the regulation of the motor activity in mammals.

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