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An anti-immobility effect of spermine in the forced swim test in mice

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ABSTRACT

Background: Spermine is one of the naturally occurring ligands that influence the function of the *N*-methyl-D-aspartate receptor. Similar to other endogenous polyamines present in micromolar concentration in the brain, it may play a role in the modulation of depression. Thus, the present study investigated the suggested antidepressant effect of spermine.
Methods: The mouse forced swim test (FST) was used as a reliable tool that allowed us to determine the antidepressant activity.
Results: Spermine, administered intracerebroventricularly (*icv*), significantly and dose-dependently reduced the immobility time in the FST within the dose range of 5–20 μg without changing the spontaneous locomotor activity. The pre-treatment of the animals with ifenprodil (an antagonist of the polyamine binding site of the NMDA receptor), given intraperitoneally at a dose of 20 mg/kg, thoroughly reversed the anti-immobility effect of spermine (5 μg, *icv*).
Conclusion: Our preliminary study revealed the anti-immobility activity of centrally administered spermine in the FST in mice, with a probable involvement of the polyamine-binding site at the NMDA receptor complex.

Introduction

The discovery and description of the glutamate receptors brought better understanding of the pathophysiology of depression as well as opened new possibilities for the proper management of this disease. Ionotropic *N*-methyl-D-aspartate receptors (NMDA receptors), regulated by a number of exogenous and endogenous ligands, are still widely studied. They consist of several subunits (NR1, NR2A, NR2B, NR2C, NR2D and two types of NR3), organised in tetramers built of two NR1 and two NR2 or two NR3 elements, distributed non-uniformly throughout the brain [6]. The sequence homology of these three NMDA receptor subtypes is limited, reaching only 27–31% [19]. Multiple binding sites for molecules from structurally distinct groups have been recognised within the NMDA receptor complex, i.e., the sites for glutamic acid (acting as an agonist), glycine (acting as a co-agonist), zinc (acting as an allosteric modulator), magnesium, phencyclidine, redox agents, and polyamines [19].

Spermine is one of the naturally occurring polyamines present in the brain, playing an essential role in cell growth as well as in differentiation and modulation of the ion channel receptors. It is released in the hippocampus from presynaptic terminals. Similar to other endogenous polyamines, it may interact directly with the NMDA receptor either enhancing or inhibiting its function (negative versus positive allosteric modulation). Spermine binds to the NMDA receptor with a rapid on/off kinetics [14]. Literature data highlight that extracellular spermine influences the NMDA receptor in several different ways: (i) it increases NMDA current in the presence of saturating concentrations of glycine ("glycine-independent" stimulation), (ii) potentiates the response to NMDA increasing the affinity of NMDA receptors for glycine ("glycine-dependent" stimulation) and (iii) it may also partially block NMDA channels

Abbreviations: FST, forced swimming test; *icv*, intracerebroventricularly; *ip*, intraperitoneally; NMDA, *N*-methyl-D-aspartate.

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in a voltage-dependent manner, as well as (iv) reduce the affinity of NMDA receptors for glutamate [33,35]. According to Monaghan and Jane [19], polyamines have no effect on the activity of NMDA receptor complex in the absence of glutamate and glycine. The enhancement of NMDA-induced currents are observed at lower polyamine concentrations whereas NMDA-evoked current amelioration or even inhibition are connected with high polyamine levels [34]. Similarly, high concentrations of spermine and spermidine (>100 μM) may reduce the binding of [^{3}H]-MK-801 (dizocilpine maleate) or exert no influence on it, while their lower levels (3–100 μM) are known to improve [^{3}H]-MK-801 binding [27,34]. This heterogenous effect most probably results from the stimulation of different subunits of the NMDA receptor complex. The type of the involved NR2 subunits seems to be most essential [19,26]. According to Williams et al. [35], there are at least three independent polyamine-binding sites in the NMDA receptor; within a given subunit, the amino acid residues involved in spermine stimulation have a distinct location than the ones associated with voltage-dependent block [13]. The glycine-dependent stimulation and voltage-dependent block were seen at receptors containing NR1 with NR2A or NR2B subunits [35]. The glycine-independent and voltage-independent forms of stimulation by spermine were noted in the combination of NR1 and NR2B subunits. Most probably, an interface between NR1 and NR2B N-terminal domain (NTD) lower lobes highly enriched in acidic residues is the locus of potentiating spermine binding. According to literature data, spermine does not affect NMDA receptors containing either NR2C or NR2D subunits [14], it may only influence them to a lesser extent [33].

The investigations performed by Genedani et al. [8] and Zomkowski et al. [36], imply a possible role of spermine in depression. Thus, the main objective of our research was to observe and analyse the effect of spermine in the mouse forced swim test (FST) which is a behavioural despair assay widely used in the screening of antidepressants as a reliable tool for prediction of their potency in the human body [22]. Moreover, we investigated whether ifenprodil, a negative modulator of the NMDA receptor, alters the anti-immobility effect produced by spermine. Although there are a few other compounds frequently used in experimental studies as relatively safe agents with the antidepressant-like activity selectively targeting NR2B subunit of the NMDA receptor complex [4,16,25], ifenprodil seemed to be the most suitable NR2B antagonist for our studies, since it acts at the specific site interplaying via an allosteric mechanism with a polyamine binding site.

Materials and methods

Animals

Experiments were conducted on naïve adult male Albino Swiss mice (25–30 g). The animals were maintained in the environmentally controlled rooms under a 12 h night/day cycle. They had free access to food and water except for the short time during which they were removed from their home cages for testing. The experimental groups consisted of 7–10 randomly assigned animals. Each mouse was tested only once. Separate groups of animals were used in the locomotor studies. All experimental procedures involving animals were performed in accordance with the National Institute of Health Animal Care and Use Committee guidelines and had been approved by the Local Ethics Committee at the Medical University of Lublin.

Drug administration

Spermine (*N,N′*-bis(3-aminopropyl)-1,4-diaminobutane, Abcam Biochemicals, Oxford, United Kingdom), imipramine (Polfa, Kraków, Poland) and ifenprodil (Sigma) were dissolved in 0.9% saline, immediately prior to the experiment. In the experiments designed to assess the antidepressant-like activity of spermine, four different doses of spermine were administered intracerebroventricularly (*icv*) 15 min before the tests: 2.5, 5, 10 or 20 μg per mouse (equivalent to 12.50, 25, 50 or 100 nmol per mouse, respectively). The control animals received *icv* injections of saline (vehicle). As the *icv* injection can be stressful and may activate glutamatergic system by itself [31], an additional control group that received intraperitoneal (*ip*) injection of saline (vehicle) was included in the study. The volume of drug solution and vehicle given *icv* was 5 μl per mouse, while the volume of vehicle for *ip* administration was 10 ml/kg. The *icv* administration was performed according to a modified method described by Lipman and Spencer [17]. A 10 μl glass Hamilton microsyringe (type 701) with a needle (26 G) shortened to the length of 7 mm was used. A rigid PVC tubing was put on the needle to limit its penetration to 3 mm. The injection site was approximately 2 mm posterior and 1 mm lateral (right) to bregma. In the experiments designed to assess the antidepressant-like activity of ifenprodil, four different doses of ifenprodil were administered *ip* 60 min before the tests: 5, 10, 20 or 40 mg/kg. The control animals received *ip* injections of saline (vehicle) or imipramine at a dose of 30 mg/kg (as a positive control). The dose of imipramine was selected on the basis of the outcomes of the previous experiments [23]. In the study designed to observe the effect of NMDA receptor antagonist interplaying with the polyamine binding site on the anti-immobility activity of spermine, ifenprodil was administered *ip* at a dose of 20 mg/kg 60 min before the tests and spermine was given *icv* at a dose of 5 μg per mouse 15 min before the tests. The pretreatment times were selected on the basis of the outcomes of the preliminary experiments. Each animal in the experiment received an *icv* injection – either spermine or the vehicle, depending on the tested group.

Forced swimming test

The forced swimming test was carried out on the untrained mice according to the method of Porsolt et al. [24]. Each mouse was placed individually into the glass cylinders (height 25 cm, diameter 10 cm) containing 10 cm of water at a temperature of 23–25 °C. The animals were left in the cylinder for 6 min. The total duration of immobility was recorded during the last 4 min of the 6-min testing period. The mouse was judged to be immobile when it ceased struggling and remained floating motionless in the water, making only the movements necessary to keep its head above the water level.

Spontaneous locomotor activity

In order to avoid the risk of obtaining false positive/negative effects in the FST caused by a possible influence of spermine on the locomotor activity, the spontaneous locomotor activity was measured using an animal activity metre Opto-Varimex-4 Auto-Track (Columbus Instruments, Columbus, OH, USA). This automatic device consists of four transparent cages with a lid, a set of four infrared emitters (each emitter has 16 laser beams) and four detectors monitoring animal movements. The mice were placed individually in the cages for 30 min. The activity was evaluated between the 2nd and the 6th minute, which corresponds with the time interval analysed in the FST. The spontaneous locomotor activity was measured by determining the amount of distance travelled in centimetres.

Statistical methods

The obtained data were assessed by the one-way analysis of variance (ANOVA) followed by Dunnett's or Student–Newman–Keuls *post hoc* test, depending on the experimental design. All

results are presented as mean ± standard error of the mean (SEM). $p < 0.05$ was considered as a statistically significant difference. In order to compare the results recorded for both control groups, receiving *ip* or *icv* injections of saline, the *t*-test was used. The same statistical method was used for comparing the results obtained for imipramine (a positive control) and saline (vehicle).

Results

The results illustrated in Fig. 1 show that spermine, administered *icv*, considerably reduced the immobility time in the FST within the dose range of 5–20 μg. The concentration of 2.5 μg appeared to be too low to produce any anti-immobility effect. The one-way ANOVA revealed statistically significant differences between the tested groups ($F(4,39) = 16.54$, $p < 0.0001$). Based on the findings presented in Table 1, none of the tested doses of spermine significantly changed the locomotor activity between the 2nd and the 6th minute, as compared with the vehicle-treated group.

Analyses by the unpaired *t*-test indicated no significant variation between the groups, demonstrating the following values for the FST and spontaneous locomotor activity, respectively: $t(18) = 0.01787$, $p = 0.9859$ and $t(14) = 0.8717$, $p = 0.3981$ (data not shown).

As presented in Fig. 2, ifenprodil possesses a significant antidepressant-like activity at the doses of 20 mg/kg and 40 mg/kg, which was confirmed by the results of the one-way ANOVA ($F(4,43) = 5.025$, $p = 0.0021$). However, 40 mg/kg of ifenprodil produced hypolocomotion of the tested animals between the 2nd and the 6th minute of the experiment. Ifenprodil at a dose of 20 mg/kg did not change the locomotor activity of mice as compared with the control group (Table 2). The outcomes obtained for imipramine confirmed the correctness of the applied methodology. As shown in Fig. 2, imipramine at a dose of 30 mg/kg exerted a significant anti-depressant activity ($t(14) = 5.315$, $p = 0.0001$) and did not influence the locomotor activity of the mice (data not shown).

The pretreatment of mice with ifenprodil given *ip* at an active dose of 20 mg/kg reversed the anti-immobility effect of the lowest effective dose of spermine (5 μg per mouse, *icv*) in the FST, which was illustrated in Fig. 3. The one-way ANOVA pointed out statistically significant differences between the tested groups

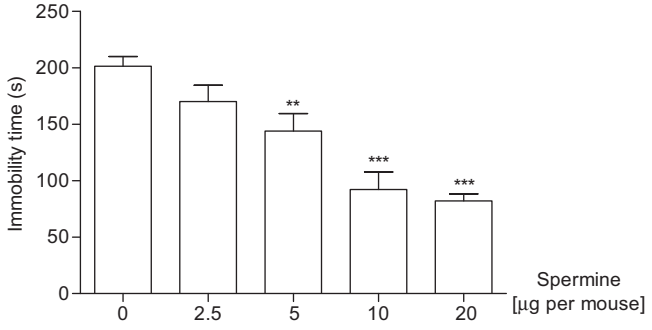

Fig. 1. Effect of acute administration of spermine in the forced swimming test in mice. Spermine was administered *icv* 15 min before the test at the following doses: 2.5, 5, 10 or 20 μg per mouse. The values represent the mean + SEM ($n = 8$–10 mice per group). $^{**}p < 0.01$, $^{***}p < 0.001$ versus vehicle-treated group (*icv*) (Dunnett's post hoc test).

Table 1
Effect of acute administration of spermine on spontaneous locomotor activity in mice.

Treatment	Dose (μg per mouse)	Activity counts between the 2nd and the 6th minute
Saline *icv*	–	763.6 ± 109.4
Spermine	2.5	546.9 ± 114.0
Spermine	5	549.6 ± 92.06
Spermine	10	663.8 ± 115.6
Spermine	20	481.1 ± 122.0

Data represent the mean ± SEM ($n = 8$–10 mice per group). Spermine was administered *icv* 15 min before the test at the following doses: 2.5, 5, 10 or 20 μg per mouse. $F(4,38) = 0.9888$, $p = 0.4253$.

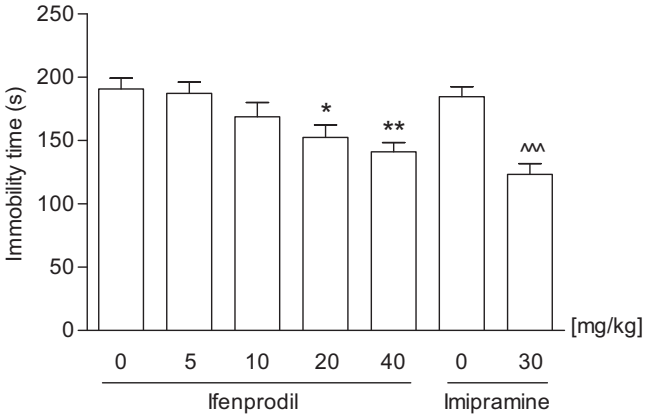

Fig. 2. Effect of acute administration of ifenprodil (IF) in the forced swimming test in mice. IF (5, 10, 20 or 40 mg/kg) and imipramine (IMI, 30 mg/kg) were administered *ip* 60 min before the tests. The values represent the mean + SEM ($n = 8$–10 mice per group). $^{*}p < 0.05$, $^{**}p < 0.01$ versus vehicle-treated group (*ip*) (Dunnett's post hoc test), $^{\wedge\wedge\wedge}p < 0.001$ versus vehicle-treated group (*ip*) (*t*-test).

Table 2
Effect of acute administration of ifenprodil on spontaneous locomotor activity in mice.

Treatment	Dose (mg/kg)	Activity counts between the 2nd and the 6th minute
Saline *ip*	–	797.9 ± 74.27
Ifenprodil	5	655.3 ± 60.99
Ifenprodil	10	576.8 ± 55.52
Ifenprodil	20	553.8 ± 78.56
Ifenprodil	40	374.0 ± 134.0*

Data represent the mean ± SEM ($n = 8$–10 mice per group). Ifenprodil was administered *ip* 60 min before the test at the following doses: 5, 10, 20 or 40 mg/kg. $F(4,35) = 3.278$, $p = 0.0220$.
* $p < 0.05$ versus control.

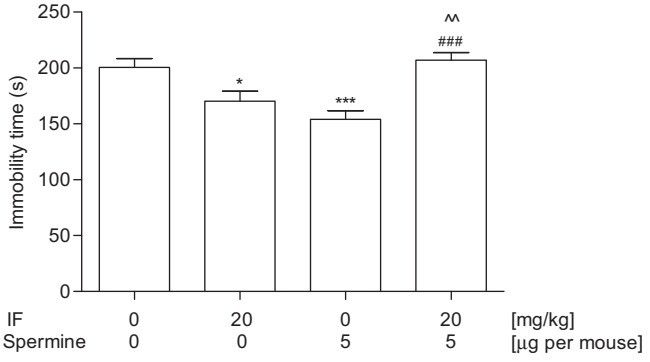

Fig. 3. Effect of joint administration of ifenprodil (IF) and spermine in the forced swimming test in mice. IF (20 mg/kg) was administered *ip* 60 min before the test and spermine (5 μg per mouse) was administered *icv* 15 min before the test. Each animal in the experiments was given an *icv* injection – either spermine or vehicle, depending on the tested group. The values represent the mean + SEM ($n = 8$–9 mice per group). $^{*}p < 0.05$, $^{***}p < 0.001$ versus control, $^{\#\#\#}p < 0.001$ versus spermine, $^{\wedge\wedge}p < 0.01$ versus IF (Student–Newman–Keuls *post hoc* test).

Table 3
Effect of joint administration of ifenprodil and spermine on spontaneous locomotor activity in mice.

Treatment	Activity counts between the 2nd and the 6th minute
Saline	690.6 ± 153.4
Spermine 5 μg/mouse	378.4 ± 74.11
Ifenprodil 20 mg/kg	546.3 ± 111.9
Ifenprodil/spermine	260.6 ± 48.72

Data represent the mean ± SEM (n = 7–8 mice per group). Ifenprodil and spermine were administered *ip* 60 min and *icv* 15 min before the test, respectively. Each animal in the experiment received an *icv* injection – either spermine or vehicle, depending on the tested group. $F(3,27) = 3.394$, $p = 0.0322$

($F(3,30) = 10.22$, $p < 0.0001$). Neither spermine nor ifenprodil given alone disturbed animals' locomotion between the 2nd and the 6th minute. The acute combined therapy considerably induced the shortening of the distance travelled by the mice compared with the vehicle-treated group. This effect was not significant as compared with the groups which received only one of the tested substances (Table 3).

Discussion

In the last decades a number of preclinical studies have linked the endogenous polyamines with the development of some neuropsychiatric disorders including stress-related conditions and suicidal behaviour [5,15]. Diminished polyamine levels (e.g., spermine) were observed in hippocampus of the rats exhibiting a depression-like behaviour [8]. Moreover, it has appeared that polyamines modulate fear conditioning as well as acquisition and/or early consolidation of inhibitory avoidance. These effects are associated with regulation of the NMDA receptor functions [28].

Since the polyamines do not seem to pass the blood–brain barrier [30], we decided to administer spermine as an *icv* injection, despite several reports confirming the centrally mediated effects of polyamines given systemically [10,29]. An additional activation of glutamatergic system after exposure to *icv* administration, as a stressful event, was not observed when compared with the *ip* treatment in relation to both immobility period and spontaneous locomotor activity.

On the basis of our findings, spermine shortens the immobility time of animals in a dosage-dependent manner. Presumably, this anti-immobility activity of spermine may engage the NMDA receptors, since it was reversed by the atypical inhibitor of the NMDA receptor complex, ifenprodil. However, some authors [9] concluded that the nature of polyamines–ifenprodil interactions could be partially independent of the NMDA receptors, i.e., affect G-proteins. Until recently, ifenprodil was thought to be a competitive antagonist at the stimulatory polyamine-binding site at the NMDA receptor. However, several thorough studies revealed that it exerts an inhibitory effect on the NMDA receptor functions also in the absence of polyamines. It was recognised that spermine and ifenprodil act at distinct but linked to each other via the allosteric interactions sites [12,14]. Experiments conducted by Han et al. [12] showed that spermine attaches to NTD domains (also referred to as the regulatory domains) of NR1, NR2A and NR2B subunits, while high ifenprodil affinity was observed only towards NR1 and NR2B regulatory domains. Tomitori et al. [32] along with Mony et al. [20] indicated that the position of spermine binding sites on both subunits are distinct from the position of ifenprodil binding sites. They also admitted that the bidirectional allosteric regulation of NR2B subunit may explain the negative interaction between ifenprodil and spermine that was discovered in the course of several studies. Observations made in our experiments are in compliance with the notion that spermine may block the glutamatergic transmission through involvement of NR1 and NR2B subunits. Kew and Kemp [14] noted an allosteric reduction of the NMDA receptor affinity to spermine following the application of ifenprodil – it induced dissociation of the polyamine from the NMDA receptor. On the other hand, in the presence of spermine the blockage of NMDA receptor by ifenprodil was remarkably lower than in its absence. Spermine binding resulted in allosteric reduction of ifenprodil affinity which in turn caused the antagonist unbinding from the NMDA receptor or slowing its binding rate down. Thus, most probably, this two-way reduction of the NMDA receptor affinity was predominantly responsible for the reversal of the antidepressant-like activity of both spermine and ifenprodil when given concurrently, as was observed in our study. An additional evidence for the allosteric mode of interplay between spermine and ifenprodil (and Zn^{2+}) can also be taken from the experiments performed by Berger and Rebernik [2] who showed that spermine counteracted ifenprodil inhibition of [^{3}H]-MK-801 binding to rat neuronal membranes by mechanisms other than the competitive ones, as this reversibility was compromised in the presence of Zn^{2+}. And vice versa, ifenprodil prevented the spermine reversal of Zn^{2+} inhibition. Hackman and Holohean [11] reported that addition of ifenprodil blocked spermine potentiation of NMDA-induced motoneuronal depolarisation correspondingly to arcaine, a spermine antagonist.

Our findings are consistent with the observations made by Zomkowski et al. [36], who investigated the antidepressant-like activity of putrescine – another endogenous polyamine, the precursor of spermidine and spermine. The anti-immobility effect of putrescine evaluated in the forced swim test and the tail suspension test was comparable to those of imipramine, a well-known antidepressant medication.

Since it is widely-known that the antidepressant-like effect in the FST may be also evoked by the substances which induce hyperactivity [18], the influence of spermine on the spontaneous locomotor activity was evaluated. In our studies spermine did not significantly affect the locomotor parameters, which confirmed the previous observations made by other authors [3]. No stereotyped behaviour was observed either. Moreover, Bertrand and Cazalets [3] reported that spermine antagonised the inhibitory action of arcaine on locomotion. Therefore, it could be suspected that the decrease in the immobility after the spermine treatment was not encouraged by any motor effect. Similarly, mice treated with putrescine did not exhibit the enhanced open-field locomotor activity [36]. On the other hand, Gimenez-Llort et al. [10] as well as Sakurada et al. [29], who investigated the effect of polyamines on motor activity after their subcutaneous (*sc*) administration to mice, found out that spermine, spermidine and putrescine produced a dose-dependent motor depression. The ED_{50} value of spermine that caused a motor depression was 38 mg/kg; however, this amount of spermine given as a *sc* injection did not significantly modify its brain level. The high doses of the tested polyamines caused a long-lasting alteration in the motor activity along with severe toxicity. The polyamine-mediated motor depressant effect was counteracted by MK-801, the NMDA receptor blocker [10]. Literature data indicate that an immediate hypothermia and sedation followed by hyperexcitability, convulsions and death within 24 h may occur after high doses (72–216 nmol) of spermine given *icv* to mice [1]. The convulsant properties and excitotoxic damage in rodents associated with the NMDA receptor transmission may be strengthened by *icv* injections of polyamines [21]. On the other hand, De Vera et al. [7] found the protective effect of ifenprodil against the NMDA-related neuronal damage induced by spermine. Nevertheless, no change in the general behaviour/condition of the animals was recorded during the course of the present investigation.

In conclusion, our preliminary experiments convincingly revealed that centrally administered spermine exerts the anti-immobility

effect in the mice FST which, most probably, can be attributed to the interaction with the polyamine recognition site at the NMDA receptor complex.

Conflict of interest

The authors have no conflict of interest in relation to the presented work.

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