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Original research article

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