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N^1-Dansyl-spermine: a potent polyamine antagonist

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Abstract

The potential polyamine antagonist action of N^1-dansyl-spermine (a potent NMDA antagonist) was assessed in two *in vivo* mouse models of polyamine action. Co-administration of N^1-dansyl-spermine (2–10 μg, i.c.v.) with spermine (100 μg, i.c.v.) resulted in a dose-dependent antagonism of the spermine-induced CNS excitation (body tremor and fatal tonic convulsions). In addition, the same dose of N^1-dansylspermine antagonised spermine's enhancement of NMDA-induced convulsions. These results suggest that N^1-dansyl-spermine is *in vivo* a potent antagonist of the CNS effects of spermine and of its action at the positive polyamine modulatory site on the NMDA receptor.

Theme: Neurotransmitters, modulators, transporters and receptors
Topic: Excitatory amino acids: excitotoxicity
Keywords: Spermine; *N*-Methyl-D-aspartate; N^1-dansyl-spermine; Convulsion; Intracerebroventricular

1. Introduction

The polyamines (putrescine, spermidine and spermine) are a family of di-, tri- and tetra-amines found in heterogeneous concentrations throughout the body, including within the brain [16]. Intense research over recent years has elucidated a number of physiological roles for the polyamines within the body [5,13]. They interact, among others, with different ion channels, and are likely to control membrane excitability by inward rectification of ion channels (for review see Ref. [20]).

The *N*-methyl-D-aspartate (NMDA) receptor, one of the ionotropic glutamate receptors made up of two different subunit groups (NR1 and NR2), is responsible for slow excitatory neurotransmission. Two polyamine recognition sites have been identified on the NMDA receptor macrocomplex, a positive modulatory and negative modulatory site [11,12]. At physiological concentrations, spermine and spermidine act mainly as activators of the NMDA receptor [18,21]. In agreement with these observations, spermidine has been shown to enhance *N*-methyl-DL-aspartate (NMDLA)-induced convulsions [4,17]. Thus, the antagonism of polyamine enhancement of NMDA-induced seizures provides an *in vivo* model for examination of polyamine antagonists.

The injection of spermine (100 μg) into the cerebral ventricles of mice (i.c.v.) results in the development of CNS excitation and convulsions [7]. The initial behavioural response consists of hypothermia, sedation, scratching and face washing and, occasionally, clonic convulsions. This first phase of effects lasts for approximately 1 h with a second distinct phase commencing about 2 h after injection. During this second phase, CNS excitation is manifest in all animals as a tremor, which increases in intensity with time, culminating in a fatal tonic convulsion, usually within 6–8 h [7]. While these excitatory effects of spermine are well recognised [1], their underlying mechanism still remains to be fully understood. The presence of polyamine binding sites on the NMDA receptor macrocomplex [11,12] suggests that potentiation of the action of glutamate may, at least partially, explain the excitatory effects of spermine. However, neither the putative polyamine antagonist, ifenprodil [7], nor other polyamine analogues tested to date [8] were fully effective antagonists of spermine's CNS excitatory action *in vivo*, especially against the second phase effects.

N^1-Dansyl-spermine is a lipophilic polyamine sulfonamide that may be regarded as a simple structural analogue of the polyamine amide spider and wasp toxins [14]. The latter are known to activate and antagonise NMDA receptors [10]. It has

been shown that N^1-dansyl-spermine induces a voltage-dependent channel block in recombinant NMDA receptors at EC_{50} in the range of 0.3–16 μM, depending on the subunit composition of the receptor [3]. It is the most potent blocker of the NMDA receptor currently known. However, this direct action, mediated through the negative polyamine site on the NMDA receptor, which may not be relevant at physiological concentrations, does not rule out a polyamine antagonist action at the positive site on the NMDA receptor.

The aim of the present study was to investigate the effects of N^1-dansyl-spermine on general behaviour and its antagonist activity *in vivo*, both against the direct CNS excitatory effects of spermine and against spermine-evoked enhancement of NMDA-induced seizures. Previous studies of seizure enhancement have used spermidine. However, the administration of spermidine (i.c.v.) does not produce marked CNS excitation [1]. Therefore, the use of spermine was deemed more appropriate.

2. Materials and methods

Female *Laca* mice (20–25 g) were obtained from the Bioresources Unit, Trinity College. During the experiment, they were housed in groups of five to six (12-h light/dark cycle) with standard laboratory diet and water available *ad libitum*.

N^1-Dansyl-spermine trihydrochloride was synthesised as described by Seiler *et al.* [14]. Spermine tetrahydrochloride and *N*-methyl-D-aspartic acid (NMDA) were obtained from Sigma UK. All compounds were dissolved in 0.9% sterile saline for administration and the doses shown refer to the free base.

For evaluation of the direct effects of spermine, mice were given 100 μg spermine (dose volume 20 μl) directly into the left cerebral ventricle [2] and displayed the characteristic two-phase behavioural profile as described above. During the second phase, CNS excitation is manifest in all animals as a tremor, which increases in intensity with time and culminates in fatal tonic convulsions within 8 h [7]. For the evaluation of CNS excitation, the following scoring system was used: (1) slight tremor; (2) moderate tremor; (3) severe tremor; (4) tonic convulsion - survived; (5) fatal tonic convulsion. The CNS excitation was quantified every 30 min (from 1 to 7.5 h after injection) by an observer blind to treatment. In order to examine the potential polyamine antagonist effects of N^1-dansyl-spermine, increasing doses (2, 5 and 10 μg) were coadministered with the spermine in the same dose volume.

In order to examine the effects of a polyamine on NMDA-induced convulsions, a sub-convulsive dose of spermine (25 μg) was administered (i.c.v.) in a volume of 20 μl either 30 min or 3 h before administration of NMDA (6.25–400 mg/kg, i.p., dose volume 0.1 ml/10 g). N^1-Dansyl-spermine was co-administered with spermine in the same dose volume. The animals were observed for 30 min after the injection of the NMDA and the latency to the first tonic episode was determined (this was the only behavioural effect that appeared with absolute reliability). The ED_{50} for NMDA was calculated using the method described by Weil [19]. If no response was produced in 30 min, a latency score of 30 was assigned since previous observations indicated that if convulsions were not observed within 30 min they would not occur.

2.1. Data analysis

2.1.1. Spermine-induced CNS excitation

The median second phase CNS excitation scores and interquartile ranges (IQR) of the spermine and test groups were calculated. Results are shown as plots of median CNS excitation scores versus time. Statistical significance between test and control subjects was calculated by the Mann–Whitney *U*-test. The percentage of mice developing a tonic convulsion was also calculated and the differences between the groups analysed using a proportionality test (Primer of Biostatistics, McGraw-Hill, 1992).

2.1.2. Spermine-induced potentiation of NMDA

The statistical significance of the differences between treatments was determined using a logistic regression model (Dr. M. O'Regan, Department of Statistics in Trinity College, Dublin).

3. Results

Although results are not presented in detail for the first phase of the two phases of spermine action [1], following its i.c.v. injection, N^1-dansyl-spermine (5–10 μg) reduced the number of clonic convulsions and other behavioural effects of spermine that are typical for the first phase. However, at the same doses, N^1-dansyl-spermine, administered alone, had no effect on body weight and general behaviour over 4 days.

3.1. Effect on spermine-induced tremor and tonic convulsions — second phase

The lowest dose of N^1-dansyl-spermine (2 μg, i.c.v.) showed a trend towards a reduction in body tremor, which was not statistically significant. However, the higher doses of N^1-dansyl-spermine (5, 10 μg, i.c.v.) caused a highly significant reduction in the development of both body tremor and tonic convulsions (Fig. 1). Similarly, there was a dose-dependent reduction in the number of mice exhibiting a tonic convulsion (Table 1).

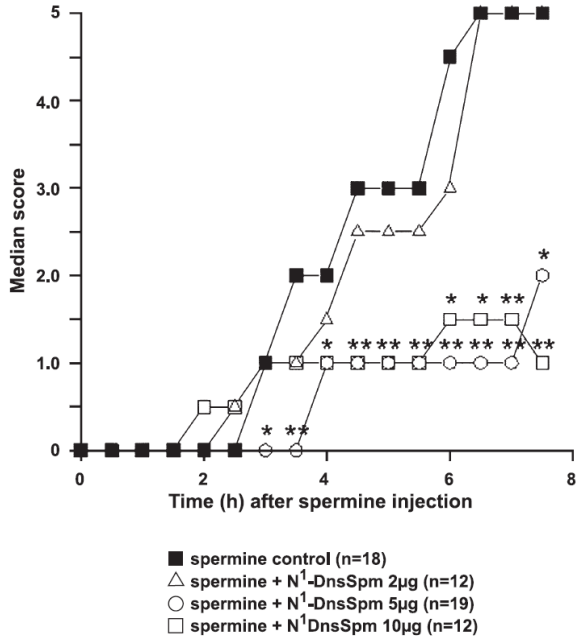

Fig. 1. *The effect of administration of N^1-dansyl-spermine (2, 5, 10 μg, i.c.v.) (n = 12, 19, 12, respectively) on median CNS excitation score after injection of 100 μg of spermine i.c.v. (n = 18); *p < 0.01, **p < 0.001, Mann–Whitney U-test vs. spermine control.*

Table 1: *The effect of N^1-dansyl-spermine on the percentage of mice developing tonic convulsions within 7.5 h of spermine injection.*

Drug treatment	n	Percent of animals showing tonic convulsions by 7.5 h after spermine injection
Spermine 100 μg (i.c.v.)	18	72.2
Spm + 2 μg N^1-DnsSpm (i.c.v.)	12	75
Spm + 5 μg N^1-DnsSpm (i.c.v.)	19	36.8*
Spm + 10 μg N^1-DnsSpm (i.c.v.)	12	16.7**

*Statistical significance was assessed using a proportionality test (Primer of Biostatistics, McGraw-Hill, 1992) (*p < 0.1; **p < 0.01 vs. spermine alone).*

3.2. Effect on spermine's potentiation of NMDA

In agreement with the slow development of the CNS excitatory effect of spermine, a non-convulsive dose of spermine (25 μg, i.c.v.) potentiated NMDA-induced convulsions when given 3 h before, but not when given 30 min before (Table 2 and Fig. 2). Co-administration of N^1-dansyl-spermine (5 μg) with the spermine (25 μg) substantially antagonised the enhancement (Table 2 and Fig. 3). However, 5 μg N^1-dansyl-spermine alone was without significant effect on NMDA-induced convulsions (Table 2).

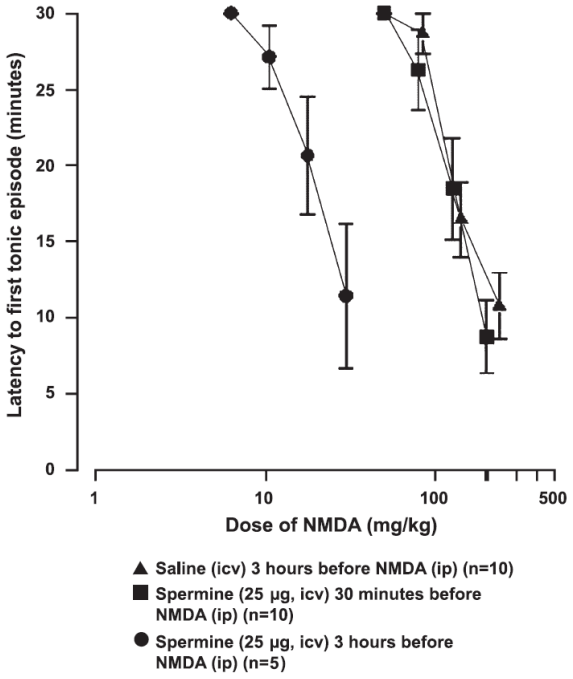

Fig. 2. *The effect of time of administration of spermine on enhancement of NMDA-induced convulsions. Values shown are mean latency values with the S.E.M. shown by vertical bars. The n values refer to the number of animals at each data point.*

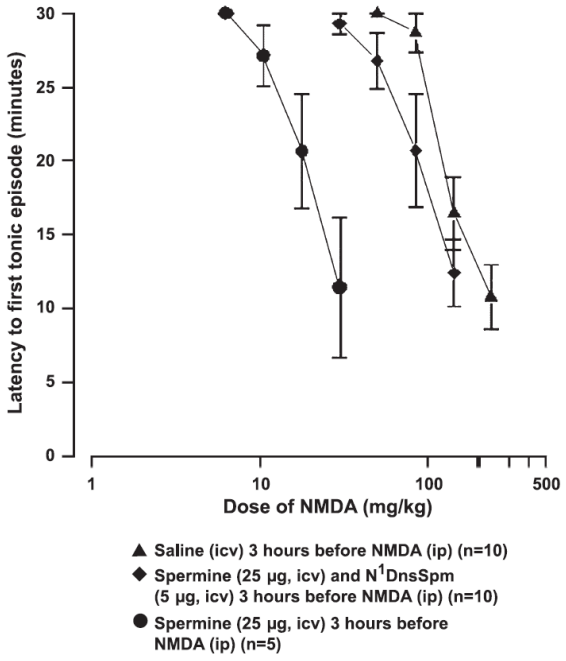

Fig. 3. *The effect of N^1-dansyl-spermine on the spermine enhancement of NMDA-induced convulsions. Values shown are mean latency values with the S.E.M. shown by vertical bars. The n values refer to the number of animals at each data point.*

Table 2: *The effect of administration of spermine, N^1-dansyl-spermine or a combination of both on the ED_{50} for NMDA induced convulsions.*

Drug	Dose (i.c.v.)	ED_{50} (mg/kg)	95% confidence interval (mg/kg)
Saline control (n = 40)	20 μl	118.9	96.7 – 146.3
Spermine (n = 20)	25 μg	13.57***	8.4 – 21.9
N^1-dansyl-spermine + spermine (n = 40)	5 + 25 μg	78.8###	57.7 – 107.7
N^1-dansyl-spermine (n = 40)	5 μg	141.42	101.5 – 197.1

*In all cases shown above, treatments were administered (i.c.v.) 3 h prior to NMDA (i.p.). ***p < 0.001 vs. saline control; ###p < 0.001 vs. spermine alone, logistic regression analysis.*

Analysis using a logistic regression revealed the following differences between groups: between saline control and spermine alone (Z= + 5.15, p < 0.001), between spermine alone and spermine co-administered with N^1- dansyl-spermine (Z = – 4.70, p < 0.001) and between the saline control and spermine with N^1-dansyl-spermine (Z= + 2.29, p < 0.05). No significant difference was found between the saline control and the N^1-dansyl-spermine alone (Z = – 0.89, p = 0.373), demonstrating that N^1-dansyl-spermine has no effect on NMDA-induced convulsions alone.

4. Discussion

This study demonstrates the polyamine antagonist activity of N^1-dansyl-spermine in two *in vivo* models. First, it suppressed early effects of spermine on clonic convulsions and general behaviour, and it reduced in a dose-dependent manner the late (second phase) CNS excitation (body tremor and tonic seizures). Second, N^1-dansyl-spermine antagonised the spermine enhancement of NMDA-induced convulsions in mice, while having no effect on NMDA-induced convulsions, when given alone. Finally, the lack of effect of N^1-dansyl-spermine, when given alone, on general behavioural parameters up to 4 days after administration rules out any spermine-like or even spermidine-like action at the doses used. Spermidine has been shown to produce a behavioural syndrome that develops over 4 days culminating in quadriplegia [1,6].

From both models it is clear that there are two phases of spermine's action (indeed, this is exploited in the timing of the second model). The delay in the development of body tremor and tonic convulsions after administration of spermine is not well understood. The most likely explanation is that spermine sets off an as yet unidentified cascade of events to enhance the effect of extracellular glutamate. In favour of this idea is that the second phase of spermine's action is susceptible to antagonism by NMDA receptor antagonists. In contrast, the first phase is susceptible to a wider range of anticonvulsants [7]. More recently, work has shown that different Ca^{2+} channels play a role in the second phase effects [9], though this effect may be a downstream result of the general CNS excitation rather than a direct effect of spermine. However, metabolic transformation of spermine cannot be excluded. It is known that extracellular spermine is highly cytotoxic if it is exposed to oxidative degradation [15]. In favour of metabolite formation is also the observation that MDL 72527, an inactivator of polyamine oxidase, reduced the neurotoxicity of spermidine [6].

Other putative polyamine antagonists, arcaine, 1,10-diaminodecane, diethylenetriamine and ifenprodil, have been examined using the first model described here [7,8]. These antagonists were found to be effective against either the first or second phase, but none were effective, as monotherapy, against both phases, unlike N^1-dansyl-spermine in the present study.

Taking this and the fact that N^1-dansylspermine effectively antagonised the spermine enhancement of NMDA induced convulsions into account, it is most likely that the actions of N^1-dansyl-spermine were mediated through a polyamine antagonist effect at the positive polyamine modulatory site on the NMDA receptor macrocomplex. In addition, the small doses of N^1-dansyl-spermine used here illustrate the potency of this polyamine antagonist, especially when compared to the polyamine analogue, arcaine, which was effective at doses of 25 μg and higher [8].

Interest has increased over the last number of years in exploiting the role of the polyamines and polyamine antagonists for potential therapeutic uses. N^1-Dansyl-spermine and related lipophilic polyamine derivatives may serve as a lead in the systematic exploration of the therapeutic potential of the polyamine backbone as a pharmacophore.

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