British Journal of Pharmacology (1998) 124, 386–390
http://www.stockton-press.co.uk/bjp

Investigation of the actions and antagonist activity of some polyamine analogues *in vivo*

[1]Karen M. Doyle & Graham G. Shaw

Department of Pharmacology, School of Pharmacy, Trinity College, 18 Shrewsbury Road, Dublin 4, Ireland

1 The ability of three putative polyamine antagonists to antagonize behavioural changes induced by spermine was assessed.

2 Injection of an excitotoxic dose of spermine (100 μg, i.c.v.) in mice results in the development of a characteristic behavioural profile, which has two temporally distinct phases. The early events include clonic convulsions, and the later, more general excitation, includes tremor and culminates in the development of a fatal tonic convulsion.

3 Co-administration of arcaine (25 μg, i.c.v.) potentiated the early phase effects after spermine injection, but antagonized the development of spermine-induced tonic convulsions. A larger dose of arcaine (50 μg, i.c.v.) given alone resulted in the development of spermine-like body tremor and convulsions. It therefore appears that arcaine is not a pure polyamine antagonist *in vivo*, but may be a partial agonist.

4 Similarly, 1,10-diaminodecane appeared to act as a partial agonist *in vivo*, although it was less potent than arcaine.

5 In contrast, diethylenetriamine (DET) effectively inhibited the development of the early effects of spermine, but was ineffective against the spermine-induced CNS excitation and tonic convulsions.

6 It is concluded that none of the putative polyamine antagonists tested behaved as effective polyamine antagonists *in vivo*, although each produced some antagonism.

Keywords: Spermine; polyamine; CNS excitation; convulsion; N-methyl-D-aspartate receptor; arcaine; 1,10-diaminodecane; diethylenetriamine

Introduction

Injection of spermine (100 μg) directly into the cerebral ventricles of female *Laca* mice leads to the development of CNS excitation and convulsions (Doyle & Shaw, 1996). Initially, within minutes of injection, the mice are hypothermic, slightly ataxic and show signs of CNS stimulation in the form of scratching and face washing. Some mice ($\sim$11%) develop clonic convulsions (Doyle & Shaw, 1996). This stage of spermine-mediated effects generally lasts for approximately 1 h. The mice can then appear normal until about 2 h after spermine injection, when they begin to develop signs of the second phase of spermine induced effects in the form of body tremor. This body tremor worsens with time and culminates in a fatal tonic convulsion, generally within 8 h of injection (Doyle & Shaw, 1996).

Although the neurotoxic effects of spermine have been known for some time (Anderson *et al*., 1972), the mechanisms underlying these events remain to be fully elucidated. Ransom and Stec (1988) were the first to demonstrate the existence of a specific polyamine binding site. They demonstrated a positive modulatory site specific for polyamines on the N-methyl-D-aspartate (NMDA) receptor (Ransom & Stec, 1988). This evidence sparked much interest in the polyamines as potential therapeutic targets for stroke, epilepsy and neurodegenerative disease.

It now appears that there may be more than one polyamine binding site on the NMDA receptor macrocomplex (Sacaan & Johnson, 1990a; Marvizon & Baudry, 1994) and that the interaction of the polyamines at the macrocomplex may not simply be a direct modulation of the opening of the ion channel as first proposed, but may also involve complex allosteric interactions between the polyamine, glutamate and glycine sites on the macrocomplex (Pullan & Powel, 1991; Carter *et al*., 1992; Doyle & Shaw, 1996). Evidence has also come to light which has demonstrated NMDA receptor heterogeneity (Reynolds & Palmer, 1991), a property which may result from varying combinations of different NMDA receptor subunits (Katsuwada *et al*., 1992). The polyamines appear to act predominantly on the NR1a/2B subunits (Lynch *et al*., 1995). Furthermore, activation of the NMDA receptor macrocomplex is known to induce polyamine synthesis through stimulation of ornithine decarboxylase (Kish *et al*., 1991), thus the polyamines may play a large role in the vicious cycle of events underlying NMDA receptor associated neurotoxicity.

Taken together, these findings paint a complex picture. However, mitigation of the role of the polyamines in activating the NMDA receptor macrocomplex has significant therapeutic potential. Several compounds have been proposed as polyamine antagonists. Non-competitive polyamine antagonists, such as ifenprodil and eliprodil, do not structurally resemble the polyamines and appear to bind to a site close to, and perhaps overlapping the polyamine binding site (Williams *et al*., 1991). A second family of putative polyamine antagonists are structurally similar to the polyamines and diethylenetriamine (DET). As receptor binding is subject to structure-activity relationships, it seems likely that structural analogues of the polyamines may prove to be effective antagonists of the polyamines.

The aim of this investigation was to evaluate the antagonist potential of these readily available polyamine analogues *in vivo*. The ability of arcaine, 1,10-diaminodecane and DET to inhibit the development of spermine-induced neurotoxicity was studied.

[1]Author for correspondence at present address: Department of Biosciences, School of Natural Sciences, University of Hertfordshire, College Lane, Hatfield, Hertfordshire AL10 9AB, U.K.

Methods

Female *Laca* mice (20–25 g) were obtained from the Bioresources Unit, Trinity College and were housed in groups of 4–6 under a twelve hour light/dark cycle (07 h 00 min, 19h 00min) with food and water *ad libitum*.

Spermine-induced behavioural changes

Mice given 100 μg of spermine directly into the left cerebral ventricle, by use of the method described by Brittain (1966), displayed a distinct behavioural profile of effects which could be divided into two main stages (for full description of effects see Doyle & Shaw, 1996). The first phase of effects developed within minutes after injection and generally lasted for approximately one hour. Most mice showed scratching of the upper body and frequent face-washing and some ($\sim$11%) developed clonic convulsions. By about two hours after injection, the second phase of spermine-induced effects began to develop in the form of body tremor which worsened with time. The CNS excitation culminated in the onset of tonic convulsions, which were ultimately fatal and generally occurred within eight hours of injection.

Assessment profile

The methodology used was essentially that described by Doyle & Shaw (1996). Briefly, the mice were studied in a blind fashion at 10, 20, 30 and 40 min after i.c.v. injection over 2 min periods for the occurrence of clonic convulsion episodes induced by spermine.

For assessment of the second phase of spermine-induced CNS excitation the mice were observed at 30 min intervals over a period of up to 7.5 h after injection. A simple behavioural profile which scored the development of body tremor, and subsequent tonic convulsions was used. The scoring system was as follows: (1) slight tremor; (2) moderate tremor; (3) severe tremor; (4) tonic convulsion – survived; (5) fatal tonic convulsion. The extent of body tremor was assessed by lifting the mouse by the tail and feeling the degree of tremor. The scoring system only recognizes three different grades of body tremor in order to reduce scope for assessment error.

Drugs/dosage

Spermine (100 μg) (Sigma, Dorset, U.K.) was administered as a hydrochloride salt dissolved in 0.9% sterile saline. Arcaine (10, 25, 50 or 100 μg) (Sigma, Dorset, U.K.), 1,10-diaminodecane (10, 20, 30, 40 or 50 μg) (Semat (RBI), Herts, U.K.) and DET (50, 100 or 200 μg) (Fluka, Switzerland) were dissolved in 0.9% sterile saline. None of these compounds readily cross the blood brain barrier and they were therefore administered by intracerebroventricular injection in a final dose volume of 20 μl, either alone or as a co-administered mixture.

Behavioural changes produced by putative antagonists

The behavioural effects produced by each of the putative polyamine antagonists administered alone were observed for a period of up to seven days following injection. The effects observed are described for each compound in the results section.

Data analysis

The effect of the putative polyamine antagonists on the mean number of clonic convulsions observed over the total observation time window per animal in the first phase of spermine-induced effects was assessed. Statistical significance of differences between spermine controls and drug treated groups was calculated by means of a proportionality test (Multistat, Biosoft, 1988).

The median second phase CNS excitation scores and interquartile ranges (IQR) of the spermine control group and test groups were calculated. Results are expressed in graph form as plots of median CNS excitation scores versus time (hours). Statistical significance of the difference between test and control subjects was calculated by the Mann–Whitney U-test. The effect of the drugs on the latency to the development of tonic convulsions in the second phase was also assessed.

Results

Animals treated with low doses of arcaine alone (10 and 25 μg, i.c.v.) appeared normal in every respect up to four days after injection. However, mice which received 50 μg of arcaine appeared slightly hyperactive for several hours and within 24 h approximately half of the mice tested developed an abnormal gait with high carriage which lasted for two to three days. Mice which received the highest dose of arcaine tested (100 μg) were hyperactive, ataxic, hyperexcitable and some vocalized for several hours after dosing. Within two to four hours after arcaine administration approximately half of the mice at this dose level developed face-washing and head-scratching behaviour and experienced non-fatal clonic and extensor tonic convulsions. These convulsions began to ease approximately 6 h after injection but within 24 h the mice then developed behavioural signs of neurotoxicity in the form of tip-toe walking, which progressed within two to three days to front or hind limb paralysis and eventually a moribund state. This paralysis resembled the effects observed after administration of the polyamine, spermidine (Doyle & Shaw, 1994). The mice were killed immediately when they reached the quadriplegic stage to avoid unnecessary suffering.

In the presence of spermine, arcaine significantly potentiated the mean number of clonic episodes per animal in response to spermine at all three doses tested (10, 25, 50 μg, i.c.v.) (Table 1). The development of the second phase of spermine-induced effects in the form of body tremor and tonic convulsions was significantly potentiated by 10 μg of arcaine

Table 1 The effect of arcaine, 1,10-diaminodecane and DET on the development of clonic convulsions in spermine-treated mice

Drug treatment	n	*Mean number of clonic episodes per animal*
Spermine control (Spm)	35	0.80 ± 0.45
Spm + Arcaine 10 μg, i.c.v.	23	2.43 ± 0.93**
Spm + Arcaine 25 μg, i.c.v.	34	4.76 ± 0.77**
Spm + Arcaine 50 μg, i.c.v.	19	3.32 ± 1.31**
Spm + 1,10-diaminodecane 10 μg, i.c.v.	12	3.08 ± 1.62**
Spm + 1,10-diaminodecane 20 μg, i.c.v.	12	10.08 ± 1.23**
Spm + 1,10-diaminodecane 30 μg, i.c.v.	12	4.00 ± 1.24**
Spm + 1,10-diaminodecane 40 μg, i.c.v.	12	5.17 ± 0.80**
Spm + DET 100 μg, i.c.v.	18	0**

Data shown are means ± s.e.mean. **$P < 0.01$; proportionality test (MultiStat, Biosoft, 1988).

(Figure 1). Conversely, higher doses of arcaine (25 and 50 μg) significantly reduced the development of the behavioural changes seen in this phase (25 μg dose effect shown in Figure 1).

Low doses of 1,10-diaminodecane (10 and 20 μg, i.c.v.) administered alone induced no behavioural changes and test animals appeared normal in every respect up to four days after injection. A larger dose of 30 μg caused a longer lasting sedation than saline treatment, and the development of slight body tremor which lasted for approximately 2 h, after which time the animals appeared normal. However, within 24 h of injection the mice developed high carriage and tip-toe walking which resembled that seen in spermidine- or arcaine-treated mice (see above). However, in the case of 1,10-diaminodecane treated animals, the behavioural signs of neurotoxicity did not worsen with time. Higher doses of 1,10-diaminodecane of 40 and 50 μg i.c.v. caused similar effects to those observed with the 30 μg dose, although the severity of the effects increased dose-dependently. With the highest dose tested (50 μg), signs of CNS stimulation lasted for approximately two hours and were characterized by slight body tremor and wild-running. Some mice developed clonic and forelimb extensor tonic convulsions which were generally non-fatal. Within 24 h the mice developed high carriage and tiptoe walking behaviour and some mice developed the early stages of front or hind limb paralysis. These effects did not worsen with time and the mice generally recovered fully within a period of seven days.

When co-administered with spermine, all doses of 1,10-diaminodecane tested (10, 20, 30, 40 μg, i.c.v.) significantly potentiated the mean number of clonic episodes observed per animal (Table 1). 1,10-Diaminodecane (10 and 20 μg, i.c.v.) was largely ineffective against the development of body tremor and tonic convulsions (data not shown), but a larger dose of 30 μg, i.c.v. caused a significant reduction in the development of the second phase effects at some time points (Figure 2).

Administration of 50 μg of DET to mice did not cause the development of any behavioural effects. Mice which received 100 μg were sedated and ataxic immediately after injection and showed short-lived signs of CNS stimulation in the form of wild jumping. A higher dose of 200 μg produced similar initial effects to the 100 μg dose, but mice also developed high carriage and tip-toe walking within 24 h of injection, which they recovered from within two to three days.

Unlike arcaine and 1,10-diaminodecane, DET caused a statistically significant reduction in the mean number of clonic convulsions per animal (Table 1) when co-administered with spermine. Conversely, injection of 100 μg of DET potentiated the development of spermine-induced body tremor and tonic convulsions (Figure 3).

As DET completely inhibited the development of the first phase of spermine-induced effects and actually potentiated the second phase of spermine effects at a dose of 100 μg, it was decided that it was not necessary to test the inhibitory potential of higher doses against the development of spermine-induced CNS excitation and convulsions, particularly as DET itself causes the development of polyamine-like behavioural effects.

Discussion

Arcaine, 1,10-diaminodecane and DET, which resemble the polyamines structurally, have been proposed as polyamine antagonists through the results of binding studies, although conflicting findings have shed doubt on a pure competitive antagonism of polyamine effects by any of these compounds. Arcaine is a naturally occurring structural analogue of the polyamines. It produces a rightward shift of the increase in [^{3}H]-dizocilpine binding produced by spermidine (Reynolds, 1990a; Sacaan & Johnson, 1990b), which suggests that it may be a competitive antagonist at a polyamine binding site on the NMDA receptor macrocomplex. However, other evidence has suggested that arcaine may not be a pure competitive antagonist of the polyamines. Arcaine has been suggested to

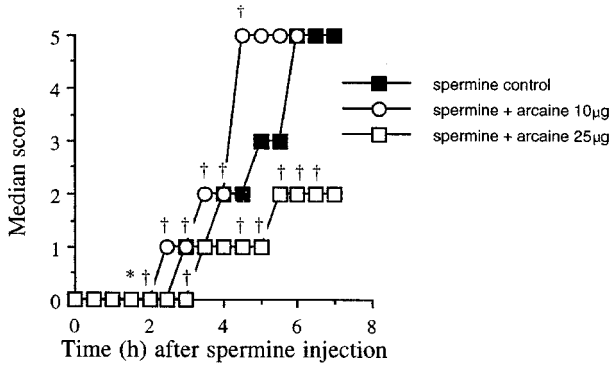

Figure 1 The effect of administration of arcaine 10 μg, i.c.v. ($n=23$), or arcaine 25 μg, i.c.v. ($n=25$), on median CNS excitation score after injection of 100 μg of spermine i.c.v. ($n=36$). $^*P<0.05$; $^\dagger P<0.01$, Mann–Whitney U-test.

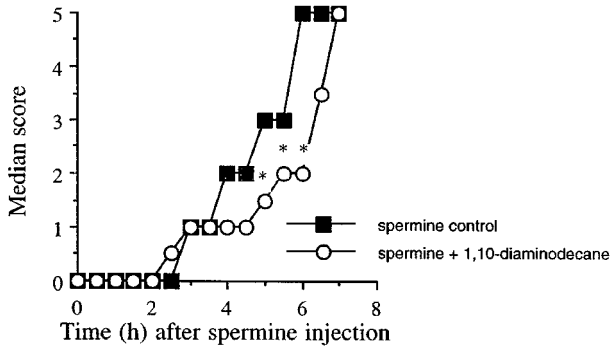

Figure 2 The effect of the administration of 1,10-diaminodecane 30 μg, i.c.v. ($n=12$) on median CNS excitation score after injection of 100 μg of spermine i.c.v. ($n=36$), $^*P<0.05$; $^\dagger P<0.01$, Mann–Whitney U-test.

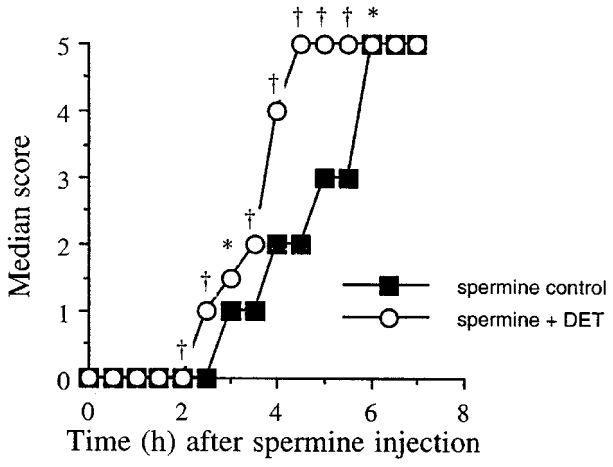

Figure 3 The effect of the administration of diethylenetriamine 100 μg, i.c.v. ($n=18$), on median CNS excitation score after injection of 100 μg of spermine i.c.v. ($n=36$). $^*P<0.05$; $^\dagger P<0.01$, Mann–Whitney U-test.

be an inverse agonist (Reynolds, 1990b) of the polyamine site, or to act in a non-competitive, allosteric fashion (Sacaan & Johnson, 1990c; Maciver *et al*., 1991).

Similarly, 1,10-diaminodecane reduces [^{3}H]-MK801 binding in cultured hippocampal neurones, both in the presence and absence of polyamines and has been proposed to be an inverse agonist at the polyamine binding site (Williams *et al*., 1990; 1992). However, conflicting evidence has suggested that 1,10-diaminodecane may mediate its effects by blocking the open NMDA receptor channel directly (Donevan *et al*., 1992; Rock & MacDonald, 1992; Subramaniam *et al*., 1992).

DET has also been proposed to be a polyamine antagonist. It was shown to inhibit the increase of [^{3}H]-MK801 binding produced by spermine in rat brain membranes and to have no effect in the absence of polyamines (Williams *et al*., 1989), which would satisfy the criteria of a competitive antagonist. However, in another study it was found that DET increased [^{3}H]-MK801 binding in rat brain membranes when tested under basal conditions (Reynolds, 1990b). DET is a very small cationic molecule and it is unlikely that it would interact solely with the polyamine receptor site in an *in vivo* situation, but would also bind to other negatively charged particles such as membrane phospholipids.

In the present study, arcaine, 1,10-diaminodecane and DET produced polyamine-like behavioural effects when injected alone. This suggests that these polyamine analogues have some agonist-like activity *in vivo*. In keeping with this, arcaine and 1,10-diaminodecane potentiated the development of the first phase of spermine-induced effects and DET potentiated the development of the second phase of effects, which also suggests agonist activity. However, these polyamine analogues also showed some evidence of antagonism. Arcaine and 1,10-diaminodecane inhibited the development of the second phase of spermine-induced effects and DET inhibited the development of clonic convulsions. Despite this antagonist action, arcaine, 1,10-diaminodecane and DET clearly do not act purely as competitive antagonists of the polyamines *in vivo*.

These results also support the previously published suggestion that the two temporally distinct phases of spermine-induced effects may be mediated by different pharmacological mechanisms of action (Doyle & Shaw, 1996). In the present study, arcaine and 1,10-diaminodecane demonstrated a similar profile of effects, which suggests that these agents act through the same mechanism. Both arcaine and 1,10-diaminodecane potentiated the first stage of spermine-induced effects, but inhibited the development of the second stage effects. However, conversely, DET demonstrated the opposite profile of effects to the other putative antagonists tested. DET was effective against the development of the first stage of spermine induced effects, but potentiated the second stage of effects. Little is known about the pharmacokinetics of arcaine, 1,10-diaminodecane and DET, but evidence of their effects several hours after administration suggests that they are sufficiently persistent. These findings are further *in vivo* evidence supporting a heterogeneity of polyamine binding sites.

The polyamines exert a biphasic bell-shaped effect on [^{3}H]-dizocilpine binding (Ransom & Stec, 1988). At lower doses, polyamines stimulate dizoclipine binding, but at higher doses this gives way to the development of an inhibition. Arcaine has been demonstrated to inhibit the stimulant phase of the curve selectively but not affect the inhibitory phase of effects (Reynolds, 1990a). This suggests that polyamines may have two binding sites on the NMDA receptor macrocomplex, one which is arcaine-sensitive and the other arcaine-insensitive. The present results suggest that the second phase of spermine induced effects may be mediated, at least in part, through an action at the arcaine-sensitive polyamine binding site on the NMDA receptor. DET may mediate its actions predominantly through an action at the arcaine-insensitive polyamine binding site on the NMDA receptor.

NMDA receptor heterogeneity may explain the inconsistencies in findings suggesting antagonist, partial agonist or inverse agonist activities of polyamine analogues. The modulatory effects of polyamines on NMDA receptor activity may differ greatly depending on the subunit composition of the NMDA macrocomplex. Polyamine receptors with different properties may result from different combinations of NMDA receptor subunits, or the influence of the polyamine sites on NMDA receptor activity may be greatly altered through the effects of variations in the expression of the glutamate and glycine receptor sites. As mentioned in the Introduction, it is known that the tonicity of glutamate, glycine and polyamine receptors can greatly influence the activity of polyamine antagonists (Pullan & Powel, 1991; Carter *et al*., 1992; Doyle & Shaw, 1996).

In conclusion, none of the putative polyamine antagonists tested behaved as effective polyamine antagonists *in vivo*. The analogues tested all showed some intrinsic polyamine-like agonist activity. Nevertheless, the finding of some antagonist activity in these readily available simple molecules suggests that the development of other polyamine structural analogues may lead to the discovery of a better polyamine antagonist.

References

ANDERSON, D.J., CROSSLAND, J. & SHAW, G.G. (1972). The actions of spermidine and spermine on the central nervous system. *Neuropharmacology,* **14,** 571 – 577.

BRITTAIN, R.T. (1996). The intracerebral effects of noradrenaline and its modification by drugs in the mouse. *J. Pharm. Pharmacol.,* **18,** 621 – 623.

CARTER, C., MINISCLOU, C. & RIVY, J.P. (1992). Glycine receptor status determines the effects of ifenprodil and spermidine on [^{3}H]-TCP binding to the NMDA receptor. *Br. J. Pharmacol.,* **105,** 18P.

DONEVAN, S.D., JONES, S.M. & ROGAWSKI, M.A. (1992). Arcaine blocks N-methyl-D-aspartate receptor responses by an open channel mechanism: whole-cell and single-channel recording studies in cultured hippocampal neurons. *Mol. Pharmacol.,* **41,** 727 – 735.

DOYLE, K.M. & SHAW, G.G. (1994). The mechanism of the neurotoxic effects of spermidine. *Biochem. Soc. Trans.,* **22,** 386S.

DOYLE, K.M. & SHAW, G.G. (1996). Investigation of the involvement of the N-methyl-D-aspartate receptor macrocomplex in the development of spermine-induced CNS excitation in vivo. *Br. J. Pharmacol.,* **117,** 1803 – 1808.

KATSUWADA, T., KASHIWABUCHI, N., MORI, H., SAKIMURA, K., KUSHIYA, E., ARAKI, K., MEGURO, H., MASAKI, H., KUMANISHI, T., ARAKAWA, M. & MISHINA, M. (1992). Molecular diversity of the NMDA receptor channel. *Nature,* **358,** 36 – 41.

KISH, S.J., WILSON, J.M. & FLETCHER, P.J. (1991). The polyamine synthesis inhibitor α-difluoromethylornithine is neuroprotective against N-methyl-D-aspartate induced brain damage in vivo. *Eur. J. Pharmacol.,* **209,** 101 – 103.

LYNCH, D.R., LAWRENCE, J.J., LENZ, S., ANEGAWA, N.J., DICHTER, M. & PRITCHETT, D.B. (1995). Pharmacological characterisation of heterodimeric NMDA receptors composed of NR 1a and 2B subunits: differences with receptors formed from NR 1a and 2A. *J. Neurochem.,* **64,** 1462 – 1468.

MACIVER, C.R., BEDNAR, D.L. & KARBON, E.W. (1991). Opposite effects of spermine and arcaine on responses of N-methyl-D-aspartate receptors expressed in *Xenopus* oocytes. *Neurosci. Lett.,* **132,** 146 – 150.

MARVIZON, J.-C. & BAUDRY, M. (1994). [^{3}H]Dizocilpine association kinetics distinguish stimulatory and inhibitory polyamine sites of N-methyl-D-aspartate receptors. *J. Neurochem.,* **63,** 963 – 971.

PULLAN, L.M. & POWEL, R.J. (1991). Spermine reciprocally changes the affinity of NMDA receptor agonists and antagonists. *Eur. J. Pharmacol.,* **207,** 173 – 174.

RANSOM, R.W. & STEC, N.L. (1988). Cooperative modulation of (^{3}H)MK801 binding to the N-methyl-D-aspartate receptor-ion channel complex by L-glutamate, glycine and polyamines. *J. Neurochem.,* **51,** 830 – 836.

REYNOLDS, I.J. (1990a). Arcaine is a competitive antagonist of the polyamine site on the NMDA receptor. *Eur. J. Pharmacol.,* **177,** 215 – 216.

REYNOLDS, I.J. (1990b). Arcaine uncovers dual interactions of polyamines with the N-methyl-D-aspartate receptor. *J. Pharmacol. Exp. Ther.,* **255,** 1001 – 1007.

REYNOLDS, I.J. & PALMER, A.M. (1991). Regional variations in [^{3}H]MK801 binding to rat brain N-methyl-D-aspartate receptors. *J. Neurochem.,* **56,** 1731 – 1740.

ROCK, D.M. & MACDONALD, R.L. (1992). The polyamine diaminodecane (DA-10) produces a voltage-dependent flickery block of single NMDA receptor channels. *Neurosci. Lett.,* **144,** 111 – 115.

SACAAN, A.I. & JOHNSON, K.M. (1990a). Characterisation of the stimulatory and inhibitory effects of polyamines on [^{3}H]N-(1-[thienyl]cyclohexyl) piperidine binding to the N-methyl-D-aspartate receptor ionophore complex. *Mol. Pharmacol.,* **37,** 572 – 577.

SACAAN, A.I. & JOHNSON, K.M. (1990b). Competitive inhibition of magnesium-induced [^{3}H]N-(1-[thienyl]cyclohexyl) piperidine binding by arcaine: evidence for a shared spermidine-magnesium binding site. *Mol. Pharmacol.,* **38,** 705 – 710.

SACAAN, A.I. & JOHNSON, K.M. (1990c). Spermidine reverses arcaine's inhibition of N-methyl-D-aspartate induced hippocampal [3H]norepinephrine release. *J. Pharmacol. Exp. Ther.,* **255,** 1060 – 1063.

SUBRAMANIAM, S., DONEVAN, S.D. & ROGAWSKI, M.A. (1992). 1,10-Diaminodecane and 1,12-diaminodecane block NMDA receptor currents by an open channel mechanism. *Neurosci. Lett.,* **147,** 213 – 216.

WILLIAMS, K., ROMANO, C. & MOLINOFF, P.B. (1989). Effects of polyamines on the binding of [3H]MK801 to the N-methyl-D-aspartate receptor: pharmacological evidence for the existence of a polyamine recognition site. *Mol. Pharmacol.,* **36,** 575 – 581.

WILLIAMS, K., DAWSON, V.L., ROMANO, C., DICHTER, M.A. & MOLINOFF, P.B. (1990). Characterisation of polyamines having agonist, antagonist, and inverse agonist effects at the polyamine recognition site of the NMDA receptor. *Neuron,* **5,** 199 – 208.

WILLIAMS, K., ROMANO, C., DICHTER, M.A. & MOLINOFF, P.B. (1991). Modulation of the NMDA receptor by polyamines. *Life Sci.,* **48,** 469 – 498.

WILLIAMS, K., PULLAN, L.M., ROMANO, C., POWEL, R.J., SALAMA, A.I. & MOLINOFF, P.B. (1992). An antagonist/partial agonist at the polyamine recognition site of the N-methyl-D-aspartate receptor that alters the properties of the glutamate recognition site. *J. Pharmacol. Exp. Ther.,* **262,** 539 – 544.

(Received September 12, 1997
Revised January 28, 1998
Accepted February 17, 1998)