0026-895X/92/010083-06$03.00/0

MOLECULAR PHARMACOLOGY, 41:83-88

The Polyamine Spermine Has Multiple Actions on *N*-Methyl-D-aspartate Receptor Single-Channel Currents in Cultured Cortical Neurons

DAVID M. ROCK[1] and ROBERT L. MACDONALD

Neuroscience Program (D.M.R.) and Departments of Neurology and Physiology (R.L.M.), University of Michigan, Ann Arbor, Michigan 48104-1687

Received May 24, 1991; Accepted September 6, 1991

SUMMARY

Spermine potentiates the action of *N*-methyl-D-aspartate (NMDA) at micromolar concentrations but is less effective at millimolar concentrations. In cultured cortical neurons we demonstrate that spermine enhances NMDA receptor currents in a unique manner. At low concentrations (1–10 μM) spermine enhances NMDA receptor current by increasing channel opening frequency, and at higher concentrations (>10 μM) it produces, in addition, a voltage-dependent decrease in channel amplitude and average open time that limits its enhancing action. It is likely that these two actions of spermine, due to differences in concentration and voltage dependence, are mediated by independent sites on the NMDA receptor complex.

Glutamate is one of the major excitatory neurotransmitters in the central nervous system. Postsynaptic glutamate receptors have been classically subdivided into three types, according to affinity for selective agonists NMDA, quisqualate (AMPA/metabotropic), and kainate (1, 2). There has been considerable interest in the NMDA subtype because of its role in the induction of long term potentiation in hippocampus (reviewed in Ref. 3), implying a role in learning and memory as well as in pathophysiological states such as epilepsy and ischemic neurodegeneration (reviewed in Ref. 4).

Receptor binding assays and electrophysiological studies have described several modulatory sites on the NMDA receptor ion channel complex that can either increase or decrease activity (1). These include 1) a glycine co-agonist site thought to be essential for opening of the NMDA channel (5, 6), 2) sites inside the channel where divalent cations (Mg^{2+} and Zn^{2+}) (7–9) and noncompetitive open-channel blockers (phencyclidine and MK-801) act to reduce current (10, 11), 3) a pH-sensitive site (12–14), and 4) a more recently described polyamine modulatory site (15).

Polyamines were first shown to interact with the NMDA receptor channel in binding studies by Ransom and Stec (16). Spermine and spermidine both enhance [^{3}H]MK-801 or [^{3}H] *N*-(1-[thienyl]cyclohexyl)piperidine binding at concentrations in the low micromolar range, either in the presence or in the absence of added glutamate and glycine (16–18). Higher concentrations of spermine were less effective in enhancing binding of these open-channel blockers, resulting in a biphasic concentration-response curve. Spermine has also been shown to interact with the glycine co-agonist site (19) and may even have some interaction with the NMDA recognition site (20). Structurally related analogs of spermine and spermidine interact with the polyamine site to block the action of spermine and spermidine (21, 22).

Electrophysiological studies using whole-cell voltage-clamp in cultured neurons and in poly(A)$^+$ mRNA-injected frog oocytes have shown that spermine and spermidine can enhance NMDA-evoked whole-cell currents (21, 23–25) as well as inhibit NMDA receptor current at higher polyamine concentrations (21). These effects were independent of NMDA and glycine concentrations (24, 25). Both binding and electrophysiological studies indicate that the polyamine enhancement of NMDA receptor current occurs through a site distinct from the glutamate recognition or glycine sites.

The basis for the enhancement of NMDA receptor currents by spermine is unclear at present. The following studies using whole-cell voltage-clamp and single-channel recording techniques were carried out to investigate the mechanism of modulation of the NMDA receptor channel by spermine.

Materials and Methods

Culture preparation. Cultures of fetal rat cortical neurons were prepared by standard methods (26). Briefly, cortices from E-18 fetuses

This work was supported by United States Public Health Service Grant NS19613 to R.L.M.
[1]Present address: Pharmacology Department, Parke-Davis Research, 2800 Plymouth Road, Ann Arbor, MI 48106-1047.

ABBREVIATIONS: NMDA, *N*-methyl-D-aspartate; AMPA, α-amino-3-hydroxy-5-methylisoxazole-4-propionate; GABA, γ-amino-*n*-butyric acid; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; EGTA, ethylene glycol bis(β-aminoethyl ether)-*N,N,N′,N′*-tetraacetic acid.

were enzymatically treated and then mechanically dissociated. Cells were suspended in Eagle's minimum essential medium supplemented with 30 mM glucose, 26 mM bicarbonate, 10% fetal calf serum, and 10% horse serum. Cells were plated on poly-L-lysine-coated 35-mm plastic culture dishes. On the fifth day after plating, a combination of 15 μg/ml 5-fluoro-2-deoxyuridine and 35 μg/ml uridine, in minimum essential medium lacking fetal calf serum, was added to suppress glial/astrocyte growth. Cells were maintained in culture for 2–5 weeks before experiments.

Solutions. The same external and internal solutions were used for whole-cell recording and excised outside-out single-channel recordings. The external solution consisted of 142 mM NaCl, 1.5 mM KCl, 1 mM $CaCl_2$, 10 mM glucose, 10 mM Na-HEPES (pH 7.4), and 500 nM tetrodotoxin (added for whole-cell recordings) (osmolarity, 320–300 mOsm). The internal pipette solution consisted of 153 mM CsCl, 10 mM Cs-HEPES, and 5 mM EGTA (pH 7.4) (osmolarity adjusted to 305–280 mOsm with water). Chemicals were obtained from Sigma Chemical Co. (St. Louis, MO).

Drug application. NMDA (Sigma), glycine (Sigma), and spermine tetrahydrochloride (United States Biochemicals) were dissolved in external solution at a concentration between 10 mM and 100 mM and were diluted via serial dilutions to final concentrations as indicated. Addition of 100 mM spermine tetrahydrochloride to the external solution had no effect on pH. Glycine concentrations were expressed as the amount of glycine added to the external solution. Drugs were applied to whole cells or patches by pressure ejection (0.25–1.0 psi) from blunt-tipped (15–25-μm) glass micropipettes positioned within 50 μm of the cell or patch.

Electrophysiological recording. For experiments, cultures were placed on the stage of an inverted phase contrast microscope at room temperature (20–23°) and were superfused at approximately 1 ml/min during recordings. High-resistance gigaohm seals were obtained with low-resistance glass micropipettes (5–10 MΩ), with procedures for whole-cell voltage-clamp and outside-out excised patch recordings similar to those of Hamill *et al.* (27). The intrapipette potential for whole-cell and single-channel recordings was −75 mV, unless otherwise stated. Recordings were performed with a L/M EPC-7 amplifier (LIST Medical Instruments), and currents were recorded on a videocassette system via a digital audio processor (PCM II; Medical Systems, Inc.) (16 bit, 44 kHz). Currents were simultaneously displayed on a chart recorder (Gould Inc), using a low-pass (−3 db at 1 kHz) eight-pole Bessel filter (Frequency Devices). For single-channel analysis, the data were played back from the videocassette system, through a low-pass filter (−3 db at 2 kHz), and digitized (20-kHz, 16-bit Tecmar analog/digital converter) for computer analysis. This setup resulted in a system dead-time of 70 μsec, so only openings of >140 μsec (2 times the system dead time) were considered valid openings.

Single-channel analysis. Single-channel data were analyzed by computer, using a locally written channel detection program (50% threshold-crossing criterion) and locally written analysis programs to determine opening frequency and open and closed durations by methods previously described (28). Percentage of change in opening frequency was determined in those patches where control solution (NMDA alone) was applied before and after coapplication of spermine and NMDA. The software analysis package IPROC (Axon Instruments) was used to generate amplitude histograms, which were fit by eye with Gaussian curves to determine peak amplitude of the main conductance. Although NMDA has been shown to activate a channel or channels that exist in several conductance states (29, 30), openings were predominantly of 50-pS conductance (see amplitude histogram; Fig. 4). In this study, openings from the two higher conductance levels (40 and 50 pS) were analyzed. Concentrations of spermine above 10 μM resulted in a decrease in channel amplitude. Detection windows were adjusted to compensate for these changes in amplitude.

Bursts can be defined as groups of openings separated by relatively long closed periods. For the purpose of this analysis, a critical closed time (t_c) was chosen such that all openings separated by closures longer than this critical closed time were considered to belong to a burst. A modification of the equal proportion of misclassification method was used to select a t_c of 15 msec (28).

Significant changes in single-channel parameters by spermine were determined using a two-tailed *t* test versus paired control observations.

Results

Spermine enhanced whole-cell voltage-clamped NMDA currents. Spermine produced a concentration-dependent enhancement of whole-cell NMDA receptor current, which was observed over a wide concentration range (0.1 μM to 1 mM). Spermine did not enhance NMDA receptor current on all neurons tested and, although not systematically studied, spermine enhancement of NMDA receptor current appeared to be consistent among neurons in a given culture dish. Seventy percent of neurons (n = 34) tested showed enhancement of NMDA current by micromolar concentrations of spermine. In spermine-responsive neurons, the peak enhancement of NMDA receptor current was observed at 10 μM spermine (mean ± standard error of increase of 175 ± 15%, n = 13) (Fig. 1A). At concentrations above 10 μM, spermine was less effective in enhancing NMDA receptor currents (Fig. 1B, *upper trace*). Spermine enhancement of NMDA receptor currents occurred in the presence of saturating concentrations of NMDA (100 μM) and glycine (10 μM), with 1 mM spermine increasing NMDA receptor currents by 38 ± 12% (n = 8) under these conditions.

The reduction in enhancement of whole-cell current at higher concentrations of spermine was voltage dependent and not observed in neurons voltage clamped at +75 mV. Fig. 1B shows the difference in relative enhancement at +75 mV, compared with −75 mV. In this example, 10 μM spermine caused a 100% increase in amplitude of the NMDA receptor current at a −75 mV holding potential, whereas 1 mM spermine caused a 66% increase (Fig. 1B, *upper trace*). In the same cell clamped at +75 mV, both 10 μM and 1 mM spermine caused a 100% increase in the amplitude of the current (Fig. 1B, *lower trace*). In neurons where spermine did not enhance NMDA receptor currents, high concentrations of spermine (100 μM and 1 mM) caused a small voltage-dependent reduction in the NMDA receptor current (n = 4). In contrast to its effects on NMDA receptor currents, spermine (1 mM) had no effect on 5 μM AMPA (97 ± 1.4% of control; n = 5) or 5 μM GABA (97 ± 0.9% of control; n = 4) receptor currents.

Spermine had multiple effects on NMDA single-channel currents. Application of 5 μM NMDA (0.5 μM glycine) to excised outside-out patches resulted in an average opening frequency of 10 ± 2.6 openings/sec (n = 18) (Fig. 2). Addition of spermine (1–1000 μM) resulted in a concentration-dependent increase in channel opening frequency, with a 45% increase at 1 μM (2,067 openings, six applications, four patches) and 140% increase at 100 μM (22,714 openings, 12 applications, six patches), at a holding potential of −75 mV. At a holding potential of +50 mV, spermine increased opening frequency in three of four patches at 10 μM and in three of five patches at 100 μM. Because of the variability of the spermine effect (whole-cell data) and differences in opening frequency among patches, it was difficult to compare the percentage changes in opening frequency between different holding potentials.

The increase in opening frequency at 100 μM spermine at −75 mV holding potential was not dependent on glycine con-

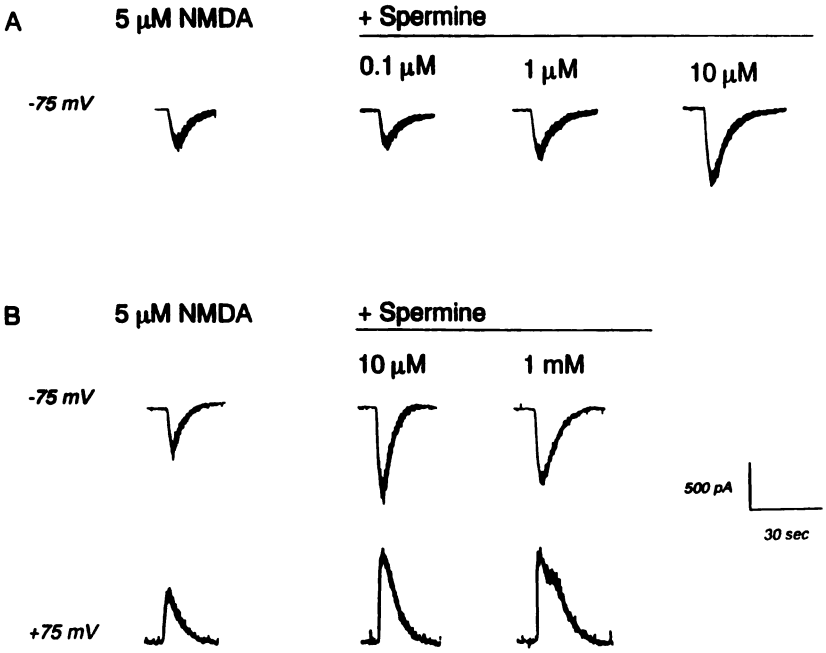

Fig. 1. Whole-cell voltage-clamp recordings of NMDA receptor currents were enhanced by spermine. A, Enhancement of 5 μM NMDA receptor currents by the coapplication of 0.1, 1, and 10 μM spermine was concentration dependent (−75 mV holding potential). B, The limitation in enhancement at higher spermine concentrations was voltage dependent. *Upper trace*, reduction in enhancement seen at 1 mM spermine, compared with 10 μM spermine, at a holding potential of −75 mV. *Lower trace* (same neuron), no difference in enhancement at the same concentrations at +75 mV holding potential.

centration, because opening frequency increased approximately 2-fold in the absence of or at a saturating concentration of glycine (0–10 μM). Addition of 10 μM glycine resulted in a 223% (n = 6) increase in opening frequency, compared with control applications of 5 μM NMDA in the absence of glycine, and a 45% increase over 5 μM NMDA with 0.5 μM added glycine. These results indicate that spermine enhanced NMDA receptor current by increasing opening frequency though a mechanism independent of the glycine site.

At concentrations of 10–1000 μM, spermine caused a voltage-dependent decrease in single-channel amplitude at a holding potential of −75 mV (Table 1). Application of 5 μM NMDA to patches voltage-clamped at −75 mV resulted in openings with a mean amplitude of 3.6 pA (Fig. 3, *left*). Addition of spermine at 100 μM reduced the mean amplitude to 2.8 pA, and at 1 mM spermine further reduced openings to a mean amplitude of 1.5 pA (Fig. 3, *left*). The reduction of channel amplitude was voltage dependent. Spermine did not alter channel amplitude at either concentration at +50 mV holding potential (Fig. 3, *right*; Table 1). A histogram of average amplitudes of channel openings illustrates the shift in channel amplitude seen with the application of 100 μM spermine at −75 mV holding potential (Fig. 4). In this patch, the mean current amplitude of the main conductance state was decreased by approximately 26%. The decrease in mean current amplitude was proportional to spermine concentration, with a 10% decrease at 10 μM spermine and a 60% decrease at 1 mM spermine. Preliminary analysis of recordings at higher bandwidths (Fig. 5) (filtered at 4 kHz) did not indicate that the reduction in channel amplitude was associated with an increase in open-channel noise. The reduction in single-channel current amplitude seen at higher spermine concentrations is consistent with the reduction in current noise noted in whole-cell voltage-clamp recordings.

In addition, spermine produced a concentration-dependent reduction of average open time at a holding potential of −75 mV (Table 1). Average open time for control NMDA applications in different patches ranged from 3.6 to 5 msec (n = 2,000–

TABLE 1

Effect of spermine on single-channel parameters at −75 mV and +50 mV holding potentials

Data are represented as mean ± standard error of 4–23 observations.

Holding potential	Spermine	Main state amplitude	Average open time	Burst duration
	μM	pA	msec	msec
−75 mV	0	3.6 ± 0.05	4.4 ± 0.4	15 ± 1.1
	10	3.2 ± 0.06[a]	4.6 ± 0.3	15 ± 1.2
	100	2.8 ± 0.05[a]	2.6 ± 0.3[a]	13 ± 1.5
+50 mV	0	3.1 ± 0.11	7.3 ± 0.9	17 ± 2.1
	10	3.0 ± 0.09	9.1 ± 2.4	13 ± 3.1
	100	3.0 ± 0.19	8.0 ± 1.1	21 ± 2.2

[a] $p \leq 0.05$ (two-tailed t test).

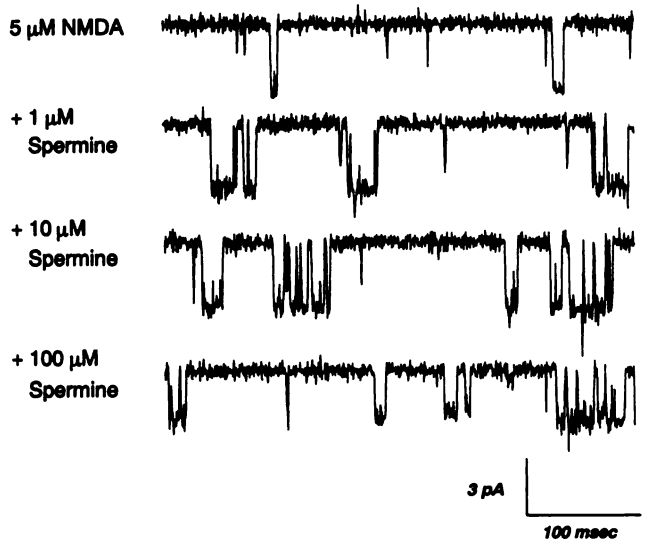

Fig. 2. Low concentrations of spermine caused a concentration-dependent increase in opening frequency of NMDA receptor single-channel currents. Application of NMDA (5 μM) (*top trace*) to an excised outside-out patch resulted in channel opening at a frequency of approximately 5 sec^{-1}. Addition of spermine (1, 10, and 100 μM) (*lower three traces*) caused a concentration-dependent increase in channel opening frequency. Data were taken from the same patch at a holding potential of −75 mV.

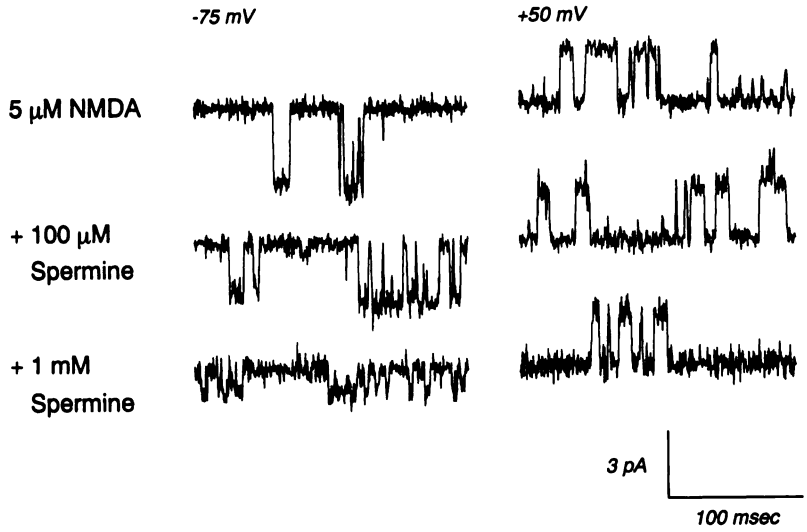

Fig. 3. High spermine concentrations resulted in a voltage-dependent decrease in NMDA single-channel amplitude. NMDA (5 μM) evoked single-channel openings of around 3.8 pA at −75 mV (*left, top trace*). Addition of 100 μM spermine resulted in a decrease in channel amplitude to 2.8 pA (*left, middle trace*) and a further reduction to 1.5 pA with 1 mM spermine (*left, bottom trace*). The reduction in amplitude of NMDA single-channel currents was voltage dependent. At +50 mV holding potential (*right, top trace*), NMDA evoked channels with an amplitude of 2.9 pA. Current amplitude was not changed by the addition of 100 μM or 1 mM spermine (*right, middle* and *bottom traces*).

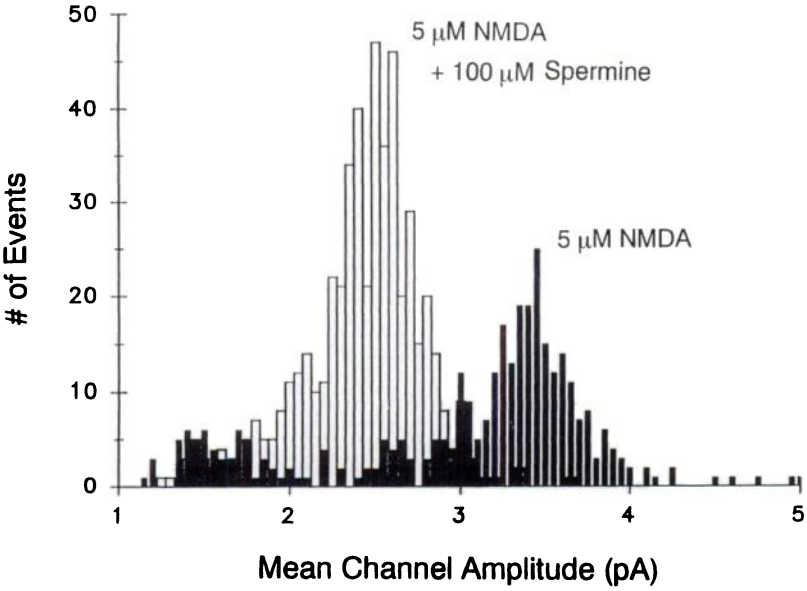

Fig. 4. Spermine (100 μM) reduced the mean single-channel amplitude. Histograms of mean event amplitude were generated using a wide detection window to accept events with amplitudes between 1.2 and 5 pA and durations longer than 0.5 msec. Application of 5 μM NMDA for 30 sec (■) resulted in a histogram with the main peak at 3.5 pA. The combination of 5 μM NMDA and 100 μM spermine applied for 30 sec (□) resulted in a histogram with the main peak at 2.5 pA.

22,000 openings), with an average of 4.4 msec (n = 10,257). An 8% decrease in average open time was first observed at 10 μM (5.0 to 4.6 msec), with an 83% decrease (3.6 to 0.6 msec) seen at 1 mM. The reduction in average open time at 100 μM spermine was voltage dependent, with no decrease in average open time observed at a +50 mV holding potential (Table 1). Burst durations were unaffected by the addition of spermine at holding potentials of −75 and +50 mV (Table 1).

Discussion

Spermine enhanced NMDA receptor currents over a wide concentration range. These results are similar to previously reported results (21, 23–25). The effect of spermine on channel opening frequency occurred at low micromolar concentrations, whereas the voltage-dependent reduction in enhancement occurred at higher concentrations. Because spermine is present in central nervous system tissue at high micromolar concentrations (31), these actions of spermine on single-channel currents may be important in the modulation of NMDA receptor currents.

Mechanism of enhancement of NMDA receptor cur-

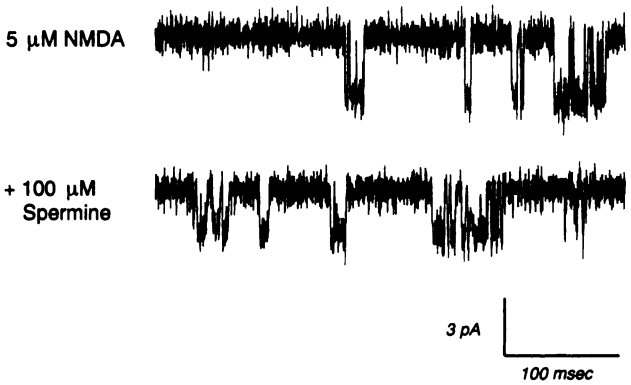

Fig. 5. Spermine reduction of channel amplitude was not associated with an increase in open-channel noise. The cutoff frequency of the low pass filter was increased to 4 kHz during digitization of these data. Open-channel noise associated with openings evoked by 5 μM NMDA (*upper trace*) was not different from that associated with openings evoked by 5 μM NMDA and 100 μM spermine (*lower trace*). Similar results were obtained with a filter cutoff frequency of 6 kHz.

rent by spermine. Enhancement of whole-cell current can arise from a variety of changes in single-channel properties. These include an increase in mean channel current amplitude, an increase in average channel open time, or an increase in the frequency of channel opening. The single-channel experiments show that, at low concentrations, spermine increased channel opening frequency without increasing average open time or amplitude. Although this action was similar to the effect of glycine on NMDA single-channel currents (5), the spermine-induced increase in opening frequency occurred in the presence of saturating glycine concentrations (both whole-cell and single-channel data). This finding indicates that the polyamine binding site that modulates opening frequency is independent of the glycine site, which agrees with receptor binding assays and other electrophysiological studies (17, 24, 25) and argues against an indirect action at the glycine site, which has been proposed for Zn^{2+} (32).

This mechanism of enhancement of NMDA receptor currents by spermine, increasing opening frequency, is similar to the one proposed for the benzodiazepine enhancement of GABA receptor currents (33, 34). However, because the single-channel recordings were obtained primarily from multichannel patches, it was not possible to determine the basis for the increased opening frequency. Possibilities include an increase in opening frequency of a single channel or an increase in the number of channels opening.

Mechanism of the limitation in enhancement of NMDA receptor currents by high concentrations of spermine. The single-channel experiments suggest that the reduction in enhancement of NMDA receptor current at high spermine concentrations was likely due to a decrease in single-channel amplitude and average channel open time. These voltage- and concentration-dependent effects occurred over the same concentration range in both single-channel and whole-cell voltage-clamp studies.

Reduction in the amplitude of single-channel current may occur in several different ways. The NMDA receptor has been shown to open to a variety of subconductance states or conductance levels (29, 30, 35), and a shift to any one of these subconductance states would result in a decrease in mean channel amplitude. This shift to a different conductance state would cause discrete changes in amplitude histograms, predicted by the amplitudes of the subconductance states. Because the spermine-induced change in mean channel amplitude was concentration dependent and to amplitudes not seen in control NMDA applications, it is not likely that spermine caused a shift from the main state to subconductance states of the NMDA receptor.

Divalent cations have been shown to affect NMDA receptor currents by a variety of mechanisms, including voltage-dependent effects on average open time and channel amplitude. Because spermine is a polyvalent cation at physiological pH, its effects on average open time and channel amplitude may be similar. Block of NMDA currents by extracellular Mg^{2+} was first shown by Ault *et al.* (36). Ascher and Nowak (7) attributed the effect of Mg^{2+} to open-channel block, similar to the actions of local anesthetics on the nicotinic acetylcholine channel described by Neher and Steinbach (37). The Mg^{2+} block of NMDA single-channel currents was associated with a voltage-dependent decrease in average open time but no change in current amplitude. Therefore, although the reduction in average open time by spermine may be explained by an open-channel block, spermine clearly has a second voltage-dependent effect, reduction of channel amplitude, that cannot be explained by a simple open-channel block mechanism similar to that of Mg^{2+}.

The blocking actions of external Zn^{2+} on inward NMDA receptor currents (9) and of internal Mg^{2+} on outward (38) NMDA receptor currents have been attributed to fast open-channel block. With fast open-channel block, the open NMDA channel becomes rapidly blocked and unblocked, which, due to the limited resolution of the recording system, leads to an apparent reduction in current amplitude and an increase in open-channel noise (39). Preliminary analysis of data recorded with higher bandwidths (see Fig. 5) does not support the idea that spermine reduces channel amplitude by fast open-channel block.

Divalent cation effects on surface charges have been shown to affect the amplitude of NMDA single-channel currents. High external Ca^{2+} has been shown not only to shift the reversal potential for NMDA currents but also to reduce the amplitude of inward currents carried by Na^+ and Ca^{2+} (7). This rectification of inward current may be due to an interaction of the divalent Ca^{2+} ions with negative charges on the external surface of the receptor, thus altering inward current flow (40). The effect of high Ca^{2+} on NMDA single-channel current amplitude is voltage dependent, with no effect on outward Cs^+ current (7, 40). Because a voltage-dependent effect on the amplitude of single-channel currents has been shown to occur by changing negative charges lining the external vestibule of the nicotinic acetylcholine receptor (41), it is possible that an interaction of the positively charged groups of spermine with surface charges at the mouth of the NMDA receptor ion channel may underlie the reduction of single-channel current amplitude.

The multiple actions of spermine on single-channel currents may thus explain the biphasic nature of concentration-response curves seen in electrophysiological (21, 24) and receptor binding assays (16, 17). Low concentrations of spermine increase opening frequency, thereby enhancing both NMDA receptor currents and the binding of open-channel blockers that preferentially bind to an open state of the channel (see Ref. 10). As the concentration of spermine is increased above 10 μM, opening frequency continues to increase but the decrease in average open time observed at these spermine concentrations begins to limit the percentage of time the channel spends in the open state, which results in a limitation of the enhancement of NMDA receptor currents and binding of open-channel blockers. Because decreased average open time occurs only at higher spermine concentrations, a biphasic concentration-response curve may result.

The reduction in channel amplitude seen at spermine concentrations above 10 μM also limits the enhancement of NMDA receptor current, but the effect of reduced channel amplitude on the binding of open channel blockers is unclear. An effect of spermine on surface charge at the mouth of the ion channel or fast open-channel block may affect access of the blockers to the internal binding site, resulting in a reduction of enhancement of binding.

In summary, we suggest that the polyamine spermine enhances NMDA receptor current in a unique way. Spermine increases channel opening frequency to enhance current, but the enhancement is limited, due to a voltage-dependent decrease in average open time and single-channel amplitude. The

polyamine binding site that modulates opening frequency appears to be independent of the glycine site. The voltage-dependent actions of spermine to reduce channel amplitude and average open time may be due to a complex interaction with surface charges at the ion channel. Because of the difference in voltage-dependence and the separation in concentration range for the effects of spermine on channel amplitude and average open time, compared with opening frequency, it is likely that spermine produces these effects by interacting with two independent sites on the NMDA receptor ion channel complex.

Acknowledgments

The authors wish to thank Mr. Gregory Campbell and Mr. Sean Hanson for preparation and maintenance of cell cultures. We also wish to thank Dr. Roy Twyman for assistance with data analysis and Dr. Twyman, Dr. Robert Gross, and Dr. Nada Porter for useful comments on this manuscript.

References

1. Monaghan, D. T., R. J. Bridges, and C. W. Cotman. The excitatory amino acid receptors: their classes, pharmacology, and distinct properties in the function of the central nervous system. *Annu. Rev. Pharmacol. Toxicol.* **29**:365–402 (1989).
2. Watkins, J. C., P. Krogsgaard-Larsen, and T. Honore. Structure-activity relationships in the development of excitatory amino acid receptor agonists and competitive antagonists. *Trends Pharmacol. Sci.* **11**:25–33 (1990).
3. Nicoll, R. A., J. A. Kauer, and R. C. Malenka. The current excitement about long-term potentiation. *Neuron* **1**:97–103 (1988).
4. Choi, D. W. Glutamate neurotoxicity and diseases of the nervous system. *Neuron* **1**:623–634 (1988).
5. Johnson, J. W., and P. Ascher. Glycine potentiates the NMDA response in cultured mouse brain neurons. *Nature (Lond.)* **325**:529–531 (1987).
6. Kleckner, N., and R. Dingledine. Requirement for glycine for activation of NMDA-receptors in *Xenopus* oocytes. *Science (Washington D. C.)* **241**:835–837 (1988).
7. Ascher, P., and L. Nowak. The role of divalent cations in the *N*-methyl-D-aspartate responses of mouse central neurons in culture. *J. Physiol. (Lond.)* **399**:247–266 (1988).
8. Westbrook, G. L., and M. L. Mayer. Micromolar concentrations of Zn^{2+} antagonize NMDA and GABA responses of hippocampal neurons. *Nature (Lond.)* **328**:640–643 (1987).
9. Christine, C. W., and D. W. Choi. Effect of zinc on NMDA receptor-mediated channel currents in cortical neurons. *J. Neurosci.* **10**:108–116 (1990).
10. MacDonald, J. F., and L. M. Nowak. Mechanism of blockade of excitatory amino acid receptor channels. *Trends Pharmacol. Sci.* **11**:167–172 (1990).
11. Lodge, D., and K. M. Johnson. Noncompetitive excitatory amino acid receptor antagonists. *Trends Pharmacol. Sci.* **11**:81–86 (1990).
12. Tang, C., M. Dichter, and M. Morad. Modulation of the *N*-methyl-D-aspartate channel by extracellular H^+. *Proc. Natl. Acad. Sci. USA* **87**:6445–6449 (1990).
13. Traynelis, S. F., and S. G. Cull-Candy. Proton inhibition of *N*-methyl-D-aspartate receptors in cerebellar neurons. *Nature (Lond.)* **345**:347–350 (1990).
14. Vyklicky, L., V. Vlachova, and J. Krusek. The effect of external pH changes on responses to excitatory amino acids in mouse hippocampal neurones. *J. Physiol. (Lond.)* **430**:497–517 (1990).
15. Williams, K., C. Romano, M. A. Dichter, and P. B. Molinoff. Modulation of the NMDA receptor by polyamines. *Life Sci.* **48**:469–498 (1991).
16. Ransom, R. W., and N. L. Stec. Cooperative modulation of [^{3}H]MK-801 binding to the *N*-methyl-D-aspartate receptor-ion channel complex by L-glutamate, glycine, and polyamines. *J. Neurochem.* **51**:830–836 (1988).
17. Williams, K., C. Romano, and P. B. Molinoff. Effects of polyamines on the binding of [^{3}H]MK-801 to the *N*-methyl-D-aspartate receptor: pharmacological evidence for the existence of a polyamine recognition site. *Mol. Pharmacol.* **36**:575–581 (1989).
18. Sacaan, A. I., and K. M. Johnson. Characterization 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 (1990).
19. Sacaan, A. I., and K. M. Johnson. Spermine enhances binding to the glycine site associated with the *N*-methyl-D-asparate receptor complex. *Mol. Pharmacol.* **36**:836–839 (1989).
20. Pullman, L. M., R. J. Powel, R. J. Stumpo, M. Britt, A. B. Klika, B. A. Meiners, and A. I. Salama. Stereoselective enhancement by (*R*)-HA-966 of the binding of [^{3}H]CPP to the NMDA receptor complex. *Eur. J. Phamacol.* **189**:237–240 (1990).
21. Williams, K., V. L. Dawson, C. Romano, M. A. Dichter, and P. B. Molinoff. Characterization of polyamines having agonist, antagonist and inverse agonist effects at the polyamine recognition site of the NMDA receptor. *Neuron* **5**:199–208 (1990).
22. Reynolds, I. J. Arcaine is a competitive antagonist of the polyamine site on the NMDA receptor. *Eur. J. Pharmacol.* **177**:215–216 (1990).
23. Sprosen, T. S., and G. N. Woodruff. Polyamines potentiate NMDA induced whole-cell currents in cultured striatal neurons. *Eur. J. Pharmacol.* **179**:477–478 (1990).
24. McGurk, J. F., M. L. V. Bennett, and R. S. Zukin. Polyamines potentiate the responses of *N*-methyl-D-aspartate receptors expressed in *Xenopus* oocytes. *Proc. Natl. Acad. Sci. USA* **87**:9971–9974 (1990).
25. Brackely, P., R. Goodnow, Jr., K. Nakanishi, H. L. Sudan, and P. N. R. Usherwood. Spermine and philanthotoxin potentiate excitatory amino acid responses of *Xenopus* oocytes injected with rat and chick brain RNA. *Neurosci. Lett.* **114**:51–56 (1990).
26. Ransom, B. R., E. Neale, M. Henkart, P. N. Bullock, and P. G. Nelson. Mouse spinal cord in cell culture. I. Morphology and intrinsic neuronal electrophysiological properties. *J. Neurophysiol. (Bethesda)* **40**:1132–1150 (1977).
27. Hamill, O. P., M. E. Neher, B. Sakmann, and F. J. Sigworth. Improved patch-clamp techniques for high resolution current recording from cells and cell-free membrane patches. *Pfluegers Arch.* **391**:85–100 (1981).
28. Macdonald, R. L., C. J. Rogers, and R. E. Twyman. Kinetic properties of the $GABA_A$ receptor main conductance state of mouse spinal cord neurones in culture. *J. Physiol. (Lond.)* **410**:479–499 (1989).
29. Cull-Candy, S. G., and M. M. Usowicz. Multiple-conductance channels activated by excitatory amino acids in cerebellar neurons. *Nature (Lond.)* **325**:525–528 (1987).
30. Jahr, C. E., and C. F. Stevens. Glutamate activates multiple single channel conductances in hippocampal neurons. *Nature (Lond.)* **325**:522–525 (1987).
31. Shaw, G. G., and A. J. Pateman. The regional distribution of the polyamines spermidine and spermine in brain. *J. Neurochem.* **20**:1225–1230 (1973).
32. Yeh, G. C., D. W. Bonhaus, and J. O. McNamara. Evidence that zinc inhibits *N*-methyl-D-aspartate receptor-gated ion channel activation by noncompetitive antagonism of glycine binding. *Mol. Pharmacol.* **38**:14–19 (1990).
33. Study, R. E., and J. L. Barker. Diazepam and (−)pentobarbital: flucuation analysis reveals different mechanisms for potentiation of γ-aminobutyric acid responses in cultured central neurons. *Proc. Natl. Acad. Sci. USA* **78**:7180–7184 (1981).
34. Twyman, R. E., C. J. Rogers, and R. L. Macdonald. Differential regulation of γ-aminobutyric acid receptor channels by diazepam and phenobarbital. *Ann. Neurol.* **25**:213–220 (1989).
35. Cull-Cancy, S. G., and M. M. Usowicz. On the multiple-conductance single channels activated by excitatory amino acids in large cerebellar neurones of the rat. *J. Physiol. (Lond.)* **415**:555–582 (1989).
36. Ault, B., R. H. Evans, A. A. Francis, D. J. Oakes, and J. C. Watkins. Selective depression of excitatory amino acid induced depolarizations by magnesium ions in isolated spinal cord preparations. *J. Physiol. (Lond.)* **307**:413–428 (1980).
37. Neher, E., and J. H. Steinbach. Local anaesthetics transiently block currents through single acetylcholine-receptor channels. *J. Physiol. (Lond.)* **277**:153–176 (1978).
38. Johnson, J. W., and P. Ascher. Voltage-dependent block by intracellular Mg^{2+} of *N*-methyl-D-aspartate-activated channels. *Biophys. J.* **57**:1085–1090 (1990).
39. Yellen, G. Ionic permeation and blockade in Ca^{2+}-activated K^+ channels of bovine chromaffin cells. *J. Gen. Physiol.* **84**:157–186 (1984).
40. Ascher, P. Divalent cations and the NMDA channel. *Biomed. Res.* **9 (Suppl. 2)**:31–37 (1988).
41. Imoto, K., C. Busch, B. Sakmann, M. Mishina, T. Konno, J. Nakai, H. Bujo, Y. Mori, K. Fukuda, and S. Numa. Rings of negatively charged amino acids determine the acetylcholine receptor channel conductance. *Nature (Lond.)* **335**:645–648 (1988).

Send reprint requests to: Robert L. Macdonald, M.D., Ph.D., Neuroscience Laboratory Building, 1103 East Huron, Ann Arbor, MI, 48104-1687.