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Spermine and Related Polyamines Produce a Voltage-Dependent Reduction of *N*-Methyl-D-aspartate Receptor Single-Channel Conductance

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SUMMARY

Several polyamines have been shown to interact with a site on the *N*-methyl-D-aspartate (NMDA) receptor that regulates the binding of open channel blockers. Spermine (SP) and spermidine (SD), polyamine agonists, enhanced binding of open channel blockers, whereas arcaine (ARC), diethylenetriamine (DET), and putrescine (PUT), polyamine antagonists, reduced the polyamine enhancement of open channel blocker binding. We previously showed that SP had multiple actions on NMDA receptor single-channel currents that underlie its effect on whole-cell NMDA receptor current. At high concentrations, SP produced a voltage-dependent decrease in NMDA receptor single-channel conductance and average open time. In the present study, another polyamine agonist (SD) produced a similar reduction of NMDA receptor single-channel conductance at higher concentrations. The polyamine antagonists (ARC, DET, and PUT), however, produced a voltage-dependent reduction in NMDA receptor whole-cell currents and reductions in single-channel conductance and average open time, even in the absence of polyamine agonists. The rank order of potency for reduction of NMDA receptor single-channel conductance by polyamines was ARC > SP > SD > PUT = DET, a rank order similar to that for the inhibitory actions of polyamines in receptor binding assays and for the effects of the antagonists on NMDA receptor whole-cell currents. The polyamine antagonist DET did not block the reduction of single-channel conductance by the polyamine agonist SP. In fact, the effects of SP and DET on single-channel conductance were additive. DET also showed a variable enhancement of NMDA receptor whole-cell currents in some neurons, suggesting polyamine agonist-like properties. These results are not consistent with the standard pharmacological profile for agonists and antagonists acting at the same site. Potential mechanisms for the effects of the polyamines on single-channel conductance are discussed.

SP, SD, and PUT (Fig. 1) are naturally occurring polyamines that are the product of ornithine metabolism (1-3). Although the role of polyamines is unclear, several lines of evidence indicate that polyamines may be neuromodulators in the central nervous system. This evidence includes 1) the presence of high intracellular concentrations of polyamines (1, 2), 2) Ca^{2+}-dependent release of SP evoked by K^+ or electrical stimulation (4), 3) the presence of a high affinity uptake system for polyamines (5), and 4) a recently described modulatory effect on the NMDA receptor channel.

Polyamines interact with the NMDA subtype of postsynaptic glutamate receptors. In receptor binding assays, SP and SD enhanced binding of the open channel blockers [^{3}H]MK-801 and [^{3}H]TCP to the NMDA receptor channel (6-8). This enhancement occurred at low micromolar concentrations either in the presence or in the absence of added glutamate and glycine. Higher concentrations of SP, however, were less effective in enhancing binding, resulting in a biphasic concentration-response curve. SP also has been shown to affect binding at the glycine coagonist site (9) and may even have some modulatory action at the NMDA recognition site (10, 11). Recent studies with [^{3}H]SD have indicated the presence of high and low affinity binding sites for SP in rat brain membranes (12).

DET, ARC, and PUT, structurally related analogs of SP and SD, interact with the polyamine site to block the effects of SP and SD on the binding of open channel blockers and, thus, have been suggested to be antagonists at the polyamine binding site (7, 8, 13-18). DET and ARC blocked SP and SD enhancement of binding of open channel blockers to the NMDA receptor in a competitive manner, although ARC may have had additional actions (16-18). PUT has also been considered a polyamine antagonist, because it inhibited the SP- and SD-enhanced MK-801 and TCP binding, but the interaction was

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ABBREVIATIONS: SP, spermine; NMDA, *N*-methyl-D-aspartate; PUT, putrescine; DET, diethylenetriamine; ARC, arcaine; SD, spermidine; TCP, *N*-(1-[thienyl]cyclohexyl)piperidine; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; EGTA, ethylene glycol bis(β-aminoethyl ether)-*N,N,N',N'*-tetraacetic acid.

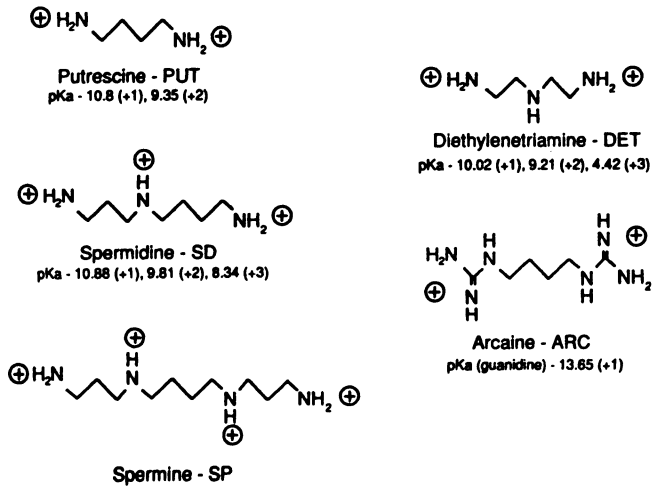

Fig. 1. Structure and charge of polyamines at physiological pH. Net charge at pH 7.4 for PUT, SD, and DET was estimated from pK_a values (32, 34). Net charge value for SP was estimated from that of SD, and the value for ARC was estimated from the pK_a of guanidine (33).

not competitive (8). SP, SD, DET, and PUT all displaced binding of [^{3}H]SD from a low affinity binding site (12).

Voltage-clamp recordings from cultured neurons and poly(A)$^+$ mRNA-injected frog oocytes showed that SP and SD enhanced NMDA-evoked whole-cell currents, whereas higher concentrations of polyamines produced less enhancement or inhibited NMDA receptor current (13, 19–23). Single-channel studies indicated that the enhancement of NMDA receptor current by SP was due to an increase in opening frequency of NMDA receptor single channels, whereas at higher concentrations SP decreased average open duration and conductance of the NMDA single-channel currents at negative holding potentials (22).

Because SP has multiple actions on NMDA receptor single-channel currents, it is possible that structurally related polyamines may also produce similar effects on NMDA receptor single-channel currents. The following studies, using whole-cell voltage-clamp and single-channel recording techniques, were carried out to investigate the effects of SD, ARC, DET, and PUT on NMDA receptor currents in rat cortical neurons in culture and to compare the effects of these polyamines with those of SP.

Materials and Methods

Culture preparation. Cultures of fetal rat cortical neurons were prepared by standard methods (24). Briefly, cortices from E-18 fetuses were enzymatically treated and then mechanically dissociated. Cells were suspended in Eagle's minimum essential medium supplemented with 30 mM glucose, 26 mM sodium 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 10 μg/ml 5-fluoro-2-deoxyuridine and 25 μg/ml uridine, in Eagle's 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 voltage-clamp 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), 500 nM glycine, 200 nM strychnine, and 500 nM tetrodotoxin (added for whole-cell recordings). Osmolarity was adjusted to 320 mOsM by the addition of sucrose. The internal pipette solution consisted of 153 mM CsCl, 10 mM Cs-HEPES, and 5 mM EGTA (pH 7.4). Osmolarity was adjusted to 305 mOsM with water. Chemicals were obtained from Sigma Chemical Co. (St. Louis, MO).

Drug application. NMDA (Sigma), glycine (Sigma), SP tetrahydrochloride (US Biochemicals), SD tetrahydrochloride (US Biochemicals), and PUT dihydrocholide (Sigma) were dissolved in saline or external solution. ARC sulfate (Sigma) was dissolved in saline, with the addition of 1 N HCl. Stock solutions were made up at concentrations between 10 mM and 100 mM and were serially diluted to final concentrations. The concentration of free Mg^{2+} was measured in the external solution by atomic absorption spectroscopy and was 0.9 μM. Addition of 100 mM SP tetrahydrochloride into the external solution had no effect on free Mg^{2+} (1 μM) or pH of 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) using procedures for whole-cell voltage-clamp and outside-out excised patch recordings similar to those of Hamill *et al.* (25). 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 video cassette system via a digital audio processor (Medical Systems, Inc. PCM II, 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 video cassette system through a low-pass filter (−3 db at 2 kHz) and were digitized (20 kHz, 16 bit, Tecmar A/D converter) for computer analysis. System dead time was 70 μsec. Openings of >140 μsec (2 × the system dead time) were considered valid openings.

Data analysis. In whole-cell recordings, peak current was measured during 5-sec applications of 5 μM NMDA or 5 μM NMDA plus test compound. Data were converted to a percentage of the amplitude of control NMDA responses for that neuron.

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 described previously (26). The software analysis package IPROC (Axon Instruments) was used to determine amplitudes of single-channel openings. A wide detection window (1.2–6 pA) was used and openings of <500 μsec in duration were rejected. Lists of amplitudes were compiled and used to generate amplitude histograms in pSTAT (Axon Instruments). Gaussian curves were fit to the amplitude histograms, to determine average amplitude of openings to the main conductance level. Although NMDA has been shown to activate a channel or channels with several different conductance levels (27–29), openings were predominantly of 50-pS conductance. In this study, openings from the two higher conductance levels (40 and 50 pS) were analyzed. Application of polyamines often resulted in a decrease in channel amplitude at negative holding potentials. Detection windows were adjusted to compensate for these amplitude changes.

Concentration-response curves for changes in whole-cell current and single-channel conductance were fit by using a four-parameter logistic equation to determine the IC_{50} concentrations for drug effects, using the following equation: $f(x) = ((a - d)/1 + (x/c)^b) + d$, where a is the maximal effect, d is the minimum effect, c is the IC_{50} concentration, x is the drug concentration, and b is the slope (analogous to Hill number) (30).

A Woodhull analysis (31) was performed to estimate the voltage dependence of the polyamine effects on single-channel conductance. The following equation was used to fit current-voltage relationships:

$i_o/i_b = 1 + (x/K_d(0)) \cdot \exp(-zdVF/RT)$, where i_o/i_b is the ratio of control (i_o) and drug-treated (i_b) current amplitude, x is the blocker concentration, $K_d(0)$ is the dissociation constant of the drug at 0 mV, z is the valence of the blocker, d is the fraction of the membrane field that influences the blocker, and RT/F is a ratio of constants (25.4 mV at 22°). Valence estimates for the polyamines at physiological pH are illustrated in Fig. 1 and were estimated either from pK_a values or from literature values for similar compounds (SP and ARC) (32–34).

Results

Polyamines reduced NMDA receptor whole-cell current. ARC, PUT, and DET reduced NMDA receptor current at a holding potential of −75 mV (Fig. 2). The reduction of whole-cell current was concentration dependent (Fig. 3), with an IC_{50} of 95 μM for ARC and 700 μM for PUT. Reduction of NMDA receptor whole-cell current was nearly complete with 1 mM ARC (10 ± 1.2% of control, $n = 6$), but inhibition of NMDA receptor current by PUT was incomplete. PUT at concentrations above 3 mM produced only a small additional reduction of NMDA receptor current (24–32% of control; Fig 3).

DET produced a variable enhancement of whole-cell NMDA receptor current in two of five neurons at 100 μM and two of five neurons at 1 mM, at negative holding potentials. This variable enhancement of NMDA receptor current by DET made calculation of potency for inhibition less accurate, but an estimated IC_{50} for reduction of whole-cell current by DET was 970 μM. Concentrations of DET above 3 mM resulted in an induction of an inward current ($n = 3$), so concentrations above 3 mM DET were not tested. SP and SD also showed enhancement of NMDA receptor current over this concentration range, making estimation of their potency for reduction of NMDA receptor currents difficult. However, at higher concentrations (>10 μM) the enhancement of NMDA receptor current by SP was reduced, relative to that seen at 10 μM SP (22).

The reduction in NMDA receptor whole-cell current by ARC, DET, and PUT was voltage dependent. The reduction with 100 μM ARC was decreased from 60% at −75 mV to 19% at +75 mV ($n = 5$; Fig. 2B). Reductions of NMDA receptor current with 1 mM DET (43% at −75 mV to −6% at +75 mV, $n = 4$) and 1 mM PUT (39% at −75 mV to 4% at +75 mV, $n = 2$) were also decreased at positive holding potentials.

Polyamines reduced NMDA receptor single-channel conductance and average open duration. SP, SD, ARC, DET, and PUT all reduced NMDA single-channel conductance at negative holding potentials. At a holding potential of −75 mV, 5 μM NMDA evoked single-channel openings with an average amplitude of 3.5 pA. Addition of 10 μM ARC, 100 μM SP, 1 mM SD, or 1 mM PUT reduced the conductance of NMDA receptor single-channel currents by 25–38% (Fig. 4A).

The effect of polyamines on single-channel conductance was concentration dependent. Openings evoked by 5 μM NMDA (Fig. 4B, *left top trace*) were reduced in a concentration-dependent manner by the addition of 10 and 100 μM ARC (Fig. 4B, *left lower traces*). The reduction in amplitude of NMDA single-channel currents by ARC was quantified by measuring the shift in the amplitudes of openings of the main conductance level, measured from Gaussian fits of amplitude histograms for applications of 5 μM NMDA alone or in combination with various concentrations of drugs (Fig. 5). In the example in Fig. 5, ARC produced a 28% reduction with 10 μM and a 62%

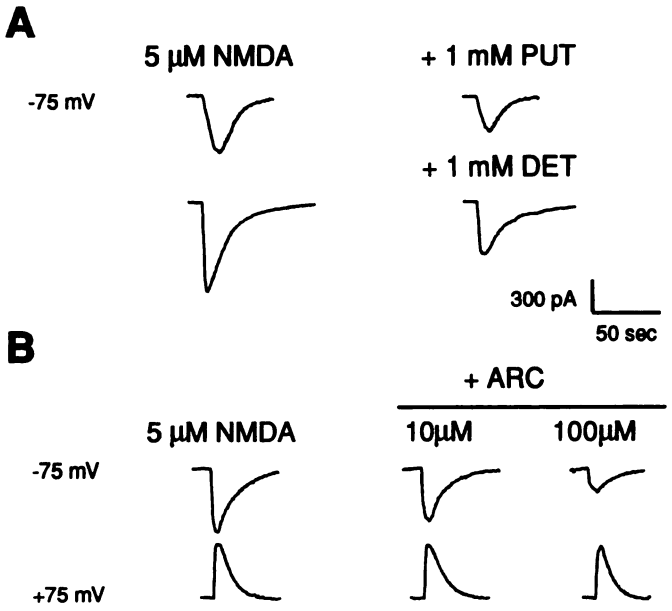

Fig. 2. Whole-cell NMDA currents were reduced by polyamines in a voltage-dependent manner. A, Reduction of 5 μM NMDA currents (5-sec application) was caused by the coapplication of 1 mM PUT and 1 mM DET at a holding potential of −75 mV. B, Reduction of NMDA receptor current by ARC was concentration and voltage dependent. *Upper trace*, reduction of NMDA currents by 10 and 100 μM ARC at a holding potential of −75 mV. *Lower trace* (same neuron), little or no reduction with ARC at a holding potential of +75 mV.

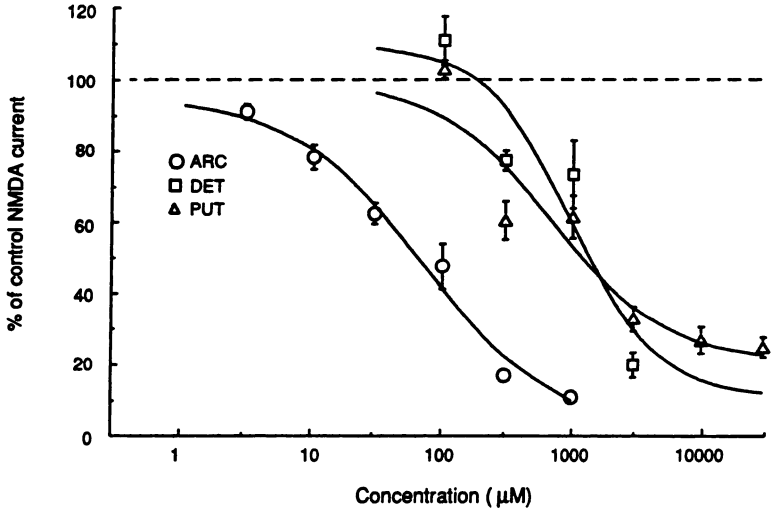

Fig. 3. Reduction of NMDA receptor current was concentration dependent. ARC (○) reduced NMDA receptor current with an IC_{50} of 95 μM. PUT (△) and DET (□) were less potent and did not completely block NMDA receptor current, even at high concentrations. Data are represented as the percentage of control and are the mean ± standard error of observations from three to seven neurons.

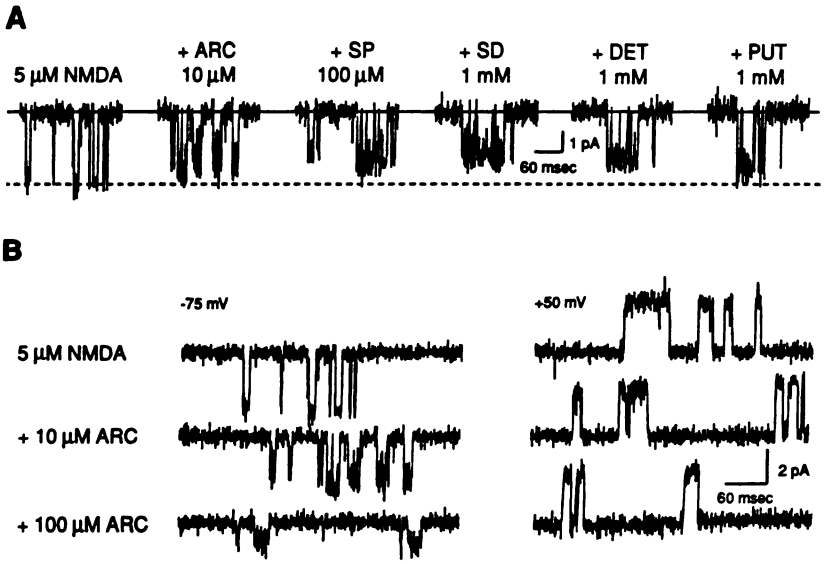

Fig. 4. Conductance of NMDA receptor single-channel currents was reduced by the polyamines. A, Application of 5 μM NMDA to excised outside-out patches resulted in openings that averaged around 3.8 pA at a holding potential of −75 mV. Coapplication of 10 μM ARC, 100 μM SP, 1 mM SD, 1 mM DET, and 1 mM PUT all reduced the amplitude of NMDA receptor single-channel openings. Data were taken from different patches. B, Effect of ARC on NMDA single-channel conductance was voltage and concentration dependent. Application of 10 and 100 μM ARC caused a concentration-dependent reduction of NMDA receptor single-channel amplitude at a holding potential of −75 mV (*left traces*). No effect of 10 and 100 μM ARC was seen at a potential of +50 mV (*right traces*).

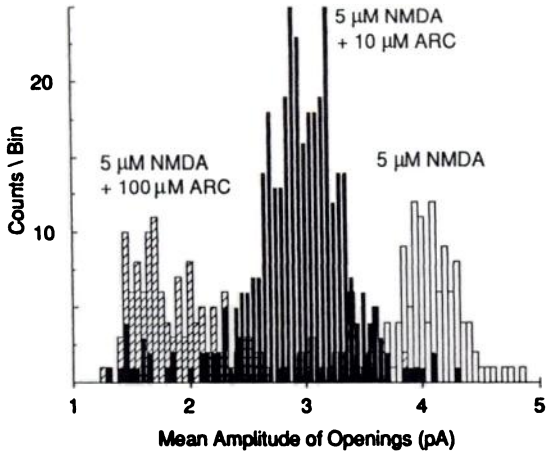

Fig. 5. ARC (10 and 100 μM) reduced the mean amplitude of NMDA single-channel currents. Histograms of mean amplitudes of openings were generated using a wide detection window, to accept openings with amplitudes between 1.2 and 6 pA and durations longer than 0.5 msec. Application of 5 μM NMDA for 30 sec (□) resulted in a histogram with the main peak at 3.9 pA. The combination of 5 μM NMDA and 10 μM ARC (■) resulted in a histogram with a main peak 2.8 pA. Addition of 100 μM to 5 μM NMDA (▨) further reduced the amplitude of openings. Data were taken from the same patch at a holding potential of −75 mV.

reduction with 100 μM. IC_{50} concentrations were determined for the polyamine reduction of amplitude of NMDA receptor single-channel currents, from concentration-response curves (Fig. 6). The rank order of potency (IC_{50} concentrations) for reducing NMDA single-channel conductance by the polyamines was ARC (65 μM) > SP (200 μM) > SD (580 μM) > PUT (1 mM) = DET (1.2 mM) (Table 1). This rank order of potency was similar to that for reduction of NMDA whole-cell currents (ARC > PUT = DET; Fig. 3).

The reduction in amplitude of NMDA receptor single-channel openings with ARC was qualitatively different than with the other polyamines. High concentrations of ARC (300 μM) reduced the amplitude of single-channel openings to an undetectable level; however, SD at high concentrations (3 and 10 mM) did not reduce the amplitude of the openings to same extent (Fig. 7). The other polyamines (SP and PUT), even at high concentrations, did not reduce the amplitude of NMDA receptor single-channel openings below 20% of control amplitudes (Fig. 6). ARC also increased open channel noise at low concentrations, compared with control (Figs. 4 and 7).

The reduction of NMDA single-channel conductance by polyamines was voltage dependent. At a holding potential of +50 mV, NMDA single-channel openings had a mean amplitude of 3.0 pA (Fig. 4B, *right top trace*). Addition of 10 and 100 μM ARC had no effect on the amplitude of NMDA-evoked single-channel openings (Fig. 4B, *right lower traces*). PUT, DET, SP, and SD also had no effect on NMDA single-channel conductance at a holding potential of +50 mV. Current-voltage relationships for ARC, SP, SD, and PUT showed a similar pattern, with reduction of amplitudes at negative potentials and no reduction at positive potentials (Fig. 8). A Woodhull analysis (see Materials and Methods), estimating the voltage dependency of the block by polyamines, indicated that the site of interaction for the compounds was shallow in the membrane field ($d < 0.25$) with ARC ($d = 0.24$) deeper than the other polyamines (SP and SD, $d = 0.13$; PUT, $d = 0.06$). No change in reversal potentials was noted with ARC, SP, SD, or PUT, suggesting that these compounds did not permeate NMDA channels.

The effects of SP and DET on NMDA single-channel conductance were additive (Fig. 9). Application of 10 μM SP caused a 9% reduction ($n = 4$) of NMDA single-channel conductance. DET (100 μM) caused a similar reduction (8% reduction). Combination of SP (10 μM) and DET (100 μM) reduced NMDA single-channel conductance by 15% in the same patches ($n = 4$).

SD, DET, and ARC had voltage-dependent effects on the average open duration of NMDA single-channel currents. At a holding potential of −75 mV, the range of average open durations of NMDA single-channel currents in control applications of 5 μM NMDA was 3.3–4.4 msec ($n = 2181$–6049). To evaluate the effect of polyamines on average open durations, concentrations of polyamine that caused an equivalent percentage reduction (25–38%) of NMDA single-channel conductance were chosen. Average open durations of NMDA single-channel currents were reduced 53% by 1 mM DET, 28% by 100 μM SP, 30% by 1 mM SD, and 35% by 10 μM ARC, compared with paired controls. PUT (1 mM) had no effect on average open duration. None of the polyamines altered average open duration at a holding potential of +50 mV.

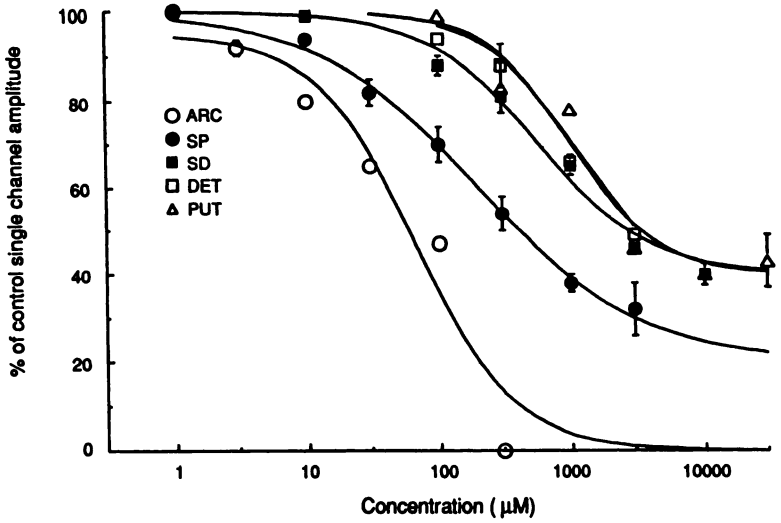

Fig. 6. Polyamines reduced the amplitude of NMDA receptor single-channel openings in a concentration-dependent manner. The rank order of potency for reduction of amplitude was ARC > SP > SD > PUT = DET. Data points represent the percentage of control amplitude and are the mean from 2–22 patches. *Error bars* are the standard error and were calculated for data with $n > 2$ but were often smaller than the symbol size.

TABLE 1

Comparison of the concentrations of polyamines effective in reducing the amplitude of single-channel openings with concentrations effective in low affinity [^{3}H]SD binding and the inhibition of open channel blocker binding

Concentration ranges for effects on single-channel amplitude were determined from concentration-response curves (Fig. 6) and are estimates of concentrations that caused 20–60% reduction in single-channel amplitude. Concentration ranges for inhibitory effects in receptor binding assays were determined from the inhibitory portion of biphasic concentration-response curves for the polyamine agonists SP and SD or were concentrations effective in the inhibition of polyamine-enhanced binding of open channel blockers for ARC, DET, and PUT.

	Amplitude reduction IC_{50}	Amplitude reduction concentration range	Receptor binding inhibition concentration range[a]	[^{3}H]SD IC_{50}[b]
	μM	μM	μM	μM
ARC	65	10–110	10–100	
SP	200	40–1,000	30–1,000	23
SD	580	300–10,000	300–1,000	150
DET	1,200 (est).	1,000 (est).	30–1,000	2,500
PUT	1,000	300–30,000	300–1,000	1,200

[a] From Refs. 6–8, 13, 15–18, and 51–53.
[b] From Ref. 12.

Discussion

Polyamine agonists and antagonists have a common effect on NMDA receptor current. A variety of polyamines have been shown to interact at a common site on the NMDA receptor complex, to regulate binding of the open channel blockers MK-801 and TCP. Polyamine agonists (SP and SD) enhanced the binding of open channel blockers at low concentrations but reduction of the enhancement occurred at higher concentrations, leading to biphasic concentration-response curves. Polyamine antagonists (ARC, DET, and PUT) reduced polyamine agonist-enhanced binding (8, 13, 14, 16, 17). We previously showed that the polyamine agonist SP had multiple effects on NMDA receptor current (22). At lower concentrations SP enhanced NMDA receptor whole-cell current by increasing channel opening frequency. At high concentrations the enhancement was reduced by a voltage-dependent reduction in single-channel conductance and average open duration.

In the present study, another polyamine agonist, SD, had an action similar to that of SP. SD at low concentrations enhanced NMDA receptor whole-cell current (data not shown) but at high concentrations also produced a voltage-dependent reduction of NMDA single-channel conductance and average open duration. The polyamine antagonists ARC, DET, and PUT, however, produced unexpected results on NMDA receptor currents. These antagonists were predicted to have minimal or no effect on NMDA receptor current. However, all three antagonists reduced NMDA receptor whole-cell currents and reduced

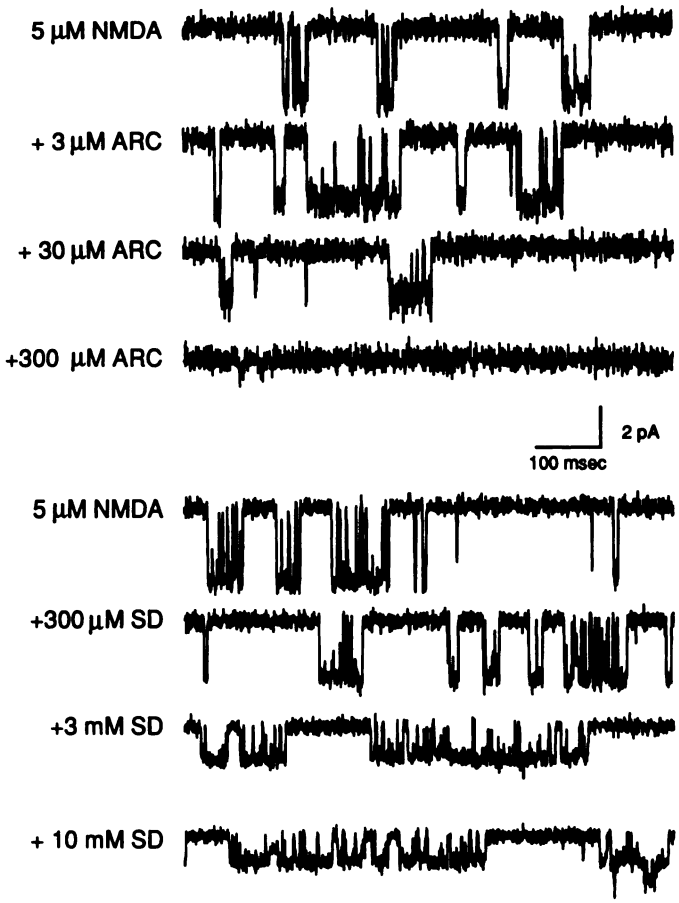

Fig. 7. ARC reduced the amplitude of NMDA receptor single-channel currents in a concentration-dependent manner and reduced currents to an undetectable level at 300 μM (*upper traces*). SD also reduced the amplitude of channel currents in a concentration-dependent manner but did not block single-channel openings to the same extent, even at high concentrations (10 mM) (*lower traces*). Data for ARC and SD were taken from separate patches at a holding potential of −75 mV.

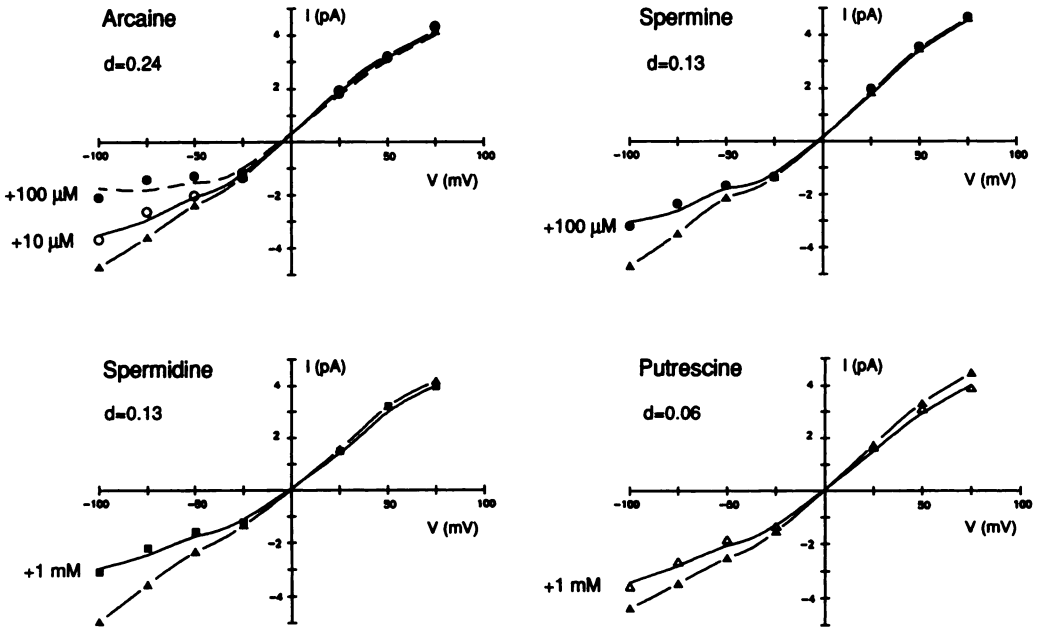

Fig. 8. Current-voltage relationships for the reduction in channel amplitudes by ARC, SP, SD, and PUT were similar. All of the compounds reduced the amplitude of single-channel openings at negative potentials, with little or no effect at positive potentials. The Woodhull analysis was used to fit curves for drug-induced reductions of NMDA receptor currents and indicated that all compounds were influenced by <25% of the membrane electrical field ($d < 0.25$). I-V curves were taken from single patches, with each data point representing the amplitude of the main conductance level, as determined from a Gaussian fit of an amplitude histogram for a 10-sec applications of NMDA or NMDA plus compound at each patch voltage.

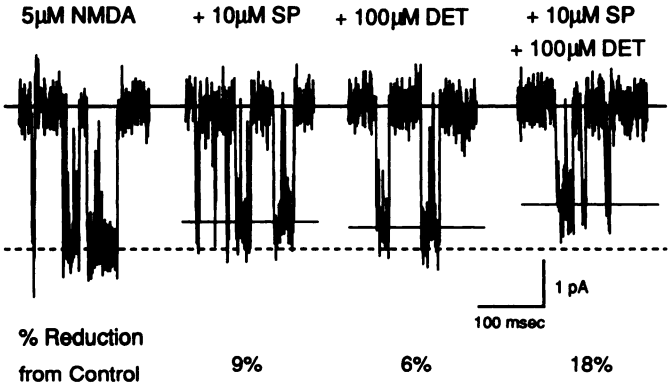

Fig. 9. Effects of SP and DET on NMDA single-channel conductance were additive. On the same patch at a holding potential of −75 mV, SP (10 µM) and DET (100 µM) reduced the amplitude of NMDA receptor single-channel currents by 9 and 6%, respectively (estimated from amplitude histograms). The combination of SP and DET resulted in an 18% reduction in this patch.

the conductance of NMDA receptor single-channel currents, even in the absence of polyamine agonists. In addition, DET did not block the reduction of NMDA receptor single-channel conductance by SP; the effects of SP and DET on single-channel conductance were additive. DET also had polyamine agonist-like actions on some neurons. These observations were not consistent with the action of agonists and antagonists acting at the same site but indicated that polyamine agonists and antagonists have similar actions on NMDA receptor currents.

The basis for the apparent discrepancy between polyamine actions in receptor bindings assays and on whole-cell/single-channel recordings is unclear. Concentrations that were effective in reducing single-channel conductance were similar to concentrations that were effective in receptor binding assays for inhibition of polyamine-enhanced open channel blocker binding (ARC, DET, and PUT) or in the inhibitory portion of biphasic polyamine agonist concentration-response curves (SP and SD) (Table 1). In addition, the IC_{50} values for displacement of [^{3}H]SD from a low affinity binding site were similar to the IC_{50} values for the effects of polyamines on single-channel conductance (Table 1). These data suggest that the low affinity SD binding site may be involved in the inhibitory action of polyamines and that the inhibitory actions of the polyamines in receptor binding assays and in the reduction of single-channel conductance may be due to action at a common site.

In summary, we believe that the polyamines act at two separate sites to modulate NMDA receptor currents (22). Polyamine agonists interacted with a site to increase single-channel opening frequency, to enhance NMDA receptor current, but also caused a voltage-dependent reduction in NMDA receptor single-channel conductance by interacting with a separate site. The polyamine antagonists may interact with this second site to cause their effect on single-channel currents. It has been proposed that the ARC-induced shift in polyamine agonist-enhanced receptor binding is due to reduction of the enhancing properties of polyamine agonists, without an effect on the inhibitory actions (16–18). We demonstrate here that ARC produced an effect on single-channel conductance that was similar to the inhibitory action of the polyamine agonists and, further, that the inhibitory action of SP and DET were additive. The data presented here are consistent with the hypothesis that polyamine antagonists may be adding to the inhibitory actions of the polyamine agonists, to shift the biphasic concentration-response curves for enhancement of receptor binding.

Reduction of NMDA receptor single-channel conductance may occur by two different mechanisms. The reduction of NMDA receptor currents by polyamines was similar to reduction produced by divalent cations. Because polyamines are positively charged at physiological pH (Fig. 1), the mechanism for the polyamine reduction of single-channel conductance may be similar to those described for divalent cations. The voltage-dependent reduction of NMDA receptor single-channel conductance by divalent cations has been attributed mainly to either fast open channel block or screening of negative surface charges.

Divalent inorganic cations have a voltage-dependent effect on both the conductance and the average open duration of

NMDA receptor currents. The block of inward NMDA receptor currents by externally applied Mg^{2+} was attributed by Nowak and Ascher (35, 36) to open channel block. This block of the NMDA receptor current did not result in a reduction of single-channel conductance at concentrations that had effects on average open time (35). One of the actions of Zn^{2+}, i.e., the voltage-dependent reduction of amplitude of NMDA single-channel currents (37, 38), and the effect of internal Mg^{2+} on outward currents through the NMDA receptor (39) have also been attributed to fast open channel block.

Fast open channel block may produce an apparent reduction in channel conductance, due to unresolved openings and closings of single channels (40). The reduced amplitude of channel openings occurs because openings and closings are shorter than the system dead time, leading to an "average" open amplitude instead of fully resolved openings and closings. This type of block would result in channel flicker or an increase in open channel noise (40, 41) and would also produce a nearly complete block of single-channel currents at high concentrations (42).

The mechanism for the effect of ARC on NMDA receptor single-channel openings was consistent with fast open channel block. ARC at high concentrations (300 μM) reduced the amplitude of single-channel openings to an undetectable level. Although it was not systematically analyzed, ARC at lower concentrations also increased open channel noise, compared with control (Figs. 4 and 7). SP, SD, and PUT at high concentrations did not reduce the amplitude of single-channel openings to undetectable levels (Figs. 6 and 7), and SP did not appear to increase open channel noise (22). These data suggest that ARC may reduce the amplitude of NMDA receptor single-channel currents by producing a fast open channel block. The mechanism of fast open channel block, however, was not consistent with the actions of the other polyamines.

Divalent cations may also affect the conductance of NMDA single-channel currents by screening fixed negative charges near the opening of the channel pore (42, 43). High concentrations (10–30 mM) of the permeant divalent cation Ca^{2+} not only caused a shift of reversal potential for NMDA receptor currents but also produced a voltage-dependent reduction in the conductance of NMDA single-channel currents at negative holding potentials (35, 36). This voltage-dependent reduction in conductance by Ca^{2+} may be due to an interaction with negative charges that, by analogy with other cation channels (44), may be located near the opening of the channel pore. Actions of Ca^{2+} and Zn^{2+} on sodium channels have also been attributed to charge screening (45). This reduction by divalent cations was characterized by a voltage-dependent block that occurred primarily at negative potentials, a shallow electrical depth, as calculated from a Woodhull analysis ($d = 0.21$ for Zn^{2+} and $d = 0.18$ for Ca^{2+}), and no observed increase in open channel noise (45).

Recent molecular biological studies have indicated that at least one subunit of the NMDA receptor has several amino acid residues with negatively charged side chains that have a tentative location in the extracellular vestibule area of the NMDA receptor (46). These negatively charged groups are thought to be important in determining channel conductance and Ca^{2+} permeability (46). In nicotinic acetylcholine receptors, replacement of negative charges with neutral or positively charged groups in the extracellular domains, by site-directed mutagenesis, decreased conductance of the nicotinic acetylcholine channel, and complete replacement of all the negative charges greatly reduced channel conductance but did not reduce the conductance to zero (44). Mutation of similar sites in voltage-gated K^+ channels resulted in a large reduction in inward currents, with only a small reduction in outward K^+ current (47). Removal of negative surface charges by chemical modification reduced single-channel conductance of voltage-gated Na^+ channels (48) and nicotinic acetylcholine receptor channels (49). Screening of similar surface charges in the NMDA receptor may have a similar effect on channel conductance.

The polyamines might reduce NMDA receptor single-channel conductance by screening negative charges in the NMDA receptor. Screening of charges near the channel pore should result in an incomplete block of single-channel currents (42). SP, SD, and PUT all produced incomplete blocks of NMDA receptor single-channel conductances, even at high concentrations. The concentration dependency of charge-screening effects should also be related to the valence of the blocker (42). The rank order of potency for reduction of single-channel conductance was related to charge at physiological pH [SP (+4) > SD (+3) > PUT (+2) = DET (+2)]. The concentration dependencies for reduction of NMDA receptor conductance with PUT (+2) and DET (+2) were similar to that of Ca^{2+}.[2] The rectification of current-voltage relationships and shallow electrical depth calculated by the Woodhull analysis are also consistent with charge screening (42, 45, 50). This evidence, along with the apparent lack of increase in open channel noise with SP (22), indicates that some of the effects of the polyamines on NMDA single-channel conductance may be due to screening of negative surface charge on the channel.

In conclusion, SP and related polyamines produced a voltage-dependent reduction in NMDA receptor single-channel conductance and average open duration. The reduction in conductance of NMDA receptor currents appears to be due to screening of negative charge near the channel pore (SP, SD, PUT, and DET) or fast open channel block (ARC). Because the enhancing action of polyamine agonists on NMDA receptor currents may occur by a mechanism distinct from the inhibiting action, it is possible that the polyamine antagonists ARC, DET, and PUT do not block the enhancing action of polyamine agonists but, instead, may simply add to the inhibitory action of the polyamine agonists. In fact, the concentrations of the polyamines that are effective in reducing single-channel amplitude and average open duration correlate with those in the inhibitory phase of the biphasic agonist concentration-response curves and those that inhibit polyamine-enhanced open channel blocker binding. Further studies must be done to evaluate the effect of these polyamines on SP-evoked changes in opening frequency of NMDA single-channel currents, to determine whether the polyamine antagonists interact with the enhancement site.

[2] D. M. Rock and R. L. Macdonald, unpublished observations.

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