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Sensitivity of the *N*-Methyl-D-Aspartate Receptor to Polyamines Is Controlled by NR2 Subunits

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SUMMARY

The endogenous polyamine spermine has multiple effects on the *N*-methyl-D-aspartate (NMDA) receptor. These include an increase in the magnitude of NMDA-induced whole-cell currents that is seen in the presence of saturating concentrations of glycine ("glycine-independent" stimulation), an increase in the affinity of the receptor for glycine ("glycine-dependent" stimulation), and voltage-dependent inhibition. Although many of the properties of native NMDA receptors are seen with homomeric NR1 receptors expressed in *Xenopus* oocytes, we have found that the effects of spermine are differentially regulated by NR2 subunits in heteromeric NR1/NR2 receptors. Glycine-independent stimulation by spermine occurred at homomeric NR1A receptors, which lack the amino-terminal insert in NR1, and at heteromeric NR1A/NR2B receptors but not at heteromeric NR1A/NR2A or NR1A/NR2C receptors. Glycine-independent stimulation was not seen at homomeric NR1B receptors, which include the amino-terminal insert in NR1, or at heteromeric receptors containing NR1B. Thus, glycine-independent stimulation by polyamines requires the presence of an NR1 variant, such as NR1A, that lacks the amino-terminal insert, but the manifestation of the stimulatory effect is controlled by the type of NR2 subunit present in a heteromeric complex. Glycine-dependent stimulation was seen at NR1A/NR2A and NR1A/NR2B receptors and may therefore involve a second polyamine binding site distinct from that which produces glycine-independent stimulation. The voltage-dependent inhibitory effect of spermine, which is more pronounced at hyperpolarized membrane potentials, occurred with similar magnitudes at NR1A/NR2A and NR1A/NR2B receptors but was absent at NR1A/NR2C receptors. Thus, NR2 subunits control both the stimulatory and inhibitory effects of spermine at NMDA receptors. Stimulation but not inhibition by spermine was seen at NR1A/NR2B receptors in the presence of extracellular Mg^{2+}. Stimulation, seen in the presence of physiological concentrations of Ca^{2+} and Mg^{2+}, may be the predominant effect of polyamines at NMDA receptors in the intact nervous system.

The endogenous polyamines spermine and spermidine are found in high concentrations in the brain but their functions remain largely unknown (1). Polyamines have been shown to have both stimulatory and inhibitory effects on the NMDA receptor, as studied biochemically (2–4) and electrophysiologically (5–9). "Glycine-independent" stimulation, seen in the presence of saturating concentrations of glycine, represents one form of stimulation by spermine (5–9). Spermine also induces a small increase in the affinity of the receptor for glycine, so-called "glycine-dependent" stimulation (9–11). The inhibitory effect of spermine is voltage dependent, being more pronounced at hyperpolarized membrane potentials, and may be due to an open-channel block or to screening of surface charges (7–9). The degree of both stimulation and inhibition by spermine at NMDA receptors on cultured neurons is highly variable (5–9). This may be due to the expression of different forms of the NMDA receptor composed of different subunits.

Multiple NMDA receptor subunits have been cloned (12). The NMDAR1 (NR1) gene is transcribed as eight alternatively spliced mRNAs containing combinations of one 5′ exon and/or two alternatively spliced 3′ exons, one of which alters the carboxyl terminus of NR1 (13–17). Homomeric NR1 receptors expressed in frog oocytes show many of the properties of native NMDA receptors, including stimulation by glutamate and glycine and voltage-dependent block by Mg^{2+} (14). These receptors have been used to try to define the properties of native NMDA receptors with regard to polyamine sensitivity (18). Glycine-independent stimulation by spermine is seen at homomeric NR1 receptors expressed from variants, such as NR1A, without the amino-terminal (5′) insert but not from variants, like NR1B, that contain the amino-terminal insert (18). A second family of NMDA receptor subunits, NR2A-NR2D (also termed ε1-ε4); (19, 20), do not form functional homomeric channels but,

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ABBREVIATIONS: NMDA, *N*-methyl-D-aspartate; BAPTA, 1,2-bis(2-aminophenoxy)ethane-*N*,*N*,*N*′,*N*′-tetraacetic acid; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid.

when coexpressed with NR1, form heteromeric receptors that produce much larger whole-cell currents than do homomeric NR1 receptors (12, 19–22). Inclusion of NR2 subunits in heteromeric NMDA receptors also markedly alters some of their pharmacological properties. For example, NR1/NR2B receptors have a 10-fold higher affinity for glycine and a 400-fold higher affinity for the atypical antagonist ifenprodil than do NR1/NR2A receptors (19, 23). In the present work we have examined the effects of spermine on heteromeric NMDA receptors expressed in *Xenopus* oocytes from NR1 plus NR2A, NR2B, or NR2C subunits. The results indicate that inclusion of different NR2 subunits in a heteromer can control both the stimulatory and inhibitory effects of polyamines. Glycine-independent stimulation by spermine occurs at NR1A/NR2B receptors but not at NR1A/NR2A or NR1A/NR2C receptors, whereas voltage-dependent inhibition by spermine occurs at NR1A/NR2A and NR1A/NR2B receptors but not at NR1A/NR2C receptors.

Materials and Methods

Oocyte injection and recording. Defolliculated stage V-VI oocytes were prepared from *Xenopus laevis* as described previously (24) and were injected with 4–8 ng of NR1A or NR1B cRNA plus 20–40 ng of NR2A, NR2B, or ε3 (NR2C) cRNA synthesized *in vitro* from NR1A, NR1B (gifts from Dr. S. Nakanishi, Kyoto University, Kyoto, Japan), NR2A, NR2B (gifts from Dr. P. H. Seeburg, University of Heidelberg, Heidelberg, Germany), and ε3 (the mouse NR2C cDNA; a gift from Dr. M. Mishina, Niigata University, Niigata, Japan) cDNAs. NR1 and NR2 subunits were coinjected in a ratio of 1:5. Most of the 5′ untranslated region was removed from the NR2A and NR2B clones before synthesis of cRNAs (23). Macroscopic currents were studied by two-electrode voltage-clamp recording 2–8 days after injection of cRNAs, as described previously (23). Oocytes were continuously superfused (≈10 ml/min) with a saline solution (96 mM NaCl, 2 mM KCl, 1.8 mM $CaCl_2$ or $BaCl_2$, 10 mM Na-HEPES, pH 7.5), and NMDA and spermine were applied in the same solution. Unless otherwise indicated, NMDA was applied in solutions containing 10 μM glycine. In some experiments the extracellular solution contained $BaCl_2$ rather than $CaCl_2$ and oocytes were injected with BAPTA on the day of recording to eliminate Ca^{2+}-activated Cl^- currents (23, 25).

Materials. NMDA was purchased from Research Biochemicals Inc. (Natick, MA). Glycine was purchased from Sigma Chemical Co. (St. Louis, MO). Spermine tetrahydrochloride was purchased from Aldrich Chemical Co. (Milwaukee, WI). High performance liquid chromatographic analysis with fluorescence detection did not reveal any glycine in a 2.5 mM stock solution of the batch of spermine used for these studies (limit of detection, 0.3 μM). Therefore, a 100 μM solution of spermine contains <0.01 μM glycine, and glycine contamination of spermine solutions does not contribute to the observed effects of spermine.

Results and Discussion

NR2 subunits control polyamine sensitivity. Many of the properties of native NMDA receptors are seen with homomeric NR1 receptors expressed in *Xenopus* oocytes (14). Spermine enhances NMDA-induced currents at homomeric NR1A receptors, which lack the amino-terminal insert (Fig. 1A and Ref. 18), but not at homomeric NR1 receptors expressed from variants, such as NR1B, that contain the amino-terminal insert (Fig. 1B and Ref. 18). However, when effects of spermine were studied using heteromeric receptors, stimulation was seen at NR1A/NR2B but not at NR1A/NR2A receptors (Fig. 1A). Stimulation was not seen at heteromeric NR1B/NR2B or NR1B/NR2A receptors (Fig. 1B). This suggests that NR2 subunits can alter the properties of polyamine-responsive NMDA receptors.

Studies of homomeric NR1 receptors and our initial studies of heteromeric receptors were carried out in the presence of a physiological concentration (1.8 mM) of extracellular Ca^{2+} (Fig. 1). Under these conditions, Ca^{2+}-activated Cl^- conductances contribute to the macroscopic current induced by NMDA (23, 25). To facilitate quantitative studies of the effects of spermine, subsequent experiments were carried out in the presence of extracellular Ba^{2+}, using oocytes injected with BAPTA to eliminate Ca^{2+}-activated Cl^- currents (23). Experiments were carried out with heteromeric NR1A/NR2 receptors containing NR2A, NR2B, and NR2C to look for each of the three reported effects of spermine, i.e. glycine-independent stimulation, glycine-dependent stimulation, and voltage-dependent inhibition, to determine whether NR2 subunits differentially control one or more of these phenomena.

Glycine-independent effects of spermine at NR1A/NR2 receptors were studied in the presence of 10 μM glycine (Figs. 2 and 3). At NR1A/NR2B receptors, stimulatory and inhibitory effects of spermine were seen (Fig. 2B). These effects were concentration and voltage dependent. Stimulation and inhibition were seen over a similar range of concentrations of spermine (10–300 μM). The voltage dependence of the effects of spermine was studied by using cells that were voltage-clamped at different holding potentials (Fig. 2) and by applying voltage ramps (Fig. 3). Current-voltage curves were constructed by ramping the command potential from −100 to +40 mV over a period of 12 sec during steady state responses induced by NMDA or NMDA plus spermine at −100 mV (Fig. 3). Leak currents, measured in ramps before and after test ramps, were digitally subtracted. A slow ramp was used because the voltage-dependent block was not fully relieved during fast (<3-sec) ramps from hyperpolarized to depolarized potentials, presumably because the rate of unblocking of spermine is relatively slow. During fast ramps part of the stimulatory effect of spermine is masked by the voltage-dependent inhibition.

In studies of steady state currents (Fig. 2B) or of current-voltage relationships measured by voltage ramps (Fig. 3B), stimulatory effects at NR1A/NR2B receptors were seen at relatively depolarized membrane potentials, whereas inhibition was more pronounced at hyperpolarized membrane potentials. The results shown in Fig. 2B probably reflect the existence of separate stimulatory and inhibitory effects of spermine, with stimulatory effects being seen at depolarized potentials where the magnitude of the voltage-dependent block by spermine is reduced. Thus, glycine-independent stimulation and voltage-dependent inhibition by spermine occur at NR1A/NR2B receptors.

At NR1A/NR2A receptors, voltage-dependent inhibitory effects of spermine were observed (Figs. 2A and 3A). Spermine stimulation was not seen at NR1A/NR2A receptors (Fig. 2A). Using voltage ramps, stimulation by spermine was not seen even at a membrane potential of +40 mV (Fig. 3A). We also examined the effects of spermine on NMDA responses in oocytes in which the command potential was stepped from −7 mV (close to the reversal potential) to +40 mV for 10–40 sec. Using this protocol, spermine markedly enhanced responses of NR1A/NR2B receptors but had no effect at NR1A/NR2A receptors (data not shown). Thus, NR1A/NR2A receptors exhibit

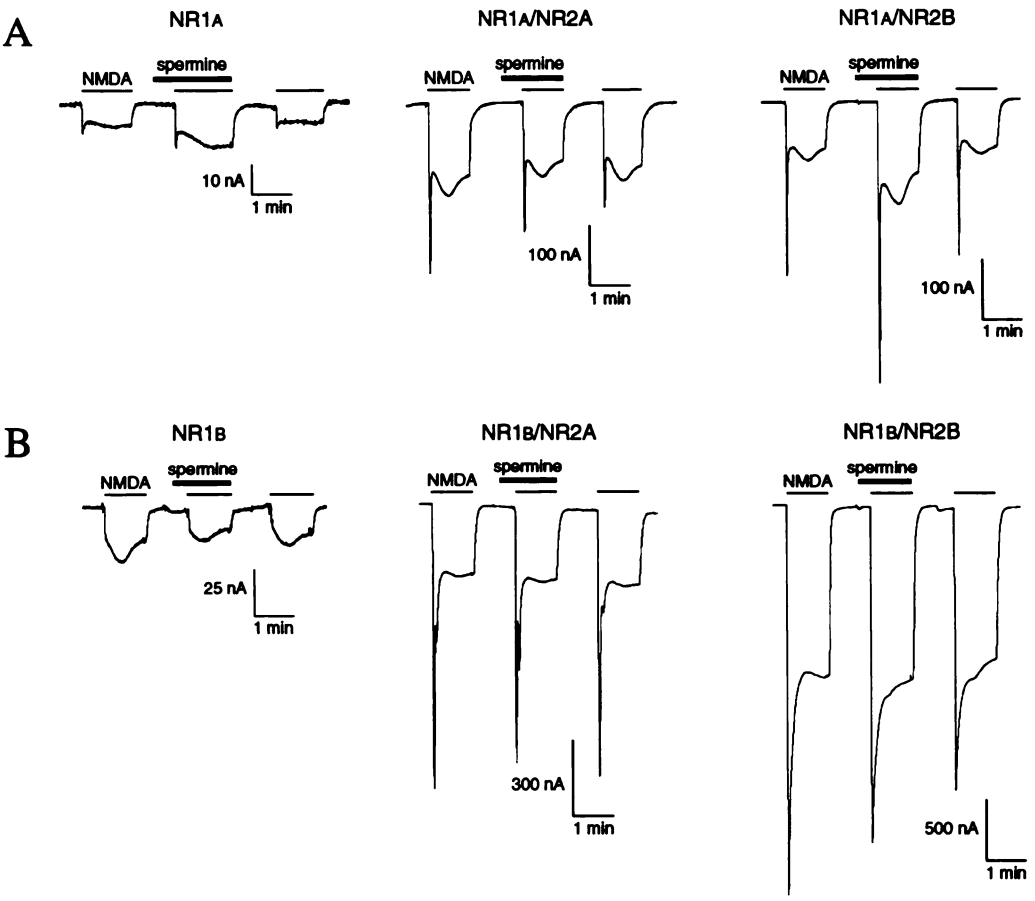

Fig. 1. Effects of spermine on recombinant NMDA receptors. *Traces*, inward currents induced by NMDA (100 μM, with 10 μM glycine) or NMDA plus spermine (100 μM) in oocytes expressing homomeric NR1A and NR1B receptors and heteromeric NR1A/NR2 (*A*) and NR1B/NR2 (*B*) receptors. Oocytes were voltage-clamped at −70 mV, and the extracellular solution contained 1.8 mM $CaCl_2$.

voltage-dependent inhibition but not glycine-independent stimulation by spermine.

The results shown in Figs. 2 and 3 suggest that inhibitory but not stimulatory effects of spermine are voltage dependent, although one cannot unequivocally exclude the possibility that spermine stimulation, seen at NR1A/NR2B receptors, is also voltage dependent and is more pronounced at depolarized membrane potentials. However, the degree of stimulation by spermine was largely voltage independent when studied by briefly stepping the command potential from −20 mV or −70 mV to more negative values (data not shown) or by using fast voltage ramps from +40 to −100 mV (Fig. 4). Using these protocols, the voltage-dependent block would not have time to fully develop at hyperpolarized potentials, and under these conditions spermine stimulation appears to be independent of membrane potential (Fig. 4). It is likely that the voltage-dependent inhibition by spermine is common to both NR1A/NR2B and NR1A/NR2A receptors and that glycine-independent stimulation, seen only at NR1A/NR2B receptors, is mediated through a separate extracellular site on the receptor complex.

At NR1A/ϵ3(NR2C) receptors, spermine had no effect on NMDA-induced currents (Fig. 2C). Thus, NR1A/NR2C receptors show neither glycine-independent stimulation nor voltage-dependent inhibition by spermine at concentrations up to 300 μM. This is consistent with the proposal that voltage-dependent inhibition by spermine is due to an open-channel block, because other channel blockers, including Mg^{2+} and MK-801, have lower potencies at NR1/NR2C receptors than at NR1/NR2A and NR1/NR2B receptors (19, 21, 22). The binding site responsible for voltage-dependent inhibition by spermine may be equivalent to or overlap with the binding sites for Mg^{2+} and MK-801.

Glycine-dependent effects of spermine were studied by measuring concentration-response relationships for glycine in the absence and presence of spermine (Fig. 5). In the absence of spermine, the affinity of NR1A/NR2B receptors for glycine (EC_{50} = 0.25 μM) was 10-fold higher than that of NR1A/NR2A receptors (EC_{50} = 2.5 μM). The affinities at each receptor type were very similar to those reported for the mouse homologue subunits, where ζ1/ϵ2 (NR1/NR2B) receptors have a 10-fold higher affinity for glycine than do ζ1/ϵ1 (NR1/NR2A) receptors (19). Spermine caused a small increase (2–3-fold) in the apparent affinity of both NR1A/NR2B and NR1A/NR2A receptors for glycine (Fig. 5). Thus, glycine-dependent stimulation by spermine, which is seen at both NR1A/NR2A and NR1A/NR2B receptors, is likely to be mechanistically distinct from glycine-independent stimulation, which is seen only at NR1A/NR2B receptors, and may be mediated at a separate site or involve different domains of the NMDA receptor-channel complex. This is consistent with results reported by Benveniste and Mayer (9), who found that the degree of glycine-dependent stimulation at individual neurons did not correlate with the degree of glycine-independent stimulation by spermine and who proposed that the two forms of stimulation are mechanistically different.

Interaction with Mg^{2+}. The properties of NMDA receptors are often studied in the absence of extracellular Mg^{2+}, because Mg^{2+} causes a voltage-dependent block of the channel (26, 27).

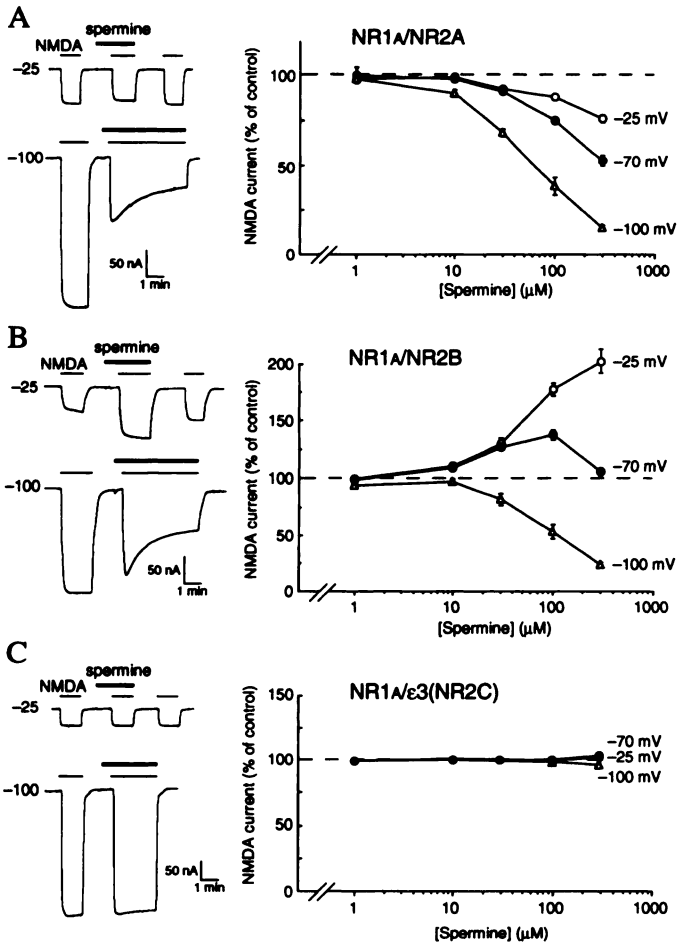

Fig. 2. Effects of spermine on heteromeric NMDA receptors. The effects of spermine (100 μM) on responses to NMDA (100 μM, with 10 μM glycine) were measured in oocytes expressing NR1A/NR2A receptors (A), NR1A/NR2B receptors (B), or NR1A/ε3(NR2C) receptors (C). *Left*, inward currents induced by NMDA or NMDA plus spermine in oocytes voltage-clamped at −25 and −100 mV. *Right*, similar protocols were used to measure concentration-response relationships at each receptor subtype in oocytes voltage-clamped at −25, −70, or −100 mV (means ± standard errors from four to 11 oocytes). Oocytes were injected with BAPTA, and the extracellular solution contained 1.8 mM $BaCl_2$.

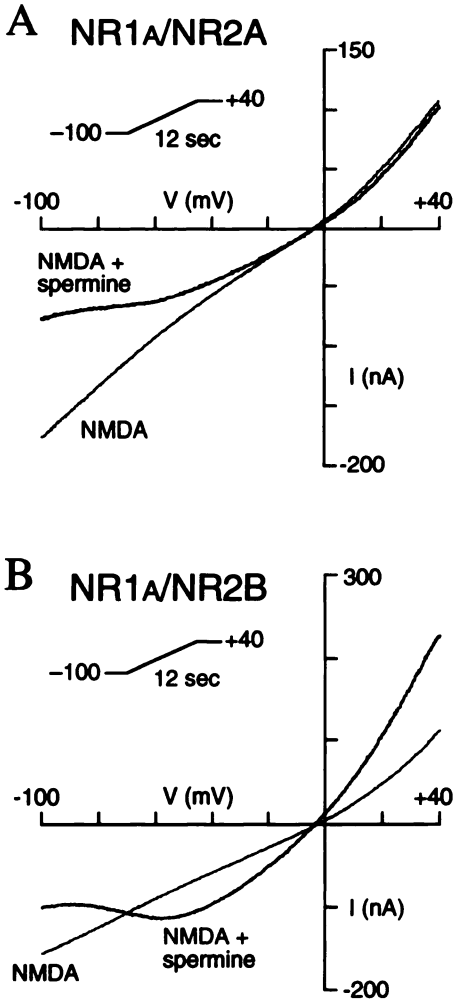

Fig. 3. Current-voltage relationships at NR1A/NR2A and NR1A/NR2B receptors. Current-voltage curves were constructed by using slow (12-sec) voltage ramps from −100 to +40 mV during steady state responses induced by NMDA (100 μM, with 10 μM glycine), in the absence and presence of 100 μM spermine, in an oocyte expressing NR1A/NR2A receptors (A) and an oocyte expressing NR1A/NR2B receptors (B). Oocytes were injected with BAPTA, and the extracellular solution contained 1.8 mM $BaCl_2$. Leak currents have been subtracted. Similar results were obtained in four or five oocytes for each combination of subunits.

Based on results of ligand-binding assays, it has been suggested that Mg^{2+} or Ca^{2+} present at physiological concentrations may normally compete with polyamines at the extracellular stimulatory polyamine site on the NMDA receptor, thus reducing or negating potential stimulatory effects of endogenous polyamines (28). However, this hypothesis has never been tested directly. Therefore, the effects of spermine on NR1A/NR2B receptors were studied in the presence of Ca^{2+} (1.8 mM) or Ca^{2+} and Mg^{2+} (1 mM). Glutamate was used to stimulate the NMDA receptors because it is likely to be the endogenous agonist at these receptors.

In oocytes voltage-clamped at −70 mV, in the presence of Ca^{2+} and Mg^{2+}, macroscopic currents were small due to block of the channel by Mg^{2+} (Fig. 6A), but spermine enhanced the response to glutamate even under these conditions (Fig. 6, A and B). Current-voltage relationships for glutamate were studied in the presence of Ca^{2+} and Mg^{2+} using slow (12-sec) voltage ramps. In the presence of Ca^{2+}, stimulatory and inhibitory effects of spermine were seen (Fig. 6C), presumably reflecting a voltage-independent stimulation and an overlapping voltage-dependent block. Stimulation by spermine also occurred in the presence of Mg^{2+} (Fig. 6D). The inhibitory effect of spermine, which is voltage dependent over a membrane potential range similar to that which influences Mg^{2+} block, was not seen in the presence of Mg^{2+} (Fig. 6D), and under these conditions stimulatory effects of spermine did not appear to be voltage dependent.

The results show that spermine stimulation is not affected by a physiological concentration of Mg^{2+}, indicating that Mg^{2+} does not bind to the stimulatory polyamine site, whereas inhibitory effects of spermine are masked by Mg^{2+}. Thus, under physiological conditions in the presence of Mg^{2+}, inhibitory effects of high micromolar concentrations of spermine would be negligible or absent, and stimulatory effects would predominate at native NMDA receptors that are sensitive to spermine stimulation.

General discussion and conclusions. The results of this work may help to clarify some of the variable effects of spermine reported from studies with cultured neurons (5–9). Based on

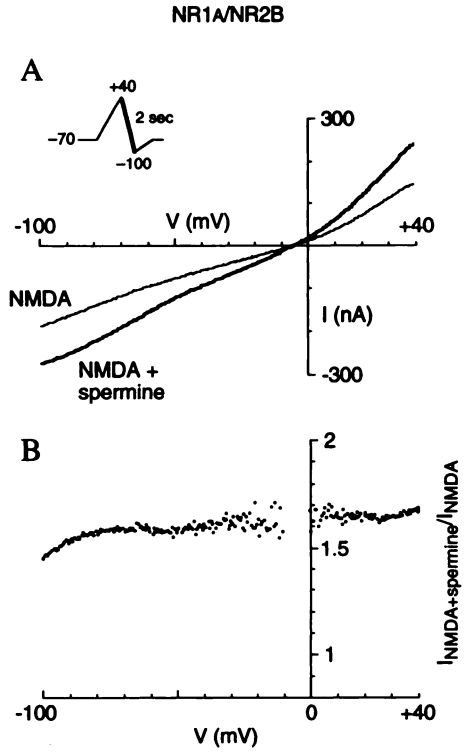

Fig. 4. Glycine-independent stimulation by spermine is not voltage dependent. A, Current-voltage curves were constructed by fast (2-sec) voltage ramps from +40 to −100 mV during steady state responses induced by NMDA (100 μM, with 10 μM glycine), in the absence and presence of 100 μM spermine, in an oocyte expressing NR1A/NR2B receptors. Leak currents have been subtracted. B, Currents measured in the presence of NMDA plus spermine are expressed as a fraction of the control (NMDA) current (control = 1.0); data are replotted from A.

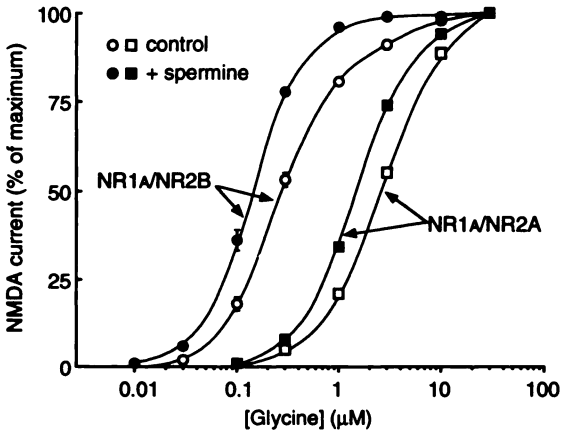

Fig. 5. Spermine increases the affinity of NR1A/NR2A and NR1A/NR2B receptors for glycine. Currents induced by NMDA (100 μM) were measured in the presence of different concentrations of glycine, in the absence and presence of 100 μM spermine, in oocytes voltage-clamped at −70 mV. Oocytes were injected with BAPTA, and the extracellular solution contained 1.8 mM $BaCl_2$. Values are mean ± standard error from four to eight oocytes and are expressed as a percentage of the maximum response seen with 30 μM glycine.

studies of homomeric NR1 receptors, it has been suggested that different splice variants of NR1 with or without the amino-terminal insert may control the polyamine sensitivity of native NMDA receptors (18). However, our results have shown that coexpression of NR1A with particular NR2 subunits can markedly alter properties associated with the NR1 subunit. Thus,

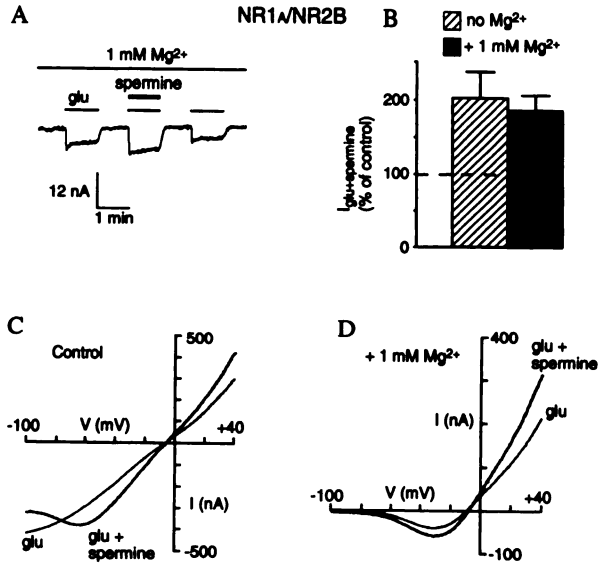

Fig. 6. Mg^{2+} does not block the stimulatory effect of spermine. A, The effects of spermine (100 μM) on responses to glutamate (*glu*) (10 μM, with 10 μM glycine) were measured in an oocyte expressing NR1A/NR2B receptors and voltage-clamped at −70 mV. The extracellular solution contained 1.8 mM $CaCl_2$ and 1 mM $MgCl_2$. B, Currents measured in the presence of glutamate plus spermine are expressed as a percentage of control currents in the absence and presence of 1 mM Mg^{2+} (mean ± standard error from four to six oocytes). C and D, Current-voltage curves were measured by voltage ramps (12 sec, −100 to +40 mV) during steady state responses induced by glutamate or glutamate plus spermine in an oocyte expressing NR1A/NR2B receptors. The extracellular solution contained 1.8 mM $CaCl_2$ in the absence (C) and presence (D) of 1 mM $MgCl_2$. Leak currents have been subtracted. Similar results were obtained in five oocytes.

inclusion of an NR1 variant, such as NR1A, without the amino-terminal insert is necessary for polyamine stimulation, but the manifestation of this effect is controlled by the type of NR2 subunit present in a heteromeric complex. The amino-terminal insert (21 amino acids) is located in the putative extracellular domain of the NR1 subunit. It is possible that this insert, which contains excess positively charged amino acids, interferes with the binding of polyamines or that the properties of a polyamine binding site are directly altered or abolished by this insert.

mRNAs encoding NR1 splice variants without the amino-terminal insert represent 80–90% of NR1 mRNA in cultured cortical neurons (18a) and in rat cerebral cortex,[1] suggesting that differential expression of NR1 variants may play only a minor role in controlling polyamine sensitivity of NMDA receptors in cortical neurons. On the other hand, NR2A and NR2B are the predominant NR2 mRNAs expressed in rat forebrain and in cultured cortical neurons, and their levels are altered during postnatal development *in vivo* and over time in cultured neurons (18a–22, 29).[1] After 1–7 days in primary culture, neurons from embryonic neocortex express relatively high levels of mRNAs encoding NR1 and NR2B but only a very low level of NR2A mRNA. Expression of NR2A mRNA increases during days 7–21 *in vitro*, but levels of NR2A remain lower than those of NR2B (18a). Thus, time-dependent and cell-specific expression of different NR2 subunits may contribute markedly to the variable effects of spermine seen at native NMDA receptors on cultured forebrain neurons. In other brain regions, such as

[1] J. Zhong, D. B. Pritchett, P. B. Molinoff, and K. Williams, unpublished observations.

cerebellum, where there is a greater proportion of NR1 containing the amino-terminal insert (16), alternative splicing of NR1 and expression of NR2 subunits may both be major determinants of sensitivity to polyamines.

Understanding the effects of polyamines on recombinant heteromeric receptors may be useful in elucidating the structural and functional properties of different forms of the NMDA receptor. It is possible that in homomeric NR1A receptors the stimulatory polyamine binding site is located on the NR1A subunit and that in heteromeric receptors its properties are altered by NR2 subunits, resulting in a loss of glycine-independent spermine stimulation at NR1A/NR2A and NR1A/NR2C receptors. This could be due to an allosteric interaction that, for example, reduces the affinity of the binding site for polyamines. Alternatively, the polyamine binding site may be formed by the structural interaction of two or more NR1A subunits in homomeric NR1A receptors and could involve parts of both NR1A and NR2 subunits in a heteromeric complex. In this case, the stimulatory polyamine site would be formed by an interaction of NR1A with NR2B but not with NR2A or NR2C. It is also possible that the differential effects of spermine seen at heteromeric receptors are due to intrinsic differences in receptor/channel properties, such as gating or desensitization, rather than differences in polyamine binding sites.

The results of this work also provide an explanation for the apparent antagonism of polyamine effects by the atypical antagonist ifenprodil, which has been suggested to act as a competitive antagonist at the stimulatory polyamine site (30), although ifenprodil does not appear to competitively inhibit the effects of spermine, and ifenprodil inhibition is seen in the absence of extracellular polyamines (23, 24, 31, 32). Ifenprodil binds with high affinity to NR1A/NR2B receptors, which are sensitive to polyamine stimulation, but has only very low affinity at NR1A/NR2A receptors, which are insensitive to polyamine stimulation (23). Mixed populations of NMDA receptors, having high and low affinities for ifenprodil, are seen in rat brain and in cultured neurons (24, 32). Thus, the reported interactions between polyamines and ifenprodil are probably coincidental and receptor subtype dependent.

It is not known whether endogenous polyamines act at NMDA receptors *in vivo*. Polyamines are present in high concentrations in the brain, and uptake and depolarization-induced release of polyamines have been reported (33–36). The results of the present work suggest that high micromolar concentrations of spermine present in the synaptic cleft would increase the activation of some subtypes of NMDA receptor. Thus, if levels of polyamines change because they are released tonically or by neuronal activation at glutamatergic synapses, then polyamines may play a role in synaptic transmission and plasticity involving NMDA receptors. Studies of the effects of polyamines at excitatory synapses using dissociated neurons or brain slices have been hampered by the lack of selective, high affinity ligands for polyamine sites on the NMDA receptor and by the dual stimulatory and inhibitory effects of spermine. Because inhibitory effects of spermine at the NMDA receptor are masked in the presence of extracellular Mg^{2+}, studies using physiological concentrations of Mg^{2+} may be a valuable approach to investigating the effects of polyamines at intact synapses.

Acknowledgments

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