Spermine potentiation of recombinant N-methyl-D-aspartate receptors is affected by subunit composition

(excitatory amino acid receptors/glutamate receptors/recombinant receptors/$Xenopus$ oocyte expression system/polyamines)

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ABSTRACT   The present study shows that both the NR1 and NR2 subunits critically affect spermine potentiation of heteromeric recombinant N-methyl-D-aspartate receptors. $NR1_{011}$, the most prominent NR1 splice variant in rat forebrain, and $NR1_{100}$, prominent in midbrain, were expressed in $Xenopus$ oocytes singly and in combination with NR2A, NR2B, and NR2C subunits. As for $NR1_{011}$ homomers, $NR1_{011}$/NR2B receptors exhibited spermine potentiation by two mechanisms: by increasing glycine affinity and by increasing current through receptors with bound N-methyl-D-aspartate and glycine. $NR1_{011}$/NR2A receptors exhibited only the increase in glycine affinity, and $NR1_{011}$/NR2C receptors exhibited neither. As for $NR1_{100}$ homomers, $NR1_{100}$/NR2B and $NR1_{100}$/NR2A receptors exhibited spermine potentiation only by increasing the glycine affinity. Spermine produced no potentiation of $NR1_{100}$/NR2C receptors. Thus, the NR2B subunit "permits" both forms of spermine potentiation, the NR2A subunit permits spermine potentiation only by increasing the glycine affinity, and the NR2C subunit permits neither form of potentiation. Spermine actions on NR1/NR2 showed little voltage dependence. These observations are of interest because the NR1 and NR2 subunits are differentially distributed and developmentally regulated. At early postnatal ages, NR2B subunit mRNA was more highly expressed than NR2A and NR2C mRNAs in hippocampus, neocortex, and caudate-putamen. These findings account for many of the observed differences among neurons in polyamine actions and suggest that these actions will vary in a cell-specific and age-related manner.

Diverse forms of the N-methyl-D-aspartate (NMDA) receptor subunit NR1 arise by alternative RNA splicing. Differential splicing of three exons (two exons and an exon segment) generates as many as eight NR1 splice variants, seven of which have been identified in cDNA libraries (1–4). The alternatively spliced exons encode a 21-amino acid sequence in the N-terminal domain (termed N1) and adjacent sequences of 37 and 38 amino acids in the C-terminal domain (termed C1 and C2). Splicing out of the C2-encoding exon segment removes a stop codon and opens a new reading frame encoding an unrelated sequence of 22 amino acids (C2') before a stop codon is reached. We denote the splice variants by subscripts indicating the presence or absence of the three alternatively spliced exons from 5' to 3'; thus, $NR1_{011}$ lacks the N1 insert and has the C1 and C2 inserts, and $NR1_{100}$ has the N1 insert and lacks the C1 and C2 inserts (2). In $Xenopus$ oocytes, each of these variants forms functional channels (1–4) with a number of the properties of native NMDA receptors, including activation by glutamate and NMDA, a coagonist requirement for glycine, inhibition by micromolar

Zn^{2+}, voltage-dependent block by Mg^{2+} and phencylidine, and permeability to Ca^{2+}.

Homomeric NR1 receptors differ in a number of their properties (2, 4). Splicing in of N1 decreases agonist affinity and potentiation by spermine at high glycine and potentiation by submicromolar Zn^{2+}; N1 increases potentiation by activators of protein kinase C. Oocytes expressing NR1 subunits containing N1 exhibit larger currents than do oocytes expressing the corresponding subunit lacking N1 (4, 5). These observations led us to hypothesize that splice variants containing the N1 insert exhibit less spermine and zinc potentiation, because the insert itself produces a conformational change similar to that produced by spermine and by zinc; i.e., potentiation by the insert occludes potentiation by spermine and by zinc (5). Exchanging alanines for the six positively charged amino acids in N1 rescues spermine and Zn^{2+} potentiation and reduces response amplitudes to the level of NR1 receptors lacking N1 (5).

Diversity of NMDA receptors also arises by differential association with members of the NR2 gene family. NR2A/NR2D subunits do not form functional NMDA receptors by themselves but assemble with NR1 to form channels with enhanced NMDA-induced responses (6–9). Subunit-specific antibodies coprecipitate NR1 subunits with NR2A and/or NR2B subunits, indicating the presence $in vivo$ of both two and three component heteromeric receptors (10). For recombinant NMDA receptors, the NR2 subunit affects Mg^{2+} block (6, 11), glycine affinity (8, 12), and potentiation by activators of protein kinase C (13). These differences are likely to be physiologically relevant, because the NMDA receptor NR2A–NR2D subunits are differentially expressed and developmentally regulated (11, 14).

Spermine potentiates NMDA-induced currents in $Xenopus$ $laevis$ oocytes expressing rat brain (15, 16) or recombinant (1, 2) NMDA receptors and in cultured neurons from striatum (17), cortex (18, 19), hippocampus (20, 21), and spinal cord (22). Potentiation occurs by two mechanisms: (i) by increasing the glycine affinity (apparent only at low glycine) and (ii) by increasing current through receptors to which glutamate and glycine have bound (16, 21). Spermine potentiation of receptors with bound agonists is fast ($\tau < 10$ ms); potentiation at low glycine has a slow component consistent with the kinetics of glycine binding (21). Spermine has inhibitory and potentiating actions on neuronal (18–21) and recombinant (1, 2) NMDA receptors. At relatively negative membrane potentials, spermine can decrease whole cell currents and single-channel conductance in a voltage-dependent manner (20, 21).

The diversity of polyamine actions on NMDA receptors and the observation that polyamines are present at high concentrations and released from presynaptic terminals in

the brain (23–25) suggest that polyamines may be physiological modulators of NMDA receptors. The present study shows that differential association of NR1 splice variants and NR2 subunits alters spermine actions on NR1/NR2 heteromeric receptors. These observations may explain some of the differences seen among neuronal responses to polyamines. We also demonstrate that NR2 isoforms exhibit developmental and regional specificity.

MATERIALS AND METHODS

RNA Synthesis. $NR1_{011}$ cDNA was a gift of S. Nakanishi (Kyoto University); $NR1_{100}$ cDNA was cloned in this laboratory (1). Clones of $\varepsilon1–\varepsilon3$ of mouse (99% identical to the rat NR2A–NR2C cDNAs and herein referred to as NR2A–NR2C) were a gift of M. Mishina (Niigata University). To generate templates for transcription, circular plasmid cDNAs were linearized with *Not* I ($NR1_{011}$ and NR2A) or *Bam*HI (wild-type and mutant $NR1_{100}$, NR2B, and NR2C). Transcription reactions were performed with T7 or T3 polymerase [Ambion (Austin, TX) MEGAscript transcription kit; 4 h at 37°C]. For *in situ* hybridization, ^{35}S-labeled antisense RNA probes were transcribed from NR2A, NR2B, and NR2C cDNAs.

Electrophysiological Experiments in *Xenopus* Oocytes. Oocytes from *Xenopus* were prepared as described (26, 27). Selected stage V and VI oocytes were injected with *in vitro*-transcribed RNA [NR1 RNA (10 ng) and one or two NR2 RNAs (each at 20 ng) per cell] and maintained at 18°C. Three to 7 days after injection, oocytes were recorded from in Mg^{2+}-free frog Ringer's solution (116 mM NaCl/2.0 mM KCl/1.0 mM $CaCl_2$/10 mM Hepes, pH 7.2) by two-microelectrode voltage clamp with a Dagan amplifier (Dagan Instruments, Minneapolis); drugs were bath-applied. Statistical analyses employed one-sample *t* tests (STATVIEW, version 4.01) and ANOVA (CLR ANOVA, version 1.3).

In Situ Hybridization. *In situ* hybridization was performed by a modification of our described methods (28, 29). Cryostat sagittal sections (20 μm) of rat brain of various postnatal ages [postnatal day 4 (P4), P8, P14, and adult] were hybridized with ^{35}S-labeled RNA probes (10^6 cpm per section, 1 ng/μl) under conditions of high stringency (5–8 h at 60°C). Slides were apposed (72 h) to Kodak XAR5 film. Sense RNA probes did not label, and treatment with RNase A (100 μg/ml) prior to hybridization prevented labeling. Cross-hybridization among NR2A, NR2B, and NR2C was found to be <5% by competition experiments with unlabeled NR2A, NR2B, and NR2C RNAs (data not shown). The NR1 probe shares <20% sequence identity with NR2A–NR2C (6, 8) and is, therefore, unlikely to cross-react.

RESULTS

The NR2 Subunit Affects Spermine Potentiation of $NR1_{011}$/NR2 Receptors at High Glycine. The $NR1_{011}$ splice variant (which lacks the N1 insert) is the most prominent NR1 isoform expressed in forebrain (3, 30) and exhibits spermine potentiation at saturating glycine (1, 2). To determine the effect of NR2 subunits on spermine actions on heteromers with $NR1_{011}$, $NR1_{011}$/NR2 receptors were expressed in *Xenopus* oocytes. Responses to 300 μM NMDA with 10 μM glycine were measured under voltage clamp at −40 mV, −60 mV, and −100 mV in the presence and absence of 250 μM spermine, a concentration that elicits near maximal potentiation. At −60 mV, spermine potentiated NMDA responses of $NR1_{011}$/NR2B receptors to 185 ± 4% of control (mean ± SEM, *n* = 15) (Fig. 1). At −60 mV, spermine produced little or no change in the NMDA responses of $NR1_{011}$/NR2A or $NR1_{011}$/NR2C receptors (97 ± 5%, *n* = 9; 97 ± 4%, *n* = 4, respectively). Spermine potentiation of $NR1_{011}$/NR2B receptors was significant (*P* < 0.01) and significantly different from that of the other two heteromeric receptors. This finding
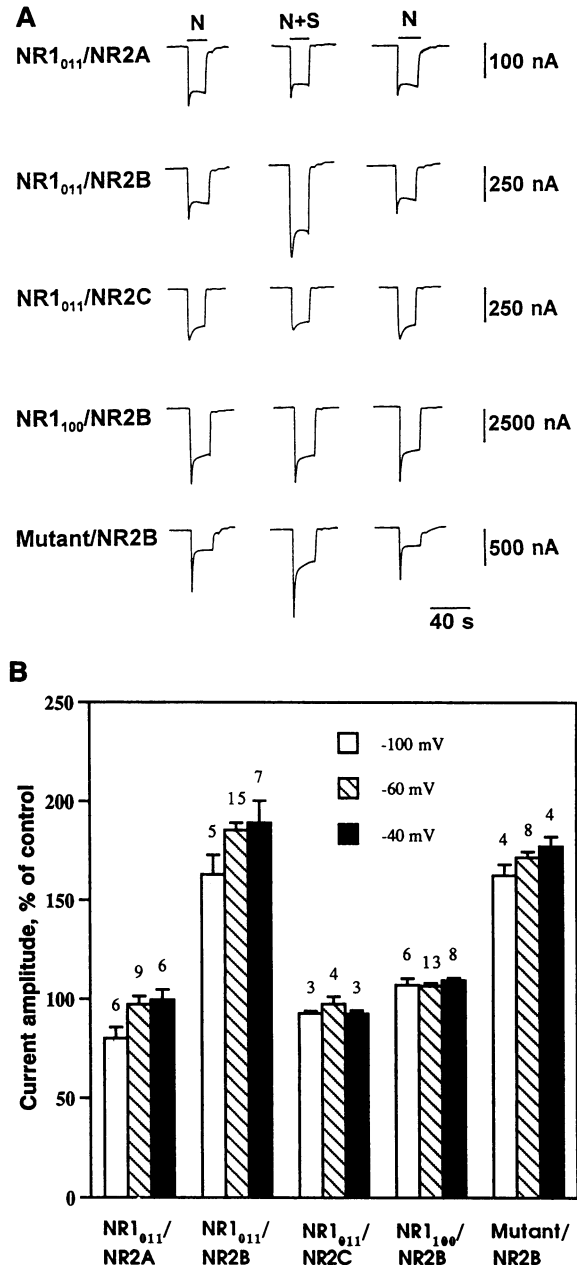
FIG. 1.   Subunit composition of NR1/NR2 heteromeric receptors affects spermine potentiation at high glycine. *Xenopus* oocytes were injected with $NR1_{011}$ RNA (10 ng) and NR2A, NR2B, or NR2C RNA (each at 20 ng) or with NR2B RNA (20 ng) and $NR1_{100}$ or mutant $NR1_{100}$ (each at 10 ng). Responses to NMDA (300 μM with 10 μM glycine) were recorded in the presence and absence of spermine (250 μM) at −40, −60, and −100 mV. (*A*) Representative sample records at −60 mV in the presence (N+S) and absence (N) of spermine. Spermine potentiated responses of $NR1_{011}$/NR2B receptors but not of $NR1_{011}$/NR2A, $NR1_{011}$/NR2C, or $NR1_{100}$/NR2B receptors. Spermine potentiation was rescued in (mutant $NR1_{100}$)/NR2B receptors. (*B*) Voltage dependence of spermine action on NR1/NR2 receptors. Spermine action was measured as the ratio of NMDA-induced currents in the presence and absence of spermine. Holding potential over the range examined had little effect on spermine action. At −100 mV, there was a small reduction in potentiation of $NR1_{011}$/NR2B receptors. Data are the mean ± SEM of values from 3 to 15 oocytes, as indicated above the bars.

indicates that the NR2B subunit "permits" spermine potentiation at saturating glycine but that NR2A and NR2C do not.

The NR1 Splice Variant also Affects Spermine Potentiation of NR1/NR2 Heteromers at High Glycine. As in $NR1_{100}$ homomers, the presence of the N1 insert greatly reduced

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spermine potentiation at high glycine of $NR1_{100}/NR2B$ receptors compared to $NR1_{011}/NR2B$ receptors. Spermine produced a modest, but significant, potentiation only at -40 mV (to 109% of control; $n = 8$; $P < 0.01$) (Fig. 1B). Spermine produced no significant potentiation of responses of $NR1_{100}/$ $NR2A$ or $NR1_{100}/NR2C$ receptors (Fig. 2 D and H).

As for homomeric $NR1_{100}$ receptors (5), exchanging alanines for the six positively charged amino acid residues in the N1 insert of $NR1_{100}$ (mutant $NR1_{100}$) rescued spermine potentiation at high glycine of (mutant $NR1_{100}$)/NR2B receptors (Fig. 1). At -60 mV, spermine (250 μM) potentiated (mutant $NR1_{100}$)/NR2B receptors to 172 ± 3% of control (Fig. 1B; $P < 0.01$; $n = 8$). Spermine potentiation of (mutant $NR1_{100}$)/ $NR2B$ receptors did not differ significantly from that of $NR1_{011}/NR2B$ receptors. This finding suggests that the reduction in spermine potentiation exhibited by $NR1_{100}/NR2B$ heteromers is due entirely to the positive charges in the N1 insert of the NR1 subunit and not to the insert's acting as a 21-amino acid "spacer."

Spermine Action on NR1/NR2 Heteromers Exhibits Little Voltage Dependence. The inhibitory action of spermine on NR1 homomers expressed in *Xenopus* oocytes is greatly reduced at more depolarized potentials (1, 2) as for some (21) but not all (20, 22) native NMDA receptors. NR1/NR2 receptors showed relatively little voltage dependence between -40 mV and -100 mV; the trend was toward less potentiation at hyperpolarized potentials, but the effect was significant only for $NR1_{011}/NR2B$ receptors (potentiation to 196 ± 20% of control at -40 mV and potentiation to 154 ± 3% of control at -100 mV; Fig. 1B; $n = 4$; $P < 0.001$, ANOVA using only those oocytes tested at -40 mV, -60 mV, and -100 mV).

Spermine Increases Glycine Affinity of Homomeric NR1 Receptors. At low glycine (0.1 μM), spermine potentiates NR1 homomers, whether or not they have the N1 insert (1, 2). To examine the mechanism of this potentiation, we determined glycine dose–response curves at -60 mV for $NR1_{011}$ and $NR1_{100}$ homomers with NMDA (300 μM) in the presence and absence of spermine (250 μM). For $NR1_{011}$ homomers, spermine shifted the dose–response curve to the left and increased I_{max} (Fig. 2A, the dotted line represents the spermine data scaled to the control I_{max}). As for NMDA receptors expressed from forebrain message (16), spermine potentiation of $NR1_{011}$ homomers was greatest at low glycine, presumably due to the combined effects of increased glycine binding and increased current through receptors with bound NMDA and glycine. Thus, potentiation was to 287 ± 24% of control at 100 nM glycine vs. 175 ± 6% of control at saturating glycine.

For homomeric $NR1_{100}$ receptors, spermine shifted the glycine dose–response curve to the left but had little effect on I_{max} (Fig. 2B). Potentiation at 0.1 μM glycine was to 197 ± 15% of control. These data suggest that the N1 insert does not affect the increase in glycine affinity caused by spermine but prevents the increase in current through receptors with bound NMDA and glycine.

Subunit Composition of NR1/NR2 Heteromeric Receptors Affects Spermine Potentiation at Low Glycine. To test whether the NR2 subunit affects the degree of potentiation at low glycine, we generated glycine dose–response curves for NR1/NR2 heteromers in the presence and absence of spermine. For $NR1_{011}/NR2B$ heteromers, spermine shifted the glycine dose–response curve to the left and increased I_{max} (Fig. 2E). Potentiation by spermine at 0.1 μM glycine, to 338 ± 67% of control, was greater than that at saturating glycine, to 171 ± 13% of control, consistent with the action of spermine on homomeric $NR1_{011}$ receptors. For $NR1_{011}/$ $NR2A$, $NR1_{100}/NR2A$, and $NR1_{100}/NR2B$ heteromers, spermine shifted the glycine dose–response curve to the left but caused no change or a small decrease in I_{max} (Fig. 2 C, D, and F). Evidently, spermine increased current through channels

with bound NMDA and glycine for only two of the receptors examined, $NR1_{011}$ homomers and $NR1_{011}/NR2B$ heteromers; spermine increased glycine affinity for both NR1 monomers and their heteromers with NR2A and NR2B. For
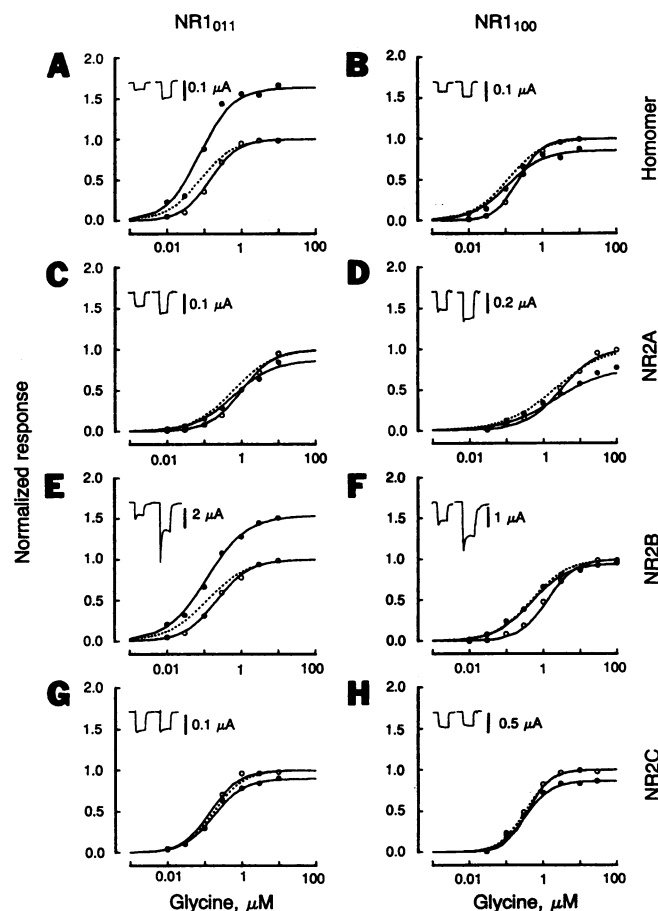
FIG. 2. Subunit composition of NR1/NR2 heteromeric receptors affects spermine potentiation at low glycine. Glycine dose–response curves are shown for homomeric $NR1_{011}$ and $NR1_{100}$ receptors and for their heteromers with NR2A–NR2C as indicated. Responses induced by NMDA (300 μM) in the presence (solid circles) and absence (open circles) of spermine (250 μM) at -60 mV. Concentration–response curves were fitted to the Hill equation: $I = I_{max}/\{1 + (K_d/[G])^{n_H}\}$, where I_{max} is maximal current, K_d is glycine affinity, [G] is glycine concentration, and n_H is the Hill coefficient. Responses normalized to the calculated maximum value, I_{max}, at saturating glycine in the absence of spermine are plotted. Dotted lines are responses in the presence of spermine normalized to the calculated maximum response in the presence of spermine. (*Insets*) Sample records at 0.1 μM glycine to NMDA applications in the presence (*Right*) and absence (*Left*) of spermine lasting ≈25 s. Each graph is for a representative oocyte; values of K_d and n_H (mean ± SEM) in the presence and absence, respectively, of spermine and I_{max} only in the presence of spermine are as follows. (*A*) $NR1_{011}$ homomers. K_d = 142 ± 77 nM and 262 ± 78 nM; I_{max} = 169 ± 6%; n_H = 1.0 ± 0.2 and 1.2 ± 0.2 (n = 4). (*B*) $NR1_{100}$ homomers. K_d = 127 ± 21 nM and 296 ± 40 nM; I_{max} = 92 ± 5% of control; n_H = 1.0 ± 0.1 and 1.3 ± 0.1 (n = 4). (*C*) $NR1_{011}/NR2A$ receptors. K_d = 1.1 ± 0.5 μM and 1.7 ± 0.5 μM; I_{max} = 85 ± 2%; n_H = 1.0 ± 0.2 and 1.1 ± 0.1 (n = 2). (*D*) $NR1_{100}/NR2A$ receptors. K_d = 3.8 ± 0.7 μM and 6.1 ± 1.2 μM; I_{max} = 84 ± 6%; n_H = 1.0 ± 0.2 and 1.0 ± 0.1 (n = 3). (*E*) $NR1_{011}/NR2B$ receptors. K_d = 238 ± 72 nM and 415 ± 114 nM; I_{max} = 178 ± 2%; n_H = 1.1 ± 0.2 and 1.2 ± 0.1 (n = 2). (*F*) $NR1_{100}/NR2B$ receptors. K_d = 0.8 ± 0.2 μM and 1.4 ± 0.2 μM; I_{max} = 99 ± 5%; n_H = 1.0 ± 0.1 and 1.3 ± 0.4 (n = 3). (*G*) $NR1_{011}/NR2C$ receptors. K_d = 177 ± 3 nM and 160 ± 19 nM; I_{max} = 88 ± 2%; n_H = 1.1 ± 0.04 and 1.1 ± 0.04 (n = 2). (*H*) $NR1_{100}/NR2C$ heteromers. K_d = 373 ± 81 nM and 352 ± 48 nM; I_{max} = 82 ± 1%; n_H = 1.4 ± 0.2 and 1.2 ± 0.3 (n = 3).
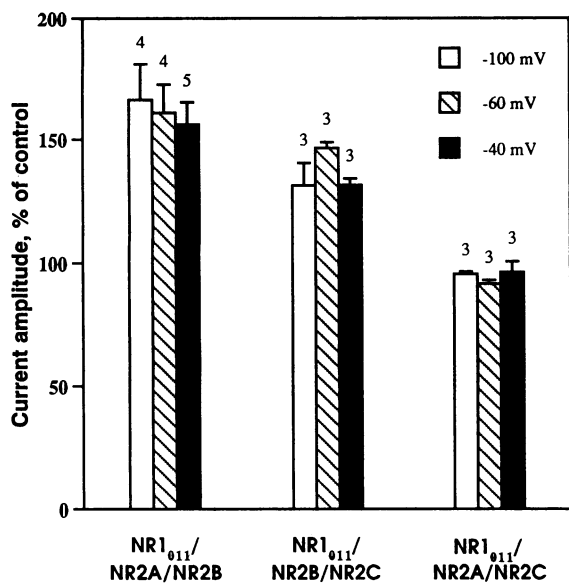
FIG. 3. NR2B subunit may be dominant in determining spermine potentiation of three-component NMDA receptors at high glycine. Oocytes were coinjected with 10 ng of $NR1_{011}$ RNA and two NR2 RNAs (each at 20 ng); responses were recorded and data were plotted as for Fig. 1B. Spermine potentiation was marked for putative $NR1_{011}$/NR2A/NR2B and $NR1_{011}$/NR2B/NR2C receptors and absent for $NR1_{011}$/NR2A/NR2C receptors. For all three classes, spermine action was not noticeably voltage-dependent.

$NR1_{011}$/NR2C and $NR1_{100}$/NR2C receptors, spermine neither decreased the glycine K_d value nor potentiated NMDA responses at any concentration of glycine (Fig. 2 G and H); in these heteromers neither potentiating action of spermine was present.

The apparent K_d values for glycine were higher for $NR1_{011}$/NR2A and $NR1_{100}$/NR2A receptors than for the other NR1/NR2 heteromers (Fig. 2), as reported for $NR1_{011}$/NR2A and $NR1_{011}$/NR2B receptors (8, 12).

Spermine Potentiation in Receptors Formed of Three Types of Subunit. *In situ* hybridization studies demonstrate that NR2A and NR2B are expressed in overlapping regions (see below). Moreover, immunoprecipitation studies indicate that NR1/NR2A/NR2B receptors are formed *in vivo* (10). Thus, it was of interest to test whether spermine potentiation would be observed in oocytes injected with NR1 mRNA and two different NR2 mRNAs (Fig. 3). Spermine potentiation at high glycine of putative $NR1_{011}$/NR2A/NR2B and $NR1_{011}$/NR2B/NR2C receptors was comparable to that of $NR1_{011}$/NR2B receptors. These findings suggest that if three component receptors were formed, the NR2B subunit may be dominant in determining sensitivity to spermine.

Regional Specialization of NR2 Expression in Adult Rat Brain. In adult rat brain, the three NR2 mRNAs exhibited differential, but overlapping, distribution patterns determined by *in situ* hybridization (Fig. 4; see also refs. 6, 7, 9, 11, and 14). NR2A mRNA was expressed prominently in neocortex, hippocampus, thalamus, and cerebellum. NR2B mRNA largely overlapped NR2A expression but was lower in cerebellum and prominent in the striatum and olfactory cortex. NR2C was largely restricted to the cerebellum with some labeling in the thalamus. In white matter, labeling with all three probes approached background.

Regulated Expression of NR2 During Development. Expression of NR2 mRNAs varies during postnatal development (Fig. 4). NR2A expression was near background throughout the brain at P4. The adult pattern began to emerge by P8 and was fully developed at P14. NR2B was expressed earlier than the other NR2 subunits. At P4, NR2B labeling was present in neocortex and caudate-putamen and prominent in the pyramidal and granule cell layers of the hippocampus. The general
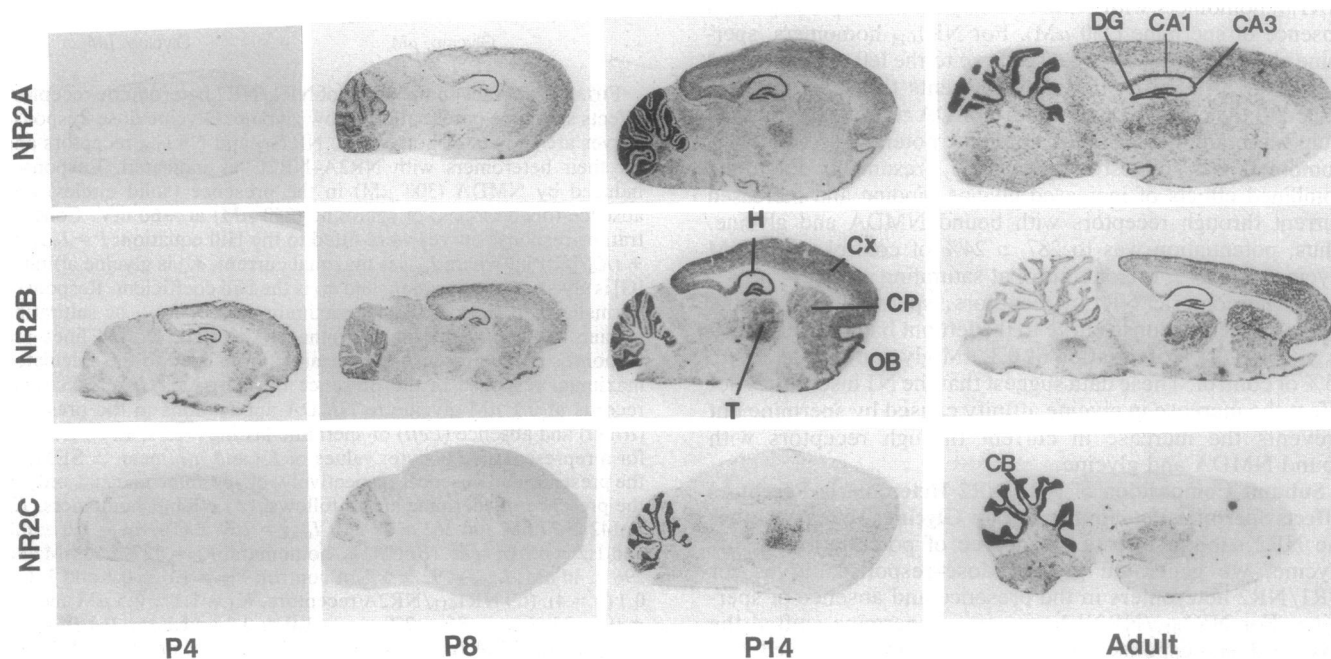

FIG. 4. Developmental and regional regulation of NR2A–NR2C mRNAs in rat brain. NR2A expression was negligible at P4, low at P8, and prominent in neocortex, hippocampus, cerebellum, and thalamus at P14. NR2A expression in neocortex declined somewhat in the adult. NR2B expression was prominent in hippocampus at all ages examined (P4–adult). NR2B was already detectable in neocortex at P4 and was present also in cerebellum and caudate-putamen at P8, suggesting that this subunit may play an important role in early postnatal development. At P14, NR2B was prominent in neocortex, olfactory bulb, caudate-putamen, thalamus, and cerebellum; labeling of the cerebellum was lower in the adult. NR2C mRNA was negligible at P4, just detectable in the cerebellum at P8, and prominent in cerebellum at P14 and in the adult; the thalamus was labeled at P14 and to a minor extent in the adult. CA1 and CA3, hippocampal fields of Ammon's horn; CB, cerebellum; CP, caudate-putamen; Cx, neocortex; DG, dentate gyrus; H, hippocampus; OB, olfactory bulb; T, thalamus.

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pattern of NR2B distribution showed little change with age, except the cerebellar granule cell layer was more densely labeled at P14 than in the adult or at the earlier stages. NR2C mRNA was first detected at P8 in the cerebellum, and this labeling was increased at P14 and in the adult. NR2C mRNA was also present in the thalamus at P14 and in the adult.

DISCUSSION

Our results indicate that both the NR1 and NR2 subunits critically affect spermine potentiation of heteromeric NMDA receptors. Spermine potentiation at high glycine is due to an increase in the current through receptors with bound NMDA and glycine. This form of potentiation is exhibited by NR1 homomers lacking the N1 insert but is greatly reduced in homomers with the N1 insert (1, 2, 5). We show here that spermine potentiates $NR1_{011}$ and $NR1_{100}$ homomers by increasing the glycine affinity, suggesting that this form of potentiation is independent of the N1 insert (Fig. 2). For NR1/NR2 heteromers, potentiation at high glycine was observed only in $NR1_{011}$/NR2B receptors; spermine at high glycine produced little or no change in responses of $NR1_{011}$/NR2A or $NR1_{011}$/NR2C receptors or of any of the $NR1_{100}$/NR2 heteromers. Spermine potentiation by increasing the glycine affinity was observed in $NR1_{011}$/NR2B and $NR1_{100}$/NR2B receptors. Potentiation of $NR1_{011}$/NR2B receptors was greater at low glycine than at high glycine, because spermine increased both the glycine affinity and the current through receptors with bound NMDA and glycine. Spermine potentiation at low glycine was also observed in $NR1_{011}$/NR2A and $NR1_{100}$/NR2A heteromers. Neither form of potentiation was observed in NR1/NR2C receptors. In summary with respect to two component heteromers, the $NR1_{011}$ and NR2B subunits "permit" both mechanisms of spermine potentiation, the $NR1_{100}$ and NR2A subunits permit only the increase in glycine affinity, and the NR2C subunit permits neither mechanism.

These findings are of particular interest, because the NR2 subunits are developmentally regulated. Since NR2B (present study) and N1 lacking NR1 (30) subunits predominate in the neocortex during the neonatal period (P4), homomeric and heteromeric receptors formed of these subunits will be sensitive to regulation by polyamines. The interstitial concentration of glycine in the central nervous system and the role of glycine in regulating NMDA receptors under physiological conditions are uncertain. The glycine site of the NMDA receptor may be normally saturated, in which case potentiation by increasing the glycine affinity would have no physiological significance. However, glycine uptake systems may reduce glycine locally or there may be physiological antagonists at the glycine site that decrease the effective glycine concentration. The lower glycine affinity of NR2A heteromers also increases the likelihood of potentiation of these receptors by increasing the glycine affinity.

The existence of NR1/NR2 heteromers is indicated by immunoprecipitation studies with subunit-specific antibodies (10) and the enhanced responses in cells expressing exogenous NR1 and NR2 subunits together compared to those expressing single NR1 subunits (6–9). The present study may account for variability of spermine effects on neuronal NMDA receptors. Variable potentiation by spermine has been reported in some whole cell recording studies (18, 21). Neuronal receptors that exhibit spermine potentiation at high glycine are likely to contain NR1 subunits that lack N1, either as homomers or as heteromers with NR2B. Neuronal receptors that exhibit spermine action with marked voltage dependence (21) are likely to be homomers (or heteromers) of NR1 subunits without NR2 subunits. Neuronal receptors that exhibit relatively voltage-independent spermine actions (20, 22) are likely to be heteromers of NR1 and NR2 subunits. The present study demonstrates that both NR1 and NR2 subunits

control spermine potentiation of NMDA receptors. Spermine potentiation is likely to be developmentally regulated through differential expression of NR1 and NR2 subunits. In conclusion, spermine may be an important endogenous modulator of NMDA receptor activity.

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