Splice variants of the N-methyl-D-aspartate receptor NR1 identify domains involved in regulation by polyamines and protein kinase C

(excitatory amino acid receptors/glutamate receptors/receptor cloning/Xenopus oocyte expression/phorbol esters)

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ABSTRACT   The N-methyl-D-aspartate (NMDA) receptor *NR1* gene encodes RNA that is alternatively spliced to generate at least seven variants. The variants arise from splicing in or out of three exons; one encodes a 21-amino acid insert in the N-terminal domain, and two encode adjacent sequences of 37 and 38 amino acids in the C-terminal domain. Splicing out of the second C-terminal exon deletes a stop codon and results in an additional open reading frame encoding an unrelated sequence of 22 amino acids before arriving at a second stop codon. We denote the *NR1* variants by the presence or absence of the three alternatively spliced exons (from 5' to 3'); thus, $NR1_{111}$ has all three exons, $NR1_{000}$ has none, and $NR1_{100}$ has only the N-terminal exon. We report here electrophysiological characterization of six splice variants of the *NR1* receptor expressed in *Xenopus* oocytes. *NR1* receptors that lacked the N-terminal exon ($NR1_{000}$, $NR1_{010}$, and $NR1_{011}$) exhibited a relatively high affinity for NMDA ($EC_{50} \approx 13 \ \mu M$) and marked potentiation by spermine. In contrast, those receptor variants with the N-terminal insert ($NR1_{100}$, $NR1_{101}$, and $NR1_{111}$) showed a lower agonist affinity and little or no spermine potentiation at saturating glycine. All six variants showed spermine potentiation at low glycine and inhibition by spermine at more negative potentials. Variants differing only in the C-terminal domain differed little in agonist affinity and spermine potentiation. These findings indicate that the N-terminal insert either participates in agonist and polyamine binding domains or indirectly modifies their conformations. The splice variants differed in the extent to which they could be potentiated by activators of protein kinase C (PKC) from 3- to 20-fold. Presence of the N-terminal insert and absence of the C-terminal sequences increased potentiation by PKC. These findings identify the contributions of the separate polypeptide domains to modulation by polyamines and PKC and provide further support for the concept that subunit composition determines functional properties of NMDA receptors.

The N-methyl-D-aspartate (NMDA)-type of glutamate receptor is thought to play a role in long-term potentiation, memory formation, and control of brain development (1–3). NMDA receptor-mediated neurotoxicity is implicated in the neurodegeneration associated with epilepsy, ischemia, Huntington chorea, Alzheimer disease, and AIDS encephalopathy (4–6).

To date, two gene families encoding NMDA receptor subunits have been identified in rat brain. One family is composed of the *NR1* gene. *NR1* encodes RNA that undergoes alternate splicing to yield at least seven receptor variants (7–10). These variants arise from splicing in or out of three alternative exons, which we designate (from 5' to 3') α, β, and γ (see Fig. 1). Exon α encodes 21 amino acids that can be inserted into the N-terminal domain. Exons β and γ are adjacent and encode the last portion of the C-terminal domain; exon β encodes 37 amino acids; exon γ encodes 38 amino acids before reaching a stop codon followed by an additional 239 nucleotides of 3' noncoding region. The splicing out of exon γ removes the first stop codon, yielding an open reading frame that encodes an unrelated sequence of 22 amino acids before a second stop codon is reached. We propose a nomenclature in which we denote the splice variants by the presence (subscript 1) or absence (subscript 0) of the three exons (in 5' to 3' order). Thus, $NR1_{011}$ has the first (N terminal) exon spliced out and the second and third (C terminal) exons spliced in, and $NR1_{100}$ has only the N-terminal exon. The exon structure, new terminology, and correspondence with previous names are indicated in Fig. 1. In expression systems, each of these variants forms functional homomeric channels with many of the electrophysiological and pharmacological properties of native NMDA receptors (7–10).

The second gene family is composed of the *NR2A-C* subunits (11); these subunits do not form functional NMDA receptors by themselves but apparently can coassemble with *NR1* subunits to give enhanced responses to NMDA. Mouse homologs of two rat *NR1* variants and *NR2A-C* have been identified and have similar properties (Fig. 1; refs. 12–14).
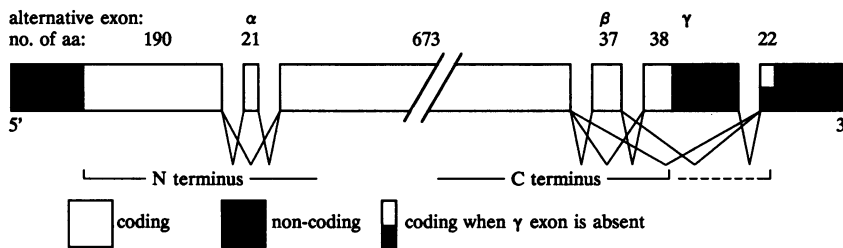
Electrophysiological studies indicate that NMDA receptors have a number of binding domains including a distinct extracellular recognition site for polyamines. Spermine potentiates NMDA-induced currents in *Xenopus laevis* oocytes injected with rat brain mRNA (15, 16) or with synthetic mRNAs encoding NMDA receptor subunits (8, 17), as well as in cultured neurons from rat striatum (18), neocortex (19), hippocampus (20, 21), and spinal cord (22). Potentiation occurs by an increase in maximum response amplitude in the presence of saturating concentrations of NMDA and glycine (indicating an independent site of action for polyamines) and, at lower concentrations of glycine, by increasing NMDA affinity for glycine (16, 21).

Recent studies involving whole-cell and single-channel recording show that spermine also has an inhibitory action on NMDA receptors in cortical and hippocampal neurons (16, 19–21). As well as increasing channel-open probability, spermine reduces single-channel conductance by fast open-channel block and/or charge screening (19, 20). The reduction in single-channel conductance is voltage dependent and virtually absent at potentials more positive than −40 mV. Thus, the potentiating effect of spermine can be seen in isolation at less-negative potentials (at whole-cell as well as single-channel level) and the contribution of inhibition can be determined at more-negative potentials. This dual action is also observed for homomeric *NR1* receptors expressed in *Xenopus* oocytes (8). These findings, together with studies showing the presence of high concentrations of polyamines in

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Abbreviations: NMDA, N-methyl-D-aspartate; PKC, protein kinase C; PMA, phorbol 12-myristate 13-acetate.
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FIG. 1. Proposed gene structure and nomenclature of the *NR1* receptor. Diagram showing three putative exons that can be spliced in or out to form the mature mRNA. Exclusion of exon γ removes a stop codon and yields a different open reading frame that encodes an additional 22 amino acids before a new stop codon is reached. We denote the *NR1* splice variants by subscripts indicating the presence or absence (1 or 0, respectively) of the three exons in 5' to 3' order.

New name for splice variant	Exon presence: α β γ	Old names by cloning group: Sugihara et al. (9)	Durand et al. (8)	Anantharam et al. (7)	N. Nakanishi et al. (10)	Yamazaki et al. (12)(mouse)
$NR1_{000}$	− − −	R1E	NR1c			
$NR1_{001}$	− − +	R1C		SS	NMDA-R1C	ζ 1-2
$NR1_{010}$	− + −	R1D				
$NR1_{011}$	− + +	R1A	NR1a	SL	NMDA-R1A	ζ 1
$NR1_{100}$	+ − −	R1G	NR1b			
$NR1_{101}$	+ − +	R1F		LS		
$NR1_{110}$	+ + −	(not yet found)				
$NR1_{111}$	+ + +	R1B		LL	NMDA-R1B	

the brain, suggest that polyamines may act as physiological modulators of NMDA receptors.

Protein kinase C (PKC) has also been shown to modulate NMDA receptors. Phorbol esters such as phorbol 12-myristate 13-acetate (PMA), which activate PKC, selectively enhance the amplitude of NMDA currents in oocytes (8, 12, 23), CA1 neurons (24), and spinal cord neurons (25). Activation of μ opioid, metabotropic glutamate, and muscarinic receptors, which activate PKC, also potentiate NMDA currents (for review, see ref. 26).

In a previous study, we showed that $NR1_{011}$ and $NR1_{100}$ differed markedly in agonist affinity and in the extent of potentiation by spermine and by activators of PKC (8). In the present study, we analyzed the functional properties of homomeric channels formed by these two and four additional splice variants. We identified receptor domains contributing to differences in function.

MATERIALS AND METHODS

RNA Preparation. $NR1_{100}$ and $NR1_{000}$ receptor subunit cDNAs were previously isolated from a ventral midbrain cDNA library constructed in the Lambda ZAP vector (8). $NR1_{011}$ and $NR1_{010}$ cDNAs were gifts of S. Nakanishi (Kyoto, Japan); $NR1_{101}$ and $NR1_{001}$ cDNAs were from S. Treistman (Worcester, MA); $NR1_{111}$ cDNA was from Richard Axel (New York). *NR1* cDNAs were linearized with *Not* I endonuclease ($NR1_{011}$, $NR1_{101}$, $NR1_{111}$, and $NR1_{010}$), *Bam*HI ($NR1_{100}$), or *Cla* I ($NR1_{000}$). Transcription reactions were performed with T7, T3, or SP6 polymerases (Ambion, Austin, TX) at 37°C for 4–6 h.

Oocyte Methods. Oocytes were collected from anesthetized *X. laevis* as described (27, 28). After incubation for 1 h in Ca^{2+}-free ND-96 medium (96 mM NaCl/2 mM KCl/1 mM $MgCl_2$/5 mM Hepes buffer, pH 7.5) containing collagenase (2 mg/ml), penicillin (100 units/ml), and streptomycin (0.1 mg/ml), the follicular layer was removed manually. Stage V and VI oocytes were injected with *in vitro* transcribed RNA (10 ng per cell). After 2–4 days at 18°C oocytes were placed in a recording chamber (0.1-ml volume) in Mg^{2+}-free ND-96 solution (116 mM NaCl/2.0 mM KCl/2.0 mM $CaCl_2$/10 mM Hepes buffer, pH 7.2) and voltage clamped with two microelectrodes. Drugs were bath applied. Factorial ANOVA was carried out with the SYSTAT program (SYSTAT, Evanston, IL).

RESULTS

Functional Characterization of the *NR1* Clones. NMDA homomeric channels were expressed in *Xenopus* oocytes and NMDA-induced currents were studied by voltage-clamp analysis. Steady-state responses at different NMDA concentrations were obtained for six of the cloned *NR1* receptors and fit with the Hill equation to determine apparent K_D values (inverse of affinities) and Hill coefficients. In our hands one clone, $NR1_{001}$, did not lead to generation of sufficiently large currents for analysis, even if the amount of RNA injected was increased from 10 to 50 ng per oocyte. Receptor variants with the N-terminal insert, $NR1_{1XX}$ ($NR1_{100}$, $NR1_{101}$, and $NR1_{111}$), exhibited apparent K_D values for NMDA that ranged from 42 to 67 μM (Fig. 2A). Variants that lacked the N-terminal insert, $NR1_{0XX}$ ($NR1_{000}$, $NR1_{010}$, and $NR1_{011}$), exhibited a 3- to 5-fold higher affinity, $K_D \approx 12$ μM. Factorial ANOVA applied to the N- and two C-terminal inserts showed that the presence of the N-terminal insert (encoded by exon α) significantly increased the apparent affinity of the receptors for NMDA ($P < 0.001$) independently of the structure of the C-terminal domain. For those variants with the N-terminal insert, the presence of each C-terminal insert significantly modified the K_D ($P < 0.05$ for exon β; $P < 0.001$ for exon γ). For variants lacking the N-terminal insert, the structure of the C-terminal domain did not have a significant effect on agonist affinity. Hill coefficients for all variants were near unity and differences between them were not significant.

Potentiation of *NR1* receptor variants by polyamines was examined at high (10 μM) and low (0.1 μM) glycine concentrations. Oocytes expressing $NR1_{0XX}$ variants exhibited robust spermine potentiation at high glycine (to 220–263% of control at −40 mV and to 178–229% of control at −60 mV; Figs. 2B and 3A). Only at very negative holding potentials (e.g., −100 mV) did spermine inhibit the steady-state NMDA response (22–57% decrease). In some oocytes the initial peak of the NMDA response at −100 mV did show potentiation (Fig. 3A); the delayed onset of inhibition suggests use dependence of spermine channel block. In oocytes expressing $NR1_{1XX}$ variants, spermine (250 μM) elicited little or no potentiation of NMDA responses at high glycine and −40 mV (Figs. 2B and 3B), as previously reported for $NR1_{100}$ (8). At −60 mV, spermine modestly inhibited responses to 72–92% of the control value. At −100 mV, spermine markedly inhibited the steady-state NMDA response to 18–40% of the control response to NMDA alone. The peak was less inhib-
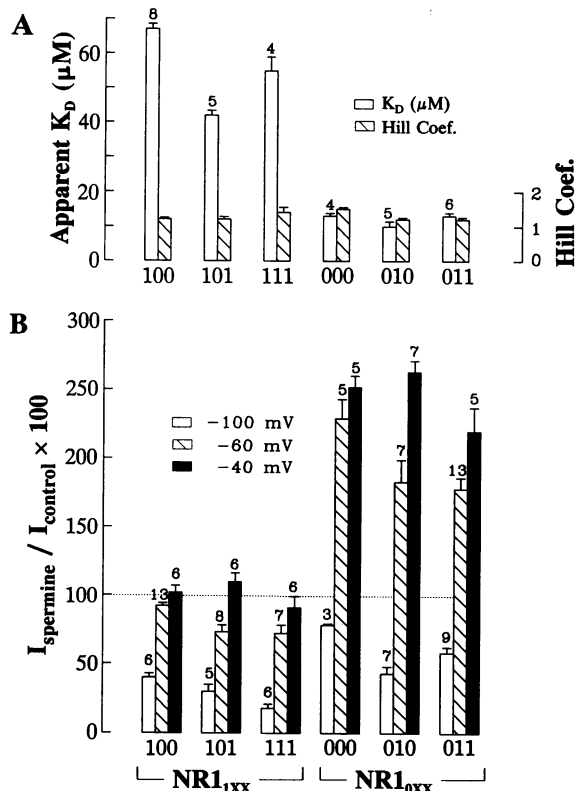
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FIG. 2. *NR1* variants with the N-terminal insert *NR1*ₓₓ show reduced agonist affinity and lack of spermine potentiation at saturating glycine concentration compared to variants without the N-terminal insert *NR1*ₓₓ. Error bars represent SE. Numbers above bars indicate number of oocytes. (*A*) Apparent K_D values (μM) and Hill coefficients. *NR1*₁₀₀ and *NR1*₀₁₁ include data from Durand *et al.* (8). (*B*) Spermine potentiation at high glycine concentration. Spermine (250 μM) was applied with 300 μM NMDA and 10 μM glycine, and the responses were compared to control responses to NMDA and glycine alone. Responses of *NR1*₁ₓₓ variants were little affected at -40 mV, were modestly inhibited at -60 mV, and were markedly inhibited at -100 mV. *NR1*₀ₓₓ variants were potentiated at -40 mV, less potentiated at -60 mV, and inhibited at -100 mV.

ited than the plateau, again suggesting use dependence of block.

All responses in the presence of spermine were significantly different from their controls except for *NR1*₁ₓₓ receptors at -40 mV ($P < 0.001$ by t test). For all three voltages, factorial ANOVA indicated that the presence of the N-terminal insert significantly reduced potentiation by spermine ($P < 0.001$). The structure of the C terminus modified spermine potentiation at the more positive potentials. Of the two 3' end exons taken separately, only exon β produced receptor variants showing a significant decrease in potentiation at -60 mV ($P < 0.05$) and at -100 mV ($P < 0.001$). To estimate the effect of both C-terminal domains together, a three-way interaction was not possible because of the sample size, and we performed a post hoc contrast (30). This method indicated that the presence of both C-terminal inserts significantly decreased spermine potentiation at both -60 and -100 mV ($P < 0.001$).

A glycine level of 10 μM is saturating for *NR1*₁₀₀, *NR1*₀₁₁, and neuronal NMDA receptors (8, 29), and we presume it is saturating for all the *NR1* variants. At reduced glycine concentration (0.1 μM), spermine (250 μM) markedly potentiated NMDA responses in oocytes expressing any one of the six receptor variants, up to 300% of control at -40 and -60 mV for oocytes expressing *NR1*₁ₓₓ homomeric channels (for *NR1*₁₀₁ in Fig. 2C). Potentiation was not significantly higher
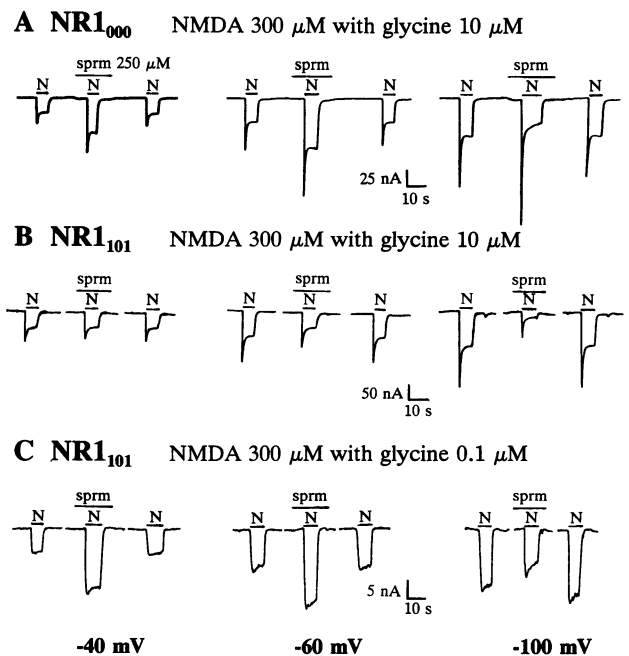

FIG. 3. Spermine modulation of NMDA responses of *NR1* receptor variants without and with the N-terminal insert. The insert reduces potentiation at saturating glycine but not at low glycine. Control, test, and control responses for each splice variant (*A*, *B*, and *C*) are shown at three different voltages (-40, -60, and -100 mV) indicated at the bottom. (*A*) *NR1*₀₀₀. At -40 and -60 mV, spermine (sprm; 250 μM) potentiated the response to NMDA (N) (300 μM) with glycine (10 μM). At -100 mV, spermine reduced the plateau phase of the NMDA response, but the peak was increased. (*B*) *NR1*₁₀₁. At all three potentials spermine (250 μM) decreased the response to NMDA (300 μM) with 10 μM glycine. Inhibition was more prominent at more negative potentials. (*C*) *NR1*₁₀₁. At -40 and -60 mV and low glycine (0.1 μM), spermine (250 μM) potentiated the response to NMDA (300 μM). At -100 mV, the NMDA response was inhibited by spermine.

in the *NR1*₀ₓₓ than in the *NR1*₁ₓₓ variants (data not shown). At low glycine concentration, *NR1* receptor variants exhibited little desensitization of NMDA responses, in contrast to oocytes injected with brain message (31) or hippocampal neurons (29).

NR1 variants differed in their sensitivity to the phorbol ester PMA, an activator of PKC (Fig. 4; Table 1). A 10-min treatment with PMA potentiated NMDA responses in *NR1*₁₀₀-injected oocytes to $\approx$20 times control; NMDA responses in *NR1*₀₁₁-injected oocytes were potentiated to a much smaller degree, to $\approx$3 times control. These results corroborate our previous findings (8). The other *NR1* variants showed a range of intermediate potentiation. Ratios of degree of potentiation are shown in Table 1 for pairs differing by the presence of the N-terminal insert or of both C-terminal inserts. Presence of the N-terminal insert increased potentiation of NMDA responses (*NR1*₁₀₀/*NR1*₀₀₀ = 1.8; *NR*₁₁₁/*NR*₀₁₁ = 3.3). Absence of both C-terminal exons increased PMA potentiation (*NR1*₁₀₀/*NR1*₁₁₁ = 2.0; *NR1*₀₀₀/*NR1*₀₁₁ = 3.7). Factorial ANOVA showed that the increase caused by the N-terminal insert was significant ($P < 0.001$). The presence of the C-terminal insert encoded by exon γ by itself produced a channel with a significantly lower potentiation ($P < 0.001$). The effect of exon β by itself was not significant. Moreover, the post hoc contrast indicated that the presence of both 3' end exons formed receptor variants with significantly decreased potentiation ($P < 0.001$) independently of the N terminus.

The time course of potentiation of steady-state NMDA responses by PMA was evaluated in oocytes expressing
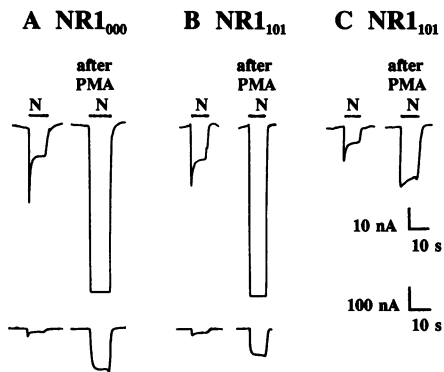
FIG. 4. PMA differentially potentiates NMDA responses of the NR1 receptor variants. (A) PMA potentiated the NRl_{100} response to NMDA (N) to $\approx$20 times control. Responses to NMDA (300 μM) and glycine (10 μM) before and after a 10-min treatment with 100 nM PMA; high and low gains are shown in upper and lower traces, respectively. (B) PMA potentiated the NRl_{111} response to $\approx$10 times control. (C) PMA potentiated the NRl_{010} response to only 4 times control. All responses were obtained in the same batch of oocytes.

NRl_{100} receptors (Fig. 5). Application of NMDA (300 μM) after a 15-min treatment with 100 nM PMA showed potentiation to >17 times the control response in the absence of PMA. During subsequent test applications of NMDA (300 μM for 10-20 s), the responses declined to a level about twice that of control; this level was maintained for at least 1 h. When test applications of NMDA were made during PMA treatment, potentiation was detectable by 30 s after application; under these conditions potentiation increased to $\approx$10 times control at 10 min. Potentiation declined by 15 min in the continued presence of PMA and on washing with saline continued to fall to a maintained value of about twice control. The decrease in potentiated responses caused by repeated NMDA application is slow compared to the desensitization during a single NMDA application (31) and presumably involves different mechanisms.

DISCUSSION

A major finding of our study was that the 21-amino acid insert in the N-terminal domain reduced the apparent affinity of homomeric *NR1* receptors for NMDA and nearly abolished potentiation by spermine at saturating glycine. For both properties, the N-terminal insert was the main determining structural feature; the C-terminal domains produced at most a minor effect. Since the NMDA binding site is presumably contained within the extracellular N-terminal domain of the *NR1* receptor, although probably not at the exact location of the 21-amino acid insert (10), the effect on agonist affinity

Table 1. Both inclusion of the N-terminal insert and omission of the C-terminal inserts increase PMA potentiation ($I_{PKC}/I_{control}$)

Receptor	Potentiation*	Ratios of potentiation†
NRl_{100}	20 ± 2 (8)	N-terminal change
NRl_{000}	11 ± 1 (8)	
NRl_{101}	7 ± 0.5 (7)	NRl_{100}/NRl_{000} = 1.8
NRl_{010}	5 ± 1 (9)	NRl_{111}/NRl_{011} = 3.3
NRl_{111}	10 ± 1 (7)	C-terminal change
NRl_{011}	3 ± 0.5 (5)	NRl_{100}/NRl_{111} = 2.0
		NRl_{000}/NRl_{011} = 3.7

* Potentiation values are mean ± SE. Numbers in parentheses are number of oocytes tested. Responses to NMDA (300 μmM with 10 μM glycine) were recorded before and after a 10-min treatment with PMA (100 nM) for each *NR1* variant.
†Ratios of degree of potentiation for pairs differing by the presence of the N-terminal insert or of both C-terminal inserts.
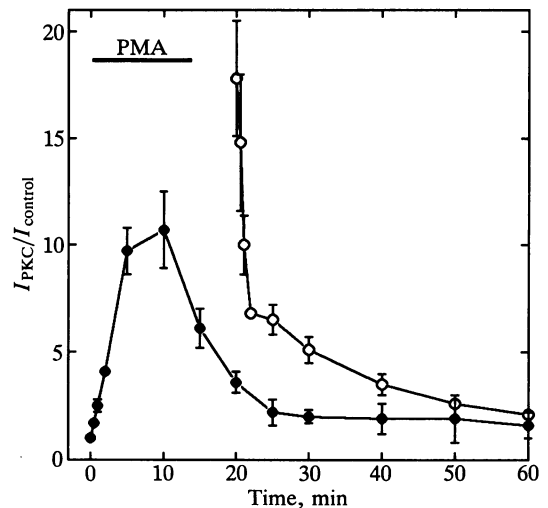

FIG. 5. Time course of PMA potentiation of NMDA responses of NRl_{100} receptors. NMDA (300 μM) with glycine (10 μM) was applied for 10-20 s repeatedly during and after ($\bullet$) or after (o) a 15-min treatment with PMA (100 nM). Error bars represent SE. For each set of experiments, n = 3.

may be the result of a delocalized conformational change in the N-terminal domain caused by the insert. The binding site for spermine potentiation is also extracellular (16, 21, 22), but its exact location in relation to the N-terminal insert is unknown.

It has been shown that the polyamine spermine has a dual action on NMDA-activated channels: it reduces single-channel currents at inside negative potentials and in some patches potentiates by increasing open probability (19, 20). In channels from hippocampal neurons, potentiation results primarily from an increase in burst length; open and closed times within bursts and burst frequency are little changed (20). In our study of *NR1* receptors, steady-state responses of all the variants were reduced by spermine at very negative potentials (−100 mV); the reduction was greater for the NRl_{1XX} variants than for the NRl_{0XX} variants, presumably because it was not opposed by potentiation. The polyamine inhibitory site is likely to be within the channel, since the inhibition is voltage dependent (and may be use dependent).

In hippocampal cells, all single NMDA channels showed similar voltage dependence of spermine inhibition whether or not they showed spermine potentiation (20). If the same were true of *NR1* variants that do and do not show potentiation, one could evaluate whether or not potentiation were voltage dependent. For the NRl_{1XX} variants, spermine inhibition was negligible at −40 mV; at −100 mV it averaged 71%. For the NRl_{0XX} variants the mean response at −40 mV was 247% of control. Inhibition of NRl_{0XX} receptors at −100 mV by 71%, the same percentage as for NRl_{1XX} receptors at this voltage, would give a response that was 72% of control, in reasonable agreement with the observed mean value of 56%. Thus, pending single-channel measurements, we tentatively conclude that spermine inhibition is the same function of voltage for all variants, that spermine potentiation is not very dependent on voltage, and that the effects of the two processes are simply multiplicative in generating the net response. [Benveniste and Mayer (21) also concluded that spermine potentiation exhibited little voltage dependence.]

NRl_{1XX} variants that were not potentiated by spermine at saturating glycine could still undergo substantial potentiation at low glycine. These results suggest two distinct potentiating actions of spermine on NMDA receptors and are consistent with Benveniste and Mayer (21). They proposed that polyamine potentiation of NMDA-mediated currents in hippo-

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campal neurons has two components—one fast and one slow. At saturating glycine concentrations, polyamines exert a fast (<20 ms) potentiation of the NMDA currents ("glycine-independent" potentiation; in spite of the name, this component is reduced at low glycine concentration). Spermine also produces an increase in the affinity of the receptor for glycine, which at low glycine increases the amount of glycine bound, which reduces desensitization, a slower process. At low glycine the decrease in desensitization accounts for at least part of the potentiation ("glycine-sensitive" potentiation). These separate effects do not necessarily require separate binding sites. Benveniste and Mayer observed that the degree of potentiation at saturating glycine and positive potentials was variable from neuron to neuron, although potentiation at low glycine was always present. Our finding that NRI_{1XX} variants exhibit little or no potentiation at saturating glycine but that all variants show potentiation at low glycine could explain their data if cells differed in the relative amount of NRI_{1XX} and NRI_{0XX} expression (and heteromeric receptors *in vivo* are similar in this respect to the corresponding homomeric NRI channels). Thus, the variation in polyamine effects on NMDA-activated currents may be related to differential expression of splice variants in different cells.

A second major finding of our study was the striking difference among receptor variants in the degree of potentiation by the phorbol ester PMA. That potentiation of NRI receptors occurs by activation of PKC is supported by its slow onset (Fig. 5) and by our previous pharmacological data (8). Stimulation ranges from 3-fold for NRI_{011} to 20-fold for NRI_{100} in corroboration of our earlier study (8). The present study showed that both the N-terminal and C-terminal sequences influence degree of potentiation by PKC.

The time-dependent increase in NMDA responses in the presence of PMA is likely to be due to an increase in channel-open probability rather than an increase in single-channel conductance. Neuronal NMDA receptors at saturating agonist have an open probability, p_o, of ≈0.3 before desensitization (32), which permits a maximum potentiation of ≈3. Thus, 20-fold potentiation by PKC, measured as the ratio of p_o in potentiated and control conditions, requires a low value of control p_o compared to the value in (the single example of) neurons. PKC potentiation may also increase responses by recruitment of receptors—e.g., for individual receptors p_o may be 0 in the unphosphorylated state and maximal in the phosphorylated state, or new receptors may be inserted into the membrane. The relatively small responses obtained in oocytes after injection of single NR1 subunit mRNAs compared to brain mRNA (17) is consistent with a low value of p_o for NR1 homomeric receptors but may also reflect differences in rates of formation and turnover.

Several observations suggest that phosphorylation of the C terminus of NRI receptors does not mediate PKC potentiation. First, the NRI splice variant with the shortest C-terminal domain, NRI_{100}, showed maximal potentiation. Although there are several potential phosphorylation sites present on the "omitted" 75 amino acids of NRI_{X00} subunits, there is no likely site for phosphorylation within the 22-amino acid "replacement" encoded by the new open reading frame in this variant. Conversely, one of the variants with the longest C-terminal domain, NRI_{011}, exhibits the least potentiation by PMA. Since presence of the N-terminal insert and absence of the C-terminal inserts have similar effects on the degree of potentiation, it is likely that exon inclusion or omission can affect PKC action through allosteric interactions of the resulting domains.

As noted above, NMDA receptors in neurons are likely to be heteromers composed of NRI and $NR2$ subunits; furthermore, heteromers of the mouse homologs of NRI_{011} and

$NR2A$ subunits exhibit potentiation by PMA but heteromers of the homologs of NRI_{011} and $NR2C$ subunits do not (14). The extent to which the dramatic differences in potentiation observed among NRI variants are retained in heteromers containing $NR2$ subunits remains to be determined. One may infer that tissue- and cell-specific expression of NRI and $NR2$ subunits accounts for regional differences in function. Electrophysiological analysis of heteromers of known subunit composition should provide strong evidence for or against such inferences.

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