was the single base pair deletion found in *amn*, suggesting that random sequence polymorphisms are rare or absent in this reading frame.
21. A. Miyata *et al.*, *Biochem. Biophys. Res. Commun.* **164**, 567 (1989); K. Ogi, C. Kimura, H. Onda, A. Arimura, M. Fujino, *ibid.* **173**, 1271 (1990); C. Kimura *et al.*, *ibid.* **166**, 81 (1990).
22. *Drosophila* appears to possess several neuropeptides homologous to mammalian peptides [R. Nichols, S. A. Schneuwly, J. E. Dixon, *J. Biol. Chem.* **263**, 12167 (1988); T. Lundquist and D. R. Nässel, *J. Comp. Neuro.* **294**, 161 (1990); D. R. Nässel, T. Lundquist, A. Höög, L. Grimelius, *Brain Res.* **507**, 225 (1990)], and an *amn* homolog may occur in mammals (M. B. Feany, unpublished observations). This homolog may not be identical to PACAP given the restricted homologies of the two proteins. *Drosophila* contains a highly related PACAP homolog that is distinct from *amn* [Y. Zhong and L. A. Peña, *Neuron*, **14**, 527 (1995)].

23. M.B.F. thanks M. S. Livingstone and W. W. Bender for their generous intellectual and material support of this work. We thank W. W. Bender and E. Folkers for comments on the manuscript. W.G.Q. was supported by grants BNS-9021698 and BNS-9410934 from NSF, and W. W. Bender by a grant from NIH. B. Hamilton, T. Schwarz, N. Brown, and G. Rubin generously donated cDNA libraries.

9 January 1995; accepted 17 March 1995

Control of Proton Sensitivity of the NMDA Receptor by RNA Splicing and Polyamines

Stephen F. Traynelis,* Melissa Hartley, Stephen F. Heinemann

The function of the *N*-methyl-D-aspartate (NMDA)–preferring glutamate receptor can be regulated by extracellular pH, a process that may be important during ischemia in the brain or during seizures. Protons inhibit NMDA receptor function by 50 percent at pH 7.3 through interactions with the NR1 subunit, and both polyamines and NR1 exon 5 potentiate receptor function through relief of the tonic proton inhibition present at physiological pH. A single amino acid (lysine 211) was identified that mediates the effects of exon 5 in the rat brain. Electroneutral substitutions at this position restored pH sensitivity and, consequently, polyamine relief of tonic inhibition. This effect, together with the structural similarities between polyamines and the surface loop encoded by exon 5, suggest that exon 5 may act as a tethered pH-sensitive constitutive modulator of NMDA receptor function.

NMDA receptors serve many functions in the developing and adult central nervous system (*1*). However, activation of these receptors also can contribute to the pathophysiology of epilepsy (*2*) and stroke (*3*). One way the brain protects itself from the potentially harmful actions of NMDA receptors is to tightly regulate their function. Indeed, NMDA receptors are controlled by many endogenous substances as well as second messenger systems (*1*). Of the effects of endogenous ions on NMDA receptor function, inhibition by extracellular protons (*4*, *5*) is particularly interesting for three reasons. First, ion-selective electrodes have provided information that describes changes in extracellular pH during normal brain function (*6*). For example, the acidic and alkaline transients associated with synaptic transmission are sufficient to alter synaptic NMDA receptor activation (*6*). Second, the acidification of the interstitial spaces that occurs during both seizures and ischemia (*7*) will inhibit NMDA receptors. Because NMDA receptor activation is critical to both seizure development and stroke-induced neuronal damage, receptor inhibition by falling pH should serve as negative feedback (*5*, *8*). Third, the sensitivity of NMDA receptors to physiological concentrations of protons suggests that NMDA receptors are tonically inhibited at physiological pH (*5*).

We studied the proton sensitivity of recombinant rat NMDA receptors expressed in *Xenopus laevis* oocytes (*9*) and in HEK 293 cells (*10*). Similar to native NMDA receptors (*5*), rat homomeric NR1 (*11*) function is strongly inhibited by physiological concentrations of protons (Table 1). This observation suggests that the NR1 subunit contains molecular entities that regulate receptor function in a pH-sensitive manner.

At least three exons (Fig. 1A) can be

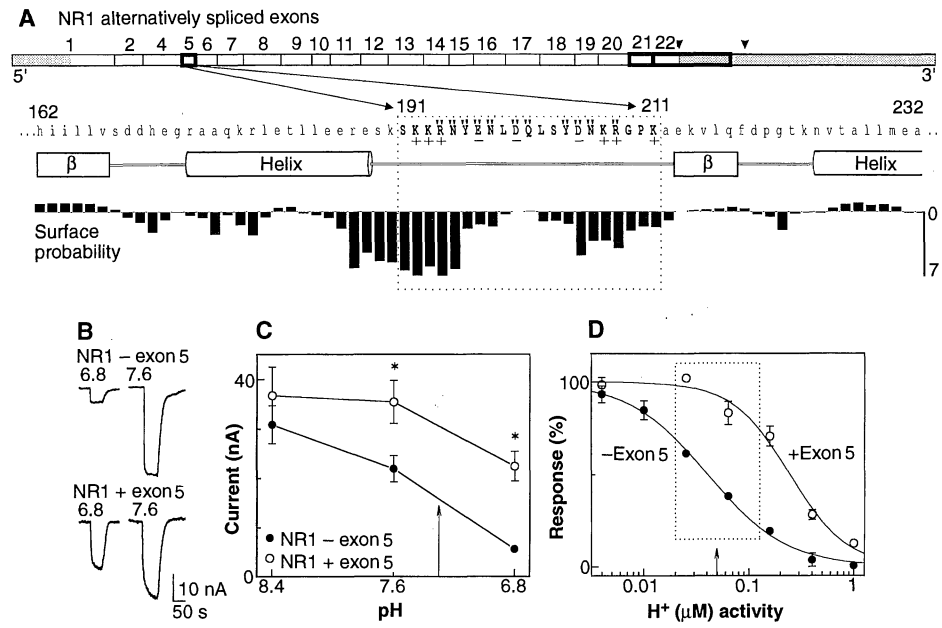

Fig. 1. Control of NMDA receptor proton inhibition by NR1 exon 5. (**A**) NR1 is constructed from 22 exons, four of which can be alternatively spliced (bold) to form nine isoforms (*11*, *21*); stop codons are shown as arrowheads. The third exon, which contains a stop codon, is not shown. Many of the amino acids encoded by exon 5 (uppercase letters) possess side chains that contain π molecular orbital systems (••). Helix and β sheet regions shown were predicted (*12*) according to Rost and Sander; accuracy was <72% if this region of NR1 behaved as a soluble protein. Other algorithms predict helices at Ala^{174} to Ser^{189}, Val^{225} to Arg^{238} (Chou and Fasman), His^{171} to Lys^{193}, and Val^{225} to Leu^{242} (Garnier *et al.*), with an estimated accuracy of about 50 to 55%. β sheets were predicted at Lys^{214} to Phe^{218} (Chou and Fasman) and Glu^{213} to Phe^{218} (Garnier *et al.*). Nearly identical results were obtained for NR1 – exon 5. The surface probability index was calculated from the amino acid surface probabilities (*12*). Downward denotes the increasing likelihood that residues are accessible to water. (**B**) Current responses at −60 mV in Ba^{2+} to glutamate + glycine (pH 6.8 and pH 7.6) are shown from oocytes injected with NR1 – exon 5 and NR1 + exon 5. Scale bars are 10 nA and 50 s. (**C**) Mean agonist-induced responses were determined for NR1 ± exon 5. Identical quantities of splice variant cRNA (from four preparations) were injected into oocytes pairwise (n = 19 cells per point; the asterisk denotes $P < 0.05$). For all figures, vertical arrows mark physiological pH (7.3) and error bars are SEM (whenever larger than the symbol). (**D**) The composite H^+ inhibition curves for NR1 ± exon 5 are shown as a percent of the fitted maximum (see Table 1 for IC_{50} values). The physiological range is superimposed as a box. Data are from 77 oocytes.

alternatively spliced to produce eight different NR1 proteins (*11*). Two of these alternative exons encode many charged amino acid residues and are predicted (*12*) to exist as flexible loops on the surface of the NR1 protein (Fig. 1A). Because the extracellular (*5*) pH sensitivity of the NMDA receptors implies that the proton sensor is accessible to H_3O^+, we tested whether the above-mentioned exons influence the pH regulation of receptor function.

We compared two NR1 isoforms that differ only by the presence or absence of exon 5. The presence of exon 5 increased both the 50% inhibition concentration (IC_{50}) and the Hill slope for proton inhibition (Fig. 1, B through D). The sum of these effects is a change in the proton sensitivity of exon 5–containing receptors within the physiological pH range (superimposed as a box in Fig. 1D). This relief of tonic inhibition by physiological concentrations of H^+ doubled the current (Fig. 1C), which could explain reports of increased current amplitudes for splice variants that contain exon 5 (*11*). Both proton inhibition and its modulation by NR1 exon 5 were insensitive to changes in voltage ($P > 0.2$; $n = 40$ oocytes), which distinguishes this regulation from the effects of pH on membrane surface charge. Examination of the proton sensitivity of receptors encoded by each of the eight alternatively spliced NR1 complementary DNAs (cDNAs) indicated that only exon 5 controls H^+ inhibition (*13*).

Coexpression studies suggest that the rat NR1 and NR2 subunits assemble to form heteromeric receptor complexes (*14*). Because the expression of NR2 subunits is widespread, we also examined exon 5–mediated control of the pH sensitivity of heteromeric NMDA receptors. Coexpression of NR1 and NR2 (Table 1) revealed that exon 5 relieved pH inhibition for heteromeric receptors comprised of NR1 plus NR2A, NR2B, and NR2D but not NR2C (*15*). The effects of exon 5 were unrelated to agonist concentration (*16*), Ca^{2+} concentration (9), or potentiation (*11*) by 1 μM Zn^{2+} ($P < 0.1$; $n = 6$). Recombinant non-NMDA receptors were insensitive to pH (*17*).

How does exon 5 modulate the pH sensitivity of NMDA receptors? Exon 5 could perturb the NR1 tertiary structure and alter the partial charge distribution within the ionizable residues that may comprise the pH sensor. Alternatively, amino acids encoded by exon 5 might be close enough to the proton sensor to either directly shield or alter access to it.

Although evaluation of potentially subtle rearrangements requires currently unavailable structural data, there are three testable predictions of the shielding hypothesis. First, if exon 5 directly shields a surface proton sensor, it should not perturb the underlying secondary structure. Results from three different secondary structure prediction algorithms all suggest that the inclusion of exon 5 into NR1 expands a surface loop located near the splice site (Fig. 1A). Second, the Debye length describing the spatial effect of electric fields produced by individual ions is

Table 1. Oocytes were injected with NMDA receptor cRNA, and currents were recorded under voltage clamp in response to glutamate + glycine. The expression

$$\text{Response} = (\text{maximum} - \text{minimum})/\{1 + ([H^+]/IC_{50})^n\} + \text{minimum}$$

was fitted (least squares) to the averaged data. IC_{50} values are given in pH units and concentration to the nearest 5 nM (with the use of an activity coefficient of 0.8); n is the Hill slope. Measurements were made at 5 to 10 pH values from 235 oocytes. Exon 5 increased IC_{50} values in all cases ($P < 0.002$) (*27*). The H^+ sensitivity was identical in NR1 and NR1 + NR2 receptors ($P > 0.01$). Equivalent results were obtained for NR1 + NR2A or -B subunits coexpressed in HEK 293 cells ($n = 28$ cells). Heteromeric receptors that contained NR2C were insensitive to pH (*15*) regardless of the exon configuration of NR1 ($P < 0.2$; $n = 27$ oocytes).

Subunit	Agonist	NR1 − exon 5			NR1 + exon 5		
		IC_{50} (nM)	IC_{50} (pH)	Slope	IC_{50} (nM)	IC_{50} (pH)	Slope
NR1	Aspartate	65	7.3	1.0	220	6.8	1.9
NR1	NMDA	60	7.3	1.1	225	6.7	1.9
NR1	Glutamate	50	7.4	1.2	305	6.6	1.6
NR1 + 2A	Glutamate	80	7.2	1.5	270	6.7	2.3
NR1 + 2B	Glutamate	60	7.3	1.2	270	6.7	1.6
NR1 + 2D	Glutamate	65	7.3	1.0	285	6.6	1.6

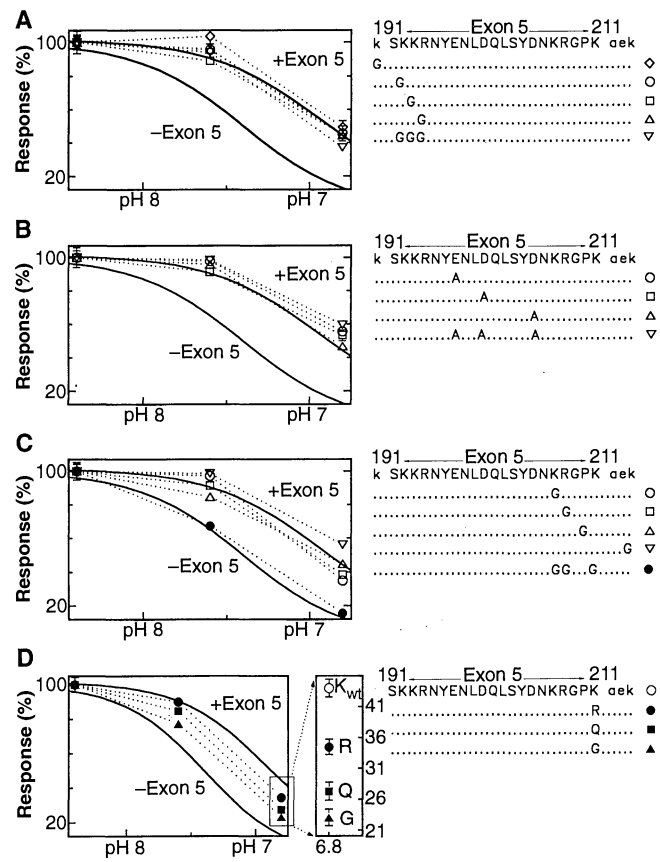

Fig. 2. Structural determinants of the control of proton sensitivity by exon 5 in NR1. (**A**) Site-directed mutagenesis (*19*) was used to convert the indicated codons (right) to glycine codons (*21*). We examined H^+ inhibition by recording homomeric receptor-mediated currents at pH 6.8, 7.6, and 8.4 (in Ba^{2+}). All responses are shown as a percent of that at pH 8.4 ($n = 49$ oocytes). These mutations had either no effect (K190G, K192G, K193G, and R194G; $P > 0.2$) or very slight effects (K192-K193-R194; $P < 0.02$) on pH sensitivity. Solid lines are fitted H^+ inhibition curves for wild-type responses ($n = 40$; see Table 1 for equation). (**B**) Mutant receptors were either identical in sensitivity to pH as wild-type receptors were (D200A and D205A; $P > 0.05$) or less sensitive (E197 and E197-D200-D205A; $P < 0.05$) (data from 42 oocytes). (**C**) Mutants P210G, K214G, K207G, and R208G had minimal effects on H^+ sensitivity ($P < 0.02$). By contrast, the mutant K207-R208-K211G completely restored pH sensitivity ($P < 0.001$). Data are from 56 oocytes. (**D**) The single mutation K211G largely restored pH sensitivity, and the rank order of effectiveness with which amino acids at this position relieved proton inhibition (K > R > Q > G; $P < 0.01$ for all) matched the expected proton exchange rates for side chain functional groups for these amino acids in free solution ($-NH_3^+ > -NHC(NH_2)_2^+ > -CONH_2 >> \alpha\text{-}CH_2$). Data at pH 6.8 are expanded ($n = 41$); the open symbol is the wild-type response.

S. F. Traynelis, Department of Pharmacology, Emory University, Atlanta, GA 30322, USA.
M. Hartley and S. F. Heinemann, Molecular Neurobiology Laboratory, Salk Institute, La Jolla, CA 92037, USA.
*To whom correspondence should be addressed.

inversely proportional to the square root of the ionic strength (I) of the solution. Consequently, alterations in I can be used to probe the degree of shielding at the proton site. If the relief of proton inhibition by NR1 exon 5 involves intraprotein shielding, then the exon 5–mediated increase in the IC_{50} value for protons should be greater when I is reduced and attenuated by larger values of I. Consistent with these predictions, we found that ionic strength was significantly correlated with the ability of exon 5 to relieve H^+ inhibition (*18*).

Third, if any of the charged amino acids within exon 5 act to shield the pH sensor, then replacement of the determinant amino acids by less electronegative residues should decrease this shielding. We used site-directed mutagenesis (*19*) to alter 12 amino acids. Only substitutions for Lys^{211} markedly reduced the effects of exon 5 on pH sensitivity (Fig. 2). For instance, exchanging three charged amino acids that included Lys^{211} completely restored the proton sensitivity of NR1 + exon 5 without altering fundamental receptor properties (*20*). Furthermore, the individual mutation K211G (*21*) restored most of the pH sensitivity to receptors containing exon 5, whereas more electronegative substitutions (K211Q and K211R) were less effective (Fig. 2D). These results are consistent with the idea that exon 5 increases the shielding of the proton sensor.

Given that the amino acids encoded by exon 5 are predicted to comprise a surface loop with structural similarities to polyamines, we wanted to know if this exon acts in a manner analogous to that of polyamines (*22, 23*). Two previous reports are consistent with this idea. First, only NMDA receptors that lack NR1 exon 5 (that is, only H^+-sensitive receptors) are potentiated by the endogenous polyamine spermine; second, polyamines increase the median effective concentration (EC_{50}) for glutamate to the same extent as the fifth exon itself (*23*). Because heteromeric receptors containing the NR2B subunit are far more sensitive to spermine than other NR2-containing receptors (*24*), we tested the effect of polyamines on proton inhibition using this receptor combination. Furthermore, although spermine inhibits native NMDA receptors in a voltage-dependent manner (*22*), NR1-NR2B receptors exhibited no inhibition at −40 mV by 1 mM spermine (*24*). If spermine relieves the tonic inhibition of NMDA receptors by protons at physiological pH, then (i) spermine should increase the IC_{50} values for proton inhibition only for receptors lacking exon 5, (ii) spermine potentiation should be greater at reduced pH, and (iii) spermine potentiation should be partially restored by mutations that inactivate exon 5.

Figure 3 presents results that confirm these predictions. Submaximal concentrations of spermine shifted the proton inhibition curve to the right only for receptors that contained the proton-sensitive NR1 −exon 5 subunit (Fig. 3, A and B). Furthermore, the proton sensitivity of such receptors was abolished by extracellular spermine with an EC_{50} value (163 μM) (Fig. 3C) that is similar to the value reported for spermine potentiation of native neuronal receptors (*22, 23*). Spermine potentiation is greater for receptors inhibited by low pH (Fig. 3D), and mutations that restore proton sensitivity to exon 5–containing receptors also restore their sensitivity to spermine (Fig. 3E). Mutations that fully restore proton inhibition do not restore the level of spermine potentiation to that of the wild-type receptor, which is consistent with the idea that the actions of spermine (but not of highly mobile H^+) might be hindered by the inactivated fifth exon in a complex manner (Fig. 3, D through F) that involves more than direct competition.

Our results—that proton inhibition of the NMDA receptor is determined by the presence or absence of exon 5 in the NR1 subunit and that the relief of proton inhibition by exon 5 explains potentiation by extracellular polyamines—have several implications. For example, the mechanism of polyamine potentiation of NMDA receptors that lack exon 5 (*23*) is simply the relief of tonic inhibition present at physiological pH. In addition, the attenuation of proton inhibition by either polyamines or inclusion of NR1 exon 5 enhances neuronal excitability and also reduces the negative feedback of extracellular acidification secondary to seizure or stroke on NMDA receptor activation (*5, 7, 8*). It follows that brain regions that express exon 5 (that is, the brainstem, thalamus, cerebellum, colliculi, hippocampal CA3, pontine nucleus, sensorimotor cortex, and subthalamic nucleus) (*25*) may be uniquely vulnerable to

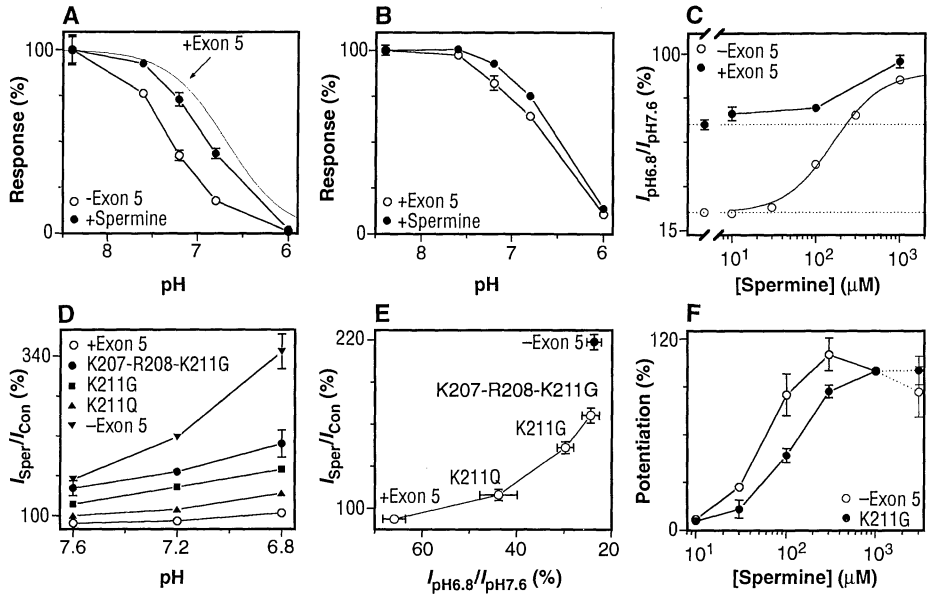

Fig. 3. Relief of tonic proton inhibition by polyamines. (**A**) Composite pH inhibition curves are shown for (NR1 − exon5)NR2B receptors in the absence and presence of 100 μM spermine. This submaximal concentration of spermine increases the IC_{50} value 2.5-fold ($P < 0.001$; $n = 53$ oocytes). The thin line indicates the inhibition curve for (NR1 + exon 5)NR2B. For all panels, experiments were performed at −40 mV in Ba^{2+} with 20 to 50 μM glutamate plus 5 μM glycine. (**B**) Spermine (100 μM) has minimal effects ($P < 0.05$) on the H^+ sensitivity of (NR1 + exon 5)NR2B ($n = 44$). (**C**) Spermine relieves H^+ inhibition of (NR1 − exon 5)NR2B receptors with an EC_{50} value of 163 μM (Hill slope = 1.5); 1 mM spermine also relieves the slight H^+inhibition of (NR1 + exon 5)NR2B receptors ($n = 66$ oocytes). (**D**) Potentiation of current responses by 100 μM spermine varies with pH for (NR1 − exon 5)NR2B and (NR1 + exon 5)NR2B receptors harboring mutations that restore H^+ sensitivity ($n = 71$). Only NR1 + exon 5 receptors that contain substitutions for Lys^{211} are potentiated by 100 μM spermine at pH 7.2 to pH 7.6 ($n = 205$ oocytes; not shown in figure) (*27*), although K190G also had a slight effect ($P < 0.05$; $n = 7$). (**E**) There is a strong correlation ($r = 0.95$; $P < 0.02$) between the restoration of pH inhibition and spermine potentiation at pH 7.2 in (NR1 + exon 5)NR2B. Each point represents data from 10 to 35 oocytes (total = 108). (**F**) Spermine dose-response curves are shown for the relief of H^+ inhibition at pH 6.8 for wild-type (NR1 − exon 5)NR2B (open symbols) and [NR1 + exon 5(K211G)]NR2B (filled symbols); relief of H^+ inhibition by concentrations below 1 mM (solid lines) is insensitive to voltage (*24*). Spermine potentiation of glutamate-induced currents is expressed as $(I_{Sper} - I_{Con})/(I_{Max} - I_{Con}) \times 100$, where I_{Con} is the current in the absence of spermine, I_{Max} is the current in the presence of 1 mM Spermine, and I_{Sper} is the test current. The concentration-effect curve for K207-R208-K211G ($n = 5$; not shown in figure) was similar (*28*) to that for the wild-type receptor + exon 5. EC_{50} values were not determined given the potential receptor block by spermine at concentrations greater than 1 mM. Data are from 30 oocytes.

glutamate-induced neuropathologies (*2*, *3*). Finally, our data raise the intriguing possibility that the predicted surface loop encoded by exon 5 acts as a tethered modulator of receptor function (*26*), binding near the polyamine recognition site (*14*) to constitutively potentiate receptor function. If this hypothesis proves to be correct, then compounds that competitively antagonize polyamine potentiation of NMDA receptors may restore tonic pH inhibition by interfering with the association of exon 5 with its binding site.

REFERENCES AND NOTES

1. M. Hollmann and S. F. Heinemann, *Annu. Rev. Neurosci.* **17**, 31 (1994); C. J. McBain and M. L. Mayer, *Physiol. Rev.* **74**, 723 (1994).
2. R. Dingledine, C. J. McBain, J. O. McNamara, *Trends Pharmacol. Sci.* **11**, 334 (1990); B. S. Meldrum, *Epilepsy Res.* **12**, 189 (1992).
3. D. W. Choi, *J. Neurosci.* **10**, 2493 (1990); *J. Neurobiol.* **23**, 1261 (1992).
4. R. G. Giffard, H. Monyer, C. W. Christine, D. W. Choi, *Brain Res.* **506**, 339 (1990); C.-M. Tang, M. Dichter, M. Morad, *Proc. Natl. Acad. Sci. U.S.A.* **87**, 6445 (1990); L. Vyklicky, V. Vlachova, J. Krusek, *J. Physiol. (London)* **430**, 497 (1990).
5. S. F. Traynelis and S. G. Cull-Candy, *Nature* **345**, 347 (1990); *J. Physiol. (London)* **433**, 727 (1991).
6. M. Chesler, *Prog. Neurobiol.* **34**, 401 (1990); ——— and K. Kaila, *Trends Neurosci.* **15**, 396 (1992).
7. B. K. Siesjö, *Prog. Brain Res.* **63**, 121 (1985); M. Balestrino and G. G.Somjen, *J. Physiol. (London)* **396**, 247 (1988); T. P. Obrenovitch *et al.*, *J. Neurophysiol.* **64**, 1125 (1990).
8. G. C. Tombaugh and R. M. Sapolsky, *Brain Res.* **506**, 343 (1990); *J. Neurochem.* **61**, 793 (1993); D. A. Kaku, R. G. Giffard, D. W. Choi, *Science* **260**, 1516 (1993).
9. *Xenopus* oocytes were prepared and injected with complementary RNA (cRNA) as described (*11*). Because of the many designations for NR1 splice variants (*1*), we refer explicitly to the configuration of exon 5. Our prototypical constructs correspond to splicing variants NR1-1a and NR1-1b (*11*), which include exons 21 and 22. Recording solutions contained 90 mM NaCl, 3 mM KCl, 1.5 mM $CaCl_2$ or $BaCl_2$, and 10 mM Hepes. Ionic strength at pH 7.55 (I) was 103 mM with the use of the net charge on Hepes [P. W. Atkins, *Physical Chemistry* (Freeman, San Francisco, 1982)]. The internal solution was 0.3 or 3 M KCl. Voltage-clamp recordings (*11*) were digitized (1 to 5 Hz), and sustained current amplitudes were measured off-line. Unless stated otherwise, responses are to 20 to 50 μM glutamate + 5 μM glycine (in Ca^{2+}) applied for 1 to 3 min at 3- to 5-min intervals after pre-wash at the new pH; responses are expressed as a percentage of the mean of the control and recovery. Uninjected or water-injected oocytes did not respond to glutamate, NMDA, aspartate, kainate, or α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) (pH 5.6 to 9.2; $P > 0.1$; $n = 80$). NMDA receptors were insensitive to Hepes (2 to 50 mM; $P < 0.6$; $n = 5$). The actions of NR1 + exon 5 were independent of Ca^{2+} concentration (0.5 to 2.5 mM; $P > 0.5$; $n = 29$) and internal BAPTA (1 nmol; $P > 0.05$; $n = 10$); exon 5 was slightly less effective in Ba^{2+} ($P < 0.01$; $n = 201$). Current-voltage (I-V) relationships were obtained from 1- to 4-s voltage ramps (+ or − slope) digitized at 0.5 to 2 kHz. Complementary DNAs correspond to GenBank accession numbers U08261, U08263, U08262, U08264, U08265, U08266, U08267, U08268, U11418, D13211, U11419, D13212, U08260, X17184, M85035, M85036, and S94371.
10. HEK 293 cells (American Type Culture Collection CRL 1573) were transfected with 1 to 4 μg/ml of NR1-NR2 cDNA (ratio = 1:2) [C. Chen and H. Okayama, *Mol. Cell. Biol.* **7**, 2745 (1987)] and maintained as described [M. Cik, P. L. Chazot, F. A. Stephenson, *Biochem. J.* **296**, 877 (1993)]. The recording solution contained 140 mM NaCl, 3 mM KCl, 1.5 mM $CaCl_2$, 10 mM Hepes, and 15 to 20 mM mannitol or sucrose; I = 153 mM at pH 7.55. The electrode solution was 30 mM CsCl, 100 mM CsF, 5 mM Hepes, 5 mM EGTA, and 0.5 mM $CaCl_2$ at pH 7.3. Whole-cell current responses were recorded with a patch-clamp amplifier and digitized (0.2 to 1 kHz) for off-line analysis.
11. K. Moriyoshi *et al.*, *Nature* **354**, 31 (1991); V. Anantharam *et al.*, *FEBS Lett.* **305**, 27 (1992); N. Nakanishi, R. Axel, N. A. Shneider, *Proc. Natl. Acad. Sci. U.S.A.* **89**, 8552 (1992); G. M. Durand *et al.*, *ibid.*, p. 9359; H. Sugihara *et al.*, *Biochem. Biophys. Res. Commun.* **185**, 826 (1992); M. Hollmann *et al.*, *Neuron* **10**, 943 (1993).
12. P. Y. Chou and G. D. Fasman, *Adv. Enzymol.* **47**, 45 (1978); J. Garnier, D. J. Osguthorpe, B. Robson, *J. Mol. Biol.* **120**, 97 (1978); E. A. Emini, J. V. Hughes, D. S. Perlow, J. Boger, *J. Virol.* **55**, 836 (1985); P. A. Karplus and G. E. Schulz, *Naturwissenshaften* **72**, 212 (1985); B. Rost and C. Sander, *J. Mol. Biol.* **232**, 584 (1993).
13. Fitted H^+ IC_{50} values for 3′ splice variants were from pH 7.2 to pH 7.3 for NR1 − exon 5 (NR1-1a, -2a, -3a, and -4a), and from pH 6.6 to pH 6.7 for NR1 + exon 5 (NR1-1b, -2b, -3b, and -4b; $P > 0.4$; $n = 129$ oocytes). The Hill slopes were 1.2 to 1.8.
14. H. Monyer *et al.*, *Science* **256**, 1217 (1992); T. Ishii *et al.*, *J. Biol. Chem.* **268**, 2836 (1993); J. M. Sullivan *et al.*, *Neuron* **13**, 929 (1994).
15. All receptors that contained NR2C had IC_{50} values near pH 6.2 (minimum fixed at 0%). The H^+ inhibition was insensitive to voltage ($P > 0.1$) (*27*), Ca^{2+} concentration, divalent ion (Ca^{2+} or Ba^{2+}), or agonist structure and concentration ($P > 0.4$). The EC_{50} values for glutamate (0.6 μM) and glycine (0.7 μM) were insensitive to pH. Data are from 155 oocytes.
16. The equation

$$\text{Response} = (\text{maximum} - \text{minimum})/\{1 + [EC_{50}/(\text{concentration} + Z)]^n\} + \text{minimum}$$

was fitted (least squares) to the dose-response curves, where n is the Hill slope and Z is the contaminant agonist concentration; for glutamate, $Z = 0$, and for glycine

$$Z = EC_{50}[(Z_R - \text{minimum})/(\text{maximum} - Z_R)]^{1/n}$$

where Z_R was the measured response to nominally glycine-free glutamate. The EC_{50} value for glutamate was 1.5 or 1.2 μM (pH 7.6 versus pH 6.6) for receptors composed of the NR1 − exon 5 and NR2B subunits [(NR1 − exon 5)NR2B], and 2.3 or 2.6 μM (+exon 5; pH 7.6 versus pH 6.6). The EC_{50} value for glycine was 230 or 350 μM (−exon 5; pH 7.6 versus pH 6.6), and 310 or 260 μM (+exon 5; pH 7.6 versus pH 6.6). Hill slopes were 1.3 to 1.7 ($n = 76$).
17. As in neurons (*5*) and cone cells [B. N. Christensen and E. Hida, *Neuron* **5**, 471 (1990)], GluR1 to GluR4 [flop; B. Sommer *et al.*, *Science* **249**, 1580 (1990)] were inhibited at nonphysiological pH in a voltage-independent fashion ($P > 0.5$). Mean IC_{50} values for kainate and AMPA were pH 6.3 for GluR1, pH 6.4 for GluR2 (R607Q) (*21*), pH 6.2 for GluR3, and pH 6.0 for GluR4c. Hill slopes were 0.7 to 1.5. Data are from 138 oocytes.
18. Ionic strength (I) was altered by addition or removal of NaCl, KCl, and Hepes {pH was corrected for junction potential errors [R. G. Bates, C. A. Vega, D. R. White, *Anal.Chem.* **50**, 1295 (1978)]}; sucrose was added to hyposomotic solutions. The H^+ IC_{50} value in Ba^{2+} for (NR1 ± exon 5)NR2B differed by 1.8-fold for I = 201 mM ($P < 0.05$), 3.2-fold for I = 103 mM ($P < 0.001$), and 5.5-fold for I = 37 mM ($P < 0.001$; $n = 76$ oocytes; $r = -0.97$).
19. S. Ho *et al.*, *Gene* **77**, 51 (1989). Amino acids are numbered so that the initiating methionine is 1.
20. The mutation K207-R208-K211G in NR1 + exon 5 did not alter the shape or the reversal potential (V_{rev}) of the I-V relationship for glutamate responses in Ba^{2+} ($P < 0.5$; $n = 15$ oocytes). Mutant receptors were sensitive to 200 μM Mg^{2+} ($n = 11$) and were potentiated by dithiothreitol (152 ± 6%; $n = 4$) and glycine (EC_{50} = 240 nM; Hill slope = 1.4; $n = 5$). However, the glutamate EC_{50} value for these mutant receptors was reduced, reminiscent of the EC_{50} value for NR1 − exon 5 ($n = 10$) (*11*). Whereas 8 of 17 exon 5 mutations slightly ($P < 0.05$; $n = 156$) altered the 117% potentiation of homomeric wild-type NR1 + exon 5 receptors (*11*) by 1 μM Zn^{2+}, only mutations at K211 restored Zn^{2+} potentiation to the levels ($P < 0.2$) observed in NR1 − exon 5 (168%; $n = 56$) (*28*).
21. Abbreviations for the amino acid residues are as follows: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr. Mutations are indicated with the single-letter code; thus, $Lys^{211} \rightarrow$ Gly is indicated by K211G.
22. Polyamines cause a rapid potentiation of function (the subject of this report), a glycine-dependent potentiation of receptor response, and a voltage-dependent block of receptor function [R. W. Ransom and N. L. Stec, *J. Neurochem.* **51**, 830 (1988); I. J. Reynolds, *J. Pharmacol. Exp. Ther.* **255**, 1001 (1990); K. Williams *et al.*, *Neuron* **5**, 199 (1990); D. M. Rock and R. L. MacDonald, *Mol. Pharmacol.* **41**, 83 (1992); M. Benveniste and M. L. Mayer, *J. Physiol. (London)* **464**, 131 (1993)].
23. G. M. Durand, M. V. L. Bennett, R. S. Zukin, *Proc. Natl. Acad. Sci. U.S.A.* **90**, 6731 (1993); R. C. Araneda, R. S. Zukin, M. V. L. Bennett, *Neurosci. Lett.* **152**, 107 (1993); K. Williams, *Mol. Pharmacol.* **46**, 161 (1994); L. Zhang *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **91**, 10883 (1994).
24. Spermine (100 μM; in Ba^{2+}) did not alter the responses of NR1 − exon 5 coexpressed with NR2A, -C, and -D (pH 7.2; −40 mV; $P > 0.05$; $n = 28$ oocytes), in contrast to potentiation of NR1 − exon 5 and (NR1 − exon 5)NR2B (194 ± 8% and 219 ± 5%, respectively; $n = 14$) (*23*). These and other data (*14*) suggest that the action of spermine on the NMDA receptor is influenced by other protein domains. Spermine had minimal effects on NR1 + exon 5 alone or when it was coexpressed with NR2A, -B, -C, or -D ($n = 34$). Potentiation of (NR1 − exon 5)NR2B currents by 300 to 1000 μM spermine was independent of voltage between −40 and +20 mV (that is, when V_{rev} ± 30 mV; $P > 0.05$; $n = 10$) (*27*). Spermine alone evoked no current ($n = 5$); reported pK_4 values (where K_4 is the fourth acid constant) are 8.0 to 8.9 [S. Z. Hirschman, M. Leng, G. Felsenfeld, *Biopolymers* **5**, 227 (1967); D. M. Templeton and B. Sarkar, *Can. J. Chem.* **63**, 3122 (1985)]. $Spermine^{3+}$ is 3.5% (pH 6.8) and 18.5% (pH 7.6) of the total for pK_4 = 8.3; 0.1 mM spermine increases the ionic strength by 1% (pH 7.2).
25. D. G. Standaert, C. M. Testa, J. B. Penney, A. B. Young, *Neurosci. Lett.* **152**, 161 (1993); D. J. Laurie and P. H. Seeburg, *J. Neurosci.* **14**, 3180 (1994).
26. T. Hoshi, W. N. Zagotta, R. W. Aldrich, *Science* **250**, 533 (1990).
27. The mean IC_{50} value for H^+ inhibition or the ratio of agonist-induced current at pH 6.8 to pH 7.6 was used to compare NMDA receptor pH sensitivity. Changes in the I-V relationships for agonist-induced currents were determined from the shape of the curve, the interpolated (regression) or fitted (polynomial) reversal potential (V_{rev}), and the ratio of current at V_{rev} − 30 mV to the current at V_{rev} + 30 mV. Data were compared with the use of paired or unpaired t tests or analysis of variance; values are the mean ± SEM. Sample sizes gave a minimum detectable difference of <10% for $\alpha = 0.05$ and a power ($1 - \beta$) of 0.75. We rejected the null hypothesis at $P < 0.05$.
28. X. Zheng, L. Zhang, G. M. Durand, M. V. L. Bennett, R. S. Zukin, *Neuron* **12**, 1 (1994).

29. Supported by the American Epilepsy Society and the Milken Family Medical Foundation (S.F.T.), NIH grants NS08549 (S.F.T.) and NS28709 (S.F.H.), and the McKnight Foundation (S.F.H.). We thank R. Allen, B. Bettler, J. Boulter, A. Buonanno, J. Egebjerg, M. Hollmann, E. Liman, S. Nakanishi, R. Papke, and J. Sullivan for sharing advice and cDNA clones or vectors. We are also grateful to J. Boulter and R. Dingledine for comments on the manuscript, C. Stevens for providing laboratory space, B. Rost and C. Sander for structure prediction algorithms, and C. Boyer, J. Kiefer, C. McGraw, J. Nolan, and L. O'Leary for technical assistance.

10 November 1994; accepted 10 February 1995