0026-895X/94/020324-06$03.00/0

MOLECULAR PHARMACOLOGY, 45:324-329

Differential Ethanol Sensitivity of Recombinant *N*-Methyl-D-aspartate Receptor Subunits

KHALID MASOOD,[1] CHIPING WU,[1] ULRIKE BRAUNEIS,[1] and FORREST F. WEIGHT

Laboratory of Molecular and Cellular Neurobiology, National Institute on Alcohol Abuse and Alcoholism, National Institutes of Health, Bethesda, Maryland 20892

Received September 1, 1993; Accepted November 29, 1993

SUMMARY

The recombinant *N*-methyl-D-aspartate (NMDA) receptor subunit ζ1 and the heteromeric subunit combinations ε1/ζ1, ε2/ζ1, and ε3/ζ1 were expressed in *Xenopus* oocytes and their sensitivities to ethanol were investigated using the two-electrode voltage-clamp technique. NMDA-activated currents in oocytes expressing subunit combinations ε1/ζ1 or ε2/ζ1 were significantly inhibited by 50 mM ethanol, whereas NMDA-activated currents associated with the homomeric expression of ζ1 or the heteromeric ε3/ζ1 combination were not significantly affected by 50 mM ethanol. Ethanol decreased the maximal amplitude (E_{max}) of the concentration-response curve for NMDA-activated current, without significantly affecting the EC_{50}. The values of percentage inhibition by ethanol were not significantly different, regardless of the amplitude of current activated by NMDA concentrations from 10 to 250 μM. Different NMDA receptor subunits and subunit combinations exhibited differences in the concentration-response curves for ethanol. NMDA-activated current associated with the ε1/ζ1 subunit combination was increasingly inhibited by increasing concentrations of ethanol from 25 to 100 mM, whereas 25 mM ethanol elicited nearly maximal inhibition of NMDA-activated current associated with the ε2/ζ1 subunits, i.e., the inhibition by 50 or 100 mM ethanol was not significantly different. NMDA-activated current associated with the ε3/ζ1 subunit combination, on the other hand, was significantly inhibited only by 100 mM ethanol, and NMDA-activated current associated with the homomeric ζ1 subunit was not significantly affected by ethanol concentrations of ≤100 mM. Because NMDA receptor subunits are differentially distributed throughout the brain, the observations suggest that the differential sensitivity of NMDA receptor subunits to ethanol may contribute to the differences in ethanol sensitivity observed in different types of neurons.

Glutamate is now recognized as the major excitatory neurotransmitter in the mammalian central nervous system. Studies on the neuronal actions of glutamate have revealed that it can activate at least three types of ligand-gated ion channels, designated NMDA, kainate, and AMPA receptors on the basis of agonists that activate them (1–3). There has been considerable recent interest in the NMDA receptor because of its possible roles in important physiological and pathophysiological aspects of nervous system function. NMDA receptors have been implicated in certain types of synaptic plasticity, such as long term potentiation (4), long term depression (5), neuronal development (6), developmental plasticity (7), and learning (8, 9). In addition, NMDA receptor-mediated neurotoxicity is thought to play a role in neural damage associated with conditions such as ischemia, hypoglycemia, and epilepsy (10, 11). The function of NMDA receptors has also been found to be affected by several psychoactive drugs, such as ketamine, phencyclidine, and ethanol, possibly contributing to the behavioral effects of these drugs (12, 13).

cDNAs encoding rat (NMDAR1) and mouse (ζ1) NMDA receptor subunits have been cloned, and the physiological and pharmacological properties of the expressed receptors have been described (14, 15). Further cloning of other subunits from mice (ε) and rats (NMDAR2) and of splice variants of NMDAR1 has revealed a molecular diversity of NMDA receptors (16–22). The ζ1 (NMDAR1) subunit is widely distributed throughout the brain and can constitute functional homomeric NMDA receptor channels (14, 15). The ε (NMDAR2) subunits exhibit differential localization in different parts of the brain and must be combined in heteromeric configurations with ζ1 for expression (16–20). These heteromeric configurations show diversity in the physiological and pharmacological properties of the expressed NMDA receptors, suggesting that differences in the molecular structure of the ε (NMDAR2) subunit family contribute to the functional heterogeneity of NMDA receptors. Ethanol, in concentrations associated with intoxication in humans (5–50 mM), has been found to inhibit neuronal responses to NMDA in a variety of preparations; however, the sensitivity to ethanol appears to vary in different types of neurons (13). These observations raised the question of whether differences

U.B. is a National Research Council Senior Associate.
[1] These authors contributed equally.

ABBREVIATIONS: NMDA, *N*-methyl-D-aspartate; AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate; ACh, acetylcholine; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; DRG, dorsal root ganglia; ANOVA, analysis of variance.

in NMDA receptor subunit composition might be responsible for differences in NMDA receptor sensitivity to ethanol in different types of neurons. To address this question, we used electrophysiological techniques to investigate the effect of ethanol on NMDA-activated current, using recombinant homomeric ζ1 and heteromeric ε1/ζ1, ε2/ζ1, and ε3/ζ1 NMDA receptors, and we report here a differential sensitivity to ethanol with different subunit combinations.

Materials and Methods

Synthesis of mRNAs. NMDA receptor cDNA clones pBKSAζ1, pBKSAε1, pBKSAε2, and pBKSAε3 were kindly provided by Dr. Masayoshi Mishina, Niigata University (Niigata, Japan). Subunit-specific mRNAs were prepared by *in vitro* transcription with T3 RNA polymerase in the presence of cap dinucleotide ^{7}meGpppG, using *Not*I-linearized pBKSAζ1, pBKSAε1, and pBKSAε2 and *Xba*I-linearized pBKSAε3 as templates. Briefly, 5 μg of plasmid DNA were linearized by either *Not*I or *Xba*I, in a total volume of 50 μl, for 1–2 hr at 37°, extracted with phenol/chloroform, precipitated with ethanol, dried, and suspended in an appropriate volume of RNase-free distilled water. One microgram of template was used for *in vitro* capping reactions, which consisted of 40 mM Tris, pH 7.5, 50 mM NaCl, 8 mM $MgCl_2$, 2 mM spermidine, 0.4 mM concentrations of ribonucleoside triphosphates except rGTP (0.125 mM), 0.3 mM dithiothreitol, 0.3 mM cap analogue ^{7}meGpppG, 1 unit of RNA guard (Pharmacia, Piscataway, NJ), and 10 units of T3 RNA polymerase, in a total volume of 25 μl. The reaction was allowed to proceed for 1 hr at 37° and DNA template was digested by addition of 10 units of DNase I for 15 min. The reaction was stopped by addition of 100 μl of RNase-free distilled water and the phenol/chloroform extraction procedure was repeated, as described above. *In vitro* synthesized capped mRNA transcripts were analyzed on 1.2% formaldehyde-agarose gels, to check the size and quality of the transcripts.

Oocyte preparation and microinjection. Mature female *Xenopus laevis* frogs (Xenopus I, Ann Arbor, MI) were housed in dechlorinated tap water at 19–21°, with 12/12-hr light/dark lighting, and were fed with beef liver at least twice each week. A small piece of ovary was surgically excised from frogs anesthetized with 0.15% tricaine (Sigma Chemical Co., St. Louis, MO) and was manually dissected into thin strips in a solution containing 88 mM NaCl, 1 mM KCl, 2.4 mM $NaHCO_3$, 0.8 mM $MgSO_4$, and 10 mM HEPES, pH 7.5. The follicular cell layer of the oocytes was removed by treatment for 2 hr with 0.2% collagenase A (Boehringer Mannheim, Indianopolis, IN), in a solution containing 83 mM NaCl, 2 mM KCl, 2.1 mM $MgCl_2$, and 5 mM HEPES, pH 7.5. Stage V and VI oocytes were selected from the denuded oocytes and transferred to modified Barth's saline solution, containing 88 mM NaCl, 1 mM KCl, 2.4 mM $NaHCO_3$, 0.3 mM $Ca(NO_3)_2$, 0.9 mM $CaCl_2$, 0.8 mM $MgSO_4$, and 10 mM HEPES, pH 7.5, to await mRNA injection. The prepared oocytes were injected with 16–20 ng of mRNA/oocyte, using a micropipette with a 10-μm tip and a Pneumatic Picopump PV 800 microinjection pump (World Precision Instruments, New Haven, CT). Subunit-specific mRNAs were injected either homomerically (ζ1) or in equimolar combinations, as described in the Results Section. The oocytes were incubated for 2–3 days at 19–21° in modified Barth's saline solution supplemented with 2 mM sodium pyruvate, 10,000 units/liter penicillin, 10 mg/liter streptomycin, 50 mg/liter gentamycin, and 0.5 mM theophylline.

Electrophysiological recording. After incubation, the oocytes were placed in a recording chamber (volume, ~100 μl) and superfused constantly at the rate of ~2.5 ml/min. The bathing solution consisted of 95 mM NaCl, 2 mM KCl, 2 mM $CaCl_2$ or 2 mM $BaCl_2$, and 5 mM HEPES, pH 7.5. The cells were impaled at the animal pole with two microelectrodes filled with a 3 M KCl solution (1–10 MΩ) and were voltage-clamped at a holding potential of −70 mV using an Axoclamp IIA amplifier (Axon Instruments Inc., Burligame, CA). Membrane ion currents were measured using the standard two-electrode voltage-clamp method and were recorded with a Gould 2400s rectilinear pen recorder (Gould Inc., Cleveland, OH). The experiments were performed at room temperature. Agonists and other drugs were dissolved in the bathing solution and applied from a macropipette (external diameter, ~1.0 mm) placed within 1 mm of the cell under study. These macropipettes were connected to reservoirs containing appropriate solutions placed above the preparation, which permitted superfusion of the cells by gravity-induced flow of solution; the superfusion rate for applying agonist and/or drugs was ~2.5 ml/min. Usually agonist and/or drugs were superfused for 20–30 sec, with a period of 5–10 min between applications, depending upon the time required for full recovery from the action of each substance. All solutions containing NMDA also contained 10 μM glycine.

Results

Fig. 1 illustrates the effects of ethanol on NMDA-activated currents in *Xenopus* oocytes expressing recombinant NMDA receptors. The records in Fig. 1, *left*, show control currents activated by the application of 100 μM NMDA. The current activated by NMDA was considerably larger in oocytes expressing heteromeric ε1/ζ1, ε2/ζ1, and ε3/ζ1 subunit combinations, compared with oocytes expressing the homomeric ζ1 subunit,

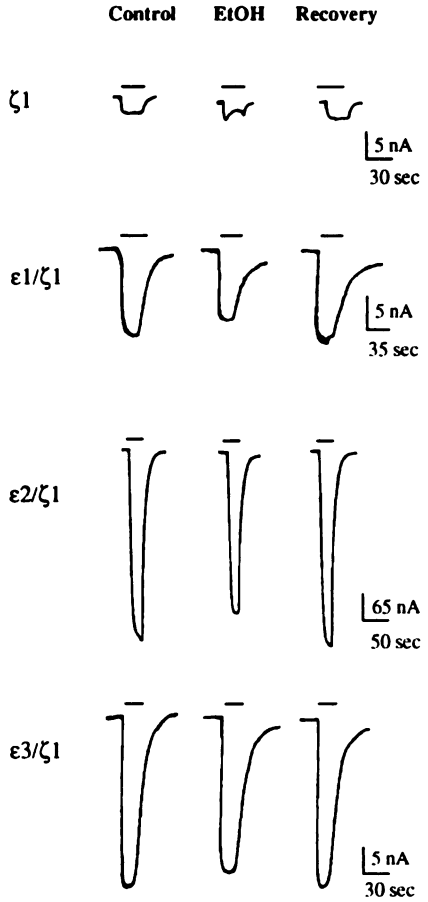

Fig. 1. Ethanol (*EtOH*) effect on NMDA-activated currents in *Xenopus* oocytes expressing different NMDA receptor subunits. Each *horizontal set of traces* shows a sequential series of current records, illustrating the effect of 50 mM ethanol on currents activated by the application of 100 μM NMDA in oocytes expressing the NMDA receptor subunits indicated. *Bar above each record*, time period of NMDA or NMDA plus ethanol application. The currents were recorded in an extracellular bathing solution containing 2 mM Ba^{2+}; similar results were obtained in extracellular bathing solutions containing 2 mM Ca^{2+}. All solutions containing NMDA also contained 10 μM glycine. Calibrations apply to each set of traces.

as reported previously (16, 17, 20). The records in Fig. 1, *middle*, show that 50 mM ethanol reduced the amplitude of NMDA-activated current to a greater extent in oocytes expressing $\epsilon 1/\zeta 1$ and $\epsilon 2/\zeta 1$ subunit combinations than in oocytes expressing $\zeta 1$ and $\epsilon 3/\zeta 1$ subunit combinations. On average, 50 mM ethanol reduced the amplitude of current activated by 100 μM NMDA by 31 and 26% for $\epsilon 1/\zeta 1$ and $\epsilon 2/\zeta 1$ subunit combinations, respectively; these inhibitions are statistically significant (ANOVA, $p < 0.001$). On the other hand, 50 mM ethanol did not significantly affect the amplitude of current activated by 100 μM NMDA for the $\epsilon 3/\zeta 1$ subunit combination or the homomeric $\zeta 1$ subunit (ANOVA, $p > 0.05$). In some oocytes, NMDA-activated current exhibited a slow increase in amplitude; this phenomenon was not observed in a Ca^{2+}-free extracellular bathing solution containing 20 mM Ba^{2+}, suggesting that it is a Ca^{2+}-dependent current (data not shown). The records in Fig. 1, *right*, show that, after ethanol was washed out for several minutes, the ethanol-induced inhibition of NMDA-activated current recovered fully in *Xenopus* oocytes, as reported previously for neurons (23, 24).

Fig. 2 illustrates the relationship between the amplitude of current activated by NMDA and the inhibition by ethanol for the $\epsilon 1/\zeta 1$ subunit combination. The graph in Fig. 2 plots concentration-response curves for NMDA-activated current in the absence and presence of 100 mM ethanol. With NMDA concentrations from 10 to 250 μM, NMDA-activated current amplitude increased in a concentration-dependent manner. The curves shown are the best fit of the data to the logistic equation $y = E_{max}/[1 + (x/EC_{50})^{-n}]$. The apparent Hill coefficient for the NMDA concentration-response curve was 1.6 and the EC_{50} was 57 μM. At NMDA concentrations of $\geq$500 μM, the amplitude of NMDA-activated current was attenuated by the rapid onset of desensitization (data not shown). In the presence of ethanol, the maximal amplitude of the NMDA concentration-response curve (E_{max}) was significantly reduced to 63% (ANOVA, $p < 0.001$), but there was no significant change in the EC_{50} or the apparent Hill coefficient (ANOVA, $p > 0.05$). In addition, the values of percentage inhibition by ethanol were not significantly different for currents activated by NMDA concentrations from 10 to 250 μM (ANOVA, $p > 0.05$).

Fig. 3 shows a concentration-response curve for ethanol inhibition of NMDA-activated current for the $\epsilon 2/\zeta 1$ subunit combination. Each data point represents the average percentage inhibition of NMDA-activated current by different ethanol concentrations, as indicated. Ethanol concentrations of 5, 10, 25, 50, and 100 mM inhibited the NMDA-activated current by 3, 9, 23, 26, and 28%, respectively. The numbers indicate that increasing concentrations of ethanol produced increasing inhibition of NMDA-activated current; however, the effects of 5 and 10 mM ethanol were not statistically significant (ANOVA, $p > 0.05$) and the effects of 25, 50, and 100 mM ethanol were not significantly different (ANOVA, $p > 0.05$). NMDA-activated currents in oocytes expressing the $\epsilon 2/\zeta 1$ subunit combination also exhibited a range of different sensitivities to ethanol. Thus, although in most oocytes 25 mM ethanol inhibited NMDA-activated current by 20–30%, 25 mM ethanol inhibited NMDA-activated current in some oocytes by as much as 60% and in other oocytes by as little as 10%. Such differences in ethanol sensitivity have been observed with the same batch of mRNA and with oocytes from the same frog, suggesting that they may involve some type of post-translational processing or perhaps differences in the number of $\epsilon 2$ and $\zeta 1$ subunits comprising the expressed receptor. A wide range of different sensitivities of NMDA-activated current to ethanol was not observed for the other NMDA subunits or subunit combinations tested.

Fig. 4 compares concentration-response curves for ethanol effects on NMDA-activated currents in oocytes expressing dif-

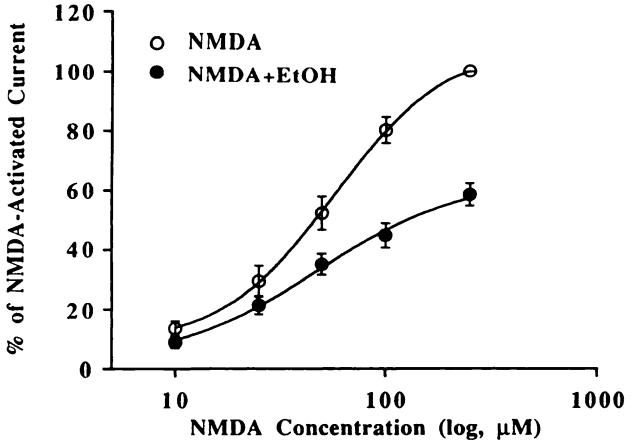

Fig. 2. Relationship between the amplitude of current activated by NMDA and the inhibition by ethanol (*EtOH*). For the $\epsilon 1/\zeta 1$ subunit combination the graph plots concentration-response curves for NMDA-activated current in the absence (○) and presence (●) of 100 mM ethanol. *Data points*, NMDA-activated current amplitude (mean ± standard error; $n = 6$) for the NMDA concentrations indicated. Ethanol reduced E_{max} for the NMDA concentration-response curve (ANOVA, $p < 0.001$) but did not significantly affect the EC_{50} (ANOVA, $p > 0.05$). The percentage inhibition by 100 mM ethanol of current activated by 10, 25, 50, 100, and 250 μM NMDA was 34, 28, 33, 44, and 41%, respectively; these values are not significantly different (ANOVA, $p > 0.05$). The currents were recorded in an extracellular bathing solution containing 2 mM Ca^{2+}; similar results were obtained in an extracellular bathing solution containing 2 mM Ba^{2+}. All solutions containing NMDA also contained 10 μM glycine. Error bars not visible are smaller than the size of the symbols.

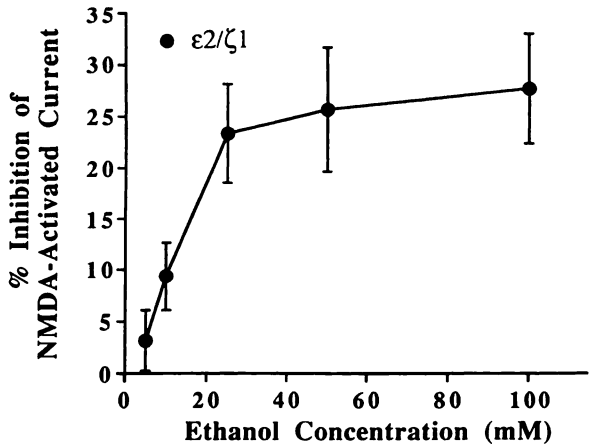

Fig. 3. Concentration-response curve for ethanol effect on NMDA-activated current in oocytes expressing the $\epsilon 2/\zeta 1$ combination of NMDA receptor subunits. *Data points*, percentage inhibition (mean ± standard error) by 5, 10, 25, 50, and 100 mM ethanol of currents activated by 100 μM NMDA; the averages are 3, 9, 23, 26, and 28%, respectively ($n = 6$ for 5 and 10 mM ethanol and $n = 17$ for 25, 50, and 100 mM ethanol). The extracellular bathing solution contained 2 mM Ba^{2+}. The effect of ethanol is not statistically significant for ethanol concentrations of 5 and 10 mM (ANOVA, $p > 0.05$). The effect of ethanol is significant for ethanol concentrations of 25, 50, and 100 mM (ANOVA, $p < 0.001$). The inhibitions by 25, 50, and 100 mM ethanol are not significantly different (ANOVA, $p > 0.05$).

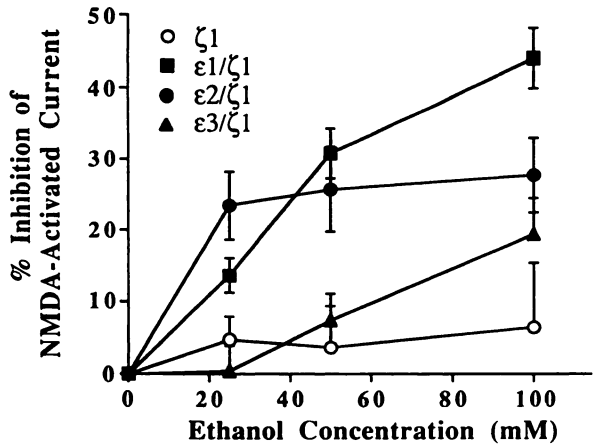

Fig. 4. Comparison of concentration-response curves for ethanol effects on NMDA-activated currents in oocytes expressing different NMDA receptor subunits or subunit combinations. *Data points*, percentage inhibition (mean ± standard error) by 25, 50, and 100 mM ethanol of currents activated by 100 μM NMDA; the numbers of oocytes studied were as follows: ζ1, $n = 5$; ε1/ζ1, $n = 8$; ε2/ζ1, $n = 17$; and ε3/ζ1, $n = 5$. The extracellular bathing solution contained 2 mM Ba^{2+} for the experiments with all heteromeric combinations and 2 mM Ca^{2+} for the experiments with ζ1, because of the small size of the ζ1 currents with Ba^{2+}. Experiments comparing the effects of 50 mM ethanol in extracellular bathing solutions containing Ca^{2+} or Ba^{2+} showed that there was no significant difference in the percentage inhibition of NMDA-activated currents between extracellular solutions containing 2 mM Ca^{2+} or 2 mM Ba^{2+}, for any of the tested NMDA receptor subunits or subunit combinations (data not shown). All solutions containing NMDA also contained 10 μM glycine. For both the ε1/ζ1 and ε2/ζ1 subunit combinations, ethanol inhibition of NMDA-activated current is significant for ethanol concentrations of 25, 50, and 100 mM (ANOVA, $p < 0.001$). However, for the homomeric ζ1 subunit the effect of ethanol on NMDA-activated current is not significant for any of the ethanol concentrations of ≤100 mM (ANOVA, $p > 0.05$), and for the ε3/ζ1 subunit combination ethanol had a significant inhibitory effect on NMDA-activated current only at a concentration of 100 mM (ANOVA, $p < 0.001$).

ferent NMDA receptor subunits or subunit combinations. Each data point represents the average percentage inhibition of NMDA-activated current by 25, 50, and 100 mM ethanol for different NMDA receptor subunits, as indicated. For the ε1/ζ1 subunit combination, and as noted above for the ε2/ζ1 subunit combination, ethanol inhibition of NMDA-activated current was significant for these three concentrations of ethanol (ANOVA, $p < 0.001$). In addition, although the NMDA-activated current associated with the ε2/ζ1 combination was inhibited nearly maximally by 25 mM ethanol, i.e., the inhibition by 50 or 100 mM ethanol was not significantly different from the inhibition by 25 mM ethanol (see above), the NMDA-activated current associated with the ε1/ζ1 combination was increasingly inhibited by increasing concentrations of ethanol over the concentration range of 25–100 mM, with 100 mM ethanol exhibiting maximal inhibition of NMDA-activated current (44% of control). Additionally, ethanol concentrations of 25 and 50 mM ethanol did not significantly affect NMDA-activated current associated with the ε3/ζ1 subunit combination (ANOVA, $p > 0.05$); however, 100 mM ethanol inhibited this current by 19%, which was statistically significant (ANOVA, $p < 0.001$). The NMDA-activated current associated with the homomeric expression of the ζ1 subunit was not significantly affected by ethanol concentrations of ≤100 mM (ANOVA, $p > 0.05$).

Discussion

The observations reported here indicate that recombinant NMDA receptor subtypes expressed in *Xenopus* oocytes exhibit differential sensitivity to ethanol. The differences in ethanol sensitivity are not correlated with the amplitude of NMDA-activated current, because NMDA activated the largest currents in oocytes expressing the ε2/ζ1 subunit combination but 100 mM ethanol inhibited NMDA-activated current to the greatest extent in oocytes expressing the ε1/ζ1 subunit combination. In addition, the concentration-response curve for NMDA revealed that the percentage inhibition by ethanol was similar for different amplitudes of NMDA-activated current. A differential sensitivity to ethanol has also been observed for NMDA-mediated responses in different types of neurons. For example, NMDA-activated current in adult DRG neurons has been found to be considerably more sensitive to ethanol (23) than NMDA-activated current in cultured hippocampal neurons (24) or NMDA-mediated synaptic excitation in hippocampal slices (25). Similarly, differences in the concentration-response curves for ethanol inhibition of NMDA-activated current have also been reported for different types of neurons. In adult DRG neurons, 25 mM ethanol was found to be nearly maximal for inhibition of NMDA-activated current, and 50 and 100 mM ethanol concentrations were not significantly different (23), whereas there was a more gradual increase in the potency of 25–100 mM ethanol for inhibiting NMDA-activated current in cultured hippocampal neurons (24). In addition, NMDA-activated neuronal spike firing has been reported to be inhibited by ethanol in medial septal nucleus, inferior colliculus, and hippocampus (26, 27), but NMDA-activated spike firing has been found to be insensitive to ethanol in lateral septum (27).

It has been suggested that the ε subunits are responsible for the functional heterogeneity of NMDA receptors, because a diversity of physiological and pharmacological properties were observed when different ε subunits were expressed heteromerically with ζ1 (16–18, 22). Consistent with this hypothesis, the ε subunits appear to contribute to the diversity of responses of NMDA receptors to ethanol, because we found that NMDA-activated currents associated with homomeric ζ1 expression were not significantly inhibited by ethanol, whereas different ε subunits expressed heteromerically with ζ1 manifested a diversity of ethanol sensitivities. The ethanol sensitivity of NMDA-activated current associated with the heteromeric expression of ε/ζ1 subunits appears to be qualitatively similar to the ethanol sensitivity of NMDA-activated current in mammalian neurons; however, there are quantitative similarities and differences. For example, the concentration-response curve for ε1/ζ1 is quantitatively similar to the concentration-response curves for cultured cortical neurons, observed recently in our laboratory (28). On the other hand, maximal inhibition of NMDA-activated current by 50 mM ethanol with heteromeric ε/ζ1 subunits was 31% (ε1/ζ1), compared with 83% for adult DRG neurons (23). The differences between the ethanol sensitivity of the NMDA-activated current associated with the heteromeric subunits expressed in the present experiments and that of NMDA-activated current in certain types of neurons may be due to the combinations of subunits studied. In the experiments reported here, only one ε subunit, either ε1, ε2, or ε3, was expressed with ζ1. The combinations of different NMDA subunits normally found in different types of neurons are not known. There is, however, some information on the subunit composition of

another ligand-gated ion channel, the nicotinic ACh receptor. For example, the nicotinic ACh receptor in *Torpedo* electric organ is a pentameric protein composed of two α, one β, one γ, and one δ subunit (29, 30). Because NMDA receptors belong to the same superfamily of receptors as nicotinic ACh receptors, it is possible that NMDA receptors may also be composed of several different subunits, which in combination may confer different physiological and pharmacological properties. An $\epsilon 4$ subunit of the NMDA receptor has also been cloned (19), but its sensitivity to ethanol has not yet been studied. In addition, a number of splice variants of NMDAR1 ($\zeta 1$) have been reported (21, 22, 31, 32), and these splice variants may also contribute to differences in the ethanol sensitivity of different types of neurons (33). It is also possible that there are NMDA receptor subunits that have not yet been cloned.

NMDA receptor subunits are differentially distributed throughout the brain. *In situ* hybridization experiments have revealed that $\zeta 1$ (NMDAR1) mRNA is widely distributed throughout the brain (14, 16, 18, 20). The mRNA for the $\epsilon 1$ (NMDAR2A) subunit is found predominantly in pyramidal cells of the hippocampal gyrus and granule cells of the dentate gyrus and cerebellar cortex (16, 18, 20). The $\epsilon 2$ (NMDAR2B) subunit mRNA is localized predominantly in forebrain, with little hybridzation being detected in cerebellum, whereas the $\epsilon 3$ (NMDAR2C) subunit hybridization is predominantly in cerebellar granule cells, with little hybridization being detected in cerebrum and brainstem (16, 18, 20). Despite these studies, information on the distribution of NMDA receptor subunits in brain is not yet sufficiently detailed for correlation of the cellular receptor subtype localization with neuronal NMDA receptor sensitivity to ethanol. However, the differential sensitivity of different NMDA receptor subunits to ethanol suggests that the diversity of NMDA receptor subunit distribution in different brain regions may contribute to the diversity of ethanol sensitivity of NMDA receptors in neurons from different brain regions. Moreover, variation in the expression of different NMDA receptor subunits in different brain regions may contribute to biological variability in the behavioral effects of alcohol.

The mechanism by which ethanol inhibits NMDA-activated current has not been established. Previous whole-cell patch-clamp experiments on neurons indicated that the effect of ethanol on NMDA-activated current is not voltage dependent, it does not result from a change of the ion selectivity of the channel, and it does not appear to be due to an alteration of one of the regulatory sites on the NMDA receptor channel (34). Recent single-channel experiments suggested that ethanol inhibits NMDA-activated current by altering gating of the channel (28). This effect appears to be due to an interaction of ethanol with a hydrophobic pocket on the NMDA receptor channel protein (35). The observation of differential sensitivities of recombinant NMDA receptor subunits may provide a basis for future studies on the molecular site of ethanol action on NMDA receptors.

Acknowledgments

We are grateful to Dr. Masayoshi Mishina for providing NMDA receptor subunit cDNA clones, and we thank Dr. Robert W. Peoples for technical assistance.

References

1. Mayer, M. L., and G. L. Westbrook. The physiology of excitatory amino acids in the vertebrate central nervous system. *Prog. Neurobiol.* **288**:197–276 (1987).
2. Collingridge, G. L., and R. A. J. Lester. Excitatory amino acid receptors in the vertebrate central nervous system. *Pharmacol. Rev.* **40**:143–210 (1989).
3. Monaghan, T. D., R. J. Bridges, and C. W. Cotman. The excitatory amino acid receptors: their classes, pharmacology, and distinct properties in the function of the central nervous system. *Annu. Rev. Pharmacol. Toxicol.* **29**:365–402 (1989).
4. Madison, D. V., R. C. Malenka, and R. A. Nicoll. Mechanisms underlying long-term potentiation of synaptic transmission. *Annu. Rev. Neurosci.* **14**:379–397 (1991).
5. Ito, M. Long-term depression. *Annu. Rev. Neurosci.* **12**:85–102 (1989).
6. Komuro, H., and P. Rakic. Modulation of neuronal migration by NMDA receptors. *Science (Washington D. C.)* **260**:95–97 (1993).
7. McDonald, J. W., and M. V. Johnson. Physiological and pathophysiological roles of excitatory amino acids during central nervous system development. *Brain Res. Rev.* **15**:41–70 (1990).
8. Morris, R. G. M. Synaptic plasticity and learning: selective impairment of learning in rats and blockadge of long-term potentiation *in vivo* by the NMDA receptor antagonist AP5. *J. Neurosci.* **9**:3040–3057 (1989).
9. Davies, S. N., R. A. J. Lester, K. G. Reymann, and G. L. Collingridge. Temporally distinct pre- and post-synaptic mechanisms maintain long-term potentiation. *Nature (Lond.)* **338**:500–503 (1989).
10. Choi, D. W. Glutamate neurotoxicity and diseases of the nervous system. *Neuron* **1**:623–634 (1988).
11. Meldrum, B., and J. Garthwaite. Excitatory amino acid neurotoxicity and neurodegenerative disease. *Trends Pharmacol Sci.* **11**:379–387 (1990).
12. MacDonald, J. F., M. C. Bartlett, I. Mody, P. Pahapill, J. N. Reynolds, M. W. Salter, J. H. Schneiderman, and P. S. Pennefather. Actions of ketamine, phencyclidine and MK-801 on NMDA receptor currents in cultured mouse hippocampal neurones. *J. Physiol. (Lond.)* **432**:483–508 (1991).
13. Weight, F. F. Cellular and molecular physiology of alcohol actions in the nervous system. *Int. Rev. Neurobiol.* **33**:289–348 (1992).
14. Moriyoshi, K., M. Masu, T. Ishii, R. Shigemoto, N. Mizuno, and S. Nakanishi. Molecular cloning and characterization of the rat NMDA receptor. *Nature (Lond.)* **354**:31–37 (1991).
15. Yamazaki, M., H. Mori, K. Araki, K. J. Mori, and M. Mishina. Cloning, expression and modulation of a mouse NMDA receptor subunit. *FEBS. Lett.* **300**:39–45 (1992).
16. Meguro, H., H. Mori, K. Araki, E. Kushiya, T. Kutsuwada, M. Yamazaki, T. Kumanishi, M. Arakawa, K. Sakimura, and M. Mishina. Functional characterization of a heteromeric NMDA receptor channel expressed from cloned cDNAs. *Nature (Lond.)* **357**:70–74 (1992).
17. Kutsuwada, T., N. Kashiwabuchi, H. Mori, K. Sakimura, E. Kushiya, K. Araki, H. Meguro, H. Masaki, T. Kumanishi, M. Arakawa, and M. Mishina. Molecular diversity of the NMDA receptor channel. *Nature (Lond.)* **358**:36–41 (1992).
18. Monyer, H., R. Sprengel, R. Schoepfer, A. Herb, M. Higuchi, H. Lomeli, N. Burnashev, B. Sakmann, and P. H. Seeburg. Heteromeric NMDA receptors: molecular and functional distinction of subtypes. *Science (Washington, D. C.)* **256**:1217–1221 (1992).
19. Ikeda, K., M. Nagasawa, H. Mori, K. Araki, K. Sakimura, M. Watanabe, Y. Inoue, and M. Mishina. Cloning and expression of the $\epsilon 4$ subunit of the NMDA receptor channel. *FEBS. Lett.* **313**:34–38 (1992).
20. Ishii, T., K. Moryoshi, H. Sugihara, K. Sakurada, H. Kadotani, M. Yokoi, C. Akazawa, R. Shigemoto, N. Mizuno, M. Masu, and S. Nakanishi. Molecular characterization of the family of the *N*-methyl-D-aspartate receptor subunits. *J. Biol. Chem.* **268**:2836–2843 (1993).
21. Sugihara, H., K. Moriyoshi, T. Ishii, M. Masu, and S. Nakanishi. Structure and properties of seven isoforms of the NMDA receptor generated by alternative splicing. *Biochem. Biophys. Res. Commun.* **185**:826–832 (1992).
22. Durand, G. M., P. Gregor, X. Zheng, M. V. L. Bennett, G. R. Uhl, and R. S. Zukin. Cloning of an apparent splice variant of the rat *N*-methyl-D-aspartate receptor NMDAR1 with altered sensitivity to polyamines and activators of protein kinase C. *Proc. Natl. Acad. Sci. USA* **89**:9359–9363 (1992).
23. White, G., D. M. Lovinger, and F. F. Weight. Ethanol inhibits NMDA-activated current but does not alter GABA-activated current in an isolated adult mammalian neuron. *Brain Res.* **507**:332–336 (1990).
24. Lovinger, D. M., G. White, and F. F. Weight. Ethanol inhibits NMDA-activated ion current in hippocampal neurons. *Science (Washington, D. C.)* **243**:1721–1724 (1989).
25. Lovinger, D. M., G. White, and F. F. Weight. NMDA-receptor mediated synaptic excitation selectively inhibited by ethanol in hippocampal slices from adult rat. *J. Neurosci.* **10**:1372–1379 (1990).
26. Simson, P. E., H. E. Criswell, K. B. Johnson, R. E. Hicks, and G. R. Breese. Ethanol inhibits NMDA-evoked electrophysiological activity *in vivo*. *J. Pharmacol. Exp. Ther.* **257**:225–231 (1991).
27. Simson, P. E., H. E. Criswell, and G. R. Breese. Inhibition of NMDA-evoked electrophysiological activity by ethanol in selected brain regions: evidence for ethanol-sensitive and ethanol-insensitive NMDA-evoked responses. *Brain Res.* **607**:9–16 (1993).
28. Wright, J. M., R. W. Peoples, and F. F. Weight. Ethanol decreases both mean open time and frequency of opening of NMDA receptor-channels. *Soc. Neurosci. Abstr.* **19**:716 (1993).
29. Noda, M., H. Takahash, T. Tanabe, M. Toyosato, S. Kikyotan, T. Hirose, H. Takashima, S. Inayama, T. Miyata, and S. Numa. Structural homology of

Torpedo californica acetylcholine receptor subunits. *Nature (Lond.)* **302**:528–532 (1983).
30. Mishina, M., T. Kurosaki, T. Tobimatsu, Y. Morimoto, M. Noda, T. Yamamoto, M. Terao, J. Lindstrom, T. Takahashi, M. Kuno, and S. Numa. Expression of functional acetylcholine receptor from cloned cDNAs. *Nature (Lond.)* **307**:604–608 (1984).
31. Ananthraram, V., R. G. Panchal, A. Wilson, V. V. Kolchine, S. N. Treistman, and H. Bayley. Combinatorial splicing alters the surface charge on the NMDA receptor. *FEBS. Lett.* **305**:27–30 (1992).
32. Nakanishi, N., R. Axel, and N. A. Shneider. Alternative splicing generates functionally distinct *N*-methyl-D-aspartate receptors. *Proc. Natl. Acad. Sci. USA* **89**:8552–8556 (1992).
33. Koltchine, B., V. Anantharam, W. Wilson, H. Bayley, and S. N. Treistman. Homomeric assemblies of NMDAR1 splice variants are sensitive to ethanol. *Neurosci. Lett.* **152**:13–16 (1993).
34. Weight, F. F., R. W. Peoples, J. M. Wright, D. M. Lovinger, and G. White. Ethanol action on excitatory amino acid activated ion channels. *Alcohol & Alcoholism* (Suppl. 2) 353–358 (1993).
35. Peoples, R. W., and F. F. Weight. Aliphatic alcohols exhibit a cutoff in potency of inhibition of NMDA-activated ion current. *Soc. Neurosci. Abstr.* **19**:277 (1993).

Send reprint requests to: Khalid Masood, Laboratory of Molecular and Cellular Neurobiology, NIH/NIAAA, 12501 Washington Avenue, Rockville, MD 20852.