Reduced Ethanol Inhibition of N-Methyl-D-aspartate Receptors by Deletion of the NR1 C0 Domain or Overexpression of α-Actinin-2 Proteins*

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The depressant actions of ethanol on central nervous system activity appear to be mediated by its actions on a number of important membrane associated ion channels including the N-methyl-D-aspartate (NMDA) subtype of ionotropic glutamate receptor. Although no specific site of action for ethanol on the NMDA receptor has been found, previous studies suggest that the ethanol sensitivity of the receptor may be affected by intracellular C-terminal domains of the receptor that regulate the calcium-dependent inactivation of the receptor. In the present study, co-expression of the NR2A subunit and an NR1 subunit that lacks the alternatively spliced intracellular C1 cassette did not reduce the effects of ethanol on channel function as measured by patch-clamp electrophysiology. Full inhibition was also observed in cells expressing an NR1 subunit truncated at the end of the C0 domain (NR1ΔCisstop). However, the inhibitory effects of ethanol were reduced by expression of an NR1 C0 domain deletion mutant (NR1ΔC0ΔC1), truncation mutant (NR1ΔCisstop), or a triple-point mutant (Arg to Ala, Lys to Ala, and Asn to Ala at 859–861) previously shown to significantly reduce calcium-dependent inactivation. A similar reduction in the effects of ethanol on wild-type NR1/2A but not NR1/2B or NR1/2C receptors was observed after co-expression of full-length or truncated human skeletal muscle α-actinin-2 proteins that produce a functional knockout of the C0 domain. The effects of ethanol on hippocampal and cortical NMDA-induced currents were similarly attenuated in low calcium recording conditions, suggesting that a C0 domain-dependant process may confer additional ethanol sensitivity to NMDA receptors.

Behaviorally relevant concentrations of ethanol (0.05–0.4 g/dl; approximately 10–100 mM) inhibit NMDA receptors expressed in neurons and in recombinant expression systems (1–7). The ethanol sensitivity of native neuronal NMDA receptors also varies with age and with the brain region studied, suggesting that differences in the subunit expression pattern of NMDA subunits may be an important determinant of ethanol sensitivity (8, 9). This is supported by findings from several studies with recombinant NMDA receptors that show that receptors composed of NR1/2A or NR1/2B subunits are more sensitive to ethanol than those composed of NR1/2C or NR1/2D subunits (3, 4, 6). Although the ethanol sensitivity of NMDA receptors may be determined in part by the subunit makeup of the receptor, there is also evidence that posttranslational processes may influence the overall sensitivity of these receptors to ethanol.

Previous studies in this laboratory showed that the inhibition of ethanol by recombinant NR1/2A receptors can be modulated by a calcium-dependent process involving the C terminus of the NR1 subunit (10). In oocytes, the ethanol inhibition of NMDA receptors composed of NR1/2A subunits was dramatically enhanced when recordings were performed in extracellular medium containing elevated concentrations of calcium. This effect was observed in receptors expressing the NR1-1a splice variant, which contains both the C1 and C2 cassettes, as well as with those receptors containing the NR1-4a splice variant, which lacks these domains. In contrast, deletion of the entire intracellular C terminus of the NR1-1a subunit or injection of oocytes with the fast calcium chelator BAPTA (1,2-bis(2-aminophenoxy)ethane-N,N,N,N-tetraacetic acid) abolished the enhanced ethanol inhibition of NMDA responses observed under normal (10 mM) calcium containing solution. A similar reduction in ethanol sensitivity was observed in HEK 293 cells expressing an NR1 subunit lacking the C0 domain. Taken together, these results suggest that a portion of the ethanol sensitivity of NMDA receptors is calcium-dependent and involves amino acids contained between the fourth transmembrane region of the receptor and the alternatively spliced C1 cassette of the NR1 subunit.

This 30 amino acid region of the NR1 subunit has been termed the C0 domain and is conserved among all NR1 splice variants. It has been shown to bind cytoskeleton-associated proteins, such as α-actinin-2, in a calcium-dependent manner, suggesting that it is important for communication between the receptor and the actin cytoskeleton. Displacement of α-actinin-2 binding to the NR1 subunit by calmodulin during receptor activation has recently been suggested to underlie the calcium-dependent inactivation of NMDA receptor currents observed in both neurons and transfected cells (11–15).

In the present study, we have further investigated the role of the C0 domain in regulating the ethanol inhibition of recombinant NMDA receptors using whole cell patch clamp analysis of transfected HEK 293 cells. This approach was selected because HEK 293 cells display no noticeable endogenous calcium-activated currents that can interfere with the analysis of calcium permeable recombinant ion channels such as NMDA receptors. This is especially important with respect to the study of the mechanisms of action of ethanol because oocytes express...
Ethanol and NMDA Receptor Function

EXPERIMENTAL PROCEDURES

Cell Culture—HEK 293 cells were obtained from the American Type Culture Collection (Manassas, VA) and were grown in Dulbecco's modified Eagle's medium (Sigma) supplemented with 10% fetal bovine serum (Fetal-Clone II; HyClone, Logan, UT). Cells were harvested from feeder flasks 24–48 h prior to transfection and plated onto sterile 35 mm culture dishes for whole-cell patch clamp analysis. Hippocampal neuronal cultures were prepared exactly according to Smothers et al. (17).

Molecular Biology and Cell Transfections—The rat NR1 (NR1a), NR2A, and NR2B cDNA clones containing the pBlueScript vector were generous gifts of Dr. S. Nakaniishi, and the rat NR1-2a splice variant cDNA was obtained from Dr. S. Heinemann. These cDNAs were subcloned into the mammalian expression vector pcDNA3 (Invitrogen, La Jolla, CA). The C0 deletion mutant (NR1Δ344-745) generated from the NR1-2a splice variant was a gift from Dr. R. Huganir. The NR1Δ555 and NR1ΔPM1 (Arg to Ala, Lys to Ala, and Asn to Ala at 859–861) mutants were kindly provided by Dr. B. Vissel and Dr. J. J. Krupp. The NR1 C-terminal deletion mutant NR1Δ555 was constructed by replacing the indicated amino acid on the full-length NR1-1a subunit with a stop codon using site-directed mutagenesis as described previously (10). The human skeletal muscle a-actinin-2 cDNA in pcDNA3 was kindly provided by Dr. A. Beggs. This cDNA was used as a polymerase chain reaction template to construct a green fluorescent protein (GFP)-tagged fusion protein in the pGFP-N3 vector (CLONTECH). The fusion protein consisted of the central rod domain of a-actinin-2 (amino acids 344–745) followed in-frame by the coding sequence of the GFP. The pGFP-N3 vector (CLONTECH) had been previously altered by site-directed mutagenesis (S65T; iGFP) to intensify and red-shift the fluorescent signal (18).

For transfections, HEK 293 cells grown to approximately 50% confluency were incubated with 1 µg each of the desired cDNAs for 6 h in serum-free Dulbecco's modified Eagle's medium in the presence of the LipofectAMINE reagent (Life Technologies, Inc.). Except in those studies with the truncated a-actinin-2/GFP fusion protein, the iGFP vector was co-transfected with the other subunits in order to easily identify transfected cells. Following transfections, cells were washed with serum-free Dulbecco's modified Eagle's medium and incubated in serum-containing minimal essential medium for 24–48 h prior to analysis. The minimal essential medium was supplemented with ketamine (0.5 mM; Fort Dodge Laboratories, Inc.; Fort Dodge, IA) to prevent excitotoxicity due to expression of functional NMDA receptors (19). The ketamine solution was removed by extensive washing prior to electrophysiological analysis.

Electrophysiological Recordings, Data Collection, and Analysis—Transfected HEK 293 cells or neurons were bathed in extracellular medium at room temperature containing the following: NaCl, 135 mM; KCl, 5.4 mM; CaCl2, 1.8 mM; HEPES, 5 mM; glucose, 10 mM; pH 7.2, adjusted to 325 mosM with sucrose. In experiments with cultured neurons, tetrodotoxin (100 nM) was included in the external solution to block spontaneous activity. For HEK 293 cells, patch electrodes (2–6 MΩ) were filled with recording solution containing the following: CsCl, 40 mM; MgCl2, 2 mM; EGTA, 10 mM, pH 7.2; and anositol polyphosphate (Warner Instruments, SF-77A) was used to switch between control and solutions containing NMDA (10–100 µM) in the absence or presence of ethanol. Glycine (10–50 µM) was added to all solutions to prevent glycine-dependent desensitization, and recordings were generally completed within 5–10 min of seal formation, which reduced the extent of glycine-independent desensitization. Ethanol was applied to cells 2 min prior to application of the test solution. The test solution required for solution exchange was measured using the change in electrode tip potential when stepping between extracellular solutions of different ionic strength. The 10%/90% rise time in currents recorded when switching between these different solutions was approximately 8 ms. Currents were obtained using Pulse control software running under the IgorPro graphics platform on a Macintosh G3 computer (Apple Computer, Cupertino, CA) in combination with an Axon 200 B amplifier (Axon Instruments, Foster City, CA). Whole-cell currents were filtered at 5 kHz, low-pass-filtered at 0.2 kHz, and digitized at 1 kHz. Currents were measured using Axograph 4.0 software (Axon Instruments, Foster City, CA). Data were analyzed for statistical significance using one-way analysis of variance with post hoc testing at a significance level of 0.05.

RESULTS

Ethanol Inhibition Is Not Altered by C1 Domain Deletion—As shown in Fig. 1A, HEK 293 cells transfected with NMDA subunits produce functional receptors that are activated by the agonists NMDA and glycine. Wild-type NR1-1a/2A receptors show desensitizing responses during agonist application (steady state to peak ratio, 0.56 ± 0.03; n = 33). Under the recording conditions used in this study, the majority of this desensitization is due to calcium-dependent inactivation (15, 21) because it is largely lost in cells transfected with NR1 subunits that lack a functional C0 domain (Fig. 1A, NR1Δ555, NR1Δ859-863, NR1Δ555αtop, and NR1-PM1) or under conditions of reduced extracellular calcium (data not shown). Fig. 1A also shows that ethanol inhibits peak and steady state NMDA-mediated currents and that this inhibition is readily reversed upon washout. NMDA currents displayed a modest rundown because the amplitude of the washout current was usually slightly less than that of the first control current. The extent of this rundown for wild-type NR1-1a/2A receptors averaged 5% over the three stimulation protocol used (S.E. = 0.03; range, 0–25%; n = 23). To account for this rundown, amplitudes of control and washout currents were averaged and used to determine the percentage of inhibition produced by ethanol.

As shown in Fig. 1B, steady state inhibition of NMDA-induced currents by 100 mM ethanol was approximately 44% in cells expressing wild-type NR1-1a/2A subunits. Ethanol (100 mM) inhibited NR1-1a/2A receptors to a similar degree (42.3 ± 2.3%; n = 14; steady state to peak ratio, 0.61 ± 0.04; n = 14; mean ± S.E.) when currents were activated by 10 µM NMDA and 10 µM glycine. Under these conditions, current inactivation is mediated almost entirely by calcium-dependent inactivation (21). The NR1-1a splice variant used in these experiments contains all three domains of the C terminus and is expressed in most brain neurons. The NR1-2a splice variant lacks the C1 cassette that has been shown to contain consensus phosphorylation sites for both protein kinase C and protein kinase A, as well as a binding site for calmodulin (11, 22). Fig. 1B shows that expression of NR1-2a/2A subunits that lack this domain did not significantly alter the inhibitory effect of 100 mM ethanol on steady state NMDA currents. The extent of current desensitization of NR1-2a/2A receptors was also not significantly different from that obtained with NR1-1a subunits (steady state to peak ratio, 0.59 ± 0.04; n = 15; mean ± S.E.).

C-terminal Truncation of the NR1 Subunit Reduces Ethanol Inhibition—Our previous study showed that that truncation of the entire C terminus of the NR1 subunit significantly reduced the inhibition of NMDA-activated currents by 100 mM ethanol (10). It has also been shown that the calcium-dependent inactivation of NR1/2A receptors is abolished by deletion of just the last five residues (859–863) of the NR1 C0 domain (15). The effect of 100 mM ethanol was tested on cells expressing NR2A and an NR1 subunit truncated just prior to this five-amino acid stretch (NR1Δ555αtop). These receptors also displayed little current inactivation (steady state to peak ratio, 0.95 ± 0.03; n = 11; mean ± S.E.), as well as a reduced inhibition by 100 mM ethanol (31.6 ± 2.3%; n = 11; mean ± S.E.) as compared with wild-type receptors.

The reduction in the ethanol sensitivity of NMDA receptors expressing a C-terminal truncated NR1 subunit was also observed at lower ethanol concentrations. For example, wild-type NR1/2A receptors were inhibited by 25 and 50 mM ethanol by 22.2 ± 2.8% (mean ± S.E.; n = 9) and 29.6 ± 4.5% (mean ± S.E.) when stepped from a control solution with 100 mM NMDA and 100 µM glycine to one containing 100 mM ethanol with 10 µM NMDA and 10 µM glycine. This effect was reduced by 10 mM ethanol to 15.0 ± 2.4% (mean ± S.E.; n = 10).

1 The abbreviations used are: GFP, green fluorescent protein; NMDA, N-methyl-D-aspartate.
The C0 Domain Mediates the Loss of Ethanol Sensitivity—In order to verify that the decrease in ethanol inhibition was specifically associated with loss of the C0 domain, a deletion mutant lacking the entire C0 domain but containing the C2 domain (NR1_{839–863}) was expressed with the NR2A. As with other NR1 subunits lacking a functional C0 domain, these receptors also showed no appreciable current decay during agonist application (Fig. 1B). In addition, currents carried by this C0 deletion mutant were significantly less sensitive to 100 mM ethanol as compared with the NR1-2a splice variant that contains the C0 and C2 domains. Overall, there was no significant difference in ethanol inhibition between the C0 domain truncation (NR1_{858stop}) or deletion (NR1_{863stop}) mutants.

To further explore the requirement for a functional C0 domain, a triple-point NR1 C0 mutant (NR1-PM1) was expressed with the NR2A subunit and tested for ethanol sensitivity. This mutant contains alanines at positions 859, 860, and 861 of the NR1 C0 domain (Fig. 1A, top panel). These substitutions have been shown to substantially reduce both calmodulin binding and calcium-dependent inactivation (15). In the present study, NR1-PM1/2A receptors also displayed reduced current decay (steady state to peak ratio, 0.78 ± 0.02; n = 8) as well as a reduced sensitivity to ethanol (Fig. 1B). Finally, to demonstrate that a functional C0 domain alone is sufficient to confer high ethanol sensitivity, a truncation mutant containing just the C0 domain (NR1_{863stop}) was co-expressed with the NR2A subunit. As shown in Fig. 1, this mutant displayed wild-type current inactivation (steady state to peak ratio, 0.51 ± 0.05; n = 8) and full sensitivity to ethanol.

Co-Expression of α-Actinin-2 Mimics Deletion of the C0 Domain—These results suggest that a functional C0 domain of the NR1 subunit is important in mediating a significant fraction of the ethanol inhibition of steady state NMDA currents under conditions of normal extracellular calcium. Co-expression of α-actinin-2 has previously been shown to significantly reduce or eliminate the degree of calcium-dependent inactivation of NR1/2A receptors expressed in HEK 293 cells (14, 15).

HEK 293 cells transfected with the full-length α-actinin-2 and probed with a monoclonal antibody directed against α-actinin-2 (EA-53; Sigma) showed robust expression of this protein, whereas non-transfected cells displayed little to no staining.2 As previously reported (15), the degree of NMDA current inactivation was significantly attenuated in cells expressing the full-length or truncated forms of the α-actinin-2 protein, as shown by the tracings in Fig. 2. The degree of ethanol inhibition of NMDA currents in these cells was also reduced as compared with non-actinin-transfected cells and resembled that for NR1 subunits lacking a functional C0 domain (Fig. 2 and Fig. 1B).

The α-Actinin-2 Effect Is NR2 Subunit-dependent—The effect of ethanol on NR1/2B and NR1/2C receptors expressed with and without the α-actinin-2/GFP fusion protein was determined to assess the role of the NR2 subunit in mediating this effect. Note that 200 μM glutamate (with 50 μM glycine) was used to activate currents in cells expressing NR1/2C subunits because those elicited by 100 μM NMDA were sometimes...
small and variable. As shown in Fig. 3, ethanol (100 mM) inhibited NR1/2B receptors by approximately 34% and those composed of NR1/2C subunits by approximately 20%. Co-expression of the α-actinin-2/GFP fusion protein did not significantly alter the ethanol inhibition of either NR1/2B or NR1/2C receptors.

Reduced Extracellular Calcium Attenuates Ethanol Inhibition in Neurons and HEK Cells—Finally, we investigated whether the ethanol inhibition of native NMDA receptors expressed in cultured neurons would be altered under conditions designed to reduce the extent of calcium-dependent inactivation. As shown in Fig. 4, pyramidal neurons in hippocampal cultures were exposed to a 5-s stimulation with NMDA (100 μM) and glycine (10 μM) in the absence or presence of 100 mM ethanol. In extracellular media containing 2 mM calcium, NMDA stimulation induced a significant degree of current desensitization. This desensitization was significantly reduced when the extracellular solution was switched to one containing 0.2 mM calcium, suggesting that it was due primarily to calcium-dependent inactivation. In normal (2 mM) calcium-containing extracellular media, ethanol (100 mM) inhibited steady state NMDA currents in hippocampal neurons by approximately 35%. This was reduced to approximately 22% when neurons were recorded in solutions containing 0.2 mM calcium. A similar reduction in ethanol sensitivity was observed when HEK 293 cells expressing NR1-1a/2A subunits were tested under conditions of reduced extracellular calcium (Fig. 4, shaded bars).

**DISCUSSION**

Previous studies from this laboratory showed that the effect of ethanol on NR1/2A receptors expressed in oocytes was attenuated under conditions that prevented or reduced increases in intracellular calcium upon activation of the NMDA receptor (4, 10). Thus, expression of calcium-impermeable NR1 mutants (NR1(E60G)), recording with a divalent cation-free extracellular solution or co-injection of oocytes with BAPTA all significantly reduced the inhibition of NMDA-mediated currents by ethanol. These treatments did not alter the ethanol sensitivity of NR2C containing receptors that show appreciably less inhibition to ethanol than NR2A containing receptors under normal recording conditions (4, 6). The results from these studies suggested that a calcium-dependent process involving an intracellular domain of an NMDA subunit was required for the expression of the full ethanol sensitivity of NR1/2A receptors. This hypothesis was strengthened by the observation that NMDA receptors composed of NR2A and a C-terminal truncated NR1 receptor (NR1Δ481stop) showed reduced inhibition to ethanol in either oocytes or HEK cells under conditions of normal or elevated extracellular calcium (10).

The intracellular C terminus of the NR1 subunit is characterized by several domains that appear to play an important role in receptor localization and function of the NMDA receptor. The C1 cassette, which is the product of the alternatively
cultured hippocampal neurons, suggesting that native NMDA receptors are subject to this calcium-dependent modulation of ethanol sensitivity as well. The effect of C-terminal truncation in the HEK cells was C0-domain-dependent because the ethanol sensitivity was not altered by expression of receptors lacking just the C1 domain that has also been shown to contain a high affinity calmodulin binding site (11). Interestingly, truncation of the NR1 subunit just past the last transmembrane domain did not completely abolish the inhibitory effect of ethanol, suggesting additional sites of action for ethanol on the receptor. Results from mutagenesis studies have identified several amino acids located in transmembrane domains 2 and 3 of \( \gamma \)-aminobutyric acid, type a and glycine receptors that regulate the alcohol and anesthetic sensitivity of those receptors (23). Although the overall amino acid similarity between \( \gamma \)-aminobutyric acid, type A/glycine receptors and NMDA receptors is small, it is possible that similar transmembrane sites are important in defining an additional site of action for ethanol on ionotropic glutamate receptors.

The reduction in the ethanol sensitivity of NR1 containing NMDA receptors was correlated with the loss of amino acids previously shown to be required for both calmodulin and \( \alpha \)-actinin-2 binding to the C0 domain. A previous study showed that residues 859–863 of the NR1 subunit were essential for both calmodulin and \( \alpha \)-actinin-2 binding to the C0 domain (15). Mutants lacking these amino acids or those containing alanine substitutions in this region (NR1PM1) also showed a loss of calcium-dependent inactivation. In the present study, deletion or substitution of these C0 domain amino acids by alanine also reduced the inhibitory effects of ethanol on NMDA-mediated currents. Importantly, expression of NR1 subunits truncated at the end of the C0 domain showed full inactivation as well as high ethanol sensitivity, supporting the hypothesis that the C0 domain is both necessary and sufficient to confer additional ethanol sensitivity to NR1/2A receptors.

Single channel studies have shown that the effects of ethanol on wild-type NMDA receptors are due primarily to decreases in open probability (\( P_o \)) and mean open time of the channel (24). The estimated open probability of a series of C0 truncation mutants varied up to 2-fold depending on the length of the C0 domain that remained (15). The high range of \( P_o \) was found for wild-type NR1 subunits and those truncated just past the C0 domain (NR1ssi, stop). Those lacking amino acids required for calmodulin and \( \alpha \)-actinin-2 binding (amino acids 859–863) had a lower \( P_o \) and were suggested to be permanently inactivated. Paradoxically, deletion of the entire C0 domain (NR1sSS, stop) resulted in receptors that did not exhibit calcium-dependent inactivation but had a \( P_o \) similar to that of the wild-type NR1. In this and our previous study, the reduction in the effects of ethanol on NMDA currents was similar for NMDA receptors truncated either near the last membrane spanning region (NR1sSS, stop) or those that were truncated just prior to the last five amino acids of the C0 domain (NR1ss, stop). These results suggest that the reduction in ethanol sensitivity of mutant NR1 subunits is correlated with loss of the ability of the receptor to inactivate rather than a result of the receptor being permanently inactivated.

The requirement for a functional C0 domain to confer high ethanol sensitivity in wild-type NR1/2A receptors is also suggested from the results of studies involving co-expression of \( \alpha \)-actinin-2 proteins. In those studies, the effects of ethanol on HEK cells expressing wild-type NR1/2A NMDA receptors and full-length or truncated \( \alpha \)-actinin-2 proteins was similar to that produced by deletion or truncation of the NR1 C0 domain. These results suggest that overexpression of \( \alpha \)-actinin-2 in these cells may have produced a functional block of the C0
domain and reduced ethanol inhibition by interfering with the calcium-dependent inactivation of NMDA receptors. The loss of high ethanol sensitivity in cells co-transfected with full-length or truncated α-actinin-2 proteins was associated with a partial reversal of the inactivation of the current, as measured by the steady state to peak ratio. The magnitude of the effect of α-actinin-2 co-expression on NMDA receptor inactivation was nearly identical to that reported in two studies (14, 15) that both used the same α-actinin-2 proteins as in the present study.

These results indicate that the ethanol sensitivity of NR1/2A receptors may be significantly affected by the ability of α-actinin-2 to interfere with the calcium-mediated inactivation of the NR1 subunit. Interestingly, α-actinin-2 protein is highly expressed in dendritic spines of brain neurons and is localized in the postsynaptic density of glutamate containing synapses but not to those containing γ-aminobutyric acid (25). Neuronal expression of the α-actinin-2 protein also shows significant regional variability and is high in pyramidal neurons of the dentate gyrus but low in the CA1 pyramidal neurons of the hippocampus and the granule cells of the cerebellum.

Previous electrophysiological and biochemical studies have demonstrated considerable variability in the ethanol sensitivity of NMDA-mediated responses in neurons from different brain regions (8, 26). Some of these differences may be explained by differences in the subunit composition of the NMDA receptors, particularly those that contain the NR2C subunit, because NMDA receptors containing this subunit generally show less sensitivity to ethanol than those containing either NR2A or NR2B subunits (3–6). However, in addition to variations in subunit-composition, the results from the present study suggest that differences in α-actinin-2 expression may also influence the ethanol sensitivity of neuronal receptors containing NR1 and NR2A subunits.

In summary, results from the present study demonstrate that a portion of the ethanol sensitivity of NR1/2A receptors involves a calcium-dependent interaction with the C0 domain of the NR1 subunit. It is not clear at this point how this effect is produced, although one simple possibility is that ethanol potentiates the degree of calcium-dependent inactivation of the receptor via a direct enhancement of calmodulin binding to the C0 domain. It is also possible that ethanol does not directly alter the degree of inactivation of the receptor but rather has more pronounced effects on receptors that are capable of inactivating. This seems consistent with the reduced ethanol sensitivity observed for NR1/2B or NR1/2C receptors that do not show appreciable calcium-dependent inactivation and by the lack of effect of the α-actinin-2 protein on these receptors. If the enhanced sensitivity of NR1/2A receptors to ethanol requires a functional calmodulin binding site on the NR1 subunit, it can be hypothesized that other proteins that are also regulated by calmodulin may show sensitivity to ethanol. A recent study found that the ethanol sensitivity of various isoforms of plasma membrane calcium ATPases was positively correlated with their affinity for calmodulin which acts to regulate enzyme activity (27). Furthermore, the ability of ethanol to enhance calcium ATPase activity in one isoform was lost after deletion of the calmodulin binding domain of the enzyme. It is of interest to note that many ion channels that appear particularly sensitive to ethanol, including NMDA receptors, calcium-activated potassium channels (28), and voltage-gated l-type calcium channels (29), may also be regulated by calcium in a calmodulin-dependent manner (30). Based on the results of the present study, it can be speculated that these calmodulin-dependent processes may confer an additional degree of ethanol sensitivity to these proteins.

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