Acute Effects of Ethanol on Kainate Receptors with Different Subunit Compositions

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ABSTRACT

Previous studies showed that recombinant homomeric GluR6 receptors are acutely inhibited by ethanol. This study examined the acute actions of ethanol on recombinant homomeric and heteromeric kainate (KA) receptors with different subunit configurations. Application of 25 to 100 mM ethanol produced inhibition of a similar magnitude of both GluR5-Q and GluR6-R KA receptor-dependent currents in Xenopus oocytes. Ethanol decreased the KA \( E_{\text{rev}} \) without affecting the EC50 and its effect was independent of the membrane holding potential for both of these receptors subtypes. Ethanol also inhibited homomeric and heteromeric receptors transiently expressed in human embryonic kidney (HEK) 293 cells. In these cells, the expression of heteromeric GluR6-R subunit-containing receptors was confirmed by testing their sensitivity to 1 mM \( \alpha \)-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid. Ethanol inhibited to a similar extent KA-gated currents mediated by receptors composed of either GluR6 or GluR6 + KA1 subunits, and to a slightly lesser extent receptors composed of GluR6 + KA2 subunits. Acute ethanol’s effects were tested on GluR5 KA receptors that are expressed as homomers (GluR5-Q) or heteromers (GluR5-R + KA1 and GluR5-R + KA2). Homomeric and heteromeric GluR5 KA receptors were all inhibited to a similar extent by ethanol; however, there was slightly more inhibition of GluR5-R + KA2 receptors. Thus, recombinant KA receptors with different subunit compositions are all acutely inhibited to a similar extent by ethanol. In light of recent reports that KA receptors regulate neurotransmitter release and mediate synaptic currents, we postulate that these receptors may play a role in acute ethanol intoxication.

Ionotropic glutamate receptors are cation-conducting channels that belong to a superfamily of ligand-gated ion channels. The three major families of ionotropic glutamate receptors have been denoted as the N-methyl-D-aspartate (NMDA), \( \alpha \)-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA), and kainate (KA) receptor families. The KA family of glutamate receptors comprises the GluR5–7 and KA1–2 subunits (Hollmann and Heinemann 1994). Similar to GluR2 AMPA receptor subunits, RNA editing takes place in the M2 domain Q/R site of both GluR5 and GluR6 subunits (reviewed in Ozawa et al., 1998). GluR5-Q, GluR6-Q, and GluR6-R subunits can form homomorphic channels, whereas GluR5-R subunits only form functional channels when coexpressed with KA1 or KA2 subunits (Herb et al., 1992). KA1 and KA2 subunits do not form homomorphic channels but coexpress with GluR5–7 subunits forming receptors with new pharmacological and functional properties. For instance, GluR6 homomeric receptors are not activated by 1 mM AMPA, whereas it activates heteromeric receptors composed of GluR6 plus either KA1 or KA2 subunits (Herb et al., 1992; Sakimura et al., 1992). Although the subunit composition of native KA receptors in most neurons remains unknown, a number of biochemical and functional studies suggest that some KA receptors exist in vivo as heteromers (Sahara et al., 1992; reviewed in Ozawa et al., 1998).

Previous studies showed that KA receptors are acutely modulated by ethanol. Dildy-Mayfield and Harris (1995) demonstrated that concentrations of ethanol as low as 35 mM inhibited KA-gated currents in Xenopus oocytes expressing recombinant GluR6 receptors. We recently reported that recombinant GluR6 receptors expressed in human embryonic kidney (HEK) 293 cells are also inhibited by ethanol and that its mechanism of action does not involve protein phosphorylation (Valenzuela et al., 1998b). Moreover, we recently showed that pharmacologically isolated native KA receptors are acutely inhibited by ethanol in cerebellar granule neurones (Valenzuela et al., 1998a). However, ethanol’s effects on KA receptors with different subunit compositions are unknown. In this article, we report the results of electrophysiological studies on the acute effects of ethanol on homomeric and heteromeric recombinant KA receptors composed of...
GluR5 or R6 subunits in the absence or presence of KA1 or KA2 subunits.

Materials and Methods

Cell Culture and DNA Transfections. HEK 293 cells were purchased from American Type Tissue Culture Collection (ATCC, Rockville, MD). Rat GluR5-Q, GluR5-R, GluR6-R, KA1, and KA2 receptor subunit cDNAs were subcloned in cytomegalovirus promoter-containing vectors and were generously provided by Drs. Yael Stern-Bach and Steve Heinemann (Salk Institute, La Jolla, CA). HEK 293 cells were maintained in minimum essential media (HyClone, Logan, UT) plus 10% fetal bovine serum (Gemini Bioproducts, Calabasas, CA), 2 mM glutamine, 100 U/ml penicillin, and 0.1 mg/ml streptomycin (all from Sigma Chemical Co., St. Louis, MO) at 37°C 5% CO2 in a humidified atmosphere; these cells were transiently transfected by the calcium phosphate precipitation method (Chen and Okayama 1987).

Experiments with Xenopus Oocytes. The techniques for injection, culture, and two-voltage clamp electrophysiological recording from Xenopus oocytes used in this study are described in detail elsewhere (Dildy-Mayfield and Harris, 1995). Oocytes were injected with rat GluR5-Q or GluR6-R receptor cRNAs, which were transcribed in vitro with the mRNA capping kit from Stratagene (La Jolla, CA). Concanavalin A (2 μM) was preapplied for 1 min before KA application to prevent desensitization. Ethanol was coapplied with KA for 30 s and effects of drugs were calculated as the percentage of change from an average of control and washout responses.

Patch-Clamp Electrophysiological Recording. For electrophysiological recording, HEK 293 cells were plated on sterile 12-mm glass round coverslips coated with poly-i-lysine and transfected as described above. Cells were used for recording 48 to 72 h after transfection. Immediately before recording, coverslips were transferred to a perfusion chamber (Warner Instruments, Hampten, CT) and visualized under a Leitz Fluovert inverted microscope (Wetzlar, Germany) equipped with Hoffman modulation optics (Greenvale, NY). Currents were measured in the whole-cell patch clamp configuration. Membrane potential was clamped at −60 mV with an Axopatch 200 amplifier (Axon Instruments, Foster City, CA). Recording pipettes (borosilicate capillaries with filament, A, 1.5 mm, Sutter Instruments, Novato, CA) were prepared with a two-step puller (Narishige Instrument Co, Tokyo, Japan) and had resistances between 5 and 7 MΩ. The external solution (all chemicals were from Sigma) contained: 130 mM NaCl, 5 mM KCl, 2 mM CaCl2, 1 mM MgCl2, 10 mM HEPES (pH 7.3), 11 mM glucose, and 1 μM concanavalin A. The internal solution contained: 130 mM KCl, 10 mM HEPES, 0.1 mM CaCl2, 1 mM EGTA, 2 mM ATP, and 0.2 mM GTP (all chemicals were from Fluka, Milwaukee, WI). Drugs were applied with a fast-exchange flow-tube perfusion system that was driven by motor (Warner Instrument Co.) and controlled by a Master-8 stimulator (A.M.P.I, Jerusalem, Israel). Agonists were applied at 60-s intervals. Ethanol was coapplied with agonist and was also present in the buffer saline. Data was acquired and analyzed with the Neuropro software package (RC Electronics, Santa Barbara, CA).

Results

Effects of Ethanol on KA Receptors Expressed in Xenopus Oocytes. Application of KA in the presence of concanavalin A (2 μM) activated GluR5-Q receptors with an EC50 of 27 μM (95% confidence interval 22–33 μM) and a Hill slope of 0.7 (95% confidence interval 0.6–0.8; n = 6–7; Fig. 1), and GluR6-R receptors with an EC50 of 1.7 μM (95% confidence interval 0.9–3.2 μM) and a Hill slope of 0.8 (95% confidence interval 0.5–1; n = 7–8; Fig. 1). Ethanol (75 mM) inhibited GluR5-Q receptor-dependent KA currents by 40 ± 7, 28 ± 6, 31 ± 3, 24 ± 4, and 27 ± 3% at KA concentrations of 1 μM, 5 μM, 25 μM, 100 μM, and 5 mM, respectively (Fig. 1, inset). Ethanol (75 mM) inhibited GluR6-R receptor-dependent KA currents by 42 ± 6, 36 ± 4, 27 ± 4, 22 ± 3, and 19 ± 2% at KA concentrations of 0.5, 1, 3, 10, and 100 μM, respectively (Fig. 1, inset). It should be noted that ethanol did not change the KA EC50 or Hill coefficient for these receptors. For GluR5-Q receptors, the EC50 value in the presence of ethanol was 34 μM (95% confidence interval 20–59 μM) and the Hill coefficient was 0.7 (95% confidence interval 0.5–0.9; n = 6–7). For GluR6-R receptors, the EC50 value in the presence of ethanol was 2.7 μM (95% confidence interval 1.5–5.3 μM) and the Hill coefficient was 0.8 (95% confidence interval 0.5–1; n = 8). The GluR5Q receptor Emax was not change the KA EC50 or Hill coefficient for these receptors.
decreased to 72% of control (95% confidence interval 63–82%) and the GluR6 receptor $E_{\text{max}}$ was decreased to 80% of control (95% confidence intervals 68–98%).

We then tested the effects of ethanol on KA responses at or below the EC$_{50}$ (Fig. 2). Appropriate KA concentrations were determined for each individual oocyte by measuring maximal KA responses and choosing a concentration that produced currents corresponding to 50% or less of these responses. In GluR5-Q receptors, ethanol concentrations of 25, 50, 75, and 100 mM decreased the amplitude of KA activated currents by 76%, 19%, 27%, and 40%, respectively ($n = 4$; Fig. 2). In GluR6-R receptors, ethanol concentrations of 25, 50, 75, and 100 mM decreased the amplitude of KA activated currents by 13%, 19%, 24%, and 35%, respectively ($n = 4–5$; Fig. 2). Statistical analysis (one-sample Student’s $t$ test versus a theoretical mean of zero and one-way ANOVA followed by Dunnett’s multiple comparison test) revealed that ethanol produced significant ($p < .05$) inhibition of GluR5-Q and GluR6-R receptor-dependent KA currents at all concentrations tested. Two-way ANOVA revealed that ethanol did not produce significantly different inhibition of GluR5-Q versus GluR6-R receptors.

The effect of ethanol on GluR5-Q and GluR6-R receptor function was independent of the membrane holding potential (Fig. 3). Ethanol (75 mM) inhibited GluR5-Q receptor function by 22%, 31%, and 24% (n = 5) at −90, −60, and −30 mV membrane holding potentials, respectively (Fig. 3). Ethanol inhibited GluR6-R receptor function by 24%, 25%, and 27% (n = 5–7) at −90, −60, and −30 mV membrane holding potentials, respectively (Fig. 3).

**Effects of Ethanol on Receptors Expressed in HEK 293 Cells.** HEK 293 cells transfected with GluR6-R, GluR6-R1 KA1, or GluR6-R1 KA2 subunits were tested for sensitivity to 100 μM KA and 1 mM AMPA (Fig. 4). Homomeric

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**Fig. 2.** Comparison of ethanol’s effect on GluR5-Q versus GluR6-R receptors expressed in Xenopus oocytes. Upper panel, representative tracings of effects of 50 mM ethanol. Lower panel, summary of effects of 25 to 100 mM ethanol. Ethanol was tested on receptors activated by concentrations at or below the KA EC$_{50}$. Each point represents mean ± S.E.M. of four oocytes. Scale bar, 50 nA by 30 s.

**Fig. 3.** Effects of ethanol on current/voltage relationships for GluR5-Q and GluR6-R receptors expressed in Xenopus oocytes. Currents were measured at indicated membrane holding potentials in the absence (■) and presence (□) of 75 mM ethanol. Each point represents mean ± S.E.M. of five to seven determinations. KA concentration was 5 μM. Currents were normalized with respect to responses obtained at −90 mV in the absence of ethanol. Inset, summary of percent change induced by ethanol at different holding membrane potentials.
GluR6 receptors were virtually insensitive to 1 mM AMPA. Conversely, 1 mM AMPA produced significant currents in cells transfected with GluR6-R plus either KA1 or KA2 (Fig. 4). It should be noted that all of the results reported in this article on heteromeric GluR6-R, KA1 or KA2 receptors were obtained from cells that significantly responded to 1 mM AMPA.

KA activated GluR6-R receptors with an EC$_{50}$ of 2.7 μM (confidence interval 1.9–3.9 μM) and a Hill slope of 1.0 (confidence interval 0.7–1.3; n = 3–5; Fig. 5). KA activated GluR6 + KA1 receptors with an EC$_{50}$ of 1.7 μM (confidence interval 1.2–2.4 μM) and a Hill slope of 1.0 (confidence interval 0.7–1.2; n = 5–6; Fig. 5), and it activated GluR6 + KA2 receptors with an EC$_{50}$ of 0.45 μM (confidence interval 0.38–0.54 μM) and a Hill slope of 1.4 (confidence interval 1–1.6; n = 3–4; Fig. 5). Two-way ANOVA indicated that the dose-response curve for GluR6 + KA2 was significantly different from the curves for both GluR6 and GluR6 + KA1 receptors (p < .001).

We then tested the effects of ethanol on KA responses at or below the EC$_{50}$, where the responses do not show desensitization under our recording conditions (i.e., in the presence of concanavalin A) and the effect of ethanol is independent of the KA concentration used (Fig. 1; Valenzuela et al., 1998b). Appropriate KA concentrations were determined for each individual cell by measuring maximal KA (100 μM) responses and choosing a KA concentration that produced currents corresponding to ≈50% of these responses. In cells expressing GluR6 receptors, ethanol concentrations of 25, 50, 75, and 100 mM inhibited KA currents by 11 ± 6, 15 ± 2, 27 ± 4, and 30 ± 4% (n = 10–14), respectively (Figs. 4 and 5). In cells expressing GluR6 + KA1 receptors, the same ethanol concentrations inhibited KA currents by 4 ±...
Effects of ethanol on heteromeric GluR5 receptors were tested with GluR5-R receptors plus KA1 or KA2, because GluR5-R receptors produce functional currents only as heteromers. We did not test GluR5-Q plus KA1 or KA2 subunits because there is not a straightforward test to determine whether the receptors are indeed heteromeric on each cell where ethanol’s effects are tested; i.e., GluR5-Q receptors in the presence or absence of KA1 or KA2 subunits are activated to a similar extent by 1 mM AMPA (Herb et al., 1992; Koreczak et al., 1995). Reproducible currents were obtained by activating these receptors with KA; the EC_{50} for activation of GluR5-R + KA1 receptors was 4.2 μM (95% confidence interval 3.4–5.1 μM) and the Hill coefficient was 0.9 (95% confidence interval 0.7–1.0; n = 3; Fig. 7). The EC_{50} for activation of GluR5-R + KA2 receptors was 6.6 μM (95% confidence interval 5.0–8.7 μM) and the Hill coefficient was 0.9 (95% confidence interval 0.7–1.0; n = 5–6; Fig. 7). Ethanol concentrations of 25, 50, 75, and 100 mM inhibited GluR5-R + KA1 receptor currents (EC_{20–50}) by 4 ± 2, 14 ± 3, 14 ± 5, and 16 ± 3%, respectively (n = 5–6; Fig. 7). Ethanol concentrations of 25, 50, 75, and 100 mM inhibited GluR5 + KA2 receptor currents (EC_{20–50}) by 8 ± 4, 21 ± 3, 23 ± 4, and 27 ± 7%, respectively (n = 5; Fig. 7). Statistical analysis (one-sample Student’s t test versus a theoretical mean of zero and one-way ANOVA followed by Dunnett’s multiple comparison test) revealed that ethanol produced significant (p < .05) inhibition of KA currents mediated by these receptors at all concentrations tested. It also indicated that there was not any significant difference in the inhibition produced by the different concentrations of ethanol tested. Two-way ANOVA revealed that GluR5-R + KA2 receptors were inhibited significantly more by ethanol than GluR5-Q and GluR5-R + KA1 receptors (p < .001).

Discussion

The results of the present study demonstrate that the function of recombinant KA receptors with different subunit compositions is acutely inhibited by ethanol. Previous studies on the actions of ethanol on recombinant KA receptors focused on homomeric GluR6 receptors expressed in Xenopus oocytes or HEK 293 cells, and these studies reported that 25 to 100 mM ethanol acutely inhibited the function of these receptors by 10 to 30% (Dildy-Mayfield and Harris 1995; Valenzuela et al., 1998b). We now report the effects of ethanol on homomeric KA receptors composed of GluR5-Q and heteromeric KA receptors composed of GluR6-R or GluR5-R plus KA1 or KA2 subunits. We found that ethanol inhibits, to
a similar extent, KA receptors with all of these subunit compositions. We detected statistically significant differences in only two cases: GluR6 \( + KA2 \) receptors were inhibited significantly less than GluR6 and GluR6 \( + KA1 \) receptors, and GluR5-R \( + KA2 \) receptors were inhibited significantly more than GluR5-Q and GluR5-R \( + KA1 \) receptors. However, the differences between receptors with different subunit compositions were relatively small.

Present results with recombinant KA receptors are in agreement with a recent study from our laboratory with native KA receptors. We reported that acute exposure to 25 to 100 mM ethanol inhibited pharmacologically isolated KA receptor currents by 5 to 25\% in cerebellar granule cells (Valenzuela et al., 1998a). In these cells, the majority of KA receptors were reported to contain either GluR5-R, GluR5-Q, or GluR6-Q subunits; GluR6-Q subunits appear to associate to KA2 subunits in these cells (Savidge et al., 1997; Pember-ton et al., 1998). In cerebellar granule cells, we found that ethanol acutely inhibited NMDA receptors to a greater extent than either AMPA or KA receptors (Valenzuela et al., 1998a). Thus, NMDA receptors may be more important targets than KA receptors in the acute actions of pharmacologically relevant concentrations of ethanol. It should be emphasized, however, that the magnitude of ethanol’s acute effects on KA receptor function might depend on the brain region studied. We recently found that KA receptor-mediated synaptic currents in the CA3 region of the hippocampus are inhibited by 11 to 50\% in the presence of 20 to 80 mM ethanol (J. L. Weiner, T. V. Dunwiddie, and C. F. Valenzuela, unpublished observation). It would be interesting to study ethanol’s actions on KA receptors expressed in neurons from central nervous system (CNS) regions other than the hippocampus.
and cerebellum and to establish the factors that determine this differential sensitivity of KA receptors to ethanol. This study and a previous one (Valenzuela et al., 1998b) suggest that neither subunit composition nor protein phosphorylation modulate acute ethanol’s actions on recombinant KA receptors. Whether these factors, or other factors such as receptor clustering, localization and/or association with neuronal specific proteins, play a role in determining the sensitivity of native KA receptors to ethanol remains to be elucidated.

A challenging question for future study will be to locate the molecular site of action of ethanol on KA receptors and other ionotropic glutamate receptors. In the case of γ-aminobutyric acidA (GABA\textsubscript{A}) and glycine receptors, ethanol was reported to interact with a specific binding pocket located between transmembrane domains M2 and M3 (Mihic et al., 1997; Wick et al., 1998). Minami et al. (1998) found that in GluR6 receptors a specific amino acid, residue Gly-819 in M4, is important for the actions of the volatile anesthetics halothane, isoflurane, and enfurane. However, this residue does not appear to play a role in ethanol’s actions on GluR6 KA receptors. Thus, more work will be required to determine the mechanism of action of ethanol on KA receptors at the molecular level.

Another question for future research will be to determine whether the acute effects of ethanol on KA receptors contribute significantly to the pathophysiology of acute intoxication.
also affects the function of NMDA, GABA<sub>A</sub>, voltage-gated ion channels, and/or metabotropic receptors, could contribute to the pathophysiology of acute ethanol intoxication. Clearly, more work will be required to test this hypothesis. For instance, it would be interesting to measure the acute effects of ethanol on the GluR6 knockout mice developed by Mulle et al. (1998).

In conclusion, we found that recombinant KA receptors composed of GluR5 or GluR6 subunits in the absence and presence of KA1 or KA2 subunits are all acutely inhibited by ethanol. Ethanol inhibited to a similar extent these recombinant KA receptors with different subunit compositions. It would be interesting to determine whether alcohol modulates in a similar manner native KA receptors with different subunit configurations in neurons of various CNS regions.

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References


