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Ethanol Inhibition of Nicotinic Acetylcholine Type α7 Receptors Involves the Amino-Terminal Domain of the Receptor

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SUMMARY

Recent studies have suggested that alcohols can affect the function of neurotransmitter-gated ion channels by a direct interaction with the receptor protein. However, the molecular region of the receptor protein that mediates the alcohol action is not known. To address this question, we studied the effect of ethanol on the function of recombinant nicotinic acetylcholine type α7 ($nACh_{\alpha7}$) receptors, 5-hydroxytryptamine (serotonin) type 3 ($5\text{-}HT_3$) receptors, and a chimeric receptor constructed from these two receptors. The receptors were expressed in *Xenopus* oocytes and their function was studied using the two-electrode voltage-clamp technique. Ethanol inhibited the response of $nACh_{\alpha7}$ receptors in a concentration-dependent manner over the concentration range of 5–100 mM; the EC_{50} for this inhibition was 33 mM ethanol. Ethanol decreased the maximal amplitude (E_{max}) of the $nACh_{\alpha7}$ receptor agonist concentration-response curve, without significantly affecting the EC_{50}. In contrast, ethanol potentiated $5\text{-}HT_3$ receptor-mediated responses at low agonist concentrations. The potentiation was concentration-dependent over the concentration range of 10–100 mM; the EC_{50} for this potentiation was 57 mM ethanol. The magnitude of the ethanol potentiation of $5\text{-}HT_3$ receptor-mediated responses decreased with increasing agonist concentration. The chimeric receptor had the amino-terminal domain from the $nACh_{\alpha7}$ receptor and the transmembrane and carboxyl-terminal domains from the $5\text{-}HT_3$ receptor. Ethanol was found to inhibit the function of this chimeric receptor in a manner similar to that of $nACh_{\alpha7}$ receptors. Because the inhibition transfers with the amino-terminal domain of the receptor, the observations suggest that the amino-terminal domain of the receptor is involved in the inhibition.

Traditionally, alcohol effects on membrane proteins have been thought to be secondary to perturbation of membrane lipids (1). A number of different types of neurotransmitter-gated ion channels have been found to be sensitive to the effect of alcohols (2), and recent studies have suggested that alcohol effects on the function of some neurotransmitter-gated ion channels are mediated by direct interaction with the receptor protein. The inhibition of an ATP-gated ion channel by a series of alcohols exhibits a distinct cutoff effect (3). The inhibitory potency of alcohols with a molecular volume of ≤42.2 ml/mol is correlated with their hydrophobicity (1-propanol = trifluoroethanol > monochloroethanol > ethanol > methanol), whereas alcohols with a molecular volume of ≥46.1 ml/mol (1-butanol, 1-pentanol, trichloroethanol, and dichloroethanol) do not affect the function of the ATP-gated channels. These observations have been interpreted as indicating that the alcohols inhibit the function of this receptor by interacting with a hydrophobic pocket, of circumscribed dimensions, on the receptor protein. Alcohol inhibition of NMDA receptors also exhibits a cutoff effect (4). However, in contrast to the ATP-gated channels, the cutoff for inhibition of NMDA receptors is observed for *n*-alcohols with a molecular volume greater than that of 1-octanol. These observations have been interpreted as evidence that alcohols inhibit the function of NMDA receptors by interaction with a hydrophobic pocket on NMDA receptors, although that pocket appears to be larger than the hydrophobic pocket on ATP-gated channels. In addition, several preliminary studies have found that other types of neurotransmitter-gated ion channels also exhibit a cutoff effect in response to a series of *n*-alcohols, and the cutoff appears to be different for each

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ABBREVIATIONS: NMDA, *N*-methyl-D-aspartate; nACh, nicotinic acetylcholine; $nACh_{\alpha7}$, nicotinic acetylcholine type α7; 5-HT, 5-hydroxytryptamine; HEPES, *N*-2-hydroxyethylpiperazin-*N'*-2-ethanesulfonic acid; ANOVA, analysis of variance.

receptor type studied (5–9). Although these observations suggest that alcohols affect the function of certain neurotransmitter-gated ion channels by direct interaction with the receptor protein, they do not provide evidence on the molecular region of the protein involved in this interaction.

Recombinant chimeric membrane proteins have been extremely valuable for determining relationships between functional properties and defined structural domains of the protein (10–13). A chimeric receptor has been constructed from two different types of neurotransmitter-gated ion channels, the $nACh_{\alpha 7}$ receptor and the 5-HT_3 receptor (14). In this chimera, the amino-terminal domain is from the $nACh_{\alpha 7}$ receptor and the transmembrane and carboxyl-terminal domains are from the 5-HT_3 receptor. This chimeric receptor manifests activation by nicotinic agonists and the channel specificities of 5-HT_3 receptors. It seemed likely that, if alcohols have different effects on $nACh_{\alpha 7}$ receptors and 5-HT_3 receptors, the chimera of these receptors should reveal whether the alcohol action is associated with the amino-terminal or the transmembrane and carboxyl-terminal domains of the receptor. Alcohols have previously been found to potentiate 5-HT_3 receptor-mediated responses (15, 16). In the study reported here, we used *Xenopus laevis* oocytes as an expression system and the two-electrode voltage-clamp technique to characterize the effect of ethanol on recombinant $nACh_{\alpha 7}$ and 5-HT_3 receptors. We found that ethanol inhibited the $nACh_{\alpha 7}$ receptor-mediated responses and potentiated the 5-HT_3 receptor-mediated responses. Because these effects are opposite, we studied the effect of ethanol on the chimera constructed from these two receptors. We found that ethanol inhibited the function of the $nACh_{\alpha 7}$-5-HT_3 chimeric receptor, with properties that were similar to the properties of the ethanol inhibition of $nACh_{\alpha 7}$ receptor-mediated responses. These observations suggest that the amino-terminal domain of the receptor is involved in the mediation of this inhibitory effect of ethanol. Some of this work has been presented previously in preliminary form (17, 18).

Materials and Methods

Construction of the chimeric receptor. The chimeric $nACh_{\alpha 7}$-5-HT_3 receptor was constructed using two methods. One method of construction of the receptor has been described previously (14). For the second method, the $nACh_{\alpha 7}$ and 5-HT_3 receptor cDNAs were generously provided by Drs. Jon Lindstrom (University of Pennsylvania, Philadelphia, PA) (19) and David Julius (University of California, San Francisco, CA) (20), respectively. The switch point between the amino-terminal domain of the $nACh_{\alpha 7}$ receptor and the transmembrane and carboxyl-terminal domains of the 5-HT_3 receptor was made at V201 of the $nACh_{\alpha 7}$ receptor, as described previously (14). Two primers (5′-CCGCGGAACAATGGGCCTCCGGGCG-3′ and 5′-TGATCACTGTGAATGTGATATCTGG-3′) were made to synthesize a fragment within the amino-terminal portion of the $nACh_{\alpha 7}$ receptor, using the polymerase chain reaction. To replace the amino-terminal fragment of the 5-HT_3 receptor with a *Bcl*I site at the 3′-end and a *Sac*II site at the 5′-end, the polymerase chain reaction product generated from these two primers was digested with *Bcl*I and *Sac*II and ligated with the fragment of the 5-HT_3 receptor in Bluescript SK(+) plasmid digested with the same enzymes. The *Bcl*I-digested fragment left over from the 5-HT_3 receptor cDNA was then ligated back to the construct. The chimera was confirmed by sequence analysis. The effects of ethanol on the chimeras constructed by these two methods were not significantly different.

Preparation of cRNA and expression of receptors. Complementary RNA was synthesized *in vitro* from linearized template cDNA with a mMACHINE RNA transcription kit from Ambion Inc. (Austin, TX). Mature *X. laevis* frogs were anesthetized by submersion in 0.2% 3-aminobenzoic acid ethyl ester (Sigma Chemical, St. Louis, MO), and a group of oocytes was surgically excised. The oocytes were separated and the follicular cell layer was removed by treatment with type I collagenase (Boehringer Mannheim, Indianapolis, IN) for 2 hr at room temperature. Each oocyte was injected with a total of 10 ng of RNA in 50 nl of diethylpyrocarbonate-treated water and was incubated at 19° in modified Barth's solution [88 mM NaCl, 1 mM KCl, 2.4 mM $NaHCO_3$, 0.3 mM $Ca(NO_3)_2$, 2.2 mM $CaCl_2$, 0.8 mM $MgSO_4$, 10 mM HEPES, pH 7.5].

Electrophysiological recording. After 2–5 days of incubation, the oocytes were studied at room temperature (23 ± 2°) in a 90-μl chamber. The oocytes were superfused at a rate of approximately 8 ml/min, with a bathing solution containing 82.5 mM NaCl, 2.5 mM KCl, 2.5 mM $CaCl_2$, 1 mM $MgCl_2$, 5 mM HEPES, and 0.5 μM atropine. In some experiments, the 2.5 mM $CaCl_2$ was replaced with an equimolar concentration of $BaCl_2$, as noted. Agonists and ethanol were diluted in the bathing solution and applied to the oocytes for a specified time, using a solenoid valve. Membrane currents were studied under two-electrode voltage-clamp conditions at a holding potential of −70 mV, using an Axoclamp 2A amplifier (Axon Instruments, Foster City, CA). The recording microelectrodes were filled with 3 M KCl and had resistances of 0.5–3.0 MΩ. Data were routinely recorded on a chart recorder (Gould 2400S), and in experiments on receptor desensitization the data were also filtered at 2 kHz, digitized at 1–5 kHz, and collected on a microcomputer, using pClamp software (Axon Instruments), for later analysis.

Data analysis. Values are expressed as mean ± standard error, and data were statistically compared by the paired *t* test or ANOVA, as appropriate. Concentration-response curves were obtained by fitting the data to a logistic equation, $y = \{(E_{max} - E_{min})/(1 + [x/EC_{50}]^{-n})\} + E_{min}$, where y is the response, x is the concentration, E_{max} is the maximal response, E_{min} is the minimal response, EC_{50} is the concentration producing 50% of the maximal response, and n is the slope factor (apparent Hill coefficient).

Results

Effect of ethanol on $nACh_{\alpha 7}$ receptors. Fig. 1 illustrates the effect of ethanol on the function of $nACh_{\alpha 7}$ receptors. The records in Fig. 1A show that the inward current activated by 10 μM nicotine was markedly decreased in amplitude by 100 mM ethanol. In the agonist concentration-response curve (Fig. 1B), the amplitude of the nicotine-activated inward current (control) was concentration-dependent over the concentration range of 1 to 100 μM nicotine. The amplitude of current activated by 200 μM nicotine was not significantly different from the current activated by 100 μM nicotine (ANOVA, $p > 0.05$). The EC_{50} for the agonist-activated concentration-response curve was 13 ± 3.5 μM nicotine, and the apparent Hill coefficient was 1.2. In the presence of 100 mM ethanol, E_{max} was significantly decreased (47 ± 9% of control; $p < 0.001$), whereas the EC_{50} (15.6 ± 1.7 μM nicotine) and apparent Hill coefficient (1.2) were not significantly different from control (ANOVA, $p > 0.05$). The inhibition by 100 mM ethanol at 1, 5, 10, 50, 100, and 200 μM nicotine was 58 ± 4, 55 ± 1, 63 ± 9, 56 ± 2, 53 ± 9, and 53 ± 7%, respectively; these values are not significantly different (ANOVA, $p > 0.05$). The concentration-response curve for ethanol inhibition of $nACh_{\alpha 7}$ receptor-mediated responses (Fig. 1C) indicates that the inhibition was concentration-dependent over a concentration range of 5 to 100 mM ethanol.

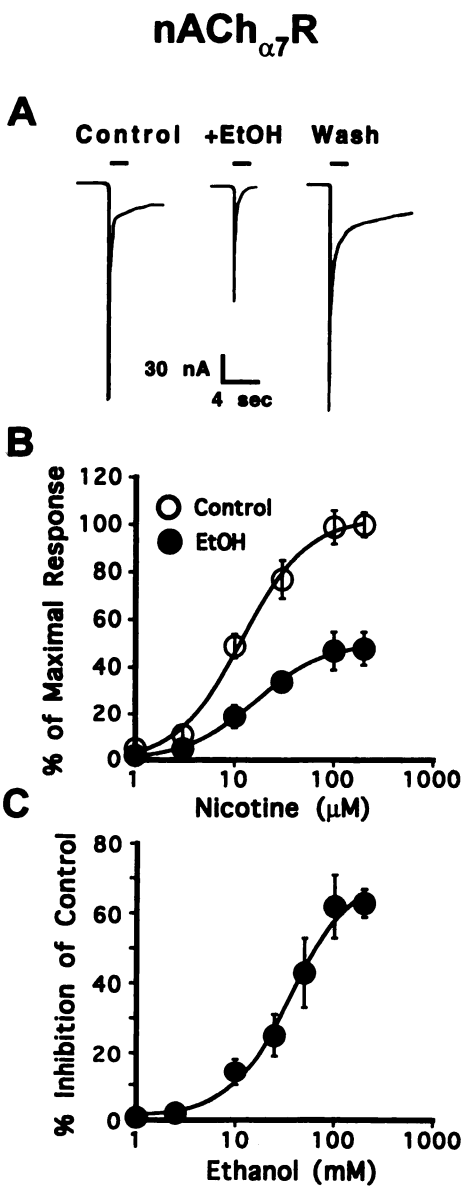

Fig. 1. Ethanol inhibition of nACh$_{\alpha7}$ receptor (*nACh$_{\alpha7}$R*) responses. A, Records of nACh$_{\alpha7}$ receptor-mediated ion current activated by the application of 10 μM nicotine and the inhibition of that current by 100 mM ethanol (*EtOH*). *Bar above each record*, time of agonist application. B, Agonist concentration-response curves for nACh$_{\alpha7}$ receptor-mediated current in the absence (○) and presence (●) of 100 mM ethanol. C, Concentration dependence of ethanol inhibition of nACh$_{\alpha7}$ receptor-mediated current. Current was activated by 10 μM nicotine. In A–C, agonist application was every 10 min. Each data point in B and C represents the mean ± standard error of seven oocytes; error bars not visible are smaller than the size of the symbols. The *curves* in B and C were obtained by fitting the data to the logistic equation given in Materials and Methods.

The inhibition by 200 mM ethanol was not significantly different from the inhibition by 100 mM ethanol ($p > 0.05$). The EC_{50} for ethanol inhibition of nACh$_{\alpha7}$ receptor-mediated responses was 33 mM, and the apparent Hill coefficient was 1.5.

The nACh$_{\alpha7}$ receptor channels have been found to be highly permeable to Ca^{2+}, so that the agonist-activated nACh$_{\alpha7}$ receptor-mediated current in *X. laevis* oocytes is the sum of the inward cation current carried by the nACh$_{\alpha7}$ receptor channels and a Cl^- current carried by Ca^{2+}-activated Cl^- channels endogenous in the oocyte membrane (21–23). Because Ba^{2+} does not activate these Ca^{2+}-activated Cl^- channels (24), to determine whether the inhibition of nicotine-activated current by ethanol is the result of inhibition of nACh$_{\alpha7}$ receptor channel-mediated current or Ca^{2+}-activated Cl^- current, we replaced the 2.5 mM Ca^{2+} in the extracellular bathing solution with 2.5 mM Ba^{2+}. Fig. 2A illustrates nACh$_{\alpha7}$ receptor-mediated current in the presence of Ca^{2+} (Fig. 2A, *left*) and in the Ca^{2+}-free bathing solution containing 2.5 mM Ba^{2+} (Fig. 2A, *right*). The amplitude of current activated by 10 μM nicotine in the Ca^{2+}-free/Ba^{2+} solution was smaller than the current activated by 10 μM nicotine in the presence of Ca^{2+} in the bathing solution (455 nA versus 777 nA). However, even though the amplitude of the nACh$_{\alpha7}$ receptor-mediated current was smaller in the Ca^{2+}-free solution, the percentage inhibition of nACh$_{\alpha7}$ receptor-mediated current by 100 mM ethanol was not significantly different between the Ca^{2+}-containing solution and the Ca^{2+}-free solution (63 ± 9% versus 59 ± 4%; $p > 0.05$).

The functional response of the muscle-type nACh receptor is potentiated by ethanol (25–28). However, the muscle-type

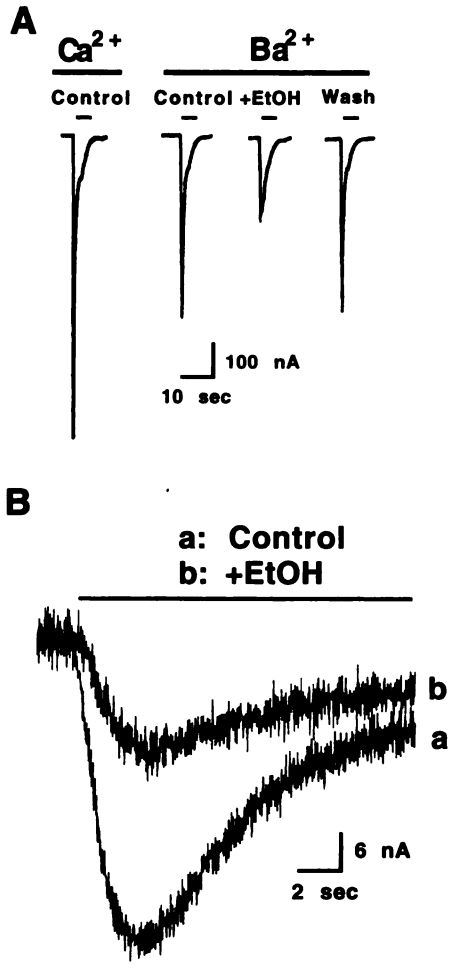

Fig. 2. A, Effect of Ca^{2+}-free bathing solution on ethanol inhibition of nACh$_{\alpha7}$ receptor-mediated current. *Left record*, current activated by 10 μM nicotine in normal extracellular solution containing 2.5 mM Ca^{2+}. *Right set of records*, current activated by 10 μM nicotine in a Ca^{2+}-free extracellular bathing solution containing 2.5 mM Ba^{2+}, in the absence and presence of 100 mM ethanol (*EtOH*). B, Desensitization of nACh$_{\alpha7}$ receptor-mediated current in the absence (*a*) and presence (*b*) of 100 mM ethanol. Current was activated by 1 μM nicotine. *Solid bars above traces*, duration of agonist application.

nACh receptor can be inhibited by long-chain alcohols, and this inhibition is associated with an increased rate of decay of the agonist-activated response (27, 29). To evaluate whether an increased decay rate might account for the ethanol inhibition of $nACh_{\alpha 7}$ receptors, we examined whether ethanol inhibition of $nACh_{\alpha 7}$ receptor-mediated current is associated with a change in the decay of the $nACh_{\alpha 7}$ receptor-mediated current. Fig. 2B illustrates currents activated by 1 μM nicotine in the absence and presence of 100 mM ethanol. The decay time constant (τ) for the current activated by 1 μM nicotine was 8.4 ± 0.8 sec in the absence of ethanol (control) and 8.8 ± 0.9 sec in the presence of 100 mM ethanol; these values are not significantly different ($p > 0.05$).

Effect of ethanol on 5-HT_3 receptors. Fig. 3 illustrates the effect of ethanol on the function of 5-HT_3 receptors. The records in Fig. 3A show that, in oocytes expressing 5-HT_3 receptors, the amplitude of current activated by 0.5 μM 5-HT was potentiated to an increasing extent by 20, 40, and 80 mM ethanol. The ethanol concentration-response curve for current activated by 0.5 μM 5-HT (Fig. 3B) indicates that the potentiation of 5-HT_3 receptor-mediated current was concentration-dependent over the concentration range of 10 to 100 mM. The potentiation of 5-HT_3 receptor-mediated current by 200 mM ethanol was not significantly different from that observed for 100 mM ethanol ($p > 0.05$). The maximal increase of 5-HT-activated current was 78 ± 8% ($p < 0.001$). The EC_{50} for the ethanol augmentation was 57 mM, and the apparent Hill coefficient was 2.2. The records in Fig. 3C

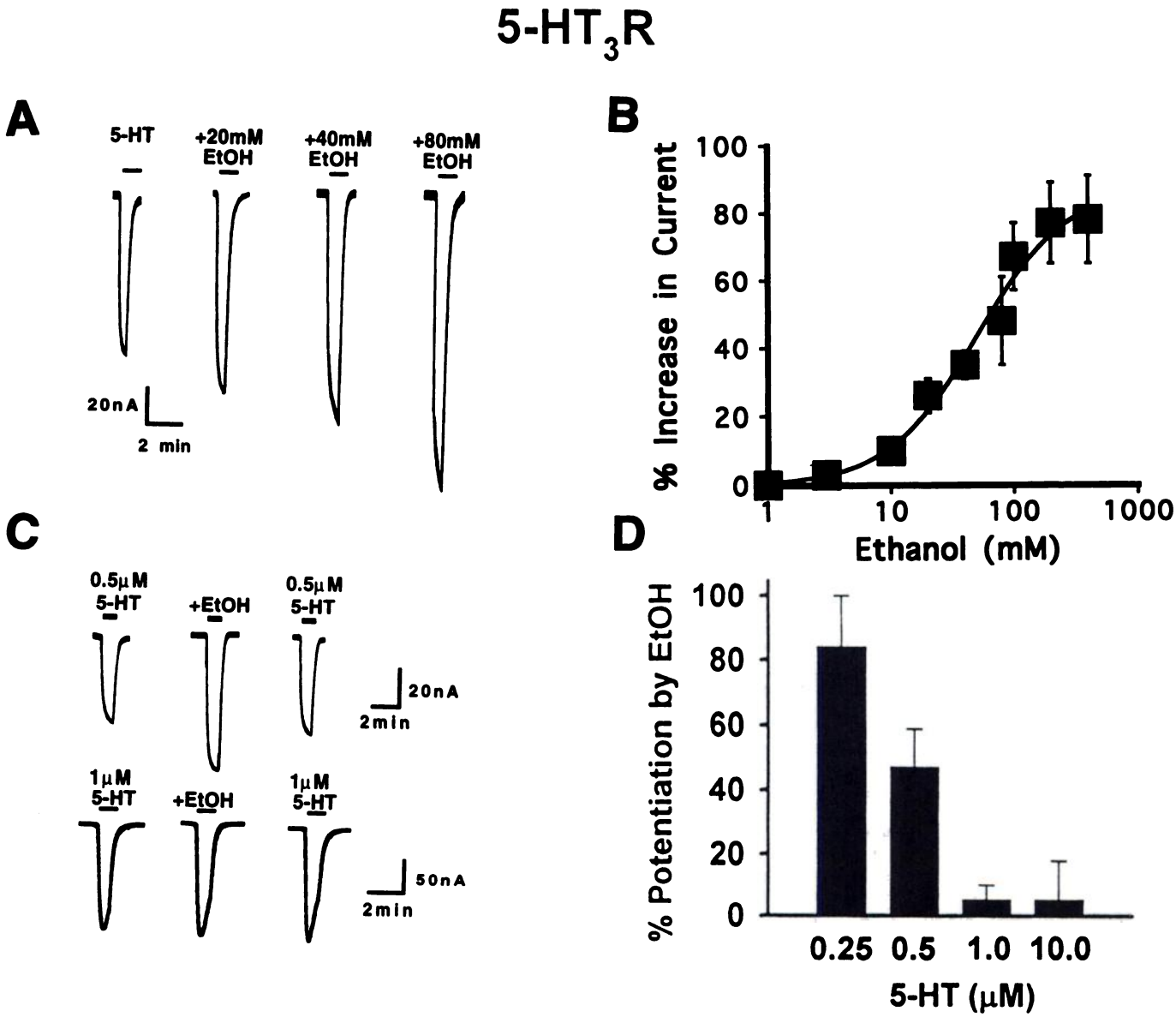

Fig. 3. Ethanol potentiation of 5-HT_3 receptor (*5-HT_3R*) responses. A, Records of 5-HT_3 receptor-mediated ion current activated by the application of 0.5 μM 5-HT, and the potentiation of that current by 20, 40, and 80 mM ethanol (*EtOH*). B, Concentration dependence of ethanol potentiation of 5-HT_3 receptor-mediated current. Current was activated by 0.5 μM 5-HT. Each data point represents the average of 13 oocytes. C, Records of 5-HT_3 receptor-mediated current activated by 0.5 and 1 μM 5-HT, and the effect of 80 mM ethanol on those currents. Note that ethanol potentiated the current activated by 0.5 μM 5-HT but had little effect on the current activated by 1 μM 5-HT. D, Dependence of ethanol potentiation of 5-HT_3 receptor-mediated current on agonist concentration. *Bars*, mean ± standard error of percentage potentiation of 5-HT_3 receptor-mediated current activated by 0.25, 0.5, 1, and 10 μM 5-HT; each bar represents the average of 10 oocytes.

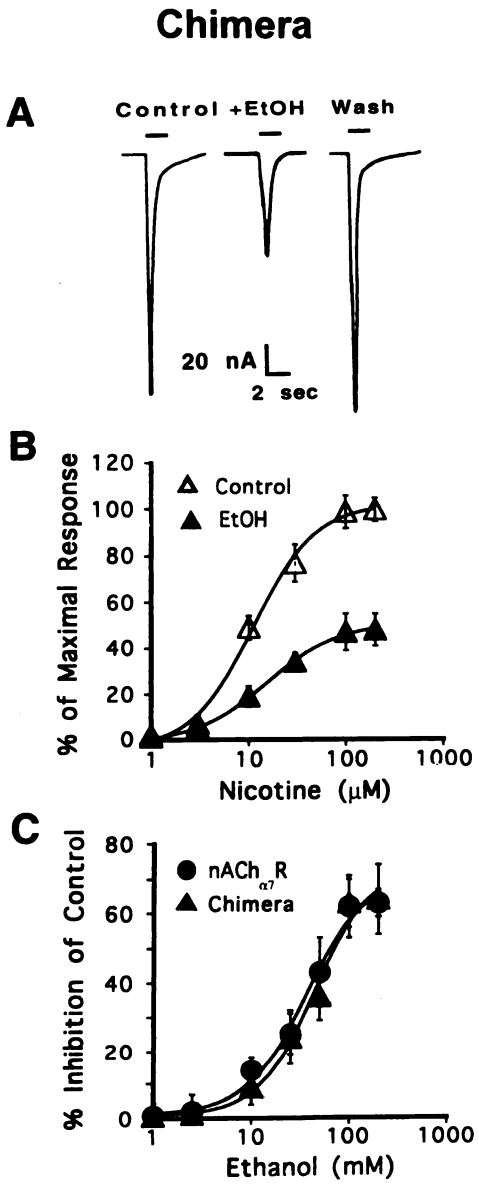

Fig. 4. Ethanol inhibition of the nACh$_{\alpha7}$-5-HT$_3$ chimeric receptor, with the extracellular amino-terminal domain from the nACh$_{\alpha7}$ receptor and the transmembrane and carboxyl-terminal domains from the 5-HT$_3$ receptor. A, Records of chimeric receptor-mediated ion current activated by 10 μM nicotine and the inhibition of that current by 100 mM ethanol (*EtOH*). B, Agonist concentration-response curves for chimeric receptor-mediated current in the absence (△) and presence (▲) of 100 mM ethanol. Each data point represents the average of seven oocytes. C, Concentration dependence of ethanol inhibition of chimeric receptor-mediated current (▲). Current was activated by 10 μM nicotine. Each data point represents the average of seven oocytes. For comparison, the concentration dependence for ethanol inhibition of nACh$_{\alpha7}$ receptor-mediated current (from Fig. 1C) is also shown (●). The data points for 100 and 200 mM ethanol are superimposed.

illustrate that, although 80 mM ethanol increased the amplitude of 5-HT$_3$ receptor-mediated current activated by 0.5 μM 5-HT, this concentration of ethanol had little effect on the current activated by 1 μM 5-HT. The bar graphs in Fig. 3D plot the average potentiation of 5-HT$_3$ receptor-mediated current induced by 80 mM ethanol at several 5-HT concentrations. On average, 80 mM ethanol increased 5-HT$_3$ receptor-mediated current activated by 0.25 μM 5-HT by 84 ± 16% ($p < 0.001$) and current activated by 0.5 μM 5-HT by 47 ± 12% ($p < 0.001$) but did not significantly affect the current activated by 1 or 10 μM 5-HT ($p > 0.05$). Because ethanol potentiated only 5-HT$_3$ receptor-mediated current activated by low agonist concentrations, it did not significantly affect the EC_{50} or E_{max} of the agonist concentration-response curve (data not shown; $p > 0.05$). The EC_{50} for the 5-HT concentration-response curve was 3.1 μM.

Effect of ethanol on the nACh$_{\alpha7}$-5-HT$_3$ chimeric receptor. As described in Materials and Methods, a chimeric receptor was constructed with the extracellular amino-terminal domain from the nACh$_{\alpha7}$ receptor and the transmembrane and carboxyl-terminal domains from the 5-HT$_3$ receptor. Fig. 4 illustrates the effect of ethanol on the function of this chimeric receptor. The records in Fig. 4A show that this chimeric receptor was activated by a nicotinic agonist, as reported previously (14). Fig. 4A also shows that the chimeric receptor-mediated ion current was markedly inhibited by 100 mM ethanol. In the agonist concentration-response curve (Fig. 4B), the amplitude of the agonist-activated current (control) was concentration-dependent over the concentration range of 1 to 100 μM nicotine. The amplitude of the current activated by 200 μM nicotine was not significantly different from that of the current activated by 100 μM nicotine (ANOVA, $p > 0.05$). The EC_{50} for the agonist-activated concentration-response curve was 7.4 ± 3.5 μM nicotine, and the apparent Hill coefficient was 1.2. In the presence of 100 mM ethanol, E_{max} was significantly decreased (46 ± 7% of control; $p < 0.001$), whereas the EC_{50} (6.3 ± 3.2 μM nicotine) and apparent Hill coefficient (1.7) were not significantly different from control (ANOVA, $p > 0.05$). The inhibition by 100 mM ethanol at 1, 5, 10, 50, 100, and 200 μM nicotine was 66 ± 5%, 57 ± 6%, 62 ± 6% 56 ± 7%, 54 ± 8%, and 53 ± 6%, respectively; these values are not significantly different (ANOVA, $p > 0.05$). The concentration-response curve for ethanol inhibition of the chimeric receptor-mediated responses (Fig. 4C) indicates that the inhibition was concentration-dependent over a concentration range from 5 to 100 mM ethanol. The inhibition by 200 mM ethanol was not significantly different from the inhibition by 100 mM ethanol ($p > 0.05$). The EC_{50} for ethanol inhibition of chimeric receptor-mediated responses was 41 mM, and the apparent Hill coefficient was 1.6. The comparison of the inhibitory effects of ethanol on chimeric and nACh$_{\alpha7}$ receptor-mediated currents in Fig. 4C shows that the ethanol inhibition of the chimeric receptor-mediated current appears similar to the ethanol inhibition of nACh$_{\alpha7}$ receptor-mediated current. In this regard, the ethanol inhibition of the chimeric receptor-mediated current was not significantly different from the ethanol inhibition of nACh$_{\alpha7}$ receptor-mediated current with respect to E_{max}, EC_{50}, and apparent Hill coefficient (ANOVA, $p > 0.05$).

Discussion

In this study, we found that ethanol inhibited the functional response of nACh$_{\alpha7}$ receptors in a concentration-dependent manner over the concentration range of 5 to 100 mM ethanol, with an EC_{50} of 33 mM ethanol. The ethanol inhibition of nACh$_{\alpha7}$ receptor-mediated responses was characterized by a decrease in the E_{max} of the agonist concentration-response curve, without a significant change in the EC_{50} or the apparent Hill coefficient. This indicates that the ethanol inhibition of nACh$_{\alpha7}$ receptor-mediated responses involves a

noncompetitive type of inhibition. The observation that the E_{max} of the ethanol concentration-response curve showed an inhibition of ~64% indicates that ethanol does not totally inhibit agonist gating of this ion channel. The allosteric inhibition of $nACh_{\alpha 7}$ receptor-mediated responses by ethanol is similar to that observed with NMDA receptors, in that it also involves a noncompetitive type of inhibition (30). In contrast, the inhibition of $nACh_{\alpha 7}$ receptor-mediated responses by ethanol differs from the ethanol inhibition of ATP-gated ion channel responses, because ethanol shifts the EC_{50} for the ATP concentration-response curve to the right without significantly affecting the E_{max} of this response (31). The effect of ethanol on $nACh_{\alpha 7}$ receptor-mediated responses also differs from the effect of ethanol on muscle-type nACh receptors. As noted above, the responses of muscle-type nACh receptors are potentiated by ethanol (25–28), whereas we found only concentration-dependent inhibition of $nACh_{\alpha 7}$ receptor-mediated responses by ethanol. In addition, although long-chain alcohols can inhibit the response of muscle-type nACh receptors, this inhibition is associated with an increased rate of decay of the agonist-activated responses (27, 29), whereas we did not find a change in the decay rate of $nACh_{\alpha 7}$ receptor-mediated responses associated with the ethanol inhibition of these receptors. The difference between ethanol effects on the function of muscle-type nACh receptors and the neuronal $nACh_{\alpha 7}$ receptors is no doubt the result of the structural differences based on subunit composition; the muscle-type nACh receptors are composed of four different types of subunits, whereas the $nACh_{\alpha 7}$ receptors are homomeric (32).

We also confirmed that ethanol can potentiate the functional response of $5\text{-}HT_3$ receptors. The ethanol potentiation of $5\text{-}HT_3$ receptor-mediated responses was maximal at low agonist concentrations and decreased in magnitude as agonist concentration was increased. With 0.5 μM 5-HT, the ethanol potentiation of $5\text{-}HT_3$ receptor-mediated responses was concentration-dependent over the concentration range of 10 to 100 mM ethanol, with an EC_{50} of 57 mM ethanol. The reported effects of ethanol on $5\text{-}HT_3$ receptor-mediated responses in NCB-20 cells and nodose neurons in previous studies were similar to the effects of ethanol on the recombinant $5\text{-}HT_3$ receptor-mediated responses reported here in that ethanol potentiated $5\text{-}HT_3$ receptor-mediated responses at low agonist concentrations and the magnitude of the potentiation decreased with increasing agonist concentration (15). However, the ethanol effects on $5\text{-}HT_3$ receptor-mediated responses in NCB-20 cells and nodose neurons differ from the effects on the recombinant receptor responses reported here in that ethanol potentiated currents activated by 1 and 2 μM 5-HT in the NCB-20 cells and nodose neurons (15), whereas we found that ethanol did not potentiate the response to $\geq$1 μM 5-HT concentrations for the recombinant $5\text{-}HT_3$ receptors. On the other hand, our observations on recombinant $5\text{-}HT_3$ receptors are comparable to those of another study of ethanol effects on recombinant $5\text{-}HT_3$ receptors (16), except that the EC_{50} for potentiation in that study was 100 mM ethanol for current activated by 0.75 μM 5-HT, whereas the EC_{50} for potentiation in our experiments was 57 mM for current activated by 0.5 μM 5-HT. The mechanism responsible for the ethanol potentiation of $5\text{-}HT_3$ receptor-mediated responses is not known. Potentiation does not appear to be the result of an alteration of the ion permeance ratio of the channel, because ethanol does not change the reversal potential of 5-HT-activated currents (15). In addition, it has been reported that ethanol does not alter binding to $5\text{-}HT_3$ receptors in membranes from NCB-20 cells and rat cerebral cortex (33). The potentiation has been attributed to an increase in the apparent potency of 5-HT for activating ion current for $5\text{-}HT_3$ receptors (15).

As noted above, the chimeric neurotransmitter receptor constructed with the amino-terminal domain from the $nACh_{\alpha 7}$ receptor and the transmembrane and carboxyl-terminal domains from the $5\text{-}HT_3$ receptor exhibits distinct functional properties associated with these structural elements [i.e., activation by nicotinic agonists and the channel properties of $5\text{-}HT_3$ receptors (14)]. The observations that ethanol can inhibit $nACh_{\alpha 7}$ receptor-mediated ion current and potentiate $5\text{-}HT_3$ receptor-mediated ion current provided the opportunity to investigate the structural element associated with these modulations of receptor function. Considering that in the chimeric receptor only the amino-terminal domain is derived from the $nACh_{\alpha 7}$ receptor, our observation that ethanol inhibited the chimeric receptor-mediated current in a manner that was not significantly different from the ethanol inhibition of $nACh_{\alpha 7}$ receptor-mediated current suggests that the ethanol inhibition of the $nACh_{\alpha 7}$ receptor involves the amino-terminal domain of the receptor. However, the possibility that ethanol binds to another region and produces an allosteric action requiring the amino-terminal domain cannot be excluded. Because the chimeric receptor does not contain the transmembrane domains of the $nACh_{\alpha 7}$ receptor, these observations also suggest that the inhibition of this neurotransmitter-gated ion channel by ethanol does not involve a primary interaction of ethanol with the membrane lipids, which by their perturbation secondarily affect the function of the transmembrane domains of the receptor. Thus, the observations reported here provide evidence, independent of a cutoff effect, that supports the protein hypothesis of alcohol action. The chimeric receptor also does not contain the $nACh_{\alpha 7}$ receptor ion channel pore, which is formed by the transmembrane domains of the receptor. Consequently, it seems unlikely that the ethanol inhibition of this receptor involves an action of ethanol in the ion channel pore. However, it has been suggested that alcohols inhibit the function of muscle-type nACh receptors and Shaw2-type K^+ channels by an action in or near the ion channel pore (34, 35).

With respect to the sequence elements of the $5\text{-}HT_3$ receptor involved in the ethanol potentiation of the responses of these receptors, because the chimeric receptor is composed of the transmembrane and carboxyl-terminal domains from the $5\text{-}HT_3$ receptor, if the potentiation involves the transmembrane or carboxyl-terminal domains of this receptor, then ethanol would be expected to either increase the amplitude of the chimeric receptor response or decrease the magnitude of ethanol inhibition of the chimeric receptor responses at low agonist concentrations. Because we found 1 μM nicotine to be the threshold for the chimeric receptor agonist concentration-response curve (Fig. 4B) and we did not observe either ethanol potentiation or a significant change of the ethanol inhibition of chimeric receptor responses activated by 1 μM nicotine, these observations suggest that ethanol potentiation of $5\text{-}HT_3$ receptor responses does not involve the transmembrane or carboxyl-terminal domains of the receptor. Additional evidence for the identification of the sequence

elements responsible for the potentiation of 5-HT_3 receptor function by ethanol could be obtained from a similar study on the reverse chimera (i.e., containing the amino-terminal domain from the 5-HT_3 receptor and the transmembrane and carboxyl-terminal domains from the $nACh_{\alpha 7}$ receptor). Despite considerable effort, we have not been able to obtain any functional reverse chimeras. However, the experimental evidence presented for the allosteric potentiation by ethanol of 5-HT_3 receptor-mediated responses argues in favor of the involvement of the extracellular amino-terminal domain of the receptor in the ethanol potentiation of these responses. Thus, our observations favor the view that the opposite allosteric modulatory actions of ethanol on $nACh_{\alpha 7}$ and 5-HT_3 receptors involve the amino-terminal domains of these receptors.

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