Dual Action of n-Alcohols on Neuronal Nicotinic Acetylcholine Receptors

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Received March 23, 2001; accepted June 13, 2001

This work was supported by National Institutes of Health Grant AA07836.

ABSTRACT

Alcohol is known to modulate the activity of a variety of neurotransmitters and ion channels. Recently, neuronal nicotinic acetylcholine receptors (nnAChRs) have become a specific focus of study because not only are they potently modulated by alcohol but also they regulate the release of various transmitters, including γ-aminobutyric acid (GABA) and dopamine, which play an important role in the behavioral effects of ethanol. Whereas the potency of normal alcohols (n-alcohols) to potentiate GABA_A receptors and to inhibit N-methyl-D-aspartate receptors increases with carbon chain length, we have found that n-alcohols, depending on the carbon chain length, exert a dual action, potentiation and inhibition, on nnAChRs in primary cultured rat cortical neurons. The mechanism of dual action of n-alcohols on nnAChRs was further analyzed using human embryonic kidney cells expressing the α4β2 subunits. Shorter chain alcohols from methanol to n-propanol potentiates acetylcholine (ACh)-induced currents, whereas longer chain alcohols from n-pentanol to n-dodecanol inhibited the currents. n-Butanol either potentiated or inhibited the currents depending on the concentrations of ACh and butanol. The parameters for both potentiation (log EC200) and inhibition (log IC50) were linearly related to carbon number, albeit with different slopes. The slope for potentiation was −0.299, indicating a change in free energy change (ΔΔG) of 405 cal/mol/methyl group, whereas the slope for inhibition was −0.584, indicating a ΔΔG of 792 cal/mol. These results suggest that potentiating and inhibitory actions are exerted through two different binding sites. Ethanol decreased the potency of n-octanol to inhibit ACh currents, possibly resulting from an allosteric mechanism.

Alcohols act on many neuronal receptors and ion channels in the central nervous system. Some of them are inhibited by alcohols, including N-methyl-D-aspartate (NMDA) receptors, α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid receptors, and voltage-gated calcium channels, whereas some others are potentiated, including γ-aminobutyric acid (GABA) type A receptors, glycine receptors, and type III 5-hydroxytryptamine receptors (Mullikin-Kilpatrick and Treistman, 1993; Crews et al., 1996; Lovinger, 1997, 1999; Mihic, 1999; Walter and Messing, 1999; Woodward, 1999). The differential modulation of neuroreceptors embedded in the same neuronal membrane points to the specific action of alcohol on these receptors.

Neuronal nicotinic acetylcholine receptors (nnAChRs) have recently received much attention because the cholinergic system plays an important role in modulating many other transmitter systems. nnAChRs are located in postsynaptic, preterminal, and presynaptic regions of GABAergic and other interneurons in the cortex and hippocampus. The modulation of nnAChRs can lead to a cascade of synaptic events involving multiple neurotransmitters. Ethanol has been found to potently modulate nnAChRs (Covernton and Connolly, 1997; Aistrup et al., 1999a; Cardoso et al., 1999; Narahashi et al., 1999). At concentrations of 3 mM and above, ethanol potentiates α-bungarotoxin (α-BuTX)-insensitive ACh-induced currents but weakly inhibits α-BuTX-sensitive currents in rat cortical neurons (Aistrup et al., 1999a). Thus, α-BuTX-insensitive nnAChRs might be an important target of alcohol action.

The action of normal alcohols (n-alcohols) on ligand-gated receptor channels depends on carbon chain length. Whereas the potency of n-alcohols to modulate the activity of GABA, glycine, and NMDA receptors increases with an increase in alkyl chain length (Nakahiro et al., 1991, 1996; Mascia et al., 1996; Peoples and Weight, 1999), the modulatory action of n-alcohols on nonneuronal nicotinic AChRs, both skeletal muscle and Torpedo californica nicotinic AChRs, changes from potentiation to inhibition as the alcohol chain length increases (Bradley et al., 1984; Wood et al., 1991). We also found that n-alcohols exerted a...
dual action on nAChRs depending on the carbon chain length.

We now report the results of analyses of the mechanism of the dual action of n-alcohols on nAChRs. In α4β2-type AChRs of cortical neurons and in human α4- and α2-containing AChRs expressed in human embryonic kidney (HEK) cells, we found that short-chain alcohols potentiated ACh-induced currents, whereas long-chain alcohols inhibited the currents. The dual action of n-alcohols was analyzed in detail using HEK cells expressing the human α4β2 nAChRs. Both inhibition (log IC50) and potentiation (log EC200) were linearly related to carbon number, albeit with different slopes. These results suggest that n-alcohols exert the two different effects by acting at two different sites.

Materials and Methods

Cell Preparations. Rat cortical neurons were isolated and cultured by a procedure slightly modified from that described elsewhere (Marszalec and Narahashi, 1993). In brief, rat embryos were removed from a 17-day pregnant Sprague-Dawley rat under methoxyflurane anesthesia. Small wedges of frontal cortex were excised and subsequently incubated in phosphate-buffered saline solution containing 0.25% (w/v) trypsin (type XI, Sigma, St. Louis, MO) for 20 min at 37°C. The digested tissue was then mechanically triturated by repeated passages through a Pasteur pipette and the dissociated cells were suspended in Neurobasal medium with B-27 supplement (Invitrogen, Carlsbad, CA) and 2 mM glutamine. The cells were added to 35-mm culture wells containing 3-ml aliquots at a concentration of 100,000 cells/ml. Each well contained five 12-mm coverslips [previously coated with poly-l-lysine] overlaid with confluent glia that had been plated 2 to 4 weeks earlier. Plated neurons were used for electrophysiological experiments after 4 to 9 weeks in culture. Both the neuron/glia cocultures and the HEK cells were maintained in a humidified atmosphere of 95% air and 5% CO2 at 37°C.

Human α4β2 subunit combination was stably expressed in the HEK 293 cell line. Cells were cultured in Dulbecco’s modified Eagle’s medium supplemented with 2 mM l-glutamine, 100 U/ml penicillin, 100 μg/ml streptomycin (Invitrogen), 6% iron supplemented calf serum (Sigma), and 100 μg/ml G418 (Mediatech, Herndon, VA). Cells were kept at 37°C in an air + CO2 (95% + 5%, by volume). For patch-clamp experiments, cells were plated on glass coverslips coated with poly-l-lysine and cultured for 1 to 5 days.

Electrophysiological Recording. Whole-cell currents were recorded with an Axopatch 200 patch-clamp amplifier (Axon Instruments, Foster City, CA) at room temperature (20–25°C). Recorded currents were directly digitized at 1 to 10 kHz via a Digitdata 1200 ADC/DAC interface to a microcomputer under control of the ClampEx module of the PC clamp6 software package (Axon Instruments). The holding potential was +50 mV. The external solution contained 150 mM NaCl, 5 mM KCl, 2.5 mM CaCl2, 1 mM MgCl2, 10 mM glucose, 5.5 mM HEPES acid, 2 mM Mg2+ ATP, and 0.2 mM Na+ GTP, at pH 7.3, and osmolarity 320 mOsM. In addition, 0.1 μM tetrodotoxin was added to block the sodium channel. The internal solution contained 140 mM K gluconate, 2 mM MgCl2, 1 mM CaCl2, 11 mM EGTA, 10 mM HEPES acid, 2 mM Mg2+ ATP, and 0.2 mM Na+ GTP, at pH 7.3 titrated with KOH, and osmolarity 300 mOsM. The patch-clamp pipettes were pulled from Clark Patch Glass capillaries (PG120T-10, 1.2 mm o.d., 0.933 mm i.d., 10 cm long; Warner Instrument Corp., Hamden, CT) and lightly fire-polished to a final resistance of 1.5 to 2 MΩ when filled with internal solution.

Drug Application. All drugs were applied to the cell by a modified computer-operated U-tube system (Marszalec and Narahashi, 1993) having a solution exchange rise time of 10 to 15 ms as detected by changes in junction potential or by U-tube application plus a 1- to 2-min bath preapplication. The solution exchange on the cell surface was found to complete within around 200 ms by measuring the rate of changes in ACh-induced current in response to changes in sodium ion concentration (Liu and Díger, 1991; Mori et al., 2001). U-tube application exposure time was 250 ms, if not otherwise mentioned. The test solution was applied at intervals of 2 min. In this study, the term “coapplication” is referred to as the simultaneous application of alcohol and ACh through a U-tube, whereas the term “preperfusion” is referred to as the application of alcohol through the external bathing solution before coapplication of alcohol and ACh.

Control currents evoked by application of 30 μM or 3 mM ACh alone were checked before and after each experiment with test drug. The ethanol used in the experiments was absolute ethyl alcohol USP (Pharmco Products, Brookfield, CT). Methanol, n-pentanol, n-hexanol, n-heptanol, n-octanol, n-decanol, and n-dodecanol were all obtained from Sigma. n-Propanol and n-butanol were from Aldrich Chemical Co. (Milwaukee, WI).

Data Analysis. Recorded currents were initially analyzed by the Clamp-Fit module of the PClamp6 to assess whole-cell current amplitudes and decay kinetics. Statistical analysis was performed with Excel, Office 2000. Data were expressed as the mean ± standard error of mean unless otherwise stated. The concentration-response data were subsequently compiled for graphical analysis in SigmaPlot 5.0. Student’s t tests were performed to assess significance of differences between test and control measurements at the P value < 0.05.

Kinetic simulation of the receptor/channel activity was carried out with a C++ program for numerical solution. Short-chain alcohols were considered to modify the kinetic parameters, and long-chain alcohols were considered to reduce ACh binding to the receptor and to block the open ACh channel.

Results

Comparison of n-Alcohols with Various Carbon Chain Lengths. Based on the reported relationship between the ability of n-alcohols to modulate ion channel function and the length of carbon chain (Nakahiro et al., 1991, 1996; Mascia et al., 1996; Peoples and Weight, 1999), we selected concentrations of n-alcohols that gave the equivalent efficacy to compare the modulating action of various n-alcohols on α-BuTX-insensitive, α4β2-type currents in rat cortical neurons. The concentration of each alcohol was decreased by a factor of 3 with an addition of one carbon to the alcohol, and currents were induced by 10 μM ACh (3 × EC50).

Similar to the results with nonneuronal nAChRs (Bradley et al., 1984; Wood et al., 1991), the effects of n-alcohols on nAChRs in cortical neurons were not monophasic in nature. For short-chain alcohols, a potentiating action was observed, whereas for longer chain alcohols, an inhibitory action was observed. The currents were potentiated by 1000 mM methanol, 300 mM ethanol, 100 mM propanol, and 30 mM butanol, although the degree of potentiation was decreased by increasing the alcohol carbon chain length (Fig. 1). In contrast, the currents were suppressed by 10 mM pentanol, 3 mM hexanol, 1 mM heptanol, and 0.3 mM octanol (Fig. 1). Thus, the current potentiation was converted to inhibition when the carbon chain was lengthened from butanol to pentanol.

To elucidate the mechanism underlying the conversion phenomenon, more detailed and quantitative analyses of n-alcohol modulation were undertaken using HEK cells stably expressing the α4β2 subunit combination. This combination was selected because it constitutes the major component of nAChRs in the vertebrate brain (Gotti et al., 1997). Three types of analyses were performed. First, the dose-response relationship for ACh activation of the receptor was obtained

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and the EC\textsubscript{50} value was determined. Second, the dose-response relationship for ACh current potentiation by each of the short-chain alcohols was measured, and the concentration to increase the current to 200% of control (EC\textsubscript{200}) was estimated. Third, the dose-response relationship for ACh current suppression by each of the long-chain alcohols was obtained, and the IC\textsubscript{50} and Hill coefficient (n\textsubscript{H}) were determined.

**ACh Dose-Response Relationship for α4β2 HEK Cells.** The α4β2 AChRs expressed in HEK cells behaved similarly to the α-BuTX-insensitive AChR of cortical neurons. Both of them were very sensitive to ACh, and the α4β2 receptor responded to even 1 μM ACh with an appreciable inward current (Fig. 2A). The currents reached a maximum at an ACh concentration of 3 mM and decreased at higher ACh concentrations. The bell-shaped dose-response relationship was also observed with nonneuronal nicotinic AChRs (Tonner et al., 1992; Wu et al., 1994). At ACh concentrations higher than 1 mM, the current decayed rapidly and a tail current was generated upon termination of ACh application (Fig. 2A).

The dose-response relationship for ACh-induced currents followed a biphasic curve (Fig. 2B). A simple fit of the dose-response curve up to 3 mM gives an EC\textsubscript{50} value of 38.8 ± 9.6 μM and an n\textsubscript{H} value of 0.65 ± 0.10. The fit to the whole range of data could be improved by a more complicated model in which there were two dose-response relationships for ACh activation and one dose-response relationship for ACh inhibition that occurred at high ACh concentrations. In most of the subsequent experiments, the current induced by 30 μM ACh was used as the control for normalizing test responses, for it was close to the EC\textsubscript{50}.

**n-Alcohols Exert Either Potentiating or Inhibiting Action on ACh-Induced Currents.** To examine the direct modulating action of alcohols on the α4β2 nAChRs expressed in HEK cells, n-alcohols were coapplied with ACh or preapplied for 2 min before coapplication because a prolonged exposure to alcohol using bath application techniques increases the possibility of indirect effects via intracellular regulatory systems (Diamond and Gordon, 1997). ACh (30 μM) was coapplied with different concentrations of various alcohols for 250 ms at intervals of 2 min. The 2-min interval in general gave the receptor enough time to recover from a previous exposure to ACh and alcohols, unless otherwise stated. The types of alcohols used and the concentration ranges covered in the experiment are given in Table 1.

![Fig. 1. Potentiating and inhibitory actions of n-alcohols on α-bungarotoxin-insensitive nAChRs of rat cortical neurons. n-Alcohols from methanol to n-octanol were coapplied with 10 μM ACh (3× EC\textsubscript{50}) for 500 ms, and currents were recorded at a holding potential of −70 mV. In each case, for the clarity of comparison among alcohols, the response to 10 μM ACh is scaled to 100% as control. Changes in peak current amplitude from control current without alcohols are plotted for each n-alcohol (mean ± S.E.M., n = 6). Conversion (flip-flop) from potentiation to inhibition occurs between n-butanol and n-pentanol.](image-url)
The current evoked by 30 μM ACh was significantly enhanced upon coapplication of high concentrations of short-chain alcohols: methanol (≥1000 mM), ethanol (≥300 mM; Fig. 3A), and propanol (≥100 mM). These potentiating effects were completely reversible after a 2-min washout as shown for ethanol in Fig. 3A.

In contrast to short-chain alcohols, long-chain alcohols exhibited an inhibitory effect on ACh-induced currents in the α4β2 receptors. An example for octanol is shown in Fig. 3B. After inhibition by long-chain alcohols at very high concentrations, it sometimes took a long time for the current to return to the original amplitude. In each case washing for 15 to 20 min was necessary. For example, octanol at 3 mM sometimes needed 15 min for recovery, and in several other cases it could recover only about 75% of the control.

Figure 4 illustrates the dose-response relationship for the potentiating and inhibitory actions of various n-alcohols. The potency of potentiation for the short-chain alcohols (from methanol to propanol) increased as the carbon number increased, with the EC200 values of 1900 ± 500 mM for methanol, 1000 ± 200 mM for ethanol, and 500 ± 100 mM for propanol (Table 1).

Pentanol and longer chain alcohols seemed to exert only inhibitory effects. With an increase in the carbon chain length, the inhibitory dose-response curves were shifted to lower alcohol concentrations. Each of the dose-response data was fit by the Hill regression and the IC50 values are given in Table 1. The IC50 values of alcohols decreased as the carbon number increased: 2.39 ± 0.08 mM for pentanol, 0.37 ± 0.02 mM for hexanol, 127 ± 4 μM for heptanol, 22.1 ± 0.8 μM for octanol, and 2.7 ± 0.4 μM for decanol. However, the IC50 value of dodecanol was 7.5 ± 1.2 μM and larger than that of decanol, indicative of a cut-off phenomenon.

Butanol lies between the short-chain and long-chain alcohols and showed a biphasic effect on ACh-induced currents (Fig. 4). The dual action is illustrated in Fig. 5. The inhibitory effect was observed at a concentration as low as 1 mM (=5% inhibition) and reached the largest inhibition at 100 mM (=50% inhibition). Above this concentration, butanol caused less inhibition at high concentrations (Fig. 4), and in some cases increased the current beyond the control level (Fig. 5). It seemed that the potentiating action overcame the inhibitory action, a result suggesting that the two actions are not independent of each other.

### Table 1

<table>
<thead>
<tr>
<th>n-Alcohol</th>
<th>Carbon No.</th>
<th>Concentration Range</th>
<th>Effect on Currents</th>
<th>IC50 (mM)</th>
<th>EC200 (mM)</th>
<th>EC200/IC50</th>
<th>nH (Inhibition)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methanol</td>
<td>1</td>
<td>30–3000</td>
<td>Potentiation</td>
<td>390</td>
<td>1900 ± 500</td>
<td>5.0</td>
<td></td>
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<tr>
<td>Ethanol</td>
<td>2</td>
<td>1–3000</td>
<td>Potentiation</td>
<td>100</td>
<td>1000 ± 200</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>Propanol</td>
<td>3</td>
<td>3–1000</td>
<td>Potentiation</td>
<td>26</td>
<td>500 ± 100</td>
<td>19</td>
<td></td>
</tr>
<tr>
<td>Butanol</td>
<td>4</td>
<td>1–3000</td>
<td>Potentiation</td>
<td>6.8</td>
<td>250</td>
<td>37</td>
<td></td>
</tr>
<tr>
<td>Pentanol</td>
<td>5</td>
<td>0.1–100</td>
<td>Inhibition</td>
<td>2.39 ± 0.08</td>
<td>130*</td>
<td>53</td>
<td></td>
</tr>
<tr>
<td>Hexanol</td>
<td>6</td>
<td>0.01–3</td>
<td>Inhibition</td>
<td>0.37 ± 0.02</td>
<td>63*</td>
<td>170</td>
<td></td>
</tr>
<tr>
<td>Heptanol</td>
<td>7</td>
<td>0.003–3</td>
<td>Inhibition</td>
<td>127 ± 4 μM</td>
<td>32*</td>
<td>250</td>
<td></td>
</tr>
<tr>
<td>Octanol</td>
<td>8</td>
<td>0.001–3</td>
<td>Inhibition</td>
<td>22.1 ± 0.8 μM</td>
<td>16*</td>
<td>720</td>
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<tr>
<td>Decanol</td>
<td>10</td>
<td>0.0001–0.1</td>
<td>Inhibition</td>
<td>2.7 ± 0.4 μM</td>
<td>4.0*</td>
<td>1500</td>
<td></td>
</tr>
<tr>
<td>Dodecanol</td>
<td>12</td>
<td>0.00001–0.01</td>
<td>Inhibition</td>
<td>7.5 ± 1.2 μM</td>
<td>1.0*</td>
<td>140</td>
<td></td>
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</table>

* Obtained by extrapolating inhibition/potentiation-carbon number relationship.
Chain Length Dependence of Potentiating and Inhibitory Effects. The IC$_{50}$ and EC$_{200}$ values estimated from the dose relationships for potentiating and inhibitory effects of alcohols illustrated in Fig. 4 were plotted as a function of the chain length of n-alcohols (Fig. 6). The IC$_{50}$-carbon chain relationship from pentanol to decanol is shown in Fig. 6A. The plot of log IC$_{50}$ versus alcohol carbon number had a slope of $-0.58 \pm 0.04$. The IC$_{50}$ value decreased by a factor of 3.3 per methylene increase, which is equivalent to a change in Gibb’s free energy change ($\Delta G$) of 790 $\pm$ 50 cal/mol/CH$_2$ group as calculated from $\Delta G = -RT \ln K$, where R is the gas constant, T is the absolute temperature, and K is the dissociation constant ($R = 1.987 \text{cal} \cdot \text{K}^{-1} \cdot \text{mol}^{-1}$; $T = (273.16 + 23) \text{K}$; $RT = 588.47 \text{cal} \cdot \text{K}^{-1} \cdot \text{mol}^{-1}$). This value of $\Delta G$ is similar to that obtained from muscle nAChRs (881 cal/mol/CH$_2$) (Wood et al., 1991). Thus, the potency of the long-chain alcohols to inhibit ACh current increased with an increase in carbon chain, reached a maximum at decanol, and then declined at dodecanol. The last effect is called cut-off. From methanol to propanol, the log EC$_{200}$ values decreased linearly with the carbon chain length with a slope of $-0.30 \pm 0.02$ (Fig. 6B). The decrease in EC$_{200}$ by a factor of 1.99 per methylene moiety gives a $\Delta G$ of 400 $\pm$ 30 cal/mol/CH$_2$ group.

The extrapolation of carbon chain length dependence of EC$_{200}$ obtained for shorter chain alcohols to those for longer chain alcohols gave an EC$_{200}$ value at least 50-fold larger than the experimentally obtained IC$_{50}$ values of the corresponding longer chain alcohols (Table 1). Thus, long-chain alcohols from pentanol to dodecanol exhibited mainly inhibitory actions on nACh receptors in the range of concentrations used in our experiments. Similarly, the IC$_{50}$ values of shorter chain alcohols were calculated by extrapolation from the IC$_{50}$-chain length relationship for longer chain alcohols. These extrapolated IC$_{50}$ values were 5- to 30-fold smaller than their EC$_{200}$ values. Therefore, if shorter chain alcohols were exerting potentiating and inhibitory actions on two separate sites independently, we would not have observed a potentiating action at any concentration.

To test whether the effect of alcohols reaches equilibrium in the coapplication experiments, bath application of alcohols for longer duration was also used. Alcohols were preperfused through external bath solution for 1 to 2 min and then coapplied with 30 $\mu$M ACh from the U-tube for 250 ms. The effects of short-chain alcohols by coapplication with ACh reached equilibrium within 250 ms. For instance, no significant difference in inhibitory and potentiating actions was seen between bath application and coapplication of butanol (Fig. 7). Preapplied longer chain alcohols ranging from pentanol to octanol inhibited currents induced by 30 $\mu$M ACh in a dose-dependent manner and the dose-response relationship was shifted toward lower concentrations with increasing carbon chain length (Fig. 8A). The IC$_{50}$ values still followed the linear relationship with the increase in carbon number, giving a slope of $-0.70 \pm 0.06$ and a $\Delta G$ of 950 $\pm$ 70 cal/mol/CH$_2$ group (Fig. 8B). These values are similar to those obtained by coapplication only, which yielded a slope from pentanol to octanol of $-0.58 \pm 0.04$ and a $\Delta G$ of 790 $\pm$ 50 cal/mol/CH$_2$ group. However, for these longer chain alcohols,
some differences in IC₅₀ values and n_H values were noted between coapplication and pre- and coapplication, depending on the concentration of ACh. At a low ACh concentration of 30 μM, the IC₅₀ values were slightly lower and the n_H values were slightly higher with pre- and coapplication than with coapplication (Table 2). However, there is about 10-fold difference in alcohol blocking potency between coapplication only and coapplication together with preperfusion at 3 mM ACh. This is because no equilibrium was reached in the coapplication experiments at the time when the current induced by 3 mM ACh reached the peak, 30 to 100 ms, which was much shorter than the time to peak of 200 ms of the 30 μM ACh-induced currents. Because the time required to reach the equilibrium in our experiments is around 200 ms, the peak amplitude measured in the coapplication experiment does not reflect the full action of alcohol.

**Potentiating Action of Short-Chain Alcohol.** To further elucidate the mechanism of enhancement of ACh-induced currents by short-chain alcohols, their effects on the agonist dose-response curve must be evaluated. Thus, we studied the effects of ethanol (100 and 300 mM) and propanol (100 mM) on the ACh dose-response curve. Alcohol was co-applied with different concentrations of ACh for 250 ms using the U-tube system at 2-min intervals. The ACh dose-response curves with and without ethanol are plotted by normalizing all currents to the current induced by 3 mM ACh in the same cell (Fig. 9). Both the affinity and efficacy of ACh increased with the increase in ethanol concentration. The EC₅₀ values for ACh were 43.5 ± 8.1 μM without ethanol, 24.0 ± 4.1 μM with 100 mM ethanol (P < 0.10), and 19.0 ± 6.9 μM with 300 mM ethanol (P < 0.05). The maximum response was increased by 13.0 ± 4.0% (P < 0.05) by 300 mM alcohol blocking potency between coapplication only and coapplication together with preperfusion at 3 mM ACh. This is because no equilibrium was reached in the coapplication experiments at the time when the current induced by 3 mM ACh reached the peak, 30 to 100 ms, which was much shorter than the time to peak of 200 ms of the 30 μM ACh-induced currents. Because the time required to reach the equilibrium in our experiments is around 200 ms, the peak amplitude measured in the coapplication experiment does not reflect the full action of alcohol.
ethanol. A 2-fold reduction in EC_{50} value and a small but significant increase (5.0 ± 1.2%, P < 0.05) in the maximum response were observed in the presence of 100 mM propanol (data not shown). Alcohol enhancement of current amplitude at high concentrations of ACh is qualitatively similar to that observed with the α4β2-type ACh receptor of cortical neurons (Aistrup et al., 1999a). The results that the short-chain alcohols cause a reduction in EC_{50} values of ACh to activate nnAChRs accompanied with an increase in the maximum response differ from their potentiating action on GABA_{A} receptors. In the latter case, alcohols reduce GABA EC_{50} values without changing the maximal response (Marszalec et al., 1994).

**Interactions of Ethanol and Octanol.** The potentiating and inhibitory actions exerted by butanol suggest that there is an interaction between the potentiating site and the inhibitory site. Experiments were performed to test such interactions using ethanol as a potentiator and octanol as an inhibitor at nnAChRs. A protocol of 2-min alcohol bath-application followed by 250-ms alcohol and ACh coapplication was used. Ethanol at 300 mM potentiated the current induced by 30 μM ACh to 140 ± 2% of control, 30 μM octanol inhibited the current to 34 ± 3% of control, and coapplication of ethanol and octanol inhibited the currents to only 68 ± 10% of control. However, if octanol inhibited the nnAChRs independently of ethanol potentiating action, one would have expected that coapplication of ethanol and octanol would reduce the ACh current to 48% of the control. Similar experiments were also performed at 3 mM ACh, which produced a saturating response. Ethanol (300 mM) potentiated the current to 113 ± 4% of control, 30 μM octanol inhibited it to 28 ± 2% of control, and the coapplication of ethanol and octanol inhibited it to 61 ± 4% of control, which was much higher than the estimated 32% of control. These results suggest that octanol is less effective in inhibiting the ethanol-potentiated nnAChR currents.

If the apparent effect of alcohols represents a mixture of inhibition and potentiation, potentiation is diminished at high concentrations of ACh that give saturating responses so that the underlying inhibition is disclosed. Ethanol-octanol interactions were studied by using two concentrations of ACh (Fig. 10). The potency of octanol inhibition did not change with the ACh concentration, with an IC_{50} value of 14.3 ± 1.3 μM at 30 μM ACh, and an IC_{50} value of 18.3 ± 2.7 μM at 3 mM ACh. This suggests that octanol neither acts as a pure open channel blocker nor as a simple competitive antagonist on ACh receptors. With increasing agonist concentration, the IC_{50} value of octanol would increase with a pure receptor antagonist model and decrease with an open channel block model. However, at 30 μM ACh coapplication of 300 mM ethanol significantly decreased the potency of octanol inhibition by increasing the IC_{50} value from 14.3 ± 1.3 to 31.9 ± 4.3

**TABLE 2**

<table>
<thead>
<tr>
<th>Alcohol</th>
<th>Carbon No.</th>
<th>Conc. Range</th>
<th>Coapplication</th>
<th>Pre- and Coapplication</th>
</tr>
</thead>
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<tr>
<td></td>
<td></td>
<td>μM</td>
<td>IC_{50}</td>
<td>n_{H}</td>
</tr>
<tr>
<td></td>
<td></td>
<td>μM</td>
<td>μM</td>
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</tr>
<tr>
<td>30 μM ACh</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pentanol</td>
<td>5</td>
<td>100–30,000</td>
<td>2400 ± 100</td>
<td>0.74 ± 0.02</td>
</tr>
<tr>
<td>Hexanol</td>
<td>6</td>
<td>10–3000</td>
<td>370 ± 20</td>
<td>0.65 ± 0.03</td>
</tr>
<tr>
<td>Heptanol</td>
<td>7</td>
<td>3–3000</td>
<td>127 ± 4</td>
<td>0.81 ± 0.02</td>
</tr>
<tr>
<td>Octanol</td>
<td>8</td>
<td>1–3000</td>
<td>31 ± 3</td>
<td>0.69 ± 0.04</td>
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<tr>
<td>3 mM ACh</td>
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<td></td>
</tr>
<tr>
<td>Hexanol</td>
<td>6</td>
<td>10–3000</td>
<td>4600 ± 600</td>
<td>0.73 ± 0.04</td>
</tr>
<tr>
<td>Octanol</td>
<td>8</td>
<td>1–3000</td>
<td>380 ± 20</td>
<td>0.39 ± 0.01</td>
</tr>
</tbody>
</table>

Fig. 7. Butanol has similar inhibitory action by coapplication (A) and by pre- and coapplication (B) in α4β2 HEK cells. Butanol at different concentrations (1–300 mM) was coapplied or both coapplied and bath-applied with 30 μM ACh. Coapplication lasted for 250 ms and bath application began at 1 to 2 min before the start of recording. Currents were recorded at a holding potential of ±50 mV at intervals of 2 min. The percentage of inhibition is calculated by normalizing to the control current induced by 30 μM ACh. No significant difference between the two methods of applications was observed at any butanol concentration. Mean ± S.E.M., n = 3 to 6.

Fig. 8. Inhibitory effects of preperfusion of alcohols exhibit the same chain length dependence as that by coapplication in α4β2 HEK cells. A, inhibitory dose-response curves for long-chain alcohols from pentanol to octanol. Alcohols were preperfused for 1 to 2 min before 250-ms coapplication with 30 μM ACh. The recordings were made at a holding potential of ±50 mV. The control current in each cell was induced by 30 μM ACh. Mean ± S.E.M. (n = 3–6). The data were fit to a logistic equation to give IC_{50} and n_{H} as shown in Table 2. B, logarithm of IC_{50} is linearly related to the carbon number of n-alcohols. The slope of inhibition is −0.70 ± 0.06, corresponding to a change in free energy change (ΔΔG) of 950 ± 70 cal/mol/CH_{2}.

The potentiating action of butanol suggests that there is an interaction between the potentiating site and the inhibitory site. Experiments were performed to test such interactions using ethanol as a potentiator and octanol as an inhibitor at nnAChRs. A protocol of 2-min alcohol bath-application followed by 250-ms alcohol and ACh coapplication was used. Ethanol at 300 mM potentiated the current induced by 30 μM ACh to 140 ± 2% of control, 30 μM octanol inhibited the current to 34 ± 3% of control, and coapplication of ethanol and octanol inhibited the currents to only 68 ± 10% of control. However, if octanol inhibited the nnAChRs independently of ethanol potentiating action, one would have expected that coapplication of ethanol and octanol would reduce the ACh current to 48% of the control. Similar experiments were also performed at 3 mM ACh, which produced a saturating response. Ethanol (300 mM) potentiated the current to 113 ± 4% of control, 30 μM octanol inhibited it to 28 ± 2% of control, and the coapplication of ethanol and octanol inhibited it to 61 ± 4% of control, which was much higher than the estimated 32% of control. These results suggest that octanol is less effective in inhibiting the ethanol-potentiated nnAChR currents.
μM \((P < 0.002)\) (Fig. 10A), whereas no significant shift was observed at 3 mM ACh \((0.10 < P < 0.20)\) (Fig. 10B). The latter result agrees with that of AChRs of \(T. \text{californica}\) membrane vesicles (Wood et al., 1991); 1.0 M ethanol had no effect on the inhibitory action of octanol over 15 ms of \(^{86}\text{Rb}^+\) flux measurement at 1 mM ACh. The ACh concentration-dependent ethanol effect on octanol inhibition of ACh currents further suggests that ethanol and octanol do not act independently. This point will be elaborated by model simulation as described under Discussion.

**Discussion**

**Bell-Shaped Dose-Response Relationship of ACh-Induced Currents.** The ACh-induced current increased in amplitude with increasing ACh concentration, reached a peak, and then declined at very high concentrations of ACh. Such a bell-shaped dose-response relationship was seen with nicotine, as reported previously on \(T. \text{californica}\) nicotinic receptors (Tonner et al., 1992; Wu et al., 1994) and depicted in Scheme 1.

In the presence of high agonist concentrations, the decline in response has been attributed partly to the desensitization of nnAChRs and partly to ACh self-block of the receptors. Higher ACh concentrations increased the apparent rate of desensitization because more receptors were drawn to the open state than with low concentrations of ACh.

The observation that the ACh currents rebound upon termination of application of high ACh concentrations (Fig. 2A) is consistent with an open channel block by ACh. If the rebound current upon termination of ACh application is due to the unblock of the receptor channels, the ACh-blocked receptor must not undergo appreciable desensitization.

**Multiple Effects of \(n\)-Alcohols on ACh Receptors.** Two major actions of \(n\)-alcohols observed in nnAChRs are similar to their actions on muscle nACh receptors in the following respects. 1) The potentiating effect: this effect was seen with short-chain alcohols and the potency increased with an increase in carbon chain length. The slope for the log \(EC_{50}\) and carbon number indicates a \(\Delta G\) of 400 cal/mol/CH\(_2\). 2) The inhibitory effect: this effect was observed with long-chain alcohols and the potency increased with carbon chain length. The slope for the log \(IC_{50}\)-carbon number relationship gave a \(\Delta G\) of 790 cal/mol/CH\(_2\). This value is close to the energy required to move one CH\(_2\) group from hydrophilic environment to hydrophobic environment (800 cal/mol/CH\(_2\)). The larger free energy change involved in inhibitory action suggests that the site of inhibitory action has a stronger hydrophobic component.

The value for \(\Delta G\) for the inhibition of the \(\alpha 4/2\) nnAChRs by longer chain alcohols (790 cal/mol/CH\(_2\)) is comparable with that for the inhibition of muscle nAChRs (880 cal/mol/CH\(_2\)) (Wood et al., 1991), suggesting the possibility of a similar binding site. Ethanol was suggested to bind to muscle nAChRs at a site in the M2 region of nAChR channel based on mutagenesis studies (Forman et al., 1995; Forman, 1997; Zhou and Forman, 1998). The \(\Delta G\) value for the inhibition of the \(\alpha 4/2\) receptors is also comparable with the \(\Delta G\) for the \(n\)-alcohol potentiation of the GABA\(_A\) receptors (880 cal/mol/CH\(_2\)) (Peoples and Weigh, 1999). This may be related to the fact that nnAChRs and GABA\(_A\) receptors belong to the same ligand-gated receptor superfamily. The \(\Delta G\) for the potentiation of the \(\alpha 4/2\) nnAChRs (400 cal/mol/CH\(_2\)) is close to that for the inhibition of the NMDA receptors (300 cal/mol/CH\(_2\)) (Peoples and Weigh, 1999), yet the significance of this similarity remains to be seen.

The \(\alpha 4/2\) nnAChRs and muscle nAChRs differ in their responses to propanol. nnAChR currents induced by ACh were potentiated by 100 mM propanol, whereas muscle nAChRs were inhibited by propanol with a \(K_i\) of 270 mM (Wood et al., 1991). This suggests that alcohol actions on nAChRs are subunit-dependent. This was indeed the case from our preliminary studies: the ACh-induced currents in the \(\alpha 4/2\), \(\alpha 4/4\), and \(\alpha 3/4\) subunit combinations expressed in HEK cells were potentiated by ethanol, whereas the \(\alpha 3/2\) subunit combination was inhibited (Aistrup et al., 1999b). A more detailed investigation of subunit dependence of alcohol action is in progress.

**One-Site versus Two-Site Model.** The dual action of \(n\)-alcohols has been extensively studied on muscle nicotinic receptors. Two models were proposed to explain the dual action of alcohols: a single-site model and a two-site model. In

![Scheme 1. Activation, desensitization, and drug-induced block.](image)

**Fig. 9.** Effects of short-chain alcohols on the ACh dose-response curve. Ethanol at 100 and 300 mM was coapplied with different concentrations of ACh for 250 ms using the U-tube system at 2-min intervals. Currents were recorded at a holding potential of -50 mV. The ACh dose-response curves with and without ethanol are plotted by normalizing the currents to the control current induced by 3 mM ACh in the same cell. A, both the affinity and efficacy of ACh are increased with the increase in ethanol concentration. The \(EC_{50}\) values for ACh were 43.5 ± 8.1 μM without ethanol, 24.0 ± 4.1 μM with 100 mM ethanol \((P < 0.10)\), and 19.0 ± 6.9 μM with 300 mM ethanol \((P < 0.05)\). The maximum responses were 1.02 ± 0.01 with 100 mM ethanol \((P < 0.2)\) and 1.13 ± 0.04 with 300 mM ethanol \((P < 0.01)\).
the single-site model proposed by Bradley et al. (1984), both potentiating action and inhibitor action of all alcohols arise from their interaction with one hydrophobic site within the channel lumen. Long-chain alcohols are large enough to block the channel, masking the potentiating action, whereas short-chain alcohols are too small to block, exhibiting as the potentiating action. The alcohols can stabilize the channel at the open state, resulting in a potentiating action (Bradley et al., 1984). This theory was challenged by the two-site model for the following reasons (Wood et al., 1991): 1) ethanol does not compete with octanol for the inhibitory site on the receptor; 2) alcohol chain length dependence for flux enhancement differs greatly from that of flux inhibition; and 3) all-or-none inhibition of ACh-induced flux was observed when alcohol was switched from ethanol to propanol. Work on *T. californica* nAChRs has also shown that the alkanol site that modulates the apparent agonist affinity for channel opening is distinct from the site that results in inhibition of cation flux through the channel (Alifimoff et al., 1993).

Further evidence supporting the two-site model comes from studies using single-channel recording and site-directed mutagenesis. Based on the single-channel analysis (Murrell et al., 1991; Murrell and Haydon, 1991; Liu et al., 1994), it was concluded that alcohols have both inhibitory and excitatory actions on nAChR channels. The inhibitory action was well explained by a model in which drug molecules bind to the channel protein and block the flow of ions through the channel (Murrell et al., 1991; Dilger and Brett, 1991; Dilger et al., 1993), resulting in a decrease in the apparent single-channel conductance or a decrease in the number of conducting channels. The potentiating action of ethanol, butanol, and pentanol may at least partially be due to an increase in burst frequency as observed in muscle nAChRs by Liu et al. (1994). Consistent with this view is the observation that the gating modulation and the reduction in the single-channel conductance can be differentially modified. Mutations of the inhibitory site within the channel lumen enhanced the sensitivity to ethanol inhibition without altering the ethanol-induced gating modulation (Forman et al., 1995; Forman, 1997; Zhou and Forman, 1998). Another mutation near the agonist binding domain increased ethanol-induced potentiation, but did not affect the reduction of single-channel conductance by alcohol (Forman and Zhou, 1999). It remains to be seen whether these notions are also applicable to nAChRs.

The difference in ΔΔ*G* as shown in present study strongly suggests that the inhibitory action and potentiating action are exerted at different sites. Our studies of interaction between ethanol and octanol suggest that these two types of actions may interfere with each other. Although the potentiating and inhibitory actions occur at different sites, they may allosterically affect each other, rendering the potentiated receptors less likely to be inhibited. This notion is further supported by the following simulation.

**Simulation of Ethanol and Octanol Action.** Different kinetic models have been previously applied to analyze the drug action on muscle nAChRs, and the rate constants for channel kinetics of muscle nicotinic ACh channels were estimated from single-channel studies (Dilger and Brett, 1991; Franke et al., 1993; Liu et al., 1994; Dilger et al., 1995). In the present study, the kinetic model of AChR was used to simulate the octanol inhibition and ethanol potentiation is shown in Schemes 2 and 3. The parameters used to simulate ACh-induced currents in the absence and presence of alcohols are given in the legend of Fig. 11.

The parameters were chosen based on earlier studies on muscle nAChRs (Dilger and Brett, 1991; Franke et al., 1993). However, some modifications were made of rate constants to find the best fit with the experimental results. The simulation yielded an ACh dose-response curve with an EC50 value

![Fig. 10. Interactions between ethanol and octanol in αβ2 HEK cells. The filled symbols represent control without ethanol, and the open symbols represent the data with ethanol coapplication. A, at 30 μM ACh, ethanol 300 mM decreased the potency of octanol inhibition by increasing the IC50

value from 14.3 ± 1.3 μM (nH = 0.95 ± 0.09) to 31.9 ± 4.3 μM (nH = 0.93 ± 0.13). This shift of IC50 is significant (P < 0.002). The current induced by 30 μM ACh was used as control for the octanol dose-response curve without ethanol; the current induced by 30 μM plus 300 mM ethanol was used as control for the octanol dose-response curve with 300 mM ethanol. B, octanol inhibitory dose-response curve at 3 mM ACh with and without 300 mM ethanol. Without ethanol, IC50 = 18.3 ± 2.7 μM, nH = 1.45 ± 0.32; with 300 mM ethanol, IC50 = 26.9 ± 3.4 μM, nH = 1.17 ± 0.18. There is no significant difference between the two IC50 values (P > 0.1). The current induced by 3 mM ACh was used as control for the octanol dose-response curve without ethanol; the current induced by 3 mM plus 300 mM ethanol was used as control for the octanol dose-response curve with 300 mM ethanol. In all cases, ACh was applied using the U-tube system for 250 ms, ethanol was applied through U-tube only whereas octanol was applied both in bath for 2 min and coapplied with ACh and ethanol through U-tubes.
of 57.2 ± 2.0 µM and an n_{H1} value of 1.40 ± 0.05. The EC_{50} value is near the experimental result (38.8 ± 9.6 µM), but the n_{H1} is larger than the experimental result (0.65 ± 0.10). The simulation results are consistent with the kinetic model in which two ACh molecules are required to open the channel. The reason for the discrepancy in Hill coefficient is not clear, yet it should be noted that the interpretation of Hill coefficient in reference to stoichiometry is a matter of controversy, and that the observed values for Hill coefficient in nAChRs and GABA_{A} receptors reported in the literature are quite variable.

Our experimental results showed that ethanol potentiated the ACh currents evoked by both low and high concentrations of ACh, but with different efficacies. At 30 µM ACh, the enhancement of current was 49 ± 4% (n = 28), whereas at 3 mM ACh, the enhancement was 13 ± 4% (n = 8). These results could not be accounted for by a classical description of dose-response relationship as depicted by \( y = (100\% \times [c]^{n}) / ([c]^{n} + [EC_{50}]^{n}) \), where \( c \) is ACh concentration, \( EC_{50} \) is the ACh concentration to activate 50% of the maximum response, 100%. Alcohol reduces ACh EC_{50}, which would increase ACh response activated by low ACh concentrations but not high ACh concentrations. The modern version of dose-response relationship for an agonist to activate receptor to open the channel can be depicted as \( y = x \times [c]^{2} / [1 + 2 \times ([c] / K) + ([c]^{2} / K^{2}) + (x \times [c]^{2} / K^{2})] \), where \( K \) is the binding constant and \( x \) determines the open equilibrium (according to Scheme 2 without desensitization and agonist block). The results suggest that alcohols favor equilibrium to the open state. One possibility of ethanol potentiation is to increase the channel opening rate, leading to an increase in the open probability. When the opening rate (\( \beta \)) was increased from 2000 to 3500/s, ethanol at 300 mM caused a potentiation of 52% at 30 µM ACh and a potentiation of 15% at 3 mM ACh. Both values are similar to those of the experimental results. However, another possibility that cannot be overlooked is a decrease in the closing rate (\( \alpha \)). The decrease of \( \alpha \) from 1000 to 600/s could also cause a potentiation of 47% at 30 µM ACh and a potentiation of 14% at 3 mM ACh. Thus, similar results of simulation are obtained by either increasing the open rate constant (\( \beta \)) or decreasing the closing rate constant (\( \alpha \)). How-

![Scheme 2](image1.png)

**Scheme 2.** Octanol blocks only the open ACh channels. R is the receptor; RA and RA\_\( \beta \) are the receptors bound by one and two agonist molecules, respectively; RA\_\( \beta \)\_\( \alpha \) is the agonist-bound activated receptor; RD\_1 and RD\_2 are the desensitized receptors; and B and RB is the receptor blocked by long-chain alcohols.

![Scheme 3](image2.png)

**Scheme 3.** Octanol blocks both open and closed ACh channels. R is the receptor; RA and RA\_\( \beta \) are the receptors bound by one and two agonist molecules, respectively; RA\_\( \beta \)\_\( \alpha \) is the agonist-bound activated receptor; RD\_1 and RD\_2 are the desensitized receptors; and B and RB is the receptor blocked by long-chain alcohols.

![Fig. 11](image3.png)

**Fig. 11.** Simulation dose-response curves for octanol inhibition at 30 µM and 3 mM ACh. The filled symbols represent for the cases at 30 µM ACh, and the open symbols represent the cases at 3 mM ACh. The following parameters were chosen to simulate ACh-induced currents in the absence and presence of alcohols. The binding rate of ACh (\( k_{on} \), \( 1 \times 10^{7} \) M\(^{-1}\) s\(^{-1}\)), the unbinding rate (\( k_{off} \), 600/s), the channel opening rate (\( \beta \), 2000/s), the channel closing rate (\( \alpha \), 1000/s), the rate for fast desensitization, 1.4/s; the rate for slow desensitization, 0.28/s; the rate for resensitization from fast desensitization state, 1.4/s; and the rate for resensitization from slow desensitization, 0.034/s. For A, octanol is assumed to reduce the ACh binding in a dose-dependent manner according to the following relations: \( k_{on} = k_{on}^{0} \times ([c] / [EC_{50}]^{n}) \). For B, octanol is assumed to block open ACh channel at a rate (\( b_{1} \), \( 2.5 \times 10^{7} \) M\(^{-1}\) s\(^{-1}\)), and unblock at a rate (\( u_{b_{1}} \), 300/s) (Scheme 2). For C, octanol is assumed to block both open and close channel with equal affinity. (\( b_{1} = b_{2} = 2.5 \times 10^{7} \) M\(^{-1}\) s\(^{-1}\); \( u_{b_{1}} = u_{b_{2}} = 300/s) (Scheme 3). The symbols represent simulated data, which are fit to a logistic equation to give \( EC_{50} \) and \( n_{H} \) values as shown in the figure.
The IC50 value of octanol obtained at 30 μM ACh (nH = 1.18) (Fig. 12A) and an IC50 value of 19.7 μM at 3 mM ACh (nH = 1.00) (Fig. 12B). Both sets of simulated values are similar to the experimental results.

The possibility that octanol blocks both open and close ACh channel with the equal affinity was also tested according to the model in Scheme 3. When block occurs at the same blocking and unblocking rates at both close and open channels (b+1 = b−2 = 2.5 × 105 M−1·s−1; ub+1 = ub−2 = 300/s; koff = 1.5 × 107 M−1·s−1), during preapplication of 30 μM octanol, the percentage of the blocked receptor (B) before ACh application would reach 71% of total ACh receptors with the blocking time constant of 95 ms. In the subsequent coapplication, an additional open channel block occurs. As a result, the simulation still produces a greater blocking effect than the experimental result.

Simulation of Ethanol-Octanol Interaction. Ethanol at 300 mM reduced octanol inhibitory potency by shifting the IC50 value to a higher concentration. To simulate ethanol-octanol interaction, we first assumed that there was no interaction between the potentiating site and the inhibitory site. Namely, when octanol and ethanol were coapplied with ACh, each alcohol performed its action independently. In this model, the IC50 value was 14.3 μM (nH = 1.16) at 30 μM ACh and 17.1 μM at 3 mM ACh (nH = 1.00). There was hardly any shift of IC50 value of octanol by 300 mM ethanol. If there is a shift at all, the shift is in the direction opposite the experimental result.

Fig. 12. Simulation of octanol and ethanol interaction. The octanol inhibitory dose–response curves are shown with and without 300 mM ethanol coapplication at two ACh concentrations (A, 30 μM ACh; B, 3 mM ACh). The filled symbols represent for the cases without ethanol, and the open symbols represent the cases with ethanol coapplication. The parameters used for simulation of ACh-induced currents are the same as those given in Fig. 10 legend. With 300 mM ethanol, the channel opening rate (b) increases to 3500/s. Besides, ethanol is assumed to reduce both octanol’s effect on koff and blocking action: without ethanol, b+1 = b−2 = 2.5 × 105 M−1·s−1; ub+1 = 300/s and koff = 1 × 107 (25 μM/[25 μM + {octanol}]) M−1·s−1; with 300 mM ethanol, b+1 = 1.5 × 107 M−1·s−1, ub+1 = 300/s; koff = 1 × 107 (75 μM/[75 μM + {octanol}])/25 μM−1·s−1. The symbols represent simulated data, which are fit to a logistic equation to give IC50 and nH values as shown in the figure.
Thus, we went on to test an alternative hypothesis that there is an interaction between ethanol and octanol. Ethanol (300 mM) reduced octanol inhibitory action by decreasing octanol inhibitory action on ACh. This phenomenon is called "cut-off". The similar cut-off effect was also seen in nAChRs of isolated Lymnaea stagnalis neurons (McKenzie et al., 1995) and in other systems such as NMDA receptors (Peoples and Weight, 1999) and GABA A receptors (Nakahiro et al., 1996). The cut-off effect has been interpreted in terms of both lipid and protein theories of alcohol action. In terms of the lipid theory, the cut-off effect was previously explained as being due to the limited ability of long-chain alcohols to partition into lipid bilayers because of the low solubility (Pringle et al., 1981), but subsequent direct measurements revealed no such cut-off in membrane partition (Franks and Lieb, 1986). In terms of the protein theory, the affinity of short- to medium-chain alcohols for the receptor continues to increase as the fitting to the hydrophobic pocket improves. For longer chain alcohols, the affinity does not continue to increase because the additional increase in chain length does not contribute to binding to the hydrophobic pocket.

Acknowledgments

We thank Nayla Hasan for technical assistance, Brian O'Neil Claeps for technical assistance with the αβ32 cell line culture, Julia Irizarry for secretarial assistance, and Min-John Lee for writing the C++ program for numerical solution of kinetic simulation. HEK cell lines stably expressing the αβ32 AChR subunits were provided by SIBIA Neurosciences, Inc. (now Merck Research Laboratories–San Diego), La Jolla, CA.

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