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Alcohol action on a neuronal membrane receptor: Evidence for a direct interaction with the receptor protein

(neurotransmitter receptor/ion channel/membrane fluidity/lipid/hydrophobicity)

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ABSTRACT **For almost a century, alcohols have been thought to produce their effects by actions on the membrane lipids of central nervous system neurons—the well known "lipid theory" of alcohol action. The rationale for this theory is the correlation of potency with oil/water or membrane/buffer partition coefficient. Although a number of recent studies have shown that alcohols can affect the function of certain neuronal neurotransmitter receptors, there is no evidence that the alcohols interact directly with these membrane proteins. In the present study, we report that inhibition of a neuronal neurotransmitter receptor, an ATP-gated ion channel, by a series of alcohols exhibits a distinct cutoff effect. For alcohols with a molecular volume of ≤42.2 ml/mol, potency for inhibiting ATP-activated current was correlated with lipid solubility (order of potency: 1-propanol = trifluoroethanol > monochloroethanol > ethanol > methanol). However, despite increased lipid solubility, alcohols with a molecular volume of ≥46.1 ml/mol (1-butanol, 1-pentanol, trichloroethanol, and dichloroethanol) were without effect on the ATP-activated current. The results suggest that alcohols inhibit the function of this neurotransmitter receptor by interacting with a small hydrophobic pocket on the receptor protein.**

Traditionally, alcohols have been thought to produce their behavioral effects by actions on the membrane lipids of central nervous system neurons (1–8). This "lipid theory" of alcohol action attributes alterations in the function of membrane ion channels, receptors, and other membrane proteins to perturbation of membrane lipids (1–8). Recently, a number of studies have shown that alcohols can affect the function of certain neuronal membrane receptors (9). However, evidence that alcohols interact directly with these membrane proteins is lacking. The observation that the potency of inhibition of a purified soluble enzyme, firefly luciferase, by a homologous series of alcohols does not exceed a certain value despite increasing hydrophobicity ("cutoff" effect) has been attributed to an interaction of the alcohols with a hydrophobic pocket on this enzyme (10).

Extracellular ATP has recently been recognized to function as an excitatory neurotransmitter in both the central and peripheral nervous systems (11–13). The receptors mediating these ATP responses have been found to be ligand-gated ion channels that are activated by extracellular ATP (14–16). Recently, we found that ethanol can inhibit the function of ATP-gated ion channels (17). Here, we report a distinct cutoff in the potency of several different alcohols for inhibiting this neuronal membrane receptor; the observations suggest that alcohols inhibit the function of this receptor by interacting directly with a hydrophobic pocket on the receptor protein. Some of this work has been presented previously in preliminary form (18, 19).

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MATERIALS AND METHODS

The effect of different alcohols on an ATP-activated current was studied by using the whole-cell patch-clamp technique as described (17). Briefly, dorsal root ganglia from adult bullfrogs (*Rana catesbeiana*) were rapidly dissected, minced, and dissociated by incubation in trypsin III (0.55 mg/ml; Sigma) and collagenase 1A (1.1 mg/ml; Sigma) in Dulbecco's modified Eagle's medium (Sigma) at 35°C for ≈30 min. Soybean trypsin inhibitor I-S (1.8 mg/ml; Sigma) was added to stop enzymatic digestion.

Whole-cell patch-clamp recording from the isolated neurons was carried out at room temperature with an EPC-7 patch-clamp amplifier (List Electronics, Darmstadt, Germany) connected via a Labmaster TL-1 interface to a computer (Compaq 386/20e). Data were collected with PCLAMP software (Axon Instruments, Foster City, CA) and stored for off-line analysis. Currents were also recorded on a chart recorder (Gould 2400S). Neurons were continuously superfused at 1–2 ml/min with an extracellular medium containing 117 mM NaCl, 2 mM KCl, 2 mM $MgCl_2$, 2 mM $CaCl_2$, 5 mM Hepes, 10 mM D-glucose; pH was buffered to 7.2 with NaOH. Patch pipettes were filled with an intracellular solution containing 110 mM CsCl, 2 mM $MgCl_2$, 0.4 mM $CaCl_2$, 4.4 mM EGTA, 5 mM Hepes, 1.5 mM ATP; pH was buffered to 7.2 with CsOH. Membrane potential was held at −60 mV. ATP (Sigma) was added as the Na^+ salt and was prepared daily in external solution. Alcohols (Aldrich) were dissolved in agonist solutions. High concentrations of the more hydrophobic alcohols could not be tested because they were insoluble in aqueous solution; in addition, aqueous concentrations near saturation had a tendency to disrupt recordings, presumably by destroying the seal between the patch pipette and the cell membrane (17). Superfusion of extracellular solutions, as well as agonist and drug applications, were performed by using gravity flow from a linear barrel array consisting of fused silica tubes (i.d., ≈200 μm) connected to independent reservoirs, and rapid solution changes were effected by shifting the pipette horizontally with a micromanipulator (the time for complete solution exchange was <50 ms). At the end of agonist and drug applications, agonist and drug solutions were washed out by, and then cells were continuously superfused by, normal external solution flowing from one pipette barrel. Data were statistically compared by Student's *t* test or one-way ANOVA, as noted. Average values are expressed as mean peak current ± SE.

For straight-chain aliphatic alcohols, the membrane/buffer partition coefficients are from McCreery and Hunt (20). These values are as follows: methanol, 0.036; ethanol, 0.096; 1-propanol, 0.438; 1-butanol, 1.52; 1-pentanol, 5.02. For halogenated alcohols, the membrane/buffer partition coeffi-

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cients were calculated from the data of Leo *et al.* (21), as described by McCreery and Hunt (20). These values are as follows: monochloroethanol, 0.234; dichloroethanol, 1.44; trichloroethanol, 8.8; trifluoroethanol, 0.463. The values of the membrane/buffer partition coefficients for straight-chain aliphatic alcohols calculated from the data of Leo *et al.* (21) are equivalent to the values given by McCreery and Hunt (20).

RESULTS

ATP has been found to activate a ligand-gated ion channel in bullfrog dorsal root ganglion neurons (22, 23). We reported recently that ethanol can inhibit the ATP-activated current in these neurons, apparently by increasing the dissociation constant of the receptor for agonist (17). Here we report the effect of several straight-chain and halogenated alcohols on this ATP-activated current. Fig. 1 illustrates the effect of several straight-chain alcohols on this ATP-activated current. As shown in Fig. 1*Ab*, 100 mM ethanol markedly decreased the amplitude of inward current activated by 2.5 μM ATP. On average, 100 mM ethanol reduced the amplitude of current activated by 2.5 μM ATP by 45% ± 3% (n = 8 cells). Of 68 cells tested, 60 exhibited ATP-activated currents that were inhibited by 100 mM ethanol. Cells used in this study were those in which 100 mM ethanol inhibited the ATP-activated current by at least 20%. As shown in Fig. 1 *Aa* and *Ac*, 200 mM methanol and 50 mM 1-propanol also markedly decreased the amplitude of ATP-activated current. On average, the amplitude of current activated by 2.5 μM ATP was decreased 34% ± 3% (n = 7) by 200 mM methanol and 57% ± 5% (n = 7) by 50 mM 1-propanol. As shown in Fig. 1*A*, the onset of inhibition of ATP-activated current by alcohols from methanol to propanol was very rapid, and the alcohols did not appear to alter the kinetics of the ATP-activated current. The concentration–response curves in Fig. 1*B* show that inhibition of the ATP-activated current by methanol, ethanol, or 1-propanol was concentration-dependent, and the concentrations that produced 50% inhibition (IC_{50}) were 298 mM (Fig. 1*Ba*), 110 mM (Fig. 1*Bb*), and

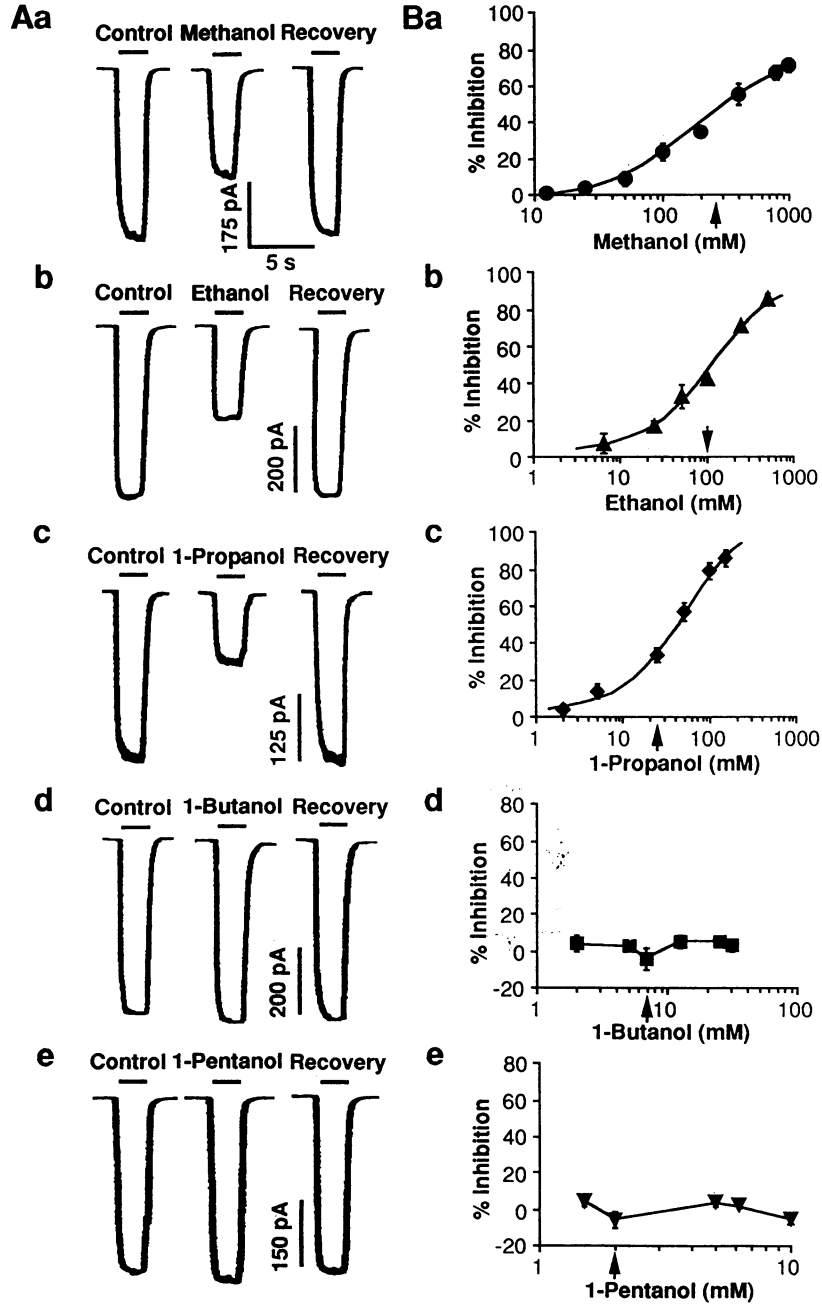

FIG. 1. Effect of straight-chain alcohols on ATP-activated current. (*A*) Effect of 200 mM methanol (*a*), 100 mM ethanol (*b*), 50 mM 1-propanol (*c*), 7 mM 1-butanol (*d*), or 2 mM 1-pentanol (*e*) on the current activated by 2.5 μM ATP. Solid bar above each record indicates time of ATP or ATP + alcohol application, as labeled. (*B*) Graphs plot percentage inhibition of current activated by 2.5 μM ATP as a function of alcohol concentration. Each point represents mean ± SE of 5–8 cells; error bars not visible are smaller than the size of the symbols. Sigmoid curves shown are the best fit of the data to the logistic equation $y = E_{max}/[1 + (x/IC_{50})^n]$, where E_{max} is the maximal response, IC_{50} is the alcohol concentration producing 50% of the maximal inhibition, and n is the slope factor. 1-Butanol (*d*) and 1-pentanol (*e*) did not significantly alter the ATP-activated current and thus yielded concentration–response curves that were poorly fitted by the logistic equation (ANOVA; $P > 0.1$; n = 5–8). Arrows on abscissae indicate the alcohol concentration that would result in a membrane concentration equivalent to that produced by 100 mM ethanol (20, 21, 24, 25).

47 mM (Fig. 1*Bc*), respectively. The slope factors of these plots are in the range 1.0–1.2; there are no significant differences among these values (ANOVA; $P > 0.1$; $n = 5–8$).

By contrast, Fig. 1*A* also illustrates that 1-butanol (7 mM) and 1-pentanol (2 mM) did not reduce the amplitude of current activated by 2.5 μM ATP in concentrations that would result in a membrane alcohol concentration equivalent to that produced by 100 mM ethanol (20, 21, 24, 25). The concentration–response curves in Fig. 1*B* illustrate that 1-butanol did not significantly affect the amplitude of ATP-activated current at any concentration between 2 and 30 mM. (Fig. 1*Bd*; ANOVA; $P > 0.1$; $n = 5–8$), and 1-pentanol did not significantly affect the amplitude of ATP-activated current at any concentration between 1.5 and 10 mM (Fig. 1*Be*; ANOVA; $P > 0.1$; $n = 5–8$). At the concentrations used in these experiments, none of the aliphatic alcohols alone induced a detectable current (data not shown).

The effects of several halogenated alcohols on the ATP-activated current were also tested. As shown in Fig. 2*A*, the amplitude of current activated by 2.5 μM ATP was markedly decreased by 90 mM monochloroethanol (Fig. 2*Aa*) or 50 mM trifluoroethanol (Fig. 2*Ab*) but not by concentrations of dichloroethanol (10 mM; Fig. 2*Ac*) or trichloroethanol (1 mM; Fig. 2*Ad*) that would result in a membrane alcohol concentration equivalent to or greater than that produced by 100 mM ethanol (20, 21, 24, 25). On average, the amplitude of the current activated by 2.5 μM ATP was decreased 46% ± 4% ($n = 5$) by 90 mM monochloroethanol and 52% ± 2% ($n = 6$) by 50 mM trifluoroethanol. The concentration–response curves in Fig. 2*B* show that inhibition of the ATP-activated current by monochloroethanol and trifluoroethanol was concentration-dependent, with IC_{50} values of 94 (Fig. 2*Ba*) and 48 (Fig. 2*Bb*) mM, respectively. The slope factors of these plots are 1.1 and 1.2 for monochloroethanol and trifluoroethanol, respectively; these values are not significantly different (Student's *t* test; $P > 0.1$; $n = 5–8$). These values are also not significantly different from those of the straight-chain alcohols (ANOVA; $P > 0.1$; $n = 5–8$). The concentration–response curves in Fig. 2*B* also show that dichloroethanol did not significantly affect the amplitude of ATP-activated current at any concentration between 2 and 50 mM (Fig. 2*Bc*; ANOVA; $P > 0.1$; $n = 5–7$), and trichloroethanol did not significantly affect the amplitude of ATP-activated current at any concentration between 0.3 and 5 mM (Fig. 2*Bd*; ANOVA; $P > 0.1$; $n = 5–8$). At the concentrations used in these experiments, none of the halogenated alcohols alone induced a detectable current (data not shown).

Because the alcohols used in the present study differ in hydrophobicity and molecular volume (20, 21, 24–26), our observations raised the question of whether the potency of different alcohols for inhibition of the ATP-activated current might be related to their hydrophobicity or molecular volume. As shown in Fig. 3*A*, the potency for inhibition of ATP-activated current increases as the membrane/buffer partition coefficient of the alcohol increases from 0.036 to 0.46 (20, 21, 24, 25). Moreover, in this range there is a

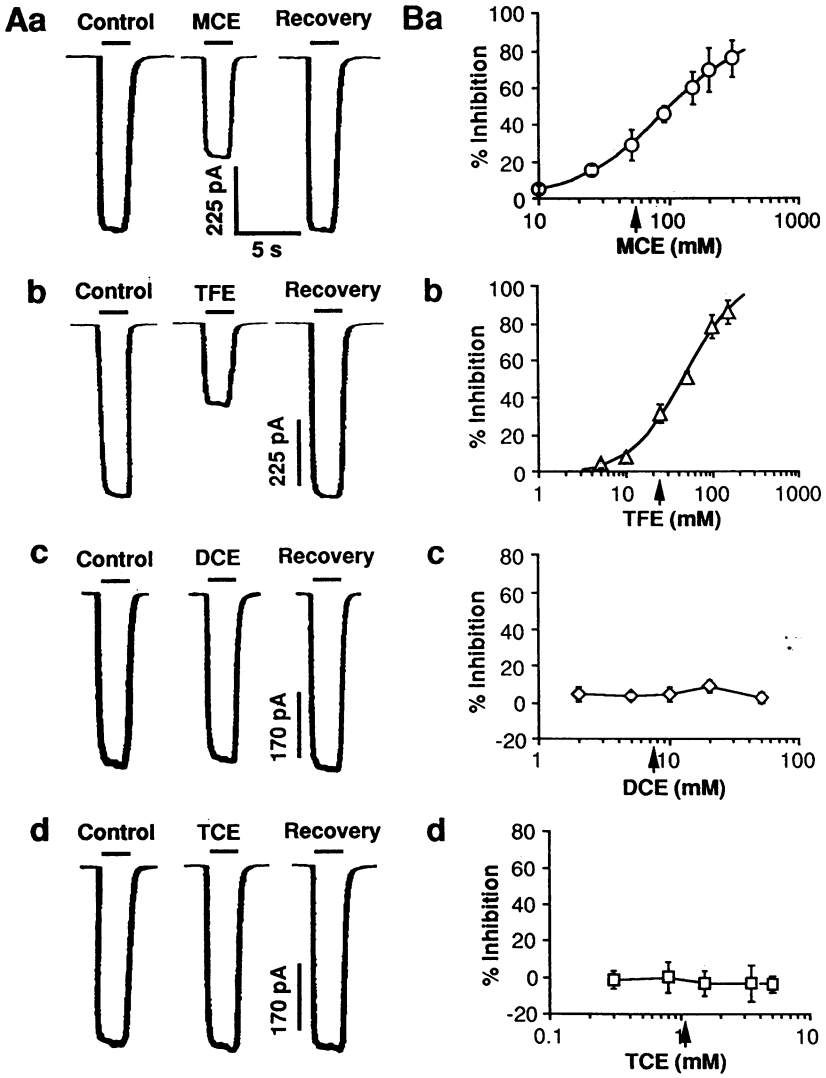

FIG. 2. Effect of halogenated alcohols on ATP-activated current. (*A*) Effect of 90 mM monochloroethanol (MCE) (*a*), 50 mM trifluoroethanol (TFE) (*b*), 10 mM dichloroethanol (DCE) (*c*), or 1 mM trichloroethanol (TCE) (*d*) on the current activated by 2.5 μM ATP. Solid bar above each record indicates time of ATP or ATP + alcohol application, as labeled. (*B*) Graphs plot percentage inhibition of current activated by 2.5 μM ATP as a function of alcohol concentration. Each point represents the mean ± SE of 5–8 cells; error bars not visible are smaller than the size of the symbols. Sigmoid curves shown are the best fit of the data to the logistic equation given in Fig. 1 legend. Dichloroethanol (*c*) and trichloroethanol (*d*) did not significantly alter the ATP-activated current and thus yielded concentration–response curves that were poorly fitted by the logistic equation (ANOVA; $P > 0.1$; $n = 5–8$). Arrows on abscissae indicate the alcohol concentration that would result in a membrane concentration equivalent to that produced by 100 mM ethanol (20, 21, 24, 25).

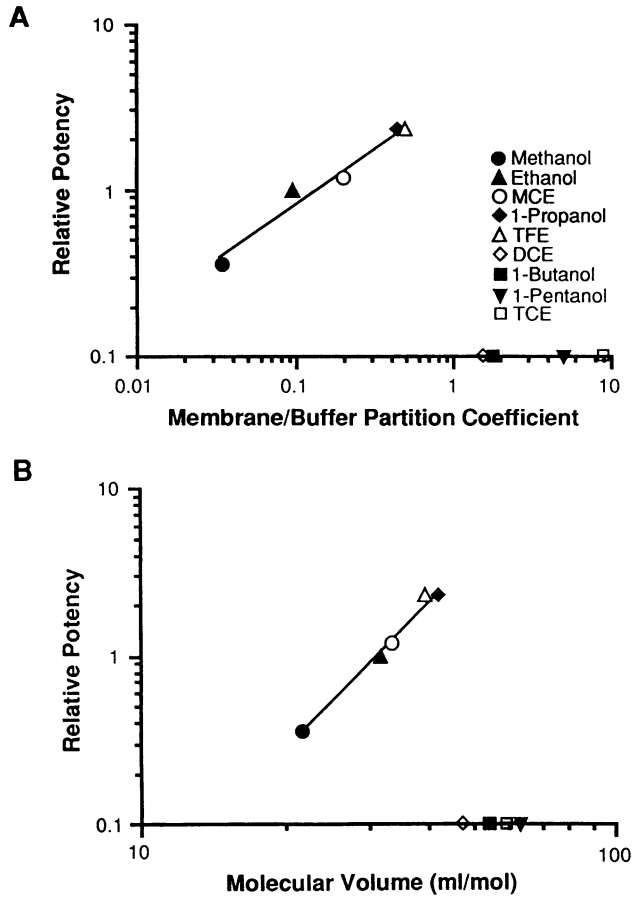

FIG. 3. (*A*) Relationship between potency of different alcohols for inhibiting ATP-activated current (IC_{50} of ethanol/IC_{50} of alcohol) and membrane/buffer partition coefficient of the alcohols. At membrane/buffer partition coefficients of ≤0.46, there is a significant correlation between potency for inhibition of ATP-activated current and membrane/buffer partition coefficient (linear regression analysis of variance; $P < 0.001$). Because IC_{50} values could not be obtained for alcohols with membrane/buffer partition coefficients of ≥1.4, the data points for these alcohols are shown on the abscissa. MCE, monochloroethanol; TFE, trifluoroethanol; DCE, dichloroethanol; TCE, trichloroethanol. (*B*) Relationship between potency of different alcohols for inhibiting ATP-activated current (IC_{50} of ethanol/IC_{50} of alcohol) and molecular volume of the alcohols. For alcohols with molecular volumes of ≤42.2 ml/mol, there is a significant correlation between potency for inhibition of ATP-activated current and molecular volume (linear regression analysis of variance; $P < 0.001$). Because IC_{50} values could not be obtained for alcohols with molecular volumes of ≥46.1 ml/mol, the data points for these alcohols are shown on the abscissa.

significant linear relationship between these two measures (linear regression analysis of variance; $P < 0.001$). Thus, the order of potency of different alcohols for inhibition of ATP-activated current is methanol < ethanol < monochloroethanol < trifluoroethanol = 1-propanol. However, alcohols with membrane/buffer partition coefficients of ≥1.4 (dichloroethanol, 1-butanol, 1-pentanol, and trichloroethanol) were without effect on the ATP-activated current. Similar results were obtained for the correlation between potency for inhibition of ATP-activated current and molecular volume of the alcohols (Fig. 3*B*). For alcohols with a molecular volume of ≤42.2 ml/mol (24, 26), potency for inhibiting ATP-activated current is correlated with molecular volume (methanol < ethanol < monochloroethanol < trifluoroethanol = 1-propanol), and there is a significant linear relationship between these two measures (linear regression analysis of variance; $P < 0.001$). However, despite increased lipid solubility, alcohols with a molecular volume of ≥46.1 ml/mol (dichloroethanol, 1-butanol, 1-pentanol, and trichloroethanol) did not inhibit the ATP-activated current.

To evaluate the possibility that alcohol inhibition of ATP-activated current might be mediated by intracellular proteins, we tested the effect of the intracellular application of ethanol on inhibition of the current by extracellularly applied ethanol. We found that the inhibition by 100 mM extracellular ethanol of the current activated by 2.5 μM ATP was not significantly different in the presence or absence of 100 mM ethanol in the intracellular solution [44% ± 4% ($n = 7$ cells) vs. 45% ± 3% ($n = 8$ cells), respectively; Student's t test; $P > 0.05$].

DISCUSSION

In the experiments reported here, we found that the potency of short-chain alcohols for inhibiting ATP-activated current increased as chain length increased from one to three carbons (methanol < ethanol < 1-propanol). However, 1-butanol and 1-pentanol did not have a significant effect on the ATP-activated current, even at concentrations that would produce membrane alcohol concentrations equivalent to that produced by 500 mM ethanol. In addition, for halogenated alcohols, dichloroethanol and trichloroethanol did not affect ATP-activated current, even at concentrations that would produce membrane alcohol concentrations equivalent to that produced by 500 mM ethanol, whereas monochloroethanol and trifluoroethanol both inhibited the current in a concentration-dependent manner. The logarithm of membrane disordering potency has been shown to increase linearly in direct proportion to the logarithm of the membrane/buffer partition coefficient for aliphatic alcohols with up to at least eight carbon atoms (24). If the effect of alcohols on ATP-activated current is secondary to their perturbation of membrane lipids, the inhibition of ATP-activated current by various alcohols would be expected to exhibit a similar linear relationship with their membrane/buffer partition coefficients. Consequently, our observation that alcohols with a membrane/buffer coefficient of ≥1.4 did not inhibit ATP-activated current is not explained by the lipid theory of alcohol action, because 1-butanol and 1-pentanol are more hydrophobic and are more potent in disordering membrane lipids than are methanol, ethanol, and 1-propanol, and dichloroethanol and trichloroethanol are more hydrophobic and are more potent in disordering membrane lipids than are monochloroethanol and trifluoroethanol (6, 7, 20, 21, 24, 25, 27, 28).

It has recently been suggested that alcohols can influence the function of intracellular second messenger systems (29, 30). However, it is unlikely that the alcohol effect on ATP-activated current in our experiments was due to an alcohol action on intracellular second messenger systems. First, the onset of the alcohol effect on ATP-activated current in our experiments was very rapid (within the time required for solution exchange; namely <50 ms), whereas equilibration of intracellular concentrations of alcohol would be expected to occur much more slowly. Second, mediation of the effect of alcohols on ATP-gated channels by second messenger systems is also unlikely because the fastest known second messenger modulators of ion channels act on a time scale of hundreds of milliseconds (31). These arguments, however, do not exclude the possibility of second messenger involvement in the alcohol inhibition of ATP-gated channel function. We therefore tested the effect of the intracellular application of ethanol on the inhibition of ATP-activated current by extracellular ethanol. The rationale for this was that if the effect of extracellular ethanol on ATP-gated channels is secondary to an action on an intracellular second messenger system, then the intracellular application of ethanol would exert its effect on that second messenger system and inhibit ATP-activated

current, thus diminishing or abolishing the effect of extracellular ethanol. Our observation that the intracellular application of ethanol had no effect on inhibition of the ATP-activated current by the extracellular application of ethanol provides evidence that the alcohol effect on ATP-gated channels is not mediated by an intracellular second messenger system.

Franks and Lieb (10) observed a cutoff effect on the inhibition of purified firefly luciferase for aliphatic alcohols with 15 or more carbon atoms. They proposed that this behavior is due to interaction of the alcohols with a hydrophobic protein pocket of circumscribed dimensions. Our observation that alcohols with a molecular volume of ≥46.1 ml/mol did not inhibit ATP-activated current is consistent with the protein hypothesis of alcohol action and suggests that alcohols inhibit ATP-activated current by interacting with a hydrophobic pocket on the ATP receptor. The cutoff in potency for aliphatic alcohols with four or more carbon atoms suggests that the size of the alcohol-sensitive hydrophobic pocket on the ATP receptor is quite small with respect to the alcohol-sensitive hydrophobic pocket on firefly luciferase. Franks and Lieb (32) also suggested that the hydrophobic pocket on firefly luciferase can accommodate only one large but more than one small alcohol molecule. Our observations that the slopes of the concentration–response curves for alcohol inhibition of ATP-activated current are not different for methanol, ethanol, 1-propanol, monochloroethanol, and trifluoroethanol is consistent with the same number of alcohol molecules acting in the putative hydrophobic pocket on the ATP receptor. It should be noted, however, that the slope factors do not necessarily indicate the precise number of alcohol molecules interacting with the receptor molecule, as they also reflect whether drug binding to its site of action is cooperative (33).

At present, the physiological role of the neuronal ATP receptor ion channel and its modulation by alcohols remains to be determined. Also, the question of whether alcohols affect other ligand-gated ion channels by acting on the membrane lipids or directly on the protein needs further investigation. However, preliminary experiments in our laboratory suggest that there are cutoffs in the potency of straight-chain alcohols for affecting the function of 5-hydroxytryptamine receptors (34) and *N*-methyl-D-aspartate receptors (35); in addition, those cutoffs differ from the cutoff for ATP-gated channels and they also differ from each other. The recognition that alcohols can affect the function of a neuronal membrane receptor by interaction with a circumscribed hydrophobic pocket should provide a basis for investigating the molecular sites of alcohol action on these membrane proteins.

1. Overton, E. (1901) *Studien Über Die Narkose* (Fischer, Jena, Germany).
2. Meyer, H. (1901) *Arch. Exp. Pathol. Pharmakol.* **46,** 338–346.
3. Meyer, K. H. (1937) *Trans. Faraday Soc.* **33,** 1062–1068.
4. Mullins, L. J. (1954) *Chem. Rev.* **54,** 289–323.
5. Seeman, P. (1972) *Pharmacol. Rev.* **24,** 583–655.
6. Janoff, A. S. & Miller, K. W. (1981) in *Biological Membranes*, ed. Chapman, D. (Academic, New York), pp. 417–476.
7. Goldstein, D. B. (1984) *Annu. Rev. Pharmacol. Toxicol.* **24,** 43–64.
8. Rall, T. W. (1990) in *The Pharmacological Basis of Therapeutics*, eds. Gilman, A. G., Rall, T. W., Nies, A. S. & Taylor, P. (Pergamon, New York), pp. 374–375.
9. Weight, F. F. (1992) *Int. Rev. Neurobiol.* **33,** 289–348.
10. Franks, N. P. & Lieb, W. R. (1985) *Nature (London)* **316,** 349–351.
11. Evans, R., Derkach, V. & Surprenant, A. (1992) *Nature (London)* **357,** 503–505.
12. Edwards, F. A., Gibb, A. J. & Colquhoun, D. (1992) *Nature (London)* **359,** 144–147.
13. Silinsky, E. M. & Gerzanich, V. (1993) *J. Physiol. (London)* **464,** 197–212.
14. Burnstock, G. (1990) *Ann. N.Y. Acad. Sci.* **603,** 1–17.
15. Bean, B. P. (1992) *Trends Pharmacol. Sci.* **13,** 87–90.
16. Illes, P. & Norenberg, W. (1993) *Trends Pharmacol. Sci.* **14,** 50–54.
17. Li, C., Aguayo, L., Peoples, R. W. & Weight, F. F. (1993) *Mol. Pharmacol.* **44,** 871–875.
18. Li, C., Aguayo, L. & Weight, F. F. (1991) *Third Int. Brain Res. Organ. World Congr. Neurosci. Abstr.*, 64.
19. Li, C., Peoples, R. W. & Weight, F. F. (1993) *Soc. Neurosci. Abstr.* **19,** 283.
20. McCreery, M. J. & Hunt, W. A. (1978) *Neuropharmacology* **17,** 451–461.
21. Leo, A., Hansch, C. & Elkins, D. (1971) *Chem. Rev.* **71,** 525–616.
22. Bean, B. P. (1990) *J. Neurosci.* **10,** 1–10.
23. Bean, B. P., Williams, C. A. & Ceelen, P. W. (1990) *J. Neurosci.* **10,** 11–19.
24. Lyon, R. C., McComb, J. A., Schreurs, J. & Goldstein, D. B. (1981) *J. Pharmacol. Exp. Ther.* **218,** 669–675.
25. Firestone, L. L., Miller, J. C. & Miller, K. W. (1986) in *Molecular and Cellular Mechanisms of Anesthetics*, eds. Roth, S. H. & Miller, K. W. (Plenum, New York), pp. 455–470.
26. Bondi, A. (1964) *J. Phys. Chem.* **68,** 441–451.
27. Pringle, M. J., Brown, K. B. & Miller, K. W. (1981) *Mol. Pharmacol.* **19,** 49–55.
28. Franks, N. P. & Lieb, W. R. (1986) *Proc. Natl. Acad. Sci. USA* **83,** 5116–5120.
29. Slater, S. J., Cox, K. J. A., Lombardi, J. V., Ho, C., Kelly, M. B., Rubin, E. & Stubbs, C. D. (1993) *Nature (London)* **364,** 82–84.
30. Snell, L. D., Tabakoff, B. & Hoffman, P. L. (1994) *Alcohol. Clin. Exp. Res.* **18,** 81–85.
31. Yatani, A. & Brown, A. M. (1989) *Science* **245,** 71–74.
32. Franks, N. P. & Lieb, W. R. (1984) *Nature (London)* **310,** 599–601.
33. De Lean, A., Munson, P. J. & Rodbard, D. (1978) *Am. J. Physiol.* **235,** E97–E102.
34. Fan, P. & Weight, F. F. (1994) *Soc. Neurosci. Abstr.* **20,** in press.
35. Peoples, R. W. & Weight, F. F. (1993) *Soc. Neurosci. Abstr.* **19,** 277.