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Ethanol Inhibits a Neuronal ATP-Gated Ion Channel

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SUMMARY

The cellular mechanisms by which ethanol affects nervous system function are poorly understood. However, evidence has been accumulating that ethanol can affect the function of neurotransmitter-gated ion channels. Extracellular ATP has recently been reported to produce excitatory actions in the peripheral and central nervous systems by activating ligand-gated ion channels. We studied the effect of ethanol on membrane ion current activated by extracellular ATP in isolated bullfrog dorsal root ganglion neurons, by means of the whole-cell patch-clamp technique. The amplitude of the ATP-activated current was decreased by ethanol in a concentration-dependent manner over the range of 3–500 mM. The average inhibition of 1 μM ATP-activated current by 100 mM ethanol was 64 ± 3%, and the concentration of ethanol that produced 50% inhibition was 68 mM. Ethanol inhibition of ATP-activated current was not dependent on membrane potential from −80 to +40 mV, and ethanol did not change the reversal potential of ATP-activated current. Ethanol (100 or 400 mM) shifted the ATP concentration-response curve to the right, increasing the EC_{50} for ATP from 3.0 μM to 6.0 μM or 22.3 μM, respectively, but did not reduce the maximal response to ATP. The results suggest that ethanol inhibits ATP-activated current by increasing the apparent dissociation constant for the ATP receptor.

Although ethanol is probably the oldest and most widely used psychoactive drug, the cellular mechanisms by which it affects nervous system function are poorly understood. Recently, attention has focused on the effects of ethanol on membrane ion channels. For example, electrophysiological experiments have shown that ethanol, in a pharmacological concentration range (5–100 mM), can affect the function of several different types of neurotransmitter-gated ion channels (1). Ethanol inhibits membrane ion current activated by NMDA in central (2, 3) and peripheral neurons (4) and potentiates current activated by GABA at $GABA_A$ receptors in some cell types (5, 6), by acetylcholine at nicotinic cholinergic receptors in frog neuromuscular junction (7), and by serotonin at 5-hydroxytryptamine type 3 channels in NCB-20 neuroblastoma cells and sensory neurons (8). These observations suggest that certain types of neurotransmitter-gated ion channels may be cellular sites of ethanol action.

Extracellular ATP has recently been reported to produce excitatory actions on neurons in both the peripheral and central nervous systems (9–11) and to mediate fast excitatory postsynaptic potentials or excitatory postsynaptic currents in peripheral (12, 13) and central (14) neurons by activating ligand-gated ion channels. To determine whether ethanol can affect the function of neuronal ATP-gated ion channels, we used the whole-cell patch-clamp technique to investigate the effects of ethanol on the membrane ion current activated by extracellular ATP in neurons freshly isolated from bullfrog DRG. We found that ethanol concentrations from 3 to 500 mM produced a concentration-dependent inhibition of ATP-activated ion current, with an IC_{50} of 68 mM. Some of this work has been presented previously in preliminary form (15).

Materials and Methods

Isolation of neurons. Adult male bullfrogs (*Rana catesbeiana*) were used in winter and spring. After decapitation and pithing, DRGs (usually six) were rapidly isolated, transferred to a Petri dish containing DMEM (Sigma) solution (1.38 g of DMEM and 0.2 g of NaCl dissolved in 100 ml of distilled water, pH 7.2; osmolarity, 250 mosmol/kg), and cut into small pieces. The DRG fragments were then placed in a flask containing 5 ml of DMEM, in which trypsin III (0.55 mg/ml; Sigma) and collagenase 1A (1.1 mg/ml; Sigma) had been dissolved, and were incubated at 35° for approximately 30 min in a water-bath shaker. Soybean trypsin inhibitor I-S (1.8 mg/ml; Sigma) then was added to stop enzymatic digestion. Neurons were then placed into a single uncoated Petri dish and used for electrophysiological recording. The neurons studied in this investigation were 30–53 μm in diameter.

Whole-cell patch-clamp recording. Neurons were observed under an inverted microscope (Diaphot; Nikon, Tokyo, Japan) using phase-contrast optics. Gigaohm seals were made using borosilicate glass microelectrodes with tip resistances of 2–4 MΩ. Whole-cell patch-clamp

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ABBREVIATIONS: NMDA, *N*-methyl-D-aspartate; DRG, dorsal root ganglion; GABA, γ-aminobutyric acid; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; EGTA, ethylene glycol bis(β-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid; DMEM, Dulbecco's modified Eagle's medium.

recordings were carried out at room temperature using a EPC-7 (List Electronic, Darmstadt, Germany) patch-clamp amplifier connected to a computer (Compaq 386/20e) via a Labmaster TL-1 interface. Data were collected and analyzed using pCLAMP software (Axon Instruments, Foster City, CA) and were stored for off-line analysis. Currents were also recorded on a chart recorder (Gould 2400S). Cells were continuously superfused at 1–2 ml/min with an extracellular medium containing (in mM) 117 NaCl, 2 KCl, 2 $MgCl_2$, 2 $CaCl_2$, 5 HEPES, and 10 D-glucose; the pH was buffered to 7.2 using NaOH. Patch pipettes were filled with an intracellular solution containing (in mM) 110 CsCl, 2 $MgCl_2$, 0.4 $CaCl_2$, 4.4 EGTA, 5 HEPES, and 1.5 ATP; the pH was buffered to 7.2 using CsOH. Series resistance in all recordings was <5 MΩ and was compensated by 50–70%. Membrane potential was usually held at −60 mV, except where indicated.

Application of solutions. Drug applications were performed using modifications of a superfusion system described previously (16, 17). We used a 12-barrel array of perfusion pipettes composed of fused silica tubes, each with an internal diameter of 200 μm. Solutions were delivered by gravity flow from independent reservoirs placed above the preparation, and rapid solution changes were effected by shifting the pipette array horizontally using a micromanipulator. Between drug applications, cells were constantly bathed in normal external solution flowing from one pipette barrel. ATP (Sigma) was added as the Na^+ salt and was prepared daily in external solution. Ethanol was purchased from Pharmco (Bayonne, NJ). In most experiments, ethanol and other compounds were added to the extracellular solution containing agonist and were delivered simultaneously to the neuron. Drug applications were spaced at least 60 sec apart.

Data analysis. Data were statistically compared using Student's *t* test and one-way analysis of variance. Statistical analysis of concentration-response data was performed using the nonlinear curve-fitting program ALLFIT (18). Average values are expressed as mean ± standard error.

Results

Extracellular ATP has recently been reported to activate inward current in bullfrog DRG neurons; the current showed inward rectification, low selective cation permeability, and a reversal potential near 0 mV (19, 20). Fig. 1 illustrates the current response to externally applied ATP in voltage-clamped bullfrog DRG neurons in our experiments. The inward current activated rapidly upon ATP application, showed slow desensitization, and decayed quickly upon removal of ATP (Fig. 1A). The amplitude of the response was concentration dependent, with an EC_{50} of 3.0 μM (Fig. 1B), which is similar to a previously reported value (19). The slope of the concentration-response curve for ATP (apparent Hill coefficient) was 1.1. Responses to ATP application were observed in 94% of the neurons studied (76 of 81 cells). Fig. 1C shows that the current activated by 2.5 μM ATP was reversibly antagonized by 100 μM suramin, a P_2 purinoceptor antagonist. Similar results were observed in five other neurons. In contrast, in four neurons in which ATP activated inward current, 200 μM adenosine, a P_1 receptor agonist, did not activate detectable current (data not shown).

Fig. 2 illustrates inhibition of ATP-activated current by ethanol. As shown in Fig. 2A, the amplitude of inward current activated by 1 μM ATP was markedly decreased in the presence of 100 mM ethanol and completely recovered after ethanol washout. On average, 100 mM ethanol inhibited the current activated by 1 μM ATP by 64 ± 3% (n = 12). As shown in Fig. 2B, the inhibition of ATP-activated current by ethanol was concentration dependent over the concentration range of 3–500 mM, and the ethanol concentration that produced 50% inhibition (IC_{50}) was 68 mM. The apparent Hill coefficient for

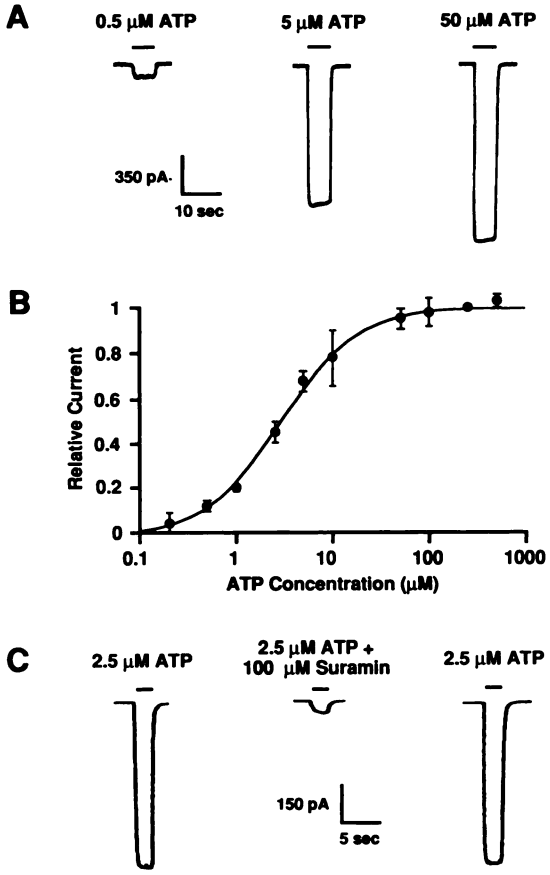

Fig. 1. ATP-activated inward currents in neurons isolated from bullfrog DRG. A, Inward currents activated by 0.5, 5, and 50 μM ATP. Records are sequential *current traces* (from *left* to *right*) obtained from a single neuron voltage-clamped at −60 mV. B, *Graph* plotting the relative amplitude of ATP-activated current as a function of ATP concentration. Amplitudes of currents activated by various concentrations of ATP were normalized to the current evoked by 250 μM ATP. Each *point* is the average of seven to 10 cells at a holding potential of −60 mV; error bars not visible are smaller than the size of the symbols. The *curve* shown is the best fit of the data to the logistic equation $Y = E_{max}/[1 + (X/EC_{50})^n]$, where E_{max} is the maximal response, EC_{50} is the agonist concentration producing 50% of the maximal response, and n is the slope factor (apparent Hill coefficient). Values obtained were E_{max} = 1, EC_{50} = 3.0 μM, and n = 1.1. C, Ion current activated by 2.5 μM ATP before, during, and several minutes after application of the P_2 purinoceptor antagonist suramin (100 μM), in a neuron voltage-clamped at −60 mV.

ethanol inhibition of ATP-activated current was 1.1. The graph in Fig. 2C plots the amplitude of the current activated by 5 μM ATP during two series of exposures to 100 mM ethanol in the same neuron. It can be seen that the inhibition of ATP-activated current by ethanol was relatively stable during the two application series. Over the concentration range of 3–500 mM, application of ethanol alone did not activate detectable current (data not shown). The effect of higher concentrations of ethanol (>500 mM to 1 M) could not be evaluated, because they result in degradation of the recording and/or rupture of the seal between the patch pipette and the cell. Of a total of 76 cells tested, we found that the current activated by 5 μM ATP was insensitive to 100 mM ethanol in eight cells.

In an attempt to elucidate the mechanism involved in the inhibitory effect of ethanol on ATP-activated current, we tested the effect of membrane potential on the inhibition of ATP-activated current by ethanol. Fig. 3A shows a current-voltage

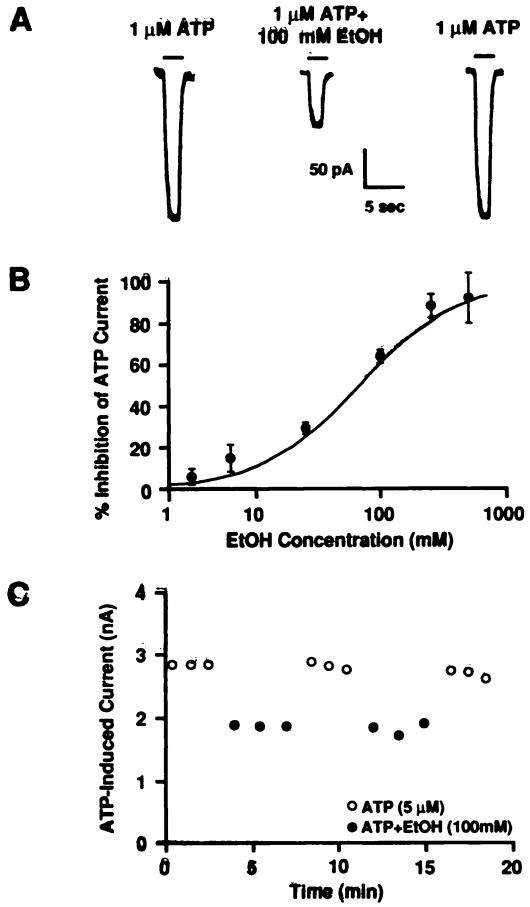

Fig. 2. Reversible inhibition by ethanol of ATP-activated current. A, Current activated by application of 1 μM ATP before, during, and after application of 100 mM ethanol (*EtOH*). Records are sequential *current traces* (from *left* to *right*) obtained from a single neuron voltage-clamped at −60 mV. B, *Graph* plotting average percentage inhibition of the amplitude of current activated by 1 μM ATP as a function of ethanol concentration. Each *point* is the average of five to eight cells at a holding potential of −60 mV; error bars not visible are smaller than the size of the symbols. The *curve* shown is the best fit of the data to the logistic equation given in the legend to Fig. 1. Values obtained were E_{max} = 100%, IC_{50} = 68 mM, and n = 1.1. C, *Graph* plotting the magnitude of individual responses to application of 5 μM ATP as a function of time in a single neuron voltage-clamped at −60 mV, illustrating two periods of exposure to 100 mM ethanol. Note the consistent inhibition of ATP-activated current in the presence of ethanol.

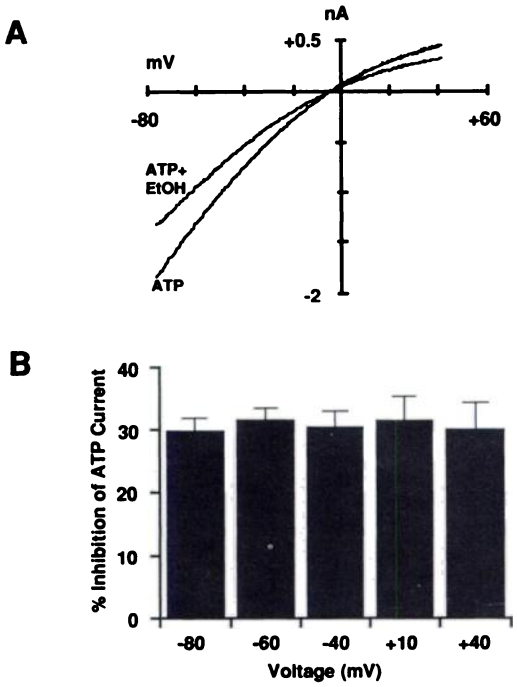

Fig. 3. Effect of membrane potential on inhibition by ethanol of ATP-activated current. A, Current-voltage relationship (I-V plot) showing the leak-subtracted amplitude of current activated by 5 μM ATP versus membrane potential, in the absence and presence of 100 mM ethanol (*EtOH*). Data were obtained using a voltage ramp from +40 to −80 mV at a rate of 150 mV/sec. Current was first recorded in the presence of 5 μM ATP in the absence and presence of 100 mM ethanol. Current was then elicited using the same ramp protocol in the presence of normal external solution. This "leak" current was subtracted from the currents measured in the presence of ATP and ATP plus ethanol to obtain the "leak-subtracted" currents shown in the I-V plot. Ethanol (100 mM) had no effect on the leak current (data not shown). Note that ethanol did not alter the reversal potential of ATP-activated current. B, *Bar graphs* showing average percentage inhibition of ATP-activated current (5 μM) by 100 mM ethanol at membrane potentials from −80 to +40 mV. Membrane potential was held at different levels for >1 min before the current was evoked in the absence and presence of ethanol. The percentage of inhibition of ATP-activated current by 100 mM ethanol was not significantly different at the holding potentials shown (analysis of variance, $p > 0.25$; n = 6–9).

relationship for current activated by 5 μM ATP, in the absence and presence of ethanol, obtained with a voltage ramp from +40 to −80 mV at a rate of 150 mV/sec. Using this protocol, we found no significant difference in the percentage of inhibition of ATP-activated current by ethanol at membrane voltages between +40 and −80 mV. For example, the percentage of inhibition of ATP-activated current by ethanol at membrane potentials of +40 and −40 mV was 29 ± 2 and 30 ± 3%, respectively ($p > 0.1$; $n = 4$). It also can be seen from this graph that ethanol did not significantly alter the reversal potential of ATP-activated current; in the absence and presence of ethanol, the average reversal potential was −1 ± 5 mV and 0 ± 4 mV, respectively ($p > 0.1$; $n = 4$). Because voltage dependence could also have a time-dependent component that is slower than the slew rate of the voltage ramp, we also studied the effect of ethanol in the steady state by holding the membrane potential at a single voltage for >1 min before activating current with ATP in the absence and presence of ethanol (Fig. 3B). On average, ethanol produced a similar reduction of current amplitude at membrane potentials of −80, −60, −40, +10, and +40 mV (30 ± 2, 32 ± 2, 30 ± 3, 32 ± 4, and 30 ± 4%, respectively; $p > 0.25$; n = 6–9).

The mechanism of the inhibitory effect of ethanol on ATP-activated current was evaluated further by studying the effect of ethanol on current activated by different concentrations of ATP. The records in Fig. 4A show current activated by 1 μM ATP before, during, and after application of 100 mM ethanol (Fig. 4A, *upper*) and current activated by 30 μM ATP under the same conditions in the same cell (Fig. 4A, *lower*). On average, 100 mM ethanol decreased the amplitude of the current activated by 1 and 30 μM ATP by 64 ± 3% (n = 12) and 12 ± 2% (n = 7), respectively. The graph in Fig. 4B shows the concentration-response curve for inhibition by 100 and 400 mM ethanol of current activated by different concentrations of ATP. As can be seen, both 100 and 400 mM ethanol shifted the ATP concentration-response curve to the right, increasing the EC_{50} for ATP from 3.0 μM in the absence of ethanol to 6.0 μM in the presence of 100 mM ethanol and 22.3 μM in the presence

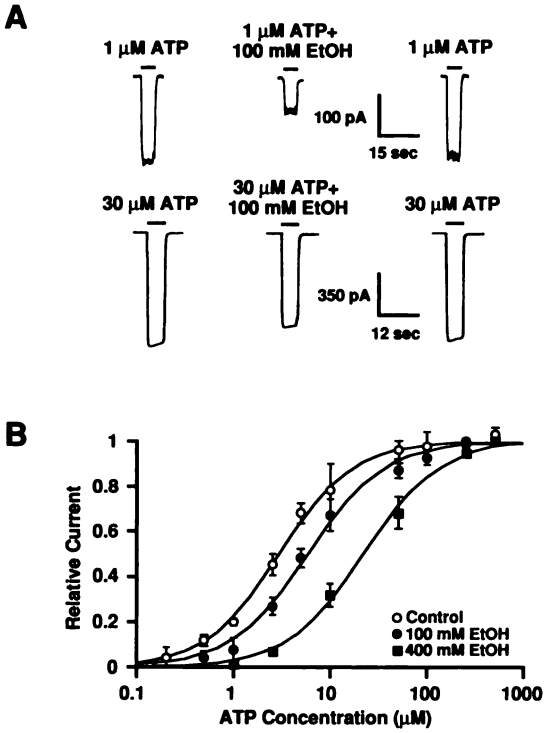

Fig. 4. Effect of ATP concentration on ethanol inhibition of ATP-activated current. A, Records illustrating effect of 100 mM ethanol (*EtOH*) on currents activated by application of 1 μM ATP (*upper*) and 30 μM ATP (*lower*). Data are from the same neuron voltage-clamped at −60 mV. B, Graph plotting the relative amplitude of ATP-activated current in the absence (○) and presence of 100 mM ethanol (●) and 400 mM ethanol (■) as a function of ATP concentration. Amplitude is normalized to the current activated by 250 μM ATP in the absence of ethanol. Each *data point* is an average of five to 10 cells. The *curves* shown are the best fit of the data to the logistic equation given in the legend to Fig. 1. Both 100 mM and 400 mM ethanol significantly increased the EC_{50} for ATP, from 3.0 μM to 6.0 μM and 22.3 μM, respectively (analysis of variance, $p < 0.001$; $n = 5$).

of 400 mM ethanol (analysis of variance, $p < 0.005$; $n = 5$). Ethanol, at either 100 or 400 mM, did not alter the slope ($p > 0.4$; $n = 5$) or maximal value ($p > 0.2$; $n = 5$) of the ATP concentration-response curve.

Discussion

Purinergic receptors have been divided into two main categories, P_1 and P_2, based on their pharmacological properties (21). P_1 purinoceptors are more sensitive to adenosine than to ATP, whereas P_2 purinoceptors are more sensitive to ATP than to adenosine and are blocked by suramin (10, 12–14). In the present study, we found that ATP activated a current with an EC_{50} of 3.0 μM, whereas 200 μM adenosine did not activate detectable current. In addition, the ATP-activated current was antagonized by suramin. These observations indicate that the receptor activated by ATP in our experiments has the characteristics of the P_2 classification of purinergic receptors. The ATP-activated current also exhibited fast activation and deactivation kinetics and a reversal potential near 0 mV. These characteristics are consistent with ATP gating a nonselective cation channel, as proposed previously (19).

The results reported here show that the inward current activated by ATP was inhibited by ethanol in a concentration-dependent manner over the concentration range of 3–500 mM and the concentration that produced 50% inhibition (IC_{50}) was 68 mM. In humans, this concentration of ethanol (0.3%) is associated with severe intoxication and loss of consciousness (22). Additionally, 3–500 mM ethanol alone did not activate a detectable ion current, indicating that the observed effect was due to an effect of ethanol on ATP-gated ion channels, rather than simultaneous direct activation of outward current by ethanol. The amplitude of ethanol inhibition was relatively stable during repeated applications over a period of 3.5 min, indicating that the effect of ethanol does not appear to desensitize over this time period, nor does it exhibit use dependence.

Ethanol might inhibit ATP-activated current by a number of possible mechanisms, including (i) a voltage-dependent channel-blocking action, (ii) alteration of the ion selectivity of the channel, (iii) interaction with an allosteric site on the channel protein, or (iv) competitive antagonism of ATP with its binding site on the receptor (23, 24). We found that the percentage of inhibition of ATP-activated current by 100 mM ethanol was similar over a range of membrane potentials from −80 to +40 mV and that 100 mM ethanol did not change the reversal potential of ATP-activated current. These observations suggest that ethanol does not inhibit the ATP-activated current by a voltage-dependent mechanism or by an alteration in the ion selectivity of the channel. Although a voltage-dependent block of the ion channel would not be expected, because ethanol is not charged, ethanol might induce a conformational change in the channel protein that could result in voltage dependence (25). Ethanol shifted the ATP concentration-response curve to the right, increasing the EC_{50} for ATP from 3.0 μM to 6.0 μM (100 mM ethanol) or 22.3 μM (400 mM ethanol), without altering the slope or maximal value of the ATP concentration-response curve. These observations are consistent with a competitive mechanism for inhibition of ATP-activated current by ethanol. However, on the basis of structural considerations, it seems unlikely that ethanol would competitively block the interaction of ATP with its binding site. The increase in IC_{50} for ATP activation of current could also be explained by an allosteric interaction of ethanol with the receptor that results in a decrease in the affinity of the receptor for ATP (24). A similar modulation of the $GABA_A$ receptor by inverse agonists at the benzodiazepine site has been reported (26). These alternative mechanisms could theoretically be distinguished by determining the effect of multiple concentrations of ethanol on the ATP concentration-response curve; if ethanol acted competitively at the ATP binding site, increasing ethanol concentrations would shift the concentration-response curve to the right in a parallel manner indefinitely, whereas, if ethanol acted at an allosteric site, the effect of ethanol would be expected to reach a maximum when the sites were saturated. However, in the present study the maximum effect of ethanol occurred at approximately 500 mM ethanol, which is close to the maximal concentration tolerated by the cells used in this study. Thus, it was not possible to evaluate the effect of supramaximal concentrations of ethanol on the ATP concentration-response curve. Although the precise mechanism by which ethanol inhibits ATP-activated current remains unclear, it is evident that ethanol increases the apparent dissociation constant (K_d) of the ATP receptor. This action of ethanol on ATP-gated channels contrasts with the inhibition of another ligand-gated ion channel, the NMDA-gated ion channel, by ethanol. Ethanol inhibition of NMDA channels is associated with a decrease in maximal NMDA-activated cur-

rent without a change in EC_{50} (27, 28). Our observations in the present study thus suggest a novel mechanism by which ethanol inhibits the function of a ligand-gated ion channel.

References

1. Weight, F. F. Cellular and molecular physiology of alcohol actions in the nervous system. *Int. Rev. Neurobiol.* **33**:289–348 (1992).
2. Lovinger, D. M., G. White, and F. F. Weight. Ethanol inhibits NMDA-activated ion current in hippocampal neurons. *Science (Washington D. C.)* **243**:1721–1724 (1989).
3. Lima-Landman, M. T. R., and E. X. Albuquerque. Ethanol potentiates and blocks NMDA-activated single-channel currents in rat hippocampal pyramidal cells. *FEBS Lett.* **247**:61–67 (1989).
4. White, G., D. M. Lovinger, and F. F. Weight. Ethanol inhibits NMDA-activated current but does not alter GABA-activated current in an isolated adult mammalian neuron. *Brain Res.* **507**:332–336 (1990).
5. Aguayo, L. G. Ethanol potentiates the $GABA_A$-activated Cl^- current in mouse hippocampal and cortical neurons. *Eur. J. Pharmacol.* **187**:127–130 (1990).
6. Wafford, K. A., D. M. Burnett, T. V. Dunwiddie, and R. A. Harris. Genetic differences in the ethanol sensitivity of $GABA_A$ receptors expressed in *Xenopus* oocytes. *Science (Washington D. C.)* **249**:291–293 (1990).
7. Bradley, R. J., K. Peper, and R. Sterz. Postsynaptic effects of ethanol at the frog neuromuscular junction. *Nature (Lond.)* **284**:60–62 (1980).
8. Lovinger, D. M., and G. White. Ethanol potentiation of 5-hydroxytryptamine$_3$ receptor-mediated ion current in neuroblastoma cells and isolated adult mammalian neurons. *Mol. Pharmacol.* **40**:263–270 (1991).
9. Burnstock, G. Purinergic mechanisms. *Ann. N. Y. Acad. Sci.* **603**:1–17 (1990).
10. Bean, B. P. Pharmacology and electrophysiology of ATP-activated ion channels. *Trends Pharmacol. Sci.* **13**:87–90 (1992).
11. Illes, P., and W. Nörenberg. Neuronal ATP receptors and their mechanism of action. *Trends Pharmacol. Sci.* **14**:50–54 (1993).
12. Evans, R., V. Derkach, and A. Surprenant. ATP mediates fast synaptic transmission in mammalian neurons. *Nature (Lond.)* **357**:503–505 (1992).
13. Silinsky, E. M., and V. Gerzanich. On the excitatory effects of ATP and its role as a neurotransmitter in coeliac neurons of the guinea-pig. *J. Physiol. (Lond.)* **464**:197–212 (1993).
14. Edwards, F. A., A. J. Gibb, and D. Colquhoun. ATP receptor-mediated synaptic currents in the central nervous system. *Nature (Lond.)* **395**:144–147 (1992).
15. Li, C., L. G. Aguayo, and F. F. Weight. Ethanol inhibits ATP-activated currents in sensory neurons. *Third IBRO World Congress Neurosci. Abstr.* 64 (1991).
16. Johnson, J. W., and P. Ascher. Glycine potentiates the NMDA response in cultured mouse brain neurons. *Nature (Lond.)* **325**:529–531 (1987).
17. Mayer, M. L., L. Vyklicky, and G. L. Westbrook. Modulation of excitatory amino acid receptors by group IIB metal cations in cultured mouse hippocampal neurones. *J. Physiol. (Lond.)* **415**:329–350 (1989).
18. De Lean, A., P. J. Munson, and D. Rodbard. Simultaneous analysis of families of sigmoidal curves: application to bioassay, radioligand assay, and physiological dose-response curves. *Am. J. Physiol.* **235**:E97-E102 (1978).
19. Bean, B. P. ATP-activated channels in rat and bullfrog sensory neurons: concentration dependence and kinetics. *J. Neurosci.* **10**:1–10 (1990).
20. Bean, B. P., C. A. Williams, and P. W. Ceelen. ATP-activated channels in rat and bullfrog sensory neurons: current-voltage relation and single-channel behavior. *J. Neurosci.* **10**:11–19 (1990).
21. Burnstock, G. A basis for distinguishing two types of purinergic receptor, in *Cell Membrane Receptors for Drugs and Hormones: A Multidisciplinary Approach* (L. Bolis and R. W. Straub, eds.). Raven Press, New York, 107–118 (1978).
22. Little, H. J. Mechanisms that may underlie the behavioral effects of ethanol. *Prog. Neurobiol.* **36**:171–194 (1991).
23. Hille, B. *Ionic Channels of Excitable Membranes*. Sinauer Associates, Sunderland, MA (1992).
24. Ross, E. M. Pharmacodynamics: mechanisms of drug action and the relationship between drug concentration and effect, in *The Pharmacological Basis of Therapeutics* (A. G. Gilman, T. W. Rall, A. S. Nies, and P. Taylor, eds.). Pergamon, New York, 33–48 (1990).
25. Magleby, K. L., and C. F. Stevens. The effect of voltage on the time course of end-plate currents. *J. Physiol. (Lond.)* **223**:151–171 (1972).
26. Kemp, J. A., G. R. Marshall, E. H. F. Wong, and G. N. Woodruff. The affinities, potencies and efficacies of some benzodiazepine-receptor agonists, antagonists and inverse-agonists at rat hippocampal $GABA_A$-receptors. *Br. J. Pharmacol.* **91**:601–608 (1987).
27. Lovinger, D. M., G. White, and F. F. Weight. Ethanol inhibition of NMDA-activated ion current is not voltage-dependent and ethanol does not interact with other binding sites on the NMDA receptor/ionophore complex. *FASEB J.* **4**:A678 (1990).
28. Peoples, R. W., and F. F. Weight. Ethanol inhibition of *N*-methyl-D-aspartate-activated ion current in rat hippocampal neurons is not competitive with glycine. *Brain Res.* **571**:342–344 (1992).

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