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SPECIAL REPORT

Ethanol-induced inhibition of a neuronal P2X purinoceptor by an allosteric mechanism

[1]Chaoying Li, Robert W. Peoples & Forrest F. Weight

Laboratory of Molecular and Cellular Neurobiology, National Institute on Alcohol Abuse and Alcoholism, National Institutes of Health, Bethesda, Maryland 20892-8115, U.S.A.

Ethanol inhibits a neuronal P2X purinoceptor by shifting the ATP concentration–response curve to the right in an apparently competitive manner. However, the underlying mechanism has not been determined. We investigated the effects of ethanol on the activation and deactivation time constants for ATP-activated current in bullfrog dorsal root ganglion neurones. Ethanol decreased the time constant of deactivation of ATP-gated ion channels without affecting the time constant of activation. The observations are not consistent with a competitive mechanism of inhibition by ethanol, but may be explained by an allosteric action of ethanol to decrease apparent agonist affinity. This represents a novel mechanism of action of ethanol on a neurotransmitter-gated ion channel.

Keywords: Ion channel; ATP; alcohol; activation/deactivation kinetics; allosteric modulation

Introduction The cellular and molecular mechanisms by which ethanol affects nervous system function have not been established. Ethanol has recently been found to affect the function of a number of neurotransmitter-gated ion channels. For example, ethanol inhibits *N*-methyl-D-aspartate (NMDA) receptor-channels (Lovinger *et al*., 1989) and P2X purinoceptors (Li *et al*., 1993). However, ethanol inhibits these channels via different mechanisms. Ethanol decreases the maximal response to NMDA without altering its EC_{50}, indicating a decrease in agonist efficacy (Peoples *et al*., 1997). By contrast, ethanol increases the EC_{50} for adenosine 5′-triphosphate (ATP) without altering its maximal response (Li *et al*., 1993). Whether this effect of ethanol on ATP-gated receptor-channels results from competitive inhibition or an allosteric action to decrease apparent agonist affinity has not been determined. To distinguish between these possible mechanisms, we investigated the effect of ethanol on the activation and deactivation time constants of ATP-activated current. Some of this work has been presented previously in preliminary form (Li *et al*., 1997a).

Methods Bullfrog dorsal root ganglion (DRG) neurones were isolated and whole-cell patch-clamp recording was carried out as described previously (Li *et al*., 1993). Animal care and use in this study were performed in accordance with NIH guidelines (protocol LMCN-SP-01). Neurones were voltage-clamped at −60 mV. Onset and offset rates of agonist-activated current were measured by use of a rapid solution application system consisting of two 300 μm i.d. fused silica tubes at $\sim 30^\circ$ angles to each other. Each exit tube was connected to a four-tube manifold. Switching the flow between the two exit tubes, by closing and opening solenoid valves under computer control, resulted in rapid solution changes (10–90% rise time of the junction potential at an open pipette tip of ~ 2 ms). Activation and deactivation time constants were determined by fitting the data to a single exponential function by use of the programme NFIT (Island Products). Fitting data to a double exponential function did not significantly improve the quality of the curve fits in any case. Statistical significance of results was assessed by analysis of variance (ANOVA).

Results With the system described above, the average time constant for solution changes in patch-clamped cells was 7 ± 1.2 ms (Figure 1a, inset; $n = 4$). To allow accurate measurement of time constants within the limits of this system, we used ATP concentrations $\leqslant 2$ μM, which activate current with time constants at least 8 fold greater than that for solution exchange.

As shown in Figure 1a, the activation time constant (τ_{on}), but not the deactivation time constant (τ_{off}), of ATP-activated current was concentration–dependent in a typical cell. In the cells used in this study, 100 mM ethanol inhibited current activation by 1 μM ATP by $65 \pm 7\%$ ($n = 12$); this agrees well with previous results (Li *et al*., 1993). Ethanol, 50 and 100 mM, decreased the τ_{off} of ATP-activated current from 210 ms to 156 and 78 ms, respectively, but did not appreciably change τ_{on} (Figure 1b). On average, τ_{on} values were highly dependent upon ATP concentration (ANOVA, $P < 0.01$; $n = 10$), but were independent of ethanol concentration (ANOVA, $P > 0.25$; $n = 10$; Figure 1c), whereas τ_{off} values were independent of ATP concentration (ANOVA, $P > 0.25$; $n = 10$), but were highly dependent upon ethanol concentration (ANOVA, $P < 0.01$; $n = 10$; Figure 1d).

Discussion Ethanol inhibits a neuronal P2X purinoceptor by shifting the agonist concentration–response curve to the right in a parallel manner (Li *et al*., 1993). This effect could result either from competitive inhibition by ethanol, or from any of a number of allosteric actions of ethanol that would result in a decrease in the apparent affinity of the receptor for ATP. Allosteric actions resulting in decreases in apparent affinity have been obtained for inhibition of $GABA_A$ receptors by benzodiazepine site inverse agonists (Kemp *et al*., 1987), and P2X purinoceptors by Mg^{2+} (Li *et al*., 1997b). It should be noted that in theory, for agonists with high intrinsic activity at ligand-gated ion channels, changes in agonist efficacy may alter apparent agonist affinity (Stephenson, 1956; Colquhoun &

[1] Author for correspondence at: Laboratory of Molecular & Cellular Neurobiology, NIAAA, National Institutes of Health, 12501 Washington Avenue, Rockville, MD 20852, U.S.A.

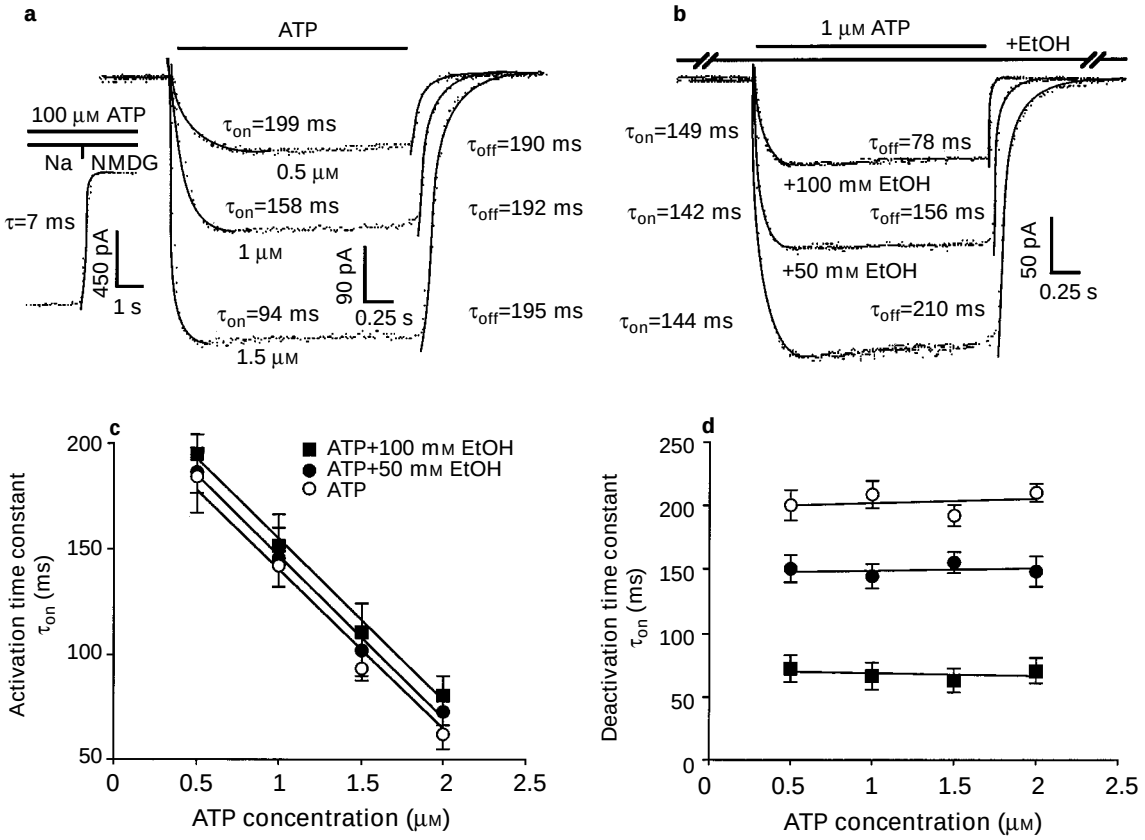

Figure 1 Effects of ATP and ethanol (EtOH) concentrations on activation (τ_{on}) and deactivation (τ_{off}) time constants of ATP-activated current. Inset: current trace showing the time constant for solution change in a patch-clamped cell. The solution bathing the cell was abruptly changed from normal external solution containing 100 μM ATP to the same solution with Na^{2+} replaced by an equimolar concentration of *N*-methyl-D-glucamine (NMDG). (a) Records of currents activated by 0.5, 1 and 1.5 μM ATP. Curves shown are fits to single-exponential equations; τ_{on} and τ_{off} values are those obtained for the records shown. (b) Records of currents activated by 1 μM ATP in the absence and presence of 50 and 100 mM EtOH, respectively. Ethanol was applied for 2 s before and after ATP application. Curves shown are fits to single-exponential equations; τ_{on} and τ_{off} values are those obtained for the records shown. (c) Graph plotting average τ_{on} values as a function of ATP concentration in the absence and presence of 50 and 100 mM EtOH. (d) Graph plotting average τ_{off} values as a function of ATP concentration in the absence and presence of 50 and 100 mM EtOH.

Farrant, 1993). Such an action cannot be excluded in the present study, because the intrinsic activity of ATP on P2X purinoceptors has not been determined. Nevertheless, allosteric mechanisms of inhibition could be distinguished from a competitive mechanism of inhibition by at least two approaches. Firstly, if ethanol acts competitively at the ATP binding site, increasing ethanol concentrations would be expected to shift progressively and indefinitely the concentration–response curve to the right, whereas if ethanol acts at an allosteric site, ethanol should cease to shift the concentration–response curve when its sites of action are saturated. However, this approach could not be used, as these cells did not tolerate ethanol concentrations >400 mM (Li *et al*., 1993). Secondly, the effect of ethanol on the τ_{on} and τ_{off} for ATP-activated current could be measured. Competitive antagonists increase τ_{on} without changing τ_{off} (Clements & Westbrook, 1994), whereas agents that decrease apparent agonist affinity by an allosteric action decrease τ_{off} without changing τ_{on} (Li *et al*., 1997b). In the present study, we found that ethanol decreased τ_{off} of ATP-gated channels without affecting τ_{on}. This observation is therefore best explained by a decrease in the apparent agonist affinity of the receptor via an action at an allosteric site. Thus, although ethanol shifted the ATP concentration-response curve to the right in an apparently competitive manner, the findings of the present study are not consistent with a competitive mechanism of inhibition by ethanol. This represents a novel mechanism by which ethanol inhibits the function of a ligand-gated ion channel.

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