Inhibition by ethanol of rat $P2X_4$ receptors expressed in *Xenopus* oocytes

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1   The effect of ethanol on the function of $P2X_4$ receptors expressed in *Xenopus* oocytes was studied using two-electrode voltage-clamp recording.

2   The amplitude of current activated by 1 μM ATP was decreased by ethanol in a concentration-dependent manner over the concentration range 1 – 500 mM. The concentration of ethanol that produced 50% inhibition (IC_{50}) of current activated by 1 μM ATP was 58 mM.

3   Ethanol inhibition of ATP-activated current was not dependent on membrane potential from -60 to $+20$ mV, and ethanol did not change the reversal potential of ATP-activated current.

4   Ethanol, 50 mM, shifted the ATP concentration-response curve to the right, increasing the EC_{50} for ATP from 9.1 to 16.0 μM, but did not reduce the maximal response to ATP.

5   The results suggest that ethanol may inhibit $P2X_4$ receptors by decreasing the apparent affinity of the binding site for ATP.

6   Since the $P2X_4$ receptor is the most abundant P2X subunit in the brain, these receptors could be important effectors of ethanol action in the central nervous system.

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Introduction

P2X receptors are ligand-gated membrane ion channels that are activated by extracellular ATP. These receptor-channels have received recent attention due to their potential importance in the central and peripheral nervous systems. P2X receptors have been reported to mediate excitatory synaptic transmission in the medial habenula (Edwards *et al.*, 1992; 1997) and locus coeruleus (Nieber *et al.*, 1997) of rat brain, rat hippocampus (Pankratov *et al.*, 1998), rat spinal cord (Bardoni *et al.*, 1997), and in tissue culture of autonomic ganglion neurons (Evans *et al.*, 1992; Silinsky *et al.*, 1992; Galligan & Bertrand, 1994). In addition, P2X receptors have been reported to mediate excitatory responses in a number of types of neurons including sensory (Krishtal *et al.*, 1983; Bean, 1990; Li *et al.*, 1993b; 1997; Khakh *et al.*, 1995), autonomic (Fieber & Adams, 1991; Cloues *et al.*, 1993; Nakazawa, 1994; Khakh *et al.*, 1995; Zhong *et al.*, 1998), and brain (Tschöpl *et al.*, 1992; Ueno *et al.*, 1992; Shen & North, 1993).

Currently, seven P2X receptor subunits, designated $P2X_1$ to $P2X_7$, have been identified (Buell *et al.*, 1996a; MacKenzie *et al.*, 1999). These subunits have been found to be widely distributed in the central nervous system, including cerebral cortex, hippocampus, thalamus, hypothalamus, midbrain, cerebellum, and spinal cord, and in sensory and autonomic ganglia in the peripheral nervous system (Collo *et al.*, 1996). Each of these subunits can form ATP-activated homomeric cation channels when expressed in *Xenopus* oocytes or cell lines.

P2X receptors in bullfrog dorsal root ganglion (DRG) neurons have been found to be inhibited by pharmacological concentrations of ethanol (Li *et al.*, 1993a; 1998a; Weight *et al.*, 1999). Although the amino acid sequence, molecular structure, and subunit composition of P2X receptors in amphibian DRG have not been identified, this ethanol-sensitive P2X receptor almost certainly does not belong to the fast desensitizing type of P2X receptors, such as the $P2X_3$ receptor, as the P2X receptor in bullfrog DRG neurons exhibits little or no desensitization to ATP. One of the possibilities is that it belongs to the $P2X_4$ type, which desensitizes more slowly than $P2X_3$ receptors and is the most abundant P2X subunit expressed in the brain (Collo *et al.*, 1996). Therefore, in the present study, we investigated the effect of ethanol on the function of $P2X_4$ receptors expressed in *Xenopus* oocytes. Some of this work has been presented previously in preliminary form (Li *et al.*, 1998b).

Methods

Preparation of cRNA and expression of receptors were performed as described previously (Xiong *et al.*, 1999b). Briefly, cRNA was synthesized *in vitro* from a linearized cDNA template using T7 RNA polymerase in the presence of the cap analogue 7 mGpppG, and was injected into *Xenopus* oocytes using a pressurized microinjection device (PV 800 Pneumatic Picopump, World Precision Instruments, Sarasota, FL, U.S.A.). Mature *Xenopus laevis* frogs were anaesthetized by immersion in water containing 3-aminobenzoic acid ethyl ester (2 g l^{-1}). Oocytes were excised, mechanically isolated into clusters of four to five oocytes, and shaken in a water bath in two changes of 0.2% collagenase A in a solution containing

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(in mM): NaCl 83, KCl 2, MgCl$_2$ 1 and HEPES 5 (pH 7.4) for 1 h each. Each oocyte was injected with a total of 10 ng of RNA in 50 nl of diethylpyrocarbonate-treated water and was incubated at 17°C for 2–5 days in Modified Barth's Saline (MBS) containing sodium pyruvate (2 mM), penicillin (10,000 U l^{-1}), streptomycin (10 mg l^{-1}), gentamycin (50 mg l^{-1}) and theophylline (0.5 mM). The care and use of animals in this study was approved by the Animal Care and Use Committee of the National Institute on Alcohol Abuse & Alcoholism (Protocol No. LMCN-SP-05) in accordance with National Institutes of Health Guidelines.

Two-electrode voltage-clamp recording was performed at room temperature using a Geneclamp Amplifier (Axon Instruments Inc., Foster City, CA, U.S.A.). Oocytes were placed in a recording chamber and impaled with two sharp microelectrodes filled with 3 M KCl. Electrode tip resistances were in the range 0.5–1.5 MΩ. Oocytes were voltage-clamped at −70 mV, except as indicated. Currents were recorded on a pen recorder (Model RS3400, Gould Inc., Valley View, OH, U.S.A.). Oocytes were constantly superfused at the rate of ∼2.5 ml min^{-1} with bathing medium containing (in mM): NaCl 95, KCl 2, CaCl$_2$ 2, HEPES 5 (pH 7.4). Solutions of ATP (added as the sodium salt) were prepared daily in extracellular medium. Solutions were delivered by gravity flow from a 0.5 mm silica tube connected to a 7-barrel manifold. Solutions superfusing the oocytes were changed *via* manually-switched solenoid valves. At least 5 min was allowed to elapse between agonist applications. All of the drugs used in these experiments were purchased from Sigma Chemical Co. (St Louis, MO, U.S.A.), except suramin and pyridoxal-phosphate-6-azophenyl-2′,4′-disulphonic acid (PPADS), which were purchased from Research Biochemicals International (Natick, MA, U.S.A.), and ethanol, which was purchased from Aldrich Chemical Company (Milwaukee, WI, U.S.A.).

Current amplitudes reported are peak values, and average values are expressed as mean±s.e.mean, with *n* equal to the number of cells studied. Data were statistically compared using Student's *t*-test or analysis of variance (ANOVA), as noted. Statistical analysis of concentration-response data was performed using the nonlinear curve-fitting program ALLFIT (DeLean *et al.*, 1978), which uses an ANOVA procedure. Values reported for concentrations yielding 50% inhibition (IC$_{50}$) or 50% of maximal effect (EC$_{50}$) and slope factor (*n*) are those obtained by fitting the data to the equation:

$$y = E_{max}/[1 + (x/EC_{50})^n]$$

where x and y are concentration and response, respectively, and E_{max} is the maximal response.

Results

It has previously been reported that purinoceptor antagonists suramin and PPADS have little or no effect on ATP-activated current in HEK293 cells mediated by P2X$_4$ receptors cloned from rat superior cervical ganglion (Buell *et al.*, 1996b). Figure 1 illustrates the ATP-activated inward current in *Xenopus* oocytes expressing P2X$_4$ receptors cloned from rat superior cervical ganglion and the effect of purinoceptor antagonists suramin, PPADS and reactive blue 2 on that current in our experiments. As can be seen, the inward current activated rapidly upon ATP application, showed slow desensitization, and decayed quickly upon removal of ATP. Moreover, suramin, PPADS, or reactive blue 2, at a concentration of 100 μM, had little or no effect on the current activated by ATP. Similar results were observed in five other oocytes expressing

P2X$_4$ receptors (Student's *t*-test; *P* > 0.5). Suramin, PPADS or reactive blue 2 alone (10–100 μM) did not activate current in any tested *Xenopus* oocytes expressing P2X$_4$ receptors (data not shown, *n* = 5). In uninjected oocytes, ATP, at concentrations up to 500 μM, did not evoke detectable current (*n* = 6, data not shown).

Figure 2 illustrates the effect of ethanol on ATP-activated current mediated by P2X$_4$ receptors. As shown in Figure 2a, the amplitude of current activated by 1 μM ATP was markedly decreased in the presence of 50 and 100 mM ethanol, and completely recovered after ethanol washout. On average, 50 and 100 mM ethanol decreased the amplitude of current activated by 1 μM ATP by 48±37% (*n* = 16) and 65±23% (*n* = 12), respectively. As shown in Figure 2b, ethanol inhibition of current activated by 1 μM ATP exhibited a clear concentration-dependence over the concentration range, 1–500 mM. The calculated ethanol concentration that produced 50% inhibition (IC$_{50}$) was 58±4.5 mM, the apparent Hill coefficient for ethanol inhibition of ATP-activated current was 1.0±0.2, and the maximal effect was 100% inhibition. Over the concentration range 1–500 mM, application of ethanol alone did not activate detectable current (*n* = 8, data not shown).

To evaluate the mechanism involved in the inhibitory effect of ethanol on ATP-activated current, we tested the effect of membrane potential on ethanol inhibition of ATP-activated current by activating current with ATP at various membrane voltages in the absence and presence of ethanol. As illustrated in Figure 3a, the average inhibition of ATP-activated current by 50 mM ethanol did not differ significantly at membrane holding potentials between −60 and +20 mV (ANOVA, *P* > 0.25; *n* = 5). In addition, as can be seen from the current-voltage relationship for current activated by 1 μM ATP, in the absence and presence of 50 mM ethanol (Figure 3b), ethanol did not change the reversal potential of ATP-activated current (−1±3 mV in the absence vs 0±4 mV in the presence of 50 mM ethanol; Student's *t*-test; *P* > 0.1; *n* = 5).

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 Figure 4a show current activated by 0.5 μM ATP before, during, and after application of 50 mM ethanol (upper series), and current activated by 30 μM ATP
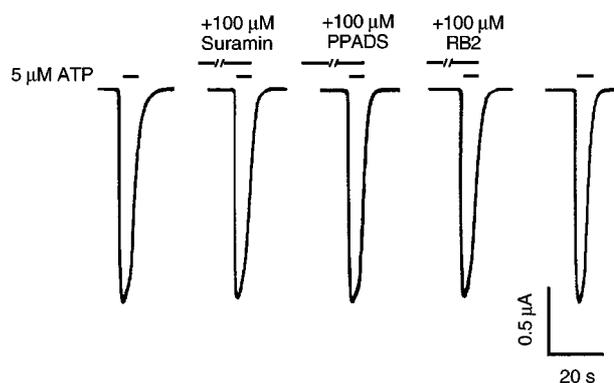

Figure 1 Effect of suramin, PPADS and reactive blue 2 on ATP-activated current mediated by P2X$_4$ receptors expressed in *Xenopus* oocytes. Records of current activated by 5 μM ATP in the absence and the presence of 100 μM suramin, PPADS or reactive blue 2 (RB2). Records are sequential current traces (from left to right) obtained from a single oocyte. Solid bar above each record indicates time of agonist application in the absence or presence of antagonists, as labelled. Suramin and reactive blue 2 were preapplied for 1 min, and PPADS was preapplied for 8 min.
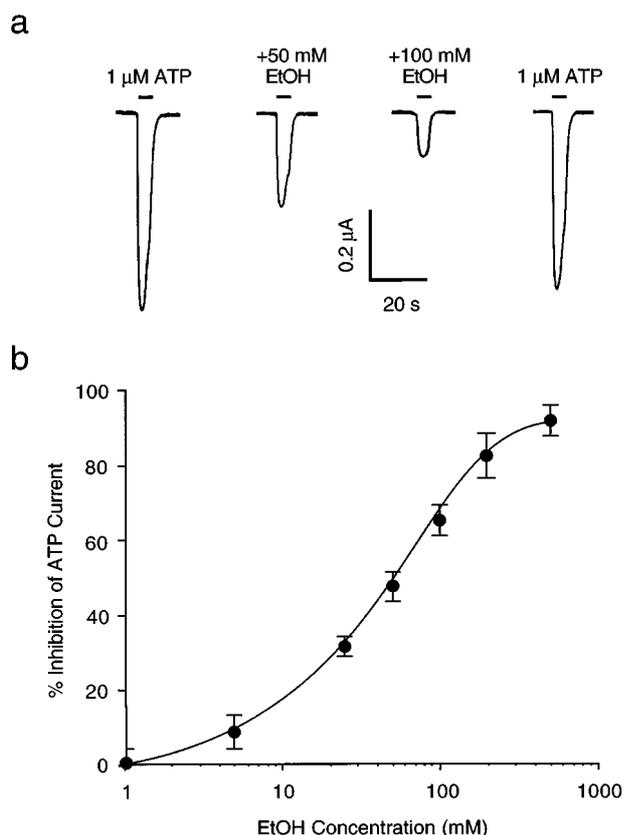
Figure 2 Effect of ethanol on ATP-activated current. (a) Current activated by application of 1 μM ATP before, during, and after application of 50 and 100 mM ethanol (EtOH). Records are sequential current traces (from left to right) obtained from a single oocyte. (b) Graph plotting average percentage inhibition of the amplitude of current activated by 1 μM ATP as a function of ethanol concentration. Each point is the average of 4–16 cells; error bars indicate s.e.mean. The curve shown is the best fit of the data to the equation in the Methods. Values obtained were: IC$_{50}$ = 58 ± 4.5 mM; n = 1.0 ± 0.2; and E$_{max}$ = 100%.

under the same conditions in the same cell (lower series). On average, 50 mM ethanol decreased the amplitude of the current activated by 0.5 and 30 μM ATP by 86 ± 3% (n = 5) and 15 ± 6% (n = 6), respectively. Figure 4b illustrates that ethanol shifted the ATP concentration-response curve to the right in a parallel manner. In the absence of ethanol, ATP had an EC$_{50}$ value of 9.1 ± 0.4 μM and a slope factor of 1.0 ± 0.1. Ethanol, 50 mM, increased the EC$_{50}$ value of ATP to 16.0 ± 0.4 μM (ANOVA, $P < 0.01$), but did not alter the slope factor (1.0 ± 0.1) or E$_{max}$ (1.0 ± 0.01) of the curve ($P > 0.5$).

Discussion

P2X$_4$ receptors have been cloned from rat brain (Bo *et al.*, 1995; Séguéla *et al.*, 1996; Soto *et al.*, 1996), mouse brain (Townsend-Nicholson *et al.*, 1999), human brain (Garcia-Guzman *et al.*, 1997), rat superior cervical ganglion (Buell *et al.*, 1996b), rat pancreatic islets (Wang *et al.*, 1996), and stomach (Dhulipala *et al.*, 1998). In most cases, P2X$_4$ receptors can be readily distinguished from the other known P2X receptors because the purinoceptor antagonists, suramin, PPADS and reactive blue 2, have little or no effect on ATP-activated current mediated by P2X$_4$ receptors (Bo *et al.*, 1995; Buell *et al.*, 1996b; Soto *et al.*, 1996). In the present study, we found that suramin, PPADS, and reactive blue 2, at a
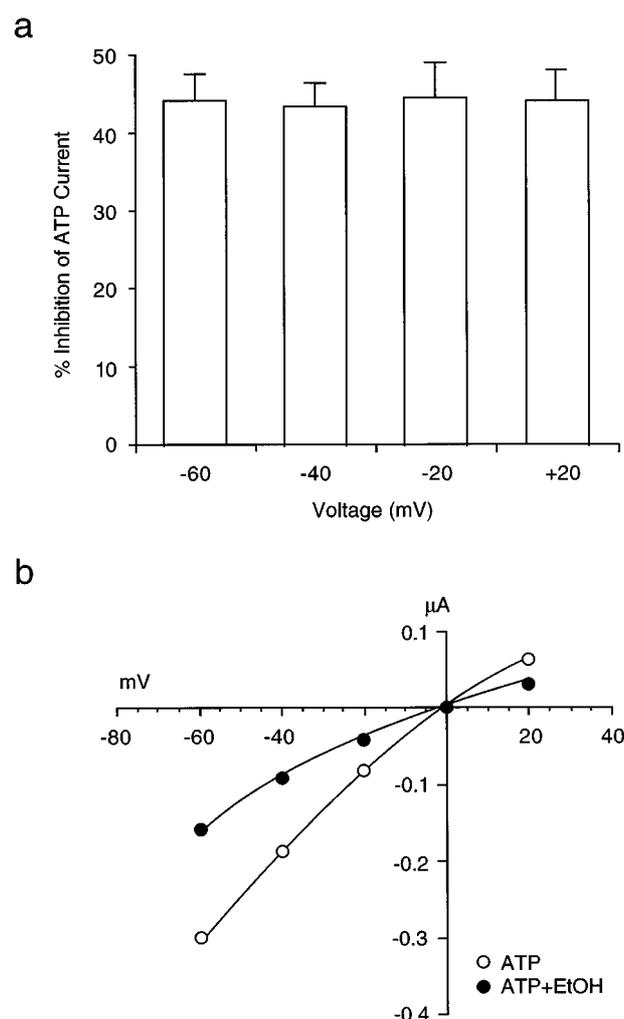

Figure 3 Effect of membrane potential on inhibition of ATP-activated current by ethanol. (a) Bar graphs showing average percentage inhibition by 50 mM ethanol of current activated by 1 μM ATP at membrane potentials from −60 to +20 mV. Current was activated by ATP at various membrane voltages in the absence and presence of ethanol. The percentage inhibition of ATP-activated current by 50 mM EtOH was not significantly different at the holding potentials shown (ANOVA, $P > 0.25$; n = 5). (b) Current-voltage relationship (I-V plot) showing the amplitude of current activated by 1 μM ATP as a function of membrane potential, in the absence and presence of 50 mM ethanol in a single oocyte. Note that ethanol did not alter the reversal potential of ATP-activated current.

concentration of 100 μM, did not antagonize ATP-activated current in *Xenopus* oocytes expressing P2X$_4$ receptors cloned from rat superior cervical ganglion (Buell *et al.*, 1996b). We also found that the EC$_{50}$ value of ATP-activated current was 9.1 μM when P2X$_4$ receptors were expressed in *Xenopus* oocytes, which is similar to the value of 10 μM observed previously for this receptor expressed in HEK293 cells (Buell *et al.*, 1996b). Thus, these observations indicate that the receptors activated by ATP in our experiments have the characteristics of the P2X$_4$ receptors cloned from rat superior cervical ganglion and expressed in HEK293 cells. It should be noted, however, that the EC$_{50}$ value of ATP for P2X$_4$ receptors cloned from a mouse brain cDNA library was reported to be 1.7 μM (Townsend-Nicholson *et al.*, 1999) and the EC$_{50}$ value of ATP for P2X$_4$ receptors cloned from a rat pancreatic islet cDNA library was reported to be 63 μM (Wang *et al.*, 1996). The reason for these differences in EC$_{50}$ values is not known.
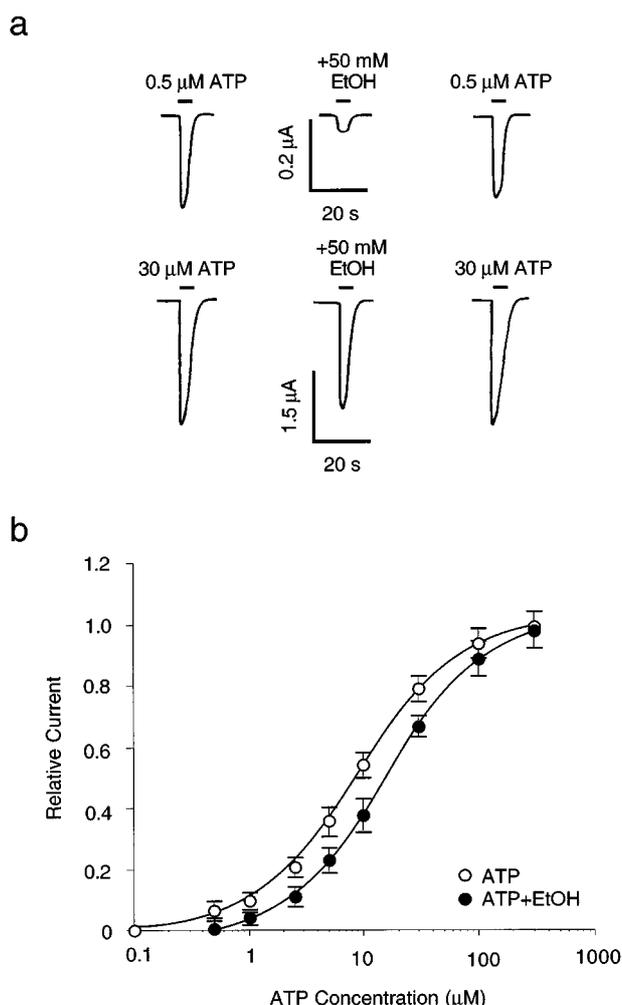
Figure 4 Effect of ATP concentration on ethanol inhibition of ATP-activated current. (a) Records showing currents activated by 0.5 μM ATP (upper traces) and 30 μM ATP (lower traces), before, during, and after application of 50 mM ethanol in a single oocyte. (b) Graph plotting the relative amplitude of ATP-activated current in the absence and the presence of 50 mM ethanol as a function of ATP concentration. Amplitude is normalized to the current activated by 300 μM ATP in the absence of ethanol. Each data point is the average current from 5–8 cells. The solid curves are the best fits of the data to the equation described in the Methods section. Ethanol significantly increased the EC$_{50}$ for ATP from 9.1 ± 0.4 μM in the absence of ethanol to 16.0 ± 0.4 μM in the presence of 50 mM ethanol (ANOVA, $P < 0.01$).

The results reported here show that ATP-activated current mediated by P2X$_4$ receptors can be inhibited by ethanol in a concentration-dependent manner over the concentration range 1–500 mM, and the concentration that produced 50% inhibition (IC$_{50}$) of current activated by 1 μM ATP was 58 mM. Additionally, 1–500 mM ethanol alone did not activate detectable current, indicating that the observed effect of ethanol was not due to an activation of outward current by ethanol.

Ethanol could inhibit the current mediated by P2X$_4$ receptors by any of several mechanisms. For example, ethanol might alter the ion permeance ratio of ATP-gated channels. We found, however, that ethanol did not alter the reversal potential of current activated by ATP, and thus this mechanism does not appear to account for the ethanol-induced inhibition of ATP-activated current that was observed in the present study. Ethanol could also inhibit ATP-activated current by decreasing the number of channels gated by ATP, but this mechanism would be expected to be reflected in a decrease in the maximal response to ATP, which was not observed. In addition, another type of inhibitory action, open-channel block, appears to be unlikely, as this mechanism would also be expected to decrease the maximal response to ATP, and this was not observed. In addition, although a voltage-dependent ion channel block 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 (Magleby & Stevens, 1972). This possibility seems unlikely since voltage-dependence of ethanol action was not observed. Finally, ethanol could decrease the apparent affinity of the receptor for ATP. Our observation that ethanol shifted the ATP concentration-response curve to the right in a parallel manner, increasing the EC$_{50}$ for ATP, is consistent with an action of ethanol to decrease the apparent affinity of the P2X$_4$ receptor for agonist. Moreover, this observation agrees well with previous findings for ethanol inhibition of P2X receptors in bullfrog dorsal root ganglion neurons (Li *et al.*, 1993a; 1998a). This action of ethanol on ATP-gated P2X receptor-channels contrasts with the observations on ethanol inhibition of another ligand-gated ion channel, *N*-methyl-D-aspartate (NMDA) receptors. Ethanol inhibition of NMDA receptors is associated with a decrease in maximal response to agonist (E_{max}), without a change in EC$_{50}$ (Peoples *et al.*, 1997).

Of the known P2X receptor subunits, the P2X$_4$ subunit has been found to be the most abundant P2X subunit in brain. Northern blot analysis and *in situ* hybridization studies have revealed extensive distribution of P2X$_4$ mRNA in rat brain with particularly strong expression in the hippocampus, Purkinje cells of the cerebellum, and spinal cord motoneurons (Bo *et al.*, 1995; Collo *et al*, 1996; Séguéla *et al.*, 1996; Soto *et al.*, 1996). In addition, the P2X$_4$ receptor and another major central subunit, P2X$_6$,- have highly overlapping mRNA distribution at both the regional and the cellular level (Collo *et al.*, 1996). The coexpression of P2X$_4$ and P2X$_6$ subunits in *Xenopus* oocytes generates a novel heteromeric ATP receptor (Lê *et al.*, 1998), which is another candidate for ethanol action in the central nervous system (Xiong *et al.*, 1999a). Moreover, in recent preliminary experiments, pharmacological concentrations of ethanol have been found to inhibit ATP-activated current in freshly isolated adult rat hippocampal neurons (Li & Weight, 1999). Thus, our observations suggest that P2X receptors could be important effectors of ethanol action in the central nervous system.

References

BARDONI, R., GOLDSTEIN, P.A., LEE, C.J., GU, J.G. & MACDER-MOTT, A.B. (1997). ATP P2X receptors mediate fast synaptic transmission in the dorsal horn of the rat spinal cord. *J. Neurosci.*, **17**, 5297–5304.

BEAN, B.P. (1990). ATP-activated channels in rat and bullfrog sensory neurons: concentration dependence and kinetics. *J. Neurosci.*, **10**, 1–10.

BO, X., ZHANG, Y., NASSAR, M., BURNSTOCK, G. & SCHOEPFER, R. (1995). A P2X purinoceptor cDNA conferring a novel pharmacological profile. *FEBS Lett.*, **375**, 129 – 133.

BUELL, G., COLLO, G. & RASSENDREN, F. (1996a). P2X receptors: an emerging channel family. *Eur. J. Neurosci.*, **8**, 2221 – 2228.

BUELL, G., LEWIS, S., COLLO, G., NORTH, R.A. & SURPRENANT, A. (1996b). An antagonist-insensitive P2X receptor expressed in epithelia and brain. *EMBO J.*, **15**, 55 – 62.

CLOUES, R., JONES, S. & BROWN, D.A. (1993). Zn^{2+} potentiates ATP-activated currents in rat sympathetic neurons. *Pflügers Arch.*, **424**, 152 – 158.

COLLO, G., NORTH, R.A., KAWASHIMA, E., MERLO-PICH, E., NEIDHART, S., SURPRENANT, A. & BUELL, G. (1996). Cloning of P2X$_5$ and P2X$_6$ receptors and the distribution and properties of an extended family of ATP-gated ion channels. *J. Neurosci.*, **16**, 2495 – 2507.

DeLEAN, A., MUNSON, P.J. & RODBARD, D. (1978). Simultaneous analysis of families of sigmoidal curves: application to bioassay, radioligand assay, and physiological dose-response curves. *Am. J. Physiol.*, **235**, E97 – E102.

DHULIPALA, P.D., WANG, Y.X. & KOTLIKOFF, M.I. (1998). The human P2X$_4$ receptor gene is alternatively spliced. *Gene*, **207**, 259 – 266.

EDWARDS, F.A., GIBB, A.J. & COLQUHOUN, D. (1992). ATP receptor-mediated synaptic currents in the central nervous system. *Nature*, **395**, 144 – 147.

EDWARDS, F.A., ROBERTSON, S.J. & GIBB, A.J. (1997). Properties of ATP receptor-mediated synaptic transmission in the rat medial habenula. *Neuropharmacology*, **36**, 1253 – 1268.

EVANS, R., DERKACH, V. & SURPRENANT, A. (1992). ATP mediates fast synaptic transmission in mammalian neurons. *Nature*, **357**, 503 – 505.

FIEBER, L.A. & ADAMS, D.J. (1991). Adenosine triphosphate-evoked currents in cultured neurons dissociated from rat parasympathetic cardiac ganglia. *J. Physiol.*, **434**, 239 – 256.

GALLIGAN, J.J. & BERTRAND, P.P. (1994). ATP mediates fast synaptic potentials in enteric neurons. *J. Neurosci.*, **14**, 7563 – 7571.

GARCIA-GUZMAN, M., SOTO, F., GOMEZ-HERNANDEZ, J.M., LUND, P.-E. & STÜHMER, W. (1997). Characterization of recombinant human P2X$_4$ receptor reveals pharmacological differences to the rat homologue. *Mol. Pharmacol.*, **51**, 109 – 118.

KHAKH, B.S., HUMPHREY, P.P.A. & SURPRENANT, A. (1995). Electrophysiological properties of P$_{2X}$-purinoceptors in rat superior cervical, nodose and guinea-pig coeliac neurons. *J. Physiol.*, **484** 385 – 395.

KRISHTAL, O.A., MARCHENKO, S.M. & PIDOPLICHKO, V.I. (1983). Receptor for ATP in the membrane of mammalian sensory neurons. *Neurosci. Lett.*, **35**, 41 – 45.

LÊ, K.-T., BABINSKI, K. & SÉGUÉLA, P. (1998). Central P2X4 and P2X6 channel subunits coassemble into a novel heteromeric ATP receptor. *J. Neurosci.*, **18**, 7152 – 7159.

LI, C., AGUAYO, L., PEOPLES, R.W. & WEIGHT, F.F. (1993a). Ethanol inhibits a neuronal ATP-gated ion channel. *Mol. Pharmacol.*, **44**, 871 – 875.

LI, C., PEOPLES, R.W., LI, Z. & WEIGHT, F.F. (1993b). Zn^{2+} potentiates excitatory action of ATP on mammalian neurons. *Proc. Natl. Acad. Sci. U.S.A.*, **90**, 8264 – 8267.

LI, C., PEOPLES, R.W. & WEIGHT, F.F. (1997). Inhibition of ATP-activated current by zinc in dorsal root ganglion neurons of bullfrog. *J. Physiol.*, **505**, 641 – 653.

LI, C., PEOPLES, R.W. & WEIGHT, F.F. (1998a). Ethanol-induced inhibition of a neuronal P2X purinoceptor by an allosteric mechanism. *Br. J. Pharmacol.*, **123**, 1 – 3.

LI, C., XIONG, K.-M. & WEIGHT, F.F. (1998b). Inhibition by ethanol of P2X$_4$ purinoceptors expressed in *Xenopus* oocytes. *Alcohol. Clin. Exp. Res. (Abstracts)*, **22**, 5A.

LI, C. & WEIGHT, F.F. (1999). Inhibition by ethanol of ATP-activated current in freshly isolated adult rat hippocampal neurons. *Alcohol. Clin. Exp. Res. (Abstracts)*, **23**, 7A.

MACKENZIE, A.B., SURPRENANT, A. & NORTH, R.A. (1999). Functional and molecular diversity of purinergic ion channel receptors. *Ann. N.Y. Acad. Sci.*, **868**, 716 – 729.

MAGLEBY, K.L. & STEVENS, C.F. (1972). The effect of voltage on the time course of end-plate currents. *J. Physiol.*, **223**, 151 – 171.

NAKAZAWA, K. (1994). ATP-activated current and its interaction with acetylcholine-activated current in rat sympathetic neurons. *J. Neurosci.*, **14**, 740 – 750.

NEIBER, K., POELCHEN, W. & ILLES, P. (1997). Role of ATP in fast excitatory synaptic potentials in locus coeruleus neurons of the rat. *Br. J. Pharmacol.*, **122**, 423 – 430.

PANKRATOV, Y., CASTRO, E., MIRAS-PORTUGAL, M.T. & KRISHTAL, O.A. (1998). Purinergic component of the excitatory postsynaptic current mediated by P2X receptors in the CA1 neurons of the rat hippocampus. *Eur. J. Neurosci.*, **10**, 3898 – 3902.

PEOPLES, R.W., WHITE, G., LOVINGER, D.M. & WEIGHT, F.F. (1997). Ethanol inhibition of N-methyl-D-aspartate-activated current in mouse hippocampal neurons: whole-cell patch-clamp analysis. *Br. J. Pharmacol.*, **122**, 1035 – 1042.

SÉGUÉLA, P., HAGHIGHI, A., SOGHOMONIAN, J.-J. & COOPER, E. (1996). A novel neuronal P$_{2X}$ ATP receptor ion channel with widespread distribution in the brain. *J. Neurosci.*, **16**, 448 – 455.

SHEN, K.-Z. & NORTH, R.A. (1993). Excitation of rat locus coeruleus neurons by adenosine 5′-triphosphate: ionic mechanism and receptor characterization. *J. Neurosci.*, **13**, 894 – 899.

SILINSKY, E.M., GERZANICH, V. & VANNER, S.M. (1992). ATP mediates excitatory synaptic transmission in mammalian neurons. *Br. J. Pharmacol.*, **106**, 762 – 763.

SOTO, F., GARCIA-GUZMAN, M., GOMEZ-HERNANDEZ, J.M., HOLLMANN, M., KARSCHIN, C. & STÜHMER, W. (1996). P2X$_4$: An ATP-activated iontropic receptor cloned from rat brain. *Proc. Natl. Acad. Sci. U.S.A.*, **93**, 3684 – 3688.

TOWNSEND-NICHOLSON, A., KING, B.F., WILDMAN, S.S. & BURNSTOCK, G. (1999). Molecular cloning, functional characterization and possible cooperativity between the murine P2X$_4$ and P2X$_{4a}$ receptors. *Mol. Brain. Res.*, **64**, 246 – 254.

TSCHÖPL, M., HARMS, L., NÖRENBERG, W. & ILLES, P. (1992). Excitatory effects of adenosine 5′-triphosphate on rat locus coeruleus neurons. *Eur.J. Pharmacol.*, **213**, 71 – 77.

UENO, S., HARATA, N., INOUE, K. & AKAIKE, N. (1992). ATP-gated current in dissociated rat nucleus solitarii neurons. *J. Neurophysiol.*, **68**, 778 – 785.

WANG, C.Z., NAMBA, N., GONOI, T., INAGAKI, N. & SEINO, S. (1996). Cloning and pharmacological characterization of a fourth P2X receptor subtype widely expressed in brain and peripheral tissues including various endocrine tissues. *Biochem. Biophys. Res. Commun.*, **220**, 196 – 202.

WEIGHT, F.F., LI, C. & PEOPLES, R.W. (1999). Alcohol action on membrane ion channels gated by extracellular ATP (P2X receptors). *Neurochem. Int.*, **35**, 143 – 152.

XIONG, K.M., LI, C. & WEIGHT, F.F. (1999a). Differential ethanol sensitive to P2X$_4$ and P2X$_6$ receptors expressed in *Xenopus* oocytes. *Alcohol. Clin. Exp. Res. (Abstracts)*, **23**, 8A.

XIONG, K., PEOPLES, R.W., MONTGOMERY, J., CHIANG, Y.-S., STEWART, R., WEIGHT, F.F. & LI, C. (1999b). Differential modulation by divalent cations of P2X$_2$ and P2X$_4$ receptor function. *J. Neurophysiol.*, **81**, 2088 – 2094.

ZHONG, Y., DUNN, P.M., XIANG, Z., BO, X. & BURNSTOCK, G. (1998). Pharmacological and molecular characterization of P2X receptors in rat pelvic ganglion neurons. *Br. J. Pharmacol.*, **125**, 771 – 781.