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Trichloroethanol inhibits ATP-induced membrane currents in cultured HEK 293-hP2X$_3$ cells

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Abstract

Membrane currents in response to the application of α,β-methylene ATP (α,β-meATP) were recorded by the whole-cell patch-clamp technique in human embryonic kidney 293 cells transfected with the human P2X$_3$ receptor (HEK 293-hP2X$_3$ cells). Trichloroethanol, the biologically active metabolite of chloral hydrate, but not ethanol itself concentration-dependently and reversibly inhibited the current responses. It was concluded that the reported analgesic effect of chloral hydrate may be due to the interruption of pain transmission in dorsal root ganglia expressing P2X$_3$ receptors. © 2000 Elsevier Science B.V. All rights reserved.

Keywords: P2X$_3$ receptor; Ethanol; Trichloroethanol

Ligand-gated ion channels are important sites of action for ethanol to interfere with the effects of various neurotransmitters in the nervous system (Korpi et al., 1998). Ethanol facilitates γ-amino-butyric acid (GABA$_A$) and 5-hydroxytryptamine (5-HT$_3$) receptor functions and inhibits the *N*-methyl-D-aspartate (NMDA) and α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) types of excitatory amino acid receptors. It has recently been reported that ethanol inhibits ATP-sensitive P2X receptors as well (Weight et al., 1999).

2,2,2-trichloroethanol (trichloroethanol) the main metabolite of the sedative-hypnotic drug chloral hydrate shares with ethanol its ability to alter the conductance of ligand-gated ion channels (Peoples and Weight, 1994; Fischer et al., 2000). Both ethanol and trichloroethanol were reported to depress ATP-induced membrane currents in bullfrog sensory neurons (Weight et al., 1999). These cells are supposed to possess slowly desensitizing heteromeric receptors composed of the P2X$_2$/X$_3$ subunits, involved in pain transmission (Burnstock and Wood, 1996). The mammalian counterparts of bullfrog sensory neurons are endowed in addition with rapidly desensitizing homomeric P2X$_3$ receptors. The aim of the present study was to investigate the effects of ethanol and trichloroethanol on human embryonic kidney 293 cells transfected with human P2X$_3$ receptors (HEK 293-hP2X$_3$).

HEK 293 cells (European Collection of Cell Cultures, Porton Down, United Kingdom) were transfected with pcDNA3 (Invitrogene, Groningen, Netherlands) carrying a human P2X$_3$ cDNA (obtained from Dr. J.N. Wood, University College London, UK) using standard transfection methods. HEK 293-hP2X$_3$ cells were cultured in Dulbecco's minimal essential medium for 1–4 days. Patch-clamp recordings of whole-cell currents were performed at room temperature (20–22°C) and at a holding potential of −70 mV by using an Axopatch 200B amplifier (Axon Instruments, Foster City, CA). Gigaohm seals were formed using electrodes with tip resistances of 3–5 MΩ. Data were filtered at 2 kHz with the Axopatch 200B, digitized at 5 kHz and stored on a laboratory computer using a Digidata 1200 interface and AxoScope software (Axon Instruments).

The extracellular medium contained (in mM): NaCl, 135; KCl, 4.5; $CaCl_2$, 2; $MgCl_2$, 2; HEPES, 10; glucose, 10; pH 7.4. The internal solution consisted of (in mM): CsCl, 140; $MgCl_2$, 1; $CaCl_2$, 2; HEPES, 10; EGTA, 11;

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pH 7.3. Solutions of all drugs were prepared in extracellular medium and were applied by pressure using a drug application device superfusion system (DAD12, Adams and List, Westbury, NY).

α,β-Methylene ATP (α,β-meATP, 10 μM; Biotrend, Köln, Germany) evoked inward currents in HEK 293-hP2X$_3$ cells (Fig. 1). The P2 receptor agonist was superfused for 1 s with an interval of 5 min between two applications and six times in a total ($T_1 - T_6$). In the absence of further drugs, this protocol yielded reproducible current amplitudes, although during the superfusion-time of 1 s a pronounced desensitization developed. Since the amplitudes of the α,β-meATP (10 μM)-induced currents largely varied within the cell population (200–4000 pA), the results were expressed as a percentage of the current recorded at T_2. Trichloroethanol depressed the α,β-meATP-induced inward currents at 3 mM (Fig. 1A) and abolished them at 10 mM ($n = 4$). Under the same conditions, ethanol (100 mM) was inactive (Fig. 1B; both compounds were from Sigma, Deisenhofen, Germany). When trichloroethanol (3, 10 mM) was washed out for 10 min, the effect of α,β-meATP partially recovered to its pre-drug level (Fig. 1B and not shown).

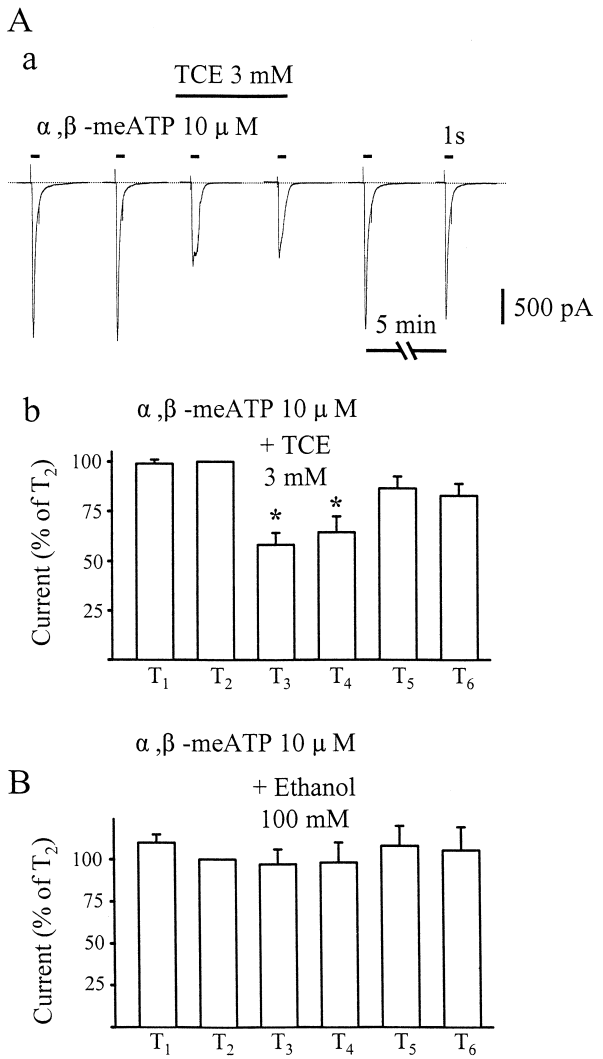

Fig. 1. Effect of trichloroethanol and ethanol on α,β-meATP-induced currents in cultured HEK 293-hP2X$_3$ cells. Whole-cell patch-clamp recordings were made at a holding potential of −70 mV. α,β-meATP (10 μM) was pressure-applied six times ($T_1 - T_6$) for 1 s each and every 5 min. Trichloroethanol or ethanol was applied 3 min after T_2. (A) Inhibition by trichloroethanol (3 mM) of the α,β-meATP (10 μM)-induced inward current. (a) Representative recording. The dotted line indicates the zero current level. (b) Mean ± S.E.M. of eight experiments similar to those shown in (a). (B) No effect of ethanol (100 mM) on the α,β-meATP (10 μM)-induced inward current ($n = 6$). $^*P < 0.05$; statistically significant difference from T_2 (one-way ANOVA followed by Bonferroni's *t*-test). The currents at T_5 and T_6 failed to differ in a statistically significant manner from the currents observed either at T_2 or at T_4 ($P > 0.05$).

In the present study, trichloroethanol (3 and 10 mM) but not ethanol (100 mM) depressed the conductance of hP2X$_3$ receptors. It has been shown previously that the inhibitory potency of ethanol on ligand-activated ion channels is much lower than that of trichloroethanol (Peoples and Weight, 1994; Fischer et al., 2000). This may be due to the fact that trichloroethanol is more lipophilic and reaches higher intramembraneous concentrations than ethanol. Subsequent to the therapeutic application of chloral hydrate the blood levels of trichloroethanol are in the low millimolar range (Owen and Taberner, 1980). Hence, the inhibition of the hP2X$_3$ receptor function by trichloroethanol occurs at pharmacologically relevant concentrations. It is noteworthy that chloral hydrate causes remarkable analgesia, which may be due to the interruption of pain transmission in dorsal root ganglia (Field et al., 1993). P2X$_4$ receptors, which are abundantly expressed in the brain, exhibit a higher sensitivity to ethanol than the P2X$_3$ receptors investigated in this study (Xiong et al., 2000).

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