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Differential effects of propofol and ethanol on P2X4 receptors expressed in *Xenopus* oocytes

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Abstract

The current study investigated the effects of propofol on P2X4 receptors expressed in *Xenopus* oocytes using two-electrode voltage clamp. We also tested the effects of 100 mM ethanol on the same oocytes used to test propofol. Propofol potentiated ATP-gated currents in a concentration dependent manner in P2X4 receptors. In agreement with our previous findings, ethanol inhibited P2X4 receptors. The opposite effects of propofol and ethanol on P2X4 receptor function suggest that these anesthetics act via different sites/mechanisms in P2X receptors as has been suggested for GABA_A and glycine receptors.

Keywords

P2X receptor; Two-electrode voltage clamp; Propofol; General anesthetic; Purinergic receptor

1. Introduction

P2X receptors, a distinct multigene family of ligand-gated ion channels, have become a focus of investigation due to their potential importance in the peripheral and central nervous system (CNS) [1]. P2X receptors are fast acting, cation-permeable ion channels that are gated by synaptically released extracellular adenosine 5'-triphosphate (ATP) [2]. In the CNS, ATP directly mediates fast excitatory synaptic transmission by acting on P2X receptors located on postsynaptic membranes. In addition, ATP acts presynaptically on P2X receptors to modulate other neurotransmitters such as GABA, glycine and glutamate [3].

Recent findings suggest that high concentrations of propofol (≥ 50 μ M) potentiate ATP-gated currents in P2X4 receptors expressed in HEK 293 cells [4]. The present study tested lower, more clinically relevant concentrations of propofol (0.54–54 μ M) on P2X4 receptors expressed in *Xenopus* oocytes.

2. Methods

Xenopus laevis frog (Nasco, Ft. Atkinson, WI) maintenance, oocyte isolation, cRNA injection and two-electrode voltage clamp (TEVC) recording of oocytes were performed according to the protocols described in our previous study [5]. Oocytes were used in electrophysiological recordings for 1–10 days after cRNA injection. Propofol was dissolved in 100% dimethylsulfoxide (DMSO) and diluted to a final concentration of 0.05%. There was no significant effect of DMSO on injected or uninjected oocytes. An ATP concentration–response curve was generated for P2X4 receptors to determine the ATP EC_{5–10} (Fig. 1A).

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3. Results and conclusions

The present study tested the effects of lower, more clinically relevant concentrations of propofol (0.54–54 μ M) on P2X4 receptors expressed in *Xenopus* oocytes using TEVC ($V_h = -70$ mV). Propofol caused a reversible and concentration dependent potentiation of ATP-activated currents (ATP EC₅₋₁₀) in oocytes expressing P2X4 receptors (Fig. 1B). In agreement with previous studies [5-7], ethanol inhibited P2X4 receptors when tested in the same oocytes that were potentiated by propofol (Fig. 1C). The current investigation is the first to demonstrate that clinically relevant concentrations of propofol modulate P2X4 receptor function and suggests that propofol induced alteration in P2X receptor function may play a role in the anesthetic and/or adverse effects of propofol.

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Fig. 1.

(A) ATP-concentration-response for P2X4 receptors. Amplitudes are normalized to the current activated by 100 μM ATP ($V_h = -70$ mV) and are shown as the mean $\pm$ S.E.M. from 6 to 12 oocytes. As determined from the curve fits, the EC_{50} ATP was 7.34 ± 0.02 μM and the Hill coefficient was 1.26 ± 0.09 . (B) Propofol (0.54–54 μM) potentiation of ATP-gated currents in P2X4 receptors. Currents were generated by application of EC_{5-10} ATP (0.75–1 μM ATP) for 25 s ($V_h = -70$ mV). Propofol was co-applied with ATP for 25 s. Current amplitudes are shown as mean $\pm$ S.E.M. from 3 to 7 oocytes. (C) Ethanol (5–200 mM) inhibition of ATP-gated currents in the P2X4 receptor. Currents were generated by the application of EC_{10} ATP (1.25–2 μM ATP) for 30 s ($V_h = -50$ mV). Ethanol was co-applied with ATP for 30 s. Current amplitudes are shown as mean $\pm$ S.E.M. from 5 to 14 oocytes. Washout time between drug applications was at least 5 min.