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Short communication

Inhibition of human 5-HT_{3A} and 5-HT_{3AB} receptors by etomidate, propofol and pentobarbital

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Abstract

The actions of intravenous anaesthetics on 5-HT_{3AB} receptors have not been studied. Using oocyte electrophysiology, the effects of etomidate, propofol, and pentobarbital on human 5-HT_{3A} and 5-HT_{3AB} receptors were studied and compared. Inhibition of peak currents by all three compounds in both receptor subtypes was anaesthetic concentration-dependant and non-competitive. Because the half-maximal inhibitory concentrations for etomidate, propofol and pentobarbital in 5-HT_{3A} and 5-HT_{3AB} receptors were all above their respective anaesthetic concentrations, the results of our study suggest that neither 5-HT_3 receptor subtype contributes to the anaesthetic actions of etomidate, propofol or pentobarbital.

Keywords: Etomidate; Propofol; Pentobarbital; 5-HT_{3A} receptor; 5-HT_{3AB} receptor

1. Introduction

5-Hydroxytryptamine type 3 (5-HT_3) receptors are cation-selective ligand-gated ion channels that belong to the anaesthetic-sensitive superfamily of cys-loop receptors. Members of this superfamily are composed of 5 subunits that share key structural features. To date, five different 5-HT_3 subunits (A, B, C, D, and E) have been identified in the human genome (Davies et al., 1999; Maricq et al., 1991; Niesler et al., 2003). Among the three 5-HT_3 receptor subunits (A, B, C) that are known to be expressed in human brain (Davies et al., 1999; Niesler et al., 2003), only the 5-HT_{3A} and the 5-HT_{3B} subunits have been shown to contribute to functional 5-HT_3 receptors (5-HT_{3A} and 5-HT_{3AB}).

Electrophysiological studies have shown that the functional characteristics of the two receptor subtypes are significantly different (Davies et al., 1999). For example, the single channel conductance of 5-HT_{3A} receptors is 20-fold lower than that of 5-HT_{3AB} receptors, whereas permeability to calcium ions is lower in 5-HT_{3AB} receptors. Pharmacological studies aimed at defining the actions of short chain anaesthetic alcohols and the inhaled general anaesthetics halothane and chloroform reveal that the sensitivities of the two 5-HT_3 receptor subtypes for these drugs differ substantially (Stevens et al., 2005). This finding may have important implications in terms of understanding the mechanisms of general anaesthesia because 5-HT_3 receptors mediate or modulate the release of synaptic neurotransmitters in the central nervous system including GABA (Zhou and Hablitz, 1999), glutamate (Funahashi et al., 2004), and acetylcholine (Consolo et al., 1994) that have been linked to important anaesthetic behavioural endpoints (e.g. immobility, hypnosis, and amnesia).

In addition to inhaled anaesthetics and anaesthetic alcohols, intravenous anaesthetics modulate the function of 5-HT_3 receptors. Barbiturates such as pentobarbital (Barann et al., 1997, 2000b) and methohexital (Barann et al., 2000b) inhibit 5-HT_3 receptors expressed endogenously in N1E-115 mouse neuroblastoma cells (Barann et al., 1997) as well as recombinant human 5-HT_{3A} receptors expressed heterologously in HEK 293 cells (Barann et al., 2000b). Similarly, propofol inhibits 5-HT_3

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receptors expressed in N1E-115 mouse neuroblastoma cells (Barann et al., 2000a) and native 5-HT_3 receptors present in rat vagus nerve (Patten et al., 2001). However, inhibition by barbiturates and propofol occurs at concentrations that exceed those required to produce anaesthesia (Krasowski and Harrison, 1999). Similarly, modulation of 5-HT_3 receptors expressed in N1E-115 neuroblastoma cells by etomidate occurs at concentrations beyond those generally considered clinically relevant (Appadu and Lambert, 1996).

As the sensitivity of the 5-HT_{3AB} receptor to intravenous anaesthetics has not been determined and previous studies have shown that the anaesthetic sensitivity of this subtype can be distinctly different from that of the 5-HT_{3A} receptor, we sought to define and compare the actions of three intravenous anaesthetic agents (propofol, etomidate, and pentobarbital) on these two 5-HT_3 receptor subtypes. To accomplish this aim, 5-HT_{3A} and 5-HT_{3AB} receptors were expressed in *Xenopus* oocytes and for each anaesthetic and receptor subtype, the half-maximal inhibitory concentration (IC_{50}) was determined.

2. Materials and methods

2.1. Animal care

Xenopus laevis maintenance and oocyte harvest procedures were approved by the local committee for animal care in research (approval # V54-19 c 20/15 c MR 20/13).

2.2. Molecular biology

cDNA encoding the human 5-HT_{3A} and 5-HT_{3B} subunits were generously provided by E. Kirkness (TIGR, Rockville, MD) and transcribed into mRNA using the mMessage mMachine High Yield Capped RNA Transcription Kit (Ambion Inc., Austin, TX).

2.3. Oocyte procedures and receptor expression

Oocytes were harvested from human chorionic gonadotropin-injected adult female *Xenopus laevis* (H. Kähler, "Bedarf für Forschung und Lehre", Hamburg, Germany). Oocyte harvest procedures, further preparation of oocytes and injection of RNA encoding the A and B subunits of 5-HT_3 receptors were done as described previously (Stevens et al., 2005).

2.4. Drugs, chemicals and preparation of solutions

Ethyl 3-aminobenzoate methanesulfonate salt (tricaine), collagenase IA, 5-Hydroxytryptamine (5-HT, serotonin) and propofol were purchased from Sigma-Aldrich. Pentobarbital was obtained as the commercially available Eutha 77 (Essex Pharma GmbH, Munich, Germany) that contains pentobarbital-sodium 400 mg/ml. Etomidate was obtained as commercially available Hypnomidate (Janssen-Cilag GmbH, Neuss, Germany) that contains R(+) etomidate 2 mg/ml dissolved in 35% propylene glycol. All electrophysiology solutions were prepared on the day of experimentation in ND-96 (96 mM NaCl, 2 mM KCl, 1.0 mM $MgCl_2$, 1.8 mM $CaCl_2$, 10 mM HEPES, pH 7.5). Concentrations of R-(+)-etomidate up to 600 μM were prepared by diluting commercial stock (2 mg/ml = 8.2 mM) into ND96. The maximum concentration of propylene glycol in the superfusate was 336 mM, a concentration that neither directly evoked nor inhibited currents elicited by maximally activating concentrations of 5-HT in either 5-HT_3 receptor subtype. Stock solutions of up to 1 mM Propofol in ND96 were prepared by diluting 100 mM Propofol in DMSO. The maximum concentration of DMSO in the superfusate was 1% (140 mM), which neither directly evoked nor inhibited currents elicited by maximally activating concentrations of 5-HT in either

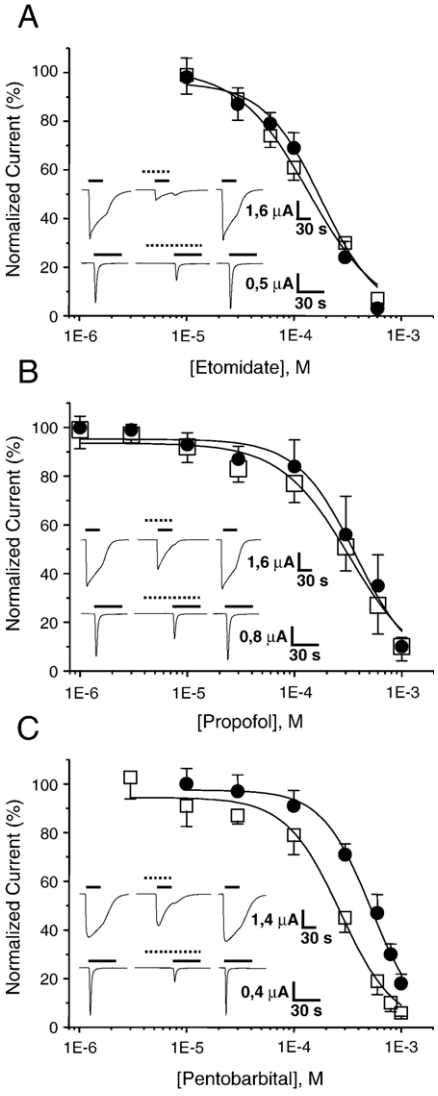

Fig. 1. Etomidate (A), propofol (B) and pentobarbital (C) concentration response relationship of inhibition of peak currents elicited by maximally activating concentrations of 5-HT (100 μM in 5-HT3$_A$ and 300 μM in 5-HT3$_{AB}$ receptors) mediated by 5-HT_{3A} (closed circles) and 5-HT_{3AB} (open squares) receptors. For each point of the 6 concentration response relationships $4<n<13$. Error bars represent the SD of the mean relative response. Insets in each panel show two sets of representative inward currents elicited by 5-HT and mediated by 5-HT_{3A} (top) and 5-HT_{3AB} (bottom) receptors. The first and the third current trace of each set show the response elicited by a maximally activating concentration of 5-HT (100 μM for 5-HT_{3A} and 300 μM for 5-HT_{3AB} receptors) and the second trace demonstrates the effect of the anaesthetic at 300 μM on 5-HT-elicited currents. Solid and dotted lines above each current trace represent the application of 5-HT for 30 and anaesthetic for 60 s, respectively.