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

Dirk Rüsch, **Hans A. Braun**, **Hinnerk Wulf**, **Anika Schuster**, and **Douglas E. Raines**
Department of Anaesthesia and Critical Care, University Hospital Giessen-Marburg GmbH, Marburg Campus, Marburg, Germany (D.R., H.W., A.S.). Institute of Physiology and Pathophysiology, Division of Neuroendocrinology and Neurodynamics, Marburg University, Marburg, Germany (H.B.). Department of Anesthesia and Critical Care, Massachusetts General Hospital, Boston, MA, USA (D.E.R.)

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$_{3A}$ receptors; 5-HT$_{3AB}$ receptors

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-HT3A and 5-HT3AB).

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

Corresponding author: Dirk Rüsch, Department of Anaesthesia and Critical Care, University Hospital Giessen-Marburg GmbH, Marburg, Campus, Baldingerstrasse, 35033 Marburg, Germany, Tel.: 49-(0)6421-2865981, Fax.: 49-(0)6421-2865971, e-mail: ruesch@staff.uni-marburg.de.

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; Barann et al., 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 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 400mg/ml. Etomidate was obtained as commercially

available Hypnomidate (Janssen-Cilag GmbH, Neuss, Germany) that contains R(+) etomidate 2mg/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 600μM 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 5-HT_3 receptor subtype. Pentobarbital solutions were prepared by diluting a 10 mM stock solution into ND 96. The pentobarbital stock solution was made by diluting the commercially available pentobarbital (400 mg/ml = 1.61 M) into ND96.

2.5 Electrophysiology

Electrophysiological experiments using the two-microelectrode voltage clamp technique were carried out at room temperature (21°C) 1 – 7 days following the injection of oocytes with mRNA encoding the 5-HT_{3A} (for studies of the 5-HT_{3A} receptors) or an mRNA mix encoding the 5-HT_{3A} subunit and the 5-HT_{3B} subunit (for studies of the 5-HT_{3AB} receptor). Oocytes were placed in a 40 μl custom-made flow chamber, impaled with two capillary glass pipettes filled with 3 mM KCl (resistance < 5MΩ), voltage clamped at −50 mV (Turbo TEC 10CX amplifier, Science Products GmbH, Hofheim, Germany), and constantly superfused with ND96 solution at a rate of 5 ml/min. The perfusion apparatus was constructed of glass syringes and teflon tubing to minimize absorptive loss of anaesthetics. Superfusion was controlled with a six-channel valve controller (Hugo Sachs Elektronik – Harvard Apparatus GmbH, March-Hugstetten, Germany). Currents were recorded on a personal computer running custom-made software (PC.DAQ1.1, developed at the Institute of Physiology, University of Marburg), filtered at 1 kHz and sampled at 100 Hz.

2.6 Experimental protocols

2.6.1. Inhibition of peak currents elicited by maximally activating 5-HT concentrations—Oocytes were preincubated for 30 s with ND96 solution containing the desired concentration of anaesthetic, followed by a co-application of anaesthetic plus a maximally activating concentration of 5-HT (100 μM in 5-HT_{3A} and 300 μM in 5-HT_{3AB} receptors) for 30 s (test experiment). Longer preincubation times did not increase inhibition by the three compounds tested (data not shown). Each test experiment was preceded and followed by a control experiment in the absence of anaesthetic and the average of these two control experiments was used to normalize each test response. To minimize the impact of desensitization and to ensure complete wash-out of anaesthetics, a recovery period of 3 min (control) or 5 to 10 min (test) was allowed between experiments.

2.6.2. Comparison of inhibition at different 5-HT concentrations—To asses the nature of anaesthetic inhibition (i.e. competitive vs. noncompetitive), anaesthetic inhibition of peak currents elicited by 100μM in 5-HT_{3A} and 300 μM in 5-HT_{3AB} receptors by etomidate, propofol and pentobarbital (each at 300μM) was compared to the inhibition of peak currents elicited by tenfold lower concentrations of agonist (10μM in 5-HT_{3A} and 30 μM in 5-HT_{3AB} receptors).

2.7 Data analysis

Concentration-response curves for inhibition of maximal 5-HT peak currents were fitted by nonlinear least squares to the following Hill equation:

$$I=I_{control}\ IC_{50}{}^{n}/([\text{anesthetic}]^{n}+IC_{50}{}^{n})$$

where I is the peak current evoked by 5-HT in the presence of anaesthetic, $I_{control}$ is the peak current elicited by 5-HT in the absence of anaesthetic, IC_{50} is the anaesthetic concentration that reduces 5-HT peak currents to 50% of the control value and n is the Hill coefficient.

2.8 Statistical analysis

Inhibition by anaesthetics of peak currents evoked by maximally activating concentrations of 5-HT were compared to those elicited by a tenfold lower concentration of agonist using a paired t-test (Prism 4.0 software; GraphPad Software Inc., San Diego, CA) with a statistical significance set at $P < 0.05$.

3. Results

For both receptor subtypes, inhibition by propofol, etomidate, and pentobarbital was concentration-dependant (Fig. 1) and the calculated IC_{50}S (table 1) greatly exceeded their EC_{50}S for general anaesthesia (etomidate: 8.7 μM in humans, propofol 0.4 μM in humans, pentobarbital: 50 μM in mice (Krasowski and Harrison, 1999)). For etomidate and propofol, the IC_{50}s did not differ significantly between the two 5-HT_3 receptor subtypes whereas for pentobarbital the IC_{50} for 5-HT_{3AB} receptors was lower than that for 5-HT_{3A} receptors.

The magnitudes of inhibition of peak currents elicited by 5-HT at maximally activating concentrations vs. tenfold lower concentrations by etomidate ($P = 0.91$), propofol ($P = 0.65$), and pentobarbital ($P = 0.65$) at 300 μM each were not statistically different in 5-HT_{3A} receptors ($n = 5$ cells for each compound). Similarly, corresponding experiments carried out in 5-HT_{3AB} receptors did not show a different magnitude of inhibition by any of the three anaesthetics ($P = 0.42$, 0.54 and 0.48, respectively with $n = 5$ cells for each compound).

4. Discussion

5-HT_3 receptors are distributed widely in many tissues and organ systems. In the central nervous system, northern blot analysis as well as RT-PCR and in situ hybridization have found mRNA encoding both 5-HT_{3A} and 5-HT_{3B} subunits in several human brain tissues (e.g. hippocampus and amygdala) suggesting the presence of 5-HT_{3AB} receptors in brain regions that are thought to be relevant to the actions of general anaesthetics (Davies et al., 1999). Similarly, studies using polyclonal antibodies in immunohistochemical experiments have detected 5-HT_{3B} subunits in interneurons (Monk et al., 2001) and, most recently, neurons in the pyramidal and molecular layers (Reeves and Lummis, 2006) of rat hippocampus. However, one study (Morales and Wang, 2002) found no evidence of 5-HT_{3B} subunit expression in rat brain and another found expression only at relatively low levels (Sudweeks et al., 2002), leading to the suggestion that the 5-HT_{3B} subunit may be highly localized in discrete populations of neurons (Reeves and Lummis, 2006).

Studies of anaesthetic action on 5-HT_3 receptors have shown that inhaled anaesthetics and anaesthetic alcohols with molecular volumes smaller than 0.12 nm^3 enhance currents evoked by low concentrations of 5-HT when mediated by 5-HT_{3A} receptors, but not 5-HT_{3AB} receptors (Stevens et al., 2005). This difference exists because the 5-HT_{3B} subunit reduces the impact that anaesthetics have on anaesthetic-channel gating efficacy (Solt et al., 2005). The two receptor subtypes also differ in their sensitivity to certain inhibitory drugs. For example, 5-

HT_{3AB} receptors are approximately fivefold less sensitive to antagonism by tubocurarine than 5-HT_{3A} receptors (Davies et al., 1999).

Propofol (Barann et al., 2000a; Patten et al., 2001) and barbiturates (Barann et al., 1997; Barann et al., 2000b) have been shown to inhibit endogenous 5-HT_3 receptors expressed in N1E-115 mouse neuroblastoma cells and recombinant 5-HT_{3A} receptors expressed in HEK 293 cells. Similarly, etomidate, propofol and thiopentone displace a selective high affinity antagonist from 5-HT_3 receptors present in N1E-115 mouse neuroblastoma cells (Appadu and Lambert, 1996). In some cases, anesthetic potencies were found to be significantly higher than those reported in the present study. For example, Barann et al (Barann et al., 2000b) reported an IC_{50} for propofol inhibition of 5-HT_{3A} receptors that is 1–2 orders of magnitude lower than that determined in this study. The same group found a similarly low IC_{50} for propofol inhibition of native 5-HT_3 receptors found in N1E-115 neuroblastoma cells (Barann et al., 2000a). However, another laboratory reported a significantly higher propofol IC_{50} for 5-HT3 receptors. At a concentration that approximates the IC_{50} reported by Barann et al. (Barann et al., 2000a), Patten et al. (Patten et al., 2001) found little or no inhibition by propofol of 5-HT_3 receptors located in rat isolated vagus nerve. At 100 μM propofol, they reported that only half of the current was inhibited in this system. Appadu and Lambert (Appadu and Lambert, 1996) reported a K_i of 819 μM for propofol's interaction with 5-HT_3 receptors. Interestingly, this was in the same system (N1E-115 neuroblastoma cells) that Barann et al. (Barann et al., 2000a) reported an IC_{50} that was more than 50-fold lower. The reason for the great variability among different laboratories, techniques, and experimental models is unclear. Nonetheless, all of the reported 5-HT_3 receptor IC_{50}s and K_is of intravenous anaesthetics are well above their respective anaesthetizing concentrations.

Our studies show that heteromeric 5-HT_{3AB} receptors are also inhibited by propofol, etomidate, and pentobarbital. There were no significant differences in the sensitivities of the two receptor subtypes to inhibition by propofol or etomidate, whereas the 5-HT_{3AB} receptor was more sensitive than the 5-HT_{3A} receptor to pentobarbital. In both receptor subtypes, the magnitudes of inhibition induced by all anaesthetics were insensitive to a 10-fold reduction in the 5-HT concentration, strongly suggesting that the mechanism of inhibition is non-competitive.

In summary, the intravenous anaesthetics propofol, etomidate, and pentobarbital significantly inhibit 5-HT_{3A} and 5-HT_{3AB} receptors at concentrations that exceed their anaesthetizing concentrations. The two receptor subtypes are equally sensitive to inhibition by propofol and etomidate whereas the 5-HT_{3AB} receptor is more sensitive to pentobarbital. Based on these findings, we conclude that inhibition of neither 5-HT_{3A} nor 5-HT_{3AB} receptors contributes significantly to the anaesthetic actions of propofol, etomidate, or pentobarbital.

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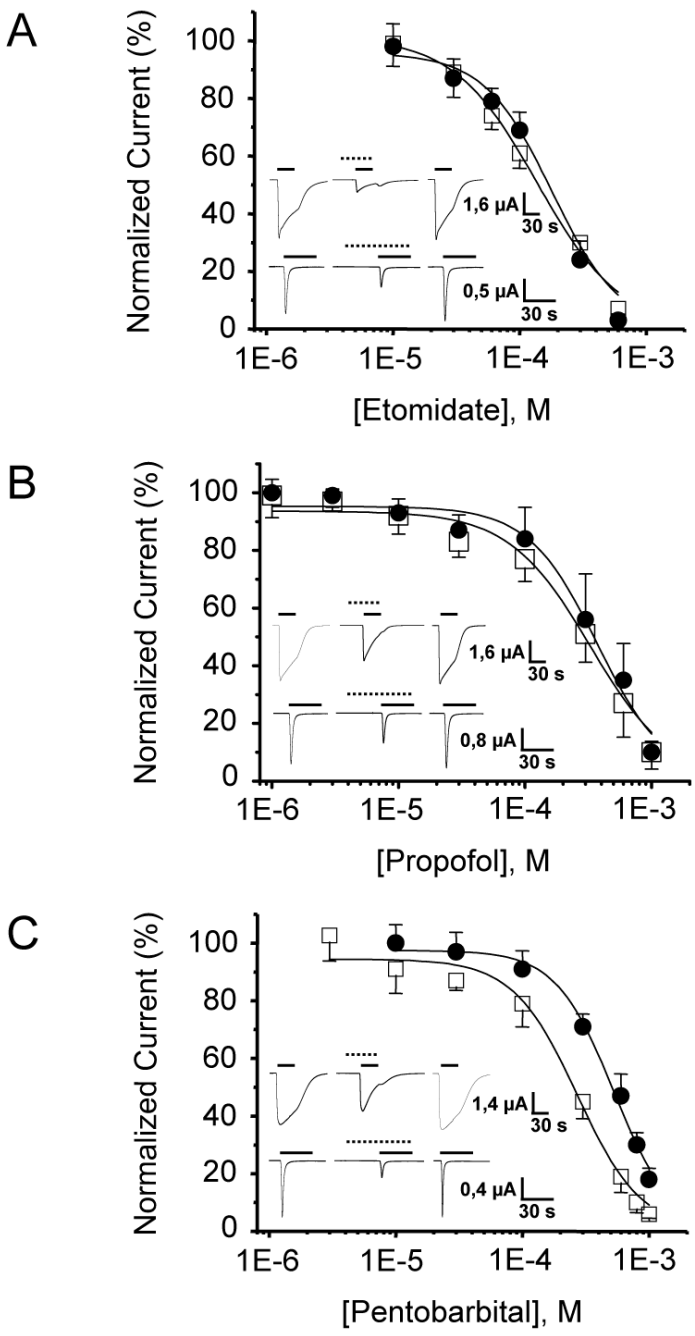

Figure 1.

Etomidate (A), propofol (B) and pentobarbital (C) concentration response relationship of inhibition of peak currents elicited by maximally activating concentrations of 5-HT (100µM in 5-HT3$_A$ and 300µM 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.

Table 1

Survey of normalized IC_{50}, I_{max} (% of maximal 5-HT current) and Hill slope data (±SD) of 5-HT in 5-$HT3_A$ and 5-$HT3_{AB}$ receptors.

DRUG	RECEPTOR	IC_{50}[μM]	I_{MAX} [%]	HILL COEFFICIENT
etomidate	5-HT_{3A}	180 ± 86	96 ± 8.5	1.6 ± 0.86
	5-HT_{3AB}	140 ± 45	101 ± 7.6	1.3 ± 0.49
propofol	5-HT_{3A}	370 ± 151	95 ± 3.3	1.6 ± 0.66
	5-HT_{3AB}	300 ± 125	94 ± 3.4	1.4 ± 0.51
pentobarbital	5-HT_{3A}	520 ± 168	97 ± 2.9	1.9 ± 0.65
	5-HT_{3AB}	270 ± 63	94 ± 3.7	1.7 ± 0.56