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TITLE: GENERAL ANESTHETIC POTENCY OF PROPOFOL IS CONSISTENT WITH THE MEYER-OVERTON-RULE

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Introduction: Although Propofol's (2,6-diisopropylphenol) blood level during anesthesia is well known[1], its free concentration is unknown because of uncertainties due to plasma protein binding, interferences with other protein bound substances on plasma proteins, the level of binding to its lipid carrier and the use of adjuvants. In this abstract we report the anesthetic potency of propofol using a method which avoids many of these problems.

Methods: Anesthetic potency was determined using *rana pipiens* tadpoles exposed to different concentrations of aqueous 2,6-diisopropylphenol (10 animals/point) until a constant response level was reached (15 min). Anesthesia was defined as loss of righting reflex (LRR). Propofol concentrations were measured spectrophotometrically at a wavelength of 272 nm.

Results: The fraction of anesthetized animals increased with increasing concentration. The logistic concentration-response-plot showed a steep sigmoidal curve (slope = 2.4 ± 0.3). Calculation of the half maximal effect resulted in an ED_{50} of 2.3 ± 0.4 μM. All animals anesthetized with concentrations below 100 μM recovered.

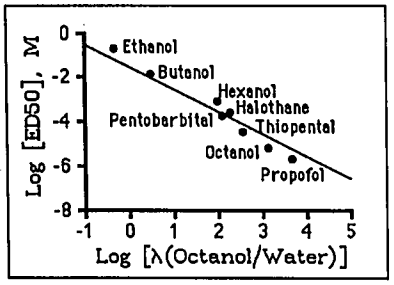

Fig. 1: Meyer-Overton-correlation for general anesthetics in tadpoles

Discussion: Figure 1 shows the Meyer-Overton-correlation between octanol/water partition coefficient and anesthetic potency. Propofol lies close to the regression line (fixed slope = -1) therefore it behaves as a typical general anesthetic. The free concentration at which halfmaximal LRR occurs in tadpoles under equilibrium conditions is 5 - 7.5 times lower than the concentrations obtained in infusion based blood concentration studies in humans. This is consistent with the presence of the known high level of protein binding[1] but the influence of other factors cannot be ruled out. Our results show that Propofol is one of the most potent anesthetics currently in clinical use. For example the free concentrations of thiopental and halothane at their ED_{50} were 30 μM [2] and 230 μM, respectively, for the LRR. This means that propofol is about 15-fold more potent than thiopental, whereas studies on blood levels[3] lead to the prediction of only a 3-fold difference in potency. This high general anesthetic potency of propofol is an additional advantage for its clinical use.

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TITLE: MODULATION OF THE $GABA_A$ RECEPTOR BY PROPOFOL: PROTEIN OR LIPID INTERACTION?

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INTRODUCTION: A variety of structurally diverse intravenous general anesthetics modulate the $GABA_A$ receptor at clinically relevant doses (1). The diversity of these compounds and their lipophilicity has led to the suggestion that modulation is achieved by membrane perturbation. Propofol has been demonstrated to potentiate GABA-evoked currents and directly activate the $GABA_A$ receptor (2) at blood concentrations observed during sedation and total intravenous anesthesia (3), respectively. In this study the patch-clamp technique was used to investigate the site(s) through which propofol modulates the $GABA_A$ receptor.

METHODS: Bovine chromaffin cells, previously demonstrated to have $GABA_A$ receptors with similar properties to those found on central neurones (4), were voltage-clamped in the whole-cell configuration at -60 mV. Cells were superfused with a solution containing (in mM): NaCl 140, KCl 2.8, $MgCl_2$ 2.0, $CaCl_2$ 1.0 and HEPES-NaOH 10 (pH 7.2). The electrode solution used to dialyze the cell interior contained (in mM): CsCl 140, $MgCl_2$ 2.0, $CaCl_2$ 0.1, EGTA 1.1 and HEPES-NaOH 10 (pH 7.2). Similar bath and electrode solutions were used in experiments involving cell-attached and outside-out membrane patches. For intracellular application of propofol and application of the drug to cell-attached patches, the anesthetic was diluted into the electrode solution from a stock solution of 10^{-2} M. The diffusional exchange rate between the patch electrode and the cell is dependent upon the molecular weight of the drug (178.3), the cell pipette access resistance (3.2 ± 0.3 MΩ, range 1.9 - 4.4 MΩ, n = 6) and the cell volume, which was estimated from the cell capacitance (7.6 ± 0.3 pF, range 6.4 - 8.6 pF, n = 6), assuming a specific membrane capacitance of 1 uF cm^2 (5). The time constant for diffusional exchange between the electrode solution and the cell interior was calculated to be 15.8 s. GABA was either applied locally by pressure ejection (1.4 X 10^5 pa at 0.05 Hz for 20 ms) from a modified patch electrode, or added to the bath solution. Chromaffin cells were maintained in tissue culture for up to 1 week and used in experiments at 17-21°C.

RESULTS: Bath applied propofol (1.7 μM) caused potentiation of GABA (100 μM)-evoked currents to 564 ± 78% (n = 4) of control amplitude. In the presence of a ten-fold higher concentration of the anesthetic inside the cell, propofol (1.7 μM) increased the size of GABA-activated currents to 550 ± 150% (n = 6) of control amplitude. The lack of effect of a high concentration of intracellular propofol (16.8 μM), on the amplitude of the potentiation achieved by a low concentration of the drug applied extracellularly, suggests that the anesthetic was unable to access its site of action from within the cell.

Propofol (16.8 μM), in the electrode solution, activated single channels recorded from chromaffin cells in the cell-attached configuration. These events exhibited cord conductances similar to those of channels evoked by bath applied propofol (30 μM), recorded from outside-out membrane patches. Propofol (30 μM)-activated channels were blocked by the $GABA_A$ receptor antagonist, bicuculline (1 μM), indicating a direct activation of the GABA receptor by the anesthetic. In the whole-cell mode, intracellular application of propofol (16.8 μM) did not cause activation of the $GABA_A$ receptor. In contrast, using the whole-cell configuration, the threshold of activation of the $GABA_A$ receptor by extracellular propofol was below 8 μM. Since propofol was unable to activate the receptor from within the cell it appears that modulation of the $GABA_A$ receptor by the anesthetic exhibits membrane asymmetry.

DISCUSSION: The ineffectiveness of intracellular propofol as a modulator of the $GABA_A$ receptor suggests that there is more to this phenomenon than membrane perturbation. We propose that a more likely site of action of propofol is a hydrophobic domain within the extracellular region of the $GABA_A$ receptor-ion-channel complex.

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