Title: ANESTHETIC OPTICAL ISOMERS ARE EQUIPOTENT IN DESENSITIZING ACETYLCHOLINE RECEPTORS

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Introduction: In anesthetized man[1] and skeletal muscle in vitro[2], volatile general anesthetics (GAs) depress synaptic transmission at the neuromuscular junction. Volatile and alcohol GAs inactivate the postsynaptic membrane, by accelerating the desensitization of acetycholine receptor (AChR)-associated ion channels[3]. The ability of GAs to desensitize AChRs has been successfully correlated with their membrane/buffer partition coefficients[4] and with their ability to disorder membrane lipids[5]. This suggests that GAs act on AChRs indirectly through the lipid bilayer, rather than directly on the receptor protein. To test this, we examined the effects of a series of anesthetic alcohol optical stereoisomers (enantiomers) on AChR-rich membranes. Stereoselectivity generally implies drug interaction with a specific receptor while lack of stereoselectivity would indicate action at a nonselective site, i.e. the lipid bilayer.

Methods: AChR-rich postsynaptic membranes were purified by differential and sucrose density gradient centrifugation from homogenates of Torpedo electric organ. The (+) and (-) isomers of 2-butanol, 2-hexanol, 2-heptanol, and (+)-2-octanol (Norse, Newbury Park, CA) were > 99% chemically pure and > 95% optically pure; (-)-2-octanol was > 85% optically pure. Desensitized AChRs were identified by their high affinity for ^{3}H-ACh (called "R_{hi}")[6]. Anesthetics were preincubated in capped vials with AChR-rich membranes (25nM sites final concentration) to steady state effect (30 min, 20°C), and ^{3}H-ACh (Amersham, Arlington Heights, IL, final 50 nM) was added for 5 sec. Under these conditions, ^{3}H-ACh does not itself induce desensitization, but is strictly a probe for the desensitized state. Then ^{3}H-ACh bound to AChR-rich membranes was separated from free ^{3}H-ACh by vacuum filtration (Whatman GF/F glass fiber filters). Bound ^{3}H-ACh was corrected for nondisplaceable binding using the irreversible nicotinic antagonist alpha-Bungarotoxin. Anesthetic concentrations were monitored by gas chromatography and remained within 10% of starting values during assays. Reversibility of effects at the highest concentrations were confirmed by separate back-dilution experiments.

Results: All agents reversibly increased the proportion of AChRs in the high affinity state (R_{hi}) as a sigmoid function of concentration; effects at the highest alcohol concentrations studied plateaued at 95% R_{hi}. Concentration-response curves were steep; the slopes ranged from 2.2±0.3 to 3.1±0.5 and were not statistically different ($p > 0.1$). Thus the R_{hi}^{50}s provide a satisfactory measure of relative potencies. There were no significant differences in desensitizing potencies between pairs of optical isomers; potency ratios [(+) R_{hi}^{50}/(-) R_{hi}^{50}, Table1] ranged from 0.89-0.95 but no systematic trends were observed. None of the potency ratios differed significantly from one ($p > 0.1$). There was however, a systematic potency increase in the series from 2-butanol to 2-octanol, such that 2-octanol was 166-times more potent than 2-butanol. R_{hi}^{50}s were logarithmically related to alcohol carbon chain length (C_n); the slope of a linear regression of log R_{hi}^{50} vs. C_n was -0.55 ± 0.02 ($r = -0.996$).

Discussion: Correlations between GA potency and physical properties strongly indicate that their site of action is hydrophobic in nature. However, membranes contain multiple hydrophobic micro-environments through which GAs could produce inexcitability. We have previously used AChR-rich membranes to demonstrate that GAs drive the receptor to a high affinity, inactive conformation in parallel with disordering the membrane's lipid.[5] Now we have shown that enantiomers of aliphatic secondary alcohols are equipotent in their ability to desensitize AChRs. Potency ratios [(+/-) R_{hi}^{50}, Table 1) were unity even for agents active in the micromolar range, which is particularly significant since stereoselectivity often increases with ligand affinity.[7] Such data indicate that the GA target in membranes is nonselective in nature, very likely the hydrocarbon core of the lipid bilayer or the lipid-protein interface, and excludes most of the common types of drug-protein interactions.

We have also found the potency ratios for loss of righting reflex (LRR) in tadpoles [(+/-)ED^{50}, Table 1] to be unity, for the same series of alcohol enantiomers. Although they were somewhat less potent when desensitizing AChRs than when producing LRR in animals the lack of stereoselectivity in both systems is an important finding since it suggests a similar site of action.

References:

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Table 1. Potency Ratios for Anesthetic Enantiomers in Nicotinic Membranes (R_{hi}^{50}s) and in Tadpoles (ED^{50}s).

Agent	$\frac{[(+)+(-)]R_{hi}^{50}}{2}$	$[+/-]R_{hi}^{50}$ (SD)	$[+/-]ED^{50}$ (SD)
2-Butanol	60 mM	0.90 (0.07)*	1.00 (0.01)*
2-Hexanol	6.6 mM	0.94 (0.07)*	0.94 (0.07)*
2-Heptanol	1.3 mM	0.95 (0.09)*	0.97 (0.07)*
2-Octanol	0.4 mM	0.89 (0.06)*	1.03 (0.09)*

* = $p > 0.1$ for a difference from a value of 1.00, using a two-tailed t-test.

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