INHIBITION OF GABA-RECEPTOR LIGAND BINDING BY ANESTHETIC ALCOHOLS: A COMPARISON WITH OBTUNDING POTENCIES

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Introduction: The GABA receptor-ion channel macromolecule (GABA-R) is a likely target for ethanol's and other general anesthetics' (GA) action, as it has been shown to possess physiologically-relevant binding sites for other classes of sedating agents (1). These agents inhibit GABA-R ligand binding (2), although entire concentration-effect relations have not yet been elucidated. Herein, we report these for the anesthetic normal alcohols, and compare their median effective inhibitory concentrations (IC50s) with respective obtunding potencies (EC50s). Moreover, since long chain alkanols were included in this study, we tested whether these agents abruptly cutoff in potency at the GABA-R, as has previously been shown to occur in animals (3).

Methods: Alkanol effects on rat brain synaptosomes were measured using an equilibrium 3H-flumazenil (3H-FMZ) binding assay (2). For very hydrophobic alcohols, gas chromatography was employed to confirm free aqueous concentrations remaining after pelleting membrane suspensions. Curvilinear concentration-effect data were fit to a logistic equation as described by Waud (4).

Results: The shorter chain alkanols (C_1OH-C_6OH) inhibited binding completely; the IC50s, and slopes of concentration-effect relations, and their respective errors, are presented in the table. In contrast, with the higher molecular weight members (C_7OH-$C_{10}OH$), efficacy gradually diminished, such that decanol was no longer able to inhibit 3H-FMZ binding despite a free aqueous concentration approaching saturation. When in vitro potencies (log IC50) were compared to in vivo potencies (log EC50) by linear regression analysis, free fit yielded a good correlation ($r = 0.991$), and a line with a slope of 0.84 ± 0.32, which is not significantly different than unity ($p > 0.6$). In addition, comparison of the regression lines of alkanol carbon chain length (C_N) vs. log IC50, and C_N vs. log EC50, were not signicantly different (-0.51 ± 0.19, and -0.60 ± 0.19, respectively, $p > 0.7$).

Discussion: For the anesthestic alcohols, comparisons between animal potencies and potencies at GABA-R, indicate a reasonably good correlation. However, the relevance of the correlation is not supported by our efficacy findings, since alcohol potency cuts off at tridecanol in animals (3), but at decanol in membrane-bound GABA-R. These data indicate that the BDZ site on the GABA-R is not an ideal model of the GA site, at least as assessed by an equilibrium binding assay.

References: (1) Int Anesthesiol Clin 26: 254, 1988; (2) Anesthesiol 71:A296,1989; (3) Br J Pharm 96: 9, 1989; (4) J Pharm Exp Ther 183: 577, 1972.

AGENT	IC50 (mM)	±SE	SLOPE	±SE
C_1OH	2739	91	-2.5	0.20
C_2OH	1623	74	-2.3	0.23
C_3OH	305	10	-1.8	0.12
C_4OH	80	4	-1.4	0.09
C_5OH	44	1.1	-1.9	0.09
C_6OH	16	1.4	-1.3	0.17