

TITLE: HALOTHANE REQUIREMENT IN MICE SELECTIVELY BRED FOR SENSITIVITY AND RESISTANCE TO DIAZEPAM

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INTRODUCTION: One approach to elucidate the general anesthetic (GA) target has employed genetic selection procedures, wherein animals are bred for sensitivity or resistance to GAs and correlations sought with a specific neuronal structural or functional defect. Such methods have yet to reveal whether the important defect underlying divergence of the selected lines involves lipids or proteins¹, or even narrow the range of possibilities. In contrast, murine strains have recently been developed that are either sensitive or resistant to the obtunding effects of diazepam² and which manifest parallel changes in the Cl channel gating properties of GABA-receptor harvested from their brains³. Thus we explored whether the defect underlying divergence of diazepam-sensitive (DS) and diazepam-resistant (DR) mice could likewise be involved in the obtunding response to GAs, by testing the requirement for inhalational anesthesia in these strains.

METHODS: Mice were bred according to their rotarod impairment time following diazepam². Requirements for halothane, enflurane and isoflurane were assessed using the endpoint of loss-of-righting-reflex (LRR) in a rotating carousel¹ and analyzed quantally by the

method of Waud⁴. GA concentrations were verified directly by mass spectroscopy

RESULTS: The slopes of all concentration-response curves were not different ($p > 0.25$), allowing comparisons of median effective concentrations (EC50) to be made between groups. For each agent, the DS group required significantly less anesthetic for LRR than did DR (Table 1), and further, that the reductions paralleled their diazepam susceptibility.

DISCUSSION: These data clearly associate a difference in diazepam-induced rotarod impairment with a difference in volatile anesthetic EC50, suggesting that these two phenotypes are mediated by a common underlying mechanism. To further strengthen this association, a replicate selected line will need to be tested⁵.

REFERENCES: 1. *Anesthesiology* 52:401-407, 1980; 2. *Psychopharmacology* 93:25-30, 1987; 3. *Brain Research* 452:118-126, 1988; 4. *J. Pharmacol Exp Ther* 183:577-607, 1972; 5. *Behavior Genetics* 19:473, 1989. Supported by UACCMF and NS-23927.

TABLE 1. Anesthetic requirement for loss-of-righting reflex in selectivity bred diazepam sensitive (DS) and resistant (DR) mice.

AGENT	DS LINE	DR LINE	n	p-value (DS:DR)
	EC50 + SE	EC50 + SE		
Halothane	0.72±0.02	0.87±0.03	24	<0.001
Isoflurane	0.64±0.01	0.71±0.01	24	<0.001
Enflurane	1.10±0.02	1.35±0.02	24	<0.001

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Title: ACTIONS OF ETHANOL AND VOLATILE GENERAL ANESTHETICS AT BOUNDARY LIPID.

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INTRODUCTION: Transmembrane excitable proteins such as the acetylcholine receptor (AChR) require bilayer-forming lipids for functional integrity¹. For example, a minimum of 40-50 phospholipids per AChR are necessary, which could correspond to a single "boundary" layer². Since loss of this boundary layer is accompanied by loss of function, it has been hypothesized that ethanol and the volatile general anesthetics (GAs) may inactivate excitable proteins by inhibiting protein-lipid interactions. To test this, the ability of these agents to diminish the spectroscopically immobilized boundary lipid was observed by electron spin resonance spectroscopy (ESR), using both reconstituted and native AChR-rich membranes derived from electric tissue of the marine ray, *Torpedo*.

METHODS: AChR-rich membranes were harvested from *Torpedo* electroplax and isolated by differential and sucrose density gradient sedimentation as previously described³. Native membranes were spin labeled with 12-doxylstearate (12-DS), at a final membrane concentration of 1 mol% (spin label : phospholipid), by gently shaking overnight at 4°C. Reconstituted membranes consisted of precisely defined mixtures of proteins and lipids, previously extracted in chloroform : methanol (2:1) from native *Torpedo* membranes. Protein-to-lipid ratios varied from 0.25 to 6.0 (by

weight). Spin label (12-DS) was also incorporated into reconstituted membranes at a ratio of 1 mol%. ESR spectra were obtained at $21 \pm 0.1^\circ\text{C}$ on a Bruker 300 spectrometer operating at 9.5 GHz with 10 mW of microwave power; digitized spectra of pure lipid extracts were subtracted from those obtained from membranes, to yield "difference" spectra representing boundary lipid. These were doubly integrated, then normalized to the double integral of corresponding membrane samples to facilitate comparisons.

RESULTS: Under control conditions, $40 \pm 2\%$ of the lipid was immobilized (τ_R , the rotational correlation time $> 50\text{ns}$), as assessed by digitized spectral subtraction (i.e., A_{11} , the hyperfine splitting parameter = 63G for 40% of the integrated 12-DS intensity). This proportion of boundary lipid was a sensitive function of the protein-lipid ratio and temperature between 15 and 45°C. In contrast, toxic concentrations of ethanol, diethylether, halothane, and octanol all failed to significantly alter the immobilized component.

DISCUSSION: Such data are not consistent with pharmacologically important GA actions at intrinsic protein-lipid interfaces. However, future studies must test GA effects using sterol and phospholipid spin labels, which may be more physiologic.

REFERENCES: 1. *Biochemistry* 26:3781, 1987; 2. *Biochemistry* 22:5523, 1983; 3. *Anesthesiology* 64:694, 1986.

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