

TITLE: TOXICITY VS. PROTECTION BY HALOTHANE DURING HYPOXIA: RELATIONSHIP TO GLUTATHIONE
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To study the intrinsic effect of halothane (H) on hypoxic cellular survival, we have utilized cultured hepatocyte monolayers, thereby eliminating the variables of cardiovascular effects and intra-organ O₂ gradients. Because the reductive metabolism of H during hypoxia yields free radicals with the potential to deplete reduced glutathione (GSH), a major intracellular antioxidant,¹ we have also determined the effect of H on GSH during hypoxia.

METHODS. Fed male Sprague Dawley hepatocytes, +/- P450 induction by phenobarbital, were harvested by collagenase perfusion and grown to confluence overnight in supplemented Dulbecco's Modified Eagle Medium (DMEM). Cells were then exposed for 2 hr at 37 C to 1% O₂, 5.6% CO₂, +/- 2% H, balance N₂, in buffer containing only the inorganic salts, glucose, and insulin of DMEM. Gas concentrations were verified by mass spectrometry. Cell death was determined by AST release into the medium. GSH was determined by HPLC analysis.²

RESULTS (see Fig.). Hypoxia alone caused a decrease in both GSH and viability. H had little additional effect in P450-induced cells. In uninduced cells, H tended to be protective during hypoxia. BCNU inhibits glutathione reductase and prevents regeneration of GSH. In induced cells, the

presence of 0.4 mM BCNU during hypoxia made H toxic, even though absolute GSH levels were not changed by H. Menadione depletes absolute levels of GSH. Surprisingly, H protected menadione-pretreated (0.025 mM, 30 min, 20% O₂), induced cells during hypoxia.

DISCUSSION. H was not intrinsically toxic to induced or uninduced hepatocytes, in contrast to another report.³ This may reflect differences in animal strains, culture, or exposure conditions. Modulation of GSH revealed two opposing effects of H during hypoxia. H toxicity with BCNU despite a small effect on GSH may reflect the greater importance of flux through glutathione reductase rather than absolute GSH levels. Protection by H after menadione pretreatment may reflect a simple reduction in metabolic O₂ demand, or other more complex effects may be involved.

REFERENCES. 1. Ann Rev Biochem 52:711, 1983. 2. Anal Biochem 114:383, 1981. 3. Anesthesiology 68:485, 1988.

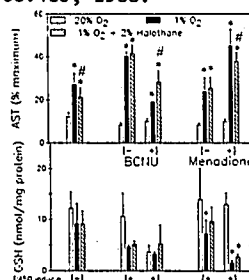


FIGURE. {} denotes single experiment with 3-5 plates each condition, typical of 3-5 similar experiments. Overlying AST and GSH values are from the same plates in the same experiments. P<0.05 by ANOVA for *(1% vs. 20% O₂), #(+ vs. - H at 1% O₂).

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TITLE: L-644,711 INCREASES ISOFLURANE MAC IN RATS
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Introduction: Isoflurane is known to alter the functional state of ion channels;¹ and it has been hypothesized that the anesthetic state may result from an influx of chloride through anion channels.² Accordingly, we evaluated the effect of the anion channel inhibitor L-644,711 on isoflurane MAC in rats.

Methods: Isoflurane MAC was determined (tail clamp method³) in age matched Sprague-Dawley rats (n=12) with chronically implanted subarachnoid catheters. After the first MAC determination, each rat was randomly assigned to one of the following groups based on intrathecal administration of L-644,711 or vehicle: 1) L-644,711-a 3000 $\mu\text{g}\cdot\text{kg}^{-1}$ bolus of L-644,711 followed by an infusion at 1500 $\mu\text{g}\cdot\text{kg}^{-1}\cdot\text{hr}^{-1}$, or 2) Control-vehicle only (equal volume of carrier solution). MAC was again determined in both groups. The data were analyzed using a paired t-test.

Results: There was no difference between groups in the

MAC requirement of isoflurane prior to intrathecal injection of L-644,711 or vehicle. The isoflurane requirement to achieve MAC was greater for the group that received L-644,711, as compared to the group that received vehicle only (p<0.05, see Table 1).

Discussion: The results of this study indicate that isoflurane MAC was $\approx 80\%$ greater in rats which received L-644,711, as compared to rats which received vehicle only. This data supports the hypothesis that isoflurane anesthesia is mediated via anion channels; and blocking these channels may effect a neurophysiologic condition in which isoflurane is pharmacodynamically less efficacious.

	Vehicle	L-644,711
Pre-injection	1.45 \pm 0.24	1.53 \pm 0.41
Post-injection	1.25 \pm 0.17*	2.28 \pm 0.22*

Table 1-MAC values prior to, and following L-644,711 or vehicle administration (mean \pm SD). *p<0.05 between the two groups.

References:

- Brett RS, et al: Anesthesiology 69:161-170, 1988
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- Cole DJ, et al: Anesth Analg 68:556-562, 1989