Title : HALOTHANE AND BRAIN HEXOKINASE ACTIVITY

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Introduction. The intracellular distribution of hexokinase (HK) activity is variable and might participate in the control of metabolism. About 75% of rat brain HK is located on the outer mitochondrial membrane. Membrane-bound HK activity increases and soluble enzyme activity decreases during ischemia or when brain ATP concentrations are lowered (1). The data reported here show that the intracellular distribution of rat brain HK activity is changed by halothane in vitro and in vivo.

Methods. In Vivo Experiments - Adult male rats were used. One group was anesthetized with 1% halothane. Body temperatures were maintained at 37°C and ventilation was controlled so that measured pH and pCO_2 were within normal limits. After fifteen minutes of anesthesia, the rats were decapitated and the brains rapidly removed. A second group of rats were decapitated without any pretreatment.

Tissue Preparation - Whole rat brains were homogenized in 10 volumes of cold sucrose 0.25M containing 0.02 M Hepes at pH 7.5. The crude homogenate was centrifuged at 41,000g x 15 min. The supernate contained soluble HK and the resuspended pellet contained membrane-bound HK activity.

In Vitro Experiments. Crude homogenates of rat brain tissue from normal rats were placed in an Erlenmeyer flask at room temperature with constant stirring. Halothane from a calibrated vaporizer perfused the gas phase above the homogenate. Aliquots of homogenate were taken after 15 min exposure to a known concentration of halothane vapor. The aliquots were centrifuged in sealed tubes to separate soluble and particulate fractions while exposed to halothane. A second group of aliquots were left open to room air to allow halothane to vaporize off prior to centrifugation.

Hexokinase Assay - HK activity was measured by the technique of Knull et al. (1), The reaction medium contained 3.3mM glucose, 6.7mM ATP, 6.7mM $MgCl_2$, 40mM Hepes pH 7.5, 10mM dithiothieitol, 0.64mM NADP and one unit of glucose-6-phosphate dehydrogenase in a total volume of 1 ml. The formation of NADPH at 340nm was recorded.

Results. Table 1 shows that in brains taken from rats anesthetized with halothane, the proportion of soluble HK activity is increased and the particulate activity is decreased compared to the HK distribution in normal awake brains.

TABLE 1. DISTRIBUTION (% ± S.E.M.) OF BRAIN HEXOKINASE

Treatment	Soluble	Particulate
None (Normal awake rats)	27 ± 0.9	73 ± 0.9
1% halothane	34.6 ± 1.4*	65.5 ± 1.4*

*p <0.05 when compared to the normal distribution by Students T-test.

In Vitro. The data in Table 2 show that the distribution of HK activity in brain homogenate changes as a function of halothane concentration. The proportion as soluble activity increases and the particulate activity decreases with increasing concentrations of halothane.

TABLE 2. IN VITRO HEXOKINASE ACTIVITY (% ± S.E.M.)

Halothane Conc.	0%	1%*	2%*	3%**	4%**
Soluble	27 ± .9	34.1 ± .6	36.4 ± 1.7	42.8 ± .6	44.6 ± .6
Particulate	73 ± .9	65.9 ± .6	63.5 ± 1.8	57.2 ± .6	55.6 ± .6

*p <0.01; **p<0.001 when compared to 0% halothane by Students T-test

In another experiment, resuspended particulate fractions were incubated with halothane in a fashion identical to the treatment of crude homogenates. The HK activity remained bound and was not solubilized.

Discussion. The mitochondrial bound HK is more active than the soluble form of the enzyme (1). The increase amount of soluble enzyme is therefore consistent with the well known decrease in cerebral metabolic rate during anesthesia. Bielicki and Krieglstein (2) have shown that thiopental anesthesia increases the soluble proportion of rat brain HK activity both in vivo and in vitro. The data reported here show that halothane concentration in vitro can alter the intracellular distribution of hexokinase. Is this a direct action of halothane on the mitochondrial membrane or is it an indirect effect of the anesthesia? That resuspended particulate fractions do not release HK when exposed to halothane, suggests that there is not a direct effect of the drug on membrane-bound enzyme. Future experiments will examine this question more closely.

References.

1. Knull HR, Taylor WF, Wells WW: Effects of energy metabolism on in vivo distribution of hexokinase in brain. J Biol Chem 248:5414-5417, 1973

2. Bielicki L, Krieglstein J: Solubilization of brain mitochondrial hexokinase by thiopental. N-S Arch Pharmacol 298:61-65, 1977