

Title: HALOTHANE AND OXYGEN DEPENDENCE OF HIGH ENERGY PHOSPHATE LEVELS IN MITOCHONDRIAL SUSPENSIONS AT STEADY STATE

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Halothane and hypoxia can each depress  $O_2$ -dependent energy generation by inhibiting electron flow within the mitochondrial respiratory chain<sup>1</sup>. Although considerable potential exists for hypoxia during halothane anesthesia, their combined influence on cellular energy metabolism has not been adequately studied, due to the inaccessibility of intact tissues for biochemical studies. This work has investigated the halothane and oxygen dependence of high energy phosphate (HEP) levels maintained in mitochondrial suspensions continuously equilibrated with a gas phase of fixed  $PO_2$ .

**Methods:** Mitochondrial fractions were isolated from the livers of adult female Sprague-Dawley rats by standard procedures. Mitochondria were suspended at 2-3 mg protein/ml in a buffered isotonic medium which also contained substrate,  $Mg^{2+}$ , phosphate and ATP (adenosine-5'-triphosphate) at typical cytoplasmic concentrations. The suspension was stirred in a thermostatted chamber, while a stream of  $N_2/O_2/\pm 1\%$  halothane was passed over the surface of the suspension. Dissolved  $O_2$  concentration ( $[O_2]$ ) was continuously measured by a polarographic electrode. Aliquots of the suspension were periodically withdrawn for determination of ATP and ADP (adenosine-5'-diphosphate) by high performance liquid chromatography.

**Results:** Following addition of the mitochondrial suspension to the incubation chamber, levels of  $O_2$ , ATP and ADP all reached constant values within 10-15 minutes (data not shown), indicating that true steady states of  $O_2$  and HEP metabolism were being attained. ATP and ADP were measured with  $[O_2]$  stabilized at a variety of possible intracellular values.  $[O_2]$  was readily shifted to new steady state values by adjusting the  $PO_2$  of the gas phase. The table shows that for  $[O_2] \geq 20$  uM (12mmHg), the ATP/ADP ratio was independent of  $[O_2]$ , averaging 60-80 in the absence of halothane and significantly

less (30-40) with 1% halothane present. At  $[O_2] = 10$  uM, ATP/ADP was significantly reduced both with and without halothane, and the latter values were not different from each other (differences significant at  $p \leq .05$ ).

$[O_2]$ (uM)	ATP/ADP	
	no halothane	1% halothane
10	21± 7	14± 3
20	63± 14	31± 10
40	76± 12	33± 10
60	75± 20	38± 17
80	59± 10	33± 14

**Discussion:** Values of  $[O_2]$  at the mitochondrial membrane *in situ* are considerably lower than at the plasma membrane, probably due to  $O_2$  diffusion gradients<sup>2</sup>. The preliminary data reported here indicate that for  $[O_2]$  well within the lower range of expected intracellular values ( $\leq 10$  uM), maintenance of extramitochondrial HEP levels by liver mitochondria was substantially  $[O_2]$ -limited, and the (further) depression of HEP by halothane was attenuated. Based on these findings, the typically lower HEP levels within intact cells, compared with mitochondrial suspensions, may reflect not only higher ATP utilization in the former but reduced  $O_2$  availability as well, especially when extracellular  $PO_2$  is low. Furthermore, the diminished effect of halothane on HEP levels when the latter are already  $[O_2]$ -limited may help to maintain hepatocellular energy stores during halothane anesthesia.

**References:**

1. Cohen PJ: Effect of anesthetics on mitochondrial function. *Anesthesiology* 39:153-164, 1973.
2. Jones DP, Mason HS: Gradients of  $O_2$  concentration in hepatocytes. *J Biol Chem* 253:4874-4880, 1978.