Title: THE PROTECTIVE EFFECT OF HALOTHANE ON ISCHEMIC ISOLATED RABBIT KIDNEY TUBULES

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Introduction. Volatile anesthetic agents have several possible protective effects on post-ischemic organs, such as membrane stabilization and blockade of calcium entry. Kidney tubule cell injury appears to be a necessary event for ischemic acute renal failure. The isolated tubule model has been shown to be an excellent and functional preparation for the study of both normal and ischemic tubules.[1] In this study non-ischemic and ischemic isolated kidney tubules were exposed to halothane and then evaluated using several viability assays.

Methods. Rabbits (3-4 kg), anesthetized with IV pentobarbital (20 mg/kg), underwent laparotomy and bilateral nephrectomy after heparin (200 u/kg) administration. Isolated kidney tubule cells were prepared by a modified Weinberg method.[2] This method consists of a heparin-collagenase flushout followed by a gradient centrifugation. The resulting tubular suspension was then divided into four groups: 1) A non-ischemia group (suspension exposed to 100% O_2); 2) a non-ischemia plus halothane group (99% O_2+1% halothane); 3) an ischemia group (100% N_2 for 30 minutes, followed by 100% O_2 for the remainder of the study); 4) an ischemia plus halothane group (100% N_2 for 30 minutes, followed by 99% O_2+1% halothane for the remainder of the study). The four groups were then allowed to equilibrate and analyzed at 60 minutes. LDH (lactate dehydrogenase) release was measured as an indicator of membrane integrity. ATP and intracellular potassium concentrations, as well as basal O_2 consumption (Gilson differential respirometer), were measured. Values are reported as mean ± SD and statistical analysis was performed using single factor ANOVA.

Results. LDH release: As shown in table 1, halothane had no effect on the non-ischemic tubules. The ischemic tubules released higher levels of LDH compared to controls, indicating membrane damage. In the presence of halothane, the amount of LDH released was reduced to control levels.

Intracellular potassium: Normal intracellular potassium concentrations were measured in both non-ischemic groups. Group 3 tubules had reduced potassium concentrations secondary to ischemic damage that was corrected in the presence of halothane.

Basal Respiration: Ischemic tubules had reduced oxygen uptake compared to both non-ischemic groups. Halothane increased oxygen consumption in the ischemic tubules.

ATP: The ATP concentrations were reduced in the ischemic group 3; however this was not corrected in the presence of halothane.

Discussion. Using an isolated rabbit kidney model, we have shown that 1% halothane maintains better cell membrane integrity following thirty minutes of ischemia. This was substantiated by reduced LDH release and increased intracellular potassium concentrations. Following renal ischemia, halothane may be protective to cell function during reflow.

References.
1. Takano T, et al: Intracellular respiratory dysfunction and cell injury in short term anoxia of rabbit renal proximal tubules. J Clin Invest 76:2377-2384, 1985.
2. Weinberg JM: Oxygen deprivation-induced injury to isolated rabbit kidney tubules. J Clin Invest 76:1193-1208, 1985.

Table 1.

Treatment		LDH (%)	K+*
Non-ischemia	(1)	18.0 ± 3.1	260 ± 58
Non-ischemia + Halothane	(2)	16.0 ± 11.6	242 ± 109
Ischemia	(3)	35.0 ± 9.8	108 ± 48
Ischemia + Halothane	(4)	12.8 ± 4.2[a]	249 ± 14[b]

Treatment		ATP*	Resp**
Non-ischemia	(1)	3.7 ± 1.7	20.4 ± 4.5
Non-ischemia + Halothane	(2)	3.4 ± .3	19.0 ± 2.7
Ischemia	(3)	.8 ± .2	8.5 ± 3.2
Ischemia + Halothane	(4)	2.6 ± 1.4	15.4 ± 2.8[c]

* nmoles/mg protein
** nmoles O_2/min/mg protein
a) p = 0.01
b) p = .008
c) p = .04

[Statistical significance was shown between groups 3 and 4].