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Title : PRESSURE REVERSAL IS NOT OBSERVED IN HALOTHANE TREATED MITOCHONDRIA

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INTRODUCTION. The ability of high pressure to antagonize anesthetic action is well documented. Restoration of light output of anesthetized luminous bacteria by 2000-4000 atmospheres (ATA),[1] recovery of swimming by tadpoles treated with 2.5% ethanol (200-300 ATA)[2] or 0.5-1.5% halothane (35-171 ATA),[3] reversal of halothane's inhibition of axonal transmission (35-69 ATA),[4] and decreased potency of nitrous oxide[5] and isoflurane[6] in the mouse (25-100 ATA) are but a few examples. However, pressure reversal is not universal. Neither halothane's inhibition of synaptic transmission in the rat superior cervical ganglion[4] nor its depression of ciliary beat in Tetrahymena pyriformis[7] is restored by pressures of 35-137 ATA. Thus, different effects of anesthetics (and pressure) may reflect actions at multiple sites.[8] Volatile anesthetics reversibly inhibit state 3 mitochondrial respiration (Q) with a potency related to MAC and lipid solubility.[9] Since not all anesthetic action appears amenable to pressure reversal, and since the interaction of anesthesia and pressure on Q has not been described, the following study was performed.

METHODS. Q was determined in air controls (n=9) and mitochondria equilibrated with 0.9% halothane in air (n=9) as previously described.[9] In each study Q was evaluated at ambient pressure (Q_A), after compression to 51 ATA (Q_{P_1}), and following decompression (Q_{P_2}). Compression was accomplished by hydrostatic pressurization of a water-filled steel chamber[3] placed in a water bath at 5°C. Immediately prior to compression, 0.5 ml of mitochondrial suspension in a 1 ml syringe was mixed anaerobically with 4.5 ml of reaction medium in a 5 ml glass syringe. Care was taken to avoid bubble formation. Neither substrate (glutamate), inorganic phosphate, O_2 nor ADP was rate-limiting. An aliquot was transferred anaerobically to a 2 ml glass syringe which was capped and placed in the chamber (sample P). The syringe with the remaining sample (A) was capped and put in the water bath. The chamber was closed but not pressurized. A sample of A was introduced into a thermostatically jacketed (perfused from the water bath) oxygen electrode (Radiometer E5046). The chamber was then pressurized within 10 sec, and the P_{O_2} of A recorded every 30-60 sec. Additional samples were added every 2-3 min to ensure that A remained thoroughly mixed. Measurements were made throughout the 7 min period of compression which was the same in control and anesthetized samples. Decompression was accomplished within 10 sec, the syringe removed and timed measurements of the P_{O_2} of sample P made for 5 min. The rate of change of P_{O_2}, determined by regression analysis ($r=0.97-1.00$), was used to calculate Q_A, Q_{P_2}, and the P_{O_2} of sample P at the beginning (extrapolating data from A) and the end (using data from P) of compression. The latter two values determined Q_{P_1}.

RESULTS. Compression and decompression had no significant effects on control and halothane Q. The inhibition of Q_A produced by halothane was similar to that reported previously[9] and persisted during and after compression.

	CONTROL	HALOTHANE	CHANGE	P*
Q_A	1.53±0.08**	1.04±0.10	-0.49±0.13	<.005
Q_{P_1}	1.26±0.11	0.93±0.08	-0.33±0.14	<.05
Q_{P_2}	1.48±0.12	0.86±0.09	-0.62±0.15	<.001
$Q_{P_1}-Q_A$	-0.27±0.14	-0.11±0.07		
$Q_{P_2}-Q_A$	-0.04±0.08	-0.18±0.10		
$Q_{P_2}-Q_{P_1}$	0.22±0.18	-0.07±0.11		
F***	1.82	1.02		
P***	>.1	>.25		

* Unpaired t-test comparing halothane and control
** Mean ± SEM; Q in nmol/mg protein/minute
*** Analysis of variance comparing Q_A, Q_{P_1} and Q_{P_2}

DISCUSSION. Prior to compression, A and P had been treated identically. Hysteresis of P_{O_2} was not observed when non-metabolizing A and P were compared. The electrode was stable (varying less than 2 torr during a 30 min period) and did not appear sensitive to halothane.[10] Assuming that the effects of compression and decompression are immediate, changes in the rate of decrease of P_{O_2} should reflect alterations of Q produced by halothane and/or pressure. These data suggest that inhibition of mitochondrial respiration may be added to those anesthetic actions not antagonized by pressure. Although 51 ATA was without effect on Q, more pronounced compression is not benign. Disruption of mitochondrial membranes with release of endogenous enzymes is produced by hydraulic compression to 483-966 ATA.[11] A significant and reversible decrease in Q is observed at 150 ATA (unpublished data). The apparent absence of pressure antagonism might possibly represent an occult depression of Q by 51 ATA in the presence of halothane interacting with a small degree of pressure reversal.

CONCLUSION. Under the experimental conditions of this study, pressure reversal of halothane's inhibition of Q was not observed.

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