Title: PRESSURE IS WITHOUT EFFECT ON HALOTHANE-INDUCED INHIBITION OF OXYGEN UPTAKE IN MONKEY KIDNEY CELLS

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INTRODUCTION. The interaction of high hydrostatic pressure and volatile anesthetics remains controversial. While the ability of high pressure to reverse anesthetic-induced unconsciousness is an exciting and remarkable phenomenon, analogous effects of pressure have not been demonstrated to occur universally with all phases of anesthetic action (1). In order to further delineate the role of pressure as a potential tool for the evaluation of anesthetic-induced cellular changes, this report details the effect of a pressure of 51 atmospheres (ATA) on the oxygen uptake (Q) of intact monkey kidney cells (Vero) exposed to anesthetizing concentrations of halothane.

METHODS. Monolayers of Vero cells were prepared by standard methods (2). Following rinsing, trypsinization, and centrifugation, cells were resuspended in Earle's Balanced Salt Solution (approximate cell count 12,000/cumm). Q was measured (1) at ambient pressure (Q_A), after compression to 51 ATA (QP_1), and following rapid decompression (QP_2). Unanesthetized controls exposed to 100% oxygen alone (C) and anesthetized samples exposed to 2% halothane in O_2 for 15 min (H) were evaluated in separate studies, while the effect of pressure on either C or H samples was studied using the same aliquot. On any given day, C cells were studied initially in half the experiments and second in the remaining studies; on the other days this order was reversed so as to obviate the effect of time. Q_A, QP_1, and QP_2 were measured and compared in four separate studies of C and four of H. Care was taken to ensure that ambient and pressurized samples were treated equivalently so that they would represent a uniform aliquot. Both samples were kept in individually ground glass syringes. Pressurization was accomplished in a water-filled Cell Disruption Bomb (Parr Instruments, Moline, Illinois) within 10 sec. Both the syringe containing the ambient sample and the pressurization apparatus were maintained at 25 C using a thermostatically controlled water bath. The PO_2 of each individual sample was measured in an ABL-2 electrode system (Radiometer, Copenhagen). Ambient PO_2 was determined at t=0, 10, 20, 30, 40, 50, 60 and 70 min. Pressurization was begun within the first minute of initiation of the study and completed within 10 sec. At t=32 min, decompression was carried out, again within 10 sec, and PO_2 measured at 35, 45, 55, 65, and 75 min. Q_A and QP_2 were obtained directly using regression analysis. QP_1 was calculated using regression analysis of QP_2 and extrapolation of these data to the 32 min point of decompression combined with extrapolation of the Q_A data to t=0 (1). Statistical analysis was facilitated using the Student t-test to compare both paired and unpaired data. Significance was felt to be achieved if p was less than 0.05.

RESULTS. This study demonstrated a significant depression of Q (parameter #1) produced by halothane. An effect of pressure alone (parameter #2) was present in 3 of the 4 samples evaluated, but was without statistical significance. Following decompression, the ratio of pressurized to decompressed Q (parameter #6) did not differ from unity. Q in Vero cells exposed to both halothane and pressure was significantly less than that that of ambient controls (parameter #4). The product of parameters 1 and 2 represents an estimation of the predicted effect of pressure and halothane were they to act independently (and without antagonism of anesthetic action). Pressure reversal might have been demonstrated had the value actually observed during exposure to halothane and pressure (parameter #4) been greater than that predicted (parameter #5). This was not the case; indeed the observed value was less than that predicted since the mean of 4 minus 5 is -0.08 $\pm$ 0.07 (SEM), a value not statistically different from 1.00.

TABLE

Parameter	Derivation	Mean	SEM
1*	$Q_A(H)/Q_A(C)$	0.63	0.09
2	$QP_1(C)/Q_A(C)$	0.77	0.13
3*	$QP_1(H)/QP_1(C)$	0.51	0.12
4*	$QP_1(H)/Q_A(C)$	0.39	0.09
5*	1 x 2	0.47	0.03
6	$QP_2(C)/Q_A(C)$	0.71	0.09

*Significantly different from 1.00.

DISCUSSION. Demonstration of pressure reversal has been inconsistent; some opposing data have been recently summarized (1). While Bedows and co-workers (3) have shown that pressure is capable of antagonizing halothane's effect on viral replication, the same group has been unable to reverse the stimulation of anaerobic metabolism produced during exposure of Vero cells to halothane, isoflurane, or enflurane (4). The latter finding is consistent with the demonstration of pressure's inability to reverse halothane's effect on aerobic metabolism of rat liver mitochondria (1). The possibility that halothane and pressure have multiple actions (effects on cellular growth vs those on metabolism) was among the factors leading to the current study. The lack of significant difference between parameters #4 and 5 raises the question of whether this investigation has demonstrated two independent actions of pressure and halothane on oxidative metabolism.

CONCLUSION. While differing concentrations of halothane and degrees of pressure remain to be examined, under the experimental conditions detailed pressure reversal was not demonstrated in this investigation.

REFERENCES

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