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HYPERBARIC PRESSURE OF 51 ATMOSPHERES IS WITHOUT EFFECT ON THE DEPRESSION OF OXYGEN UPTAKE IN KIDNEY TISSUE CULTURE PRODUCED BY HALOTHANE

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Summary

Although anesthetized animals are awakened when subjected to increased pressure, compression does not result in antagonism of all phenomena associated with these drugs. It has recently been demonstrated that halothane's inhibition of respiration of isolated rat liver mitochondria is not reversed by hydraulic compression to 51 atmospheres. In order to determine whether this phenomenon can be extrapolated to the whole cell, we have investigated the effect of hydraulic compression of intact renal cells equilibrated with halothane, and conclude that pressure does not overcome the inhibitory effect of this anesthetic.

The ability of high hydrostatic pressure to reverse clinical anesthesia is well known. Among examples of this phenomenon are the awakening of tadpoles anesthetized with 2.5 percent ethanol upon application of 200-300 atmospheres (ATA) of hydraulic pressure (1) and the reversal, also in tadpoles, of the effects of 0.5-1.5 percent halothane (2-bromo-2-chloro-1,1,1-trifluoroethane) by 35-171 ATA (2). Nitrous oxide (3) and isoflurane (4) have decreased potency in mice subjected to pressures of 25-100 ATA. It is not surprising, perhaps, that effects of general anesthetics other than narcosis are also antagonized by pressure. An early demonstration of this phenomenon was the observation that pressures of 2000-4000 ATA could restore the light output of luminous bacteria which had been treated with a variety of depressant drugs (5). Halothane's ability to inhibit axonal transmission is antagonized by 35-103 ATA (6). Recently, halothane's ability to inhibit measles virus replication was shown to be partially reversed by a pressure of 100 ATA (7). It is, however, not always possible to demonstrate the ability of pressure to antagonize *in vivo* and *in vitro* effects of anesthetics. Thus, halothane inhibits synaptic transmission in the rat superior cervical ganglion, an effect that is actually *enhanced* by application of pressure (6). Similarly, the decrease in frequency of ciliary beating of *Tetrahymena pyriformis* produced by halothane is not restored to normal by application of pressure (8).

General anesthetics have well established effects on aerobic metabolism. Volatile anesthetics decrease state 3 respiration of rat liver mitochondria in a concentration-dependent fashion. Their *in vitro* potencies are related to both *in vivo* potency (MAC) and lipid solubility (9). Anesthetic potencies are

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additive in a similar fashion *in vivo* and *in vitro* (10). For these reasons, the mitochondrion has been held to be a model (11) for the anesthetic receptor site. However, one of us (PJC) has recently demonstrated that halothane's inhibition of mitochondrial respiration cannot be antagonized by 51 ATA of hydraulic pressure (12). Because the interaction of pressure and anesthesia may be different in the intact cell, we have examined the action of high pressure on the respiratory rate of monkey kidney cells exposed to halothane.

Methods

The interaction of halothane and pressure on oxygen uptake was examined in spontaneously transformed green monkey kidney cell cultures (Vero cells). These were grown as monolayers using standard techniques (13). The Vero cells were resuspended in Earle's Balanced Salt Solution following rinsing, trypsinization, and centrifugation (approximate cell count 12,000 mm^{-3}) Hydraulic compression to 51 ATA and measurement of oxygen uptake in control and halothane-equilibrated suspensions under both ambient and hyperbaric conditions were performed as previously described (12) In this investigation, oxygen uptake was determined by timed polarographic measurements of oxygen tension carried out during a 70 minute period. In each study, two identical suspensions of Vero cells were examined. The cells were equilibrated with 2.0% halothane in oxygen or oxygen alone for 15 minutes, with the order of treatment randomized to obviate the effect of time. One suspension was used for evaluation of the respiratory rate under ambient conditions. In the other suspension, the timed measurement of oxygen tension following a 32 minute period of compression to 51 ATA allowed calculation of the rate of oxygen uptake during the period of compression. Thus, oxygen uptake under ambient conditions was measured directly while, as in previous studies (12), oxygen utilization at 51 ATA was inferred using data obtained under ambient conditions prior to compression and immediately following decompression. Four separate cell suspensions were studied. Aliquots of each were observed under control and anesthetized conditions before and after compression. Statistical analysis of the effect of pressure on oxygen uptake of control and anesthetized Vero cells was accomplished using a two-way analysis of variance. The effect of halothane on Vero cell respiration was evaluated with an unpaired t-test.

Results

TABLE I
EFFECT OF 2.0% HALOTHANE AND 51 ATA PRESSURE ON VERO CELL RESPIRATION
TEMPERATURE = 25°C

TREATMENT	OXYGEN UPTAKE PERCENT OF CONTROL MEAN ± SEM
Halothane	63 ± 9*
Pressure	77 ± 13
Halothane + Pressure (measured)	39 ± 9**
Halothane + Pressure (predicted)	47 ± 3***
Measured - Predicted	-8 ± 6***

*Significantly different from control
**Significantly different from control but not from halothane alone
***No significant difference between measured and predicted depression

Because the respiratory rate of a Vero cell suspension depends on the precise growth phase at the time measurements are made, data are presented as percentage of control. Exposure of Vero cells to 2.0% halothane (Table I) resulted in significant inhibition of oxygen uptake. Compression to 51 ATA produced a lesser degree of inhibition which was not statistically significant. However, it is possible that exposure of Vero cells to higher degrees of compression may produce significant inhibition of oxygen uptake. Oxygen uptake of mitochondria compressed to 150 ATA was significantly and reversibly decreased by 65 ± 8% when glutamate was substrate and 70 ± 9% when succinate was substrate (unpublished observations). Preliminary studies of Vero cells demonstrated respiratory rate to be approximately 30% of control during compression to 136 ATA. Thus, pressure may not be entirely devoid of depressant effects. The depression of Vero cell respiration produced by halothane was not antagonized by 51 ATA Indeed, the combination of pressure and halothane affected respiration to a greater extent than either pressure or halothane alone.

Discussion

A number of methodological questions deserve discussion at this point. The oxygen tension of both control and anesthetized Vero cell suspensions (before and after compression) decreased linearly with time in a manner similar to that observed when mitochondrial oxygen uptake is evaluated (12). Although it is possible that oxygen content does not always vary linearly with tension, the known zero-order kinetcs of oxygen utilization and the constant decrease in oxygen tension observed in this study make it reasonable to assume that oxygen uptake is proportional to the rate of change in oxygen tension. Since respiratory rate could not be measured directly during pressurization, an indirect means for its calculation was employed. The initial measurement of oxygen tension of the previously pressurized suspension was made 2-3 minutes (an average of 2.5 minutes) following decompression. Although the rate of oxygen utilization immediately following decompression might differ from that occurring 2-3 minutes later, practicalities of the study dictated that a finite but minimum time elapse between decompression and measurement. It is significant that all measurements performed after decompression showed a linear decrease of oxygen tension with time. Finally, since compression was of the liquid rather than the gas phase, it was not accompanied by changes in the partial pressure of either oxygen or halothane.

This investigation suggests that 51 ATA does not antagonize the inhibitory effects of halothane on respiration of Vero cells. Since pressure alone may inhibit cellular respiration, one might ask whether this could have hidden a lesser degree of pressure reversal. Thus, whether our findings demonstrate failure of pressure to reverse halothane-induced depression, or whether they relate to an interaction of pressure and halothane warrants further consideration. The product of the effects of halothane and pressure (e.g. 0.63 X 0.77) may be used to approximate what would be expected if anesthesia and pressure were simply additive. Individual data were evaluated for each study and yielded a mean of 47 ± 3%. This did not differ significantly from the measured value of 39 ± 9% of control. Were pressure antagonism partially masked by an independent depressant action of compression, the measured inhibition of respiration would have been less than the predicted value. That there was no significant difference between the two values suggests that the absence of pressure reversal is real, and that an occult antagonism by pressure was not hidden by its independent depressant action. A similar comparison of measured and predicted data obtained in a previous study testing the ability of pressure to antagonize halothane's inhibition of mitochondrial respiration (12) resulted in identical findings: measured oxygen uptake during exposure to halothane and pressure = 63 ± 6% of control; predicted inhibition = 56 ± 5% of control; difference = 7 ± 7%. Our data relating to aerobic metabolism are consistent

with the finding that the increased rate of lactate produced by Vero cells equilibrated with halothane is not significantly affected by pressures of 25, 50 and 100 ATA (14). The possibility of pressure-induced mechanical disruption of Vero cells should be considered Against this is the observation that Vero cell morphology is not grossly altered by compression to 100 ATA (7). Furthermore, vital staining with Trypan blue fails to show any decrease in the percentage of viable cells produced by compression (Knight PR, personal communication) However, a definite answer must await specific studies

In conclusion, clinically used concentrations of halothane result in inhibition of aerobic metabolism of both mitochondria and intact Vero cells Pressure reversal of these effects cannot be demonstrated. These findings are analogous to those of Brabec et al (14) who were unable to demonstrate reversal by 25-100 ATA of the increased lactate formation observed in Vero cells exposed to halothane. While it is always possible that yet further combination of anesthetic concentration and pressure might result in different findings, this is unlikely since the ability of various pressures to awaken animals anesthetized with several concentrations of anesthetics indicates that specific pressures and concentrations are not absolutely critical (2,7). It is therefore possible that the molecular events accompanying clinical anesthesia are not necessarily the same as those responsible for producing depression of aerobic and enhancement of glycolytic metabolism by these drugs. This conclusion is consistent with the multi-site expansion hypothesis (15).

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