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Effect of General Anesthetics and Pressure on Aerobic Metabolism of Monkey Kidney Cells

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The authors examined the inhibition of aerobic metabolism in monkey kidney cell cultures exposed to halothane, enflurane, and isoflurane. The ability of hyperbaric pressure to reverse the halothane-induced metabolic inhibition also was examined. Incubation of two monkey kidney cell lines for 24 h with clinically equipotent concentrations (2.6 MAC) of halothane, enflurane, or isoflurane vapors increased the concentration of lactate in the media by 126 to 244% relative to nonexposed control cultures. The increased rate of lactate accumulation was proportional to the concentration of halothane and was accompanied by a decrease in media *p*H. Removal of halothane restored the normal rate of lactate production. Hyperbaric pressures of 25, 50, and 100 atmospheres did not alter the halothane-stimulated rate of lactate production relative to non-anesthetic-treated controls, although pressure alone did depress the rate of lactate accumulation in all cultures. The stimulation of lactate production likely reflects the known ability of halothane to inhibit mitochondrial respiration. The failure of pressure to reverse the stimulation of lactate production by halothane suggests that inhibition of mitochondrial metabolism cannot be reversed by pressure.

(Key words: Anesthetics, volatile: enflurane; halothane; isoflurane. Cells: aerobic metabolism; lactate production. Theories of anesthesia: pressure reversal.)

PRESSURE REVERSAL of anesthesia lends experimental support for theories that involve anesthetic-induced expansion of membrane dimensions.[1] Hyperbaric pressures up to 100 atmospheres (ATA) increase the partial pressures of nitrous oxide and isoflurane required to produce anesthesia[2] and decrease the barbiturate sleeping time in mice.[3] Anesthetics also inhibit the development of high-pressure nervous syndrome in humans, a condition of tremors, and seizures associated with hyperbaric pressures.[4] Recently it was discovered that hyperbaric pressures of 100 ATA partially will reverse the inhibition of measles virus replication in cell cultures exposed to 2.0% halothane.[5]

Other effects of anesthetic exposure apparently are not reversed by pressure. Pressure does not antagonize the depression of the muscle compound action potential induced by methoxyflurane in isolated rat phrenic nerve-diaphragm[6] and does not restore respiration in isolated rat liver mitochondria inhibited by 0.9% halothane.[7] This suggests that pressure reversal may be a phenomenon exhibited only within certain domains of an intact cell or organism.

We chose to examine the combined effects of pressure and anesthetics on the metabolism of intact cells under the simplified conditions of cell culture. Respiration is inhibited in mouse cell cultures exposed to anesthetics,[8,9] while glycolysis, as measured by lactate production,[10] is stimulated. In this study, the kinetics of lactate accumulation and the ability of pressure to reverse the halothane-stimulated production of lactate by monkey kidney cells were examined.

Methods

Two cell cultures derived from green monkey kidney cells were used in this study. Vero cells, a continuous cell line, and GMK cells, a primary culture, were grown to confluency in 240 ml glass prescription bottles or 60 mm glass petri plates. Cells were grown in minimal media containing 1 g/l glucose. All cultures were incubated in one atmosphere of 95% air/5% CO_2 with or without an anesthetic vapor. Halothane was administered by directing the air mixture through a Drager vaporizer at 5 l·min^{-1} to deliver a nominal concentration of 0.5, 1, or 2%. Enflurane and isoflurane, at concentrations equivalent to 2.6 MAC (4.3% and 3.3%, respectively), were administered similarly by use of the appropriate Ohio Medical vaporizer. The concentration of the anesthetic was determined by gas chromatography.[11] Vaporizer output was passed into either a Parr disruption bomb (for the pressure studies) or via a 20-gauge needle through a septum into a 240 ml prescription bottle. Previous experiments indicated that equilibrium was obtained within 20 min.[11] The final concentration of anesthetic in the containers at the end of 24 h was within 5% of the delivered concentration. Lactate concentration in the media, or in the cell pellet, was determined with lactate dehydrogenase by the spectrophotometric method of Hohorst.[12] Each sample was assayed in duplicate or triplicate. The *p*H of

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TABLE 1. Effect of Halothane, Enflurane, and Isoflurane on Lactate Production by Vero and GMK Cell Cultures

	Anesthetic Concentration (%)		Increase in Lactate Concentration g/l	% Control
Vero cells	No anesthetic	(N = 6)	0.66 ± 0.09	100
	2.0% halothane	(N = 2)	0.85 ± 0.01	129
	4.3% enflurane	(N = 2)	0.85 ± 0.04*	129
	3.3% isoflurane	(N = 2)	0.92 ± 0.18	139
GMK cells	No anesthetic	(N = 6)	0.41 ± 0.06	100
	2.0% halothane	(N = 2)	1.00 ± 0.06	244
	4.3% enflurane	(N = 2)	0.84 ± 0.04	205
	3.3% isoflurane	(N = 2)	0.95 ± 0.07	232

Mean values (with standard deviations) and the per cent control of the lactate concentrations after 24 h are listed.

* $0.1 < P < .05$ *versus* no anesthetic; $P < .05$ for other comparisons.

the media was measured with a Corning expanded scale *p*H meter and a combination *p*H electrode.

The rate of lactate increase in Vero cells was examined in the presence and absence of 2% halothane. Sixteen plates of cells (5×10^6 cells per plate) were incubated at 37° C. Two plates were withdrawn at 1, 6, 12, and 24 h of incubation and the *p*H and lactate concentration of the media promptly determined as described above.

Lactate accumulation as a function of halothane concentration was examined following exposure of Vero cells to 0%, 0.5%, 1.0%, 1.5%, and 2.0% halothane for 24 h. The *p*H and lactate concentrations were determined on duplicate plates as described.

The reversibility of halothane-stimulated lactate production was examined by incubating plates of Vero cells in the presence or absence of 2% halothane. After 24 h, halothane was removed. The media of four of the control plates and four of the exposed plates was replaced with fresh. The incubation then was resumed with a 95% air/5% CO_2 atmosphere. Two plates of cells from each treatment group were assayed for media *p*H and lactate concentrations at 0, 24, 36, and 48 h of total incubation time.

The pressure studies were carried out in Vero cells grown in glass petri plates. Two plates were placed in each of four Parr disruption bombs and gassed with 95% air/5% CO_2 in the presence or absence of the desired concentration of vaporized halothane (0, 0.5, 1.0, 2.0%). After 20 min of gassing, the bombs were sealed. One bomb with and one bomb without halothane then were pressurized over 10 min to 25, 50, or 100 ATA with helium, maintaining the partial pressure of air and CO_2 constant. One bomb with halothane and one bomb without halothane were maintained at 1 ATA. The bombs were incubated at 37° C for 24 h, then allowed to decompress for 10 min prior to opening. Lactate concentrations and *p*H of the media were determined as described above.[12]

Within any experiment, data points each represent the average value of duplicate or triplicate determinations on duplicate samples. The number of experiments is listed in the figure legends. Graphic data were analyzed by least-squares linear regression analysis and Student's *t* comparison of slope values. Significance of the data in table 1 was assessed with a computer program utilizing one-way analysis of variance and Scheffe's comparison of means. Data of table 2 were analyzed by two-way analysis of variance via the same program, across the factors of pressure and halothane concentration.

Results

A stimulation of lactate production was observed in Vero and GMK cell cultures during a 24-h exposure to

TABLE 2. Effect of Pressure on Halothane-induced Stimulation of Lactate Production

Halothane concentration (%)	Lactate Concentration in Media (g/L) (Pressure in Atmospheres [ATA]) 1 ATA	25 ATA	50 ATA	100 ATA
0.0	0.64 ± 0.04 (N = 4)	0.54 ± 0.04 (N = 4)	0.46 ± 0.07 (N = 4)	0.46 ± 0.03 (N = 4)
0.5	0.89 ± 0.01 (N = 4)	0.78 ± 0.03 (N = 2)	0.70 ± 0.02 (N = 2)	0.72 ± 0.02 (N = 2)
1.0	0.92 ± 0.02 (N = 4)	0.78 ± 0.03 (N = 2)	0.74 ± 0.04 (N = 2)	0.77 ± 0.04 (N = 3)
2.0	0.95 ± 0.03 (N = 4)	0.92 ± 0.02 (N = 3)	0.90 ± 0.07 (N = 3)	0.77 ± 0.00 (N = 4)

Mean values of lactate concentrations (with standard deviations) were compared by two-way analysis of variance with pressure and halothane as factors.

$P < 0.001$ for the effect of pressure on lactate production (a row of values at any concentration of halothane). $P < 0.01$ for the effect of halothane on lactate production (a column of values at any pressure for increasing concentrations of halothane).

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halothane, isoflurane, or enflurane. Table 1 demonstrates that stimulation was identical for all three anesthetics at clinically equipotent concentrations (2.6 times the human minimum alveolar concentration). In no case was a change in the intracellular concentration of lactate in the cell pellet observed.

The kinetics of lactate accumulation was examined in Vero cells exposed to 2.0% halothane (fig. 1). The accumulation of lactate was linear for 24 h in control and exposed cultures (r = 0.94 and r = 0.98, respectively). However, the rate of lactate accumulation in the media exposed to 2.0% halothane was about twice the control rate. The final *p*H of the media from the nonexposed cultures also was significantly greater (7.45 ± 0.03) than the *p*H of the halothane-exposed media (7.06 ± 0.03, $P < .01$).

Figure 2 demonstrates that the stimulation of lactate production after 24 h of halothane exposure was proportional to the concentration of halothane (0.5–2.0%, r = 0.90). The *p*H of the media decreased throughout the course of the incubation.

The reversibility of the effect of halothane was examined by removing the anesthetic after 24 h of exposure and monitoring the rate of lactate accumulation and *p*H in fresh media. The rates of accumulation of lactate in fresh media in the absence of halothane were essentially the same whether or not the cells were exposed previously to the anesthetic (fig. 3, open circles). Twenty-four hours after media change, the lactate concentrations of the non-

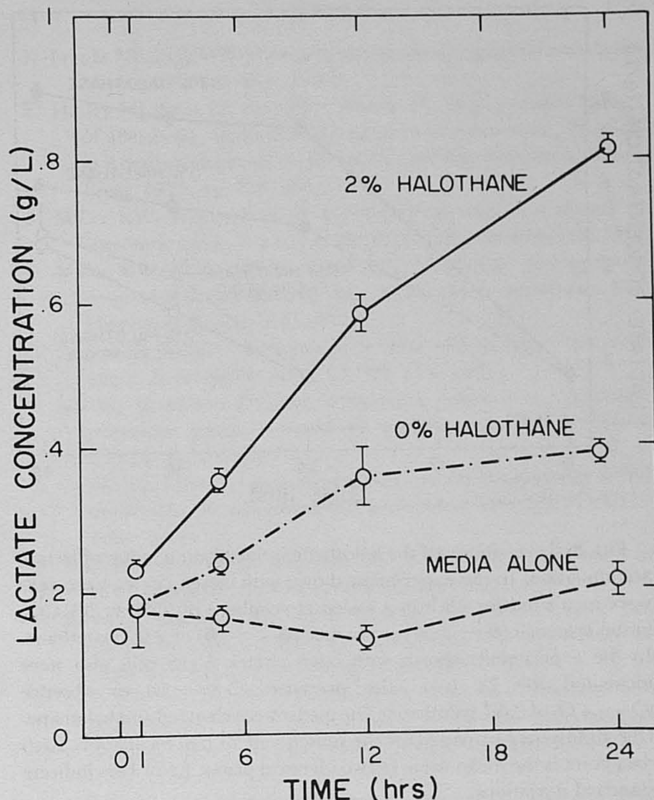

FIG. 1. Increase in lactate concentration of Vero cell media during incubation with 2.0% halothane. Data points are the means of two experiments, four plates of cells per point. Error bars indicate standard deviations.

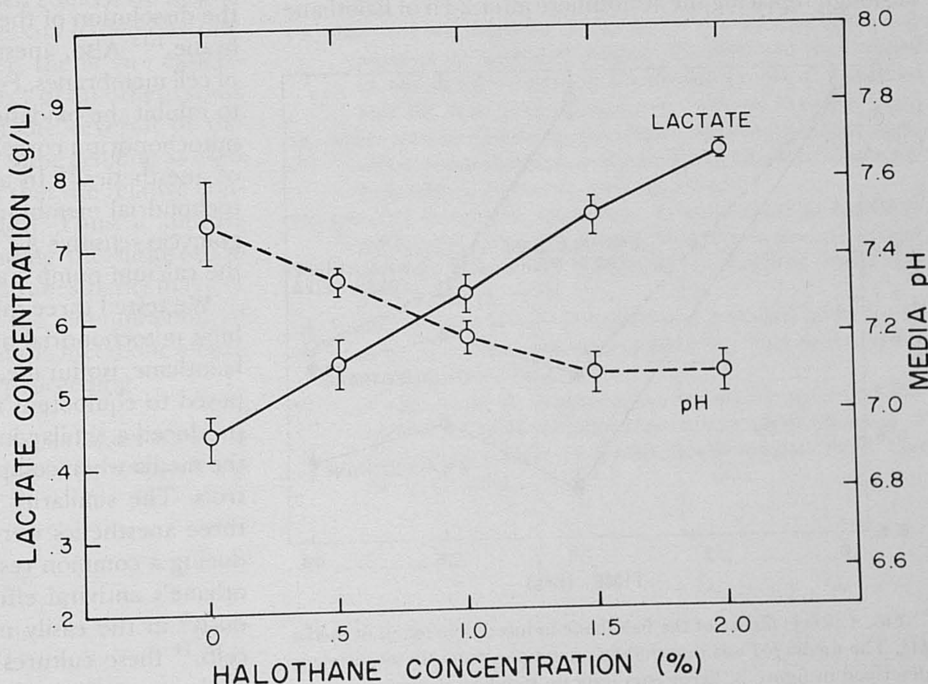

FIG. 2. Lactate accumulations and *p*H change in Vero cell media as a function of halothane concentration. Lactate concentrations and *p*H were determined in the culture media after 24 h of exposure to the indicated concentrations of halothane. Each data point is the mean value from an experiment with determinations from two plates of cells. Error bars indicate standard deviations.

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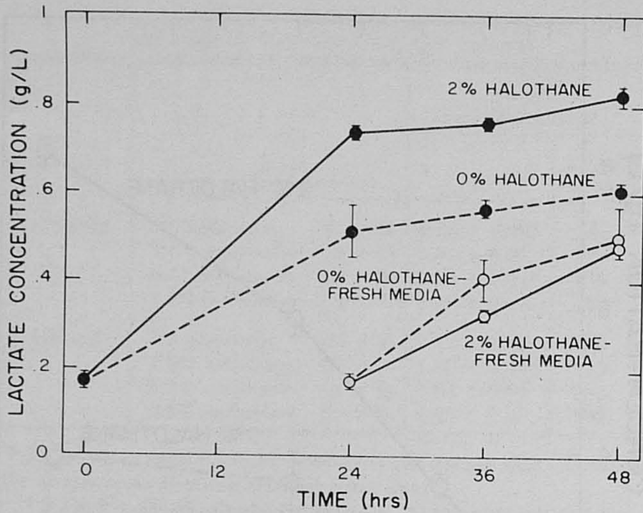

FIG. 3. Reversibility of the halothane-induced stimulation of lactate accumulation. In the experiment shown with closed circles Vero cells were incubated for 48 h in a sealed atmosphere of 95% air:5% CO_2 in the presence (● —— ●) or absence (● – – – ●) of 2.0% halothane. In the experiments shown with open circles, Vero cells also were incubated for 24 h in the presence (○ —— ○) or absence (○ – – – ○) of 2.0% halothane. The media were changed and halothane-free atmosphere provided for the subsequent 24 h of incubation. Each data point is the mean value of two identical plates. Error bars indicate standard deviations.

exposed and halothane-exposed cells were the same as in the nonexposed cells during the first 24 h of incubation ($P < 0.01$). Changes in the pH of the media (fig. 4) reflected the accumulation of an organic acid in the media, although replacing the atmosphere after 24 h of halothane exposure gave a transient increase in the media pH at 36 h.

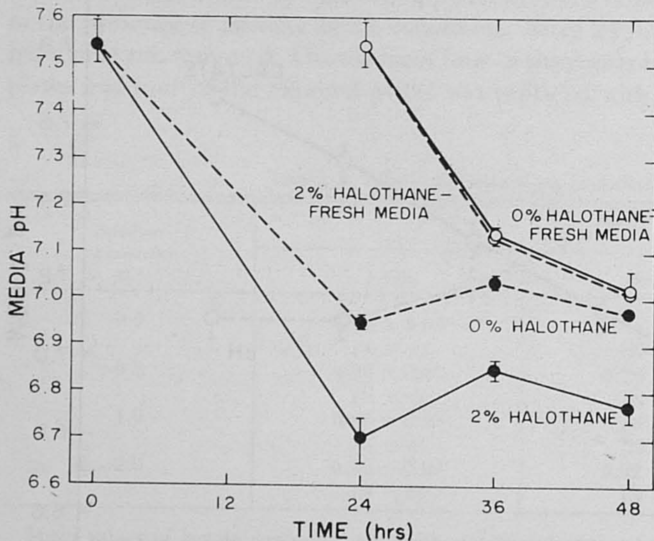

FIG. 4. Reversibility of the halothane-induced depression of media pH. The media pH was determined on samples from the experiment described in figure 3. Error bars indicate standard deviations.

Table 2 demonstrates that hyperbaric pressure failed to reverse the halothane-induced stimulation in lactate production. Halothane caused an increase in lactate levels at all concentrations and at all pressures tested ($P < 0.001$). Pressures of 25 to 100 ATA diminished the rate of lactate formation in the non-halothane-exposed cells ($P < 0.01$). However, the halothane-increased rate of lactate formation also was reduced proportionally. For example, the net increase in the media lactate concentration in the presence of 2% halothane was the same (0.31 g/l) whether the cultures were pressurized at 1 or 100 ATA. The change in media pH was inversely proportional to changes in lactate concentrations. Therefore, in the absence of halothane, the pH of the media was slightly more alkaline after pressurization (*e.g.,* 7.20 at 1 ATA *vs.* 7.52 at 100 ATA) than in the presence of halothane, due to the increased production of lactic acid. The absolute increase in lactate at all halothane concentrations tested was roughly equivalent to that produced by the exposed culture at 1 ATA. The F-value (2.37) indicated that no significant interaction between pressure and halothane occurred.

Discussion

A well-established relationship exists between the lipid solubility and potency of inhalational anesthetics. Several theories are based on a mechanism of narcosis that involves an alteration of neuronal membrane properties by the dissolution of the anesthetic in the lipid of the membrane.[1,13] Also, anesthetics may disturb other functions of cell membranes. For example, the ability of anesthetics to inhibit the oxidation of NAD-linked substrates in the mitochondrion correlates well with the narcotic potency of anesthetics.[14] In addition, the activities of other mitochondrial membrane-bound enzymes, such as the oligomycin-sensitive ATPase,[15] cytochrome c oxidase,[16] and the calcium pump[17] are affected.

We tested three inhalation anesthetics reported to inhibit mitochondrial oxidation of NAD-linked substrates: halothane, isoflurane, and enflurane.[14] Two cell lines exposed to equipotent anesthetic concentrations for 24 h produced a similar increase in lactate concentrations in the media when compared with that of nonexposed controls. The similarity of the response suggests that the three anesthetics were acting at a similar site and producing a common result. Since pressure reversal of halothane's antiviral effect had been demonstrated previously[5] in the easily maintained and manipulated Vero cells,[11] these cultures were selected for more intensive study.

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The data in figure 1 demonstrate that the stimulation of lactate production was a linear function of exposure over the dose tested (0.5–2.0%). The rate of production of lactate was constant for 24 h (fig. 2), after which the production dramatically decreased (fig. 3). The abrupt cessation of lactate production may reflect the decrease in *p*H of the media to a limiting value of 6.6–6.7. Restoration of normal *p*H by replacement of the media (fig. 4) restored normal rates of lactate production. Similarly, Lust *et al.*[10] reported that the uptake of glucose decreased and the production of lactate increased in glioma cells as the *p*H of the media was increased. The effects of *p*H, the CO_2 concentration, and bicarbonate concentration on the metabolism and growth of the Vero cells is presently under investigation.

Cohen[7] found that pressure was unable to reverse the inhibition of State 3 respiration of NAD-linked substrates produced by exposure of isolated rat liver mitochondria to halothane. The halothane-induced stimulation of lactate production, presumably as a consequence of inhibition of aerobic metabolism, offered the opportunity of testing whether pressure could antagonize this aspect of anesthetic action. Because the reversal may represent the delicate counterpoising of compression of subcellular molecular structures by hyperbaric pressure against either the increased fluidity or expansion produced by the anesthetic molecules,[1] a range of pressures and anesthetic concentrations was examined. Reversal was not apparent at any combination of halothane concentration and pressure (table 2). It still may be argued that the pressures and inhibitory concentrations we used could have missed the correct, critical proportions necessary to demonstrate the reversal phenomenon. However, the *in vivo* experiments in which the narcotizing effect of anesthetics was reversed in newts[4] and mice[3] and the reversal of the inhibition of viral replication in the same cell line as used in the present study[5] would suggest that the relative concentrations are not absolutely critical. Thus, it appears that the molecular events surrounding the suppression of aerobic metabolism by inhalational anesthetics may not share common mechanisms with the phenomenon of anesthesia. Further investigations of the metabolic effects of the inhalational anesthetics are proceeding.

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References

1. Franks NP, Lieb WR: Molecular mechanisms of general anesthesia. Nature 300:487–493, 1982
2. Halsey MJ, Eger EI, Kent DW, Warne PJ: High pressure studies of anesthesia, Molecular Mechanisms of Anesthesia, Progress in Anesthesiology, vol. 1. Edited by Fink BR. New York, Raven Press, 1975, pp 353–361
3. Miller KW, Wilson MW: The pressure reversal of a variety of anesthetic agents in mice. ANESTHESIOLOGY 48:104–110, 1978
4. Miller KW, Paton WDM, Smith RA, Smith EB: The pressure reversal of anesthesia and the critical volume hypothesis. Mol Pharmacol 9:131–143, 1973
5. Bedows E, Knight PR: Pressure reversal of halothane's antiviral effect. ANESTHESIOLOGY 59:109–112, 1983
6. Kendig JJ, Cohen EN: Neuromuscular function at hyperbaric pressures: pressure–anesthetic interactions. Am J Physiol 230:1244–1249, 1976
7. Cohen PJ: Effect of hydrostatic pressure on halothane-induced depression of mitochondrial respiration. Life Sci 32:1647–1650, 1983
8. Fink BR, Kenny GE: Metabolic effects of volatile anesthetics in cell culture. ANESTHESIOLOGY 29:505–516, 1968
9. Kowal J, Harano Y: Adrenal cells in tissue culture. The effect of papaverine and amytal on steroidogenesis, respiration and replication. Arch Biochem Biophys 163:466–475, 1974
10. Lust WD, Schwartz JP, Passonneau JV: Glycolytic metabolism in cultured cells of the nervous system. I. Glucose transport and metabolism in the C-6 glioma cell line. Mol Cell Biochem 8:169–176, 1975
11. Knight PR, Nahrwold ML, Bedows E: Anesthetic action and virus replication: Inhibition of measles virus replication in cells exposed to halothane. Antimicrob Agents Chemother 17:890–896, 1980
12. Hohorst HJ: L(+)-Lactate determination with lactate dehydrogenase and NAD. Methods of Enzymatic Analysis. Edited by Bergmeyer HU. Weinheim, Verlag Chemie, 1965, pp. 266–270
13. Nahrwold ML, Rapiejko JA, Cohen PJ: The mitochondrion as a model of the anesthetic receptor site, Molecular Mechanisms of Anesthesia, Progress in Anesthesiology, vol. 1. Edited by Fink BR. New York, Raven Press, 1975, pp 431–438
14. Nahrwold ML, Cohen PJ: Anesthetics and mitochondrial respiration, Molecular Aspects of Anesthesia. Edited by Cohen PJ. Philadelphia, FA Davis, 1975, pp 25–44
15. Lenaz G, Curatola G, Mazzanti L, Parenti-Castelli G, Bertoli E: Effects of general anesthetics on lipid protein interactions and ATPase activity in mitochondria. Biochem Pharmacol 27:2835–2844, 1978
16. Vanderkooi G, Chazotte B: Cytochrome c oxidase inhibition by anesthetics: Thermodynamic analysis. Proc Natl Acad Sci USA 79:3749–3753, 1982
17. Grist EM, Baum H: A possible mechanism for the halothane-induced inhibition of mitochondrial respiration: Binding of endogenous calcium to NADH dehydrogenase. FEBS Lett 48:41–44, 1974

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