MITOCHONDRIA UNCOUPLING EFFECT OF HALOTHANE DEPENDENT ON MAGNESIUM

Wiktor Rzeczycki and Enrique Valdivia

Bialystok Medical School, Department of Biochemistry, Bialystok, Poland and University of Wisconsin Medical School, Department of Anesthesiology, Madison 53706

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Summary. Halothane accelerates the respiration of beef heart mitochondria (HBHM) in metabolic state four in the presence of succinate as the substrate. This effect is dependent on the presence of magnesium ions in the medium and is also related to an increase of ATPase activity and a decrease of ATP available for the cytosol. The anesthetic also inhibits energy dependent processes, i.e. valinomycin induced mitochondrial swelling and potassium accumulation.

Introduction. Halothane, a widely used anesthetic agent, is responsible for the inhibition of respiration of mitochondria when NADH linked substrates are used (1,2,3). Respiration with succinate is not inhibited by this anesthetic (1,2). This paper reports that halothane uncouples oxidative phosphorylation in the presence of magnesium ions with succinate as a substrate. It is suggested that at anesthetic concentrations currently used in humans, development of mitochondrial uncoupling may be a cytotoxic phenomenon and may be responsible for sporadic cases of malignant hyperpyrexia (4).

Methods. Heavy beef heart mitochondria (HBHM) were prepared by the method of Hatefi and Lester (5) without EDTA. Oxygen consumption was measured polarographically (6). Potassium concentration was measured as described by Blondin and Green (7).

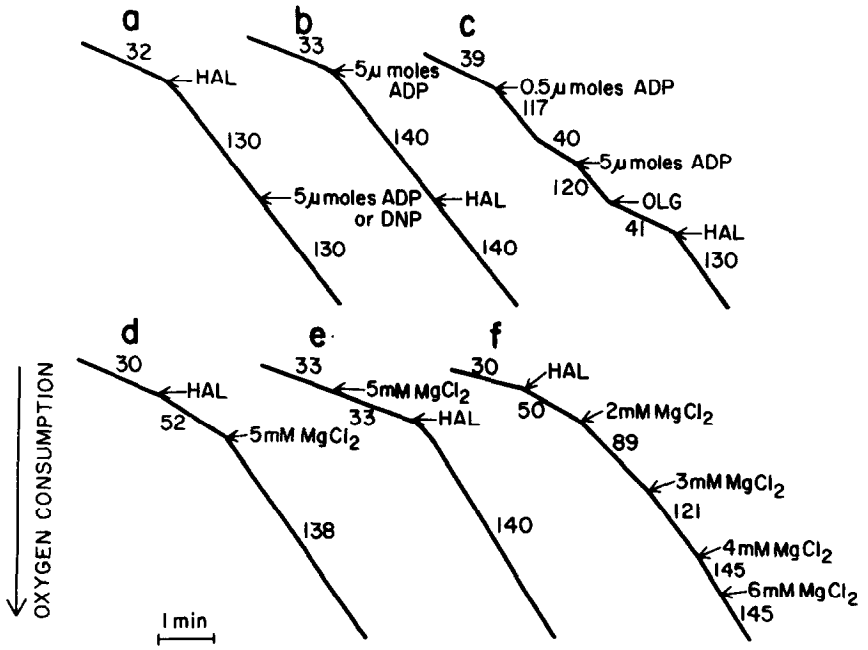

Figure 1. Rates of oxygen consumption by HBHM (3 mg protein) incubated in 5 ml medium containing 0.25 M sucrose, 10 mM tris HCl, pH 7.4, succinate (tris) 5 mM and 20 μg rotenone at 30°. Oxygen consumption was expressed in natoms min/mg protein. In experiments a, b, and c, 5 mM PO_4 (K) pH 7.4 and 5 mM $MgCl_2$ were added to the medium. When indicated the following additions were made, HAL - 7 mM halothane, DNP - 5.10^{-5} M dinitrophenol, OLG - 1 μg/mg protein oligomycin, $MgCl_2$ and ADP.

Measurements of mitochondrial swelling were performed by monitoring changes in absorbance at 520 nm (7). ATPase activity was measured under conditions described in table 2. ATP formation in mitochondria was estimated using the hexokinase (8) and hexokinase activity was determined as described by McDonald (9). Halothane dissolved in ethanol (Ayerst Lab. Inc., New York) was added to the incubation medium.

Results and discussion. The influence of halothane on the rate of oxygen consumption by HBHM was studied under several experimental conditions (fig. 1 and table 1). In the presence

Table 1

Effect of various concentrations of halothane on the respiration of mitochondria.

Concentration of halothane (mM)	Oxygen consumption (natoms min/mg protein) Without $MgCl_2$	With $MgCl_2$ (4 mM)
0.0	33	33
1.8	36	46
3.6	42	112
5.5	58	135
7.2	65	140
9.0	69	138
18.0	142	142

Oxygen consumption was determined in the same medium and conditions as in figure 1.

of magnesium ions and phosphate, halothane at 7 mM concentrations stimulated the respiration at the same degree as DNP or ADP (figs. 1a and b). This effect was not inhibited by oligomycin (fig. 1c) and was also observed in the medium without phosphate (figs. 1d, e and f). The most pronounced effect of halothane was observed at 4 mM concentration of magnesium ions (fig. 1f). Halothane added without magnesium ions gave a smaller acceleration of respiration (figs. 1d and f). Magnesium ion alone was without effect on respiration rate (table 1 and fig. 1e). Experiments shown in table 1 demonstrate the following: a) Maximal effect of halothane (in the presence of magnesium ions) was observed at 7.2 mM concentration, b) Increasing concentrations of halothane above 7.2 mM did not increase respiration, c) In the absence of magnesium ions, this effect was small and only at concentrations of 18 mM halothane was the same as in the

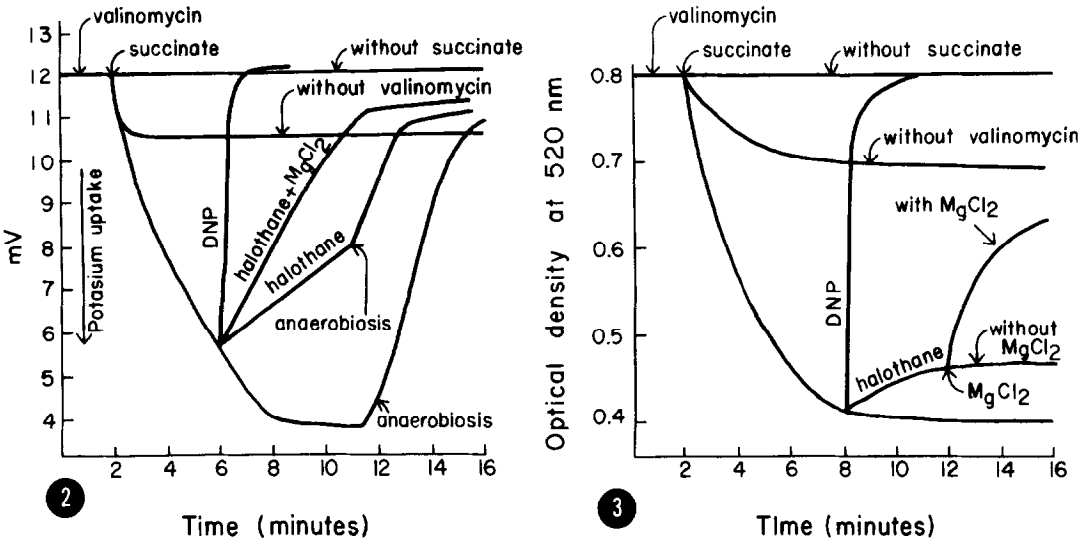

Figure 2. Rates of potassium uptake by mitochondria. HBHM (3 mg protein) were incubated at 30° in 5 ml of medium containing 0.25 M sucrose, 25 mM tris acetate pH 7.4, 2 mM potassium acetate and 20 µg of rotenone. When indicated the following additions were made: Valinomycin 0.5 µg, succinate 5 mM, DNP 5.10^{-5} M, halothane 7 mM and $MgCl_2$ 4 mM.

Figure 3. Effect of halothane on the valinomycin induced swelling of mitochondria. HBHM (1 mg protein) were incubated at room temperature in 3 ml of a medium of the same composition as in figure 2, potassium acetate was added at 5 mM concentration.

presence of magnesium ions. Halothane inhibited some energy dependent processes in mitochondria (figs. 2 and 3), i.e. accumulation of potassium ions induced by valinomycin (10) and valinomycin induced swelling of mitochondria in a medium containing potassium ions (7). In both cases the addition of magnesium ions markedly increased the effect of halothane. When magnesium ions were added without halothane these effects were not observed (experiments not shown in figures).

Results represented in table 2 showed that halothane stimulated ATPase activity which was abolished by oligomycin. In the

Table 2

Effect of halothane on ATPase activity

Incubation System	Inorganic phosphate (nmoles)
Mitochondria (mit)	100
Mit + $MgCl_2$	80
Mit + $MgCl_2$ + Olg	70
Mit + Hal	350
Mit + Hal + Olg	90
Mit + Hal + $MgCl_2$	680
Mit + Hal + $MgCl_2$ + Olg	320

HBHM (2.5 mg protein) were incubated in 1 ml medium containing 0.20 M sucrose, 10 mM tris HCl pH 7.4 and 6 mM ATP at 30° for 5 min. When added $MgCl_2$ was presented at a concentration of 4 mM, oligomycin 1 µg per mg protein (0.1 g) and halothane 7 mM (Hal). The incubation was stopped by the addition of 1 ml of 10% CCl_3COOH and inorganic phosphate was determined colorimetrically. (11)

presence of magnesium ions this activity increased seven fold. This magnesium dependent increase of ATPase activity was insensitive to oligomycin. Halothane at concentrations of 5 mM inhibited the formation of ATP by mitochondria as demonstrated by the hexokinase method (8), while the activity of this enzyme is not influenced by this anesthetic.

The experimental results presented in this study suggest that halothane is an uncoupling agent strictly dependent on the presence of magnesium ions. Snodgrass and Piras (12) report significant halothane uncoupling of mitochondria while Miller and Hunter (2) find small effects and that energy dependent mitochondrial accumulation of calcium ion is not changed by anesthetic (13). We think that these results may depend on the ionic content of the medium since magnesium ion was not present

in the negative experiments. Other factors which may influence the rates of mitochondrial respiration in the presence of halothane are the methods of halothane administration and the techniques used for mitochondrial isolation particularly the presence of chelating agents which may modify the cationic content of the organelles and affect the function of the membrane.

References

1. Harris, R.A., J. Munroe, B. Farmer, K.C. Kim and P. Jenkins, Arch. Biochem. Biophys., 142, 435 (1971).
2. Miller, R.N. and F.E. Hunter, Mol. Pharmacol. 6, 67 (1970).
3. Cohen, P.L. and B.E. Marshall, in Toxicity of Anesthetics, B.R. Fink, ed., Williams and Wilkins, Baltimore, 1970, p 24.
4. Harrison, G.G., S.J. Saunders, J.F. Biebuyck, R. Hickman, D.M. Dent, V. Weaver and J. Terblanche, Brit. J. Anaesth., 41, 844 (1969).
5. Hatefi, Y. and R.L. Lester, Biochim. Biophys. Acta, 27, 83 (1958).
6. Estabrook, R.W. in Methods in Enzymology, Vol. X, R.W. Estabrook and M.E. Pullman, eds., Acad. Press, New York 1967, p 41.
7. Blondin, G.A. and D.E. Green, Arch. Biochem. Biophys. 132, 509 (1969).
8. Slater, E.C. in Methods in Enzymology, Vol. X, R.W. Estabrook and M.E. Pullman, eds., Acad. Press, New York, 1967, p 19.
9. McDonald, M.R. in Methods in Enzymology, Vol. 1, S.P. Colowich and N.O. Kaplan, eds., Acad. Press, New York, 1955, p 371.
10. Moore, C. and B.C. Pressman, Biochim. Biophys. Res. Comm., 15, 562 (1964).
11. Fiske, C.H. and V. SubbaRow, J. Biol. Chem., 66, 375 (1925).
12. Snodgrass, P.J. and M.M. Piras, Biochem. 5, 1140 (1966).
13. Miller, R.N. and F.E. Hunter, Anesthesiol. 35, 256 (1971).