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L-CARNITINE EFFECT ON HALOTHANE-TREATED MITOCHONDRIA

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Abstract—Addition of halothane to the incubation medium is shown to lower respiratory control and transmembrane potential and to increase ATPase activity in isolated rat liver mitochondria. Evidence is presented that L-carnitine is able to substantially decrease the negative effects of halothane on the energy-linked processes of mitochondria.

The effects of halothane and the protective action of L-carnitine are discussed in the light of a possible involvement of long-chain acyl CoA in the unpairing of mitochondrial energy-linked functions.

The interaction of halothane with membranes has been studied both in artificial phospholipid bilayers and in rat brain preparations. According to Shieh *et al.* [1] addition of halothane to phosphatidylcholine liposomes causes a fluidization of the bilayer, and the first site of action of the anesthetic appears to be the choline group. The studies of Barany *et al.* [2] on rat brain preparations indicate that halothane intercalates in cellular membranes increasing the mobility of phospholipids, and that the interaction of the anesthetic with biological membranes is likely to involve also some membrane proteins.

As far as mitochondria are concerned, halothane has been reported to inhibit the oxidation of NAD-linked substrates [3–6], while its effect on succinate oxidation is still controversial [3, 6]. Moreover, according to Snodgrass and Piras [3] halothane may act as a true uncoupler of oxidative phosphorylation. On the other hand Miller and Hunter [7] reported that mitochondrial Ca^{2+} uptake and retention, typical energy-dependent processes, are unaffected by halothane.

These discrepancies, mainly due to the still unknown nature of the interaction of the anesthetic with mitochondrial membranes, prompted us to study more circumstantially the action of halothane on some energy-linked processes of rat liver mitochondria. Furthermore in consideration of the recent finding that L-carnitine protects mitochondria exposed to different kinds of stresses [8–10], the possibility that L-carnitine might prevent the halothane effects has also been considered.

Indeed, as shown by the results here reported, halothane impairs the respiratory control ratio and the transmembrane potential of rat liver mitochondria, and L-carnitine partially counteracts these adverse effects.

‡ Abbreviations used: Hepes, *N*-2-hydroxycthylpiperazine-*N'*-2-ethane-sulfonic acid; $\Delta\psi$, electrical transmembrane potential; CCCP, carbonylcyanid-m-chlorphenylhydrazon.

MATERIALS AND METHODS

Male (24 hr starved) Wistar albino rats were killed by decapitation and the livers immediately immersed in ice-cold 0.25 M sucrose, 5 mM Na–Hepes‡ (pH 7.4). The liver was minced, thoroughly washed and then homogenized in 50 ml of 0.25 M sucrose, 5 mM Na–Hepes (pH 7.4) using a Potter homogenizer with Teflon pestle. Mitochondria were then isolated by differential centrifugation in the same buffered solution. The protein content of mitochondrial suspensions was assayed according to Gornall *et al.* [11] using bovine serum albumin as the standard.

Mitochondria isolated from rat livers were tested for oxygen consumption at 20° with a Clark electrode in 2 ml of incubation mixture containing 2 mg of mitochondrial proteins. The medium composition was: 100 mM sucrose, 50 mM KCl, 10 mM KH_2PO_4, 2 mM $MgSO_4$, 1 mM EDTA, 15 mM Tris–HCl (pH 7.4), 5 mM Na-succinate and 1.25 μM rotenone. Appropriate blanks were run to avoid the possibility of halothane interferences with the electrode in our experimental conditions.

Mitochondrial ATPase activity was assayed according to Baginski *et al.* [12] in the following medium: 200 mM sucrose, 10 mM K-Hepes (pH 7.4), 2 mM $MgCl_2$, 5 mM succinate, 1.25 μM rotenone, 1 mM ATP. Mitochondria were incubated at 20° for 12 min at a concentration of 1 mg of mitochondrial proteins/ml of the incubation mixture. Samples of 300 μl were then collected, deproteinized with 10% trichloroacetic acid and assayed for inorganic phosphate.

The mitochondrial transmembrane potential was measured by monitoring the tetraphenylphosphonium distribution across the mitochondrial membrane with a tetraphenylphosphonium-selective electrode prepared in our laboratory according to Kamo *et al.* [13] using a calomel electrode (Radiometer K401) as the reference electrode. The incubations were carried out at 20° with 1 mg of mitochondrial

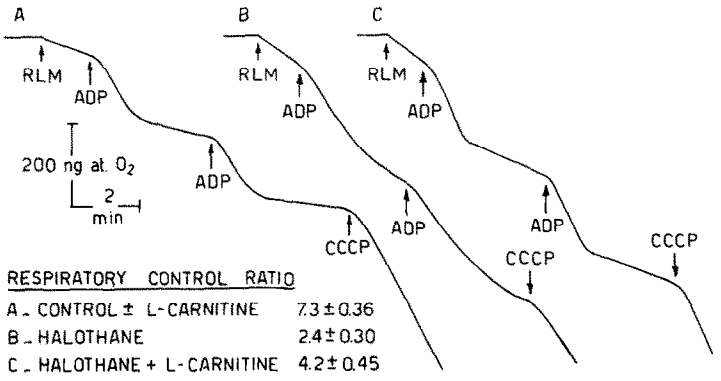

Fig. 1. Oxygen consumption traces and respiratory control ratios of rat liver mitochondria incubated in the presence and in the absence of halothane and L-carnitine. Experimental conditions are reported in the Methods section; 1 mg mitochondrial proteins/ml (RLM), 150 μM ADP or 1.6 μM CCCP were added when indicated by the arrows. When indicated 1 mM L-carnitine and/or 3 mM halothane were present in the incubation mixture. Respiratory control ratios are the mean of 10 experimental values ±SD.

proteins/ml suspension in the same incubation mixture used to monitor oxygen consumption. The electrode potential was linear with respect to the logarithm of tetraphenylphosphonium concentration with a slope of 59 mV, in agreement with Nernst equation. All the calibration tests were repeated in the presence of halothane in order to exclude any direct interference of the anesthetic with the electrodes.

Halothane was diluted with ethanol to 1 M and added to the incubation mixture immediately before beginning the experiment.

RESULTS

The oxygen traces reported in Fig. 1 indicate that 3 mM halothane significantly decreased the respiratory control ratio of rat liver mitochondria and addition of 1 mM L-carnitine to the medium appreciably improved the respiratory control ratio of halothane-treated mitochondria. L-Carnitine did not affect either the respiration rate or the respiratory control ratio of untreated mitochondria. Different compounds structurally or metabolically related to L-carnitine (deoxycarnitine, choline, succinylcholine) resulted ineffective in reversing the reported effect of halothane on mitochondrial respiration (data not shown).

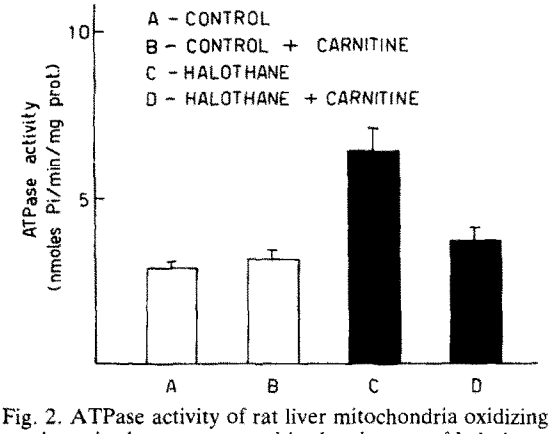

Fig. 2. ATPase activity of rat liver mitochondria oxidizing succinate in the presence and in the absence of halothane and L-carnitine. The medium composition and incubation conditions are reported in the Methods section. When indicated 1 mM L-carnitine and/or 3 mM halothane were present in the incubation mixture. The reported values are the mean of 8 different sets of experiments; standard deviations are represented by the vertical bars.

The effects of halothane and L-carnitine on the ATPase activity of mitochondria oxidizing succinate are reported in Fig. 2. In respiring mitochondria ATP hydrolysis and synthesis are concurrent processes and the resulting release of phosphate is much lower than that obtainable in the absence of any oxidizable substrate. This accounts for the relatively low ATPase activity found in our experiments. Under these conditions 3 mM halothane caused a twofold increase of the net ATPase activity and 1 mM L-carnitine almost completely reversed this effect.

The effects of halothane and L-carnitine were also evident on mitochondrial transmembrane potential (Fig. 3). Although halothane did not dramatically modify the $\Delta\psi$ attained by rat liver mitochondria during succinate oxidation in state 3, the recovery of $\Delta\psi$ following ADP addition was both decreased and prolonged in the presence of halothane. L-Carnitine partially but significantly prevented this effect. The lower steady state of transmembrane potential resulting from ADP additions is caused by the phosphorylation process [14]. The time length of this lower steady state is in fact related to the amount of added ADP and $\Delta\psi$ restoration to the original values is dependent on the coupling conditions of the system. Indeed the curves in Fig. 3 indicate that halothane has an uncoupling action which is partially prevented by L-carnitine.

DISCUSSION

The reported results show that 3 mM halothane (lower concentrations are without effect) added to liver mitochondria alters the properties of mitochondrial membranes, with a consequent decrease

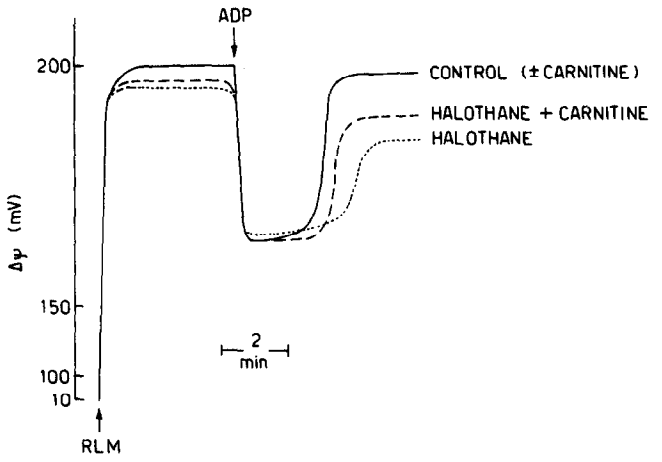

Fig. 3. Transmembrane potential ($\Delta\psi$) of mitochondria in the presence and in the absence of L-carnitine and/or halothane. Medium composition and assay conditions are described in the Methods section. When indicated 1 mM L-carnitine and/or 3 mM halothane were present in the incubation mixture. One milligram of mitochondrial proteins/ml (RLM) and 150 μM ADP were added when indicated by the arrows.

of the phosphorylation efficiency. This evidence emerges from the results relative to the respiratory control ratio (Fig. 1) as well as to $\Delta\psi$ determination (Fig. 3). The achievement of the same results with different methodologies rules out the objection of a possible interference of halothane with both the oxygen and tetraphenylphosphonium electrode. Furthermore the stimulation of the ATPase activity induced by halothane further confirms that oxidative phosphorylation is partially uncoupled. These results confirm those previously obtained with different procedures by Snodgrass and Piras [3]. The problem whether the uncoupling action of halothane might result either from an aspecific perturbation of the mitochondrial membrane or from an interaction with specific sites of the membrane is beyond the purpose of the present paper.

Whatever the mechanism of the protective action of L-carnitine on halothane-treated mitochondria, the results here reported allow some considerations on conditions which presumably render mitochondria more susceptible to the halothane damaging action.

It is known that long chain acyl CoA may be able to deform biological membrane by non specific interpolation into the lipid bilayer [15]; thereby biomembranes become more vulnerable to a variety of endogenous and exogenous noxious agents. It is then predictable that L-carnitine by removing the long chain acyl CoA through the reaction catalyzed by CoA: carnitine acyl transferase might exert a protective action. Indeed a significant protective action of L-carnitine on isolated mitochondria exposed to various stresses (heat or Ca^{2+}) has been clearly demonstrated and attributed to the removal of long chain acyl CoA [8]. On the basis of this observation it appears reasonable to assume that the observed protective action of carnitine might be attributed to a decreased mitochondria vulnerability to halothane by the removal of endogenous long chain acyl CoA. In other words it might be assumed that the damaging action of halothane potentiates that of endogenous long chain acyl-CoA and vice versa.

An alternative possibility that carnitine might counteract the intercalation of halothane into the membrane bilayer [2], thus preserving the structural and functional properties of mitochondrial membrane, seems to be unlikely, also in consideration that compounds structurally related to carnitine, deoxycarnitine in particular, are devoid of action.

The protective effect of L-carnitine against the halothane damaging action might have some clinical importance in preventing or attenuating the functional alterations which may be observed upon prolonged exposure to this anesthetic [16, 17].

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REFERENCES

1. D. D. Shieh, I. Ueda, H. Lin and H. Eyring, *Proc. Natn. Acad. Sci. U.S.A.* **73**, 3999 (1976).
2. M. Barany, Y. Chang and C. Arus, *Biochemistry* **24**, 7911 (1985).
3. P. J. Snodgrass and M. M. Piras, *Biochemistry* **5**, 1140 (1966).
4. P. J. Cohen, B. E. Marshall and J. Lecky, *Anesthesiology* **30**, 337 (1969).
5. J. F. Biebuyck, P. Lund and H. A. Krebs, *Biochem. J.* **128**, 711 (1972).
6. R. N. Miller and F. E. Hunter, *Molec. Pharmac.* **6**, 67 (1970).
7. R. N. Miller and F. E. Hunter, *Anesthesiology* **35**, 256 (1971).
8. F. Di Lisa, V. Bobyleva-Guarriero, P. Jocelyn, A. Toninello and N. Siliprandi, *Biochem. biophys. Res. Commun.* **131**, 968 (1985).
9. R. Nikula, H. Ruohola, L. Alhonen-Hougisto and J. Jarme, *Biochem. J.* **228**, 513 (1985).

10. D. Branca, F. Di Lisa, G. Scutari, A. Toninello and N. Siliprandi, *Biochem. Pharmac.* **35**, 2839 (1986).
11. A. G. Gornall, C. J. Bardawill and M. M. David, *J. biol. Chem.* **177**, 751 (1949).
12. E. S. Baginski, P. P. Foà and B. Zak, in *Methods of Enzymatic Analysis*, Vol. 2 (Eds. H. U. Bergmeyer and K. Gawehn) p. 876. Academic Press, New York (1979).
13. N. Kamo, M. Muratsugu, R. Hongoh and Y. Kobatake, *J. Membr. Biol.* **49**, 105 (1979).
14. N. Kamo, *FEBS Lett.* **142**, 63 (1982).
15. P. Brecher, *Mol. Cell Biochem.* **57**, 3 (1983).
16. H. G. Kaplan, J. Bakken, L. Quadracci and W. Schuback, *Ann. Intern. Med.* **90**, 797 (1979).
17. J. Neuberger, D. Vergani, G. Mieli-Vergani, M. Davis and R. Williams, *Br. J. Anaesth.* **53**, 1173 (1981).