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INVOLVEMENT OF LONG-CHAIN ACYL CoA IN THE ANTAGONISTIC EFFECTS OF HALOTHANE AND L-CARNITINE ON MITOCHONDRIAL ENERGY-LINKED PROCESSES

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Incubation of rat liver mitochondria in the presence of halothane induced a consistent impairment of mitochondrial oxidative phosphorylation without significantly affecting the steady-state of transmembrane electrical potential. These alterations of mitochondrial energy-linked processes were associated with a consistent accumulation of long-chain acyl CoA. Addition of L-carnitine partially prevented the effects of halothane on oxidative phosphorylation and completely abolished the halothane-induced long-chain acyl CoA accumulation. The possibility is discussed that the damaging action of halothane on mitochondrial functions might be partially ascribed to the noxious action of the excess of long-chain acyl CoA induced the anesthetic.

Halothane has been reported to inhibit mitochondrial oxidation of NAD-linked substrates, to uncouple oxidative phosphorylation supported by succinate and to stimulate ATP hydrolysis (1,5). However the mechanism by which halothane affects mitochondrial functions is still obscure. As demonstrated by Rottenberg (6) halothane does not act as an authentic protonophore, since it impairs oxidative phosphorylation without collapsing bulk $\Delta\tilde{\mu}_H$. On the other hand it is known that general anesthetics perturb biological membranes inducing alterations of their fluidity, dielectric constant and lipid-protein interactions (7); this might alter the equilibrium of charged species between different mitochondrial compartments, included the putative intramembrane pool (6).

We have previously demonstrated that some of the effects of halothane on isolated rat liver mitochondria can be prevented, or reversed, by addition of L-carnitine (8). This observation led us to assume that halothane action would be mediated in part by the generation of long-chain acyl

ABBREVIATIONS: $\Delta\tilde{\mu}_H$, transmembrane proton electrochemical gradient; LCACoA, long-chain acyl CoA; Hepes, 4-(2-hydroxyethyl)-piperazineethanesulphonic-acid; HPLC, high performance liquid chromatography; $\Delta\psi$, transmembrane electrical gradient.

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CoA (LCACoA) which, in addition to halothane itself, do negatively affect some mitochondrial functions.

Further evidence of the involvement of LCACoA in halothane induced mitochondrial damage is presented in the present paper.

MATERIALS AND METHODS

Rat liver mitochondria were prepared from 24 hours starved Wistar albino rats as previsouly described (8).

All experiments were performed at 20°C using 1 mg of mitochondrial protein/ml of an incubation medium containing 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, 1.25 μM rotenone; further additions are indicated in the legends to Figures.

Oxygen consumption was measured with a Clark electrode and mitochondrial transmembrane potential with a tetraphenylphosphonium-selective electrode prepared in our laboratory according to Kamo et al. (9).

Halothane was diluted with ethanol and added to the incubation mixtures immediately before beginning the experiment. Appropriate blanks and calibration tests were run in the presence of halothane in order to exclude any direct interference of the anaesthetic with the electrodes.

LCACoA were measured as acid-insoluble free CoA by reverse-phase HPLC. Alkaline hydrolysis was performed on 7 mg of mitochondrial proteins following the procedure described by Berge et al. (10) and CoA was assayed by HPLC using an octadecyl silane column (Altex Ultrasphere-ODS, 4.6 x 250 mm, 5 μM) and a mobile phase containing 50 mM K-phosphate (pH 5.5) and 12% methanol. The flow rate was 1 ml/min and CoA was detected at 259 nm. The proof of the nature of the CoA peak in the chromatogram was obtained as described by Ingebretsen and Farstad (11).

RESULTS

As previously observed by Rottenberg (6) and ourselves (8), halothane decreased the respiratory control ratio of mitochondria oxidizing succinate (Fig. 1), the maximum effect being attained upon addition of approximately 7 mM halothane. The simultaneous addition of 1 mM L-carnitine to the incubation mixture decreased the halothane effect at all the concentrations used.

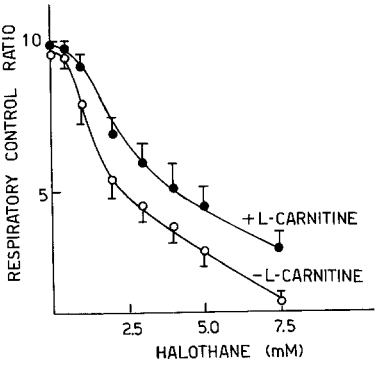

Figure 1: Respiratory control ratios of rat liver mitochondria treated with increasing concentrations of halothane in the presence and in the absence of 1 mM L-carnitine. State 3 respiration was measured upon addition of 0.2 mM ADP. Each point is the mean of 8 experiments; standard deviations are indicated by the vertical bars.

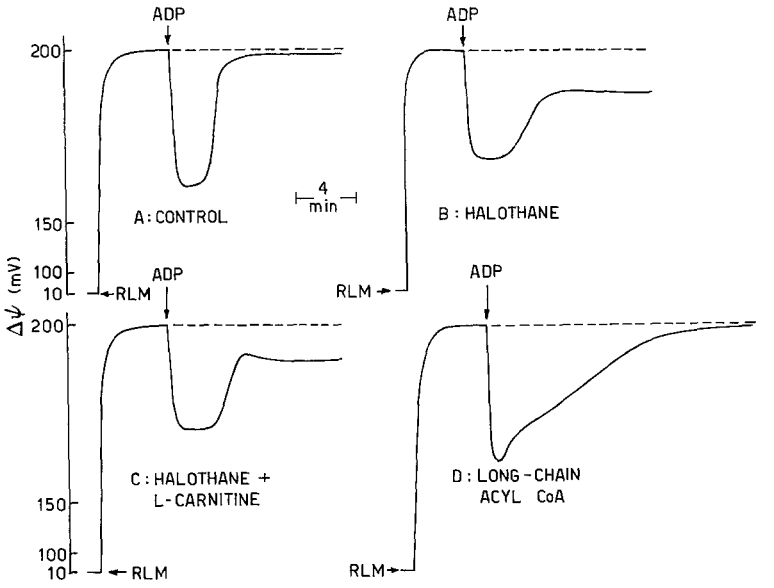

Figure 2: Transmembrane potential ($\Delta\psi$) of rat liver mitochondria incubated in the presence and in absence of halothane, L-carnitine and long-chain acyl CoA. The incubation mixture was as described in the Materials and Methods section with the addition of 5 mM halothane (curve B), 5 mM halothane and 1 mM L-carnitine (curve C), 3 μM oleoylCoA or palmitoylCoA (curve D). Rat liver mitochondria (RLM) and 0.2 mM ADP were added when indicated.

These results have been fully confirmed by measuring the recovery of transmembrane electric potential ($\Delta\psi$) upon addition of ADP which, according to Kamo (9), reflects the efficiency of the oxidative phosphorylation process. In particular it has been observed that 5 mM halothane did not affect the maximum value of $\Delta\psi$ maintained during the oxidation of succinate in the absence of added ADP (Fig. 2). However in the presence of the anesthetic the recovery of $\Delta\psi$ upon addition of 0.3 mM ADP was significantly delayed and incomplete (Fig. 2 B). Addition of L-carnitine attenuated both these negative effects (Fig. 2 C).

The amounts of LCACoA present in the mitochondrial suspension after 10 minutes of incubation in the presence and in the absence of L-carnitine and halothane are reported in Fig. 3. In all cases the concentration of LCACoA was much higher in halothane-treated than in control mitochondria and addition of L-carnitine completely abolished the increase of LCACoA. It has to be noted that addition of L-carnitine significantly decreased the concentration of LCACoA also in control mitochondria (compare in Fig. 3 the values relative to the absence of halothane).

These results show that L-carnitine reduced the effects of halothane on mitochondria energy-linked processes and prevented the accumulation of LCACoA induced by halothane. That these effects might be mutually correla-

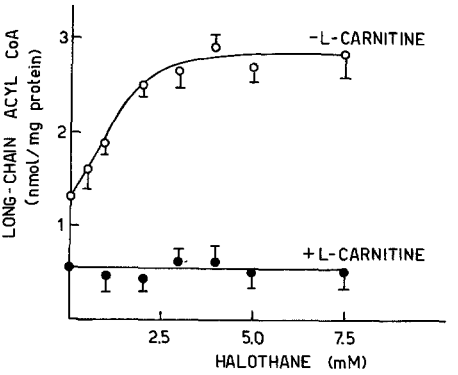

Figure 3: Long-chain acyl CoA content of rat liver mitochondria treated with increasing concentrations of halothane in the presence and in the absence of 1 mM L-carnitine. Mitochondria were incubated for 10 minutes in the presence of 0.2 mM ADP; the incubation and LCACoA assay conditions are reported in the Materials and Methods section. Each point is the mean of 6 experiments; standard deviations are indicated by the vertical bars.

ted is indicated by the results in Fig. 2 D showing that addition to liver mitochondria of amounts of LCACoA similar to those produced by halothane did not alter the $\Delta\psi$ value attained during oxidation of succinate in the absence of ADP, while it affected - though to a lesser extent with respect to halothane - the recovery of $\Delta\psi$ upon addition of ADP.

DISCUSSION

As outlined by Rottenberg (6) general anesthetics and halothane in particular impair oxidative phosphorylation without collapsing $\Delta\tilde{\mu}_H$. To stress the basic difference from $\Delta\tilde{\mu}_H$-collapsing classical uncouplers these compounds have been designated, together with long-chain fatty acids, as "decouplers" (12). Indeed the results reported in the present paper are fully consistent with this concept and furthermore suggest the existence of a functional link between general anesthetics and LCACoA.

Both these classes of compounds affect the efficiency of mitochondrial oxidative phosphorylation without appreciably reducing the $\Delta\psi$ produced by succinate oxidation (Figs. 1 and 2). The observation that both LCACoA and halothane affect mitochondrial oxidative phosphorylation in a similar way (Fig. 2) and the observed accumulation of LCACoA in halothane treated mitochondria (Fig. 3) suggest that the action of halothane on mitochondria should result from two components: the action of the anesthetic itself and that of LCACoA generated as a consequence of the interaction of halothane with mitochondrial membranes. Long-chain acyl CoA are known to have a potent inhibitory action on the activity of some mitochondrial proteins - namely adenylate translocase (13) and dicarboxylate carrier (14) - and to be able to deform mitochondrial membranes by interpolation into the lipid

bilayer (15). Thus it is conceivable that the action of free fatty acids reported by Rottenberg (12) might be ascribed, at least in part, to that of the corresponding LCACoA to which added fatty acids may be partially converted by the action of external and internal mitochondrial acyl-CoA synthetase (16).

The decrease of halothane action on mitochondria energy-linked processes induced by L-carnitine might reasonably be ascribed to the removal - through the action of CoA:carnitine palmitoyl transferase - of the LCACoA excess caused by halothane. Hence mitochondrial damages observed in the presence of halothane and L-carnitine would reflect those directly caused by the anaesthetic (Figs. 1 and 2). As to the mechanism whereby halothane induces the observed increase of LCACoA, a release of free fatty acids from membrane phospholipids by activation of phospholipase A_2 seems to be the most probable cause. An inhibitory action of halothane on the activity of CoA:carnitine acyl transferase may be ruled out in the light of the clearcut effect of added carnitine.

The results here reported might be of some relevance for the protection against the side effects of halothane in surgical patients.

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