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Uncoupling Effect of Lauryl Sulfate on Mitochondria Can Be Mediated by Release of Bound Endogenous Fatty Acids

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Abstract—The mechanism of uncoupling by lauryl sulfate (LS) has been studied. The very fact that uncoupling by low concentration of LS (a strong acid) resembles very much that by fatty acids (weak acids) was used as an argument against the fatty acid cycling scheme of uncoupling where protonated fatty acids operate as a protonophore. We have found that rat liver and heart muscle mitochondria can be uncoupled by low (70 μM) LS concentration in a fashion completely arrested by the ATP/ADP antiporter inhibitor carboxyatractylate (CAtr). On the other hand, uncoupling by two-fold higher LS concentration is not sensitive to CAtr. Addition of oleate desensitizes mitochondria to low LS so that addition of bovine serum albumin becomes necessary to recouple mitochondria. The data are accounted for assuming that low LS releases endogenous fatty acids from some mitochondrial depots, and these fatty acids are responsible for uncoupling. As to high LS, it causes a nonspecific (CAtr-insensitive) damage to the mitochondrial membrane.

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The mechanism of uncoupling of oxidative phosphorylation in mitochondria by free fatty acids has been suggested to be mediated by fatty acid cycling [1-4]. Such a scheme assumes that the fatty acid anions are electrophoretically extruded from the mitochondrial matrix to the intermembrane space where they are protonated and then return to the matrix. The latter step represents a diffusion of free fatty acid via the phospholipid part of the membrane, whereas the former requires the involvement of some proteins that facilitate transmembrane movement of the of fatty acid anions. The proteins in question belong to the family of mitochondrial anion carriers, i.e. uncoupling proteins (UCP), ATP/ADP antiporter, aspartate/glutamate antiporter, and dicarboxylate and phosphate carriers (for reviews, see [1-5]).

In line with this scheme, it was found by Garlid's and Jezek's groups that UCP1 is competent in electrophoretic translocation of strong acids like alkyl sulfonates which inhibit H^+ conductance induced by fatty acids [5]. However, in our group it was shown [6] that low concentrations of another strong acid, lauryl sulfate (LS), increase an H^+ conductance of rat liver mitochondria membranes. The increase was inhibited by carboxyatractylate (CAtr), indicating that the ATP/ADP antiporter is involved. The effect of cationic (cetyltrimethylammonium bromide) and neutral (Triton X-100) detergents were CAtr-insensitive, whereas that of such an anionic detergent as cholate was suppressed by CAtr. Later we revealed striking similarities of many features of protonophorous uncoupling induced by laurate and by lauryl sulfate (LS) [7]:

– CAtr and glutamate (or aspartate) when added together recouple the laurate- or LS-treated mitochondria almost completely;

– increase in pH is favorable for the CAtr recoupling and unfavorable for glutamate recoupling, the effect of combined action of CAtr and glutamate being pH independent;

– addition of tetraphenylphosphonium decreases recoupling by CAtr but not by glutamate;

– atractylate recoupling is weaker than that by CAtr;

– addition of atractylate before CAtr decelerates the CAtr effect;

– small concentrations of ADP have some recoupling activity, which is lower than that of CAtr;

– GDP fails to substitute for ADP.

Abbreviations: BSA) bovine serum albumin; CAtr) carboxyatractylate; LS) lauryl sulfate; UCP) uncoupling protein; $\Delta\Psi$) transmembrane electric potential difference.

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Until recently, it remained unclear how such a strong acid as LS can be protonated at neutral pH to be involved in the cycling. This fact was used by Rial et al. [8] as an argument against the fatty acid cycling scheme. However, in 2006 Jezek et al. demonstrated [5] that in a model system composed of liposomes and a fatty acid-saturated bovine serum albumin addition of undecanesulfonate causes acidification of the liposome interior just as fatty acid added without bovine serum albumin (BSA). On the other hand, addition of undecanesulfonate to liposomes incubated with the fatty acid-free BSA was without effect. The above relationships were explained by substitution of undecanesulfonate for fatty acids in the BSA molecule, and the released fatty acids being protonated and translocated into liposome down their concentration gradient. Inside liposomes, fatty acids were deprotonated, causing acidification. Similar relationships were observed in brown fat mitochondria incubated with BSA. Here undecanesulfonate was shown to induce an uncoupling mediated by fatty acids released from BSA. The above explanation was supported by the fact that BSA binds such an undecanesulfonate as LS with affinity much higher than that for fatty acids [9-12].

Taking into account these observations, we reinvestigated effect of LS on rat liver and heart mitochondria. The data obtained are consistent with an assumption that (i) at low concentrations LS substitutes for endogenous fatty acids bound to some mitochondrial proteins and (ii) the released fatty acids are responsible for the LS-induced, CAtr-insensitive uncoupling. As to high LS concentrations, the uncoupling effect is CAtr-insensitive, being most probably due to a nonspecific damage to the mitochondrial membrane.

MATERIALS AND METHODS

Isolation of mitochondria. Liver was obtained from an 150-180 g rat killed according recommendation of the Ethic Committee of Moscow State University for laboratory animal treatment, cut into 2-3 mm pieces, and homogenized in Potter Teflon–glass homogenizer with 200-μ clearance in 10 volumes (v/w) of 250 mM sucrose, 10 mM MOPS-KOH, pH 7.4, 0.5 mM EGTA, 0.1% BSA (isolation medium) for 2 min at 4°C. The homogenate was centrifuged at 1000*g* for 10 min at 4°C in a Beckman centrifuge (USA), model J2-21, rotor JA-20. Mitochondria were sedimented from the supernatant at 9000*g* for 10 min (at 4°C) and resuspended in 1 ml with subsequent dilution by the same medium but without BSA. Protein concentration was determined by biuret method. Routinely, the final mitochondrial suspension contained 100 mg/ml. Heart muscle mitochondria were isolated from four rat hearts, rinsed with isolation medium, cut

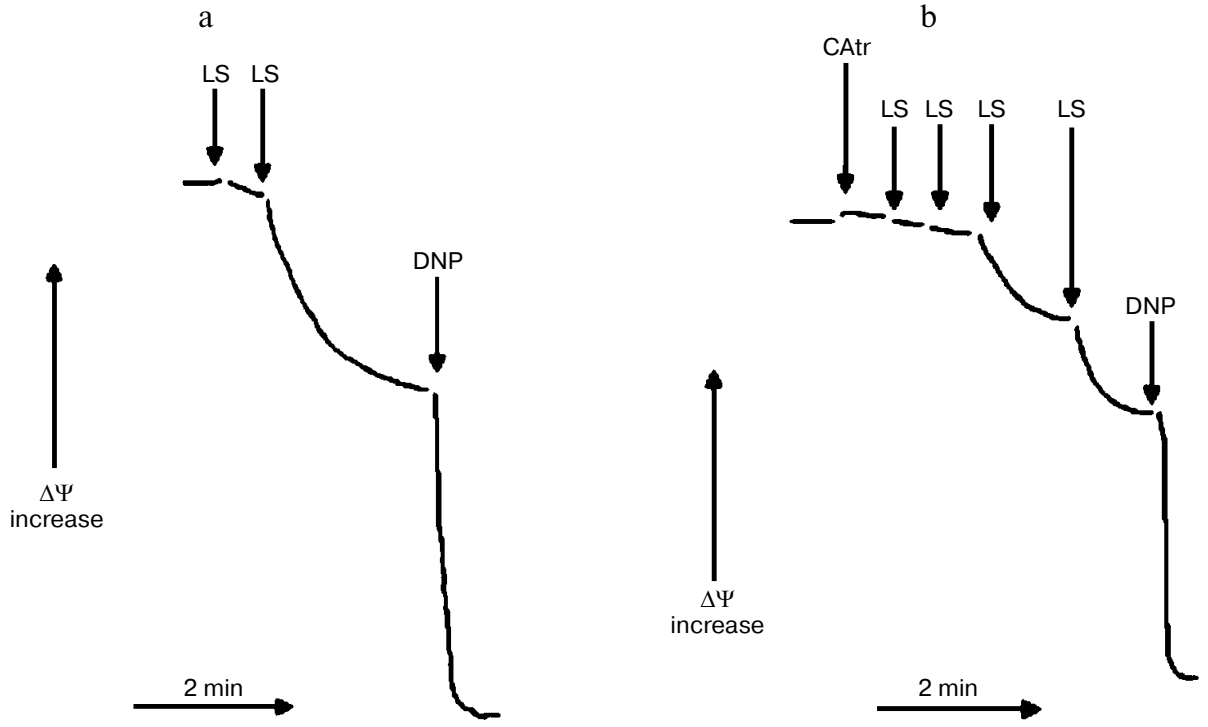

Fig. 1. Effect of carboxyatractylate (CAtr) on liver mitochondrial membrane potential decreased by lauryl sulfate (each additions of lauryl sulfate, 35 μM): a) uncoupling induced by 70 μM LS; b) prevention of uncoupling by CAtr and effect of LS addition dose. Additions: CAtr, 1 μM; LS, 35 μM; DNP, 10 μM.

into 4-5 mm pieces, and passed through a stainless meat-chopper cooled to 4°C. The subsequent treatment was the same as for liver. The final suspension of heart muscle mitochondria contained 120 mg protein/ml.

Measurement of mitochondrial ΔΨ and respiration rate. Safranin O was used as a membrane potential probe [13]. The ratio of absorptions at 555/523 nm was measured with an Aminco DW-2000 double-beam spectrophotometer. All measurements were done at 25°C in the BSA-lacking isolation medium containing 0.3-0.5 mg/ml mitochondrial protein and 15 μM safranin O. Oxygen consumption by mitochondrial suspension was measured in a closed chamber at 25°C in the BSA-lacking isolation medium with 0.4 mg/ml mitochondrial protein with Clark-type electrode on a Cyclobios oxygraph (Oroboros, Austria). In all experiments, the mitochondria were energized by 5 mM succinate in the presence of 2 μM rotenone and 1 μg/ml oligomycin. When indicated, 1 μM

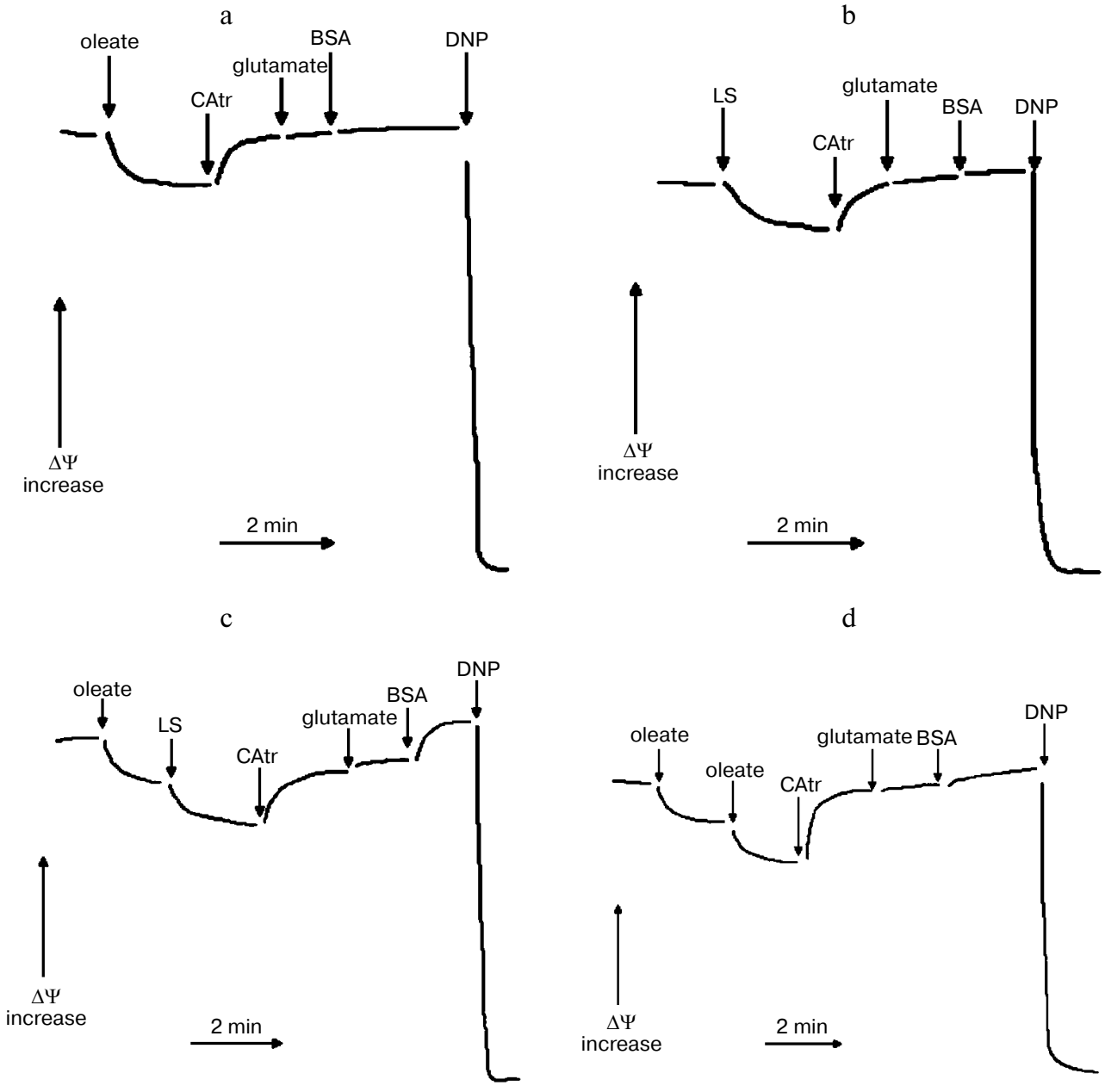

Fig. 2. Effect of carboxyatractylate, glutamate, and BSA on liver mitochondrial membrane potential decreased by oleate and lauryl sulfate. Mitochondria uncoupled by oleate (a), LS (b), oleate plus LS (c), and double amount of oleate (d). Additions: oleate, 3 μM oleate for (a) and (c) and 6 μM for (d); LS, 50 μM LS for (b) and (c); CAtr, 1 μM CAtr; glutamate, 2 mM glutamate; BSA, 0.1% BSA; DNP, 10 μM DNP.

CAtr, 2 mM glutamate, BSA (2 mg/ml), and 2 μM 2,4-dinitrophenol (DNP) were added.

Chemicals. MOPS, sucrose, oleic acid, lauryl sulfate, succinate, oligomycin, rotenone, carboxyatractylate, EGTA, DNP, essentially fatty acid free bovine serum albumin, glutamate, and safranin O were from Sigma (USA); sodium hydroxide, cuprum sulfate pentahydrate, and sodium carbonate were from Reanal (Hungary).

RESULTS AND DISCUSSION

In Fig. 1, one can see the effect of CAtr on the LS-induced lowering of ΔΨ in the rat liver mitochondria. In line with our previous observations [6, 7], CAtr almost completely prevented uncoupling by small amounts (70 μM) of LS. However, even a two-fold increase in LS concentration allows the CAtr inhibition to be overcome. The same data were obtained for rat heart mitochondria (not shown).

In Fig. 2, reversal of the LS-induced ΔΨ decrease by three different recouplers is shown. For comparison, in some samples oleate was used instead of LS as an uncoupler. It is seen that uncoupling by small concentrations of either oleate (Fig. 2a) or LS (Fig. 2b) was completely blocked by CAtr so that subsequent addition of glutamate and BSA were without effect. However, a ΔΨ decrease caused by subsequent additions of oleate and LS was reversed by CAtr only partially. In this case, addition of BSA was required to completely abolish the uncoupling (Fig. 2c). Addition of a double dose of oleate resulted in a ΔΨ decrease similar to that caused by combined action of oleate and LS, but this decrease was almost completely reversed by CAtr (Fig. 2d). Similar relationships were revealed when the rat heart muscle mitochondria were used instead of the rat liver mitochondria (data not shown).

The above observations were confirmed by polarographic experiments. Here additions of oleate or LS stimulated respiration, the effect being completely abolished by CAtr provided that the amounts of uncouplers were small. A combined addition of oleate and LS in the same concentration uncoupled in such a way that a BSA addition after CAtr was necessary to inhibit respiration to the initial (state 4) level (Fig. 3).

Taking into account the above-mentioned data of Jezek on undecanesulfonate [4], we can explain our results as follows. Without added oleate, LS substitutes for endogenous fatty acids bound to some mitochondrial proteins, and just these fatty acids are responsible for uncoupling. Apparently, the affinity of LS to these proteins is rather small so it fails to compete with added oleate for the binding site. This is why in samples with added oleate LS is not bound to the protein in question. Now an uncoupling effect of LS is due to free LS. This effect most probably consists of nonspecific damage to the mitochondrial membranes and therefore cannot be abolished by inhibiting the ATP/ADP antiporter with CAtr. On the other hand, this uncoupling is reversed by BSA, which binds LS with higher affinity than fatty acids [9].

In conclusion, the LS uncoupling in the liver and heart muscle mitochondria, like that in the brown fat mitochondria, can be simply explained by the LS-

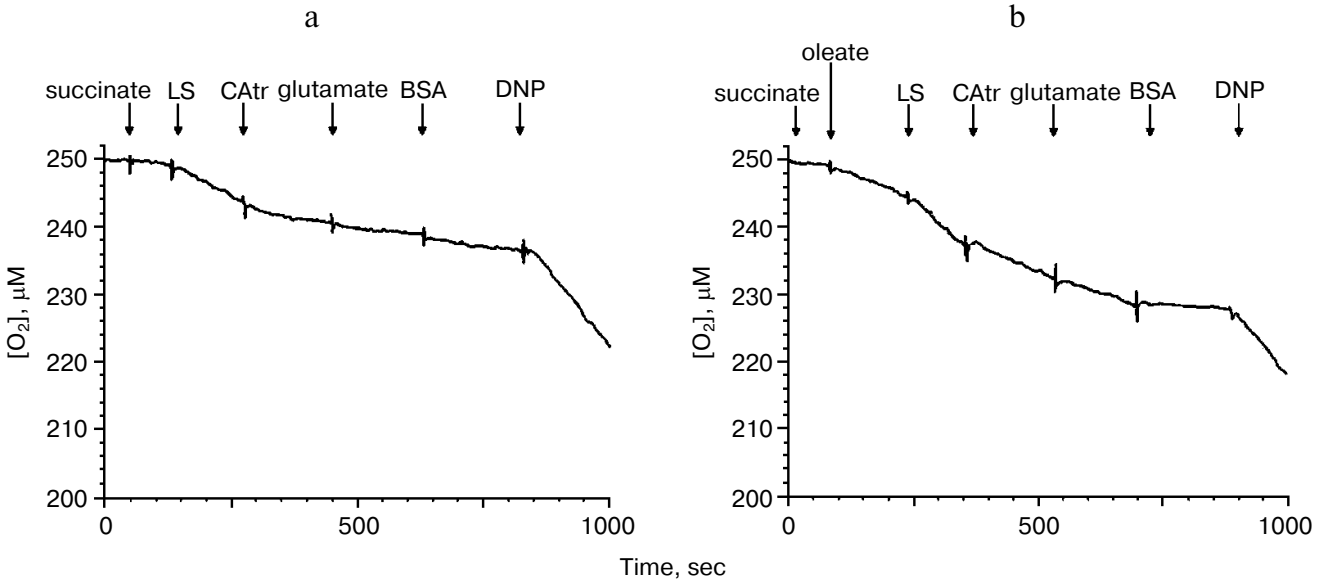

Fig. 3. Effect of carboxyatractylate (CAtr), glutamate, and BSA on liver mitochondria respiration, partially uncoupled by lauryl sulfate (LS) (a) and LS in the presence of oleate (b). Additions: LS, 50 μM LS; oleate, 10 μM oleate; succinate, 5 mM succinate. Other additions as in Figs. 1 and 2.

induced release of endogenous fatty acids from some intramitochondrial depots rather than by an involvement of LS in the H^+ conductance, as suggested by Real et al. [8]. Hence, this fact cannot be used as an argument against the fatty acid cycling scheme.

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