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Comparative study on uncoupling effects of laurate and lauryl sulfate on rat liver and skeletal muscle mitochondria

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Abstract

Uncoupling effects of laurate and lauryl sulfate have been studied in the isolated rat liver and skeletal muscle mitochondria. In the oligomycin-treated liver mitochondria, 0.02 mM laurate or 0.16 mM lauryl sulfate caused a two-fold stimulation of respiration, accompanied by a membrane potential decrease. Carboxyatractylate (CAtr) and glutamate (or aspartate) strongly decrease the effect of laurate and lauryl sulfate on respiratory rate and membrane potential (the recoupling effect). With both uncouplers, this effect is maximal for CAtr and glutamate (aspartate) at pH 7.8 and 7.0, respectively. Tetraphenyl phosphonium cations, which decrease negative membrane charges, cause an alkaline shift of these pH dependences. Small amounts of lauryl sulfate, which increase the membrane negative charge, induce the opposite shift when laurate is used as an uncoupler. ADP, but not GDP, partially recouple with both laurate and lauryl sulfate. We conclude that lauryl sulfate-induced uncoupling in rat liver, like the uncoupling induced by laurate, is mediated by the ATP/ADP and glutamate/aspartate antiporters. In skeletal muscle mitochondria uncoupled by laurate, 200 μM GDP causes partial recoupling which can be enhanced by a subsequent additions of CAtr, glutamate and serum albumin. CAtr added before GDP promotes a larger recoupling than when added after GDP and prevents the subsequent effect of GDP. ADP is effective as recoupler at lower concentrations that GDP, whereas CDP is without influence. Lauryl sulfate uncoupling of skeletal muscle mitochondria is GDP-resistant but is sensitive to ADP, CAtr, glutamate and serum albumin. Our data suggest that in skeletal muscle mitochondria a GDP-sensitive mechanism is involved in uncoupling induced by laurate. This mechanism is absent in liver mitochondria. Possible mechanisms of laurate and lauryl sulfate-induced uncoupling are discussed.

Keywords: Uncoupling; Lauryl sulfate; Fatty acid; ATP/ADP antiporter; Uncoupling protein

1. Introduction

The fatty acid-induced uncoupling in liver mitochondria [1] was proposed to be mediated by some mitochondrial anion carriers, namely the ADP/ATP antiporter [2–4], the aspartate/glutamate antiporter [5] and the dicarboxylate carrier [6]. In line with

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Abbreviations: BSA, bovine serum albumin; CAtr, carboxyatractylate; DNP, 2,4-*p*-dinitrophenol; EGTA, ethylene glycol-bis-(2aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetracetic acid; SF6847, 3,5-di(*tert*-butyl)-4-hydroxybenzylidenemalononitrile; TMPD, *N*,*N*′-tetramethyl-*p*-phenylenediamine; TPP$^+$, tetraphenylphosphonium; UCP, uncoupling protein

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this proposal, inhibitors and substrates of the abovementioned anion carriers suppress the uncoupling activity of fatty acids [4–17].

To explain the mechanism of such a phenomenon, the fatty acid cycle hypothesis was put forward [2–4]. According to the initial formulation of the hypothesis, the role of the anion carriers is confined to facilitation of transport of the fatty acid anion through the hydrophobic barrier of the inner mitochondrial membrane, whereas protonation of this anion as well as transmembrane flux of the protonated fatty acid was assumed to occur without assistance by any proteins. However, such a scheme met with difficulties when it was found in our group that lauryl sulfate (another name, dodecyl sulfate) can operate as a protonophorous uncoupler [18]. The lauryl sulfate-induced uncoupling had some properties similar to the palmitate-induced uncoupling, though much higher uncoupler concentrations proved to be needed. Uncoupling by lauryl sulfate, like that by palmitate, was strongly inhibited by carboxyatractylate (CAtr), the most powerful specific inhibitor of the ATP/ADP antiporter. Acid pulse experiments showed that lauryl sulfate, like palmitate, specifically increased the H^+ conductance of the mitochondrial membrane in a CAtr-sensitive fashion [18].

Laurate is the fatty acid structurally closest to lauryl sulfate. The only difference between these two compounds consists in that in lauryl sulfate a sulfate group substitutes for the much less acidic carboxylic group. As a result, the concentration of the protonated form of lauryl sulfate at neutral pH is negligible. It is not surprising, therefore, that dodecansulfonic acid, an analog of lauryl sulfate, is shown to be non-penetrant for liposomal membranes [19]. To explain the lauryl sulfate-mediated H^+ conductance and uncoupling, it was assumed that the ADP/ATP-antiporter not only transports the lauryl sulfate anion, but also protonates this anion by extramitochondrial H^+ ions [20].

In 1998, Wojtczak et al. [21] reported that low concentrations of lauryl sulfate fail to uncouple respiration and phosphorylation, whereas the same concentrations of fatty acids shown an uncoupling activity.

In this study, we have systematically compared the laurate and lauryl sulfate uncouplings in the liver and skeletal muscle mitochondria. It was found that in both types of mitochondria, lauryl sulfate uncouples in a fashion qualitatively similar to that of laurate, but at higher concentrations. Moreover, the laurate uncoupling proved to be partially reversed by GDP in muscle mitochondria (muscles are known to possess uncoupling proteins 2 and 3), being GDP resistant in liver mitochondria (hepatocytes contain no uncoupling proteins).

2. Materials and methods

2.1. Isolation of mitochondria

Mitochondria were isolated from liver or skeletal muscles of white rats of 180–220 g weight. The isolation medium for the liver mitochondria used in the first series of experiments (Figs. 1–5) contained 250 mM sucrose, 1 mM EGTA, 5 mM MOPS-KOH (pH 7.4). The homogenate was centrifuged at 700×*g* for 10 min. Mitochondria were sedimented at 10 000×*g* for 10 min, resuspended in 1 ml of the isolation medium supplemented with BSA (3 mg/ml), diluted with 30 ml isolation medium without BSA, and centrifuged at 10 000×*g* for 10 min. For experiments shown in Fig. 6, the rat liver mitochondria were prepared using the medium employed to isolate mitochondria from skeletal muscles (see below). The mitochondrial pellet was resuspended in the isolation medium. The final mitochondrial suspension contained about 60 mg protein/ml.

To isolate the skeletal muscle mitochondria, cooled muscles, purified from fat and tendons, were passed through a stainless meat-chopper and homogenized for 3 min with a Teflon pestle in a glass (Pyrex) homogenizer, the tissue: isolation medium ratio being 1:8. The isolation medium contained 250 mM sucrose, 10 mM MOPS (pH 7.4), 1 mM EGTA and BSA (2 mg/ml). After the first centrifugation (10 min, 700×*g*), the supernatant was decanted and filtered through gauze, then centrifuged (10 min, 16 000×*g*). The sediment was suspended in 1 ml isolation medium and centrifuged once more (10 min, 16 000×*g*). The final mitochondrial sediment was suspended in the isolation medium (70–90 mg protein/ml).

Mitochondrial protein was measured by the Biuret method.

2.2. Measurements of respiration and membrane potential

Oxygen consumption was recorded by a Clark-type oxygen electrode and LP-9 polarograph. The incubation medium for liver mitochondria contained 250 mM sucrose, 10 mM potassium succinate, 10 mM MOPS-KOH, 0.5 mM EGTA, oligomycin (2 μg/ml), 2 μM rotenone, pH 7.0 or 7.8 as indicated in the figure legends. The concentration of mitochondrial protein was about 1 mg/ml. Incubation temperature was 25°C.

Safranin O was used as a membrane potential probe [22]. The 550–523-nm light absorption was measured with an Aminco DW-2000 spectrophotometer (the double-wave length regime). The same device (the split-beam regime) was used to study swelling of mitochondria, which was estimated by a decrease in the optical density of the mitochondrial suspension at 630 nm. The suspension (1 mg mitochondrial protein/ml) was constantly stirred with a magnetic stirrer.

In the membrane potential measurements, the incubation medium contained 0.25 M sucrose, 10 mM MOPS, 1 mM EGTA, 5 mM ascorbate, 0.12 mM TMPD, 3 mM potassium phosphate, 2 μM rotenone, oligomycin (3 μg/ml), BSA (2 mg/ml), 8 μM safranin O, mitochondria (1 mg protein/ml).

In some experiments, membrane potential was estimated by TPP^+ distribution measured by means of a TPP^+-sensitive electrode. In this case, the incubation medium was supplemented with 1.6 μM TPP^+ chloride. The concentration of mitochondrial protein was about 1 mg/ml.

In Figs. 3–5, the recoupling effects of CAtr, glutamate or aspartate were expressed as a percentage of inhibition by these compounds of the mitochondrial respiration rate stimulated by laurate or lauryl sulfate (oligomycin is present). In some experiments, recoupling was monitored by measuring the membrane potential level.

2.3. Chemicals

MOPS, lauric acid, palmitic acid, lauryl sulfate, oligomycin, nigericin, succinate, glutamate, CAtr, atractylate, EGTA, and delipidized BSA were from Sigma; rotenone, from Serva; DNP and TPP^+ chloride, from Fluka; aspartate, from Reanal. Aspartate was twice recrystallized from bidistilled water. Sucrose was twice precipitated from its concentrated solution in bidistilled water with distilled ethanol.

3. Results

In the first series of experiments, we tried to optimize conditions to observe uncoupling by lauryl sulfate in rat liver mitochondria treated with oligomycin. It was found that in the sucrose incubation medium supplemented with 3 mM $MgCl_2$, some stimulation of respiration became obvious at about 40 μM lauryl sulfate. The stimulation increased at least up to 200 μM lauryl sulfate. Subsequent addition of 50 μM DNP resulted in a further stimulation of respiration. Without $MgCl_2$, the stimulation of respiration reached maximum at about 100 μM lauryl sulfate and decreased at its higher concentrations. As for DNP, it failed to stimulate respiration in the presence of 200 μM lauryl sulfate in the Mg^{2+}-free medium (Fig. 1). In further experiments, the incubation medium was supplemented with 3 mM $MgCl_2$.

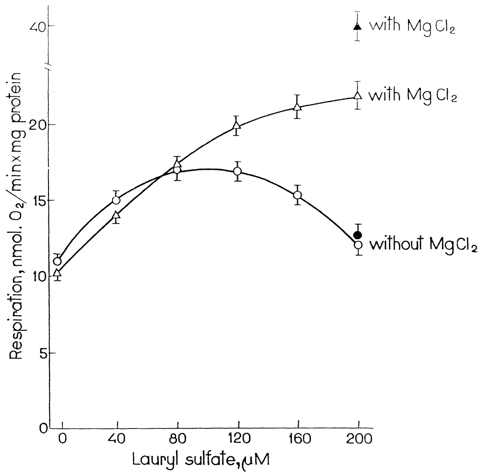

Fig. 1. Effect of $MgCl_2$ on lauryl sulfate-induced uncoupling in rat liver mitochondria. Experimental conditions, see Section 2. Incubation medium contained: 250 mM sucrose, 10 mM potassium succinate, 10 mM MOPS-KOH (pH 7.4), 0.5 mM EGTA, oligomycin (2 μg/mg mitochondrial protein), 2×10^{-4} M rotenone, BSA (0.2 mg/ml) and rat liver mitochondria (1 mg protein/ml). Where indicated, 3 mM $MgCl_2$ was added to the incubation medium before mitochondria. After addition of 2×10^{-4} M lauryl sulfate, 5×10^{-5} M DNP was added in the samples with $MgCl_2$ (dark triangle) and without $MgCl_2$ (dark circle).

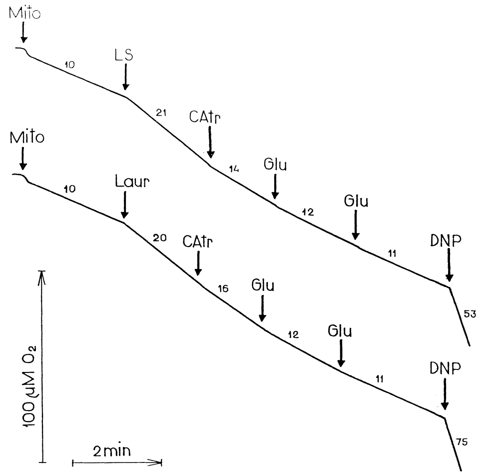

Fig. 2. Effect of CAtr and glutamate on stimulation of respiration of rat liver mitochondria by lauryl sulfate and laurate. Experimental conditions, see Fig. 1, but incubation medium was supplemented with 3 mM $MgCl_2$. Additions: Mito, rat liver mitochondria (1 mg protein/ml); LS, 1.6×10^{-4} M lauryl sulfate; Laur, 2×10^{-5} M laurate; 5×10^{-7} M CAtr; Glu, 0.4 and 2.8 mM glutamate; 5×10^{-5} M DNP. Figures above curves, oxygen consumption (nmol O_2/min/mg protein).

Under these conditions, 20 μM laurate and 160 μM lauryl sulfate were found to cause two-fold stimulation of the respiration rates. In both cases, subsequent additions of CAtr and glutamate inhibited the stimulation almost completely. DNP overcame the inhibiting effects of CAtr and glutamate (Fig. 2).

The CAtr- and glutamate-induced inhibition of respiration was shown to be accompanied by a membrane potential increase. The membrane potential level was monitored by Safranine O (see below, Fig. 6) or by TPP^+ (not shown). The very fact that CAtr and glutamate *decrease* the respiration rate and *increase* the membrane potential indicates that these compounds operate as recouplers in mitochondria uncoupled by lauryl sulfate [20]. Aspartate was found to effectively substitute for glutamate (not shown).

These data indicate that uncoupling induced by lauryl sulfate, like that by laurate, is mediated by

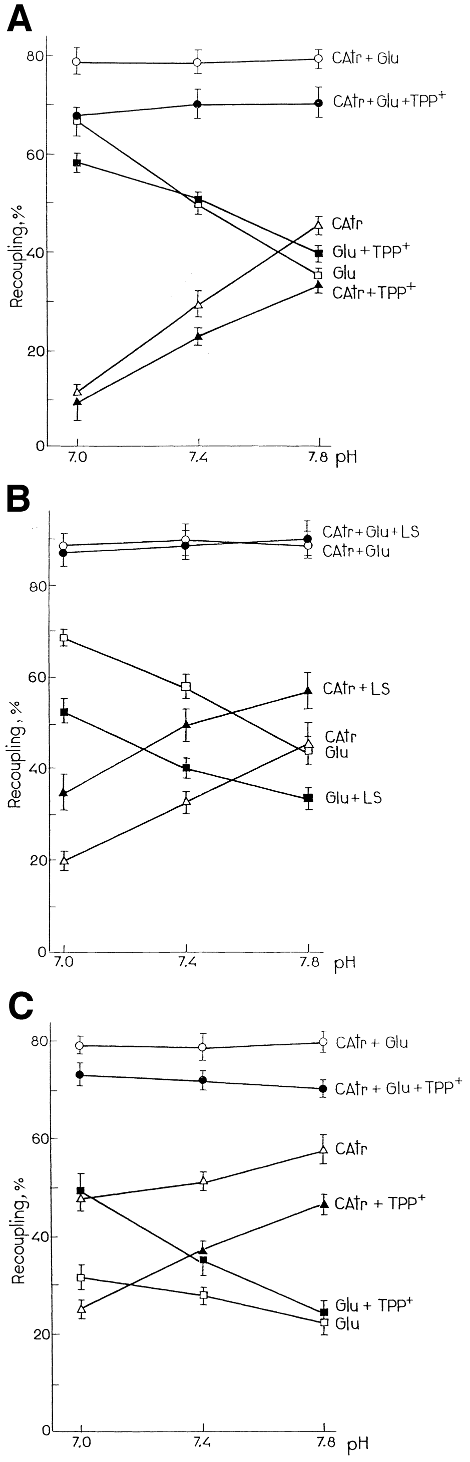

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Fig. 3. Influence of 1×10^{-5} M TPP^+ (A,C) and 4×10^{-5} M lauryl sulfate (B) on the glutamate and CAtr recoupling effects in rat liver mitochondria uncoupled with 3×10^{-5} M laurate (A), 2.5×10^{-5} M laurate (B) or 1.6×10^{-4} M lauryl sulfate (C) at different pH. Experimental conditions, see Fig. 2. Additions: Glu, 2 mM glutamate; 1×10^{-6} M CAtr; LS, 4×10^{-5} M lauryl sulfate. Here and in Figs. 4 and 5 recoupling was measured as degree of inhibition of the fatty acid- or lauryl sulfate-stimulated respiration of the oligomycin-treated mitochondria.

the ATP/ADP and aspartate/glutamate antiporters. Measurement of the mitochondrial swelling showed that 160 μM lauryl sulfate was without any dramatic effect on this process. In fact, only a small (less than 10%) biphasic decrease in the light absorbance was observed (not shown).

As was previously found in this group, the CAtr sensitivity of palmitate uncoupling increases, and the glutamate sensitivity decreases, when pH rises from 7.0 to 7.8 [17]. Similar relationships were revealed with laurate. The only difference between the two fatty acids studied consisted in that the cross-over point (the pH value when contributions of the CAtr and glutamate to the recoupling are equal) was observed at pH 7.4 and 7.7–7.8 for palmitate [17] and laurate (Fig. 3A,B), respectively. Further shift of the cross-over point to higher pH values was found to occur when the incubation mixture was supplemented with a lipophilic cation, 10 μM TPP^+ (Fig. 3A).

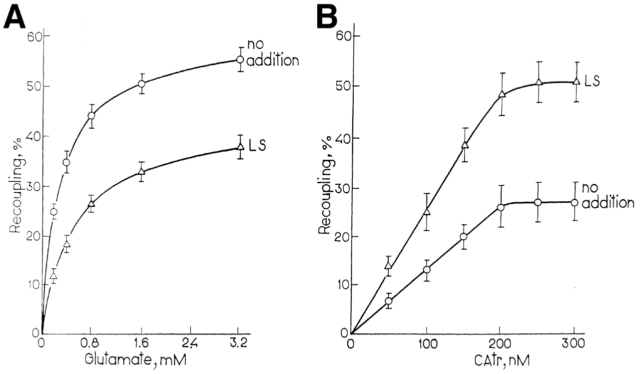

Fig. 5. Effect of 4×10^{-5} M lauryl sulfate on the glutamate (A) and CAtr (B) recoupling in rat liver mitochondria uncoupled with 3×10^{-5} M laurate. Experimental conditions, see Fig. 2.

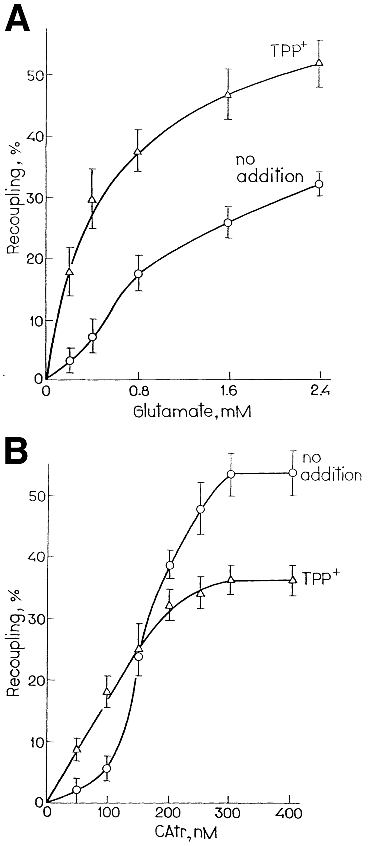

Fig. 4. Effect of 1×10^{-5} M TPP^+ on the glutamate (A) and CAtr (B) recoupling in rat liver mitochondria uncoupled with 1.6×10^{-4} M lauryl sulfate. Experimental conditions, see Fig. 2.

The opposite shift was observed when a small concentration of a lipophilic anion, lauryl sulfate, was added (Fig. 3B). In this case, the samples contained 30 μM laurate and 40 μM lauryl sulfate so that uncoupling was mainly due to laurate, a much more efficient protonophore. In Fig. 3C, one can see the pH dependence of recoupling in mitochondria uncoupled by 140 μM lauryl sulfate. Here the cross-over point seems to be at low pH (<7.0) but in the presence of TPP^+ it shifts to pH 7.4.

In the next experimental series, we studied the effect of various concentrations of recouplers on liver mitochondria uncoupled by lauryl sulfate (Fig. 4), and laurate (Fig. 5). It is shown that stimulation of the glutamate recoupling by TPP^+ is mainly due to an increase in the final level of recoupling rather than to a lowering of $C_{1/2}$ for glutamate (Fig. 4A). Similarly, a small concentration of lauryl sulfate inhibited the glutamate recoupling in the laurate-uncoupled mitochondria by decreasing the final recoupling level, $C_{1/2}$ being unaffected (Fig. 5A). Similar relationships were revealed when stimulation of CAtr recoupling by low lauryl sulfate concentration was studied (Fig. 5B). On the other hand, TPP^+ was shown to change not only the final level of recoupling, but also the form of the curve describing the concentration dependence of the CAtr recoupling in the lauryl sulfate-uncoupled mitochondria (Fig. 4B).

In the last series of experiments, the laurate and lauryl sulfate uncoupling effects were compared when

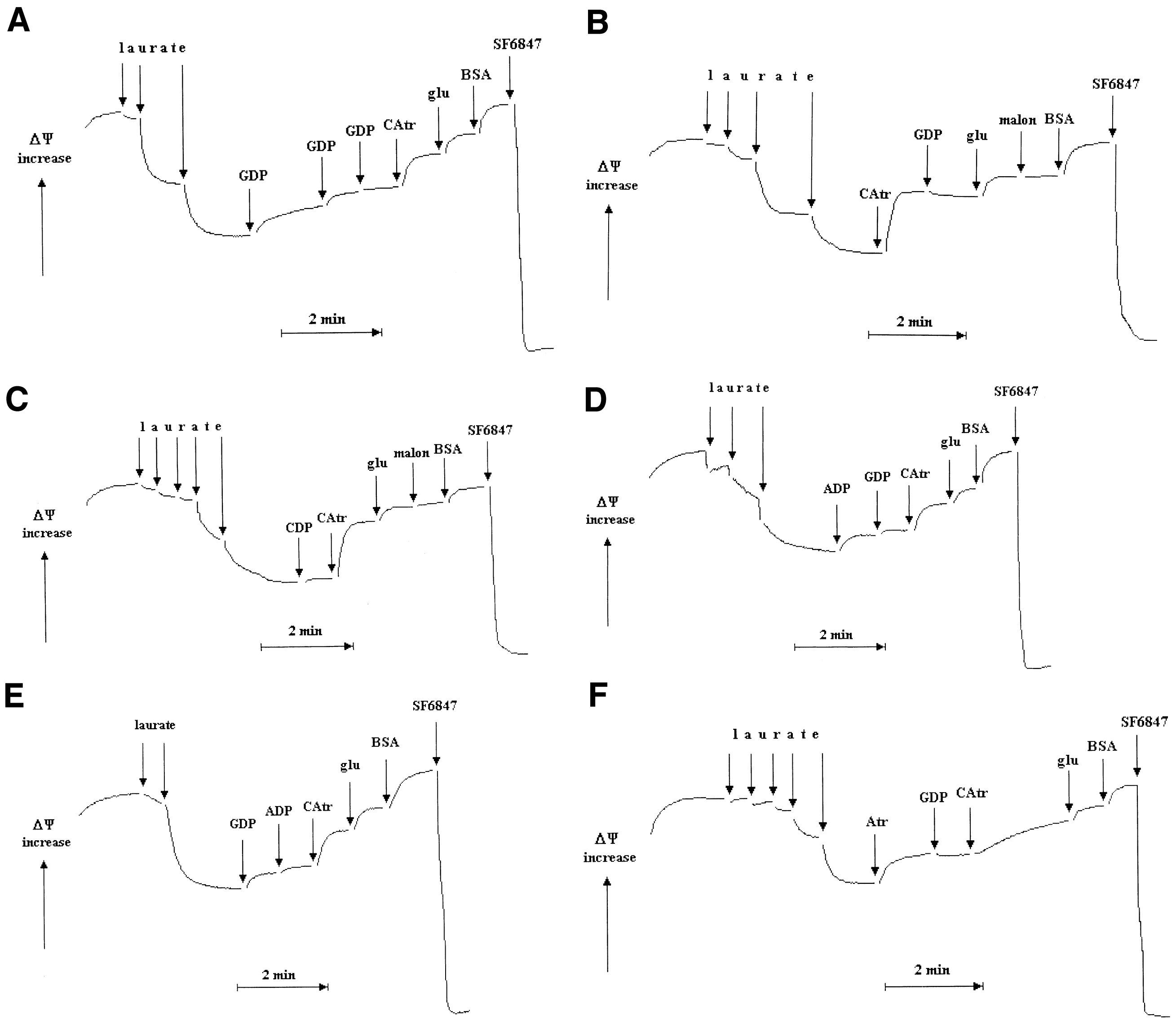

Fig. 6. Effect of uncouplers and recouplers on the membrane potential of skeletal muscle (A–I) or liver (J–L) mitochondria. Incubation medium contained 0.25 M sucrose, 10 mM MOPS (pH 7.4), 1 mM EGTA, BSA (2 mg/ml), 2×10^{-6} M rotenone, 5 mM ascorbate, 0.12 mM TMPD, oligomycin (3 μg/ml), 3 mM potassium phosphate. Laurate additions (μM): A, 20, 20 and 10; B, 20, 20 and 5; C, 20, 10, 10, 5 and 5; D, 30, 10 and 10; E, 30 and 20; F, 10 (each addition); J, 30 (each addition). Lauryl sulfate additions (μM): G, 100 (each addition); H, 150 and 100; K, 100, 100, 50 and 50; L, 200. GDP additions (μM): 50, 100 and 50 (A) or 200 (other figures). ADP additions (μM): D, E, 5; G, L, 5 and 200. Other additions: 2×10^{-6} M CAtr; 7 mM glutamate; 5 mM malonate; BSA (2 mg/ml); 6×10^{-8} M SF6847 and 2×10^{-6} M atractylate (Atr).

rat skeletal muscle (Fig. 6A–I) and liver (Fig. 6J–L) mitochondria were studied under identical conditions. BSA (2 mg/ml) was added to the homogenization, washing, storage and incubation media to avoid the uncoupling effect of endogenous fatty acids, which proved to be more pronounced in skeletal muscle than in liver. Ascorbate (with TMPD) was used as the respiratory substrate.

It is seen (Fig. 6A) that the membrane potential decrease caused by the laurate treatment of the skeletal muscle mitochondria is completely reversed by subsequent additions of GDP, CAtr, glutamate and BSA. Under conditions used, malonate is ineffective as a recoupler (Fig. 6B). CAtr added before GDP is more efficient as a recoupler than after GDP and completely prevents the GDP recoupling (Fig. 6B).

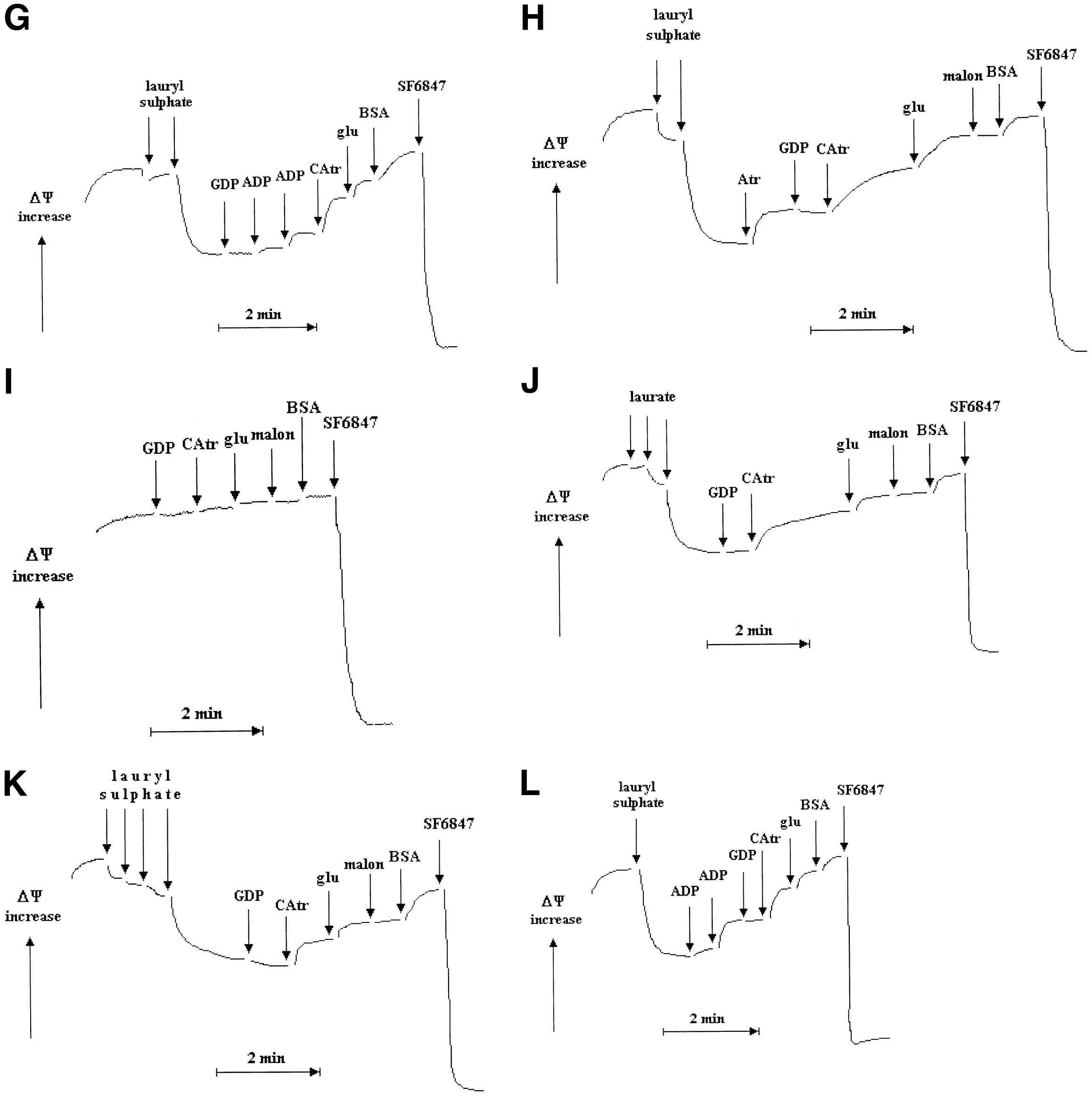

Fig. 6 (*continued*).

CDP fails to substitute for GDP (Fig. 6C). As to ADP, it appears to be efficient recoupler at concentrations lower than that of GDP (cf. Fig. 6A where the first GDP addition was 50 μM and Fig. 6D where 5 μM ADP was added). ADP (5 μM) when added after 200 μM GDP causes some additional recoupling (Fig. 6E). Atractylate is less efficient than CAtr as recoupler, but, like CAtr, prevents the GDP effect. Moreover, atractylate decelerates the CAtr recoupling action (Fig. 6F). Atractylate added after GDP is without effect (not shown).

Uncoupling by lauryl sulfate appears to be GDP-resistant but ADP, CAtr-, glutamate- and BSA-sensitive (Fig. 6G). As for atractylate, it is still less effective than CAtr. Again, CAtr added after atractylate recouples more slowly than without atractylate (Fig. 6H). None of the recouplers studied possess an effect in samples without laurate or lauryl sulfate (Fig. 6I).

In rat liver mitochondria, prepared and incubated identically to skeletal mitochondria, GDP is found to be inefficient as recoupler with both laurate (Fig. 6J) and lauryl sulfate (Fig. 6K). On the other hand, an ADP recoupling can be shown (Fig. 6L).

4. Discussion

4.1. *Lauryl sulfate, like laurate, uncouples via the antiporters but is less efficient*

The results summarized in this and previous [18] papers reveal that both the ADP/ATP and aspartate/glutamate antiporters are involved in lauryl sulfate-induced uncoupling (just as was found for the fatty acid-induced uncoupling [4–17,23]).

With both fatty acid and lauryl sulfate:

1. uncoupling is due to specific increase in H^+ conductance of the inner mitochondrial membrane [18];
2. CAtr and glutamate (or aspartate), when added together, cause 75–90% recoupling (Figs. 2, 3 and 6);
3. a pH increase is favorable for the CAtr recoupling and unfavorable for glutamate recoupling, the effect of combined action of CAtr and glutamate being pH-independent (Fig. 3);
4. addition of TPP^+ decreases the degree of the recoupling by CAtr and increases that by glutamate (Figs. 3 and 4);
5. the recoupling by CAtr is stronger than by atractylate (Fig. 6);
6. addition of atractylate before CAtr decelerates the CAtr effect (Fig. 6);
7. small concentrations of ADP have some recoupling activity (Fig. 6).

The only qualitative difference between effects of two uncouplers was revealed when recoupling by GDP in muscle mitochondria was studied. GDP found to recouple in the case of laurate but not of lauryl sulfate (for tentative explanation, see below).

On the other hand, the uncoupling efficiency of lauryl sulfate was much lower than that of laurate. For instance, to observe two-fold stimulation of respiration of the oligomycin-treated liver mitochondria, we added 160 μM lauryl sulfate or 20 μM laurate (Fig. 2). The observed difference in uncoupling efficiencies between fatty acids and lauryl sulfate may explain why Wojtczak et al. [21] failed to observe uncoupling with lauryl sulfate. They always used concentrations of lauryl sulfate as small as these of fatty acids. We employed higher lauryl sulfate concentrations that were, nevertheless, obviously too low to cause non-specific detergent or protein denaturating effects; otherwise, it is impossible to explain the above-listed specific features of the lauryl sulfate uncoupling.

In skeletal muscle mitochondria, an additional uncoupling mechanism was revealed which is (1) GDP-sensitive and (2) operative with laurate, but not with lauryl sulfate. This mechanism is absent in liver mitochondria (Fig. 6).

It is known that in skeletal muscles mRNAs of uncoupling proteins (UCP)2, 3L and 3S are present, whereas in hepatocytes are not (very small amounts of the UCP2 mRNA in liver most probably originate from Kupffer cells) [24,25]. In the sequence of the first two proteins like in that of the brown fat uncoupling protein (UCP1), a binding site for purine nucleotides is identified, whereas UCP3S lacks this site. Purine nucleoside di- and triphophates are known to be inhibitors of the UCP1 uncoupling activity (for review, see [20]). It was recently shown that these nucleotides inhibit UCP2 and UCP3L, too [26]. Concentrations of ADP and GDP required to inhibit UCP2 and UCP3L proved to be much higher than those of ADP saturating the ATP/ADP antiporter [26]. Thus, one might assume the following targets for effects of the studied recouplers on the laurate-uncoupled muscle mitochondria (see Fig. 6):

1. UCP2 and UCP3L for high GDP and ADP;
2. the ATP/ADP antiporter, UCP2 and UCP3L for CAtr, atractylate and low ADP;
3. the glutamate/aspartate antiporter for glutamate and aspartate.

As for BSA, it binds laurate and, hence, abolishes (1) the uncoupling mediated by UCP3S (if it is present in rat skeletal muscle mitochondria) and/or (2) any other uncoupling effects of laurate, resistant to GDP, CAtr and glutamate (aspartate), such as the cytochrome oxidase-mediated uncoupling [27].

It is noteworthy that, in our hands, malonate does not show any measurable recoupling. According to Wieskowski and Wojtczak [6], malonate caused small, but reproducible, recoupling when added to liver mitochondria. This was interpreted as an indication to involvement of the dicarboxylate carrier in the fatty acid-induced uncoupling. Apparently, contribution of this carrier to the uncoupling under our conditions proved to be negligible.

An additional assumption is required to explain the described relationships of the GDP and CAtr effects. According to Fig. 6A and B, CAtr effectively substitutes for GDP. In fact, (1) CAtr added before GDP shows larger uncoupling effect and (2) GDP added after CAtr cannot recouple. A possible explanation might consist in that CAtr inhibits not only the ATP/ADP antiporter, but also UCP2 and/or UCP3L. The same explanation might also be used for atractylate which, like CAtr, prevents the GDP recoupling in muscle mitochondria.

As for lauryl sulfate, GDP proved to be inefficient when added even before CAtr (Fig. 6G). This is not surprising since the protonophorous activity of lauryl sulfate cannot apparently be mediated by uncoupling proteins as was already shown for UCP1 (see below).

An alternative explanation of the above data consists in that it is the muscle isoform of ATP/ADP antiporter that is responsible for all the observed effects of GDP, CAtr and atractylate, whereas its liver isoform is GDP resistant.

4.2. *Possible mechanism of the lauryl sulfate uncoupling*

To account for lauryl sulfate uncoupling within the framework of the fatty acid cycle scheme [2–5], we need to assume that the mitochondrial antiporters not only translocate the laurate and lauryl sulfate anions from the inner to outer surface of the mitochondrial membrane, but also facilitate protonation of these anions by the external H^+ ions. Such an assumption seems necessary if we take into account that lauryl sulfate is, in contrast to laurate, a strong acid which per se cannot bind a proton and, hence, cannot operate as a protonophore, at neutral pH.

For fatty acids, it was shown that their incorporation into the liposomal membrane results in a strong alkaline shift of pK_a which becomes as high as 8.0 [28]. Thus at pH 7.4, membrane-bound fatty acids are mainly in their protonated form. As was shown in our group, this is not the case for lauryl sulfate which is mainly non-protonated at neutral pH even in a phospholipid bilayer (Yu.N. Antonenko, unpublished). Thus, to be protonated at neutral pH, the lauryl sulfate anionic group seems to require something more than being localized in the water–membrane interface.

It was suggested that cationic groups (e.g. lysine and arginine residues) of mitochondrial anion carriers are involved in binding of transported anions (A^{n-}) on one membrane side whereas protonation of A^{n-} facilitates release of A^{n-} to the water phase on the other membrane side [20]. Apparently, the protonation mechanism is also used to protonate lauryl sulfate translocated by antiporters from the inner to outer surface of the mitochondrial membrane [20]. If this is the case, the protonated lauryl sulfate, which is released from the anion carrier protein not far from the outer surface of the inner mitochondrial membrane, has two options: (1) to go to the nearest (outer) membrane surface; or (2) to diffuse to the opposite (inner) membrane surface. The majority of the protonated lauryl sulfate molecules uses option (1) as more probable whereas the minority uses option (2). This is why lauryl sulfate appears to be a much less efficient uncoupler than laurate (see above, Fig. 2). The uncoupling efficiency of lauryl sulfate would be even lower if its concentration near the outer membrane surface were equal to that near the inner one. However, this is most probably not the case. Lauryl sulfate should accumulate in the outer membrane leaflet due to electrophoretic efflux of its anionic form, mediated by the anion carriers (Fig. 7).

The postulated protonation mechanism may be essential for transport of substances containing more than one anionic group, such as ATP^{4-}, ADP^{3-}, dicarboxylic amino acids, etc. On the other hand, it is not necessary for translocation of the fatty acid monoanions since decomposition of a complex of monoanion with a cationic group of the carrier should be much easier than decomposition of a complex of, say, ATP^{4-} with four cationic groups of the ATP/ADP antiporter. It is shown that the brown fat UCP1 still binds purine nucleotides, transports them to some depth into the membrane, but cannot release the nucleotides on the opposite membrane side (probably because the protonation mechanism was lost when the ATP/ADP antiporter evolved to UCP) [20]. UCP1 seems to catalyze the fatty acid

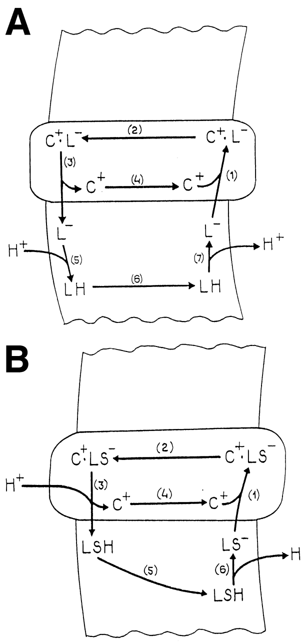

Fig. 7. Scheme explaining the uncoupling by laurate (A) and lauryl sulfate (B) mediated by some mitochondrial anion carriers (the ATP/ADP and aspartate/glutamate antiporters). It is assumed (Fig. 7A) that laurate anion (L^-) combines with a cationic residue (C^+) of the carrier near the inner surface of the inner mitochondrial membrane (step 1). The $C^+\cdot L^-$ complex crosses the membrane (step 2) and decomposes near the outer membrane surface to L^- and C^+ (step 3). The released C^+ returns to the inner membrane surface (step 4) whereas L^- combines with extramitochondrial H^+ to form lauric acid (LH) (step 5). LH diffuses to the inner membrane surface (step 6) and releases H^+ to the matrix (step 7). For lauryl sulfate (Fig. 7B), the mechanism differs in that protonation of the anion (LS^-) is obligatory carried out by the carrier (step 3). The formed LSH either releases H^+ to the outer water phase, resulting in accumulation of LS^- in the outer membrane leaflet (not shown), or goes to the inner membrane surface (step 5) to release H^+ to the intramitochondrial water. Thus, according to the scheme, the anion carrier not only facilitates translocation of the anionic form of an uncoupler, but also catalyzes its protonation near the outer membrane surface.

circuit, being involved in the fatty acid anion translocation. As for the anion protonation, it may occur spontaneously on the membrane surface due to the above mentioned alkaline shift of pK_a of fatty acid in the membrane/water interface. Such an assumption explains why fatty acids enhance, while lauryl sulfate and other alkyl sulfates and sulfonates do not, the H^+ conductance in UCP1 proteoliposomes. In fact, alkyl sulfate and sulfonate anions are transported by UCP1 and interfere with fatty acid-linked H^+-conductance. Dicarboxylates and other di- and polyanions are not transported by UCP1 [29,30]. On the contrary, the ATP/ADP antiporter was shown in our group to mediate uncoupling by dicarboxylic fatty acids in a CAtr-sensitive manner [31].

To explain a putative inhibitory effect of CAtr and atractylate on laurate-induced uncoupling mediated by UCP2 and UCP3L, we can apply the same reasoning as for that mediated by the ATP/ADP antiporter (see [2,3,20]). Apparently, these ligands are bound with the nucleotide-binding gate of these anion carriers rather than with their anion-translocating machinery shown in Fig. 7. This binding affects the machinery in some indirect way causing its inhibition which is stronger with CAtr than with atractylate although both CAtr and atractylate completely arrest the gate since they prevent the GDP action (see Fig. 6).

4.3. *pH dependence of the CAtr- and glutamate-induced recoupling: effect of the surface charge*

When studying the palmitate uncoupling, we found that the pH values equal to 7.8 or 7.0 are favorable for recoupling by CAtr or glutamate (aspartate), respectively [17]. The data of Fig. 3A–C show that such a pH effect is also inherent in uncoupling caused by both laurate and lauryl sulfate. These relationships may be due to possible involvement of lysine and histidine residues, strongly differing in pK values, in binding of the fatty acid anions by ATP/ADP- and aspartate/glutamate antiporters, respectively [17]. In this context, it should be mentioned that, according to Krämer and coworkers [32], the pH lowering from 7.4 to 6.9 decreases K_m of the aspartate/glutamate antiporter for the external glutamate from 1.8 to 0.6 mM and for aspartate from 0.17 to 0.09 mM.

At pH 7.4, addition of cations lowering the negative membrane charge (TPP^+) reduces the CAtr-sensitive recoupling and elevates the glutamate-sensitive one (see [23] and this paper, Fig. 4). Such an effect may be explained assuming that the effective pH level in the interface was shifted to alkaline values due to the cation binding to the negatively charged phospholipid and protein surface residues.

If this proposal is true, introduction of additional negative charges to the interface should produce the opposite effect. This assumption is in line with data of Fig. 5 which shows that a low lauryl sulfate concentration increases the coupling effect of CAtr and decreases that of glutamate in mitochondria uncoupled by laurate. The stronger recoupling effect of CAtr in experiments with the lauryl sulfate uncoupling than that with the laurate uncoupling at pH 7.4 (see Fig. 3) may be accounted for in the same manner.

5. Conclusion

The results summarized in this paper demonstrate that not only weak acids, but also some strong acids possessing a hydrophobic tail can operate as uncouplers of oxidative phosphorylation. This effect is mediated by mitochondrial carriers of anions bearing two or more negative charges, i.e. the ATP/ADP- and glutamate/aspartate antiporters that are also involved in uncoupling by fatty acids. It is assumed that the antiporters, besides facilitating the anion transport, carry out protonation of the transported anion bound to a cationic protein ligand.

In this respect, the antiporters differ from uncoupling proteins which seem to translocate anions of fatty acids as well as other monoanions, but cannot protonate them.

Uncoupling by relatively low concentrations of lauryl sulfate may be involved in the toxicity of this and other anionic detergents [2].

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