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Involvement of aspartate/glutamate antiporter in fatty acid-induced uncoupling of liver mitochondria

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Abstract

Effects of aspartate, glutamate and an inhibitor of the aspartate/glutamate antiporter, diethylpyrocarbonate (DEPC), on uncoupling of the energy transduction processes in rat liver mitochondria have been investigated. It is found that both the antiporter substrates and the antiporter inhibitor operate as recouplers when uncoupling is caused by free fatty acids (FFA). Recoupling consists in (1) partial inhibition of the FFA-stimulated respiration and (2) some increase in the membrane potential. Half-maximal effects are observed at concentrations of glutamate and aspartate close the K_m values of the antiporter. Recouplings by glutamate (aspartate) and DEPC are not additive. On the other hand, recoupling by any of these compounds and carboxyatractylate or ADP appears to be additive. Uncoupling by dinitrophenol is less sensitive to the recouplers whereas that by FCCP is not sensitive at all. It is concluded that uncoupling by FFA in rat liver mitochondria is mediated not only by the ATP/ADP antiporter but also by the aspartate/glutamate antiporter.

Keywords: Uncoupler; Fatty acid; ATP/ADP antiporter; Aspartate/glutamate antiporter; Diethylpyrocarbonate; Liver mitochondria

1. Introduction

The mechanism of uncoupling of oxidative phosphorylation by free (non-esterified) long-chain fatty acids (FFA) is studied since 1956 [1,2]. The studies showed that uncoupling by endogenous FFA plays an essential role in thermoregulatory heat production [3–5]. Under some pathological conditions, these natural uncouplers were found to damage cellular energetics (for references, see [6]).

Uncoupling effect of low FFA concentrations is due to their protonophoric activity. The ATP/ADP antiporter is assumed to be involved in this process since mitochondria uncoupled by FFA can be recoupled by CAtr, the most powerful and very specific inhibitor of the ATP/ADP antiporter [7]. Recently this effect was reproduced on proteoliposomes containing purified ATP/ADP-antiporter [8,9].

There is a correlation between amount of the ATP/ADP antiporter and degree of the CAtr recoupling in various tissues. Both parameters were found

Abbreviations: FCCP, *p*-trifluoromethoxycarbonylcyanide phenylhydrazone; DNP, 2,4 dinitrophenol; CAtr, carboxyatractylate; DEPC, diethylpyrocarbonate; EGTA, ethyleneglycol-bis-(2-aminoethylether)-*N*,*N*,*N*′,*N*′-tetraacetic acid; FFA, long-chain free fatty acids; TPP^+, tetraphenylphosphonium.

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to be maximal in heart and minimal in liver mitochondria [10]. It was assumed that in liver mitochondria, proteins are not involved in the CAtr-insensitive uncoupling [6,10,11].

In this group, it was proposed that the ATP/ADP antiporter facilitates uncoupling by FFA, transporting fatty acid anions from the mitochondrial matrix to the intermembrane space [5,12]. As for the CAtr-insensitive FFA uncoupling, it was suggested to be mediated by other anion carrier(s) [5].

The ADP/ATP antiporter is structurally similar to some other mitochondrial anion carriers, i.e., phosphate/OH antiporter, aspartate/glutamate antiporter and thermogenine [13]. The aspartate/glutamate antiporter was shown to resemble the ADP/ATP antiporter also in that oxidation of its SH-groups caused rather non-specific membrane permeabilization [14]. On the other hand, activity of the aspartate/glutamate antiporter in proteoliposomes was inhibited by 80 μM DEPC which did not influence the ADP/ATP antiporter activity [14].

Recently we have shown that DEPC and glutamate caused some recoupling effect (inhibition of respiration and increase in membrane potential) of the CAtr-treated liver mitochondria uncoupled by palmitate [15].

In the present paper, the recoupling effect of glutamate, aspartate, DEPC, CAtr, ADP and their combinations has been demonstrated. The results are in line with proposal that the aspartate/glutamate carrier is involved in the uncoupling effect of FFA. The relationships between the FFA-induced uncoupling and switching the anion carriers from the antiport to the uniport mode [16] is discussed.

2. Materials and methods

Mitochondria were isolated from liver of white rats of 180–220 g weight. The isolation medium contained 250 mM sucrose, 5 mM MOPS, 1 mM EGTA (pH 7.4). The homogenate was centrifuged at 700 g for 10 min. Mitochondria were sedimented at 10 000 $\times g$ for 10 min, resuspended in 1 ml of isolation medium supplemented with BSA (3 mg/ml), diluted with 30 ml isolation medium without BSA, and centrifuged at 10 000 $\times g$ for 10 min. The mitochondrial pellet was resuspended in isolation medium. In some experiments, BSA (3 mg/ml) was also added to the suspension medium. The final mitochondrial suspension contained about 60 mg protein/ml.

Mitochondrial protein was measured by the biuret method.

Oxygen consumption was recorded by a Clark type oxygen electrode and LP-9 polarograph. The incubation medium contained 250 mM sucrose, 0.5 mM EGTA, 5 mM succinate, 2 μM rotenone and 5 mM MOPS-KOH, pH 7.4. In the majority of experiments, the incubation medium was supplemented with oligomycin (2 μg/ml). The concentration of mitochondrial protein was about 1 mg/ml.

Membrane potential was estimated by TPP^+ distribution measured using a TPP^+-sensitive electrode. In these experiments, the incubation medium was supplemented with 1.6 μM TPP^+-chloride. The concentration of the mitochondrial protein was about 1 mg/ml. Incubation temperature, 25°C.

MOPS, palmitic acid, lauric acid, oligomycin, succinate, glutamate, CAtr, EGTA were from Sigma; rotenone and ADP, from Serva; DNP and TPP, from Fluka; DEPC, from Aldrich-Chemie; aspartate from Reanal. Aspartate was twice recrystallized from bidistilled water. Sucrose was twice precipitated from concentrated solution in bidistilled water with distilled ethanol. Ethanol solutions of 100 mM DEPC, 10 mM palmitic acid and 10 mM lauric acid were used.

3. Results and discussion

In Fig. 1 the time courses of oxygen consumption by rat liver mitochondria after consecutive additions of palmitic acid, CAtr, glutamate, aspartate and DEPC are shown. One can see that 15 μM palmitate causes 2.5-times stimulation of succinate oxidation in the oligomycin-treated mitochondria. All the other above mentioned substances exerted the recoupling effect, i.e., they decrease the respiration rate. As a result, about 80% palmitate-induced stimulation of respiration can be abolished (Fig. 1). Subsequent FCCP addition stimulates the oxygen consumption.

1 mM β-hydroxybutyrate, glutamine or malate, being added instead of glutamate or aspartate exerted no recoupling effect. It was also found that laurate effectively substitutes for palmitate (not shown).

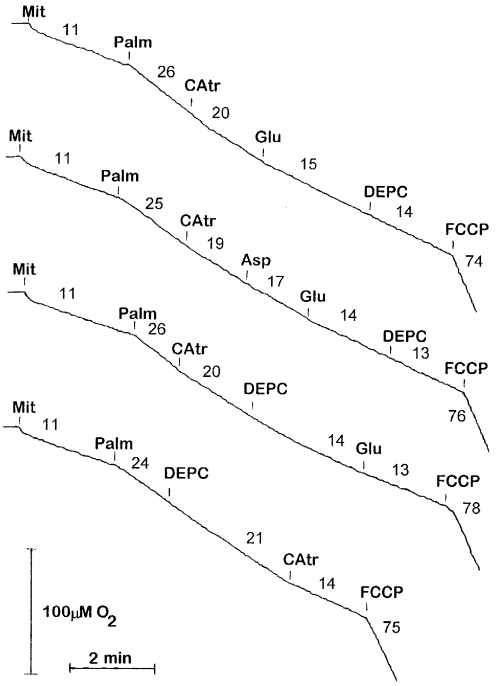


Fig. 1. Recoupling effect of CAtr, glutamate, aspartate and DEPC on the palmitate-uncoupled respiration of rat liver mitochondria. Incubation medium contained 250 mM sucrose, 5 mM succinate, 2 μM rotenone, oligomycin (2 μg/ml), 0.5 mM EGTA and 5 mM MOPS (pH 7.4), rat liver mitochondrial protein (1 mg/ml). For other experimental conditions, see Section 2. Additions, Palm, 15 μM palmitate; 1 μM CAtr; Glu, 0.6 mM glutamate; Asp, 0.6 mM aspartate; 100 μM DEPC; 200 nM FCCP. Figures near curves, rates of oxygen consumption (nmol O_2/min/mg protein).

Measurement of membrane potential in mitochondria with TPP^+-sensitive electrode (Fig. 2) showed that glutamate, aspartate and DEPC, when added after CAtr, caused some increase in the membrane potential lowered by palmitate. All these substances were ineffective when FCCP was added instead of palmitate. Thus, recouplers reversed both uncoupling effects of palmitate on respiring mitochondria, i.e., stimulation of respiration and membrane potential decrease.

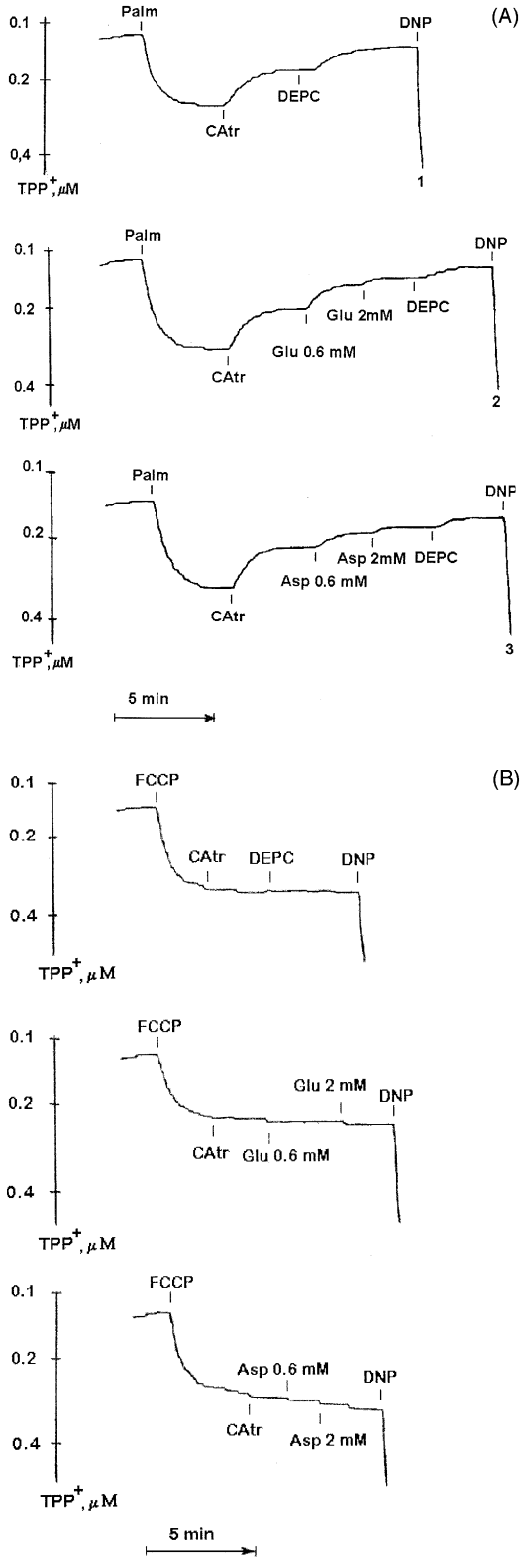


Fig. 2. Recoupling effect of CAtr, glutamate, aspartate and DEPC on membrane potential in palmitate-treated mitochondria. Incubation medium (see Fig. 1) was supplemented with 1.6 μM TPP^+ and 20 nM nigericine and BSA (0.2 mg/ml). Additions, Palm, 20 μM palmitate; 1 μM CAtr; Glu, glutamate; Asp, aspartate; 100 μM DEPC; 60 nM FCCP; 100 μM DNP.

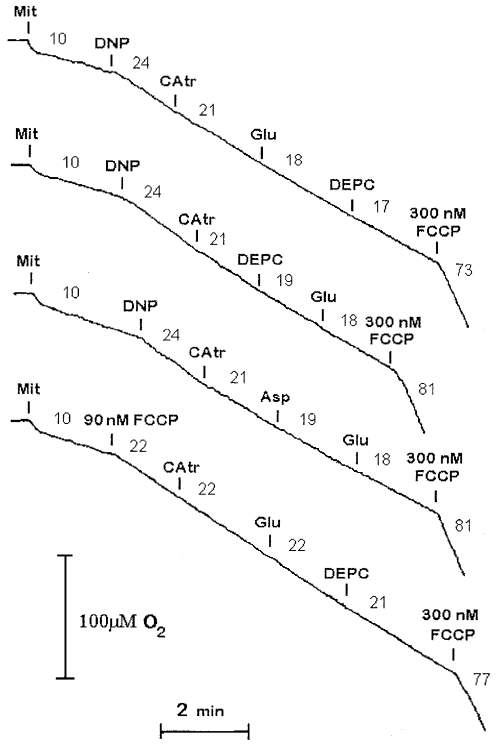


Fig. 3. Recoupling effect of CAtr, glutamate, aspartate and DEPC on the DNP-uncoupled respiration. Incubation medium and experimental conditions as in Fig. 1, but media for storage and incubation of mitochondria were supplemented with BSA (3 and 0.2 mg/ml, respectively). Addition, 8 μM DNP; other additions as in Fig. 1.

CAtr, glutamate, aspartate and DEPC also showed some recoupling effect when they were added after 8 μM DNP which stimulated the respiration rate to the same degree as 15 μM palmitate (Fig. 3). However, in this case the final recoupling effect was lower, namely about 45%. With 90 nM FCCP causing 2.2-times stimulation of the respiration rate, CAtr, glutamate and aspartate have no measurable recoupling effect; there was some DEPC inhibition but very small.

In Fig. 4 we compared the DEPC effect on the CAtr titration of the State 3 and State 3^u (palmitate) respiration rates. It is seen that DEPC inhibits the State 3 respiration only slightly (Fig. 4A). On the

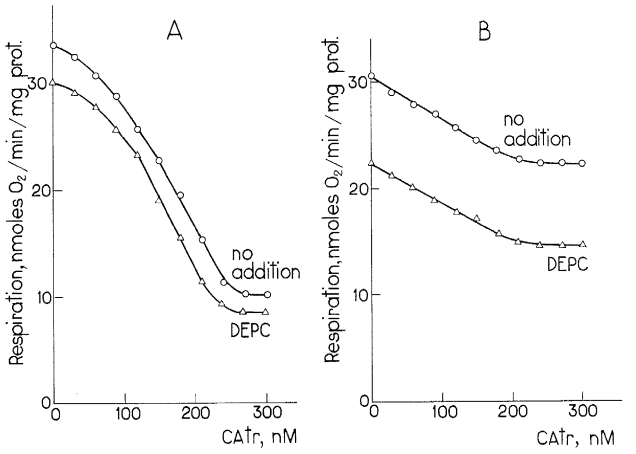
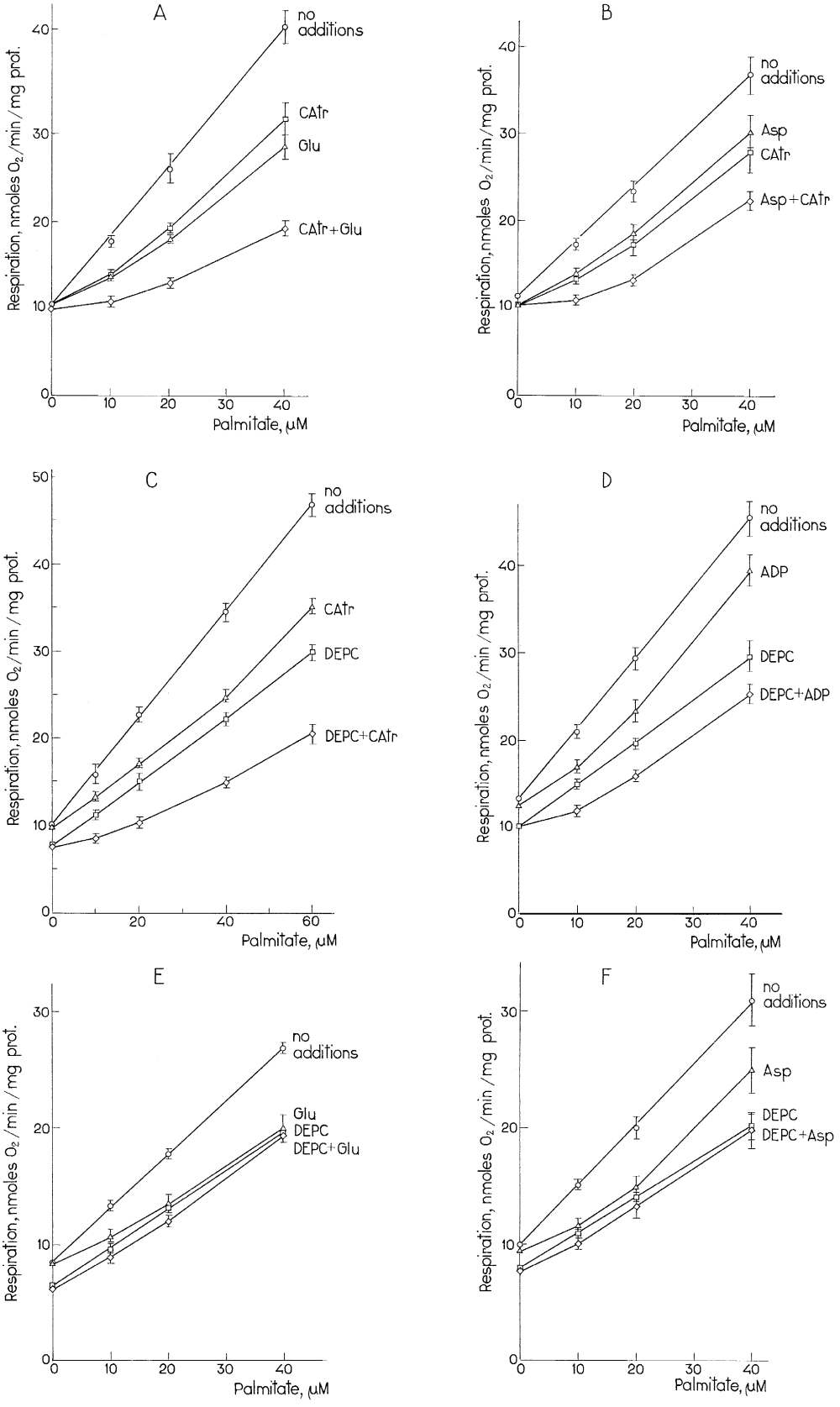


Fig. 4. Effects of CAtr and DEPC on respiration in State 3 (A) and in State 3^u with palmitate (B). Incubation medium without oligomycin (A) or with oligomycin (B) was used. Other experimental conditions as in Fig. 1. A: the medium is supplemented with 5 mM KH_2 PO_4 and 2 mM ADP; B: the medium contained 15 μM palmitate. Addition, 100 μM DEPC.

other hand, the DEPC inhibition appears to be pronounced when respiration was uncoupled by palmitate (Fig. 4B). In both cases, DEPC does not affect either level of the CAtr concentration causing half maximal inhibition, or the shape of the CAtr titration curves. Thus CAtr and DEPC, when acting as recouplers, seem to attack different targets. In this context, it should be mentioned that recoupling by DEPC (but not by CAtr) can be increased by addition of 2 mM $MgCl_2$ to the incubation medium (not shown in the figures).

Recoupling effects of glutamate, aspartate, CAtr, as well as of ADP which can partially substitute for CAtr as recoupler [7], were shown to be stronger at low palmitate concentration (Fig. 5). On the other hand, the DEPC recoupling was not dependent on the palmitate level (Fig. 5C–F). It is noteworthy that combined treatment with CAtr (or ADP) and glutamate (or aspartate, or DEPC) always resulted in stronger recoupling than that caused by single recoupler (Fig. 5A–D). This is one more indication that targets of these two groups of inhibitors are different.

Fig. 5. Effects of various recouplers at different FFA concentrations. Incubation medium and experimental conditions as in Fig. 1. Additions, 1 μM CAtr; Glu, 1 mM glutamate; Asp, 1 mM aspartate; 100 μM DEPC; 50 μM ADP. All recouplers were added before palmitate.


A
no additions
CATr
Glu
CATr+Glu
Respiration, nmoles O2/min/mg prot.
Palmitate, μM
B
no additions
Asp
CATr
Asp+CATr
Respiration, nmoles O2/min/mg prot.
Palmitate, μM
C
no additions
CATr
DEPC
DEPC+CATr
Respiration, nmoles O2/min/mg prot.
Palmitate, μM
D
no additions
ADP
DEPC
DEPC+ADP
Respiration, nmoles O2/min/mg prot.
Palmitate, μM
E
no additions
Glu
DEPC
DEPC+Glu
Respiration, nmoles O2/min/mg prot.
Palmitate, μM
F
no additions
Asp
DEPC
DEPC+Asp
Respiration, nmoles O2/min/mg prot.
Palmitate, μM

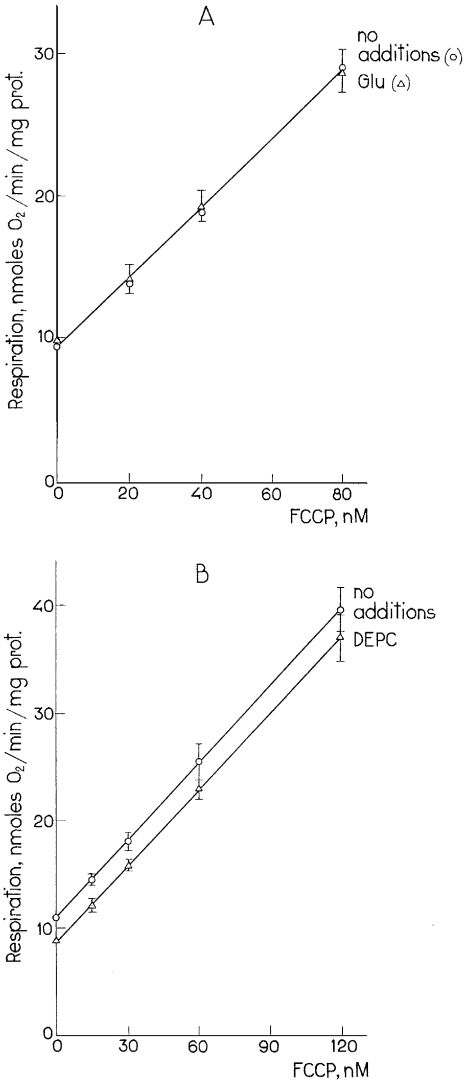


Fig. 6. The absence of the DEPC and glutamate recoupling effects when FCCP is used as uncoupler. Incubation medium and experimental conditions as in Fig. 1. 1 mM glutamate (A) or 100 μM DEPC (B) were added before FCCP.

In the case of FCCP-mediated uncoupling, all the recouplers tested were without effect or their influence was very small (Fig. 6).

Half-maximal glutamate recoupling of the palmitate-uncoupled mitochondria was observed at about 160 μM glutamate. This value did not depend upon

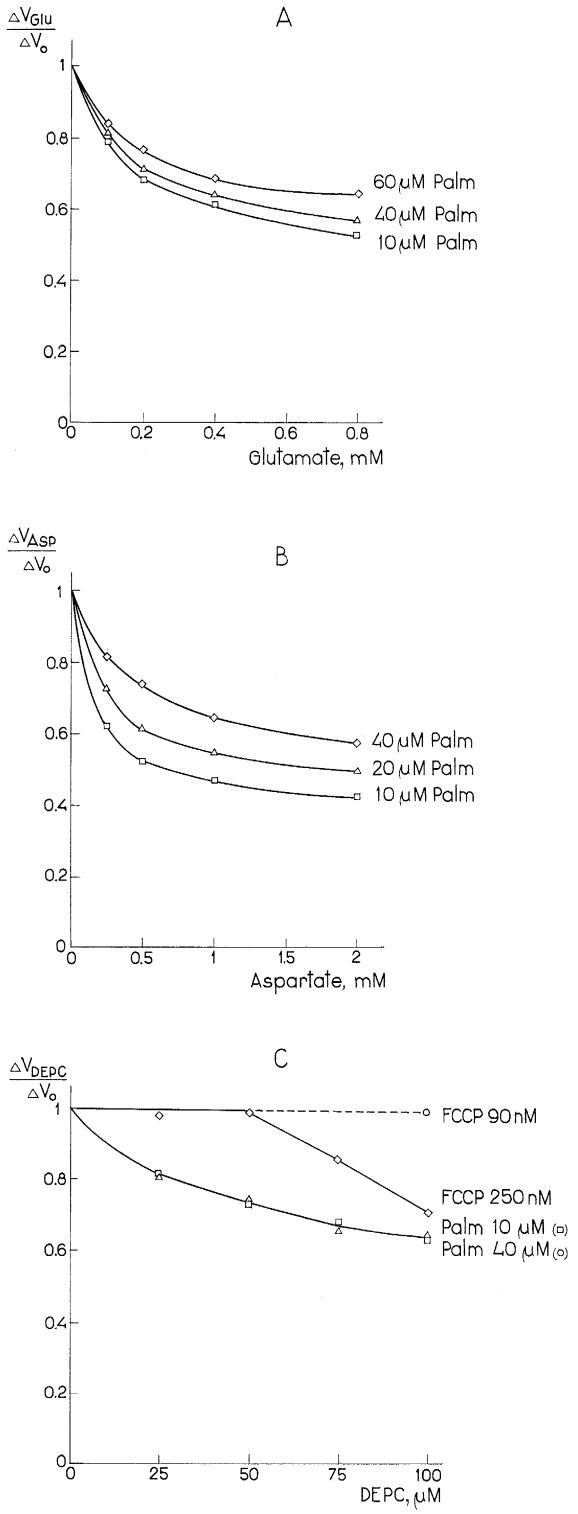


Fig. 7. Recoupling effects of glutamate, aspartate and DEPC on the palmitate-uncoupled mitochondria as a function of the recoupler concentration. Incubation medium and experimental conditions as in Fig. 1. ΔV_0, ΔV_{Glu}, ΔV_{Asp} and ΔV_{DEPC}, increase in the respiration rates caused by addition of uncoupler (palmitate or FCCP), or by uncoupler with glutamate, aspartate or DEPC, respectively. Glutamate, aspartate and DEPC were added before palmitate.

palmitate concentration within the 10–60 μM range (Fig. 7A). Similar relationships were revealed when aspartate substituted for glutamate (Fig. 7B). It should be stressed that the aspartate and glutamate concentrations causing half maximal recoupling were close to their K_m values for the aspartate/glutamate antiporter [17].

Recoupling effect of DEPC was also independent from the palmitate concentration. Half-maximal recoupling was observed at about 60 μM DEPC. Higher DEPC concentrations were required for inhibition of respiration stimulated by 250 nM FCCP. With 90 nM FCCP the DEPC inhibition was hardly measurable (Fig. 7C). The DEPC inhibition with FCCP may be explained by the direct effect on the respiratory chain [18].

In samples without added uncouplers, DEPC also showed some inhibition of respiration (Fig. 6B), K_m being the same as for sample with palmitate (not shown). Such action was most probably due to removal of the uncoupling induced by endogenous FFA.

The above data suggest that not only the ATP/ADP antiporter but also the aspartate/glutamate antiporter are involved in the FFA-induced uncoupling.

Recently, Herick and Krämer [16] showed that both the ATP/ADP antiporter and the aspartate/glutamate antiporter in proteoliposomes switch from antiport to uniport mode of functioning when treated with mersalyl. Such a treatment strongly decreased the specificity of the carriers to the transported solutes. However, uniport of anions was still much faster than that of cations [16]. One may suggest that ability to carry out non-specific anion uniport is inherent even in the intact ATP/ADP and aspartate/glutamate antiporters provided that the anion in question is hydrophobic. In this connection, we may mention that uncoupling by small concentrations of dodecylsulphate anion is sensitive to CAtr in a fashion like that by FFA [19].

Involvement of the aspartate/glutamate antiporter in the FFA-induced uncoupling indicates that CAtr-resistant constituent of this uncoupling is still mediated by some mitochondrial proteins. It would be interesting to study a possible physiological role of such an energy-dissipative mechanism in thermoregulation. In any case, the above observations are in line with the concept assuming that the protein-mediated efflux from mitochondria of the uncoupler anions is involved in the action of various protonophores [20].

Acknowledgements

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