Influence of fatty acid oxidation on the outer mitochondrial membrane permeability for ADP: dependence on the concentration of fatty acids and oncotic pressure

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Oxidation of fatty acids, palmitoyl-L-carnitine (PC) and octanoyl-L-carnitine (OC), in the mitochondria of saponin-permeabilized rat heart fibers, when applied in combination with pyruvate+malate, decreases the apparent Km of oxidative phosphorylation for ADP (app. K_m^{ADP}) three times in a concentration-independent manner. It means a high increase in the outer mitochondrial membrane permeability for ADP. Noteworthy, it occurs at a very low concentration of PC (2.2 μM). The low app. K_m^{ADP} value (77 μM) observed in case of OC oxidation was increased more than three times by 5% dextran T-70. Though the exact mechanism of the phenomenon is not clear, the morphological changes of mitochondria seem to be important.

Key words: saponin-permeabilized fibers; Michaelis-Menten constant, outer mitochondrial membrane permeability, oxidative phosphorylation, fatty acid oxidation

Abbreviations: OMM, outer mitochondrial membrane; IMM, inner mitochondrial membrane

INTRODUCTION

The last decade studies of saponin-permeabilized heart and skeletal muscle fibers have led to the observation that, in contrast to isolated mitochondria, the outer mitochondrial membrane (OMM) *in situ* possesses a low permeability for ADP (high apparent K_m of oxidative phosphorylation for ADP (app. K_m^{ADP})), and, therefore, plays a crucial role in the mechanism of regulation of mitochondrial respiration *in vivo* [1–3]. Our experiments performed on saponin-permeabilized rat cardiac fibers confirmed this observation [4, 5]. The app. K_m^{ADP} values in the above-mentioned studies were estimated by using glutamate+malate, pyruvate+malate or succinate as a respiratory substrate [1–5]. Recently we have found [6] that a very high value of app. K_m^{ADP} (236 μM ADP), which is characteristic of saponin-treated rat cardiac fibers respiring on pyruvate+malate, drastically (up to 10-fold) decreases in the case of fatty acid oxidation. Noteworthy, octanoyl-D-carnitine, as well as palmitate, palmitoyl-CoA, and palmitoyl-L-carnitine were not effective in this respect, when their oxidation was prevented by the absence of necessary cofactors or blocked with rotenone. Our data suggest that it is oxidation but not transport of fatty acids into mitochondria that induces an increase in the OMM permeability for ADP. However, the precise mechanism of this phenomenon was not elucidated. An assumption was made that it could be partly related to the fatty-acid-induced morphological changes of mitochondria. To get a deeper insight into the problem, the dependence of app. K_m^{ADP} on fatty acid concentration and oncotic pressure was investigated in the present study.

MATERIALS AND METHODS

The saponin-permeabilized myocardial fibers were prepared from the biopsied specimens of Wistar ma-

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le rat hearts, and the fiber respiration measurements (at 37 °C) were performed exactly as described earlier in [6]. The concentrations of the respiration substrates and dextran T-70 (Amersham Pharmacia Biotech AB, Sweden) are presented in the legend of the Figure.

RESULTS AND DISCUSSION

In our previous study [6], an assumption was made that the considerable increase in OMM permeability to ADP (decrease of app. K_m^{ADP}) during fatty acid oxidation could be, at least in part, related to the fatty-acid-induced swelling of the mitochondria.

Figure, A shows that app. K_m^{ADP} during pyruvate+malate oxidation in the cardiac fibers is very high (368 ± 50 μM ADP) compared to isolated mitochondria (23 μM ADP [5]) and decreases to 109 ± 14 and 122 ± 13 μM ADP, *i.e.* to 30 and 33% of control values, when 2.2 μM or 8.8 μM palmitoyl-L-carnitine, respectively, was used in combination with pyruvate+malate.

Essentially similar results were obtained in a separate group of experiments when octanoyl-L-carnitine was used instead of palmitoyl-L-carnitine (Figure, B); in these experiments, the app. K_m^{ADP} value for pyruvate+malate oxidation was much lower than that in the previous group (see Figure, A), however, the effect of octanoyl-L-carnitine on app. K_m^{ADP} was completely equal to that of palmitoyl-L--carnitine. Thus, it may be concluded that the extent of fatty acid oxidation-induced increase in the OMM permeability to ADP (decrease in K_m^{ADP} value) does not depend on the concentration of fatty acids, at least in the range used in this work. Moreover, it should be noted that the concentrations of carnitine esters of fatty acids that induce the K_m^{ADP} decrease are very low, particularly in the case of palmitoyl-L-carnitine (2.2 μM). At this concentration (2.2 μM), the rate of palmitoyl–L-carnitine oxidation in State 3 was equal to 30–40% of that estimated at 8.8 μM.

Further experiments demonstrated (Figure, C) that in the case of octanoyl-L-carnitine oxidation 5% dextran T-70 added to the incubation medium increased app. K_m^{ADP} more than three times (from 77 ± 20 μM to 243 ± 60 μM), *i.e.* dextran affected app. K_m^{ADP} in opposite direction, but by the same factor as fatty acids.

It is known that dextran or other macromolecules decrease the conductivity of porin pores in artificial membranes [7, 8], the volume of the intermembrane space in the isolated mitochondria, and

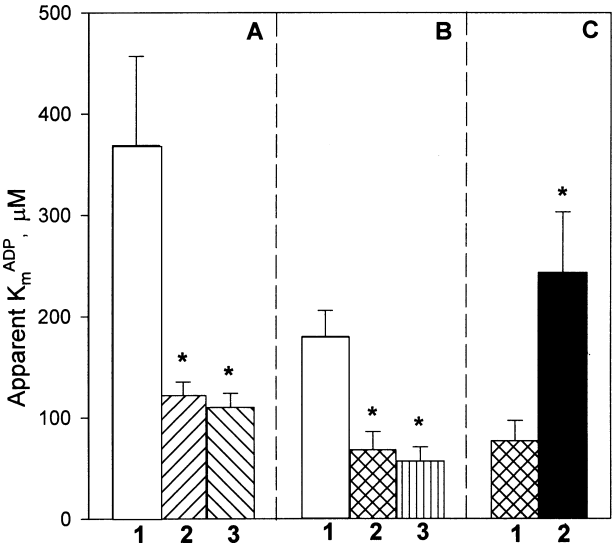

Figure. Effect of palmitoyl-L-carnitine (A), octanoyl-L-carnitine (B) and dextrane (C) on apparent K_m for ADP. **A** 1 = 6 mM pyruvate + 6 mM malate (n = 7); 2 = = 6 mM pyruvate + 6 mM malate + 8.8 μM palmitoyl-L-carnitine (n = 6); 3 = 6 mM pyruvate + 6 mM malate + 2.2 μM palmitoyl-L-carnitine (n = 9); **B** 1 = = 6 mM pyruvate + 6 mM malate (n = 7); 2 = 6 mM pyruvate + 6 mM malate + 0.36 mM octanoyl-L-carnitine (n = 3); 3 = 6 mM pyruvate + 6 mM malate + + 0.1 mM octanoyl-L-carnitine (n = 3); **C** 1 = 0.36 mM octanoyl-L-carnitine + 0.24 mM malate (n = 7); 2 = = 0.36 mM octanoyl-L-carnitine + 0.24 mM malate + + 5% dextran T-70 (n = 7). *P < 0.05 vs experiment 1. The State 3 respiratory rates (in ngatoms O_2/min/mg fiber dry weight) and respiratory control index values were as follows: **A** 1 = 198 ± 8 and 3.38 ± 0.3; 2 = 103 ± ± 6 and 3.6 ± 0.4; 3 = 108 ± 6 and 3.27 ± 0.2; **B** 1 = = 147 ± 11 and 3.52 ± 0.3; 2 = 169 ± 12 and 3.18 ± ± 0.2; 3 = 136 ± 18 and 2.67 ± 0.4; **C** 1 = 135 ± 15 and 3.27 ± 0.3; 2 = 107 ± 14 and 2.61 ± 0.2.
Note The differences revealed in app K_m^{ADP} values (pyruvate + malate, A and B) in different sets of experiments are probably due to seasonal variations.

increases the number of contact sites between both mitochondrial membranes [9]. The morphological changes of the isolated mitochondria are accompanied by a reduced OMM permeability for adenine nucleotides [9]. In conclusion, the data obtained in this work suggest that the fatty acid oxidation-induced increase in OMM permeability for ADP can be related to the morphological changes of mitochondria.

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References

1. Saks VA, Veksler VI, Kuznetsov AV et al. Mol Cell Biochem 1998; 184: 81–100.
2. Saks VA, Kuznetsov AV, Khuchua ZA et al. J Mol Cell Cardiol 1995; 27: 625–45.
3. Kay L, Li Z, Mericskay M et al. Biochim Biophys Acta 1997; 1322: 41–59.
4. Toleikis A, Majiene D, Trumbeckaite S et al. Biosci Rep 1996; 16: 513–9.
5. Liobikas J, Kopustinskiene DM, Toleikis A. Biochim Biophys Acta 2001; 1505: 220–5.
6. Toleikis A, Liobikas J, Trumbeckaite S et al. FEBS Lett 2001; 509: 245–9.
7. Gellerich FN, Wagner M, Kapischke M et al. Biochim Biophys Acta 1993; 1142: 217–27.
8. Zimmerberg J, Parsegian VA. Nature 1986; 323: 36–9.
9. Gellerich FN, Kapischke M, Kunz W et al. Mol Cell Biochem 1994; 133/134: 85–104.

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RIEBALŲ RŪGŠČIŲ OKSIDACIJOS POVEIKIS REGULIUOJANT MITOCHONDRIJŲ IŠORINĖS MEMBRANOS PRALAIDUMĄ ADENOZINO DIFOSFATUI: PRIKLAUSOMYBĖ NUO RIEBALŲ RŪGŠČIŲ KONCENTRACIJOS IR ONKOTINIO SLĖGIO

S a n t r a u k a

Nustatyta, kad oksiduojant riebalų rūgštis (palmitoil-L-karnitiną ir oktanoil-L-karnitiną) mišinyje su piruvatu ir malatu, trigubai sumažėja tariamoji K_m^{ADP} reikšmė, kuri būdinga saponinu permeabilizuotų žiurkės miokardo skaidulų mitochondrijoms, oksiduojančioms tik piruvatą ir malatą. Pažymėtina, kad ryškus mitochondrijų išorinės membranos pralaidumo ADP padidėjimas (K_m^{ADP} sumažėjimas) stebimas jau esant mažai (2,2 μM) palmitoil-L-karnitino koncentracijai. 5% dekstrano T-70 pridėjimas į terpę tris kartus padidino žemą (77 μM) K_m^{ADP} reikšmę, būdingą oktanoil-L-karnitino oksidacijai. Nors šio reiškinio mechanizmas nėra aiškus, mitochondrijų morfologiniai pakitimai yra svarbūs.