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The role of different fatty acids in the regulation of mitochondrial respiration *in situ*

Lolita Kuršvietienė[1],

Sonata Trumbeckaitė[2],

Daiva Majienė[2],

Antanas Praškevičius[1],

Adolfas Toleikis[1,2*]

[1] Department of Biochemistry, Kaunas University of Medicine

[2] Institute for Biomedical Reseach, Kaunas University of Medicine, Eivenių 4, LT-3007 Kaunas, Lithuania

The role of fatty acids (both saturated and unsaturated and of various chain length) in the regulation of mitochondrial respiration *in situ* using saponin-permeabilized rat cardiac fibers was investigated. We have found that the oxidation of both saturated nonactivated fatty acid decanoate and unsaturated fatty acid ester oleoyl-CoA induces a marked (3–4-fold) decrease in app. K_m for ADP, if compared to pyruvate + malate oxidation. In conclusion, our present and previous data show that different fatty acids (saturated and unsaturated, activated or not, long and medium chain) similarly decrease app. K_m^{ADP}.

Based on these findings and the similarity of effects obtained previously with saturated fatty acids, we conclude that this property is common to all fatty acids.

Key words: saponin-permeabilized heart muscle fibers, outer mitochondrial membrane permeability, fatty acid oxidation; oxidative phosphorylation

INTRODUCTION

A series of studies [1, 2, 3, 5] showed that, in contrast to isolated mitochondria, the outer mitochondrial membrane (OMM) in saponin-permeabilized cardiac muscle fibers exhibits a low permeability for ADP which is supposed to be regulated by some specific cytoskeleton-related protein(s) bound to porin pores [6]. It is known that fatty acids are a major energy source for heart cells. Our previous studies on saponin-permeabilized heart muscle fibers revealed that oxidation of activated saturated fatty acids, both long-chain (palmitoyl-CoA and palmitoyl-L-carnitine) and medium-chain (octanoyl-L-carnitine), substantially reduced app. K_m^{ADP} and thus increased OMM permeability for ADP [5]. The precise mechanism of this phenomenon as well as the possibility of its manifestation in case of other fatty acids oxidation is still unclear. In this regard, it is important to note that different fatty acids affect differently mitochondrial enzymes, membranes and processes of energy coupling [7].

Therefore the aim of this study was the further elucidation of the role of fatty acids differing in length and saturation in the regulation of oxidative phosphorylation in rat heart muscle fibers. We have shown that activated unsaturated long-chain oleoyl-CoA and nonactivated saturated medium-chain decanoic acid have the same property as fatty acids mentioned above, i.e. their oxidation remarkably reduces the app. K_m^{ADP}, indicating a increase in OMM permeability for ADP.

* Corresponding author. E-mail: toleikis@kmu.lt

MATERIALS AND METHODS

Preparations

Male Wistar rats weighing 250–300 g were used in the experiments. After decapitation, beating hearts were excised and rinsed in ice-cold 0.9% KCl solution.

Bundles of the heart muscle fibers, approximately 0.2–0.3 mm in diameter, were prepared (using sharpened needles) from the muscle strips cut out from the left ventricular endocardium and transferred to cooled solution A containing 20 mM imidazole, 20 mM taurine, 0.5 mM dithiothreitol, 7.1 mM $MgCl_2$, 50 mM 2-[N-morpholino]ethanesulfonic acid (MES), 5 mM ATP, 15 mM phosphocreatine, 2.6 mM CaK_2EGTA and 7.4 mM K_2EGTA (free Ca^{2+} concentration 0.1 μM) (pH 7.0 adjusted with KOH at 2 ˚C), supplemented with 50 μg/ml saponin (from Gypsophila; sapogenin content 17+%; Sigma) and incubated for 30 min. Then the bundles were washed for 10 min in solution B containing 20 mM imidazole, 20 mM taurine, 0.5 mM dithiothreitol, 1.6 mM $MgCl_2$, 100 mM MES, 3 mM KH_2PO_4, 3.0 mM CaK_2EGTA and 7.1 mM K_2EGTA (pH 7.1 adjusted with KOH at 37 °C). All procedures were carried out under intensive shaking (120 times/min). The washed bundles of fibers were once rinsed in solution

B, transferred to test tubes with the same solution and stored on ice for 6–7 h.

Assays

The rates of oxygen uptake were recorded at 37 °C by a Clark-type electrode system with intensive stirring in solution B, containing eiter pyruvate and malate (6 mM + 6 mM) or oleoyl-CoA + L-carnitine + malate (6 μM + 2.5 mM 0.24 mM) or decanoic acid + pyruvate + malate (0.3 mM + 6 mM + 6 mM). Oleoyl-CoA was dissolved in water and decanoic acid in ethanol. The medium was supplemented with 2 mg/ml of bovine serum albumin. The solubility of oxygen was taken to be 422 nmol/ml. Respiration rates were expressed as nmolO/min/mg fiber dry weight (dry weight = wet weight before respiration measurement / 4.85). The ADP regenerative system consisting of 1.2 IU/ml lyophilized yeast hexokinase (Type V; EC 2.7.1.1; Sigma) and 24 mM glucose (Sigma) was added into the oxygraph chamber before adding heart muscle fibers. Titration was made by different ADP concentration in each separate probe. The concentrations covered a range from 10 to 1000 mM. App. K_m^{ADP} was estimated from the least-squares fit to the Michaelis–Menten equation and Hanes–Woolf plot by GraphPad Prism demo v3.0.

The values in Table and figures are expressed as means ± S.E.M. Statistical analysis was performed using Student's t test, and $p < 0.05$ was taken as the level of significance.

RESULTS AND DISCUSSION

Our previous data [4, 5] showed that oxidation of palmitoyl-L-carnitine, octanoyl-L-carnitine and palmitoyl-CoA (+ L-carnitine) markedly decreased a very high value of K_m for ADP specific to pyruvate + malate oxidation. In this study, the role of saturated medium-chain fatty acid decanoate and unsaturated long-chain fatty

Table. **Kinetic properties of the oxidative phosphorylation system of rat heart muscle fibers with different respiratory substrates.**

	K_m^{ADP} (μM)	V_{max} (nmolO/min/mg dry wt)
Pyruvate + malate (6 mM + 6 mM), n = 5	217.8 ± 7.9	48.0 ± 3.7
Oleoyl-CoA+ L-carnitine + malate (6 μM + 2.5 mM + 0.24 mM), n = 3	55.7 ± 5.1	44.7 ± 3.2
Decanoic acid + pyruvate + malate (0.3 mM + 6 mM + 6 mM), n = 5	75.8 ± 7.6	37.1 ± 1.5

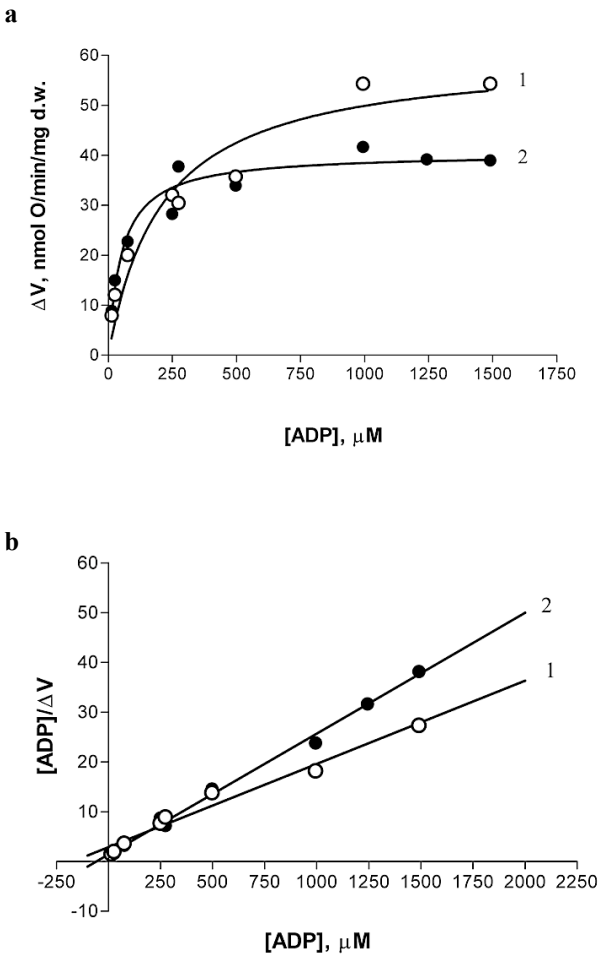

Fig. 1. Dependence of respiration rates of rat cardiac fibers on ADP concentration: **a)** according to Michaelis–Menten kinetics; **b)** the same data set by linear Hanes–Woolf transformation. **1**: pyruvate + malate (6 mM + 6 mM); **2**: decanoate (0.3 mM) + pyruvate + malate(6 mM + 6 mM). The data of one separate experiment are presented.

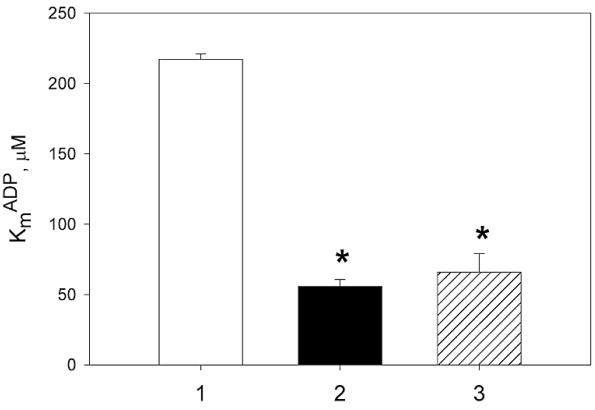

Fig. 2. Influence of respiratory substrates on app. K_m^{ADP} of saponin-permeabilized rat cardiac fibers.
1: pyruvate + malate (6 mM + 6 mM), n = 5; **2**: oleoyl-CoA + L-carnitine + malate (6 μM + 2.5 mM + 0.24 mM), n = 3; **3**: Decanoic acid + pyruvate + malate (0.3 mM + 6 mM + 6 mM), n = 5; *p < 0.05 vs control (column 1)

acid oleoyl-CoA in the regulation of mitochondrial respiration was investigated.

Figure 1 shows the results of a typical experiment on saponin-treated rat cardiac fibers when the respiration rate with different substrates (decanoic acid + pyruvate + malate and pyruvate + malate) is measured at various ADP concentrations (n = 5). The maximal respiration rate with pyruvate + malate as a substrate (curve 1) is achieved at a much higher ADP concentration (about 1 mM) than with decanoate + pyruvate + malate (about 300 μM) (curve 2). The app. K_m^{ADP} values obtained in two different ways, indicated in the legend of Fig. 1, were reasonably close, and those for pyruvate + malate as a respiratory substrate were in good agreement with the data obtained by other investigators [1].

The calculated mean app. K_m^{ADP} values and external ADP-dependent V_{max} values obtained using different respiratory substrates namely oleoyl-CoA + malate (+L-carnitine), decanoic acid+pyruvate+malate and pyruvate + malate as control, are presented in Table and Fig. 2 and are in line with our earlier findings [3, 6]. This means that OMM permeability for ADP *in situ* in case of pyruvate+malate oxidation is very low (K_m^{ADP} = 217.8 ± 7.97 μM) if compared with isolated mitochondria (K_m^{ADP} = 23 μM [6]). When rat cardiac fiber respiration with oleoyl-CoA + malate (+L-carnitine) or decanoic acid + pyruvate + malate was titrated with ADP, the mean app. K_m^{ADP} values were several times lower (55.7 ± 5.1 μM and 75.8 ± 7.6 μM, respectively), if compared with that of pyruvate + malate oxidation. It is evident that oxidation of both decanoic acid and oleoyl-CoA induces a dramatic increase in OMM permeability for ADP. Control experiments showed that effects of fatty acids described above cannot be attributed to the injury of OMM (no injury was demonstrated by cytochrome c test) or increase in the volume of mitochondria (no mitochondrial swelling was observed) (data not shown).

It is known [8] that, in contrast to oleoyl-CoA, short- and medium-chain fatty acids (up to twelve carbon atoms) cross mitochondrial membranes bypassing the carnitine-dependent transport system. Decanoate is first activated to acyl-CoA inside mitochondria before being directed into oxidation. This reaction is catalysed by the medium-chain acyl-CoA synthetase and requires both CoA and ATP. Meanwile, oleoyl-CoA does not need activation.

Despite differences in transport pathways, some reactions preceding β-oxidation and the enzyme systems responsible for β-oxidation, decanoic acid and oleoyl-CoA decreased app. K_m^{ADP} to a similar extent.

Based on these findings and the similarity of effects obtained previously with saturated fatty acids [5], we conclude that this property is common all fatty acids.

On the basis of our previous reseach [5], an assumption was made that changes in app. K_m^{ADP} could be partly related to the fatty-acid-induced morphological changes in mitochondria, which make porin more permeable for ADP. However, since different fatty acid concentrations caused similar effects on app. K_m^{ADP} [9], this assumption was denied.

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ĮVAIRIŲ RIEBALŲ RŪGŠČIŲ VAIDMUO REGULIUOJANT MITOCHONDRIJŲ KVĖPAVIMĄ *IN SITU*

S a n t r a u k a

Šiame darbe tirtas riebalų rūgščių (sočiųjų ir nesočiųjų, taip pat besiskiriančių grandinės ilgiu) vaidmuo reguliuojant kvėpavimą žiurkės širdies raumens mitochondrijose *in situ*. Kaip mitochondrijų kvėpavimo substratą naudojome oleoil-CoA (+ L-karnitiną), dekanoinę rūgštį (mišinyje su piruvatu + malatu) bei kontrolei – piruvatą + malatą.

Nustatėme, kad mitochondrijose oksiduojantis riebalų rūgštims, tiek sočiajai neaktyvuotai dekanoinei rūgščiai, tiek nesočiajai oleoil-CoA (+ L-karnitinas), ženkliai (3–4 kartus) sumažėja tariamoji K_m^{ADP}, lyginant su piruvato + malato oksidacija. Ankstesni (naudojant sočiąją riebalų rūgštį) ir dabar gauti duomenys rodo, kad oksiduojantis visoms riebalų rūgštims (įvairaus grandinės ilgio, sotumo laipsnio, aktyvuotoms ir neaktyvuotoms), K_m^{ADP} sumažėja panašiai.