FATTY ACID COMPOSTION OF MITOCHONDRIAL PHOSPHOLIPIDS OF THE ISCHEMIC HEART

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Investigations have shown [5, 6, 12, 13] that intensive hydrolysis of phospholipids takes place in the ischemic heart cells, and as a result, free fatty acids and lysophospholipids accumulate. It has been suggested on the basis of investigations on isolated liver mitochondria *in vitro* that one cause of damage to mitochondrial membranes under anoxic conditions is activation of mitochondrial phospholipase A_2 [1, 4]. Since position 2 of phospholipid molecules is occupied mainly by unsaturated fatty acids (UFA) [2, 9], which are removed by phospholipase A_2, it can be postulated that during ischemia the percentage of saturated fatty acids in the lysophospholipids should increase. However, the fatty acid composition of phospholipids during ischemia has received little study. Only one investigation is known [11], in which the fatty acid composition of phospholipids and lysophospholipids was studied in the pig heart.

The writers showed previously [5] that changes in the phospholipid composition in myocardial ischemia are more marked in the mitochondria. In the present investigation the effect of myocardial ischemia was therefore studied on the fatty acid composition of mitochondrial phospholipids.

EXPERIMENTAL METHOD

Experiments were carried out on rabbit hearts weighing 2.5-3.5 kg. Total ischemia of the heart (autolysis for 1 h) was created in a chamber saturated with vapor of physiological saline at 37°C [7]. Mitochondria were isolated by differential centrifugation in medium containing 180 mM KCl, 10 mM EDTA, and 10 mM Tris-HCl, pH 7.5 and suspended in medium with 180 mM KCl and 20 mM Tris-HCl, pH 7.5. Mitochondrial lipids were extracted by the method in [8]. Extracts of lipids from the mitochondria (4 mg of mitochondrial protein) was applied to a thin layer of silica-gel H (Merck, West Germany) in the form of a strip 5 cm long. Phospholipids were fractionated as described in [5]. To reveal spots of the phospholipids the plates were sprinkled with a 0.2% solution of 2,7-dichlorofluorescein in ethanol. Fatty acids of phosphatidylcholine (PC) and lysophosphatidylcholine (LPC) were converted into methyl esters by boiling for 2 h with a reflux condenser in a 2.5% solution of HCl in methanol [3]. Methyl esters of the fatty acids were fractionated on a Chrom 41 gas chromatograph in a conditioned glass column with 4% DEGS on Chromosorb G-AW 80-100 (Merck). Fractionation was carried out under isothermic conditions at the following temperatures: column 178°C, vaporizing chamber 250°C, detectors 200°C. The rate of flow of the gases was nitrogen 23 ml/min, hydrogen 28 ml/min, air 0.4 liter/min. Fractionation of the methyl esters of the fatty acids was checked against standrad mixtures (Sigman USA). Quantitative analysis of the chromatograms was carried out by triangulation. The statistical significance of differences was determined by the paired t test.

EXPERIMENTAL RESULTS

Analysis of the fatty acids composition of LPC in control cardiac mitochondria (Table 1) showed that UFA accounted for 95%. The greater part of them (77%) consisted of linoleic and arachidonic acids. The content of UFA in PC from the control mitochondria was considerably

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TABLE 1. Effect of Total Ischemia on Fatty Acid Composition (in %) of LPC (I) and PC (II) of Rabbit Heart Mitochondria (M ± m)

Exptl. conditions		16:0	18:0	18:1	18:2	20:4
I. Control	(8)	3,8±0,58	1,5±0,24	17,9±0,60	38,5±1,30	38,5±1,87
Ischemia	(8)	7,1±1,57*	3,0±0,73*	18,0±1,28	43,7±2,54*	27,9±3,65†
II. Control	(7)	26,8±1,40	7,1±0,49	20,3±1,51	25,7±1,42	19,7±1,77
Ischemia	(8)	29,9±2,14	6,1±0,47	21,8±1,05	25,3±1,23	16,6±1,35

Legend. *P < 0.05, †P < 0.02 compared with control. Number of experiments shown in parentheses.

less, namely 65.7%, including 20.3% of oleic, 25.7% of linoleic, and 19.7% of arachidonic acids. The main component of UFA was palmitic acid (26.8%). Incidentally, a somewhat different fatty acid composition of PC has been obtained for mitochondria of the rat heart [10], the main differences being a higher percentage content of stearic and a lower content of oleic acids than in rabbit mitochondria (our own experiments). Very probably these differences are due to dietary differences and to the species of the animals.

After 1 h of total myocardial ischemia the fatty acid composition of PC was unchanged, in agreement with results obtained [11] in a study of pig heart tissue. The percentage of saturated fatty acids in LPC showed a statistically significant increase (palmitic by 87%, stearic by 100%), whereas the percentage of UFA fell (arachidonic by 27%, linoleic by 14%). According to the results described in [11], after 40 min of ischemia no changes were found in the fatty acid composition of LPC isolated from pig heart tissue. However, it must be noted that in [11] the model of ischemia (occlusion of the coronary artery) differed from our own: In the case of total ischemia the blood flow in the heart ceases completely, whereas after ligation of the coronary vessels it usually is not reduced to zero. The possibility cannot be ruled out that the presence of a residual blood flow may affect phospholipid and fatty acids metabolism of the heart cell and may be one explanation of the difference between results obtained on different models of ischemia. Furthermore, in [11] the fatty acid composition of LPC extracted from heart tissue was studied, whereas in our case it was LPC extracted from the mitochondria of the heart.

Position 2 in the PC molecule in membranes of the sarcoplasmic reticulum of rabbit skeletal muscles is known to be occupied by UFA to the extent of about 96% [9], and a very similar percentage (about 97%) also is found in PC from liver tissue [2] and mitochondria of the rat heart (94%) [10]. Correspondingly, only about 4, 10, and 18% of UFA can be found in position 1. Incidentally, arachidonic acid is found exclusively in position 2 of PC in the liver [2] and cardiac mitochondria [10] of rats. Palmitic and stearic acids also exhibit very high positional specificity relative to position 1 [10]. During aging, the rate of accumulation of palmitic and stearic acids in rat heart mitochondria in medium without Ca^{++} was considerably greater in [10] than that of other fatty acids. The authors cited concluded that under these conditions activation of phospholipase A_1 takes place. According to our own results, LPC without a fatty acid in position 1 should predominate in control mitochrondria from rabbit heart. The fatty acid composition of LPC in the control is evidence that under physiological conditions the action of phospholipase A_1 is exhibited. At the same time, the evident increase in the content of palmitic and stearic acids in LPC and the decrease in the content of arachidonic acid during total myocardial ischemia is evidence in support of the relative intensification of degradation of mitochondrial phospholipids by phospholipase A_2.

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LITERATURE CITED

1. E. Q. Bragin, A. D. Derginov, G. L. Neugodova, et al., Vopr. Med. Khim., No. 5, 673 (1977).
2. E. V. Dyatlovitskaya, L. I. Éinisman, S. M. Mamontov, et al., Biokhimiya, 41, No. 3, 538 (1976).
3. M. Kates, Techniques in Lipidology: Laboratory Techniques in Biochemistry and Molecular Biology, Elsevier (1971).
4. V. I. Sorokovoi and Yu. A. Vladimiorov, in: Progress in Science and Technology. Series: Biophysics [in Russian], Vol. 5, Moscow (1975), p. 5.

5. A. I. Toleikis, A. I. Dagys, and A. K. Praškevičius, Vopr. Med. Khim., No. 4, 64 (1982).
6. A. J. Trumpickas, A. I. Toleikis, and A. K. Praškevičius, Nauch. Tr. Vuzov Lit. SSR, 22, 146 (1982).
7. L. C. Armiger and M. Seeley, Lab. Invest., 34, 357 (1976).
8. J. Folch, M. Lees, and G. H. Sloane-Stanley, J. Biol. Chem., 226, 497 (1957).
9. V. M. C. Madeira and M. C. Antunes-Madeira, Cienc. Biol. (Portugal), 2, 265 (1965).
10. J. W. Palmer, P. C. Schmidt, D. R. Pfeiffer, et al., Arch. Biochem., 211, 674 (1981).
11. N. A. Shaikh and E. Downar, Circulat. Res., 49, 316 (1981).
12. B. E. Sobel, P. B. Corr, A. K. Robinson, et al., J. Clin. Invest., 62, 546 (1978).
13. G. I. Van der Vusse, T. H. M. Roemen, F. W. Prinzen, et al., Circulat. Res., 50, 538 (19 (1982).

EFFECT OF ACTH ON DEHYDROGENASE ACTIVITY OF CELLS AND TISSUES

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One manifestation of the action of hormones of the pituitary-adrenocortical system in man or animals is modification of energy metabolism. Effects of glucocorticoids on intermediate metabolism of carbohydrates and lipids and on the level of high-energy compounds linked with it [1, 5], on activity of enzymes of the glycolytic [6] and respiratory chains [4, 5, 7] and on concentrations of metabolites and activity of enzymes of the Krebs' cycle [2, 3] are known. However, there is no information on the effect of ACTH on respiratory function of the tricarboxylic acid cycle.

In the investigation described below the effect of ACTH on dehydrogenation in the Krebs' cycle was studied.

EXPERIMENTAL METHOD

Experiments were carried out on male rats weighing 180-200 g. Dehydrogenase activity was determined colorimetrically, by a method based on reduction of triphenyltetrazolium chloride to formazan.

After decapitation of the rats strips of muscle weighing 100 mg were cut from the diaphragm and placed in Krebs–Ringer medium with phosphate buffer, without glucose, pH 7.4, in a volume of 3 ml, containing 0.2 ml of a 0.5% solution of 2,3,5-triphenyltetrazolium chloride and 0.01 M of the corresponding dehydrogenation substrate. ACTH (Kaunas Endocrine Preparations Factory) or Synacten ($ACTH_{1-24}$; Ciba-Geigy) was added to the incubation medium up to a concentration of 0.005-0.5 U/ml. The samples were incubated at 37°C for 60 min, after which the tubes were placed in melting ice, and the incubation medium was replaced by 3 ml of a 2% solution of Trition X-100 in a mixture of ehter and alcohol (8:2) to extract the formazan produced. Complete extraction of formazan from the diaphragm was achieved by homogenization of the muscle in a glass homogenizer with the ether–alcohol mixture. Pooled extracts were centrifuged at 10,000g for 10 min. The supernatant was decanted into graduated tubes and the volume made up with the ether–alcohol mixture to 10 ml; colorimetry was carried out at 540 nm in a 10-mm cuvette. Dehydrogenase activity was calculated by a calibration curve plotted for diformazan and expressed in nanomoles diformazan per gram tissue. Similar tests were carried out with a suspension of *Escherichia coli* serotype O III B_4 (stoke W strain), grown on nutrient agar. Dehydrogenase activity of the *E. coli* cells was determined colorimetrically by estimation of reduction of 2,3,5-triphenyltetrazolium chloride and expressed in micrograms formazan formed during incubation of a suspension containing 10^{12} microbial cells for 2 h at 37°C [8]. ACTH was added to a concentration of 0.005-0.5 U/ml of incubation medium. The results were subjected to statistical analysis.

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