CARNITINE ESTERS OF FATTY ACIDS SUPPORT MITOCHONDRIAL FATTY ACID SYNTHESIS

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SUMMARY. Acylcarnitines are active primers for mitochondrial fatty acid synthesis. It is suggested that another metabolic fate of carnitine esters of fatty acids in addition to their role in fatty acid oxidation is to provide substrates for mitochondrial fatty acid elongation.

It has been clearly established that the oxidation of fatty acids of 12 carbon units or greater is dependent on the formation of acylcarnitine esters of the fatty acids which are permeable in the inner mitochondrial membrane. Acylcarnitine synthesis is catalyzed by the enzyme carnitine palmityltransferase as is seen in the following reaction.

palmityl-CoA + carnitine $\rightleftharpoons$ palmitylcarnitine + CoA

This involves the formation of palmitylcarnitine for transport into the mitochondrial membrane. The reverse reaction presumably takes place within the inner mitochondrial membrane where the transport intermediate (palmitylcarnitine is reconverted to the CoA ester prior to β-oxidation. Although there has been extensive investigation of this carnitine pathway as it relates to fatty acid oxidation (1,2), there have been no previous studies of the capacity of carnitine esters of fatty acids to support mitochondrial fatty acid synthesis. Fatty acid synthesis by mitochondria involves the addition of 2 carbon units to fatty acids present in the system. This communication will show that carnitine esters of fatty acids are active primers for fatty acid elongation by guinea pig heart mitochondria.

METHODS AND MATERIALS. Heart mitochondria were isolated from adult guinea pigs by a modification of the method of Chance and Hagihara (5,6). Experiments were carried out with intact mitochondria and mitochondria disrupted by sonic oscillation. Disruption was accomplished using a Branson sonifier equipped with a microtip immersed for 1 minute in a 1.0 ml volume of suspended mitochondria. Incorporation of acetyl-CoA into long chain fatty acids was determined in an assay medium containing 1.0 mg of mitochondrial protein, 50 mμ moles of acetyl-CoA containing 30,000 CPM of acetyl-1-^{14}C-CoA (20 mc/mMole), 1.5 μ moles of NADH, 1.1 μ moles of NADPH, 50 μ moles of potassium phosphate, pH 6.5, 1 μg of antimycin A and water in a final volume of 1.0 ml. The concentrations of fatty acids and bovine albumin used in the assays are given in the tables. Bovine serum albumin was defatted according to the method of Chen (7). The assay mixture was incubated at 38° for one hour. Fatty acid oxidation was inhibited by a gentle flow of nitrogen over the surface of the reaction and by the presence of antimycin A in the medium.

The reaction was terminated by the addition of KOH to a final concentration of 15%. Saponification was carried out by heating the reaction mixture at 70° for 30 minutes. Following acidification with HCl, the fatty acids were extracted with pentane and evaporated to dryness in a liquid scintillation vial. The material was then counted in a Beckman scintillation spectrometer. Extracted lipids were identified as fatty acids by thin layer chromatography on silica gel using a petroleum ether, ethyl ether, acetic acid developing solvent (90:10:1). L-carnitine and l-palmitylcarnitine were generous gifts from the Otsuka Pharmaceutical Factory, Osaka, Japan. Other reagents were obtained from commercial sources.

RESULTS AND DISCUSSION. Acetyl-CoA incorporation into long chain fatty acids by guinea pig heart mitochondria was negligible unless a fatty acid substrate primer was present in the assay medium. In addition, NADH, NADPH, and ATP were required for optimal acetyl-CoA incorporation. The requirement for fatty acids may reflect a low level of those substrates in the guinea pig heart.

TABLE I

Acetyl-CoA Incorporation into Intact and Sonically Disrupted Mitochondria.

Fatty Acid Primer	mμ Moles Acetyl 1-^{14}C-CoA Incorporated	
	Intact Mitochondria	Disrupted Mitochondria
None	0	0
Palmitic Acid	0.04	0.22
Palmityl-CoA	0.54	0.89
Palmitylcarnitine	1.05	0.77
Capric Acid	0.02	0.84
Caprinylcarnitine	0.60	0.11

1.0 mg of mitochondria was used in the assay. The concentrations of palmitic acid, palmityl-CoA and palmitylcarnitine were 1.0 mM. The concentration of capric acid and caprinylcarnitine was 3.0 mM. Other conditions are as in the text.

Table 1 compares acetyl-CoA incorporation into fatty acids by intact and sonically disrupted mitochondria when 10 carbon and 16 carbon substrate primers were added to the assay. In the case of intact mitochondria, the highest rates of incorporation were consistently observed when palmitylcarnitine was the primer. The level of incorporation with palmityl-CoA was about 50% of the value seen with palmitylcarnitine and was very limited with palmitic acid. The addition of l-carnitine to the assays completely inhibited acetyl-CoA incorporation.

Sonic disruption of the mitochondria increased acetyl-CoA incorporation when palmitic acid and palmityl-CoA were primers. As mitochondria exhibit a permeability barrier to CoA esters of fatty acids, the membrane disruption probably enhanced activity by allowing the fatty acids and other substrates to penetrate the inner mitochondrial membrane more readily. The incorporation of acetyl-CoA seen with palmitylcarnitine as substrate primer was decreased after disruption and approximated the value observed with palmityl-CoA. These data indicate that the high energy fatty acid transport intermediate, palmitylcarnitine, is an effective substrate primer for long chain fatty acid elongation.

When capric acid was used as a substrate primer, acetyl-CoA incorporation

was uniformly low in experiments using intact mitochondria and relatively high after disruption. With caprinylcarnitine incorporation was much greater in the intact mitochondria as compared with that of sonically disrupted mitochondria. These results were unexpected. Experience with fatty acid oxidation by intact mitochondria (8,9) has shown that both capric acid and its carnitine ester are oxidized at comparable rates indicating that mitochondrial permeability is not rate limiting for capric acid oxidation. However, the increased primer activity of capric acid after sonic disruption may also reflect enhanced penetration of acetyl-CoA to the site of elongation.

Albumin has been shown to protect mitochondria from the detergent effect of acylcarnitines (9). Table 2 shows the effect of bovine albumin on palmitylcarnitine primer activity in experiments with intact and disrupted mitochondria. In the case of intact mitochondria, albumin resulted in approximately a 70% decrease in acetyl-CoA incorporation, irrespective of albumin concentration. Up to 1 mg of bovine albumin had little effect on the activity of disrupted mitochondria but concentrations above 1 mg/ml effectively inhibited incorporation. In the case of the disrupted mitochondria the data suggest that higher concentrations of bovine albumin may bind palmitylcarnitine and effectively remove that substrate from the reaction. With regard to the intact mito-

TABLE II

Effect of Bovine Albumin on Primer Activity of Palmitylcarnitine.

mg BSA	mμ Moles Acetyl 1-^{14}C-CoA Incorporated	
	Intact Mitochondria	Disrupted Mitochondria
None	1.75	0.47
0.40	0.53	0.72
0.80	0.65	0.62
1.20	0.57	0.08
1.60	0.62	0.07

1.0 mg of mitochondria was used in the assay. Palmitylcarnitine concentration was 1.0 mM. Other conditions are as in the text.

chondria, one can postulate the presence of an extra-mitochondrial pool of palmitylcarnitine bound to the excess albumin present in the medium and a finite dissociation of the palmitylcarnitine from the albumin during the course of the reaction.

ATP markedly enhanced the incorporation of acetyl-CoA into long chain fatty acids when both palmitylcarnitine and palmityl-CoA were substrate primers (Table 3). In the intact mitochondria, palmitylcarnitine plus ATP gave the highest incorporation observed. Incorporation of acetyl-CoA by the sonically disrupted mitochondria was similar when palmityl-CoA plus ATP and palmitylcarnitine plus ATP were utilized as primers. Others have reported previously (4,10) that addition of CoA esters of fatty acids can replace the ATP requirement seen in experiments with free fatty acids. However, under the conditions of our assays, there was consistent stimulation of acetyl-CoA incorporation by ATP when both palmityl-CoA and palmitylcarnitine were included in the assay.

Harlan and Wakil (4) showed that rat liver mitochondria incorporated acetyl-CoA into a variety of fatty acids. Acetyl-CoA incorporation by rat liver mitochondria could be markedly enhanced by the addition of fatty acids to the medium to serve as exogenous primers to which acetyl units were added.

TABLE III

Effect of ATP on Acetyl-CoA Incorporation.

	mμ Moles Acetyl 1-^{14}C-CoA Incorporated	
	Intact Mitochondria	Disrupted Mitochondria
Palmitic Acid	0.02	0.06
Palmitic Acid, ATP	0.05	0.34
Palmityl-CoA	0.08	0.19
Palmityl-CoA, ATP	0.62	0.89
Palmitylcarnitine	0.11	0.09
Palmitylcarnitine, ATP	1.26	0.82

1.0 mg of mitochondria were used in the assay. Concentrations of fatty acids and ATP were 1.0 mM and 3 mM, respectively. Other conditions are as in the text.

Similar data have been reported by Quagliariello, et al (10) who localized this elongation activity to the inner mitochondrial membrane. Dahlen and Porter (11) investigated mitochondrial fatty acid elongation in an outer membrane preparation from beef heart mitochondria. Their results have differed from ours and those of other investigators (3,4,10) in that NADPH inhibited the incorporation of acetyl-CoA into fatty acids. Disruption of mitochondria by sonic oscillation increased fatty acid elongation activity in our experiments when free fatty acids and palmityl-CoA were substrate primers. This is similar to data reported by others and may relate to disruption of the mitochondrial permeability barrier, thus, allowing substrates better access to the site of the reaction (10). L-carnitine completely inhibited fatty acid elongation activity. This is probably due to the conversion of the acetyl-CoA substrate to acetylcarnitine by mitochondrial acetylcarnitine transferase which removes it from the reaction.

Whereat, et al (11) have proposed that fatty acid elongation within mitochondria is controlled by the NADH:NAD ratio of mitochondria, with high ratios favoring synthesis rather than oxidation. The results of these experiments suggest that a metabolic fate other than β-oxidation of fatty acids transported into mitochondria as carnitine esters may be to provide fatty acid substrates for the elongation pathway. As fatty acid elongation by mitochondria is thought to occur by reversal of β-oxidation in the inner mitochondrial membrane, acylcarnitine may support either oxidation or elongation depending on metabolic demands of the cell.

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