THE EFFECT OF FATTY ACIDS ON THE SYNTHESIS OF P-ENOLPYRUVATE BY HUMAN LIVER MITOCHONDRIA

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1. Introduction

Most of the studies of the regulation of gluconeogenesis in mammalian liver have been carried out using the rat and have pointed to a key role for the enzyme P-enolpyruvate carboxykinase (E C 4. 1. 1. 2) [1–3]. In this species the hepatic enzyme is almost totally cytosolic and is induced by a variety of dietary and hormonal affectors [4–7]. More recently, work with other species such as the guinea pig [2, 8, 9], rabbit [10] and cat [11], all of which contain 50–60% of their total P-enolpyruvate in the mitochondria, has indicated that the regulation of hepatic gluconeogenesis in these animals may be different from that in rat liver. The mitochondrial formation of P-enolpyruvate is of considerable importance in gluconeogenesis in guinea pig and rabbit liver [12] and is markedly decreased by both fatty acids and β-hydroxybutyrate which act by increasing mitochondrial NADH levels [9, 10]. These compounds also inhibit gluconeogenesis from lactate and alanine in the isolated, perfused liver from both species and stimulates it in rat liver [8, 13–15]. The difference in the regulation of glucose synthesis in guinea pig and rabbit liver as compared to rat liver underlines the difficulties in generalizing about the control of important metabolic processes between species. This is of critical importance since human liver contains 60% of its total P-enolpyruvate carboxykinase in the mitochondria. In the present paper we have assessed the capacity of human liver mitochondria to synthesize P-enolpyruvate as well as the effect of fatty acids in this process.

2. Methods

Guinea pig or human liver mitochondria were isolated by differential centrifugation in 0.32 M sucrose and 2 mM HEPES† buffer at pH 7.35. After sedimentation of the nuclear pellet, the mitochondria were extracted and then washed twice with the sucrose-HEPES buffer. Oxygen consumption and ADP phosphorylation were simultaneously monitored with a Clark type oxygen electrode and Radiometer combination glass electrode with subsequent amplification of the resulting signal.

Approx. 12 mg of mitochondrial protein was incubated in 3 ml of medium and the metabolites determined after stopping the incubation with 36% perchloric acid. The extracts were chilled to 0° and neutralized with $KHCO_3$. Mitochondrial proteins were measured by ultraviolet absorption using serum albumin as a standard [16]. Analysis of ATP [17], ADP and AMP [18], P-enolpyruvate [19], malate [20] and α-ketoglutarate [21] were performed by standard enzymatic methods.

3. Results

In fig. 1 are shown results obtained with simultaneous monitoring of proton movements and oxygen

† Abbreviations: HEPES: *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid; FCCP: Carbonyl cyanide-p-trifluoromethoxyphenylhydrazone.

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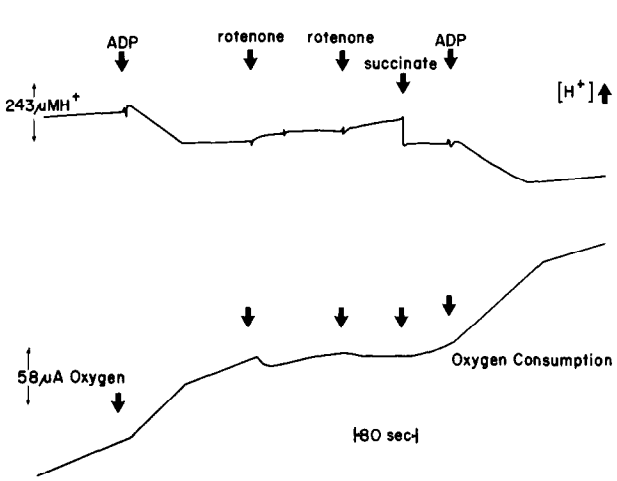

Fig. 1. Oxygen consumption and proton movement by mitochondrial suspensions from human liver. 2 mg of mitochondrial protein were incubated at 25° in 0.8 ml of 0.25 M sucrose containing 5 mM $MgCl_2$, 1.6 mM HEPES, 5 mM potassium phosphate, 5 mM malate, 5 mM glutamate at pH 7.5. At times shown by the arrows 0.15 mM ADP, twice 1.25 μM rotenone, 6.25 mM succinate and 0.15 μM ADP were added.

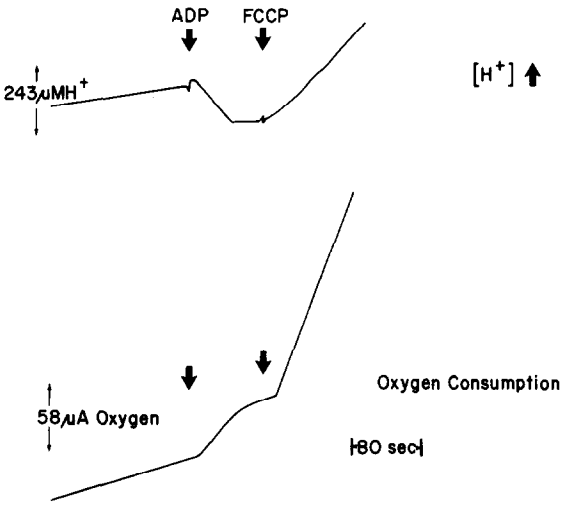

Fig. 2. Oxygen consumption and proton movement by mitochondrial suspensions from human liver. 2 mg of mitochondrial protein were incubated in conditions similar to those described under fig. 1. At times shown by the arrows, 0.15 mM ADP and 0.14 μM FCCP were added.

consumption during oxidation of malate + glutamate by human liver mitochondria. A net state 3 – state 4 transition was noticed, with a respiratory control ratio of about 3 and a calculated P/O ratio of 2.5. Also, the ratio of oxygen consumption in state 4_2 to state 4_1 was very close to 1, indicating negligible ATPase activity, as shown by the traces of proton movement. Respiration by isolated human liver mitochondria was blocked by rotenone, an inhibitor of the electron transport chain at site I, while further additions of succinate and ADP produced oxidative phosphorylation with a P/O ratio of 1.7. FCCP addition produced a typical acceleration of oxygen consumption with a marked proton release due to the uncoupling effect (fig. 2).

Table 1
Influence of fatty acids on the metabolism of human and guinea pig liver mitochondria.

	Substrate	Octanoate (0.2 mM)	PEP	ATP	AMP	ADP	Malate	α-Ketoglutarate
			(nmoles produced/mg protein)			(nmoles used/mg protein)		
Human	Malate	–	64	476	50	468	525	
		+	23	523	36	544	572	
	α-Ketoglutarate	–	44	549	38	511		192
		+	21	549	26	571		206
Guinea Pig	Malate	–	66 ± 5.6	344 ± 21	31 ± 2.3	423 ± 12	346 ± 30	
		+	35 ± 1.9	416 ± 20	33 ± 7.4	462 ± 25	362 ± 36	
	α-Ketoglutarate	–	42 ± 1.8	387 ± 25	35 ± 5.0	447 ± 13		221 ± 26
		+	19 ± 1.2	398 ± 23	29 ± 5.6	453 ± 6		233 ± 25

Approx. 12 mg of mitochondrial protein were incubated for 10 min at 30° in 3 ml of 160 mM sucrose,1 mM HEPES, 20 mM potassium phosphate at pH 7.5, 6.6 mM $MgCl_2$, 3.3 mM ADP and either 5 mM malate or 5 mM α-ketoglutarate. Values are the means ± standard error for 5 experiments with guinea pigs and the result obtained from the human sample.

Table 1 illustrates a comparison between human and guinea pig intramitochondrial P-enolpyruvate formation and the influence of octanoate on the metabolism of these mitochondria oxidizing malate or α-ketoglutarate. A striking similarity between the two species was noticed in their capacity for synthesizing P-enolpyruvate and ATP. The rate of utilization of malate, α-ketoglutarate and ADP was similar for liver mitochondria from both species. In presence of 0.2 mM octanoate, both human and guinea pig liver mitochondria synthesized much less P-enolpyruvate from malate or α-ketoglutarate, while the rate of ATP formation was not significantly affected. The observed ATP synthesis was not due to an adenylate kinase (EC 2. 7. 4. 3) since no enhancement of AMP production was observed in the presence of octanoate. Finally, the rate of utilization of malate, α-ketoglutarate or ADP was not significantly affected by the addition of this fatty acid.

4. Discussion

The results of the present study indicate a similarity in the mechanism which regulates mitochondrial P-enolpyruvate formation in human and guinea pig liver. This is of particular importance since it supports a more general model for the regulation of gluconeogenesis suggested by studies of perfused guinea pig and rabbit livers [14]. As was first demonstrated by Willms and Söling [8, 13], fatty acids inhibit gluconeogenesis in perfused guinea pig liver while stimulating this process in the rat. Garber and Hanson [9] made the assumption that the presence of mitochondrial P-enolpyruvate carboxykinase in guinea pig liver mitochondria could contribute to the differences in fatty acid effects on gluconeogenesis noted by Söling and Willms [8]. In studies of P-enolpyruvate formation by isolated guinea pig liver mitochondria, we noted that the rate of P-enolpyruvate formation from a variety of substrates could be inhibited by shifting the mitochondrial NAD/NADH ratio more reduced. The significance of this factor was underlined by the observation that freeze-clamped guinea pig liver has a normal mitochondrial NAD/NADH ratio of 13 which shifts markedly oxidized (to 33) after 48 hr of starvation [9]. In our view, the mechanism for the inhibition of glucose formation by guinea pig liver caused by fatty acids was an increased mitochondrial NADH concentration caused by fatty acid oxidation. To support this idea, Arinze et al. [14] have shown that β-hydroxybutyrate infusion into guinea pig, rabbit and cat liver (all species containing the bulk of this total hepatic P-enolpyruvate carboxykinase in the mitochondria) reduces gluconeogenesis.

If the hypothesis discussed above is correct, then it might also be applicable to the regulation of gluconeogenesis in human liver. As a first step toward testing this possibility we have isolated human liver mitochondria and studied the control of P-enolpyruvate formation by fatty acids. The results of these studies, presented in this paper, indicate an almost identical response to fatty acids by mitochondria from both human and guinea pig liver. It remains to be determined whether these mitochondrial studies can be directly applied to human liver and if fatty acids inhibits hepatic gluconeogenesis in the human. However, the guinea pig would seem to be a better model than the rat for studies of the regulation of gluconeogenesis in human liver.

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