SHORT COMMUNICATIONS

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The effect of carnitine and CoA on ketogenesis and citric acid cycle activity during long-chain fatty acid oxidation by isolated rat liver mitochondria

Recently it was found that in the presence of malate, ADP, ATP and relatively high concentrations of palmitate, maximal rates of oxygen uptake can be observed in the absence of carnitine[1]. In this case the palmitate oxidized is activated in the inner membrane–matrix compartment of the mitochondrion. In the presence of carnitine, the palmitoyl-CoA synthetase (acid:CoA ligase (AMP), EC 6.2.1.3) localized in the outer mitochondrial membrane contributes to the palmitate utilization in the inner membrane–matrix compartment[1]. In the present paper the hypothesis was tested as to whether carnitine addition influences the distribution between the end products of fatty acid oxidation in liver: CO_2 and ketone bodies. FRITZ[2] already observed a preferential stimulation of ketone body production by carnitine.

The hypothesis mentioned above was tested by comparison of oxygen uptake, CO_2 production and ketone body formation under several conditions. Table I shows the results of a representative experiment out of a series of 3 experiments. Carnitine or carnitine *plus* CoA stimulates ketogenesis 2-fold or more in the presence or absence of malate (compare also Fig. 1A). However, oxygen uptake is only stimulated by carnitine or carnitine *plus* CoA in the absence of malate (compare also ref. 1). An inhibition of $^{14}CO_2$ production from [1-^{14}C]palmitate is seen when carnitine or carnitine *plus* CoA is added. This indicates that carnitine and carnitine *plus* CoA stimulate ketogenesis and inhibit the complete oxidation of palmitate by inhibition of the citric acid cycle. During fatty acid oxidation the citric acid cycle may be inhibited at the level of the citrate synthase (EC 4.1.3.7) reaction[3–12]. It has been postulated that this inhibition is caused by a decrease of the intramitochondrial oxaloacetate concentration[7–12]. That carnitine, in the present investigation, also decreases the intramitochondrial concentration of oxaloacetate, is illustrated by the observation (Table I, Fig. 1) that carnitine addition increases the β-hydroxybutyrate/acetoacetate ratio. This correlates with an increase of the intramitochondrial malate/oxaloacetate ratio[13]. Indeed carnitine decreases the synthesis of products of oxaloacetate metabolism: citrate and phosphoenolpyruvate (Table I, Fig. 1B). In the presence of malate, the ATP concentration does not change under the influence of carnitine, and in the absence of malate, a significant hydrolysis of ATP is observed only in the absence of carnitine. It is known that a decrease of the phosphorylation state contributes to a lowering of NADH/NAD$^+$. The importance of intramitochondrial NADH/NAD$^+$ in the regulation of ketogenesis, citrate and phosphoenolpyruvate synthesis is again shown in Fig. 1 (a representative experiment out of a series of 3 is given). Here the time-course of the metabolic events is demonstrated. Ketone body production shows a lag time of about 5 min and is closely related to the β-hydroxybutyrate/acetoacetate ratio (Fig. 1A). The net synthesis of citrate levels off to zero after about 5 min, when the

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TABLE I

FACTORS AFFECTING KETOGENESIS AND THE FORMATION OF CITRATE AND PHOSPHOENOLPYRUVATE DURING PALMITATE OXIDATION

The incubation medium contained 65 mM Tris–HCl, 18.8 mM potassium phosphate buffer, 20 mM KCl, 1 mM EDTA, 25 mM sucrose, 10 mM $MgCl_2$, 7.5 mM ATP and 0.6 mM potassium [1-^{14}C]palmitate (specific activity, 0.4 mC/mmole) complexed with 0.086 mM bovine serum albumin. Where indicated, 0.5 mM L-carnitine, 0.05 mM CoA and/or 5 mM potassium DL-malate were added. Reaction volume, 2 ml; temp., 37°; pH 7.4. Heavy rat liver mitochondria were isolated as described before[16] and washed once. The reaction, carried out in Warburg vessels, was started by the addition of mitochondria (5.0 mg of protein) and incubation was carried out for 30 min in the Gilson differential respirometer. The total oxygen uptake was calculated by extrapolation. The centre well contained a KOH-soaked filter paper. The reaction was stopped by adding 0.1 ml 70% $HClO_4$ from the side arm and the vessels were allowed to shake for another 20 min. The radioactivity present on the filter paper was counted by liquid scintillation counting. The contents of the Warburg vessels was transferred to centrifuge tubes with 2 ml 4% $HClO_4$ and the protein was centrifuged off. The supernatant was neutralised in the cold with KOH, and after removing the $KClO_4$ by centrifugation, the supernatant was analysed for β-hydroxybutyrate[21], acetoacetate[22], citrate[23], phosphoenolpyruvate[24] and ATP[25].

Additions	$\Delta^{14}CO_2$ (*disint./min*)	$-\Delta O_2$ (*μmoles*)	*Δβ-Hydroxybutyrate + Δacetoacetate* (*μmoles*)	*ΔCitrate* (*μmoles*)	*ΔPhosphoenol-pyruvate* (*μmoles*)	$-\Delta ATP$ (*μmoles*)	*β-Hydroxybutyrate / Acetoacetate*
None	22 500	2.7	0.86	—	—	5.3	0.02
Carnitine	20 250	7.6	1.78	—	—	0.7	1.30
Carnitine + CoA	9 800	6.0	3.02	—	—	0.0	6.03
Malate	23 350	9.6	1.06	0.48	0.124	0.0	2.33
Malate + carnitine	10 250	9.5	2.20	0.36	0.062	0.0	9.22
Malate + carnitine + CoA	6 700	6.8	2.10	0.31	0.052	0.0	11.79

ratio increases (Fig. 1B). A less striking inversed relationship between the phosphoenolpyruvate synthesis and the β-hydroxybutyrate/acetoacetate ratio is also shown in Fig. 1B. It may be noted that the ATP concentration is constant under the conditions shown in Fig. 1 (see Table I).

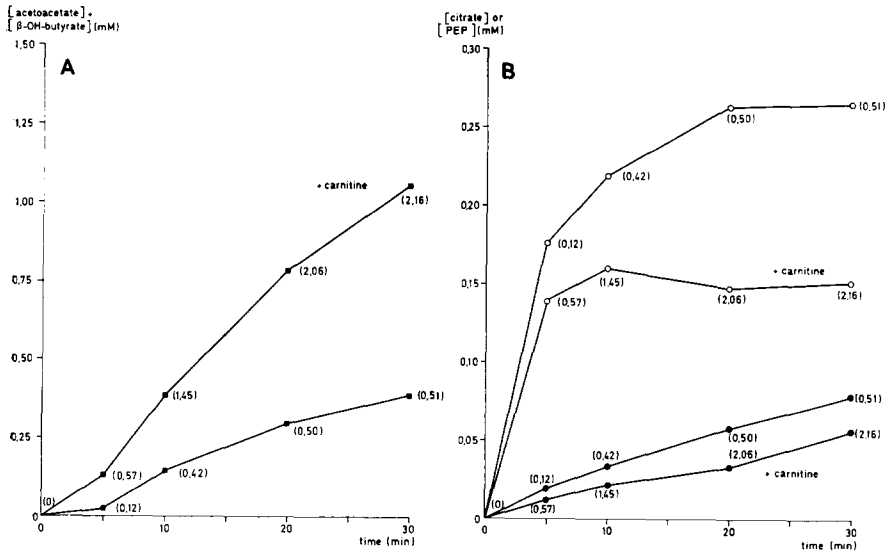

Fig. 1. Influence of carnitine on the time-course of ketogenesis (A), the β-hydroxybutyrate/acetoacetate ratio (given in parentheses) and the production of citrate and phosphoenolpyruvate (PEP) from malate (B), during palmitate oxidation. Incubation conditions were exactly the same as described in the legend to Table I (malate present). At the times indicated, samples of 2.0 ml were taken from the reaction mixture, and the metabolites were estimated in the deproteinized neutralized supernatant as described in the legend to Table I. The concentration of mitochondrial protein in the reaction medium was 2.4 mg/ml. ●—●, phosphoenolpyruvate formation; ○—○, citrate formation; ■—■, ketogenesis.

It may be of interest to note that although the bulk of the phosphoenolpyruvate carboxykinase (EC 4.1.1.32) activity in rat liver is localized in the extramitochondrial compartment[14], significant synthesis of phosphoenolpyruvate occurs in the mitochondria (Table I, Fig. 1B), confirming the observation of SCHOLTE AND TAGER[15].

In conclusion, carnitine addition influences the distribution between the end products of fatty acid oxidation in isolated rat liver mitochondria by making more activated fatty acid available to the β-oxidation system[16–18]. This results in an increased NADH/NAD$^+$ which decreases the citric acid cycle activity by lowering the intramitochondrial oxaloacetate concentration. This effect of carnitine is most pronounced in the presence of added CoA. Only in this case the full capacity of the palmitoyl-CoA synthetase present in the mitochondrial outer membrane can be used[1,19], since a suboptimal concentration of CoA exists in the sucrose space of isolated, washed rat liver mitochondria[17,19].

These present results are in agreement with the data obtained in perfusion experiments in which an inhibitor of the palmitoyl-CoA:carnitine palmitoyltransferase was employed[20].

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A rapid and simple procedure to deplete rat-liver mitochondria of lysosomal activity

Lysosomal activities that contaminate rat-liver mitochondria prepared by the conventional methods often hamper the exact localization of lysosomal and mitochondrial isoenzymes[1–5]. It has been shown[6–8] that lysosomes present in subcellular