ACTIVATION OF PROPIONATE IN RAT KIDNEY

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Received 8 August 1969

1. Introduction

Recently Wiedemann and Krebs [1] have reported that carnitine stimulates gluconeogenesis from propionate in rat kidney cortex slices, and they suggested that this effect of carnitine can be explained by a carnitine-dependent transport into the mitochondria of propionate activated in the extramitochondrial cytoplasm. This hypothesis implies an extramitochondrial activation of propionate in the kidney.

In the liver propionate is activated mainly in the matrix of the mitochondria, as are acetate and other short chain fatty acids [2]. Only long chain fatty acids are activated outside the mitochondria or in the outer mitochondrial membrane [3,4]. The acyl-CoA: carnitine acyltransferases (EC 2.3.1.7 and EC 2.3.1...) are localized in the inner membrane of the mitochondria [4,5]. These enzyme localizations are in accordance with the observation that in the liver carnitine stimulates the mitochondrial oxidation of long chain fatty acids, but not the oxidation of short chain fatty acids [6,7].

We have now studied propionate activation in subfractions of rat kidney homogenate as a test of the hypothesis of Weidemann and Krebs. The results indicate that in the kidney, as in the liver, propionate is activated in the matrix of the mitochondria. On this background an alternative explanation of the effect of carnitine on gluconeogenesis from propionate is discussed.

2. Materials and methods

Tissue homogenization and fractionation were performed according to De Duve et al. [8]. Propionate activation was assayed by trapping the propionyl-CoA formed as propionylcarnitine by addition of $(CH_3\text{-}^3H)$-carnitine and excess carnitine acetyltransferase to the incubation mixture [2]. Increasing tissue amounts gave rectilinear increase in propionylcarnitine formation up to 35 nmoles per min. In the fractionation experiments propionylcarnitine formation never exceeded 15 nmoles per min. Detergent (Triton X-100) was added to the incubation mixture to obtain maximum activity by disrupting the mitochondria. Triton had no effect on the solubilized enzyme. Carnitine palmityltransferase was assayed according to Norum [9], acid phosphatase and glucose-6-phosphatase according to Beaufay et al. [10].

3. Results

Table 1 and fig. 1 show that in subfractions of rat kidney homogenate propionate activation had a distribution corresponding almost exactly to that of carnitine palmityltransferase. For comparison are included data on the distribution of the other liver marker enzymes, glucose-6-phosphatase for the microsomal fraction and acid phosphatase for the lysosomal fraction. It is striking that the overall distribution pattern in kidney subfractions is almost identical with that of liver subfractions [2]. These results indicate therefore that in the kidney, as in the liver, both the carnitine palmityltransferase and the activation of propionate are localized in the mitochondria.

Table 2 shows that propionate activation in whole homogenate was stimulated about 2.5 times by addition of detergent. A similar stimulation was observed in the experiments with liver mitochondria [2]. These results show that most likely there is a diffusion

Table 1
Subcellular distribution of propionate activation and some marker enzymes in rat kidney.

Enzyme	Absolute values total homogenate	Percentage values						% recovery
		N+E	N	M	L	P	S	
Propionate activation	1.44	100	23	48	3	3	6	83
Acid phosphatase (total activity)	5.97	100	29	18	8	18	17	90
Acid phosphatase (free activity)	0.83	14	4	3	1	2	2	–
Glucose-6-phosphatase	17.4	100	20	7	3	44	3	77
Carnitine palmityltransferase		100	22	50	2	2	6	82
Protein (mg/g wet wt.)	157.8	100	29	16	4	15	25	89

The incubation mixture contained in a total of 1 ml of 0.1 M Tris-HCl-buffer (pH 7.5): potassium propionate, 10 mM; CoA, 0.4 mM; ATP, 7.5 mM; $MgCl_2$, 5 mM; (−)-(CH_3-^{3}H)-carnitine, 1 mM; GSH, 2.5 mM; carnitine acetyltransferase, 0.2 unit; KCN, 5 mM; Triton X-100, 0.1%; and an appropriate amount of cellular subfraction. The reaction was started with ATP. Incubation temperature was 35°, and incubation time was 15 min. The reaction was stopped by adding 2 ml of absolute ethanol. Precipitated proteins were removed by centrifugation and the water-ethanol mixture was evaporated to dryness. The (CH_3-^{3}H)-carnitine and the acyl-(CH_3-^{3}H)-carnitine were redissolved in 50% methanol and separated by chromatography on thin-layer silicic acid (after Stahl).

The absolute values for enzyme activities in the total homogenate are expressed in μmoles per min per g wet wt. of kidney, except for carnitine palmityltransferase which is expressed in arbitrary units. Protein is given in mg. The enzyme activities and the protein content in the subfractions are expressed as per cents of the total in whole homogenate (N + E). N = nuclear fraction, E = cytoplasmic extract, M = mitochondrial fraction, L = lysosomal fraction, P = microsomal fraction and S = particle-free supernatant.

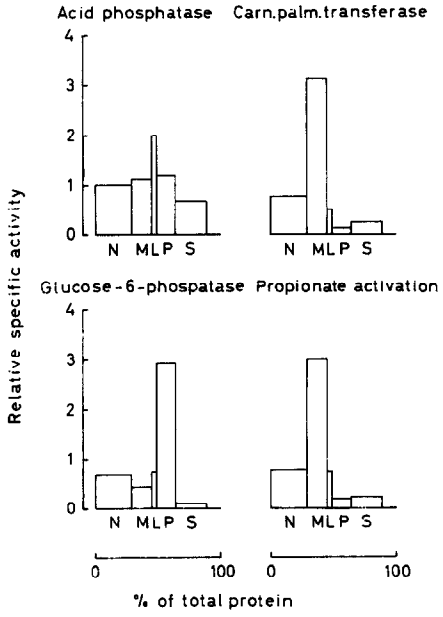

Fig. 1. The relative specific activity of the propionate activating enzyme in relation to some marker enzymes in subcellular fractions of rat kidney. Abscissa: The percentage of the total protein in each fraction (for identification of the different fractions, see legend to table 1). Ordinate: Relative specific activity (percentage of total activity/percentage of total protein) of the various fractions.

Table 2
The effect of detergent on propionylcarnitine formation in rat kidney homogenate.

Addition	Propionylcarnitine formation, nmoles	
None	99	102
0.1 % Triton X-100	223	253

Whole kidney homogenate from 5 mg of tissue wet wt. was used. Triton X-100 was added as indicated. Other conditions as described in table 1.

barrier which must be broken down to obtain maximum activity. This barrier probably is the inner mitochondrial membrane since it is established that this membrane is impermeable to CoA and carnitine [11, 12]. Thus, in contrast to the suggestion of Weidemann and Krebs [1] the propionate activating enzyme(s) in rat kidney seem(s) to be localized inside the diffusion barrier in the mitochondria, probably in the matrix. It should be noted that also the propionyl-CoA carboxylase has been found in the mitochondrial matrix [13].

4. Discussion

The present finding that the activation of propionate is localized in the same metabolic compartment as the carboxylation of propionyl-CoA, makes it unlikely that the effect of carnitine on gluconeogenesis from propionate in rat kidney slices is due to facilitated transport of activated propionate into the mitochondria.

It is striking that in the experiments of Weidemann and Krebs [1] addition of phosphate inhibited both respiration and gluconeogenesis from propionate. Both these inhibitions were relieved by carnitine, and it seems likely therefore that they have a common cause. In the absence of phosphate and at low propionate concentrations, carnitine had little or no effect.

Recently Van Tol, De Jong and Hülsmann [14] have presented evidence for an inhibitory effect of phosphate on the citric acid cycle. Such an inhibition may lower the ATP/ADP ratio in the mitochondria. As pointed out by Weidemann and Krebs an increase in the concentration of ADP together with the added inorganic phosphate will inhibit the propionyl-CoA carboxylase. This may lead to an accumulation of propionyl-CoA and a depletion of the pool of free CoA. Such a depletion of free CoA may increase the inhibition of the citric acid cycle, thus giving a vicious circle. Addition of extra carnitine may counteract the depletion of CoA by permitting an increased formation of propionylcarnitine. Weidemann and Krebs did in fact observe such an increased formation of propionylcarnitine and an increase in the concentration of free CoA.

This action of carnitine is analogous to that observed on the inhibition of α-oxoglutarate oxidation in the presence of aceto-acetate or pyruvate [15], and on the inhibition of pyruvate oxidation in the presence of acylcarnitines [16,17]. In these cases accumulation of acetyl-CoA seemed to be the cause of the inhibition. Addition of carnitine lead to acetylcarnitine formation and thus made free CoA available for the inhibited CoA-dependent reactions.

Acknowledgements

One of the authors (M.A.) is a Fellow of the Norwegian Cancer Society. The authors are indebted to Mrs. Brita Haga for skilled technical assistance.

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