CHAIN SHORTENING OF ERUCIC ACID BY SUBCELLULAR PARTICLES ISOLATED FROM LIVER AND HEART OF RAT

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1. Introduction

In several animal species, the presence in the diet of rape-seed oil rich in erucic acid (*cis*-13-docosenoic) causes various pathological disorders, especially in the heart. Many workers have dealt with the morphological aspects of the affected organs; others with the metabolic modifications brought about by the diet (reviewed [1]). As erucic acid has often been considered responsible for such disorders, many studies have been made on the metabolism of this fatty acid. The results have shown that, although erucic acid was a source of expired carbon dioxide [2,3], it was also shortened into oleic acid [4–6], particularly in the liver. Until now, studies on metabolic conversions have been carried out on the entire animal [4–7], on isolated organs or on tissue culture [8,9]. The purpose of this work was to determine in which cellular organelles, mitochondria or microsomes, these chain-shortening reactions in liver and heart occur.

2. Materials and methods

Livers and hearts from 200–250 g male Wistar rats were homogenized in a medium containing 250 mM sucrose and 10 mM triethanolamine, adjusted to pH 7.4, with 10 mM EDTA in addition for the hearts.

The homogenates were centrifuged at 800 × *g* for 10 min at 0°C and the pellet was discarded. The mitochondria were separated at 8000 × *g* for 10 min at 0°C and washed twice. The supernatant of the first mitochondrial sediment was centrifuged at 14 000 × *g* to eliminate an impure subcellular fraction and microsomes were fractionated from the latter supernatant at 105 000 × *g* for 45 min at 0°C and washed once. Mitochondrial and microsomal pellets were finally suspended in the sucrose–triethanolamine medium and their protein concentrations were measured immediately [10].

Purified potassium [14-^{14}C]erucate (35 nmol) bound to bovine serum albumin (4 nmol) was added to 2 ml of each of the media as described in the tables. The media were preincubated for 15 min at 37°C before the addition of 2 mg mitochondrial or microsomal protein and prior to incubation for 10 min at 37°C. Incubations were stopped by the addition of 10 ml 10% perchloric acid for the study of the acid-soluble products or by the addition of 2 ml 10% methanol–KOH for the analysis of total lipids.

The measurements of labelled acid-soluble products (ASP) and labelled CO_2 were performed as in [11]. The total lipids were saponified and the fatty acids were extracted as in [12], methylated, separated by gas–liquid chromatography, collected individually on glass wool as in [13] and their radioactivity was measured.

3. Results and discussion

When a medium favourable for β-oxidation was used, the results (table 1) show that the liver mitochondria activated more erucic acid than did those from heart, but the % activated erucic acid β-oxidized (ASP + CO_2) was higher with the latter. In spite of this, the calculated amounts of the cold-soluble molecules were higher with liver mitochondria (4.8 nmol) than with heart mitochondria (3.3 nmol).

Table 1
Amounts of erucic acid activated by CoA[a] and percentage[b] transformed into oleic acid (18:1) or recovered as acid-soluble products (ASP) and CO_2 in a medium[c] favourable for β-oxidation

		Liver		Heart	
		Mitochondria	Microsomes	Mitochondria	Microsomes
nmol activated erucic acid[d]		12.12	2.71	5.54	0.32
Percent found in	18:1	0.39	0.76	0.74	0
	ASP + CO_2	39.68	0	60.43	0

[a] Comprises erucoyl-CoA recovered, unacted or transformed, as long chain acyl-CoA and acylcarnitines insoluble in aqueous perchloric acid, or oxidized in acid-soluble products and CO_2
[b] Percentage of the activated erucic acid
[c] The incubation medium contained: 10 mM Tris–HCl buffer, pH 7.4; 35 mM KCl; 10 mM inorganic phosphate; 4 mM $MgCl_2$; 1 mM ATP; 0.02 mM CoA; 2 mM DL-carnitine; 1 mM sodium malate
[d] Produced in the presence of 35 nmol [14-^{14}C]erucic acid and 2 mg mitochondrial or microsomal protein

The microsomes of liver and heart were incapable of producing smaller molecules from erucate. In all cases the radioactive oleate recovered from the medium in presence of mitochondria or microsomes was very low and almost negligible. The β-oxidation reactions seem thus to proceed without any formation of intermediates. However the formation of shorter monoenes cannot be excluded since these

Table 2
Amounts of erucic acid activated by CoA[a] and percentage[b] recovered in eicosenoic acid (20:1), oleic acid (18:1), hexadecenoic acid (16:1) and in the acid-soluble products (ASP), in a medium[c] favourable for chain-shortening

		Liver		Heart	
		Mitochondria	Microsomes	Mitochondria	Microsomes
nmol activated erucic acid[d]		13.65	4.11	2.02	0.37
percent found in	20:1	5.97	2.38	7.13	
	18:1	1.35	0.29	5.41	e
	16:1	0.42	0.28	2.87	
	ASP	4.73	0.37	0.37	0

[a, b] See [a] and [b] in table 1
[c] The incubation medium contained: 33 mM KCl; 50 mM P_i, pH 7.6; 4 mM $MgCl_2$; 1 mM ATP; 0.05 mM CoA; 2 mM $NaHCO_3$; 1 mM NADP; 1 mM KCN
[d] Produced in the presence of 35 nmol [14-^{14}C]erucic acid and 2 mg mitochondrial or microsomal protein
[e] Activation and shortening did not occur to a sufficient extent to give any significant figures

Table 3
Amounts of erucic acid activated by CoA[a] and percentage[b] recovered in eicosenoic acid (20:1), oleic acid (18:1), hexadecenoic acid (16:1) and in the acid-soluble products (ASP), after shortening[c] by liver mitochondria[e]

		Shortening medium			
NADP 1 mM		–	+	–	+
DL-Carnitine 2 mM		–	–	+	+
nmol activated erucic acid[d]		13.51	12.86	17.57	17.65
Percent found in	20:1	2.12	12.33	7.56	11.78
	18:1	0.70	5.70	4.83	5.41
	16:1	0.30	3.87	5.88	3.71
	ASP	8.00	6.86	5.48	3.45

a, b, c, d See a–d in table 2
e The mitochondria were incubated 15 min at 35°C

newly formed acids could be rapidly oxidized and thus could not accumulate.

In the second series of experiments, attemps were made to determine whether chain-shortening was the reverse of the elongating reaction. Thus, oxidation was stopped by the addition of cyanide, and the absence of carnitine prevented the penetration of fatty acids into mitochondria and prevented also a possible accumulation of acylcarnitines. NADP was used in place of NADPH, the latter being necessary for the reducing reactions of elongation [12]. Under these conditions (table 2) the radioactivity of the acid-soluble products was very low in each case, but contrary to that observed previously, monounsaturated fatty acids shorter than erucic acid appeared in the medium, mainly after incubation with mitochondria. Those were eicosenoic acid (20:1 n-9), oleic acid (18:1 n-9) and hexadecenoic (16:1 n-9).

From these two series of experiments, it appears that the chain-shortening of erucic acid neither follows the same pathway of intramitochondrial β-oxidation, nor probably that of the reverse reaction of elongation, since microsomes which can actively elongate, cannot shorten erucic acid to the same extent as mitochondria. This suggests the existence of a specific chain-shortening system essentially localized in mitochondria, those from liver being particularly active if we consider their very great possibilities of activation. In absence of carnitine, erucic acid cannot be transported across the inner mitochondrial membrane, which, in addition, is impermeable to NADP. It can therefore be deduced that the chain-shortening system is not localized within the inner mitochondrial membrane, nor inside the mitochondria.

The chain-shortening reaction using NADP, may not be the only pathway of producing shorter monoenes from erucic acid. In particular these monoenes could appear in the first steps of the intramitochondrial β-oxidation, when cyanide is added. To test this hypothesis, a third series of experiments was undertaken with mitochondria incubated in presence of NADP or not and in presence of carnitine or not. Mitochondria isolated from liver were prefered, because they revealed the most active chain-shortening process in vitro and because in vivo 18:1 was found in liver [6]. The results (table 3) show that in the absence of carnitine the chain-shortening reaction was significant only when NADP was added. However the addition of carnitine to the incubation medium, in absence of NADP, increased the amount of shortened fatty acids. They were probably formed at the beginning of the β-oxidation reactions, according to the conclusions in [14] when the respiratory chain is inhibited. When both NADP and carnitine were added, it might be expected that the two chain-shortening processes would take place simultaneously. However the very low radioactivity of the acid-soluble products suggests that carnitine played no major part, in this case, facilitating the entry of erucic acid into the mitochondria towards the sites of β-oxidation. On the other hand, as the relative proportion of the three shortened fatty acids was the same, in the presence of NADP alone or associated with carnitine, and different from those observed in the presence of carnitine alone, it seems that erucic acid activated by CoA was channeled mainly towards shortening reactions, to the detriment of transfer reactions into the mitochondria. This suggests that the chain-shortening system is more likely to be localized at the outer mitochondrial membrane; this view is supported by results obtained with isolated liver cells [9].

The hypothesis that erucic acid could be used by the cardiac cells after chain-shortening, suggested in

[8], is supported by the results in table 2, since the conversion of 20:1 to 18:1 and then to 16:1 seems to be achieved more easily with heart mitochondria than with those of liver.

Proof that erucic acid is very actively shortened in the presence of NADP by liver and heart mitochondria is presented in this paper.

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