CHAIN SHORTENING OF ERUCIC ACID IN ISOLATED LIVER CELLS

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1. Introduction

C_{22} monounsaturated fatty acids are abundant in some types of rapeseed oil and some marine oils used for human consumption in many countries.

In feeding experiments rapeseed oil, rich in erucic acid (22:1, *n-9cis*) causes a fatty infiltration in the heart of rats and several other species [1].

Previous studies with isolated heart and liver mitochondria have shown that erucic acid is oxidized at a distinctly slower rate than palmitic acid. It has also been shown that erucic acid inhibits the oxidation of other fatty acids in mitochondria, probably by an inhibitory effect on acyl CoA dehydrogenase [2].

In vivo experiments with ^{14}C-labelled erucic acid given to rats have shown that part of the injected radioactivity is recovered in shorter monounsaturated fatty acids, particularly in oleic acid in liver [3]. A chain shortening of erucic acid in cultured myocytes has also been reported [4].

In the present work the metabolism of erucic acid in isolated liver cells has been studied.

2. Materials and methods

[1-^{14}C]Palmitic acid was obtained from the Radiochemical Center, Amersham. [14-^{14}C]Erucic acid (22:1, *n-9cis*) was from Centre d'Etudes Nucléaires de Saclay, Gif sur Yvette. (−)-Carnitine chloride was from Koch-Light Labs Ltd, Colnbrook Bucks. Essentially fatty acid free bovine albumin, *N*-2 hydroxyethylpiperazine-*N*-2-ethanesulfonic acid (Hepes), collagenase Type I and erucic acid were from Sigma Chemical Co., St Louis, MO. Palmitic acid was from Fluca AG, Switzerland.

Hepatocytes were prepared from livers of male Wistar-derived rats (220–250 g). The rats had free access to food and water until they were sacrified.

Isolated hepatocytes were prepared and purified as in [5]. About 100–300 × 10^6 cells were obtained from each liver, and 90–95% were viable, measured as resistance to uptake of trypan blue.

In most experiments the incubation medium was as in [6]. If not stated otherwise the fatty acid concentration was 1 mM. In some experiment the hepatocytes were preincubated for 30 min with (−)-carnitine as in [7].

The extraction of lipids, the measurements of radioactive acid-soluble products and radioactive CO_2 were performed as in [7].

The lipids were separated in silicic acid thin-layer plates (stahl H) (hexane–diethylether–glacial acetic acid, 85:15:5, v/v/v). Aliquots of the total lipid extract and of the free fatty acid, triglyceride and phospholipid fractions were transmethylated [8] and analyzed by radio-gas chromatography using a Pye 104 gas chromatograph connected to a ESI Nuclear radioactivity detector with a 1:1 outlet splitter. Fatty acid methyl-esters were separated at 185°C using 10% SP-2340 on Supelcoport 100/120 (Supelco Inc., Bellefonte, PA). The peaks were identified on the basis of the retention time compared with the standards.

3. Results and discussion

Table 1 shows that erucic acid is metabolized at a distinctly lower rate than palmitic acid in isolated liver cells. After 30 min incubation the total amount in moles of erucic acid, oxidized and esterified was only

Table 1
Oxidation and esterification of [^{14}C]erucic acid in isolated liver cells in the presence and absence of added carnitine

Additions	Incubation period (min)	Fatty acid			Ratio oxidized	Fatty acid	
		Oxidized + esterified	Oxidized	In triglycerides	in triglycerides	In phosphatidylcholine	In phosphatidylethanolamine
Erucic acid	30	28.2	11.8	9.3	1.27	3.5	1.6
	120	80	44.0	18.3	2.40	7.9	3.0
Erucic acid	30	27.2	15.0	4.8	3.13	2.5	0.8
+ carnitine	120	88.5	60.5	8.9	6.80	4.3	1.2
Palmitic acid	30	92	41.2	30.0	1.37	9.3	1.7
	120	169.1[a]	98.4	44.9	2.19	13.3	3.5
Palmitic acid	30	89.1	54.7	16.6	2.94	8.2	1.6
+ carnitine	120	170[a]	112.4	26.7	4.21	10.3	2.6

[a] Nearly all the substrate was used, since 2000 nmol, i.e., 185.2 nmol × mg protein^{-1} was added both of [^{14}C]palmitic- and of [^{14}C]erucic acid

The incubation conditions were as in section 2. Hepatocytes (10.8 mg protein) was used. Carnitine (4 mM) was added where indicated. The results are expressed as nmol [^{14}C]erucic acid metabolized × mg protein^{-1}. The amount oxidized is calculated from the sum of ^{14}C activity in the acid soluble fraction and in $^{14}CO_2$

30% of the amount metabolized with palmitic acid.

The ratio of fatty acid oxidized above fatty acids incorporated in triacylglycerol seems to be approximately the same for erucic acid and palmitic acid. The relative amounts of fatty acid channelled to oxidation and to triacylglycerol synthesis thus seems to be the same with both fatty acids.

The addition of carnitine had little effect on the total amount of fatty acid metabolized both with erucic acid and with palmitic acid. Only a very slight stimulatory effect of carnitine was observed.

It has previously been shown that addition of carnitine increased the rate of oxidation and reduced the esterification of palmitic acid in isolated liver cells [7].

Table 1 also shows that carnitine stimulates the oxidation and reduces the esterification of erucic acid to triacylglycerol. The effects of carnitine on the metabolism of erucic acid seems to be slightly more pronounced than the effect on palmitic acid. It is further shown that the incorporation into phospholipids is distinctly lower with erucic acid than with palmitic acid. The effect of carnitine on the esterification to phospholipids is less marked than the effect on the triacylglycerol synthesis both with erucic acid and with palmitic acid.

The possibility of a chain shortening of [14-^{14}C]-erucic acid to other fatty acids was studied by using radio-gas chromatography. Figure 1A shows that significant amounts of the ^{14}C label appeared in oleic acid (18:1, *n*-9), eicosenoic acid (20:1, *n*-9) and in palmitoleic acid (16:1, *n*-9) after incubation of hepatocytes with ^{14}C-labelled erucic acid. [^{14}C]Oleic acid accumulated approximately twice as fast as [^{14}C]eicosenoic acid. Only relatively small amounts of [^{14}C]-palmitoleic acid were formed. After 120 min of incubation 15–20% of the radioactivity added as [14-^{14}C]-erucic acid appeared in shorter fatty acids with approx. 10% in oleic acid alone.

Figure 1B shows that the rate of formation of [^{14}C]eicosenoic-, [^{14}C]oleic- and [^{14}C]palmitoleic acid increased with increasing concentrations of [^{14}C]erucic acid in the incubation medium until a maximal rate of chain shortening was reached at approx. 1 mM of erucic acid.

Table 2 shows the content of different ^{14}C-labelled fatty acids in the triacylglycerol and phospholipid fractions from isolated hepatocytes incubated with

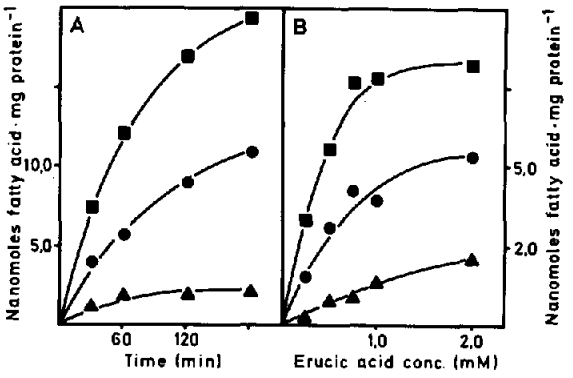

Fig.1. Formation of [^{14}C]oleic acid (18:1, *n*-9), [^{14}C]eicosenoic acid (20:1, *n*-9) and [^{14}C]palmitoleic acid (16:1, *n*-9) from [^{14}C]erucic acid in isolated liver cells. (A) Hepatocytes (12.8 mg protein) were incubated with 1 mM erucic acid as described in section 2. (B) Hepatocytes (12.0 mg protein) were incubated with different concentrations of [^{14}C]erucic acid for 30 min. (■–■) oleic acid; (●–●) eicosenoic acid; (▲–▲) palmitoleic acid.

[^{14}C]erucic acid. In the triacylglycerol fraction the labelled $C_{20:1}$, $C_{18:1}$ and $C_{16:1}$ fatty acids accounted for 40–50% of the radioactivity with the rest of the activity in erucic acid. In the phospholipid fraction 75–85% of the radioactivity was found in the shortened $C_{20:1}$, $C_{18:1}$ and $C_{16:1}$ fatty acids. Erucic acid thus seems to be less readily incorporated in the phospholipid fraction than in triacylglycerol.

These observations agree with the finding that very low density lipoproteins from rat livers perfused with [^{14}C]erucic acid contain a high proportion of labelled $C_{20:1}$, $C_{18:1}$ and $C_{16:1}$ fatty acids [10]. In the free fatty acid fraction only very small amounts of shortened fatty acids were found as this fraction mainly consists of unmetabolized extracellular erucic acid substrate.

The rapid chain shortening of erucic acid to $C_{20:1}$, $C_{18:1}$ and $C_{16:1}$ fatty acids in isolated liver cells demonstrated in the present study, may occur either in the mitochondria or by an extramitochondrial β oxidation system. In previous work with isolated liver and heart mitochondria with the carnitine ester of [^{14}C]erucic acid as the substrate, we were unable to demonstrate any accumulation of radioactive $C_{20:1}$, $C_{18:1}$ or $C_{16:1}$ fatty acids in the medium (unpublished). It is interesting that an extramitochondrial β oxidation

Table 2
Incorporation of shorter fatty acids formed from [^{14}C]erucic acid in different lipid fractions of isolated liver cells – effect of carnitine

Lipid fraction	Incubation (min)	Sum	$C_{16:1}$	$C_{18:1}$	$C_{20:1}$	$C_{22:1}$
Triglycerid	30	10.4	–	3.1	1.3	6.0
Phospholipids	30	5.6	–	2.9	1.7	1.0
FFA	30	93.2	–	1.3	0.3	91.6[a]
Triglycerides	60	15.0	0.6	4.4	2.9	7.1
Phospholipids	60	8.8	0.2	3.9	3.2	1.5
FFA	60	70.2	0.5	3.1	0.6	66.0[a]
Total lipid + carnitine	120		1.2	14.2	6.4	55.3[b]
Total lipid – carnitine	120		1.5	17.2	8.3	62.9[b]

[a] Unmetabolized free erucic acid
[b] Sum of esterified + unmetabolized free erucic acid

Hepatocytes (12.4 mg protein) were incubated with 1 mM erucic acid as in section 2. The results are expressed as nmol [^{14}C]fatty acid in each fraction × mg protein^{-1}. Carnitine (4 mM) was added where indicated

of fatty acids has recently been found and localized to the peroxisomes [9].

If the accumulation of radioactive $C_{20:1}$, $C_{18:1}$ and $C_{16:1}$ fatty acids observed with hepatocytes incubated with [^{14}C]erucic acid should be caused by mitochondrial β oxidation, it would mean that the labelled shortened fatty acids present in the triacylglycerol stems from erucic acid which had first been transported into the mitochondria and then after a chain shortening had left the mitochondria for the extramitochondrial triacylglycerol synthesis.

The stimulating effect of carnitine on the oxidation of erucic acid in liver cells, is most probably caused by an increased mitochondrial β oxidation.

If the accumulation of $C_{20:1}$, $C_{18:1}$ and $C_{16:1}$ fatty acids from [^{14}C]erucic acid was caused by regular mitochondrial β oxidation, one should expect that addition of carnitine would not significantly decrease the proportion of labelled $C_{20:1}$, $C_{18:1}$ and $C_{16:1}$ fatty acids.

The finding that carnitine reduces the content of $C_{20:1}$, $C_{18:1}$ and $C_{16:1}$ fatty acids formed from [^{14}C]erucic acid in isolated liver cells (table 2) therefore suggest that the chain shortening occurs outside the mitochondria, possibly in the peroxisomes. This view is also supported by the finding [11] that the chain shortening of erucic acid is increased in hepatocytes from rats fed chlofibrate. Chlofibrate is known to cause a proliferation of peroxisomes [9].

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