ARACHIDONIC ACID SYNTHESIS STUDIED IN ISOLATED LIVER CELLS

Effects of (−)-carnitine and of (+)-decanoylcarnitine

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1. Introduction

The biosynthesis of arachidonic acid from linoleic acid has been extensively studied in isolated liver microsomes and in vivo [1]. The mechanisms regulating the synthesis of polyunsaturated fatty acids have been little studied in isolated cells and perfused organs and are to a great extent still unknown.

The aim of this work was to study whether isolated hepatocytes can be used as a suitable in vitro system to study the regulation of arachidonic acid synthesis.

Hepatocytes were prepared from rats raised on a diet deficient in essential fatty acids since studies with microsomes and in vivo have demonstrated that the activity of the desaturases introducing new double bonds is increased by such diets.

2. Materials and methods

[1-^{14}C]Linoleic acid was obtained from the Radiochemical Center, (Amersham). (−)-Carnitine chloride was from Otsuka Pharmaceuticals (Osaka). (+)-Decanoylcarnitine was synthesised according to [2]. Essentially fatty acid-free bovine albumin, *N*-2-hydroxyethylpiperazin-*N*-2 ethanesulphonic acid (Hepes), collagenase type I and unlabelled linoleic acid were from Sigma Chemicals (St Louis MO).

Male weanling rats of the Wistar strain were from Möllegaard Laboratory (Denmark). The animals were fed a semisynthetic diet deficient in essential fatty acids according to [3] with 15 wt % hydrogenated coconut oil for ⩾60 days. Parenchymal liver cells were prepared and purified according to [4]. About 100–300 × 10^6 cells were obtained from each liver, and 90–95% were viable, as measured by resistance to uptake of trypan blue.

In most experiments the incubation medium was as in [5]. Linoleic acid with spec. act. 7 mCi/mmol was used. Cell suspension (1 ml) was mixed with 1 ml incubation medium containing the additions. The final linoleic acid level was 0.07 mM, and 5 mM glucose was present. Incubation was for 45 min. When indicated the hepatocytes were preincubated for 20 min with 1 mM (−)-carnitine or 1 mM (+)-decanoylcarnitine before the addition of linoleic acid.

The extraction of lipids, the measurements of radioactive acid soluble products and radioactive CO_2 were performed as in [6].

The lipids were separated on silicic acid thin-layer plates (stahl H+) (hexane–diethylether–glacial acetic acid, 80:20:1, by vol.). Aliquots of the total lipid extract and of the free fatty acid, triglyceride and phospholipid fractions were transmethylated [7] and analyzed by radiogas chromatography using a Varian 2100 gas chromatograph connected to a ESI Nuclear radioactivity detector with a 1:1 outlet splitter. Fatty acid methyl-esters were separated at 175°C and 200°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

Fig.1 shows that 3 fatty acid metabolities, γ-linolenic acid (18:3), homo-γ-linolenic acid ((8,11,14)-eicosatrienoic acid)) (20:3) and arachidonic acid (20:4) were present after incubation of [^{14}C]linoleic acid with isolated liver cells. Arachidonic acid was quantitatively the dominating product (fig.1, table 1). Significant amounts of γ-linolenic acid were also

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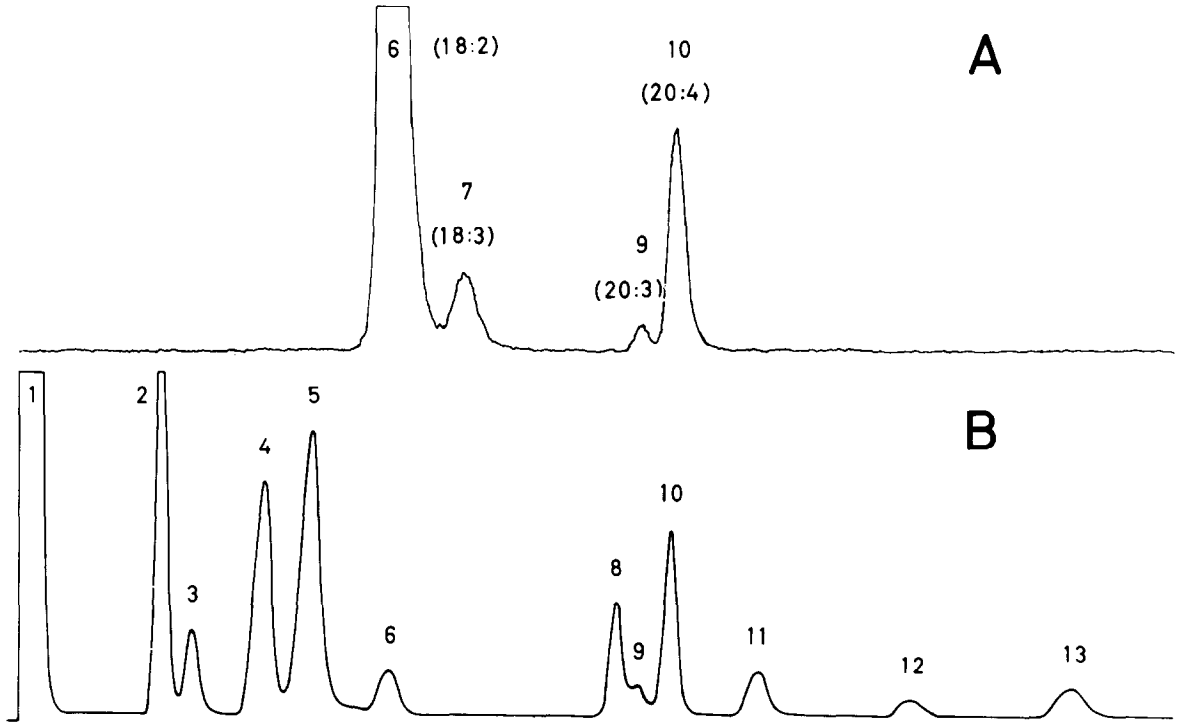

Fig.1. Gaschromatogram showing the formation of γ-[^{14}C]linolenic acid (18:3), homo-γ-[^{14}C]linolenic acid (20:3) and [^{14}C]-arachidonic acid (20:4) from [^{14}C]linoleic acid. Hepatocytes (39.4 mg protein) were incubated with 1 mM (−)-carnitine for 20 min before incubation with 0.07 mM linoleic acid as in section 2. (A) Radioactivity detector; (B) flame ionization detector. The initial column temperature of 175°C was increased to 200°C after the appearance of peak no. 7. The chromatographic peaks are: (1) solvent front; (2) 16:1; (3) 16:1; (4) 18:0; (5) 18:1; (6) 18:2; (7) 18:3; (8) 20:3 (5,8,11-eicosatrienoic acid); (9) 20:3 (homo-γ-linolenic acid (8,11,14-eicosatrienoic acid)); (10) 20:4; (11) 24:0 (internal standard); (12) 22:5; (13) 22:6.

present while only trace amounts of homo-γ-linolenic acid were detected.

Table 1 shows that the relative amounts of the 3 fatty acids formed could be varied markedly by addition of (+)-decanoylcarnitine and to a smaller extent by addition of (−)-carnitine. Addition of (+)-decanoylcarnitine caused a very marked decrease in the formation of arachidonic acid and homo-γ-linolenic acid was no longer detectable. Instead a marked increase in the amounts of γ-linolenic acid was found. (−)-Carnitine increased the formation of arachidonic acid, and increased amounts of homo-γ-linolenic acid

Table 1
Desaturation and chain elongation of [1-^{14}C]linoleic acid

Addition	$C_{18:2}$	$C_{18:3}$	$C_{20:3}$	$C_{20:4}$	Sum of 18:3, 20:3 and 20:4 *i%* of sum of all 4 fatty acids
None	2.01	0.15	0.03	0.30	20%
(−)-Carnitine	1.76	0.07	0.07	0.38	23%
(+)-Decanoyl-carnitine	2.53	0.63	0	0.04	21%

Hepatocytes (39.4 mg protein) were incubated with 0.07 mM linoleic acid as in section 2. The results are presented as nmol ^{14}C-labelled fatty acid/mg protein

Table 2
The effect of (−)-carnitine and (+)-decanoylcarnitine on [1-^{14}C]linoleate metabolism in hepatocytes

Addition	Oxidized to CO_2	Acid-soluble products	Triacyl-glycerol	Phospho-lipids	Cholesterol esters	Sum of linoleate metabolized
None	0.18	0.88	1.22	1.17	0.04	3.48
(−)-Carnitine	0.18	1.09	0.89	1.29	0.03	3.47
(+)-Decanoyl-carnitine	0.15	0.18	2.57	0.52	0.02	3.44

Nearly all the substrate was used since 140 nmol linoleate added is 3.55 nmol/mg protein; hepatocytes (39.4 mg protein) were incubated with 0.07 nM linoleate as in section 2; the results are presented as nmol [^{14}C]linoleic acid metabolized/mg protein

were present at the expense of the amounts of γ-linolenic acid found. Although the quantitative effects of carnitine on the concentration of the 3 fatty acids were moderate, these effects were constantly present in all experiments (5 expt.) performed.

Liver cells had been shown to loose a significant part of their endogenous carnitine content during the isolation procedure [8]. In experiments with palmitate, addition of carnitine increased the mitochondrial oxidation and reduced the triacylglycerol formation in isolated liver cells. These effects were also observed in the present experiments with linoleic acid as the fatty acid substrate (table 2).

In [6] the phospholipid biosynthesis in the liver cells was not affected by (+)-decanoylcarnitine while the reduction in palmitic acid oxidation was accompanied by a quantitatively equal increase in the triacylglycerol formation [6]. Here, (+)-decanoylcarnitine caused a reduction of the esterification to phospholipids in addition to a reduced mitochondrial oxidation when linoleic acid was used as the substrate (table 2) and thus caused a very marked increase in the triacylglycerol synthesis. The presence of 5,8,11-eicosatrienoic acid (fig.1, peak 8) is typical for animals fed a diet deficient in essential fatty acids [9].

4. Discussion

γ-Linolenic acid (18:3) and homo-γ-linolenic acid (20:3) have been firmly established as the main intermediates in liver biosynthesis of arachidonic acid from linoleic acid. γ-Linolenic acid is however not found in liver in vivo even after feeding animals with relatively large amounts of this fatty acid [10].

(+)-Decanoylcarnitine had been shown to be a potent inhibitor of palmitate synthesis in isolated liver cells [11]. Since (+)-decanoylcarnitine is known to be a non-metabolizable inhibitor of long-chain acyl CoA-carnitine transferase, it increases the extramitochondrial concentration of long-chain acyl CoA by inhibiting the transport of long-chain acyl groups into the mitochondria. Long-chain acyl CoA again is known to inhibit acetyl-CoA carboxylase, a regulating enzyme in the fatty acid synthesis. The effects of (+)-decanoylcarnitine on palmitic acid synthesis in hepatocytes have thus been explained by the inhibitory effect of palmityl CoA on malonyl CoA synthesis [11].

A similar mechanism may explain the inhibitory effect of (+)-decanoylcarnitine on the chain elongation of 18:3–20:3 and 20:4 here. A high concentration of long-chain acyl CoA outside the mitochondria may thus inhibit acetyl CoA carboxylase and then the chain elongation from 18:3–20:3 and 20:4 fatty acids.

Carnitine is known to stimulate fatty acid synthesis since long-chain acyl carnitine can remove the inhibitory effect of long-chain acyl CoA on acetyl CoA carboxylase [12]. Again, the stimulating effect of carnitine on the conversion of 18:3–20:3 and 20:4 here may be explained by increased concentration of long-chain acyl carnitine stimulating the acetyl CoA carboxylase activity.

These experiments cannot exclude other explanations of the effects of carnitine and (+)-decanoylcarnitine for instance effects on the transfer of acetyl groups as acetyl carnitine from the mitochondria to the sites of formation of malonyl CoA and the chain elongation of linoleic acid to arachidonic acid.

The inhibitory effect of (+)-decanoylcarnitine on the incorporation of ^{14}C-labelled fatty acids in phos-

pholipids in isolated hepatocytes may partly be explained as a secondary effect of the decreased formation of arachidonic acid which is known to be preferentially incorporated in the phospholipid fraction.

The relative amounts of γ-linolenic acid, homo-γ-linolenic acid and arachidonic acid formed from [^{14}C]linoleic acid in the absence of (−)-carnitine or (+)-decanoylcarnitine is probably also related to the supply of malonyl CoA in the hepatocytes and is probably affected by the hormonal and feeding state of the animals.

These findings support the view that isolated hepatocytes can be used to study the regulation of the synthesis of essential fatty acids.

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