INCREASED 4-ENOYL-CoA REDUCTASE ACTIVITY IN LIVER MITOCHONDRIA OF RATS FED HIGH-FAT DIETS AND ITS EFFECT ON FATTY ACID OXIDATION AND THE INHIBITORY ACTION OF PENT-4-ENOATE

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Received 20 September 1980

1. Introduction

Administration of clofibrate to rats protects hepatic fatty acid oxidation against inhibition by hypoglycin [1] and pent-4-enoate [1,2]. The inhibitory action of pent-4-enoate is related with the accumulation of pent-2,4-dienoyl-CoA or a subsequent β-oxidation metabolite in the mitochondria [2,3]. Apparently, clofibrate prevents this accumulation by increasing the activity of 4-enoyl-CoA reductase, or rather 2,4-dienoyl-CoA reductase, since the enzyme is most active with 2,4-dienoyl-CoA esters [2,4].

Here we show that partially hydrogenated marine oils in the diet also lead to increased 4-enoyl-CoA reductase activity and concomitantly prevent inhibition of fatty acid oxidation by pent-4-enoate.

2. Methods

Male Wistar rats (150 g) were fed either a normal standard pellet diet or semisynthetic high fat diets for 15–18 days. These diets have been described in [5] and were modified to contain either 15% by wt. of partially hydrogenated marine oils or soya-bean oil.

Hepatocytes were prepared according to [6] except that Krebs-Henseleit bicarbonate buffer with 0.5 mM $CaCl_2$ was used as suspension medium and incubation medium which also contained 0.5 mM fatty acid free albumine and 1 mM [U-^{14}C]palmitate.

NADPH-dependent 4-enoyl-CoA reductase was assayed as in [4] in the particle-free supernatant of isolated mitochondria treated with 0.2% deoxycholate.

Fatty acyl-CoA esters were synthesized and characterized as in [7]. Pent-2,4-dienoate was prepared according to [8].

3. Results

Table 1 shows that the activity of 4-enoyl-CoA reductase with 2,4-dienoyl-CoA esters as substrates were about doubled in mitochondria from rats fed partially hydrogenated marine oils, as compared with those from normally fed rats. Also soya-bean oil diet led to an increase in the activity of this enzyme, but

Table 1
The activity of 4-enoyl-CoA reductase in liver mitochondria from rats fed either a normal pellet diet or diets containing partially hydrogenated marine oils or soya-bean oil

Diet	Substrate	No. obs.	Activity of 4-enoyl-CoA reductase
Normal	Sorboyl-CoA	8	27.4 ± 1.8
Marine oils	Sorboyl-CoA	7	60.5 ± 3.2
Soya-bean oil	Sorboyl-CoA	4	39.4 ± 1.6
Normal	Pent-2,4-dienoyl-CoA	3	46.1 ± 5.9
Marine oils	Pent-2,4-dienoyl-CoA	3	89.5 ± 8.5

The results are given as nmol NADPH oxidized . min^{-1} . mg soluble mitochondrial protein^{-1} (means ± SEM). Sorboyl-CoA and pent-2,4-dienoyl-CoA was 0.1 mM

Table 2
The oxidation of [U-^{14}C]palmitate in hepatocytes from rats fed either a normal pellet diet or a diet containing partially hydrogenated marine oils in the presence and absence of pent-4-enoate, antimycin A or rotenone

Inhibitor	Normal rats		Rats fed marine oils	
None	20.1	23.3	55.7	64.9
Pent-4-enoate (2 mM)	6.0 (30)	9.1 (39)	38.2 (69)	45.4 (70)
Antimycin A (2 μM)	6.3 (31)	6.8 (29)	12.0 (22)	12.5 (19)
Rotenone (30 μM)	13.3 (66)	14.6 (63)	26.5 (48)	34.0 (52)

The results are given as palmitate recovered as acid-soluble radioactive products (nmol . mg protein^{-1} . 30 min^{-1}), and (in parenthesis) as palmitate oxidized in % of oxidation in the absence of inhibitor. The parallels represent results obtained with hepatocytes from two different animals

less pronounced (table 1). We had used sorboyl-CoA as substrate since pent-2,4-dienoyl-CoA was not available. As is evident from table 1, the enzyme was even more active with pent-2,4-dienoyl-CoA. The apparent K_m-value with both this substrate and sorboyl-CoA was 22 μM.

As shown in table 2, feeding partially hydrogenated marine oils stimulated palmitate oxidation and protected specifically against inhibition by pent-4-enoate.

4. Discussion

Increased peroxisomal β-oxidation in liver cells seems to be a common effect of clofibrate and high fat diets [9–11]. Feeding a clofibrate diet also leads to a 4–5-fold increase in 2,4-dienoyl-CoA reductase activity of the liver mitochondria and a complete abolition of the inhibitory action of pent-4-enoate [2]. Now we have found that high fat diets, especially with hydrogenated marine oils, also increase the activity of the reductase (table 1) and give a partial protection against pent-4-enoate (table 2). Compared with low-fat diet or normal pellet diet, peroxisomal β-oxidation and 2,4-dienoyl-CoA reduction are increased 4–7-fold by clofibrate [2,12], 2–2.4-fold by the marine oil diet (table 1) [11] and 1.4-fold by the soya-bean oil diet (table 1) [11]. Thus, high fat diets and clofibrate have several common effects.

NADPH-dependent reduction of deca-2,4-dienoyl-CoA may be a step in the degradation of linoleic acid [4]. Our results support that 4-enoyl-CoA reductase (2,4-dienoyl-CoA reductase) participates in the degradation of unsaturated fatty acids and that changes in its activity is part of an adaptation to high fat diets.

Acknowledgements

We are indebted to June Taje and Mette Østby for skillful technical assistance. This work has been supported by the Royal Norwegian Council for Scientific and Industrial Research and by the Anders Jahres Foundation.

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