INHIBITORY EFFECT OF ERUCYLCARNITINE ON THE OXIDATION OF PALMITATE BY RAT HEART MITOCHONDRIA

B.O. CHRISTOPHERSEN and Jon BREMER
Institute of Clinical Biochemistry, Rikshospitalet, University of Oslo, Oslo, Norway

Received 15 April 1972

1. Introduction

In rat and some other species a diet with a high caloric per cent of rapeseed oil induces the rapid accumulation in their heart muscles of fat droplets, consisting mainly of triglycerides [1, 2]. Macroscopically the hearts grow pale and the contractile force of the heart muscles decreases markedly after 2–3 days. The adverse effect of rapeseed oil is mainly caused by erucic acid (*cis*-13-docosenoic acid) which constitutes 30–40% of the fatty acids in rapeseed oil [2, 3]. In many countries this oil is used in small amounts for domestic consumption and as a constituent for the production of margarine [4].

The present knowledge about the effects of erucic acid stems largely from studies of the growth of rats, histopathological studies and lipid analyses of organs from animals fed on rapeseed oil [2, 5, 6]. The biochemical mechanisms of the effects have been little studied. *In vivo* studies have shown that erucic acid can be β-oxidized [7]. It has recently been shown that heart mitochondria from rats which have been fed on rapeseed oil, oxidize several substrates such as α-oxoglutarate, succinate and caprinate at a reduced rate and give low P/O ratios [8].

In the present work evidence is presented that erucic acid or some of its metabolites inhibits the oxidation of other fatty acids in the heart.

2. Materials and methods

Erucic acid of approx. 99% purity was obtained from Sigma Chemical Co., St. Louis, USA. Erucylcarnitine was prepared from erucylchloride and (−)-carnitine perchlorate in acetonitrile and purified by a general method for the preparation of carnitine esters of unsaturated fatty acids previously described [9].

Since young rats are more susceptible to the toxic effects of rapeseed oil feeding than older animals, 4 week old Wistar rats were used for the preparation of heart mitochondria. After weaning the animals had been fed conventional, pelleted laboratory diet. They had not been given rapeseed oil. The hearts were homogenized in 0.25 M sucrose which contained 10 mM neutralized EDTA (sucrose-EDTA). The homogenates were centrifuged at 800 *g* for 5 min and the pellet was discarded. The mitochondria were sedimented at 10,000 *g* for 5 min and washed once and finally suspended in sucrose-EDTA in concentrations corresponding to 5–8 mg of protein per ml.

Mitochondrial oxygen uptake was measured with a Clark oxygen electrode. Protein was measured by the method of Lowry et al. [10].

3. Results and discussion

Fig. 1 shows that the rate of oxygen uptake of rat heart mitochondria with erucylcarnitine as the substrate was only approx. one fifth of the rate with palmitylcarnitine as the substrate in the presence of dinitrophenol and malate. The rate of oxygen uptake with erucylcarnitine was only twice as high as the rate of endogenous oxygen uptake.

North-Holland Publishing Company – Amsterdam

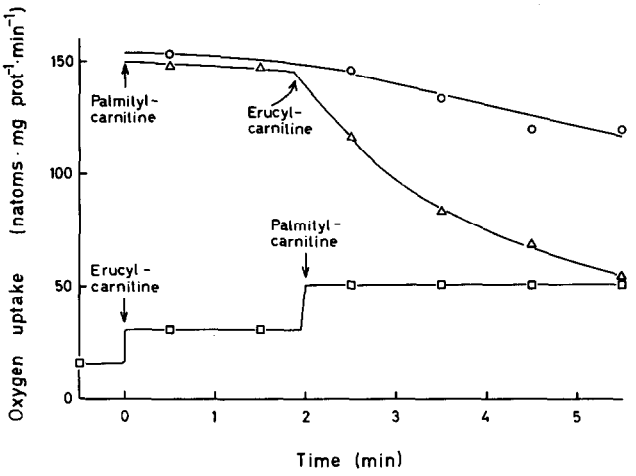

Fig. 1. Oxygen uptake of rat heart mitochondria with erucylcarnitine and palmitylcarnitine as substrates. The incubation medium contained 0.1 M KCl; 6 mM $MgCl_2$; 10 mM *N*-Tris(hydroxymethyl)-methyl-2-aminoethane sulfonic acid (TES) buffer pH 7.4; 0.5% (w/v) bovine serum albumin; 2 mM malate and rat heart mitochondria (0.5 mg of protein). 0.12 mM 2,4-dinitrophenol was added 70–90 sec before the first addition of acylcarnitine. 0.04 mM erucylcarnitine and 0.04 mM palmitylcarnitine was added as indicated. The incubation volume was 2.5 ml and the temperature was 25°.

Fig. 1 also shows that the addition of palmitylcarnitine to heart mitochondria which had been preincubated with erucylcarnitine, caused a significantly lower rate of oxygen uptake than in experiments where palmitylcarnitine was the only added substrate. In experiments with 6 different preparations of rat heart mitochondria and with experimental conditions as described in the legend to fig. 1, the rate of oxygen uptake after the addition of palmitylcarnitine to mitochondria which had been preincubated with erucylcarnitine was 41.6% ± 4.2% (mean ± 1 SD) of the rate after the addition of palmitylcarnitine as the sole substrate.

In experiments where palmitylcarnitine was added first, a subsequent addition of erucylcarnitine caused a gradual decrease in the rate of oxygen uptake (fig. 1). After 2–3 min the rate of oxygen uptake had decreased to a relatively constant level which was approximately equal to the rate observed in experiments where palmitylcarnitine was added after a preincubation with erucylcarnitine.

Because of this interval of 2–3 min from the addition of erucylcarnitine to when the maximal inhibition of the oxidation of palmitylcarnitine is obtained, it seems probable that the inhibition is not caused by erucylcarnitine itself but by some intermediate formed. In accordance with this we have found that soluble carnitine palmityltransferase is not inhibited by erucylcarnitine, and also that mitochondrial CoA is acylated by erucylcarnitine. Since oleic acid is formed after two cycles of β-oxidation of erucic acid, the inhibition most likely is due to either the erucyl CoA formed or to some intermediate formed in the first steps of β-oxidation of this compound.

The relative amounts of fatty acids in the triglycerides which accumulate in the hearts of rats fed rapeseed oil, reflect the fatty acid composition in the diet [2]. The present findings suggest that the presence of erucic acid may inhibit the oxidation of other fatty acids in the heart. This may lead to the accumulation of the fatty acids as triglycerides.

References

[1] A.M.M. Abdellatif and R.O. Vles, Nutr. Metabol. 12 (1970) 285.
[2] J.L. Beare-Rogers, E.A. Nera and H.A. Heggtveit, Can. Inst. Food.Technol. J. 4 (1971) 120.
[3] H.J. Thomasson and J. Boldingh, J. Nutr. 56 (1955) 469.
[4] J.L. Beare, Food Manufacture 32 (1957) 378.
[5] A. von Beznak, M. von Beznak and M. Hadju, Ernahrung 8 (1943) 236.

[6] P. Roine, E. Uksila, H. Teir and J. Rapola, Z. Ernahrungsw. 1 (1960) 118.
[7] B.M. Craig and J.L. Beare, Can. J. Biochem. 45 (1967) 1075.
[8] U.M.T. Houtsmuller, C.B. Struik and A. van der Beek, Biochim. Biophys. Acta 218 (1970) 564.
[9] B.O. Christophersen and J. Bremer, Biochim. Biophys. Acta 260 (1972) 515.
[10] O.H. Lowry, N.J. Rosebrough, A.L. Farr and R.J. Randall, J. Biol. Chem. 193 (1951) 265.