ERUCIC ACID OXIDATION BY BEATING HEART CELLS IN CULTURE

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1. Introduction

It has recently been reported that rats and other species fed on a diet containing a high percentage of rapeseed-oil rapidly accumulated fat in their heart muscle cells, mainly as triglycerides containing a high proportion of erucic acid [1–3]. Necrosis followed this lipid accumulation [4].

It has also been shown that erucic acid can be transformed or completely oxidized by the animal in vivo [5, 6] but it has not been proved that heart cells are able to do this. In addition it has been shown that heart mitochondria from rats which have been fed with rapeseed-oil oxidize α-oxoglutarate and succinate at a lower rate [7]. A direct inhibitory effect of erucylcarnitine on palmitate oxidation has been also reported [8].

In the present work, oxidation of erucic acid by beating heart cells in culture has been studied. Two differently labelled erucic acids were used in order to determine whether differentiated cultured cardiac cells in a tissue-like structure, are able themselves to oxidize erucic acid and whether transformation is an essential step prior to oxidation. The incorporation of erucic acic into triglycerides is also reported in order to support our proposition that transformation before oxidation is an obligatory step followed by erucic acid in the cultured myocyte.

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2. Materials and methods

Cultures were obtained from 3–4 day old rats as described elsewhere [9]. Erucic acid of purity grade 99% was purchased from Sigma Chemical Company, [14-^{14}C]erucic acid specific activity 26.5 mCi/mM and [1-^{14}C]erucic acid specific activity 40 mCi/mM were purchased from CEA Saclay (France).

For substrate preparation 0.1 mM potassium erucate containing 18% of either [14-^{14}C]erucate or [1-^{14}C]erucate was incorporated into 100 ml of fetal bovine serum by 30 min ultrasonication and 1 hr agitation at room temperature. This preparation was diluted 1/4 v/v with a Ham F10 culture medium [10] and constituted the substrate.

For $^{14}CO_2$ production measurements 5–7 day-old cultures and 5 ml of the substrate were placed in a special incubation chamber and the CO_2 produced was trapped by hyamine hydroxide placed in collecting chambers as described elsewhere [11]. Radioactivity was measured in a Packard Tricarb Spectrometer. The quenching effect was corrected by the automatic external standard method. Protein content was measured by the method of Lowry et al. [12].

The incorporation of erucic acid into different lipid classes was studied by incubating the cultures for 18 hr with [14-^{14}C]erucate substrate. Lipids were extracted by the method of Delsal [13] and submitted to a thin-layer chromatography. The different lipid classes were eluted and their butyl esters prepared for gas–lipid chromatography analysis [14]. The column was coupled to a Packard fraction collector by a 90%/10% bypass to measure radioactivity of individual fatty acids [15].

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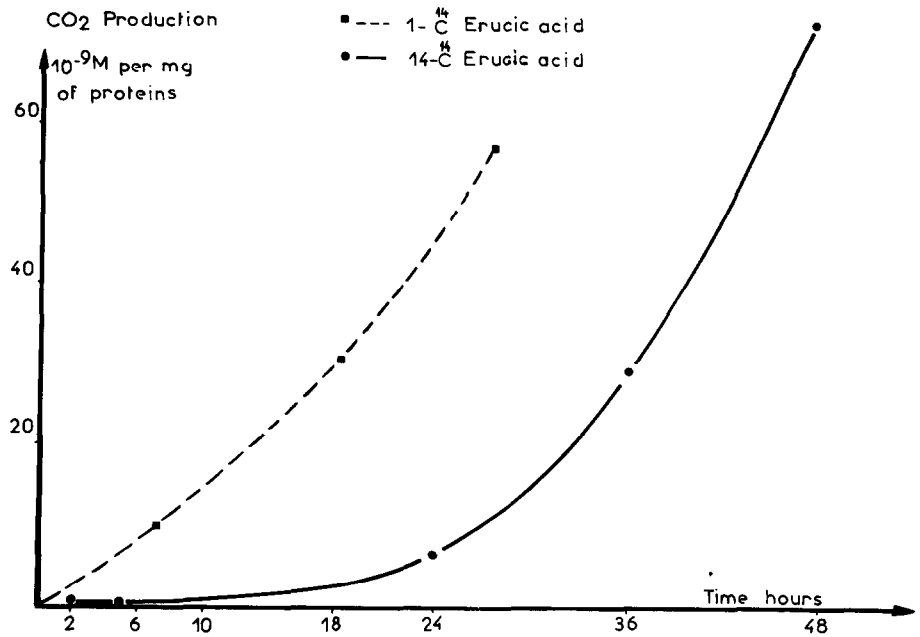

Fig. 1. Carbon dioxide production by heart cells in culture from [1-^{14}C]erucic acid (▪—▪—▪) and from [14-^{14}C]erucic acid (●—●—●).

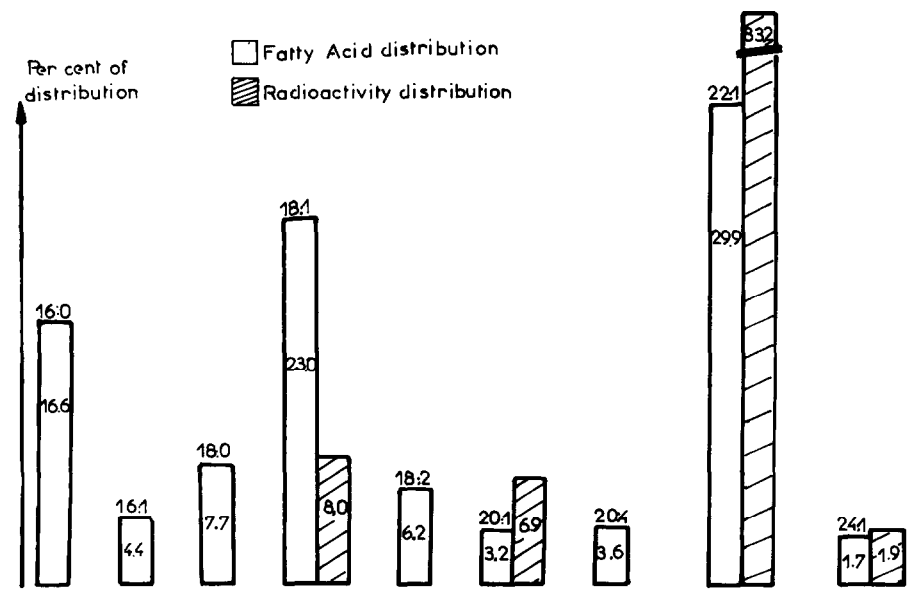

Fig. 2. Distribution of fatty acids and radioactivity originating from erucic acid in triglycerides. GLC was performed with a column of 2 m length and 4 mm internal diameter containing chromosorbe W with 25% DEGS. Nitrogen flow rate: 180 ml/min. Temperature 190°C. Sensitivity 1×10^{-11}. The column was coupled by a 90%/10% bypass to a fraction collector

3. Results and discussion

Fig. 1 shows $^{14}CO_2$ production from [1-^{14}C]erucic acid and from [14-^{14}C]erucic acid.

It shows that CO_2 originating from the carboxylic group appears rapidly while $^{14}CO_2$ originating from the 14th carbon of the erucic acid chain has a latency time of more than 12 hr. Afterwards the two curves follow approximately parallel slopes displaced over an interval of about 16 hr.

Fig. 2 shows the distribution of fatty acids and the percentage of radioactivity originating from erucic acid in triglycerides. It indicates that the erucic acid chain is only shortened to Δ-11 eicosenoic (20:1) and oleic (18:1) acids and that only a minute amount is lengthened to nevronic acid (24:1). It also shows approximately the same percentage of radioactivity in the two shortened secondary products. The early appearance of $^{14}CO_2$ originating from the carboxyl group and the late appearance of the CO_2 originating from the 14th carbon of the erucic acid chain would indicate that erucic acid cannot be oxidized by the cultured myocyte but must be transformed into Δ-11 eicosenoic (20:1) and oleic (18:1) acids prior to entering the β-oxidation cycle. In addition the fact that the two curves of $^{14}CO_2$ evolution (fig. 1) follow parallel slopes indicates that, under our experimental conditions, after 16 hr, the turnover of the substrates probably occurs at a similar rate and that an equilibrium is reached between the two pathways: transformation and oxidation.

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References

[1] Abdellatif, A.M.M. and Vles, R.O (1970) Nutr. Metabol. 12, 285–295.
[2] Beare-Rogers, J.L., Nera, E.A. and Heggtveit, H.A. (1971) Can. Inst. Food Techn. J. 4, 120–124.
[3] Rocquelin, G., Sergiel, J.P., Martin, B., Leclerc, J. and Cluzan, R. (1971) J. Am. Oil Chem. Soc. 48, 728–732.
[4] Rocquelin, G. and Cluzan, R. (1968) Ann. Biol. Anim. Biochim. Biophys. 8, 395–406.
[5] Boucrot, P. and Bezard, J. (1973) Arch. Sci. Physiol. 27, 1–24.
[6] Bach, A., Métais, P., Raulin, R. and Jacquot, R. (1969) Bull. Soc. Chim. Biol. 51, 167–175.
[7] Houtsmuller, U.M.T., Strujk, C.B. and Van der Beek, A. (1970) Biochim. Biophys. Acta 218, 564–566.
[8] Christophersen, B.O. and Bremer, J. (1972) Febs Letters, 23, 230–232.
[9] Pinson, A., Frelin, Ch. and Padieu, P., Biochimie, in press.
[10] Ham, R.G. (1963) Exptl. Cell Res. 29, 515–526.
[11] Pinson, A. and Padieu, P., submitted to Anal. Biochem.
[12] Lowry, O.H., Rosenbrough, N.J., Furr, A.L. and Randell, R.J. (1951) J. Biol. Chem. 193, 265–275.
[13] Delsal, J.L. (1944) Bull. Soc. Chim. Biol. 26, 99–105.
[14] Clément, G. and Bézard, J. (1961) C.R. Acad. Sci. Paris 253, 564–566.
[15] Bézard, J., Boucrot, Ph. and Clément, G. (1964) J. Chromatogr. 14, 368–377.