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INCREASED ACTIVITY OF PEROXISOMAL β-OXIDATION IN RAT LIVER CAUSED BY ETHYL 2{5(4-CHLOROPHENYL)PENTYL}-OXIRAN-2-CARBOXYLATE: AN INHIBITOR OF MITOCHONDRIAL β-OXIDATION

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Summary

Ethyl 2{5(4-chlorophenyl)pentyl}oxiran-2-carboxylate (POCA) is a new hypoglycaemic compound. The POCA-CoA ester strongly inhibits β-oxidation at carnitine palmitoyltransferase I. Chronic administration of POCA to rats decreases plasma concentrations of cholesterol and triacylglycerol and increases the number of hepatic peroxisomes similarly to hypolipidaemic drugs related to clofibrate. Peroxisomal fractions from rats fed a diet containing 0.2% of POCA for 4 weeks were prepared on self-generated Percoll gradients. POCA induced a 4-fold increase in catalase activity and peroxisomal β-oxidation, agreeing with the morphological data. The increase in peroxisomal β-oxidation caused by POCA feeding does not prevent accumulation of lipid following the inhibition of mitochondrial β-oxidation.

1. Introduction

Hepatic peroxisomes contain a system for the partial oxidation of long-chain acyl-CoA esters{1-3}. Both the number of peroxisomes and their capacity for β-oxidation is increased 6-12 fold by chronic administration of several compounds to rats, including some hypolipidaemic drugs (for example, clofibrate, ethyl 4-chlorophenoxy-isobutyrate {3-5}). Furthermore, high fat diets, in particular those containing a high proportion of fatty acids which are poorly oxidised

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by mitochondria (e.g. $C_{22:1}$ and trans-fatty acids), cause up to a three-fold increase in hepatic capacity for peroxisomal β-oxidation{6,7}.

Ethyl 2{5(4-chlorophenyl)pentyl}oxiran-2-carboxylate (POCA) is a new hypoglycaemic compound which is effective in starved, but not fed, animals {8,9}. We have shown that 2{5(4-chlorophenyl)pentyl}oxiran-2-carbonyl-CoA is a specific and powerful inhibitor (apparent $K_i < 1\mu M$) of mitochondrial β-oxidation at the stage of carnitine palmitoyltransferase I (EC 2.3.1.21) which is situated on the outer face of the mitochondrial inner membrane {10}. Chronic administration of POCA in the diet (0.1% or 0.2%) lowered plasma concentrations of triacylglycerols and cholesterol by approximately 50%, caused a 3-fold increase in the total CoA and carnitine concentrations in livers {11} and a 60% increase in mitochondrial glycerol 3-phosphate dehydrogenase (EC 1.1.2.1) activities {10}. These changes were reminiscent of those caused by known hypolipidaemic peroxisomal proliferators {3-5}. Indeed, quantitative electron microscopy showed that feeding male rats 0.1% or 0.2% POCA in their diets for 12 weeks caused a 2.15-fold increase in the number of peroxisomes, a decrease in their average diameter from 0.36 to 0.31μm and a 1.58-fold increase in the volume fraction (corrected for lipids and nuclei) of the hepatocytes occupied by peroxisomes {12}. In the present study we show directly that the capacity for peroxisomal β- oxidation is also increased 3-fold by administration of POCA.

2. Methods

POCA was generously supplied by Dr. Gerhard Ludwig of the BYK Gulden Lomberg Chemische Fabrik GmbH, Konstanz, G.F.R. Male Wistar rats were maintained on a standard Pelleted diet containing 0.2% (w/w) of POCA for 4 weeks. Peroxisomal fractions were prepared from the livers of fed and control rats by density gradient centrifugation on a self-generated Percoll gradient at $79,000g_{av}$ {6}. Fractions of 3.0ml volume were collected from the top of the gradient. Catalase (EC 1.11.1.6) activity, cytochrome c oxidase (EC 1.9.3.1) activity and protein were measured as described {6}. Peroxisomal β-oxidation was assayed spectrophotometrically as the palmitoyl-CoA-dependent reduction of NAD^+ at 37°C and pH 7.4 as previously described {6} except for the inclusion of 25μM FAD. The hepatic capacity for peroxisomal β-oxidation was estimated as described {6}. As judged by catalase and cytochrome c oxidase activities the distribution of organelles on the gradient was similar to that observed previously with preparations from clofibrate-treated animals {6}.

3. Results

The POCA-supplemented diet increased the total and specific activities of catalase in livers of rats (Table 1) compared with the controls. Similar results were also obtained from animals fed on some high fat diets {7}.

There was a 3-fold increase in the total capacity of the liver for peroxisomal β-oxidation and in the specific activity of the peroxisomal fractions (Table 2) in good agreement with the morphological data {12}. The control values were similar to earlier results for untreated rats {7}.

4. Discussion

The 3-fold increase in peroxisomal β-oxidation induced by POCA was similar to that caused by feeding some high fat diets {6,7}, rather than the 10-fold increase caused by clofibrate {1}. Further, POCA caused a 14% decrease in the average diameter of peroxisomes, while by contrast administration of clofibrate produced an average increase of approximately 30% {12}.

Administration of clofibrate to rats on a normal diet increased the capacity of the livers for both peroxisomal and mitochondrial

Table 1

The effects of feeding POCA on the total protein contents and catalase activities of rat liver

	Body weight (g)	Total liver protein (mg)	Total catalase activity (units)	Specific activity hepatic catalase (units per mg of protein)
Control (3)	359 ± 20	4280 ± 386	1396 ± 67	0.329 ± 0.019
POCA-fed (6)	290 ± 9***	3968 ± 299	2200 ± 97****	0.576 ± 0.061*

The number of experiments is given in parentheses ± S.E.M. for each parameter. Units of catalase activity are defined in ref. {14}. Significance of differences from controls: * P <0.05; *** P <0.005; **** P <0.001

Table 2

The effect of feeding POCA on the activity of peroxisomal β-oxidation

	Palmitoyl-CoA-dependent reduction of NAD^+ at 37°C, the rates (nmol/min) are expressed as:			
	Total liver capacity	Per g body-wt	Per g of liver	Per mg of protein
Control (3)	6,230 ± 887	173 ± 1.7	840 ± 41	1.44 ± 0.07
POCA-fed (6)	18,700 ± 1560***	650 ± 6***	2480 ± 245**	4.84 ± 0.51**

The number of experiments are given in parentheses ± S.E.M. for each parameter. Significance of difference from controls: ** P <0.05; *** P <0.001

β-oxidation {13}, with a decrease in their fat content to less than 0.03% compared with about 1% in the controls {5}. Paradoxically, POCA caused a very large increase in the fat content of up to 15% {11}, presumably because of the inhibition of mitochondrial β-oxidation. It is not known whether increased peroxisomal β-oxidation is a direct effect of POCA or is secondary to the accumulation of lipid. It is clear that the hypolipidaemic effect of POCA is not due to an increased rate of hepatic oxidation of fatty acids.

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