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Comparison of the activities of some peroxisomal and extraperoxisomal lipid-metabolizing enzymes in liver and extrahepatic tissues of the rat

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Peroxisomal (acyl-CoA oxidase and peroxisomal dihydroxyacetone-phosphate acyltransferase) and extraperoxisomal (mitochondrial fatty acid oxidation, extraperoxisomal dihydroxyacetone-phosphate acyltransferase, mitochondrial and microsomal glycerophosphate acyltransferases) lipid-metabolizing enzymes were measured in homogenates from rat liver and from seven extrahepatic tissues. Except for jejunal mucosa and kidney, extrahepatic tissues contained very little acyl-CoA oxidase activity. Peroxisomal dihydroxyacetone-phosphate acyltransferase, taken as the activity that was not inhibited by 5 mM-glycerol 3-phosphate, was present in all tissues examined, and its specific activity in liver and extrahepatic tissues was roughly of the same order of magnitude. Clofibrate treatment increased the activity of acyl-CoA oxidase in liver, and to a smaller extent also in kidney, but did not influence the activity of peroxisomal dihydroxyacetone-phosphate acyltransferase. Comparison of the activities of peroxisomal and extraperoxisomal lipid-metabolizing enzymes in extrahepatic tissues and in liver, an organ in which the contribution of peroxisomes to fatty acid oxidation and to glycerolipid synthesis has been estimated previously, suggests that, as in liver, peroxisomal long-chain fatty acid oxidation is of minor quantitative importance in extrahepatic tissues, but that in these tissues (micro)-peroxisomes are responsible for most of the dihydroxyacetone phosphate acylation and, consequently, for initiating ether glycerolipid synthesis.

In mammals typical peroxisomes are found in liver and kidney. In other organs their diameter is smaller and they are called microperoxisomes. Roughly half of the enzymes identified in rat liver peroxisomes is related to lipid metabolism (Tolbert, 1981). This suggests that peroxisomes are of importance in lipid metabolism, but their exact role remains unknown.

Like mitochondria, rat liver peroxisomes are capable of oxidizing fatty acids via β-oxidation (Lazarow, 1978). Their contribution in liver to the oxidation of long-chain fatty acids such as oleate or palmitate appears to be minor (Mannaerts *et al.*, 1979). However, there is evidence that peroxisomes may be of importance in the oxidation of dicarboxylic fatty acids (Mortensen *et al.*, 1982) and of very-long-chain fatty acids ($>C_{20}$) (Bremer & Norum, 1982; Moser *et al.*, 1984), which may be shortened in peroxisomes to C_{16}–C_{18} fatty acids before further oxidation in mitochondria. In liver the peroxisomal β-oxidation system may also be responsible for the oxidative side-chain cleavage in the synthesis of cholic acid (Kase *et al.*, 1983). Like endoplasmic reticulum and mitochondria, rat liver peroxisomes contain the enzymes that can catalyse the initial steps in the synthesis of glycerolipids (Hajra & Bishop, 1982). The enzymes responsible for the terminal reactions are not found in peroxisomes (Declercq *et al.*, 1984). Glycerolipid synthesis starts with the acylation of either glycerol 3-phosphate or dihydroxyacetone phosphate. Glycerophosphate acyltransferase (EC 2.3.1.15) is found in endoplasmic reticulum and mitochondria (see Brindley & Sturton, 1982; Bell & Coleman, 1983), whereas dihydroxyacetone-phosphate acyltransferase (EC 2.3.1.42) is mainly localized in peroxisomes and endoplasmic reticulum (Schlossman & Bell, 1977; Hajra *et al.*, 1979; Declercq *et al.*, 1984). Studies in rat liver indicated that the peroxisomal dihydroxyacetone-phosphate acyltransferase is responsible for most of the hepatic dihydroxyacetone phosphate acylation, but that the dihydroxyacetone phosphate pathway contributes only to a minor extent to overall hepatic

glycerolipid synthesis (Declercq *et al.*, 1984). This suggests that the main function of the hepatic dihydroxyacetone phosphate pathway lies in the synthesis of ether glycerolipids, which have acyldihydroxyacetone phosphate as obligatory precursor (Hajra & Bishop, 1982), and that peroxisomes play a major role in initiating ether glycerolipid synthesis.

The contribution of peroxisomes to long-chain fatty acid oxidation, overall glycerolipid synthesis and ether glycerolipid synthesis (dihydroxyacetone phosphate acylation) has not been studied in extrahepatic tissues. We therefore compared the activities of acyl-CoA oxidase, the first enzyme of peroxisomal β-oxidation, of mitochondrial fatty acid oxidation and of the various glycerophosphate acyltransferases and dihydroxyacetone phosphate acyltransferases in liver and several extrahepatic tissues of the rat.

Experimental

Tissue preparation

Male Wistar rats, maintained on a standard laboratory diet, were killed at 08:30h, the tissues were removed and 5% (w/v) homogenates were prepared in 0.25M-sucrose/0.1% (v/v) ethanol/3mM-Hepes, (pH7.2)/1mM-EDTA in a Dounce homogenizer. The small intestine was first rinsed with 0.9% NaCl and the mucosa was scraped off. Biceps femoris muscle was taken as skeletal muscle; total brain cortex was used.

Enzyme assays

Catalase (EC 1.11.1.6) was measured as described by Baudhuin *et al.* (1964). Mitochondrial fatty acid oxidation was measured with [1-^{14}C]palmitoyl-CoA as the substrate and in the presence of albumin as described by Mannaerts *et al.* (1979), except that the final volume was decreased 5-fold and that antimycin plus rotenone were used instead of KCN. Acyl-CoA oxidase was measured by a modification of the procedure of Vamecq & Van Hoof (1984).

For this, 25μl of homogenate, appropriately diluted with homogenization medium, was incubated with 25μl of 0.25mM-pargyline for 10min at room temperature to decrease monoamine oxidase activity, which, especially with homogenates from small intestine, causes high blanks. The reaction was started with the addition of 200μl of reaction mixture, consisting of 50mM-potassium phosphate buffer, pH7.5, 12.5mM-aminotriazole, 0.75mM-homovanillic acid, 25units of horseradish peroxidase/ml, 0.025% (v/v) Triton X-100, 0.25mM-palmitoyl-CoA and 1.5mg of defatted (Chen, 1967) bovine serum albumin/ml (palmitoyl-CoA:albumin molar ratio = 10). After an incubation period of 60min in the dark at 30°C, 50μl of 1.2M-$HClO_4$ was added, the mixture was centrifuged (30000*g*-min) and the supernatant was diluted 20-fold with 0.5M-Na_2CO_3/$NaHCO_3$ buffer, pH10.7. Fluorescence was read after 10min in an Aminco spectrofluorimeter (excitation wavelength 327nm; emission wavelength 420nm). The $HClO_4$-precipitation step can be omitted, but it decreases the spontaneous increase in fluorescence observed after alkalinization of the reaction mixture. Appropriate blanks without palmitoyl-CoA were always performed. The addition of FAD to the reaction mixture caused a more than 10-fold increase in fluorescence of blank and samples. This increase could be decreased 3–4-fold in the absence of aminotriazole. Since FAD caused only a slight (10–20%) increase in acyl-CoA oxidase activity, it was omitted from the reaction mixture. Standards contained, in addition to all other components except homogenate, 0.5–2nmol of uric acid and 6munits of urate oxidase per assay.

Glycerophosphate acyltransferase was measured at pH7.5, at 1mM-glycerol 3-phosphate and at a palmitoyl-CoA:albumin molar ratio of 0.65, with and without pretreatment of the enzyme source with 20mM-*N*-ethylmaleimide as described by Declercq *et al.* (1984). The *N*-ethylmaleimide-sensitive and -insensitive activities were taken as the microsomal and mitochondrial activities respectively (for references see Brindley & Sturton, 1982; Bell & Coleman, 1983). Dihydroxyacetone phosphate acyltransferase was measured at pH7.5, at 0.94mM-dihydroxyacetone phosphate and at a palmitoyl-CoA:albumin molar ratio of 0.65, in the absence and presence of 5mM-glycerol 3-phosphate (Declercq *et al.*, 1984). The glycerol 3-phosphate-sensitive and -insensitive activities were taken as the extraperoxisomal (mainly microsomal) and peroxisomal components respectively. Glycerol 3-phosphate has been shown to inhibit microsomal dihydroxyacetone phosphate acyltransferase strongly in liver (Declercq *et al.*, 1984) and in a number of extrahepatic tissues such as brain, intestinal mucosa, lung and kidney (Schlossman & Bell, 1977). The peroxisomal enzyme from liver (Jones & Hajra, 1980; Declercq *et al.*, 1984) and from brain (Hajra & Burke, 1978; Hajra & Bishop, 1982) is virtually unaffected by glycerol 3-phosphate. Although the subcellular localization of the glycerol 3-phosphate-insensitive dihydroxyacetone phosphate acyltransferase has not yet been established in other extrahepatic tissues, it is reasonable to assume that its localization in these tissues is identical with that in liver and brain.

Protein was determined as described by Peterson (1977).

Results and discussion

The main purpose of this study was to gain some insight in the role that (micro)peroxisomes may play in extrahepatic lipid metabolism. We therefore measured in liver and in several extrahepatic tissues the first enzyme of the peroxisomal β-oxidation sequence, acyl-CoA oxidase, and the first enzyme of peroxisomal glycerolipid synthesis, dihydroxyacetone-phosphate acyltransferase. At saturating substrate concentrations acyl-CoA oxidase appears to be rate-limiting for peroxisomal β-oxidation (Osumi & Hashimoto, 1979); under these conditions, the rate-limiting step in glycerolipid synthesis is unknown. We compared these activities with those of mitochondrial fatty acid oxidation and of extraperoxisomal dihydroxyacetone-phosphate acyltransferase and glycerophosphate acyltransferase.

Table 1 shows that liver, jejunal mucosa and kidney displayed comparable acyl-CoA oxidase activity. Little or no activity could be detected in other tissues. The acyl-CoA oxidase activities found in the present experiments for rat tissues are very similar to those reported by Small *et al.* (1980) for guinea-pig tissues. The activity of peroxisomal dihydroxyacetone-phosphate acyltransferase was roughly of the same order of magnitude in all tissues. The highest activity was present in testis.

Treatment of rats for 2 weeks with clofibrate [0.3% (w/w) in the diet], a hypolipidaemic compound, known to induce the proliferation of hepatic peroxisomes and to enhance hepatic acyl-CoA oxidase activity (Lazarow & de Duve, 1976), caused a 7-fold and 3-fold increase in acyl-CoA oxidase activity in liver and kidney respectively, but the drug did not enhance peroxisomal dihydroxyacetone-phosphate acyltransferase, either in liver or in extrahepatic tissues (results not shown).

In some animals the activity of the microsomal glycerophosphate acyltransferase was high in jejunum, whereas it was low in other animals. A similar phenomenon was observed for jejunal extraperoxisomal dihydroxyacetone-phosphate acyltransferase. This could be related to the possibility that microsomal glycerophosphate acyltransferase and microsomal dihydroxyacetone-phosphate acyltransferase are dual catalytic functions of a single protein (Schlossman & Bell, 1977). The animals with high jejunal microsomal acyltransferase activities also showed higher activities of mitochondrial fatty acid oxidation. The reason for these differences in enzyme activities between rats is unknown. The peroxisomal enzymes of Table 1 were also measured along the length of the small intestine. Specific enzyme activities were the same as in jejunum (results not shown).

To estimate the contribution of (micro)peroxisomes to long-chain fatty acid oxidation and glycerolipid synthesis in a particular tissue, corresponding peroxisomal and extraperoxisomal enzymes ought to be measured at physiological substrate concentrations prevailing in that tissue, and their activities compared. Since physiological substrate concentrations are not known for most extrahepatic tissues, the enzyme activities of Table 1 were measured under routine conditions as applied in our laboratory. The ratios within a given extrahepatic tissue of the activities of peroxisomal and extraperoxisomal enzymes with similar function were then compared with the corresponding ratios in liver, an organ in which the contribution of peroxisomes to long-chain fatty acid oxidation and glycerolipid synthesis has been estimated previously (Mannaerts *et al.*, 1979; Declercq *et al.*, 1984). The hepatic ratio was taken as unity, and all ratios were normalized accordingly (Table 2). Such comparison between liver and extrahepatic tissues suggests the following.

(1) Except in jejunal mucosa, the ratio of acyl-CoA oxidase to mitochondrial fatty acid oxidation was smaller in extrahepatic tissues than in liver. Since the contribution of peroxisomes to hepatic long-chain fatty acid oxidation is only minor (Mannaerts *et al.*, 1979; Foerster *et al.*, 1981; Leighton *et al.*, 1984), this indicates that the contribution of (micro)peroxisomes to long-chain fatty acid oxidation in these tissues is most likely also of little or no quantitative significance. This does not exclude the possibility that, in liver, kidney and jejunum, peroxisomal fatty acid oxidation may serve a special purpose, such as the oxidation of very-long-chain fatty acids (see the introduction).

(2) In liver, peroxisomal dihydroxyacetone-phosphate acyltransferase is responsible for most of the dihydroxyacetone phosphate acylation (Declercq *et al.*, 1984). The ratio of peroxisomal to extraperoxisomal dihydroxyacetone-phosphate acyltransferase is larger in extrahepatic tissues than in liver. This could indicate more conclusively that also in extrahepatic tissues (micro)peroxisomes are responsible for the major part of acyl-dihydroxyacetone phosphate acylation and, as a consequence, ether glycerolipid synthesis. This conclusion is strongly supported by the report by Heymans *et al.* (1983), who described that tissues from infants suffering from the Zellweger syndrome lack peroxisomes and ether glycerolipids (plasmalogens).

(3) The contribution of the dihydroxyacetone phosphate pathway to overall hepatic glycerolipid synthesis is most probably less than 10% (Declercq *et al.*, 1984). The observation that the ratio of peroxisomal dihydroxyacetone-phosphate acyltransferase to total glycerophosphate acyltrans-

Table 1. *Peroximal and extraperoxisomal enzyme activities in liver and extrahepatic tissues from rats*

Peroxisomal (catalase, acyl-CoA oxidase, peroxisomal dihydroxyacetone-phosphate acyltransferase) and a number of extraperoxisomal enzyme activities were measured in whole tissue homogenates. Dihydroxyacetone-phosphate acyltransferase (DHAPAT) was measured in the absence and presence of 5mM-glycerol 3-phosphate, and the glycerol 3-phosphate-sensitive and -insensitive activities were taken to represent the extraperoxisomal (mainly microsomal) and peroxisomal enzymes respectively. Glycerophosphate acyltransferase (GPAT) was measured with and without pretreatment of the enzyme source with 20mM-*N*-ethylmaleimide, and the *N*-ethylmaleimide-sensitive and insensitive activities were taken to represent the microsomal and mitochondrial enzymes respectively. In jejunal mucosa mitochondrial fatty acid oxidation, extraperoxisomal dihydroxyacetone-phosphate acyltransferase and microsomal glycerophosphate acyltransferase were high in some animals, whereas all three activities were low in other animals. The high and low activities are reported as separate values. Results are expressed as nmol of substrate converted/min (munits) per mg of homogenate protein, and are means ± S.E.M. for the numbers of experiments indicated in parentheses. Catalase activity is expressed in the units used by Baudhuin *et al.* (1964). Abbreviation: ND, not detectable.

Tissue	Liver	Lung	Kidney	Heart	Brain	Skeletal muscle	Jejunal mucosa	Testis
Catalase	351 ± 15 (5)	27.1 ± 1.0 (4)	151 ± 8 (5)	9.21 ± 0.91 (5)	1.48 ± 0.08 (5)	1.15 ± 0.12 (4)	7.71 ± 1.43 (5)	7.11 ± 0.49 (4)
Acyl-CoA oxidase	2.18 ± 0.20 (4)	0.087 ± 0.012 (4)	0.945 ± 0.157 (4)	0.035 ± 0.009 (4)	ND (4)	0.012 ± 0.002 (4)	2.08 ± 0.30 (4)	ND (4)
Mitochondrial fatty acid oxidation	1.47 ± 0.11 (10)	0.203 ± 0.045 (4)	1.16 ± 0.17 (4)	1.80 ± 0.24 (4)	0.082 ± 0.022 (4)	0.192 ± 0.025 (4)	0.958 ± 0.201 (3) 0.323 ± 0.067 (5)	0.431 ± 0.050 (4)
Peroxisomal DHAPAT	0.110 ± 0.005 (10)	0.170 ± 0.009 (4)	0.236 ± 0.032 (4)	0.074 ± 0.013 (4)	0.059 ± 0.005 (4)	0.031 ± 0.004 (4)	0.128 ± 0.018 (10)	0.450 ± 0.035 (4)
Extraperoxisomal DHAPAT	0.419 ± 0.019 (10)	0.255 ± 0.033 (4)	0.725 ± 0.040 (4)	0.124 ± 0.006 (4)	0.168 ± 0.013 (4)	0.061 ± 0.011 (4)	1.37 ± 0.20 (4) 0.078 ± 0.015 (6)	0.334 ± 0.064 (4)
Microsomal GPAT	0.813 ± 0.059 (10)	0.400 ± 0.061 (4)	0.587 ± 0.050 (4)	0.166 ± 0.016 (4)	0.375 ± 0.048 (4)	0.085 ± 0.005 (4)	1.51 ± 0.11 (4) 0.037 ± 0.007 (5)	0.426 ± 0.024 (4)
Mitochondrial GPAT	0.433 ± 0.038 (10)	0.082 ± 0.016 (4)	0.163 ± 0.011 (4)	0.090 ± 0.003 (4)	0.043 ± 0.006 (4)	0.028 ± 0.003 (4)	0.072 ± 0.015 (9)	0.284 ± 0.028 (4)

Table 2. *Comparison of ratios of enzyme activities in extrahepatic tissues and in liver*

In a given extrahepatic tissue the ratio of the activities of corresponding peroxisomal and extraperoxisomal enzymes was calculated from the enzyme activities described in Table 1. Ratios were also calculated in liver, the hepatic ratios were taken as unity and all ratios obtained for extrahepatic tissues were normalized accordingly. Abbreviations: DHAPAT, dihydroxyacetone-phosphate acyltransferase; GPAT, glycerophosphate acyltransferase.

Tissue	Liver	Lung	Kidney	Heart	Brain	Skeletal muscle	Jejunal mucosa	Testis
Acyl-CoA oxidase/mitochondrial fatty acid oxidation	1	0.289	0.549	0.013	(0)	0.042	1.46 4.34	(0)
Peroxisomal DHAPAT/extraperoxisomal DHAPAT	1	2.54	1.24	2.27	1.34	1.94	0.356 6.25	5.13
Peroxisomal DHAPAT/total GPAT	1	4.00	3.56	3.27	1.60	3.11	0.916 13.3	7.20

ferase is larger in extrahepatic tissues than in liver suggests that in these tissues the dihydroxyacetone phosphate pathway may contribute to overall glycerolipid synthesis to a larger extent. Interestingly, and possibly as a consequence, the ether glycerolipid content of extrahepatic tissues is markedly higher than that of liver (Diagne *et al.*, 1984).

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