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Modulation of rat liver peroxisomal and microsomal fatty acid oxidation by starvation

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In this work the microsomal lauric acid ω-hydroxylation, fatty acid peroxisomal β-oxidation, and the levels of cytochrome P-450 IVA1 were studied in liver tissue from starved rats. Starvation increased the peroxisomal β-oxidation and the microsomal hydroxylation of fatty acids. The correlation between these activities would support the proposal that both processes are linked, contributing in part to catabolism of fatty acids in liver of starved rats.

Peroxisomal β-oxidation; Microsome; Lauric acid ω-hydroxylation; Starvation; Rat liver

1. INTRODUCTION

Several cytochrome P-450 isoenzymes are present in the rat liver microsomes. The amounts of these proteins are regulated by several factors such as sex, nutritional status, administration of drugs and chemicals [1]. It has been reported that several gene families code for cytochrome P-450 and many of them have been cloned and sequenced [2]. The IVA family include genes for rat liver and kidney fatty acids ω-hydroxylases (cytochromes P-450 IVA1, IVA2 and IVA3) [3]. The cytochrome P-450 IVA1 purified from liver of clofibrate-treated rats, catalyzes the ω-hydroxylation of lauric and arachidonic acids [4,5].

Treatment with clofibrate or many other hypolipidaemic agents results in several characteristic changes in rodents. Induced hepatic responses include hepatomegaly, proliferation of smooth endoplasmic reticulum, induction of cytochrome P-450 IVA1, peroxisome proliferation and alteration in mitochondrial number and structure [6,7]. Peroxisome proliferation is always associated with an increase in several peroxisomal enzyme activities, including the fatty acid β-oxidation and this is considered to be partially responsible for the lipid lowering effect of hypolipidaemic drugs [8].

The nutritional status of animals modulates the liver microsomal metabolism of several endogenous compounds such as fatty acids and steroidal hormones [9]. Malnutrition selectively decreases all of the oxidation products of arachidonic acid, with the exception of ω-1 and ω-hydroxy derivatives [10].

It has been demonstrated that lauric acid ω-1 and ω-hydroxylation are catalyzed by different P-450 isoenzymes [11] but little is known about their physiological role. Possible functions proposed are: the increase in the polarity of liposoluble compounds including steroidal hormones, facilitating in this way their excretion from the organism [1]; the modulation of the biological activity of some lipophilic compounds such as prostaglandins and leukotrienes [12] and the catabolism of fatty acids [6].

Fatty acid oxidation is catalyzed by the mitochondrial and peroxisomal β-oxidation system. The hydroxylation in a terminal carbon (ω position) and its last oxidation catalyzed by an alcohol dehydrogenase, generates the corresponding dicarboxylic acids, preferentially chain-shortened by the peroxisomes [13]. Under normal conditions, the peroxisomal β-oxidation is only a minor pathway for fatty acid oxidation. However, during starvation, diabetes and treatment with hypolipidaemic drugs or high fat diets, this pathway is enhanced [8,14,15]. In addition to increasing aniline hydroxylation and *N*-nitrosodimethylamine demethylation activities in rat liver microsomes, starvation also modulates the content of cytochrome P-450 IIE1, DM, K-5 and several other P-450 isoenzymes [16,17]. The study of the lauric acid microsomal oxidation in starved rats would contribute to understanding the regulatory mechanism of constitutive cytochrome P-450s such as those from IVA family [18].

In this work we study fatty acid oxidation in liver of starved rats with the aim of detecting a possible link between microsomal ω-hydroxylation and the peroxisomal β-oxidation in the catabolism of fatty acids.

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Abbreviations: ω, 12-hydroxy lauric acid; ω-1, 11-hydroxy lauric acid.

2. MATERIALS AND METHODS

2.1. *Animals*

Mature male Wistar rats weighing 200–250 g were used throughout. The animals were starved for 24, 48 or 72 h, with water provided ad libitum.

2.2. *Microsomal lauric acid hydroxylation*

Livers from at least three rats were used for each microsomal sample prepared as described elsewhere [19]. The total hepatic cytochrome P-450 content was measured as described by Omura and Sato [20]. Microsomal incubations (1 mg/ml) were conducted in a constantly stirred buffer mixture of 50 mM Tris-HCl, pH 7.5; 150 mM KCl; 10 mM $MgCl_2$; 8 mM sodium isocitrate and 0.25 IU/ml isocitrate dehydrogenase. After temperature equilibration at 30°C for 3 min, [1-^{14}C]lauric acid (0.6 mCi/mmol) was added to a final concentration of 0.1 mM. One minute later, NADPH (1 mM final concentration) was added to initiate the reaction. After a 5 min incubation (a range where the reaction was linear with time) 1 ml aliquots of the reaction mixture were removed, the metabolites extracted three times with 2 ml of diethyl ether containing 0.05 ml of 1 N HCl, the organic phases combined and evaporated under nitrogen for their analysis by HPLC.

2.3. *HPLC analysis of lauric acid metabolites*

The 11- and 12-hydroxy metabolites of lauric acid were resolved by reverse phase HPLC technique developed in our laboratory using a Novapack C_{18} column (0.39 × 30 cm, 4 μm particle size from Waters Associates, Milford, MA). The metabolites were separated with 55% solution B (50% methanol/50% acetonitrile), 0.1% acetic acid and 44.9% water for a 10 min period. The lauric acid was then eluted with a linear gradient to 100% solution B over a 10 min period. A flow rate of 1.0 ml/min was used throughout. The retention times were 6 and 11 min for the ω-1 and ω derivatives, respectively, and 22 min for lauric acid. The retention times were confirmed with non-radioactive standards by conversion of hydroxylauric acids to their *p*-bromophenacyl esters and their absorbance at 254 nm was measured [21].

2.4. *Peroxisomal β-oxidation and catalase activity*

A 20% liver homogenate prepared in the reaction medium buffer (60 mM Tris-HCl, pH 8.3) was centrifuged 10 min at 500 × *g*. Supernatants were diluted 1:1 with 1% Triton X-100 and the peroxisomal β-oxidation was measured as cyanide-insensitive reduction of NAD^+ using palmitoyl CoA as substrate as described by Bronfman et al. [22]. Catalase activity was measured in the supernatants containing 1% Triton X-100 and 1% ethanol according to the method of Aebi [23].

2.5. *Western blot*

Western immunoblotting was performed using 7.5% polyacrylamide-SDS gels [24,25]. After transfer to nitrocellulose sheets blots were developed using polyclonal antibody raised against purified rat hepatic cytochrome P-450 IVA1. In each case, 20 pmol of purified P-450 IVA1 and 20 μg microsomal protein were subjected to electrophoresis and Western blotting.

2.6. *Materials*

Lauric acid, NADPH, isocitrate dehydrogenase, sodium isocitrate, palmitoyl CoA, FAD, NAD and nicotinamide were purchased from Sigma Chemical Co. [1-^{14}C]Lauric acid (56 mCi/mmol) was from Amersham (Arlington Heights, IL). Purified rat liver cytochrome P-450 IVA1 was kindly provided by Dr. G. Gordon Gibson from the University of Surrey, London, UK. All other chemicals were obtained from commercial sources and were of the highest purity available.

3. RESULTS

Starvation of Wistar rats for up to 72 h, in addition to reducing the body and liver weight by about 30% (results not shown), decreases the liver microsomal protein content to 63% of control values. The total cytochrome P-450 content, however, was not altered by more than 10% during the starvation periods used (Table I).

Table I

Effect of starvation on the microsomal protein and total cytochrome P-450 content

Group	Microsomal protein (mg/g liver)	Cytochrome P-450 (nmol/mg prot.)
Control	10.7 ± 2.4	0.64 ± 0.11
Starved 24 h	8.7 ± 2.7	0.61 ± 0.10
Starved 48 h	8.0 ± 1.7*	0.61 ± 0.13
Starved 72 h	6.8 ± 1.8*	0.57 ± 0.14

The total hepatic cytochrome P-450 content was measured as described by Omura and Sato. Values are the means ± S.D. of at least 5 different experiments.
*Significantly different from control at $P < 0.05$.

The lauric acid oxidation catalyzed by rat liver microsomes was significantly increased in the starved groups being 148% at 24 and 48 h of fasting and 139% of control values at 72 h (Table II). A good resolution in the separation of 12-OH (ω) from 11-OH lauric acid (ω-1) was obtained with our HPLC technique. In all groups studied the major metabolite produced was the ω derivative. The ratio ω/ω-1 was 1.6 in the controls increasing to 2 in the 48 h starved group.

To study the peroxisomal β-oxidation, a 48 h starvation period was used to guarantee an increased lipid catabolism. As shown in Table III, starvation increased significantly the peroxisomal β-oxidation of palmitoyl CoA to 145% of the control values. The catalase activity, however, measured as a control parameter of another peroxisomal activity, was not altered. These results would indicate that starvation specifically increases the β-oxidation activity in peroxisomes. The relation between peroxisomal and microsomal activities in the four groups studied was calculated and a good correlation ($r = 0.95$) was found between both processes.

Table II

Effect of starvation on the lauric acid oxidation catalyzed by rat liver microsomes

Group	Metabolites (nmol/min/mg prot.)		
	ω-1	ω	Total
Control	0.72 ± 0.12	1.12 ± 0.25	1.84 ± 0.35
Starved 24 h	1.00 ± 0.25	1.74 ± 0.30	2.73 ± 0.43*
Starved 48 h	0.91 ± 0.22	1.81 ± 0.40	2.72 ± 0.50*
Starved 72 h	0.87 ± 0.14	1.68 ± 0.20	2.55 ± 0.47*

Microsomes (1 mg protein/ml) were incubated for 5 min and the lauric acid metabolites were analyzed by HPLC as described in section 2.
Values are the means ± S.D. of at least 5 different experiments.
*Significantly different from control at $P < 0.05$.

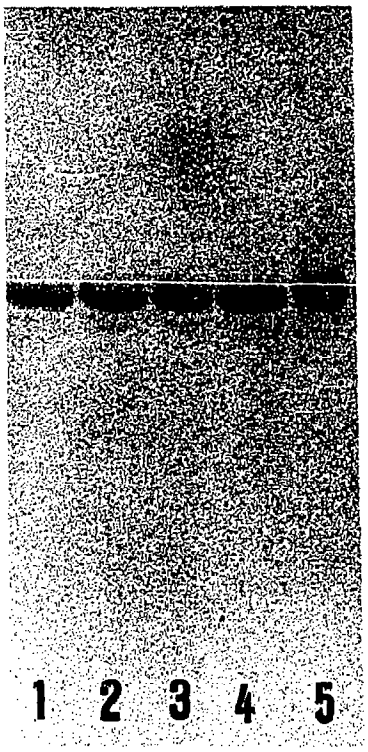

Fig. 1. Western blot analysis of purified cytochrome P-450 IVA1 and hepatic microsomes from control and starved rats. Lanes 1–4 contain 20 μg of microsomal protein from control and 24, 48 and 72 h starved rats, respectively. Lane 5 contains 20 pmol of P-450 IVA1 purified cytochrome. Liver microsomes and cytochrome P-450 IVA1 were electrophoresed in 7.5% polyacrylamide gels and transferred electrophoretically to a nitrocellulose membrane.

Sheep polyclonal antibody prepared against pure cytochrome P-450 IVA1 was used for a Western immunoblot analysis of liver microsomes. This antibody reacts with two different proteins from control and starved liver microsomes as shown in Fig. 1. The intensity of the lower molecular weight band was increased in the starved groups. The slower migrating band corresponds to P-450 IVA1, as indicated by the migration of the homogeneously pure sample of this protein (lane 5). The faster migrating band may represent another P-450 with high amino acid homology to P-450 IVA1.

Table III

Effect of starvation on peroxisomal β-oxidation and catalase activity

Activity	Control	Starved 48 h
β-oxidation	4.66 ± 0.95	6.77 ± 1.00*
Catalase	17.20 ± 3.20	15.40 ± 2.10

The peroxisomal β-oxidation (nmol/min/mg prot.) and catalase activity (k/mg protein × 10^{-2}) were measured in liver homogenates as described in section 2 (k = catalase first-order kinetic constant). Values are the means ± S.D. of at least 5 different experiments.
*Significantly different from control at $P < 0.05$.

4. DISCUSSION

Starvation has been described previously as a good model for studying the mitochondrial β-oxidation of fatty acids. It is also appropriate for studying the microsomal and peroxisomal oxidation of these compounds. Our results indicate that the food deprivation increases the peroxisomal β-oxidation of fatty acids and the microsomal activity to hydroxylate lauric acid with a good correlation ($r = 0.95$). These results are similar to those obtained with hypolipidaemic agents on rat liver, in terms of the endoplasmic reticulum changes and biochemical responses in peroxisomes [26–28].

The increase in lauric acid hydroxylation by starvation indicates that the content or the activity of ω and ω-1 hydroxylases should be incremented in these rat liver microsomes. There are several cytochrome P-450 from the IVA family that oxidize fatty acids in rat liver and kidney [2,18]. The P-450 IVA1, catalyzes the ω-hydroxylation of lauric and arachidonic acids [4,5]. The P-450 IVA3 catalyzes the ω- and ω-1-hydroxylation of lauric and palmitic acids and the ω-hydroxylation of prostaglandins E_1 and F_2 [3]. The P-450 IVA2 is constitutive in kidney and it seems to be equivalent to P-450 K-5 [29]. The existence of two bands with cross reactivity using sheep antibody against the cytochrome P-450 IVA1, would be explained by the presence of another P-450 with high amino acid homology to P-450 IVA1, probably P-450 IVA3. These results, together with those from Table II, suggest that starvation increases the activity and/or the content of other isoenzyme cytochrome P-450, probably from the IVA family, which should contribute to increase the lauric acid hydroxylation.

Numerous alterations in hepatic ultrastructure and metabolism occur during diabetes and starvation. Both metabolic states are characterized by an extensive accumulation of long fatty acids and their corresponding acyl-CoA derivatives. As a consequence, the rate of fatty acids β-oxidation is increased [15,30]. Sharma et al. [31], studying the effect of several peroxisome proliferators in kidney, observed that those compounds that maximally induce microsomal fatty acid hydroxylation are also the best inducers of peroxisomal palmitoyl-CoA oxidation. Similar results were found in liver [27]. They postulate a possible linkage between the control of ω-hydroxylase in microsomes and fatty acid β-oxidation activity in peroxisomes. Essentially this mechanistic interrelationship would involve the initial induction of microsomal ω-hydroxylation of fatty acids, under the control of transcriptional activation of P-450 IVA1 gene by the inducers [6]. These increased levels of cellular ω-hydroxy fatty acids are further oxidized to their long-chain dicarboxylic acids, which then serve as a substrate stimulus for induction of the peroxisomal β-oxidation enzymes [14,26].

In our studies, the starvation effect in increasing the lipid catabolism was similar to that obtained from rats

treated with peroxisome proliferators [8]. The induction of peroxisomal β-oxidation and microsomal activity towards lauric acid support the view that the inductive response would be triggered by endogenous factors common to metabolic state of starvation, namely high hepatic levels of free fatty acids (or their metabolites) and/or a relative endogenous excess of hormones such as glucagon. In addition, the correlation between the increase of peroxisomal β-oxidation and microsomal oxidation of fatty acids found in this work would support the theory that both processes might be linked, contributing in part to the catabolism of fatty acids in liver of starved rats.

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