Volume 322, number 1, 61–64 FEBS 12398 May 1993

Starvation effect on rat kidney peroxisomal and microsomal fatty acid oxidation

A comparative study between liver and kidney

Myriam Orellana, Oscar Fuentes and Elena Valdés

Departamento de Bioquímica, Facultad de Medicina, Universidad de Chile, Independencia 1027, Casilla 70086, Santiago 7, Chile

Received 16 March 1993

Microsomal lauric acid 12-hydroxy lauric acid (ω)-hydroxylation and fatty acid peroxisomal β-oxidation were studied in kidney tissue from starved rats. Starvation increased the microsomal ω-hydroxylation and peroxisomal β-oxidation of fatty acids with a high correlation between both processes. Earlier, we reported similar results in liver. Our results support the hypothesis that the role of microsomal fatty acids ω-hydroxylation is the generation of substrate for peroxisomal β-oxidation, with the final purpose of contributing to a catabolic or gluconeogenic pathway from fatty acids.

Lauric acid ω-hydroxylation; Microsome; Peroxisomal β-oxidation; Starvation; (Rat kidney)

1. INTRODUCTION

Cytochrome P-450s are found predominantly in the liver but also in many other tissues, such as the kidney, and catalyze the oxidative metabolism of many compounds, such as drugs and carcinogens as well as endogenous steroids and fatty acids [1,2]. It has been reported that several gene families code for cytochrome P-450 and many of them have been cloned and sequenced. The 4A family include genes for rat liver and kidney fatty acid ω-hydroxylases [3,4].

The effects of hypolipidaemic drugs, such as clofibrate, on rat liver have been extensively studied [5]. Induced hepatic responses include proliferation of hepatic endoplasmic reticulum, peroxisomes and mitochondria and induction of cytochrome P-450 4A1 [6]. By contrast, our knowledge of both the constitutive and clofibrate-inducible content and activity of kidney cytochrome P-450s remains very sparse. The levels of cytochrome P-450 in kidney have previously been shown to be much lower than those seen in liver, and the renal cortex presents the highest levels of cytochrome P-450 [7,8]. The oxidation of fatty acids such as arachidonic and lauric acids by kidney microsomes is greater than oxidation of steroid hormones [9,10].

Fatty acids $\omega-1$ and ω-hydroxylation are catalyzed by different P-450 isoenzymes [11,12], but little is known about the physiological role of these hydroxylations. Possible functions proposed are: (i) to increase the polarity of liposoluble compounds, facilitating in this way their excretion from the organism; (ii) the modulation of the biological activity of some lipophilic compounds such as prostaglandins and leukotrienes [13] 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 [14]. 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, this pathway is enhanced [5].

It has been demonstrated that food deprivation increases the gluconeogenesis and fatty acid oxidation in the body, but little is known about the microsomal fatty acid metabolism in this physiological condition. In a previous work, we showed an increase in the microsomal lauric acid ω-oxidation and the fatty acid peroxisomal β-oxidation in liver of starved rats with a high correlation between both processes [15].

A decreased susceptibility toward induction of kidney microsomes by several typical liver inductors, such as phenobarbital and clofibrate, has been shown [5,16]. Therefore, kidney response to exogenous inducers is known to be different from liver, but their response to endogenous factors is poorly known.

The aim of this study is to investigate the physiologi-

Correspondence address: M. Orellana, Departamento de Bioquímica, Facultad de Medicina, Universidad de Chile, Independencia 1027, Casilla 70086, Santiago 7, Chile. Fax: (56) (2) 735 5580.

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

cal role of the microsomal fatty acids ω-hydroxylations in kidney. For this purpose, we studied the microsomal lauric acid ω-hydroxylation and the fatty acid peroxisomal β-oxidation in kidney from starved rats. We carried out a comparative study between liver and kidney response to an endogenous factor such as starvation.

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*

Decapsulated kidneys from at least three rats were used for each microsomal sample prepared as described elsewhere [1,17]. The total kidney cytochrome P-450 content was measured as described by Omura and Sato [17]. Because of the presence of mitochondrial contamination in kidney microsomes, sodium succinate was added in order to reduce mitochondrial electron transport enzymes [19]. Microsomal incubations (1 mg/ml) were conducted in a constantly stirred buffer mixture of 50 mM Tris-Cl, 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. 1 min later, the reaction was started by the addition of NADPH (1 mM final concentration). 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 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 lauric acid and its 11-OH (ω−1) and 12-OH (ω) derivatives were resolved by a reverse-phase HPLC technique as previously described [15] using a Novapack C_{18} column from Waters (0.39 × 30 cm, 4 μm particle size). Briefly, lauric acid metabolites were separated with 55% solution B (50% methanol, 50% acetonitrile), 1% acetic acid and 44.9% water for a 10 min period. The lauric acid was then eluted with a 10 min linear gradient to 100% solution B. 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.

2.4. *Peroxisomal β-oxidation and catalase activity*

The peroxisomal β-oxidation was measured in a 20% kidney homogenate as cyanide-insensitive reduction of NAD^+ using palmitoyl CoA as substrate, as described by Bronfman et al. [20]. Catalase activity was measured according to the method of Aebi [21].

2.5. *Materials*

Lauric acid, NADPH, isocitrate dehydrogenase, sodium isocitrate, palmitoyl CoA, FAD, NAD, were purchased from Sigma Chemical Co. [1-^{14}C]Lauric acid (56 mCi/mmol) was from Amersham (Arlington Heights, IL). 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 weight, decreases the liver and kidney weight by about 30% (Table I). The kidney microsomal protein content is also affected by food deprivation, and a decrease of about 30% from the control values after 72 h was observed. In control rats, the kidney total cytochrome P-450 content is about 30% that of the liver values (0.19 and 0.64 nmol mg protein, respectively), however, this parameter was not altered during the starvation periods used (Table II).

Table I

Effect of starvation on the body, liver and kidney weight

Group	Body (g)	Kidney (g)	Liver (g)
Control	190 ± 18	1.10 ± 0.13	12.1 ± 1.5
Starved 24 h	170 ± 18	0.91 ± 0.15	10.5 ± 2.1
Starved 48 h	165 ± 7*	0.88 ± 0.18	9.5 ± 1.2*
Starved 72 h	151 ± 15*	0.81 ± 0.19*	8.5 ± 1.8*

Values are the means ± S.D. of at least twenty rats. *Significantly different from control at $P < 0.05$.

The overall rates of lauric acid metabolism (nmol/min/mg protein) catalyzed by rat kidney microsomes was significantly increased in the starved groups being 144%, 184% and 147% of control values at 24, 48 and 72 h of fasting, respectively (Table III). In all groups studied, the major metabolite produced was the 12-OH lauric acid (ω-derivative). Although kidney has a total cytochrome P-450 content lower than liver, the kidney presents a higher turnover rate than liver in all groups studied (nmol/min/nmol cytochrome P-450). As shown in Table IV, the kidney turnover rate is about three times the liver values at 48 h of starvation.

To study the peroxisomal β-oxidation, a 48 h starvation period was used to guarantee an increased lipid catabolism. As shown in Table V, starvation significantly increased the peroxisomal β-oxidation of palmitoyl CoA to 158% of control values. The catalase activity, however, measured as a parameter of another peroxisomal activity, was not altered. In contrast to microsomal lauric acid metabolism, the kidney peroxisomal β-oxidation was lower than that of liver (Fig. 1), however, starvation causes similar induction of peroxisomal β-oxidation (150% of control values). The relationship between peroxisomal and microsomal activities in the four groups studied was calculated and a high correlation ($r = 0.96$) was found between both processes.

Table II

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

Group	Microsomal protein (mg/g kidney)	Cytochrome P-450 (nmol/mg protein)
Control	9.7 ± 2.4	0.19 ± 0.04
Starved 24 h	8.3 ± 1.7	0.23 ± 0.04
Starved 48 h	6.5 ± 1.6	0.19 ± 0.03
Starved 72 h	6.8 ± 1.0	0.23 ± 0.05

The total renal cytochrome P-450 content was measured as described in section 2. Values are the means ± S.D. of at least five different experiments.

Table III

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

Group	Metabolites (nmol/min/mg protein)		
	$\omega-1$	ω	Total
Control	0.57 ± 0.12	0.81 ± 0.12	1.38 ± 0.33
Starved 24 h	0.83 ± 0.13	1.16 ± 0.26	1.99 ± 0.31*
Starved 48 h	0.94 ± 0.19	1.59 ± 0.33	2.53 ± 0.69*
Starved 72 h	0.78 ± 0.18	1.24 ± 0.30	2.02 ± 0.43*

*Significantly different from control at $P < 0.05$.
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 five different experiments.

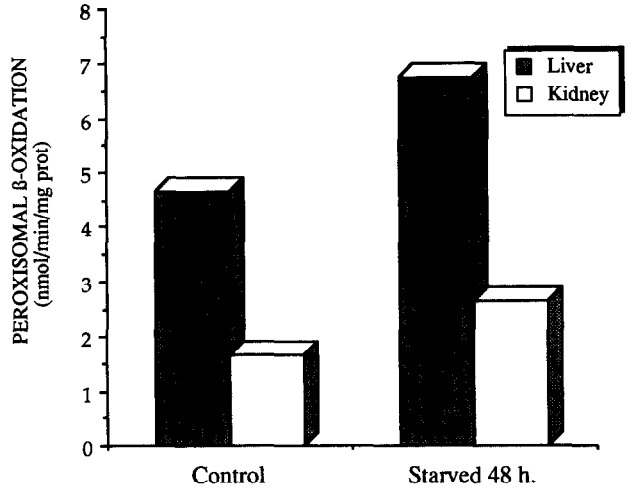

Fig. 1. Effect of starvation on liver and kidney peroxisomal β-oxidation. Liver values are those previously published in [15]. Values are the means ± S.D. of at least 5 different experiments.

4. DISCUSSION

In agreement with an earlier report performed in liver [15], starvation caused similar changes in kidney. It increased both processes, the peroxisomal β-oxidation and microsomal lauric acid ω-oxidation, with a high correlation between them. These results indicate that liver and kidney respond in a similar way to an endogenous factor such as food deprivation. However, the renal turnover rate of microsomal lauric acid metabolism is higher than the hepatic one, indicating that kidney is more adapted to ω-hydroxylate fatty acids than liver. On the other hand, the lower renal peroxisomal β-oxidation may be attributed to a lower peroxisomal content in this tissue.

Both metabolic states, diabetes as well as starvation, are characterized by an extensive accumulation of long-chain fatty acids and their corresponding acyl-CoA derivatives. As a consequence, the rate of fatty acid β-oxidation is accelerated [14]. Studies about the effect of several peroxisome proliferators demonstrate that those compounds that maximally induce microsomal fatty acid hydroxylation are also the best inducers of peroxisomal palmitoyl-CoA oxidation [11,22].

In our studies, the starvation effect in increasing the lipid catabolism was similar to that obtained from rats treated with peroxisome proliferators. In this case, 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 the metabolic state of starvation, namely high hepatic levels of free fatty acids and a relative endogenous excess of hormones such as glucagon. These may give rise to an induction of the cytochrome P-450 4A family with increased rates of fatty acid ω-hydroxylation and formation of dicarboxylic acids. In addition, the high levels of free acids or their metabolites, which are known to be accumulated during starvation, may act as endogenous peroxisomes proliferators. Our study indicates, moreover, a high correlation between the microsomal and peroxisomal oxidation of fatty acids in starvation. Taken together, our results strongly argue that the regulation of microsomal and peroxisomal fatty acid oxidation is closely related.

Certain forms of cytochrome P-450 seem to be in-

Table IV

Effect of starvation on the microsomal turnover rate of lauric acid

Group	Liver (nmol/min/nmol cytochrome P-450)	Kidney (nmol/min/nmol cytochrome P-450)
Control	2.87 ± 0.35	7.21 ± 0.33
Starved 24 h	4.47 ± 0.43*	8.61 ± 0.31*
Starved 48 h	4.46 ± 0.50*	13.31 ± 0.69*
Starved 72 h	4.47 ± 0.47*	8.78 ± 0.43*

*Significantly different from control at $P < 0.05$.
Kidney data from Tables II and III were used to calculate the turnover rate. Liver values were calculated from data previously published in [15]. Values are the means ± S.D. of at least five different experiments.

Table V

Effect of starvation on the kidney peroxisomal β-oxidation and catalase activity

Activity	Control	Starved 48 h
β-Oxidation	1.67 ± 0.31	2.64 ± 0.82*
Catalase	13.21 ± 2.14	13.29 ± 2.31

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

duced in renal microsomes by starvation to judge from the increase in lauric acid oxidation. The P-450 DM (2E1), barely detected in kidney of control rats, and P-450 K-5 (4A2), are induced by starvation [23,24]. P-450 K-5, the major kidney cytochrome P-450, is a fatty acid ω-hydroxylase. Both forms seem to be regulated by an endocrine factor but in a different way. The renal cytochrome P-450 readily uses fatty acids and prostaglandins as substrate. Renal cytochrome P-450 produces bioactive metabolites from arachidonic acid [25,26].

Ketotic rats and humans excrete considerable amounts of dicarboxylic acids in the urine. These seem to be derived from endogenous fatty acid by ω-oxidation [14]. It has been proposed that dicarboxylic acids can be completely chain shortened to succinate by peroxisomal β-oxidation. Succinate synthesized in this manner can make possible some net synthesis of glucose from fatty acids [14]. No such conversion has yet been directly demonstrated.

It is known that in the starvation state, the gluconeogenesis and lipid catabolism are incremented. In addition, it is interesting to point out that kidney and liver are two gluconeogenic organs since both have glucose 6-phosphatase, the key enzyme of the gluconeogenesis pathway.

Taken together, our results support the view that the role of microsomal fatty acid ω-hydroxylations is the generation of dicarboxylic acid, the substrate for peroxisomal β-oxidation. We propose that the final purpose of these linked fatty acid oxidations could be the catabolism of fatty acids or the generation of succinate for the synthesis of glucose from fatty acids. More experimental evidence is required to probe these hypotheses. The catabolic or gluconeogenic pathway of fatty acids by microsomal and peroxisomal oxidation would be triggered by starvation in a similar way in liver and kidney.

Acknowledgements: We thank Dr. Catherine Allende for her assistance in the preparation of the manuscript. This work was supported by Grant B-3178 from Departamento Tecnico de Investigation from the Universidad de Chile.

REFERENCES

[1] Gil, L., Orellana, M., Vasquez, H. and Silva, M. (1988) Int. J. Androl. 11, 339–348.
[2] Orellana, M., Valdes, E., Capdevila, J. and Gil, L. (1989) Arch. Biochem. Biophys. 274, 251–258.
[3] Nebert, D., Nelson, D., Coon, M., Estabrook, R., Feyereisen, R., Fujii-Kuriyama, Y., Gonzalez, F., Guenguerich, P., Gunsalus, I., Johnson, E., Loper, J., Sato, R., Waterman, M. and Waxman, D. (1991) DNA Cell Biol. 10, 1–14.
[4] Bains, S.K., Gardines, S.M., Mannweiler, K., Gillet, D. and Gibson, G.G. (1985) Biochem. Pharmacol. 34, 3221–3229.
[5] Hawkins, J.M., Jones, W.E., Bonner, F.W. and Gibson, G.G. (1987) Drug Metab. Rev. 18, 441–551.
[6] Milton, M.N., Elcombe, C.R. and Gibson, G.G. (1990) Biochem. Pharmacol. 40, 2727–2732.
[7] Ellin, A., Jakobsson, S.V., Schenkman, J.B. and Orrenius, S. (1972) Arch. Biochem. Biophys. 150, 64–71.
[8] Jakobsson, S., Thor, H. and Orrenius, S. (1970) Biochem. Biophys. Res. Commun. 39, 1073–1080.
[9] Hawke, R.L. and Welch, R.M. (1985) Pharmacology 27, 283–289.
[10] Morrison, A.R. (1986) Am. J. Med. 80, 3–11.
[11] Sharma, K., Lake, B.G., Makowsky, R., Bradshaw, T., Earnshaw, D., Dale, J.W. and Gibson, G.G. (1989) Eur. J. Biochem. 184, 69–78.
[12] Tanaka, S., Imaoka, S., Kusunose, E., Kusunose, M., Maekawa, M. and Funae, Y. (1990) Biochim. Biophys. Acta 1043, 177–181.
[13] Muerhoff, A.S., Williams, D.E., Leithauser, M.T., Jackson, V.E., Waterman, M.R. and Masters, B.S.S. (1987) Proc. Natl. Acad. Sci. USA 84, 7911–8004.
[14] Osmundsen, H., Bremer, J. and Pedersen, J.I. (1991) Biochem. Biophys. Acta 1085, 141–158.
[15] Orellana, M., Fuentes, O., Rosenbluth, H. and Valdes, E. (1992) FEBS Lett. 310, 193–196.
[16] Okey, B.A. (1990) Pharmacol. Ther. 45, 241–298.
[17] Gil, L., Orellana, M., Mancilla, J., Garcia, J. and Vásquez, H. (1986) Anal. Lett. 19, 2261–2275.
[18] Omura, T. and Sato, R. (1964) J. Biol. Chem. 239, 2379–2385.
[19] Jacobsson, S.V. and Cinti, D.L. (1973) J. Pharmacol. Exp. Ther. 185, 226–230.
[20] Bronfman, M., Inestroza, N. and Leighton, F. (1979) Biochem. Biophys. Res. Commun. 88, 1030–1036.
[21] Aebi, H. (1974) in: Methods in Enzymatic Analysis (H.U. Bergmeyer, ed.) pp. 673–684, 2nd edn., Academic Press, New York.
[22] Ortiz de Montellano, P.R., Chan, W.K., Tuck, S.K., Kaikaus, R.M., Bass, N.M. and Peterson, J.A. (1992) FASEB J. 6, 695–699.
[23] Imaoka, S., Terano, Y. and Fumae, Y. (1990) Arch. Biochem. Biophys. 278, 168–178.
[24] Imaoka, S., Yamaguchi, Y. and Funae, Y. (1990) Biochim. Biophys. Acta 1036, 18–23.
[25] Capdevila, J.H., Falk, J.R. and Estabrook, R.W. (1992) FASEB J. 6, 731–736.
[26] Sacerdoti, D., Abraham, N.G., McGiff, J. and Schartzman, M.L. (1988) Biochem. Pharmacol. 37, 521–527.