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Cytochrome P450, peroxisome proliferation, and cytoplasmic fatty acid-binding protein content in liver, heart and kidney of the diabetic rat

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Abstract

Diabetes mellitus generally results in an increased systemic fatty acid mobilization which can be associated with an increase in mitochondrial and peroxisomal β-oxidation of fatty acids in selected tissues. The latter is usually accompanied by a concomitant increase in the tissue content of cytoplasmic fatty acid-binding protein (FABP) which functions in the intracellular translocation of fatty acids. It was previously found that in liver clofibrate-induced proliferation of peroxisomes and increase in FABP expression each are dependent on the induction by cytochrome P4504A1 -mediated (CYP4A1) formation of dicarboxylic acids. We studied whether peroxisome proliferation and an increase of FABP contents in liver, heart and kidney of streptozotocin-induced diabetic rats are also accompanied by an increase of CYP4A1 activity, as this would indicate a possible regulatory role for dicarboxylic acids in peroxisome proliferation and FABP induction in diabetic organs other than liver. In livers of the diabetic rat, a concomitant increase was observed of the activities of CYP4A1 and the peroxisomal key enzyme fatty acyl-CoA oxidase (FACO) and of the FABP content. In the diabetic heart FACO activity and FABP content also increased, but there was no induction of CYP4A1 activity. Conversely, in diabetic kidney there was no increase in FACO activity nor FABP content in spite of a marked induction of CYP4A1 activity. It is concluded that streptozotocin-induced diabetes leads to increased peroxisome proliferation and increased levels of FABP in both liver and heart, which only in liver is accompanied by an induction of the cytochrome P450 system. Consequently, it is not likely that dicarboxylic acids are involved in the induction of peroxisome proliferation in the heart. (Mol Cell Biochem **192**: 53–61, 1999)

Key words: diabetes mellitus, cytochrome P450 4A, fatty acid-binding protein, peroxisome proliferation, streptozotocin, rat

Abbreviations: EC – ethoxycoumarin; FACO – fatty acyl CoA oxidase; FABP – fatty acid-binding protein; MES – β-morpholino-ethanesulfonic acid; MOPS – 3-[N-Morpholino] propane sulfonic acid; PPAR – peroxisome proliferator-activated-receptor; PPRE – peroxisome proliferator reponse element

Introduction

Diabetes mellitus is well known to lead to profound changes in whole body fatty acid metabolism. In both insulin-dependent and non-insulin dependent diabetes mellitus, glucose uptake and/or metabolism by extra-hepatic tissues is decreased, causing an increased mobilization of fatty acids from adipose tissue and a shift towards increased utilization of fatty acids by liver, heart and skeletal muscles [1]. Uncontrolled diabetes mellitus leads to a fatty acid overload, which is accompanied by an increased capacity for oxidation of fatty acids by the mitochondrial,

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peroxisomal and microsomal pathways of liver and heart [2].

Under normal circumstances, the peroxisomal β-oxidation is only a minor pathway for fatty acid oxidation relative to the mitochondrial counterpart, although it appears to be of particular importance for the β-oxidation of very-long-chain fatty acids which are poor substrates in the mitochondrial oxidative pathway [3–5]. Diets containing (very) long-chain fatty acids not only induce in liver the enzymes of both peroxisomal and microsomal oxidation [6], but also the cytoplasmic liver-type fatty acid-binding protein (L-FABP) [7]. This protein exhibits marked affinity for long-chain fatty acids and is considered to play a significant role in the cellular uptake and intracellular targeting of fatty acids [8]. Therefore, in the transport and metabolism of long-chain fatty acids during fatty acid overload, the expression of FABP and the activity of peroxisomal enzymes, respectively, each play a crucial role. However the precise mechanisms underlying the regulation of FABP expression and peroxisome proliferation are still unknown.

In liver the proliferation of peroxisomes and the induction of FABP have been demonstrated to be invariably dependent on the cytochrome P450 system localized at the endoplasmic reticulum [5, 9]. This was seen both under conditions of fatty acid overload and after administration of so-called peroxisome proliferators, such as clofibrate [9]. The mechanism has been proposed to be an adaptive response to altered intracellular fatty acid fluxes, mediated by dicarboxylic fatty acids formed via the cytochrome P4504A1 (CYP4A1) ω-oxidation pathway [9]. In this pathway long-chain fatty acids are first hydroxylated on the omega position, then oxidized via an intermediary aldehyde to a dicarboxylic acid [10] and finally converted into their CoA derivative [6, 10]. Recently, a soluble receptor termed peroxisome-proliferator-activated receptor (PPAR) belonging to the nuclear hormone receptor superfamily has been isolated [11]. These receptors are ligand-activated transcription factors and can bind to specific DNA sequences, i.e. the so-called peroxisome proliferator response elements (PPRE). The detection by Northern blotting of PPAR-specific mRNA in liver, heart, kidney, gut and brown adipose tissue of the rat, suggests its involvement in regulating aspects of peroxisomal function and fatty acid homeostasis [12]. However, none of the peroxisomal proliferators tested thus far has been shown to bind to PPAR while the endogenous ligands of this receptor remain unknown [13].

Under starved and diabetic conditions, co-induction in liver of peroxisomal fatty acid β-oxidation and microsomal fatty acid ω-oxidation has also been established [14, 15]. Conversely, conditions of hyperinsulinemia inhibit hepatic peroxisomal β-oxidation in rats [16]. Therefore, it is reasonable to hypothesize that in diabetes mellitus peroxisomal fatty acid β-oxidation and microsomal ω-oxidation in rat liver are cooperatively regulated by PPAR in order to achieve an optimal utilization of fatty acids. With respect to kidney and heart, no data are available. The notion that dicarboxylic acids might play a role in these inductive processes in diabetes is substantiated by the observation that in diabetic rats the urinary excretion of dicarboxylic acids is enhanced [10]. However, Gustafsson *et al*. [17] demonstrated in liver that the cytochrome P450 system as well as dicarboxylic acids do not play a role in peroxisome proliferation. The assembled findings indicate that the precise mechanism of the induction of peroxisome proliferation and of FABP in liver, heart and kidney is not yet fully understood.

The aim of the present study was to determine whether in experimental diabetes, as induced by streptozotocin injection, CYP4A1 activity is implicated in peroxisome proliferation, in the induction of FABP, and/or in the induction of CYP4A1 itself, as was postulated by Kaikaus *et al*. [9, 18, 19] for clofibrate administration and high-fat feeding, and, if so, whether possible organ-specific mechanisms are involved. For this, we measured in rat liver, heart, and kidney not only CYP4A1 activity, but also the activities of CYP1A1 as a generally inducible cytochrome P450-related enzyme and of CY1P2El, a cytochrome P450 subspecies which is induced particularly under diabetic conditions [20]. Peroxisome proliferation was assessed by measuring the activity of fatty acyl-CoA oxidase (FACO). FABP was determined as L(iver) and H(eart)-type FABP by ELISA's type-specific antibodies.

Our data presented in this study indicate marked differences in the response of these organs towards conditions of streptozotocin-induced hyperglycemia with respect to peroxisome proliferation, FABP levels and CYP4A1 induction, thereby questioning the general role of cytochrome P450 related metabolites, such as dicarboxylic acids, in the process of a coordinated induction.

Materials and methods

Chemicals

Streptozotocin, clofibrate, 7-ethoxycoumarin, bovine serum albumin (fraction V), lauric acid, 11-hydroxy and 12-hydroxy lauric acid, 110-hydroxy caproic acid, arachidonic acid, MES (β-morpholino-ethanesulfonic acid), MOPS (3-[N-Morpholino]propane sulfonic acid) and Tris-HCl were obtained from Sigma Chemicals (St. Louis, MO, USA). Acetonitrile, diethyl ether, and tetrahydrofuran were purchased from Rathburn (Walkerburn, UK). Panacyl bromide [p-(9-anthroyl)phenacyl bromide] was from Molecular Probes Inc. (Eugene, OR, USA). NADH, NADPH, $NADP^+$, glucose-6-phosphate and glucose-6-phosphate dehydrogenase were obtained from Boehringer Mannheim (Mannheim, Germany). All other chemicals were at least reagent grade (Merck, Darmstadt, Germany).

Animals

Experiments were performed with adult male Wistar rats (Winkelmann, Borchen, FRG) (body wt 250–300 g). Animals were fed *ad libitum* (Diet SRIVI-A, Hope Farms, Woerden, The Netherlands), had free access to water, and were kept under an artificial dark-light cycle of 12 h. The experiments were approved by the Institutional Animal Care and User Committee of Maastricht University.

Treatment

Insulin-dependent diabetes was evoked by a single intravenous injection of streptozotocin (55 mg/kg body wt) as described in detail by Glatz *et al*. [21]. The rats were sacrificed after 3 weeks.

In a subset of experiments (n = 8) clofibrate was used as peroxisome proliferator. Hereto, clofibrate was administered daily for 7 days via an oral cannula. A solution of 80 mg of the drug per ml glycerol (60%) was given so that each rat received 250 mg/kg per day of clofibrate.

Blood glucose

Blood glucose concentrations were measured with a glucose sensor electrode (Medisense Inc., Waltham, MA, USA) in whole blood obtained by orbital punction.

Tissue homogenization and isolation of microsomes

Liver, heart and kidney were dissected and treated as described by McCallum *et al*. [22]. Briefly, rats were heparinized by an intraperitoneal injection of 1000 U Thromboliquine (Organon Technika, Oss, The Netherlands). After 10 min, the animals were decapitated and liver, heart, and kidney were quickly removed and weighed. Immediately thereafter, the organs were freeze-clamped and stored at –80°C until further analysis. Tissue was pulverized in an aluminum mortar cooled with liquid nitrogen and subsequently homogenized in 0.15 M KCl and 50 mM potassium phosphate, pH 7.4, at 0–4°C using a Potter-Elvehjem glass-Teflon homogenizer. Thereafter, microsomes were prepared by differential centrifugation as described by Rutten *et al*. [23]. The pellet was resuspended carefully with a 5 ml glass-Teflon homogenizer in ice-cold 0.15 M KCl in a volume equal to the original organ weight.

For the determination of FABP freeze-clamped tissue was homogenized as a 10% (w/v) solution in 50 mM Tris-HCl, pH 7.4, with an Ultraturrax (50 Watt) during three periods of 5 sec with 10 sec intervals at 0°C.

Fatty acyl-CoA oxidase (FACO)

Fatty acyl-CoA oxidase activity was measured using palmitoyl-CoA as the substrate, as described by Vannecq [24]. Briefly, the homogenates were freshly prepared by homogenizing the freeze-clamped organ tissue in Mannaerts buffer (10 mM tetrasodium pyrophosphate (pH 9.0), 10 µM FAD^+ and 1 mM EDTA) [25]. Then, 10 µl of the homogenate (containing 50–100 µg total protein) was added to 2 ml of a reaction mixture containing 100 mM HEPES (pH 7.6), 1 mM homovanillic acid, 1 µg horse radish peroxidase, 5 µM FAD^+, 1 mM NaN_3, 240 µg bovine serum albumin (fatty acid free), and 0.06% Triton X-100. The reaction temperature was 37°C, and the cuvet was equipped with a stirring bar at the bottom. The reaction was started by addition of 100 µl of a palmitoyl-CoA solution (1 mM in 20 mM MES buffer). The increase in fluorescence was measured during 15 min on a Shimadzu RF-5001PC fluorescence spectrophotometer (settings: excitation wavelength 327 nm, and emission wavelength 428 nm).

FABP assay

Tissue contents of L(iver-type)-FABP and H(eart-type)-FABP each were measured by specific affinity-enzyme-linked immuno-sorbent assays of the antigen capture type (sandwich-ELISAs), using purified IgG antibodies directed against purified rat L- or H- FABP and the streptavidin-biotin detection system as described by Vork *et al.* [26] and Van Nieuwenhoven *et al*. [27]. The cross-reactivities of H-FABP in the assay for L-FABP, and conversely, were found to be less than 0.002%.

Spectroscopic determination of cytochrome P450

Determinations of cytochrome P450 content of the microsomal preparations was based on the reduced carbon monoxide difference spectrum, using sodium dithionite as the reducing agent, according to Onnura and Sato [28], as described by Stegeman *et al*. [29]. Microsomal suspensions containing about 1 mg of protein per ml were used. The cytochrome P-450 content was calculated from the absorbance difference between 450 and 490 nm, using a molar extinction coefficient of 91 mM^{-1} cm^{-1} [28].

CYP4A1 activity

This activity was measured as the omega-hydroxylation of lauric acid. Hereto we developed a new specific HPLC method, using panacyl bromide as the fluorescent derivative.

Reaction mixtures containing microsomes (1–2 mg of protein), 100 nM lauric acid (added as a stock solution of 10 mM in ethanol) , 0.1 ml of a NADPH regenerating system (10 mg NADH, 7.9 mg $NADP^+$, 6.8 mg glucose-6-phosphate, 14 µl glucose-6-phosphate dehydrogenase, and 6.8 mg $MgCl_2$ in 1 ml of 100 mM potassium phosphate buffer, pH 7.4) in a final reaction volume of 1 ml of 100 mM potassium phosphate buffer, pH 7.4, were incubated at 37°C for 10 min in an orbital shaking water bath (200 rpm). The reaction was stopped by the addition of 50 µl of acetic acid. The formation of the hydroxy derivatives of lauric acid was linear with time until at least 60 min. After addition of the internal standard 10-hydroxy caproic acid, the samples were extracted twice with 3 ml of diethyl ether. The organic phases were collected and dried under a stream of nitrogen at 40°C. The residue was dissolved in diethyl ether-acetic acid (100:0.5, v/v) and consecutively fractionated by silica gel chromatography, purified by reverse-phase chromatography, and derivatized with panacyl bromide as described earlier [30]. The panacyl esters were solubilized in 100 µl of acetonitrile and were analyzed by C_{18} reversed-phase HPLC with fluorimetric detection. The HPLC system consisted of a Lichrocart Supersphere 100-RP-18 column (250 × 4 mm ID, 4 µm particles with a precolumn guard cartridge) (Merck Darmstadt, Germany), a Spectroflow 400 pump (Kratos Analytical, Ramsey, NJ, USA) with a Rheodyne 7125 loop injector (Cotati, CA, USA), and a Jasco fluorescence detector (Jasco Corporation, Tokyo, Japan). The settings were: Excitation wavelength 360 nm and emission wavelength 480 nm. The mobile phase was acetonitrile-water (80:20, v/v). The injection volume was 20 µl and the flow rate 0.5 ml/min.

CYP1A1 activity

This activity was measured as the deethylation of ethoxycoumarin (EC) using a 1.0 ml of a reaction mixture containing 2 µM EC, 0.1 mM Tris (pH 8.0), 0.1 ml M NaCl, and 20 µl microsomes (3–8 mg of microsomal protein per ml). The reaction was started at 37°C by the addition of NADPH (final concentration 0.5 mM), and the appearance of coumarin was monitored with a fluorescence spectrophotometer (Shimadzu RF-5001PC). The fluorimeter settings were: Excitation wavelength 530 nm and emission wavelength 585 nm.

CYP2E activity

This activity was measured as the conversion of p-nitrophenol into 4-nitrocatechol according to Reinke [31] using a 2 ml incubation mixture containing 1.58 ml incubation buffer (50 mM Tris, pH 7.4, 5 mM $MgSO_4$), 0.20 ml of a NADPH regenerating system (see above) and 0.20 ml of microsome suspension. The reaction was started by addition of 20 µl of a p-nitrophenol solution (20 mM) and the mixture was incubated at 37°C for 30 min. The reaction was stopped by addition of 0.5 ml 0.6 M $HClO_4$. After centrifugation at 2000 × g for 10 min, 150 µl 10 M KOH was added to 1.5 ml supernatant. The tubes were placed in ice for 30 min and subsequently centrifuged at 2000 × g for 10 min. The extinction of the supernatants was measured spectrophotometrically at 546 nm. Hydroxylase activity was calculated based on the formation of 4-nitrocatechol, for which a molecular extinction coefficient of 10.28 $mM^{-1}.cm^{-1}$ was used.

Protein determination

Protein concentrations were determined by the Micro BCA (Bicinchoninic acid) assay (Pierce Chem Co., Rockford, IL, USA), using bovine serum albumin (fraction V, Sigma Chemicals) as the standard.

Statistics

Data are presented as mean values ± S.D. Comparisons between groups were made using one-way analysis of variance followed by Tukey's method for multiple comparisons [32]. P values ≤ 0.05 were considered to be statistically significant.

Results

General

The insulin-dependent diabetic state of the streptozotocin-treated adult rats manifested as a high plasma glucose concentration (20–25 mM) and a considerable loss (–27%) of body weight (Table 1). An increase of organ weight was observed only for kidney (+20%), probably due to hypertrophy [33]. In contrast, the absolute heart weight of the diabetic rats decreased significantly (–21%) when compared to the controls. No significant change in liver weight was observed.

Peroxisome proliferation

Generally, diabetes mellitus is characterized by a condition of increased mobilization of fatty acids which might lead to an increase of peroxisomal activity. Therefore, we measured peroxisomal activity based on the peroxisomal enzyme fatty acyl-CoA-oxidase (FACO) (Fig. 1). A significant increase of

Table 1. Body weight, blood plasma glucose concentration and organ weights in control and streptozotocin-induced diabetic rats

Condition	Body weight (g)	Glucose (mM)	Liver (g)	Heart (g)	Kidney (g)
Control	329 ± 25	7.0 ± 0.7	13.3 ± 1.5 (4%)	1.03 ± 0.11 (0.31%)	1.22 ± 0.10 (0.37%)
Diabetes	240 ± 39*	22.5 ± 2.7*	11.9 ± 1.7 (5%)	0.81 ± 1.7 (0.33%)	1.47 ± 0.17* (0.61%)

Values are means ± S.D. (n = 6). Values in parentheses refer to mean organ weight expressed as percentage of total body weight. Statistical significance was determined by Tukey's test. *p < 0.05.

FACO was observed in both liver (+62%) and heart (+265%). However, in kidney the FACO activity of the diabetic group did not differ from the control.

FABP

The diabetic animals showed an increase by 40–50% in the content of both L-FABP in liver and H-FABP in the heart (Table 2). In kidney from the control rats the amounts of L- and H-FABP were low when compared to those in liver and heart (Table 2). The 5–10 fold higher kidney content of H-FABP compared to L-FABP in control animals agrees with an earlier report [34]. A small but significant decrease of the L-FABP content was observed in kidney the diabetic animals, whereas the contents of H-FABP did not differ significantly between diabetic and control rats.

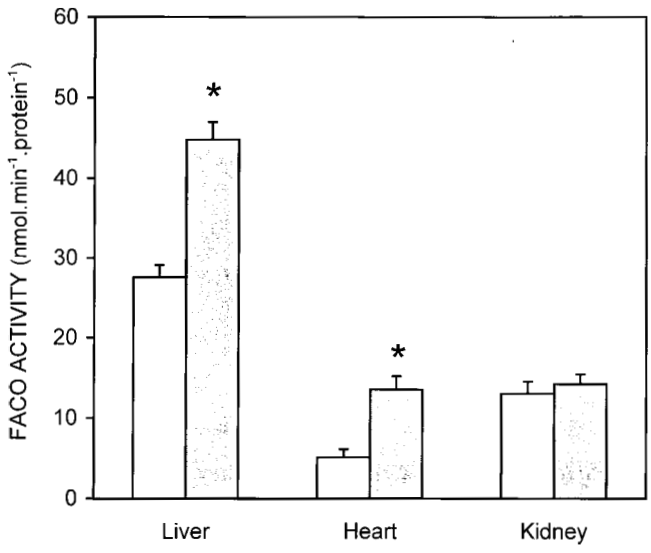

Fig. 1. Activities of the peroxisomal enzyme fatty acyl-CoA oxidase (FACO) in liver, heart and kidney of control and streptozotocin-induced diabetic rats. Activities were measured in tissue whole homogenates and are represented as means ± S.D. (n = 6). Statistical significance was determined by Tukey's test. *p < 0.05, compared with the control group. Open bars, control animals; dark bars, streptozotocin-induced diabetic animals.

Cytochrome P450

To explore whether peroxisome proliferation is a cytochrome P450-dependent process, in which the formation of di-carboxylic acids is crucial, we measured in addition to the total content of cytochrome P450 also the activity of CYP4A1 (based on ω- and ω-1 hydroxylation of lauric acid). Moreover, to assess the specificity of the cytochrome P450 induction, the activity of CYP1A1 and CYP2E was measured (Table 3). In both liver and kidney a 2 to 3-fold increase in the total content of cytochrome P450 was observed. In contrast, in heart cytochrome P450 or CYP4A1 activity (even after prolonged incubation times up to 1 h) could not be demonstrated. CYP4A1 activities were increased in liver and kidney of diabetic animals, but not in the heart. Ethoxy-coumarin deethylase (CYP1A1) and p-nitrophenol (CYP2E) hydroxylase could be demonstrated only in liver. In kidney and heart these activities were not detectable, neither in control, nor in streptozotocin-treated animals.

Since streptozotocin might be a weak inducer of the cytochrome P450 system in heart, in a separate set of animals (n = 8) we used clofibrate, a compound commonly used to

Table 2. Content of liver-type(L-) and heart-type (H-) FABP in liver, heart and kidney of streptozotocin-induced diabetic and control rats

Tissue	Condition	L-FABP (μg.mg protein^{-1})	H-FABP (μg.mg protein^{-1})
Liver	Control	8.8 ± 2.5	< 0.0002
	Diabetes	12.4 ± 2.2*	< 0.0002
Heart	Control	0.005 ± 0.012	5.1 ± 0.6
	Diabetes	0.002 ± 0.012	7.4 ± 0.8*
Kidney	Control	0.031 ± 0.010	0.12 ± 0.02
	Diabetes	0.012 ± 0.007*	0.14 ± 0.05

FABP contents were measured in tissue whole homogenates and are expressed as means ± S.D. (n = 6). Statistical significance was determined by Tukey's test. *p < 0.05.

Table 3. Cytochrome P450 content and activities of ω- and ω-1-hydroxylation, 7-ethoxycoumarine deethylase, and p-nitrophenol in microsomal preparation in liver, heart and kidney of streptozotocininduced diabetic rats.

Tissue	Condition	Cytochrome P450[a]	Omega hydroxylation[b] (CYP4A1)	Omega hydroxylation[b] (CYP4A1)	Ethoxycournarin deethylase[b] (CYP1Al)	p-Nitrophenol hydroxylase[c] (CYP2E)
Liver	control	0.60 ± 0.17	1.17 ± 0.37	0.89 ± 0.12	0.91 ± 0.20	0.65 ± 0.15
	diabetes	1.69 ± 0.37*	3.18 ± 0.51	1.41 ± 0.10*	1.79 ± 0.42*	1.82 ± 0.12*
Heart	control	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
	diabetes	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
Kidney	control	0.20 ± 0.10	1.32 ± 0.27	0.53 ± 0.10	< 0.01	< 0.01
	diabetes	0.37 ± 0.12*	2.06 ± 0.54*	0.65 ± 0.25	< 0.01	< 0.01

Content/activities were measured in tissue whole homogenates and are expressed as [a]ng.mg total protein^{-1}; [b]nmol.min^{-1}.mg total protein^{-1}; [c]pmol.min^{-1}.mg total protein^{-1}, respectively. Values are means ± S.D. (n = 6). Statistical significance was determined by Tukey's test. *$p < 0.05$.

induce peroxisome proliferation [18]. In liver a significant increase in cytochrome P450 content (3-fold) and in CYP4A1 activity (4-fold) occurred. In heart no increased activity could be demonstrated. Nevertheless, peroxisomal β-oxidation, as assessed by FACO activity, increased 14-fold in liver and 3-fold in heart (data not shown). Additional experiments with β-naphtoflavone and phenobarbital as potent inducers of cytochrome P4501A1 in heart [22, 35] neither resulted in detectable amounts of cytochrome P450, nor of CYP4A1 activity in the heart (data not shown). In contrast, in the present study these compounds gave in liver a considerable and comparable induction of omega hydroxylation (+420%), ethoxycournarin deethylation (+160%), aniline hydroxylation (+300%) and 6β-testosterone hydroxylation (+240%).

Discussion

In the present study we explored the effect of streptozotocin-induced diabetes mellitus on peroxisome proliferation, cytochrome P450 activity and the content of cytoplasmic FABP in various tissues of the rat. In liver there was a co-induction of all three parameters, which finding extends the previous observations of Kaikaus *et al*. [18, 19] in clofibrate-treated rats, and suggests that also in the liver of diabetic animals the increase in peroxisome proliferation and L-FABP content is induced by dicarboxylic acids formed in the cytochrome P450 pathway. However, in the diabetic heart we found both an increased peroxisome proliferation and H-FABP content, but no detectable CYP4A1 activity, while in kidney an induction of CYP4A1 was seen in combination with a decreased L-FABP content, an unchanged H-FABP content and peroxisomal activity. These findings strongly suggest that peroxisome proliferation and H-FABP induction in the heart take place via a mechanism not involving CYP4A1 metabolites (such as dicarboxylic acids) and, conversely, that the enhanced CYP4A1 activity, as seen in kidney, does not necessarily lead to an induction of peroxisome proliferation and induction of either cytoplasmic L- or H-type FABP.

A potential role for dicarboxylic fatty acids, produced from long-chain monocarboxylic fatty acids via CY1P4A1, as mediators of the peroxisome proliferator response and possible ligands of PPAR, has been demonstrated in rat liver [18]. In contrast, Gustafson and coworkers demonstrated that the induction of peroxisome proliferation in rat liver by long-chain fatty acids was not dependent of cytochrome P450 and that dicarboxylic acids did not activate PPAR [17]. At present, no explanation can be given for these contradictory results. Additionally, it was shown by Göttlicher *et al*. [36, 37] that PPAR can be activated by long-chain fatty acids *per se*. These workers also demonstrated that β-oxidation of the inducing fatty acid is not required and that blocking of the β-oxidative pathway increases the potency of fatty acids or of an, as yet, unidentified metabolite for activating PPAR, most likely due to enhanced levels of unmetabolized long-chain fatty acids [37]. The latter would be in agreement with the suggestion made by Isseman *et al*. [7] that a rapid metabolism of β-oxidizable fatty acids within the cell requires high concentrations of these fatty acids to activate PPAR. This notion is also supported by the observation of Kaikaus *et al*. [19] that oleic acid alone was unable to stimulate peroxisomal β-oxidation or the expression of FAI3P, but in the presence of an inhibitor of carnitine palmitoyl transferase 1, induced peroxisomal β-oxidation and the expression of FABP pre-translationally.

In general, streptozotocin-induced diabetes not only leads in liver to the induction of CYP4A1, but also of subspecies, such as CYP1A1, CYP2E [38, 39]. The most well-defined change in livers of diabetic rats with respect to cytochrome P450 is the induction of CYP2E1 and this elevation can be reversed by treatment with insulin [39]. Data from the study of Shimojo [39] indicate that during diabetes most probably the enhanced production of ketone bodies is primarily responsible for the induction of CYP1A1 and CYP2E,

whereas fatty acid overload results in an increased expression of CYP4A1.

Although it is well documented that administration of peroxisome proliferators, such as clofibrate, results in a markedly increased content of L-FABP in rat liver [18], data on tissue FABP levels under diabetic conditions are limited. In this study a significant increase in L-FABP content could be demonstrated, which most likely is related to the increased fatty acid utilization by the tissue [8]. In contrast, however, Veerkamp *et al*. [40] reported decreased L-FABP contents in the liver of streptozotocin-induced diabetic rats when compared to the control animals. Possibly, the duration of streptozotocin treatment (12 vs. 21 days) and differences in the assay procedure of FABP may have contributed to the discrepancy.

Peroxisome proliferation measured as an increase of FACO activity was relatively more pronounced in the heart than in liver (Fig. 1). This supports the marked increase in density of myocardial peroxisomes in experimental diabetes observed by others [41, 42]. Although the myocardial peroxisomal β-oxidative activity might be relatively low, when compared to that of the liver [43], it has been demonstrated to be significant for the β-oxidation of very-long chain fatty acids [44, 45]. Interestingly, no induction of the activities of cytochrome P450 subspecies (CYP1A1, CYP2E, and CYP4A1) could be demonstrated in this organ. Although, a number of other studies has provided evidence that very low levels of cytochrome P450, in particular CYP1A1, are present in the hearts of rabbit, [35], guinea pig [22] and rat [46], at present no other data are available on the occurrence of CYP4A1 activity in the heart. Since the microsomal P450 monooxygenase system is composed of a flavoprotein (NADPH-P450 reductase) and multiple isoenzymes of the hemoprotein P450 [47, 48], the observed low activities might be due not only to a low content of cytochrome P450 but also to low activities of P450-reductase in heart. This notion is substantiated by the observation that reconstitution of cardiac microsomal fractions with purified NADPH-cytochrome P450 reductase dramatically increases cytochrome P4501A activity *in vitro* [22, 49]. Therefore, it is possible that the low activity of cytochrome P450 monooxygenase in the heart, even after CYP1A1 induction, is based on a deficiency of NADPH-P450 reductase activity. The present study suggests that in cardiac cells the cytochrome P450 system at best may play a minor role in the formation of hydroxy fatty acids. It should be noted that we were not able to detect in rat heart, in contrast to liver and kidney, alcohol dehydrogenase activity (unpublished results). Thus, in cardionnyocytes the conversion of hydroxy fatty acids into the aldehyde and subsequently into the dicarboxylic form is unlikely.

The induction of CYP4A1 activity in kidney supports the observation that in this organ arachidonic acid can be converted not only into 20-HETE but also into 20-COOH-arachidonic acid [50]. It should be noticed that the cytochrome P450 system is capable of producing a variety of arachidonic acid metabolites which play an important role in electrolyte homeostasis and in the regulation of renal blood flow [50]. However, in contrast to heart and liver, no increase of FACO activity could be observed in kidney of streptozotocin-treated animals. In this respect, our data support the findings of Asayama *et al*. [41] that streptozotocin does not induce peroxisome proliferation in rat kidney. Also in mice feeding of high-fat diets failed to increase the number of peroxisomes and peroxisomal β-oxidation in kidney [51, 52].

The presence of both H- and L-FABP in kidney has been reported by various authors [8, 34], but the amounts are relatively low when compared to those observed in heart and liver, respectively. The small but significantly decreased L-FABP content of the diabetic kidney is difficult to explain, partly since the distinct functions of the two FABP types in kidney are not yet understood [8].

Our observations suggest that in kidney the role of the cytochrome P450 system is of greater importance for the conversion of fatty acids into bioactive substances regulating integrated renal function, such as participation in electrolyte homeostasis and regulation of tissue blood flow, than for the process of peroxisome proliferation which could help reducing fatty acid overload as associated with diabetes mellitus [50]. The role of FABP in the induction of these cytochrome P450-related activities has to be established.

The findings of the present study show that in the liver of diabetic rats a coordinated induction of CYP4A1, cytoplasmic FABP content and peroxisome proliferation occurs, similar to that observed in rats after administration with peroxisome proliferators (such as clofibrate) or feeding of high-fat diets. In heart, increased peroxisome proliferation and FABP content was not accompanied by an induction of any . Therefore, a role for cytochrome P4504A1 -mediated metabolites of fatty acids, such as dicarboxylic acids, in the induction of peroxisome proliferation and expression of FABP in the heart is unlikely. Finally, in kidney induction of cytochrome P450 activity was observed in absence of an increase in peroxisomal activity and in the content of FABP.

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