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CONCOMITANT INDUCTION OF CYTOSOLIC BUT NOT MICROSOMAL EPOXIDE HYDROLASE WITH PEROXISOMAL β-OXIDATION BY VARIOUS HYPOLIPIDEMIC COMPOUNDS

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Abstract—The effects of two cholesterol-lowering (probucol and 1-benzyl-imidazole), three triglyceride- and cholesterol-lowering (clofibrate, tiadenol and fenofibrate) and one triglyceride-lowering (acetylsalicylic acid) compounds on the specific activities of two lipid-metabolizing enzymes (cyanide-insensitive peroxisomal β-oxidation and palmitoyl-CoA hydrolase) and two xenobiotic metabolizing enzymes (cytosolic (cEH) and microsomal epoxide hydrolase (mEH_b)) from the livers of male Fischer F-344 rats were investigated.

With the exception of probucol and acetylsalicylic acid, all compounds tested caused a dose-dependent hepatomegaly. Taken on a weight basis fenofibrate was the most effective inducer, causing a 20-fold induction of peroxisomal β-oxidation, a 13-fold induction of cEH activity and a 16-fold induction of palmitoyl-CoA hydrolase activity. The other compounds with triglyceride-lowering activity also induced cEH as well as peroxisomal β-oxidation and palmitoyl-CoA hydrolase activity. The potency of each individual drug was similar for induction of cEH activity as compared with that of peroxisomal β-oxidation and palmitoyl-CoA hydrolase activity, but very dissimilar for mEH_b, which upon treatment with any of the triglyceride-lowering compounds was either not or only minimally (<1.5-fold) induced. 1-Benzylimidazole possessing exclusively cholesterol-lowering activity increased mEH_b much more than either cEH or peroxisomal β-oxidation.

The absence of an enhancement of cEH activity in *in vitro* studies confirmed that the increase in enzyme activity by the test compounds is not caused by activation. cEH activity was also induced in the kidney but only about 2-fold by fenofibrate, tiadenol and acetylsalicylic acid.

With hypolipidemic drugs varying in their peroxisome-proliferating potency from inactive to very potent, the effects on peroxisomal β-oxidation and cEH were similar in all instances, whilst the effects on mEH_b could be clearly dissociated. Thus, in the rat a concomitant regulation of cEH with peroxisomal β-oxidation and peroxisome proliferation by hypolipidemic drugs becomes apparent.

Lipid-lowering drugs are used in the prophylaxis of atherosclerosis, as high serum cholesterol levels have been recognized as one of the risk factors for this disease. Recently, it has been shown that several lipid-lowering drugs are tumourigenic in rats and mice [1]. As these compounds have been found to be negative as mutagens in the Ames test, hypotheses regarding their tumourigenicity are based on unique biochemical alterations induced by them [2]. In rats and mice, these drugs have been reported to cause a proliferation of peroxisomes and concomitantly a large induction (up to 20-fold) of some—mostly peroxisomal—enzymes, which are mainly involved in the metabolism of fatty acids [3]. One of the most critical events may be the induction‡ of a peroxisomal β-oxidation system. The first step of the peroxisomal pathway is, in contrast to mitochondrial β-oxidation, catalyzed by an acyl-CoA oxidase producing hydrogen peroxide. Due to only moderate induction of catalase (about 2-fold), as compared to peroxisomal β-oxidation, an increase in intracellular hydrogen peroxide may occur. Although catalase has a high molecular turnover number reactive oxygen species derived from hydrogen peroxide may therefore be responsible for the carcinogenicity of peroxisome proliferators [4].

Epoxide hydrolases metabolize epoxides, which are common products of cytochrome P-450 catalyzed oxidations of aromatic and olefinic compounds. They catalyze the addition of water to the epoxide moiety to yield dihydrodiols. The two major forms of epoxide hydrolases, mEH_b§ and cEH, are two distinct proteins and differ in most properties investigated

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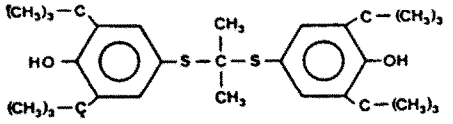
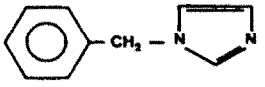
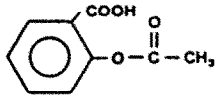
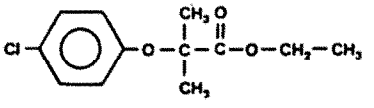
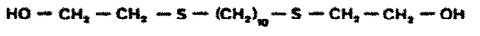
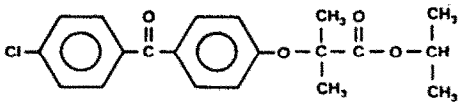
‡ The term induction is used in the present study in its broad sense to denote an increase in enzyme activity regardless of the underlying mechanism, but not mimicked by the addition of the compound to the *in vitro* preparation.

§ Abbreviations used: STO, 2-phenyloxirane (styrene 7,8-oxide); TSO, *trans*-2,3-diphenyloxirane (*trans*-stilbene oxide); mEH_b, microsomal epoxide hydrolase with broad substrate specificity for various xenobiotic epoxides, specifically including benzo(*a*)pyrene 4,5-oxide; cEH, cytosolic epoxide hydrolase.

Table 1. Effects of the tested compounds on peroxisome proliferation and serum lipid level

Compound		Hypolipidemic activity	Peroxisome proliferation	Triglyceride (T) or cholesterol (C) lowering activity
	Probucol	+	–	C
	1-Benzylimidazole	+	+	C
	Acetylsalicylic acid	+	+	T
	Clofibrate	+	+	T + c
	Tiadenol	+	+	T + c
	Fenofibrate	+	+	T + c

(for a review see [5]). Selective substrates for the cEH are *trans*-1,2-disubstituted epoxides, whereas *cis*-substituted epoxides including epoxides derived from cyclic systems are hydrolyzed by mEH_b [6, 7].

In contrast to mEH_b, cytosolic epoxide hydrolase is not induced by classical inducers of xenobiotic metabolizing enzyme systems [8, 9]. Up to now only three compounds (clofibrate, di-(2-ethylhexyl)-phthalate [9] and nafenopin [10]) are known to cause an induction (about 2-fold) of cytosolic and microsomal epoxide hydrolases in mice. All three compounds are known to cause peroxisome proliferation and to be inducers of peroxisomal β-oxidation [4]. The aim of the study was to investigate whether there is a common induction of lipid metabolizing enzymes (peroxisomal β-oxidation system, palmitoyl-CoA hydrolase) and cytosolic and/or microsomal epoxide hydrolase after treatment with several hypolipidemic drugs. The structural formulas of the compounds used in this study are shown in Table 1.

MATERIALS AND METHODS

Chemicals. [2,3-^{3}H]2-phenyloxirane ([^{3}H]STO, 11.7 GBq/mmol) and [2,3-^{3}H]*trans*-2,3-diphenyloxirane ([^{3}H]TSO, 0.41 GBq/mmol) were synthesized as described in [11] and [12], respectively.

1-Benzylimidazole and tiadenol (2,2'-(decamethylenedithio)diethanol] were purchased from Aldrich (Steinheim, F.R.G.) and clofibrate [2-(4-chlorophenoxy)-2-methylpropanoic acid ethyl ester] from Serva Feinbiochemica (Heidelberg, F.R.G.). Acetylsalicylic acid was obtained from Sigma (Deisenhofen, F.R.G.). Fenofibrate (2-[4-(4-chlorobenzoyl)phenoxy]-2-methylpropanoic acid 1-methylethyl ester) fenofibric acid, clofibric acid and probucol (4,4'-[(1-methylethylidene)bis (thio)] bis-[2,6-bis(1,1-dimethylethyl)phenol]) were provided by Laboratoires Fournier (Fontaine les Dijon, France).

Animal experiments. Male Fischer F-344 rats (150–160 g; 56–63 days old) were obtained from Charles River Wiga GmbH, Sulzfeld, F.R.G. They were kept at constant temperature, under a constant light–dark cycle with free access to water and a defined diet (Altromin). For the induction studies, animals were fed a pelleted diet containing the hypolipidemic compound. They remained on their diet for 7 days until they were killed. The compounds were added by soaking the food in an acetone solution and subsequent evaporation of the organic solvent. As the compounds investigated differ markedly in their hypolipidemic potency, lowest doses corresponding on a body weight basis to about 2-fold the human therapeutic dose and correspondingly higher doses were used. Doses of 1-benzylimidazole and acetylsalicylic acid, which are not used as hypolipidemic drugs in human therapy, were in the same order of magnitude as described in [4]. Other rats were treated with 500 mg/kg Aroclor 1254 by two i.p. injections seven and four days before sacrifice.

Preparation of subcellular fractions. Animals were killed at a constant time of day by cervical dislocation. The livers were perfused with ice-cold homogenisation buffer (10 mM Tris–HCl, pH 7.4, containing 0.25 M sucrose) and removed immediately. Homogenisation of livers and kidneys was carried

out in the same buffer, using an Ultra-Turrax to give a 25% (w/v) homogenate. The homogenate was centrifuged for 10 min at 600 *g* and for 15 min at 10,000 *g*. The resultant supernatant was centrifuged for 60 min at 100,000 *g* to give the cytosolic supernatant. The microsomal pellet was resuspended in a volume of homogenisation buffer which was equivalent to the original liver weight.

Enzyme assays. Activity of cEH was determined in the cytosol [8] and for mEH_b in the microsomal fraction as described previously [11] under the conditions described in [13], specifically in the absence of detergent.

Peroxisomal β-oxidation activity was measured in the 600 *g* supernatant [14] and palmitoyl-CoA hydrolase in the cytosol [15].

RESULTS

Weight gain and hepatomegaly

With the exception of 1-benzylimidazole, and the higher doses of acetylsalicylic acid (1%) and fenofibrate (0.25%) treatment, the gain in body weight of the treated animals was not markedly different from that of the controls (Table 2). Whilst benzylimidazole and acetylsalicylic acid (1%) caused a decrease in body weight, fenofibrate (0.25%) led only to a lower increase in weight gain. Benzylimidazole was toxic at higher doses. Probucol, clofibrate and tiadenol had no or little effect on the weight gain even at the highest doses used.

A dose-dependent increase in liver weight was observed, with clofibrate, fenofibrate or tiadenol (Table 2). The liver/body weight ratio was not, or only slightly, effected by probucol and acetylsalicylic acid.

Enzyme activities

Treatment with Aroclor 1254 did not change the activities of cEH, palmitoyl-CoA hydrolase or peroxisomal β-oxidation, but caused a 2-fold induction of mEH_b. The cholesterol-lowering drug, probucol, had no effect on peroxisomal β-oxidation and cEH at both concentrations tested, whilst mEH_b activity was slightly increased and palmitoyl-CoA hydrolase activity was doubled at the highest dose. A 5-fold increase of mEH_b activity was observed with benzylimidazole, whereas a less than 2-fold (1.7–1.8-fold) induction of cEH and peroxisomal β-oxidation activities was observed, whilst the specific activity of palmitoyl-CoA hydrolase remained unchanged. The four triglyceride-lowering compounds had no or only a very weak (<1.5-fold) influence on mEH_b activity at all doses tested (Table 3).

Treatment with a relatively high dose (1% w/w in the diet) of the rather weak triglyceride-lowering compound acetylsalicylic acid caused a 5-fold induction of both cytosolic epoxide hydrolase activity and peroxisomal β-oxidation, and a somewhat lower (2.6-fold) induction of palmitoyl-CoA hydrolase. At a lower dosage (0.2%) there was only a 1.5-fold induction of all three enzymes.

The other triglyceride-lowering compounds (clofibrate, tiadenol and fenofibrate) also caused a dose-dependent induction rate. At the lowest doses, cEH, peroxisomal β-oxidation and palmitoyl-CoA hydrolase were all induced to a similar extent (2–4-fold). If higher concentrations were used a dramatic increase of enzyme activities was observed. At the highest dose, the peroxisomal β-oxidation was increased somewhat higher than cEH or palmitoyl-CoA hydrolase activity. Treatment of rats for one week with clofibrate, tiadenol and fenofibrate (at the highest doses) caused an 8-fold, 13-fold and 13-fold induction of cEH activity, and a 13-fold, 19-fold and 20-fold induction of peroxisomal β-oxidation activity, respectively. Palmitoyl-CoA hydrolase activity was increased 8-fold (by clofibrate) and 16-fold (by fenofibrate and tiadenol).

Figure 1 illustrates the *in vitro* effect of the hypo-

Table 2. Dose schedule of tested compounds and their effect on gain in body weight and liver weight

Compound	Drug included in the diet at % (w/w)	Mean dose intake (mg/kg/day)	Gain in body weight (g/week)	Liver weight (g)	Liver/body weight ratio %
Control	—	—	28 ± 8	7.6 ± 0.3	4.1 ± 0.3
Probucol	0.025	40	31 ± 5	8.1 ± 0.4	3.8 ± 0.5
	0.125	102	36 ± 7	9.1 ± 0.7	4.3 ± 0.2
1-Benzylimidazole	0.1	64	−3 ± 5	8.7 ± 0.7	5.6 ± 0.3
Acetylsalicylic acid	0.2	190	31 ± 6	8.6 ± 0.2	4.2 ± 0.2
	1.0	630	−10 ± 5	6.8 ± 0.3	4.2 ± 0.2
Clofibrate	0.05	56	31 ± 4	8.6 ± 0.8	4.6 ± 0.3
	0.25	205	24 ± 4	11.5 ± 1.1	6.3 ± 0.5
Tiadenol	0.04	37	34 ± 3	8.4 ± 0.5	4.4 ± 0.3
	0.1	99	29 ± 2	13.8 ± 0.9	7.5 ± 0.6
	0.5	548	29 ± 4	15.4 ± 1.4	8.5 ± 0.6
Fenofibrate	0.01	8	31 ± 1	8.9 ± 0.2	4.7 ± 0.2
	0.05	43	32 ± 8	11.8 ± 0.8	6.2 ± 0.2
	0.25	216	9.3 ± 4.2	12.0 ± 1.1	7.2 ± 0.2

Results represent mean ± standard deviation from four male rats treated (control: 12 rats) and prepared individually.

Table 3. Effect of Aroclor 1254 and six hypolipidemic compounds on the specific activities of peroxisomal β-oxidation, palmitoyl-CoA hydrolase, cytosolic and microsomal epoxide hydrolase in male Fischer F-344 rat liver*

Compound	Drug included in the diet at % (w/w)	Peroxisomal β-oxidation‡	Cytosolic epoxide hydrolase§	Microsomal epoxide hydrolase‡	Palmitoyl-CoA hydrolase‡
Control	—	5.2 ± 0.3	38 ± 8	4.0 ± 0.5	11.4 ± 3.8
Aroclor 1254†	—	5.6 ± 0.9‖	33 ± 2‖	8.7 ± 2.1	13.7 ± 1.5‖
Probucol	0.025	5.2 ± 0.4‖	39 ± 2‖	4.5 ± 0.7‖	16.6 ± 1.4¶
	0.125	5.3 ± 0.2‖	36 ± 2‖	6.1 ± 1.2	23.4 ± 1.0
1-Benzylimidazole	0.1	8.8 ± 0.9	69 ± 9	19.0 ± 3.0	10.8 ± 2.4‖
Acetylsalicylic	0.2	8.2 ± 0.5	58 ± 10**	4.4 ± 0.8‖	17.8 ± 0.6**
	1.0	25.0 ± 3.0	178 ± 36	5.8 ± 0.6	29.8 ± 4.3
Clofibrate	0.05	9.3 ± 0.9	82 ± 14	5.2 ± 0.4	14.8 ± 2.8‖
	0.25	70.0 ± 7.0	312 ± 45	5.8 ± 0.6	90.0 ± 7.8
Tiadenol	0.04	15.0 ± 3.0	119 ± 12	3.5 ± 0.6‖	32.6 ± 3.5
	0.1	66.0 ± 6.0	460 ± 50	5.5 ± 0.5	144.8 ± 21.5
	0.5	98.0 ± 7.0	505 ± 34	5.7 ± 0.6	183.7 ± 27.4
Fenofibrate	0.01	14.9 ± 2.5	140 ± 21	4.9 ± 1.0‖	23.6 ± 2.9
	0.05	48.1 ± 4.4	331 ± 38	4.5 ± 0.4‖	124.0 ± 6.8
	0.25	106.0 ± 1.0	489 ± 52	5.1 ± 0.1	179.9 ± 20.9

* Results represent mean ± standard deviation for hepatic tissues from four rats treated (control: 12 rats) and prepared individually.

† Animals were treated with Aroclor 1254 by two i.p. injections of 500 mg/kg seven and four days before killing.

‡ Specific activity is given in nmol/min × mg protein.

§ Specific activity is given in pmol/min × mg protein.

‖ Not significantly different from control ($P > 0.05$).

¶ $P < 0.05$.

** $P < 0.01$.

For all other values $P < 0.001\%$ (Student's *t*-test).

lipidemic compounds on the specific activity of rat liver cytosolic and microsomal epoxide hydrolase. The activities of both enzymes are not or only slightly effected in the presence of 1 mM fenofibrate, acetylsalicylic acid and probucol. Clofibrate and tiadenol, which are strong inducers of cEH *in vivo* inhibited enzyme activity *in vitro* to 60% and 25% of control activity at concentrations of 1 mM. In contrast, mEH_b activity was enhanced 1.8-fold by tiadenol whereas benzylimidazole activated the enzyme 2.7-fold, but had no effect on cEH activity.

Clofibric acid did not decrease, and the cor-

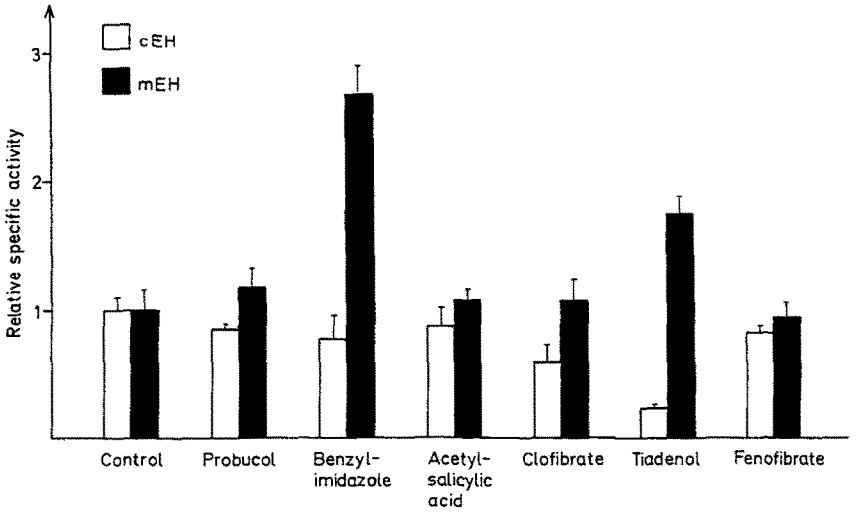


Fig. 1. *In vitro* effect of six hypolipidemic compounds on the specific activity of rat liver cytosolic and microsomal epoxide hydrolase. The compounds were incorporated into the assay mixture at final concentrations of 1 mM.

Table 4. Effect of fenofibrate, tiadenol and acetylsalicylic acid on kidney and liver cytosolic epoxide hydrolase activity of male Fischer F-344 rats*

Compound	Drug included in the diet at % (w/w)	Kidney		Liver	
		Peroxisomal β-oxidation†	Cytosolic epoxide hydrolase‡	Peroxisomal β-oxidation†	Cytosolic epoxide hydrolase‡
Control	—	0.7 ± 0.2	98 ± 15	5.2 ± 0.3	38 ± 8
Fenofibrate	0.05	6.1 ± 0.2 (8.7)	173 ± 19 (1.8)	48.1 ± 4.4 (9.2)	331 ± 38 (8.7)
Tiadenol	0.1	n.t.	203 ± 26 (2.1)	66.0 ± 6.0 (12.7)	460 ± 50 (12.1)
Acetylsalicylic acid	1.0	n.t.	178 ± 12 (1.8)	25.0 ± 3.0 (4.8)	178 ± 36 (4.7)

* Results represent mean ± standard deviation for tissues from four rats treated (number of control livers: 12; number of control kidneys: 7 for cytosolic epoxide hydrolase activity and 4 for peroxisomal β-oxidation activity) and prepared individually.
† Activity is given in nmol/min × mg protein.
‡ Activity is given in pmol/min × mg protein.
n.t. not tested. Induction factors are given in brackets.

responding acid of fenofibrate slightly decreased, the activity of cEH (to about 80% of control activity; data not shown).

Table 4 shows the effect of one weak (acetylsalicylic acid) and two potent (tiadenol, fenofibrate) triglyceride-lowering agents, on the specific activity of Fischer F-344 rat kidney cytosolic epoxide hydrolase. In comparison to the high induction of liver cEH activity, only a 2-fold induction was observed in kidney. Cyanide insensitive β-oxidation in kidney was determined only after treatment with fenofibrate. Activity in kidney from untreated animals was 14% of that in liver and was induced about 9-fold after feeding for one week a 0.05% fenofibrate diet. Interestingly the kidney specific activity of cEH was 2.5-fold higher than in liver.

DISCUSSION

Fibric acid derivatives and various structurally unrelated compounds with hypolipidemic activity have been shown to cause proliferation of peroxisomes in many species [3, 4]. Concomitant with, or even preceeding, peroxisome proliferation a huge induction of several enzymes, involved in the metabolism of fatty acids is observed. Some of the induced enzymes are thought to be exclusively localized in peroxisomes (enzymes of the peroxisomal β-oxidation, e.g. palmitoyl-CoA oxidase; catalase [16]) whereas other enzymes are also found in other subcellular compartments (palmitoyl-CoA hydrolase [15]) whilst yet others are completely absent from peroxisomes (e.g. cytochrome P-452 dependent lauryl ω-hydroxylase [17, 18]).

Recently, a moderate induction (approx. 2-fold) of cEH and mEH_b by clofibrate and nafenopin has been observed in mice [9, 10]. In contrast to mEH_b, which is present in the microsomes, nuclei, Golgi apparatus and plasma membranes [19], cEH activity is found in the cytosol and peroxisomes (unpublished observations; [20]).

In the present study, we demonstrate that there is a concomitant induction of cEH, but not of mEH_b, with other enzymes induced by peroxisome proliferators. Six hypolipidemic compounds were investigated for their effect on enzyme induction. Clofibrate and fenofibrate are fibric acid derivatives and are structurally dissimilar to the others (Table 1). Acetylsalicylic acid has only very low hypolipidemic potency, whereas fenofibrate and tiadenol are potent triglyceride-lowering drugs. 1-Benzylimidazole and probucol are cholesterol-lowering drugs exhibiting only weak (1-benzylimidazole) or no (probucol) peroxisome-proliferation activity [3].

For our investigation, we chose Fischer F-344 rats (inbred strain), because of the small interindividual differences in the cEH activity, as compared to Sprague–Dawley rats (outbred strain) [8].

On a body weight basis, the lowest doses of the various compounds were slightly (about twofold) higher than the standard doses used in human therapy (probucol: 17 mg/kg/day; clofibrate: 29 mg/kg/day; tiadenol: 27 mg/kg/day; fenofibrate: 5 mg/kg/day). However, we realize that a direct comparison of the doses, based only on weight, is not fully valid due to the difficulties inherent in species comparisons.

The effect of hypolipidemic compounds on peroxisomal β-oxidation has been thoroughly investigated [3, 4, 22, 23], but it is difficult to compare the studies due to the use of different dose schedules, application forms, animals, strains and duration of treatment. Induction of peroxisomal β-oxidation activity (in order of decreasing potency) in the liver of Fischer F-344 rats by fenofibrate, tiadenol, clofibrate, and acetylsalicylic acid agreed well with other studies with respect to the rate of induction and dose dependency [23, 24].

Rat liver cEH was increased upon treatment with these triglyceride-lowering drugs to a surprisingly high extent (Table 3). When drug intake is taken into account, the strongest induction was caused by

fenofibrate (13-fold, 0.25% w/w; Table 3). Clofibrate (0.25% w/w) enhanced the cEH activity 8-fold, whereas it was reported to be about 2-fold in mice [9], despite treatment with a higher concentration of clofibrate (2% w/w) for a longer time period (10 days). Thus, rat cEH is more sensitive than mouse cEH to induction by peroxisome proliferators. A twofold induction of mouse liver cEH was also observed with di-(2-ethylhexyl)phthalate [9] and nafenopin [10].

Probucol, which possess exclusively cholesterol-lowering activity, had no significant effect on peroxisomal β-oxidation and cEH activity. 1-Benzylimidazole caused a slight induction of both peroxisomal β-oxidation and cEH activity. This agrees well with findings that—in contrast to probucol—1-benzylimidazole is a weak peroxisome proliferator [25, 26].

Therefore, considering the evidence from this study that strong peroxisome-proliferators (fenofibrate, tiadenol, clofibrate) caused a high induction of rat liver cEH, weak peroxisome-proliferators (acetylicsalicylic acid, 1-benzylimidazole) a low induction of cEH and probucol, which does not lead to peroxisome proliferation, no induction of cEH activity, we conclude that cEH induction may be related to peroxisome proliferation in rat liver.

On the other hand, it became clear from the results of this study, that the effect of hypolipidemic drugs on mEH_b is unrelated to their peroxisome proliferating activity. In the studies in mice [9, 10] the effect of clofibrate and nafenopin was similar on cEH and mEH_b activities (both increased about 2-fold). In the present study the effect of hypolipidemic drugs on cEH and mEH_b could be clearly dissociated.

From the compounds investigated only 1-benzylimidazole caused a large increase in the mEH_b activity (4.8-fold). This increase in mEH_b activity may be partially due to activation of the enzyme as shown by an *in vitro* assay (Fig. 1). As cEH activity is not affected or even inhibited by addition of the investigated compounds to the assay mixture (Fig. 1), increase in enzyme activity is not caused by activation.

From data available from the literature [15, 23, 24] the influence of the various hypolipidemic drugs on palmitoyl-CoA hydrolase activity appear to be similar to that on peroxisomal β-oxidation. However, in our experiments the effect on palmitoyl CoA hydrolase activity in the cytosol could be dissociated from that on both peroxisomal β-oxidation and peroxisome proliferating activity. Probucol, which does not lead to peroxisome proliferation nor to an increase in peroxisomal β-oxidation, increased palmitoyl-CoA hydrolase activity, whilst no increase occurred after treatment with 1-benzylimidazole, which is a weak, but significant, peroxisome proliferator and also leads to a weak, but significant increase in peroxisomal β-oxidation.

Enhancement of palmitoyl-CoA hydrolase activity by the triglyceride-lowering drugs and the different concentrations used is within the same order of magnitude as the induction of peroxisomal β-oxidation and cEH and agrees with data reported by other studies [15, 23].

Although specific activity of palmitoyl-CoA hydrolase of control animals is lower in cytosol than in other fractions (600 *g* supernatant, 12,000 *g* pellet, and microsomes), activity was estimated in the 100,000 *g* supernatant, because in the cytosol the highest induction rate occurred. No induction occurred in the 12,000 *g* pellet, which contained most of the peroxisomes (data not shown). This is in agreement with findings described in [23]. Evidence has been presented that palmitoyl-CoA hydrolase exists in peroxisomes as well as in mitochondria and microsomes [15]. Also, it has been shown that peroxisomes are very sensitive to hydrostatic pressure [27], and it was therefore concluded that the high activity in the cytosolic fraction, after treatment with peroxisome proliferators, is due to leakage of the peroxisomal matrix enzymes as a result of increased peroxisomal fragility [15]. As an organelle-bound form of the cEH also exists in rat and mice peroxisomes (data not shown; [20]), it is possible that the increase in cEH activity in liver cytosol is due to induction of the peroxisome-localized species of EH.

In kidney, a maximal induction of cEH of about 2-fold was not exceeded whether a weak (acetylsalicylic acid, 1% w/w) or a potent (fenofibrate, 0.05% w/w; tiadenol, 0.1% w/w) peroxisome proliferator was used, whereas peroxisomal β-oxidation was enhanced 9-fold by fenofibrate. Another potent hypolipidemic agent, methyl clofenapate, caused a marked increase in peroxisomes in the cells of the P_1, P_2 and P_3 segments of the proximal convoluted tubule of mice [28], and led as well as BR-931, Wy-14643 and procetofen to a 4–7-fold increase in peroxisomal β-oxidation in the mouse kidney cortex [29].

Rat liver cEH is concomitantly induced with other enzymes induced by peroxisome proliferators, and the rate of induction of cEH correlates well with the peroxisome-proliferating potency of these compounds. The physiological role of cEH has, up to now, not been established. However, as nearly all enzymes induced by peroxisome-proliferating drugs are involved in lipid metabolism, a possible involvement of cEH in lipid metabolism seems reasonable. Interestingly, some fatty acid epoxides, which may be formed from unsaturated fatty acids [30] have been reported to be excellent substrates for cEH [31, 32]. Further work will be needed to evaluate the formation of such metabolites and the importance of cEH in their further metabolism.

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