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OXIDATION AND GLUCURONIDATION OF VALPROIC ACID IN MALE RATS—INFLUENCE OF PHENOBARBITAL, 3-METHYLCHOLANTHRENE, β-NAPHTHOFLAVONE AND CLOFIBRATE*

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Abstract—The influence of phenobarbital, clofibrate, 3-methylcholanthrene and β-naphthoflavone on ω- and β-oxidation as well as on glucuronidation of valproic acid (*n*-dipropylacetic acid) was evaluated in male Sprague–Dawley rats by determination of urinary excretion of its metabolites by GC–MS after administration of 100 mg/kg. In controls 12% of the dose was excreted within 24 hours, primarily as glucuronides; metabolites formed by oxidation amounted to about 4%.

Phenobarbital treatment led to stimulation of 4-hydroxyvalproic acid [(ω-1)-oxidation], 5-hydroxyvalproic acid and *n*-propylglutaric acid (ω-oxidation) excretion. Clofibrate enhanced the excretion of 4-hydroxyvalproic acid and 3-keto-valproic acid, a product of peroxisomal β-oxidation.

β-Naphthoflavone slightly increased the excretion of 5-hydroxyvalproic acid. The most specific effect was found for 3-methylcholanthrene, which was effective in stimulating the formation of 3-hydroxyvalproic acid which might be formed by (ω-2)-oxidation. The addition of fatty acids (olive oil in which 3-methylcholanthrene and β-naphthoflavone were suspended) led to increased excretion of 3-keto-valproic, 4-hydroxyvalproic and *n*-propylglutaric acid. The excretion of 3-hydroxyvalproic acid was completely suppressed by olive oil.

Such specific effects were not observed for glucuronidation of valproic acid and its metabolites, although stimulation was attained after phenobarbital, clofibrate and 3-methylcholanthrene treatment, because of instability of glucuronide conjugates.

Stimulation of valproic acid metabolism was also shown by increased plasma clearance after treatment with phenobarbital. In contrast, clofibrate given once 1 hr before valproic acid inhibited excretion of valproic acid, possibly by competition during renal tubular secretion.

Determination of valproic acid metabolites in urine provides a useful tool for evaluation of inducer specificity of short chain fatty acid metabolism and differentiation between microsomal and peroxisomal enzyme activity.

The branched-chain fatty acid valproic acid (*n*-dipropylacetic acid) is used as an antiepileptic drug in petit mal epilepsy and tonic clonic seizures [1, 2]. It has been reported that drug interactions with other antiepileptics, such as phenobarbital, lead to decreased valproic acid blood levels [3, 4] or increased phenobarbital levels [3] either by induction of VPA metabolism by phenobarbital or inhibition of phenobarbital metabolism by valproic acid.

Rare but severe toxicity of valproic acid has been observed in patients with symptoms of hepatic injury resembling Reye's syndrome [5]. Hepatotoxicity has also been found in rats treated with phenobarbital, suggesting increased formation of toxic metabolites of valproic acid [6].

Metabolism and excretion of valproic acid has been documented by several authors [7–10], who found valproic acid to be metabolized by β-oxidation as well as by (ω-1)- and ω-oxidation. β-Oxidation takes place in mitochondria and peroxisomes [11]. (ω-1)- and ω-oxidation occur via microsomal cytochrome P-450 [12]. Valproic acid is also excreted as glucuronide conjugate [13].

The different metabolic steps in valproic acid oxidation might be stimulated by compounds inducing peroxisomal or microsomal enzymes. Clofibrate (clofibric acid) stimulates peroxisomal proliferation. Microsomal enzyme induction occurs after treatment with phenobarbital, 3-methylcholanthrene or β-naphthoflavone with specificity towards different cytochrome P-450 isoenzymes. The present study was performed to evaluate the specificity of these different inducers in the formation of valproic acid metabolites.

MATERIALS AND METHODS

Reagents and chemicals. Phenobarbital (sodium salt) was purchased from Merck, Darmstadt, Germany. 3-Methylcholanthrene and β-naphthoflavone were obtained from Serva, Heidelberg and EGA Chemicals, Steinheim, Germany, respectively. Clofibrate was obtained

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from commercially available capsules of clofibric acid marketed by ICI, Planckstadt, Germany. Valproic acid, 3-keto-valproic acid, 3-hydroxyvalproic acid, 4-hydroxyvalproic acid, 5-hydroxyvalproic acid, valproic acid-2-ene, and *n*-propylglutaric acid were obtained from Dr. H. Schäfer, Desitin Werke, Hamburg. β-Glucuronidase-aryl sulfatase was purchased from Boehringer, Mannheim. All other agents were of analytical grade.

Animals. Male Sprague–Dawley rats (Buchner, Kienberg, Germany) weighing 150–300 g, were subjected to treatment with phenobarbital (2 × 80 mg/kg first day; 80 mg/kg the 2 following days), clofibrate (500 mg/kg for 7 days), 3-methylcholanthrene (26 mg/kg for 3 days), β-naphthoflavone (40 mg/kg for 2 days) or olive oil (0.2 ml) for 3 days. Phenobarbital was diluted in 0.9% sodium chloride solution; 3-methylcholanthrene and β-naphthoflavone were suspended in olive oil, and clofibrate was injected as clofibric acid without addition of any solvent. Valproic acid (sodium salt) was administered 24 hr after the last injection of an inducer at a dose of 100 mg/kg body weight (=602 μmoles/kg of the free acid) in 0.9% sodium chloride solution. Urine sampling was done in metabolism cages for 24 hr following valproic acid injection. All injections were given intraperitoneally. Plasma levels of valproic acid were evaluated in controls and in rats treated with phenobarbital or clofibrate; seven animals were taken for each experiment. A further group of 4 rats received valproic acid 1 hr after a single dose of 500 mg/kg of clofibrate. The rats were decapitated and blood was collected for determination of valproic acid 5, 10, 20, 30, 60 and 90 min and 2, 3 and 4 hr after valproic acid injection.

Determination of valproic acid and its metabolites. Serum concentrations of valproic acid were determined by gas–liquid chromatography after acidic extraction without derivatization using a 25-m fused silica carbowax 20 M capillary column. A Hewlett–Packard 5830 A gas chromatograph was used. Oven and FID temperatures were set at 120 and 300°, respectively. The split liner (Chrompack, Middleburg, The Netherlands) was heated at 180°; the inlet pressure (helium) was 1 bar, and a split ratio of 95:5 was adjusted. Hydrogen and air flow rates were 30 and 60 ml/min, respectively.

In urine valproic acid and its metabolites were determined by gas chromatography/mass spectrometry as described elsewhere [14]. All samples were also treated with β-glucuronidase (5 U/ml) for 1 hr at 37°. Incubation time has been found to be sufficient for cleavage of valproic acid glucuronides [14].

Statistics. The excretion of valproic acid and its metabolites was statistically compared to the respective controls by application of the U-test.

RESULTS

Effect of phenobarbital, clofibrate, 3-methylcholanthrene and β-naphthoflavone on the formation of valproic acid metabolites. The urinary excretion of valproic acid and its metabolites is given in Table 1. Determinations were all performed with and without β-glucuronidase. When evaluating changes caused by inducers in the oxidative metabolism of valproic acid, total amounts of metabolites must be taken into account.

Valproic acid is metabolized by (ω-1)-oxidation to 4-hydroxyvalproic acid and by ω-oxidation to 5-hydroxyvalproic acid, being further oxidized to *n*-propylglutaric acid. Oxidation in position 3 leads to different metabolites. β-Oxidation results in the formation of valproic acid-2-ene and 3-keto-valproic acid.

Considering the dose of 602 μmoles/kg, a relatively small amount of valproic acid was excreted within 24 hr in controls. A total of only 50.7 μmoles/kg (=8.5% of dose) of valproic acid was excreted. 4.3% of the dose (26.4 μmoles/24 hr) was found to be oxidized (Table 1).

Main oxidative pathways are represented by β- and ω-oxidation. Valproic acid-2-ene and 3-keto-valproic acid (β-oxidation), amounted to 9.5 μmoles/kg/24 hr, while 5-hydroxyvalproic acid and *n*-propylglutaric acid (ω-oxidation), added up to 15.4 μmoles/kg/24 hr in controls. 3-Hydroxyvalproic and 4-hydroxyvalproic acid are metabolites of minor importance.

Phenobarbital treatment significantly ($P < 0.001$) increased valproic acid excretion. Both (ω-1)- and ω-oxidation were stimulated by phenobarbital: 4-hydroxyvalproic acid formation was increased about 20-fold to 9.2 μmoles/kg/24 hr. 5-Hydroxyvalproic acid and *n*-propyl-glutaric acid were also excreted in significantly higher amounts of 18.5 and 44.1 μmoles/kg/24 hr, respectively. The relative increase, however, was lower than that found for 4-hydroxyvalproic acid. Valproic acid-2-ene, which represents the first step in β-oxidation, was also stimulated by treatment with phenobarbital. However, increase in 3-keto-valproic acid was not statistically significant.

Clofibrate treatment caused a 3-fold increase in the overall excretion of valproic acid and its metabolites (Table 1). The major oxidative product was due to β-oxidation of valproic acid. 11.2% (66.8 μmoles/kg/24 hr) of the entire dose was excreted as valproic acid-2-ene and 3-keto-valproic acid, amounting to 77% of all metabolites formed oxidatively. A significant 5-fold increase in the amount of 4-hydroxyvalproic acid (2.3 μmoles/kg/24 hr) was also found after treatment with clofibrate. However, this metabolite did not play an important role in overall excretion.

Two inducers tested here, 3-methylcholanthrene and β-naphthoflavone, were suspended in olive oil. Surprisingly, significant stimulation of valproic acid metabolism by olive oil was observed. Valproic acid excretion was increased 3-fold, an effect similar to that by clofibrate. Effects on formation of oxidative products differed: 3-hydroxyvalproic acid, the formation of which was not affected by phenobarbital or clofibrate, was completely inhibited by olive oil. The formation of 3-keto-valproic acid and *n*-propylglutaric acid, however, was found to be stimulated 4- to 5-fold.

Considering these effects of olive oil, 3-methylcholanthrene treatment resulted in a very specific change in the oxidation of valproic acid. Only 3-hydroxyvalproic acid, the formation of which was completely inhibited by olive oil, was stimulated by

Table 1. Excretion of valproic acid (VPA) and its metabolites in male rats after treatment with phenobarbital, clofibrate, olive oil, 3-methylcholanthrene and β-naphthoflavone

	Valproic acid		3-Hydroxy-VPA		VPA-2-ene		3-Keto-VPA		4-Hydroxy-VPA		5-Hydroxy-VPA		*n*-Propylglutaric acid	
					(μmoles/kg/24 hr)									
	free	total	free	total	free	total	free	total	free	total	free	total	free	total
Controls														
Mean	9.56	50.7	0.39	0.77	0.024	1.1	7.3	8.4	0.32	0.47	3.2	6.1	4.9	9.3
S.D.	4.50	27.7	0.44	0.60	0.031	1.1	2.7	7.8	0.41	0.61	1.3	4.4	2.7	9.0
N	7	7	7	6	7	6	7	5	7	7	7	6	7	6
Phenobarbital														
Mean	98.4**	276.6**	0.71	0.70	1.60*	2.40*	15.3	19.9	5.50	9.20**	19.4*	18.5**	14.1**	44.0**
S.D.	59.7	137.6	1.20	1.20	0.60	1.70	10.9	16.4	2.50	9.50	11.8	19.5	5.1	43.0
N	7	7	7	7	7	7	7	7	7	7	7	7	7	7
Clofibrate														
Mean	35.7	127.4**	0.00	1.00	0.58	1.00	47.2	65.8*	1.80	2.30*	1.3	3.7	5.4	12.3
S.D.	29.2	33.5	0.00	1.20	0.56	0.60	68.2	66.2	2.30	1.70	1.3	3.2	5.0	6.9
N	7	7	7	7	7	7	7	7	7	7	7	7	7	7
Olive oil														
Mean	44.0	176.0*	0.00	0.00	0.33	1.30	33.5	44.0**	2.40	2.50**	9.0	7.8	29.2	34.0*
S.D.	37.0	39.0	0.00	0.00	0.33	0.50	15.0	24.0	0.36	0.29	3.7	2.3	7.5	10.2
N	3	5	6	6	6	6	6	6	6	6	6	6	6	6
3-Methylcholanthrene														
Mean	161.5	264.0	0.11	3.00**	0.59	1.80	9.5	17.3	0.90	2.60	4.0	10.3	2.4	26.2
S.D.	66.0	193.0	0.26	1.90	0.28	0.60	7.6	9.4	1.10	1.40	3.5	4.4	4.6	26.1
N	6	6	6	6	6	6	5	6	6	6	5	6	6	6
β-Naphthoflavone														
Mean	31.9	128.0	0.00	0.02	0.50	6.80*	47.2	94.3	2.45	5.30	13.4	24.2*	22.2	65.2
S.D.	15.4	61.1	0.00	0.05	0.56	11.90	55.7	109.0	1.22	4.00	12.3	19.7	10.6	53.5
N	8	8	8	8	8	8	8	8	8	8	8	8	8	8

* $P < 0.05$
** $P < 0.001$

Valproic acid was given intraperitoneally 24 hours following the last injection of the respective inducer at a dosage of 100 mg/kg (=602 μmoles/kg of free valproic acid). Valproic acid metabolites were determined by gas chromatography/mass spectrometry as described elsewhere [14]. Urine samples were treated with β-glucuronidase for one hour at pH 5 to liberate glucuronic acid conjugates. Statistical significance is calculated by the U-test and compares phenobarbital and clofibrate to controls, or methylcholanthrene and β-naphthoflavone to olive oil.

3-methylcholanthrene. Therefore, this stimulation (3.0 μmoles/kg/24 hr) represented the most selective effect of any inducer tested here on valproic acid metabolism.

Induction by β-naphthoflavone resulted in significantly increased amounts of valproic acid-2-ene (6.8 μmoles/kg/24 hr) and 5-hydroxyvalproic acid (24.2 μmoles/kg/24 hr) excretion. Further metabolic products, 3-keto-valproic acid and *n*-propylglutaric acid, were not excreted in higher amounts. The overall effects of β-naphthoflavone, therefore, seemed to be of minor importance as compared to those obtained after phenobarbital, clofibrate or methylcholanthrene treatment.

Influence of inducers on the glucuronidation of valproic acid and its metabolites. As valproic acid and some of its metabolites are conjugated by glucuronidation, it was of interest to evaluate the effects of phenobarbital, clofibrate, 3-methylcholanthrene and β-naphthoflavone on the excretion of conjugated and unconjugated compounds. As shown in Table 1, valproic acid was excreted predominantly as glucuronide conjugate (total minus free). It must be mentioned that excretion of free valproic acid was also increased by most inducers.

Slight effects of phenobarbital, clofibrate, 3-methylcholanthrene and β-naphthoflavone on glucuronide conjugates of valproic acid metabolites were observed. In controls, only valproic acid-2-ene was excreted primarily as glucuronide conjugate. This was also found after treatment with β-naphthoflavone. All others did not significantly alter the excretion of valproic acid-2-ene-glucuronide.

n-Propylglutaric acid glucuronidation was enhanced after treatment with phenobarbital, 3-methylcholanthrene and β-naphthoflavone. Glucuronidation of all other metabolites did not seem to be altered by the inducers.

Influence of phenobarbital and clofibrate on plasma levels of valproic acid. The stimulation of valproic acid metabolism by inducers of oxidation and glucuronidation should result in alterations in plasma concentrations. As shown in Fig. 1, treatment with phenobarbital and clofibrate led to different responses in plasma concentrations. Each point represents a value measured in a different animal. Valproic acid was absorbed very rapidly after intraperitoneal injection. Maximal concentrations extrapolated to zero minutes showed differences between controls and phenobarbital-treated animals. In the latter, the maximal concentration was 80 μg/ml, in contrast to the 200 and 280 μg/ml found in clofibrate-treated rats and controls, respectively.

Half lives calculated from the decline in plasma level (Table 2) showed only minor variance. Phenobarbital resulted primarily in a decreased AUC, which was one fourth of that in controls.

No difference was found between controls and clofibrate-treated rats. This lack of any effect led to the assumption that clofibrate might interact in a more complex manner with valproic acid. Administration of 500 mg/kg of clofibrate one hour prior to valproic acid injection caused decreased clearance of valproic acid with a half-live of 88 minutes, resulting in an increased AUC and a low clearance of 5.6 ml/kg/min (Table 2).

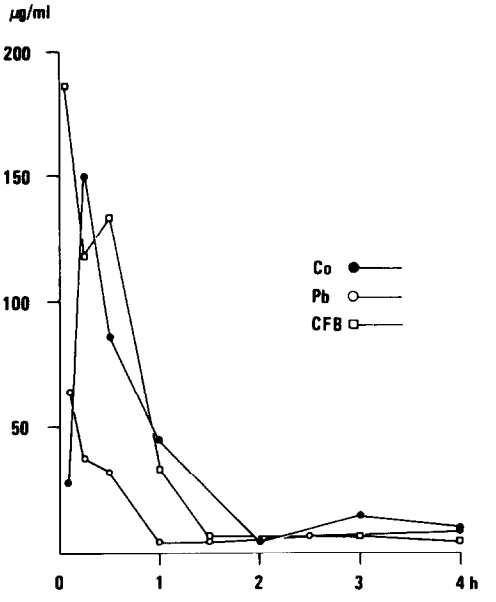

Fig. 1. Plasma concentration curve of valproic acid in male rats after treatment with phenobarbital and clofibrate. Valproic acid was measured by capilliary gas chromatography after acidic extraction without derivatization, as described under Methods. Each dot represents one rat. Co: control, Pb: phenobarbital, CFB: clofibrate.

DISCUSSION

Several authors have shown that the short branched-chain fatty acid valproic acid is metabolized by β-oxidation as well as by (ω-1)- and ω-

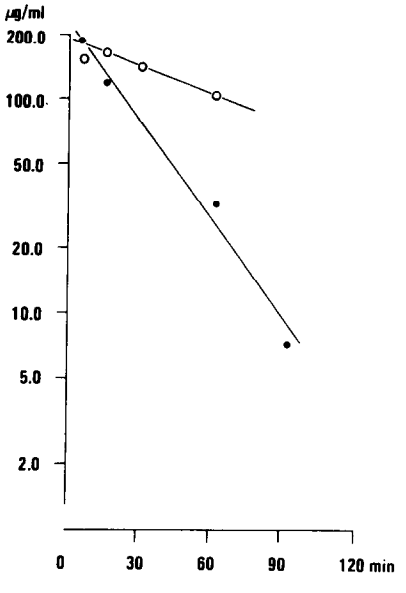

Fig. 2. Inhibition of valproic acid elimination by simultaneous administration of clofibrate in plasma. Concentrations were determined by capillary gas chromatography after injection of 100 mg/kg of sodium valproate one hour after administration of a single dose of 500 mg/kg (○——○) or 24 hours after treatment for 7 days with 500 mg/kg (●——●). Each dot represents one rat.

Table 2. Pharmacokinetic data of valproic acid in serum after treatment with phenobarbital and clofibrate

	$t_{1/2}$ min	AUC $\frac{\text{mg} \times \text{min}}{\text{ml}}$	$\frac{\text{Dose}}{\text{AUC}}$ $\frac{\text{ml}}{\text{kg} \times \text{min}}$	V_d $\frac{1}{\text{kg}}$
Control	17.5	7.05	14.2	0.36
Phenobarbital	14.0	1.74	57.5	1.17
Clofibrate (7 days)	20.0	6.68	14.9	0.43
Clofibrate*	88.0	17.79	5.6	0.71

* Clofibrate was also given one hour prior to valproic acid injection. The data are calculated from plasma level curves as shown in Figs. 1 and 2.

oxidation, representing major metabolic steps in rats, dogs and humans [7–9], similar to long- and medium-chain fatty acid ω-oxidation [12, 15]. Modifiers of the different oxidation steps should alter the metabolic pattern and provide information on the significance of microsomal and nonmicrosomal oxidation of valproic acid. This was tested by application of phenobarbital, 3-methylcholanthrene and β-naphthoflavone, all of which induce microsomal enzymes and by clofibrate, which stimulates peroxisomal enzyme activity. The investigation was done with the possibility in mind that valproic acid might become a model compound to measure the state of induction of hepatic microsomal and peroxisomal enzyme activities.

It appears that valproic acid metabolism is modified by inducers of microsomal, peroxisomal, and mitochondrial enzymes. Specific induction of different cytochrome P-450 isoenzymes is obtained using phenobarbital, clofibrate, 3-methylcholanthrene and β-naphthoflavone, resulting in a different metabolic pattern as shown in Fig. 3. ω-Oxidation is stimulated by phenobarbital (5-hydroxyvalproic acid and *n*-propylglutaric acid) and also by β-naphthoflavone (5-hydroxyvalproic acid). (ω-1)-Oxidation (4-hydroxyvalproic acid) is enhanced by phenobarbital and clofibrate. (ω-2)-Oxidation (3-hydroxyvalproic acid) is stimulated by 3-methylcholanthrene but not by β-naphthoflavone.

β-Oxidation is found to be the most important pathway of oxidative metabolism of valproic acid, being primarily stimulated by clofibrate. Phenobarbital and β-naphthoflavone only stimulated the

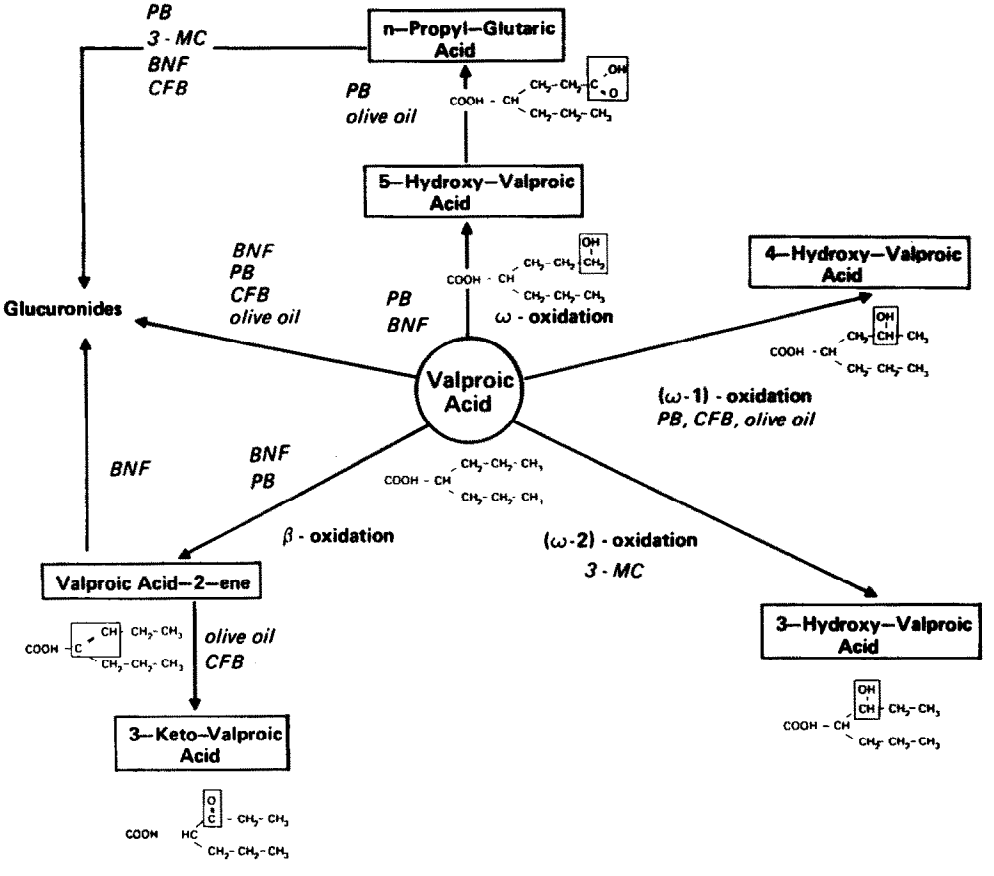

Fig. 3. Schematic representation of the metabolic pathways of valproic acid as evaluated in male rats. Alterations after administration of inducers of microsomal activity [phenobarbital (PB), 3-methylcholanthrene (3-MC), β-naphthoflavone (BNF)] and a modifier of peroxisomal and microsomal β-oxidation [clofibrate (CFB)].

first step of β-oxidation, i.e. valproic acid-2-ene formation.

Microsomal ω-oxidation. The finding that treatment with phenobarbital and β-naphthoflavone leads to different alterations of ω- as well as (ω-1)-oxidation agrees with findings obtained for medium-chain fatty acids [16] and prostaglandins [17], and leads to the assumption of an involvement of different cytochrome P-450 isoenzymes, as proposed by others [18, 19]. Clofibrate has been described [20] to stimulate a cytochrome P-450 species similar to phenobarbital which might catalyse valproic acid (ω-1)-oxidation. 3-Hydroxyvalproic acid formation was stimulated only by 3-methylcholanthrene. In previous reports, 3-hydroxyvalproic acid formation was attributed to β-oxidation [9]. Since clofibrate did not increase its formation, it should be assumed that this metabolite is formed by a P-450 isoenzyme, stimulatable by 3-methylcholanthrene and therefore by (ω-2)-oxidation, as recently described for short-chain fatty acid residues [21]. The enzyme catalyzing this reaction, however, seems to be different from that being responsible for benzo(*a*)pyrene-3-hydroxylation [22] or nitrofurantoin-oxidation [23] which are stimulated by both 3-methylcholanthrene and β-naphthoflavone. The latter, however, was ineffective in stimulating 3-hydroxy-valproic acid.

To recognize the specificity of effects of inducers of valproic acid metabolizing enzymes is complicated by the use of olive oil, i.e. 83% oleic acid, 9.4% palmitic acid, 4.0% linoleic acid, 0.7% arachidonic acid [24], as a vehicle for 3-methylcholanthrene and β-naphthoflavone. As long-chain fatty acids are oxidized by microsomes [12] and peroxisomes [12, 25], competition with valproic acid metabolism might cause changes in its metabolic pattern. 3-Hydroxyvalproic acid formation was completely inhibited by olive oil. Competition of arachidonic acid with drug metabolism has been demonstrated by Pessayre *et al.* [26]. On the other hand, stimulation of 3-ketovalproic acid, 4-hydroxyvalproic acid and *n*-propylglutaric acid was found. Hietanen *et al.* [27] reported that P-450 biosynthesis was doubled on a diet containing 45% lipids as compared to a fat-free diet. As fatty acids are able to displace valproic acid from its plasma protein binding site [28], this might also be responsible for increased oxidation rates.

Non-microsomal oxidation of valproic acid. As shown by Barnard *et al.* [29] by ultrastructural microscopy, 250 mg/kg of clofibrate caused a marked increase in liver weight associated with proliferation of peroxisomes. β-Oxidation of short chain fatty acids primarily occurs in these cell organelles. Therefore, valproic acid β-oxidation should be stimulated by clofibrate leading to 3-ketovalproic acid.

The significant stimulation of 3-keto-valproic acid formation implies that clofibrate application enables one to differentiate between microsomal and peroxisomal oxidation of valproic acid. Furthermore, the results confirm findings [25] that short chain fatty acid β-oxidation occurs in peroxisomes and not in mitochondria. Phenobarbital and β-naphthoflavone stimulated the first step of β-oxidation (valproic acid-2-ene). This might be explained by an overall increase in liver weight by treatment with these inducers. On the other hand, it has to be noted that phenobarbital is able to increase activity of intermediary metabolism enzymes [30], leading to alteration of NADP/NADPH ratios. Thus, via shuttle mechanisms, β-oxidation might be increased as shown by valproic acid-2-ene as well.

Glucuronidation of valproic acid. Increased excretion of valproic acid, valproic acid-2-ene and *n*-propylglutaric acid-glucuronide conjugates following treatment with clofibrate, phenobarbital and 3-methylcholanthrene is consistent with the observations that these compounds induce the activity of glucuronyltransferases [31, 32]. Glucuronidation of the other metabolites was not increased. Data for valproic acid glucuronides, however, might be misleading, because, in some cases, the free fraction of valproic acid exceeds the total. It could be that the composition of urine, incubation time and pH lead to non-enzymic cleavage of glucuronides. For instance, incubation time with β-glucuronidase was set for one hour at pH 5.0. This time was found to be sufficient for cleavage in earlier experiments [14]. Dickinson *et al.* [33] found that valproic acid glucuronides increased when valproic acid cleaved by alkaline hydrolysis decreased. As rat urine is alkaline, unstable valproic acid conjugates may be cleaved either by alkaline or acidic hydrolysis during sampling or extraction.

4-Hydroxyvalproic acid seems to be a stable conjugate, whereas other metabolites might be less stable, which might explain the great variety in the standard deviations.

Effects of phenobarbital and clofibrate on valproic acid plasma kinetics. Significant effects of certain drugs on valproic acid metabolism should result in changes in blood concentration. As shown by Dickinson *et al.* [33], valproic acid kinetics in rats are dose-dependent. After 15 and 150 mg/kg they found half lives of 11 and 40 min, respectively. The half-life of 17.5 min in our animals is in agreement with these data. In rats treated with clofibrate and phenobarbital, we found significant effects on glucuronides as well as metabolites formed by oxidation. Plasma curves obtained after treatment with phenobarbital are in agreement with enhanced metabolic activity. A lack of alteration of valproic acid pharmacokinetics was found after clofibrate treatment. Since simultaneous administration of clofibrate showed a delay in valproic acid excretion, it should be assumed that valproic acid and clofibric acid compete with tubular secretion. This effect might occur even 24 hours after the last injection of clofibrate, because in rats, this compound has been shown to be slowly excreted [34]. Considering a valproic acid half-life of 20 min, 24-hr urine sampling represents a nearly complete cumulative excretion of valproic acid and cannot demonstrate alterations in renal clearance.

To conclude, valproic acid metabolism is modified by inducers of microsomal, peroxisomal and mitochondrial enzymes, resulting in a mode of metabolite composition which could be helpful as a diagnostic tool. Specific cytochrome P-450 reactions have been evaluated by application of *in vivo* parameters [35] and by determination of, e.g. antipyrine metabolites [36]. It appears from data given here that peroxisomal enzyme activity can also be determined *in vivo* using valproic acid as a test compound. Because

of the instability of its glucuronide conjugates, however, valproic acid seems unsuitable for testing alterations in glucuronidation reactions.

Furthermore, determination of the valproic acid metabolic pattern might provide additional information about toxicity, since hepatic necrosis was found to be connected with induction [5]. The finding that fatty acids increased some metabolites points towards alteration of their metabolism in relation to the state of nutrition.

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