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The Effect of Valproate on the Metabolism of Phenobarbital in the Rat

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Valproate has been shown to interact with all major antiepileptic drugs. The interaction with phenobarbital is the most clinically significant. The mechanism of the interaction was evaluated in the *in vivo* rat and *in vitro* liver perfusion system. Phenobarbital and parahydroxyphenobarbital (PbOH) were administered with and without valproate treatment. *In vivo,* after administration of PbOH, valproate caused a significant inhibition of both the renal clearance of unchanged PbOH (40%) and the formation clearance (Cl_F) of its glucuronide conjugate (44%). When coadministered with phenobarbital, valproate caused a significant decrease in the total plasma clearance of phenobarbital (95.4 ± 29.0 to 65.8 ± 20.2 ml/hr/kg), with no apparent effect on the phenobarbital renal clearance or the Cl_F of PbOH. Valproate did cause a significant inhibition (50%) of formation of a minor metabolite, metahydroxyphenobarbital. The largest effect of valproate appears to be on unknown pathways of phenobarbital elimination. In the isolated perfused rat liver, the Cl_F of PbOH and its glucuronide conjugate were determined. Valproate caused a small (10%) but significant decrease in the Cl_F of PbOH. As seen *in vivo,* the most significant effect of valproate was on the Cl_F of the PbOH glucuronide (66% decrease). In conclusion, inhibition of PbOH formation by valproate cannot account entirely for the increased plasma concentrations of phenobarbital that occur when valproate is added to therapy. A complete understanding of the mechanism will require a complete accounting of the phenobarbital dose in rat or in humans.

KEY WORDS: valproate; valproic acid; phenobarbital; drug interaction; metabolic inhibition.

INTRODUCTION

Valproate has been shown to interact with all the major anticonvulsant drugs (phenobarbital, phenytoin, and carbamazepine), as well as with diazepam, salicylic acid, and chlorpromazine; many of these interactions are reciprocal. Generally, valproate affects the disposition of other drugs through two mechanisms of action: displacement from albumin binding sites and inhibition of drug metabolism (1).

The most clinically significant interaction of valproate occurs with phenobarbital. Clinically, plasma concentrations of phenobarbital have been reported to increase from 25 to 65% when valproate was added to the regimen (2,3). This strong effect of valproate on phenobarbital is surprising since phenobarbital is not highly protein bound and few, if any, metabolic inhibitors have been shown to alter phenobarbital clearance in a clinically relevant way.

Several studies have attempted to elucidate the mechanism(s) involved in the interaction between phenobarbital and valproate. Clinical studies have indicated that valproate decreased the nonrenal clearance of phenobarbital with no effect on elimination of unchanged phenobarbital (4). Kapetanovic *et al.* (5) found a decrease in the plasma clearance of phenobarbital, no change in the fraction of dose eliminated as parahydroxyphenobarbital (PbOH), and a decrease in the conjugated PbOH-to-total PbOH ration when valproate was added to phenobarbital. Using a rat liver microsomal preparation, the same investigators showed that valproate behaved as a competitive inhibitor of microsomal parahydroxylation (6). However, the inhibition constant (K_i) of valproate was significantly higher than the accepted therapeutic range for valproate.

This evidence suggests that valproate affects both the formation and the elimination of PbOH and that the interaction may involve other oxidative metabolites of phenobarbital. Therefore, studies were designed to evaluate the effect of valproate on phenobarbital, PbOH, and metahydroxyphenobarbital (MOHPB) conjugation and elimination in the rat. Initial studies were performed *in vivo* to evaluate the rat as a model for the interaction. Further studies were done in the isolated perfused rat liver system. By maintaining the intact architecture of cells in the liver, the isolated perfused rat liver allows a direct measurement of the effect of valproate on the sequential metabolism of phenobarbital (i.e., phenobarbital → PbOH → PbOH glucuronide), independent of renal elimination and enterohepatic recycling.

MATERIALS AND METHODS

Materials

Dextran, dextrose, bovine serum albumin (Fraction V), and β-glucuronidase were obtained from Sigma Chemical Co. (St. Louis, MO). Parahydroxyphenobarbital monohydrate and Alphenal, 5-allyl-5-phenylbarbituric acid, were obtained from Aldrich Chemical Co. (Milwaukee, WI) and Alltech-Applied Science, (State College, PA), respectively. Valproate was a gift from Abbott Laboratories (Chicago, IL). The spectraphor dialysis membrane used in the protein binding experiments was obtained from VWR Scientific, Inc. (Chicago, IL). The Microsorb "short-one" column was obtained from Rainin (Woburn, MA).

Synthesis of Metahydroxyphenobarbital (MOHPB)

MOHPB was synthesized according to previously published procedures (7). Conformation of identification was carried out using direct-probe mass spectrometry analysis on a VG 7070H mass spectrometer using the chemical ionization mode. The synthesized MOHPB showed a spectrum identical to the spectrum obtained with a known PbOH standard. High-field proton NMR at 500 MHZ on a Brucker Wm-500 spectrometer was used to verify the meta position of the hydroxy group. Using the high-pressure liquid chro-

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matography (HPLC) system described below, the synthesized MOHPB produced one peak.

Drug Analysis

Unchanged phenobarbital and PbOH concentrations in bile and perfusate were analyzed by a HPLC method described in the literature (8), with some modification. Two milliliters of perfusate or 0.1 ml of a 1:10 dilution of bile was acidified to pH 5 with 1 ml of a 2 *M* potassium phosphate monobasic buffer and extracted with 10 ml of ethyl acetate. Alphenal, a commercially available analogue of phenobarbital, was used as an internal standard. Ethyl acetate was evaporated under nitrogen at 40°C and samples were reconstituted with mobile phase. Total PbOH was determined after hydrolysis with 1000 U β-glucuronidase at 37°C (pH 5.0) for 24 hr and after acid hydrolysis (6 *N* HCl at 100°C for 1 hr). The *N*-glucoside of phenobarbital is resistant to hydrolysis by β-glucuronidase but is sensitive to acid hydrolysis (9). Samples were injected onto a 10-cm, 3-μm C18 column with 30:70% methanol:0.05 *M* sodium phosphate buffer (pH 8.0) as the mobile phase. Phenobarbital, MOHPB and PbOH were detected by UV absorbance at 254 nm. Retention times were 5.3, 5.8, 7.9, and 9.6 min for PbOH, MOHPB, phenobarbital, and internal standard, respectively. The limits of sensitivity were 0.1 μg/ml for both compounds. The intraday coefficient of variation (CV) was <5% and the interday CVs for phenobarbital and PbOH were less than 4 and 7%, respectively. Valproate concentrations were determined by a capillary gas chromatography method as described previously (10).

In Vivo Studies

Study I. In order to estimate accurately the PbOH formation clearance after phenobarbital administration, it was necessary to determine the elimination clearance and mass balance of PbOH in the rat. Five male Sprague–Dawley rats (246 ± 31-g body weight) were cannulated in the left femoral and right jugular veins under ether anesthesia. Each rat was used as its own control. Two PbOH doses were administered under each of two treatment conditions, saline and valproate. Rats were allowed a day to recover from surgery and then were randomized to receive a saline control or valproate infusion (7.5 mg/hr) for 20 hr. Twenty hours after initiation of the infusion, rats were placed in metabolic cages, which allowed complete collection and separation of urine and feces. A 5-mg bolus dose of PbOH was administered intravenously into the femoral vein (t = 0 min). Blood samples (200 μl) were collected from the jugular vein at 5, 20, 40, 60, 90, 120, 150, and 180 min. Urine was collected during the next 12 hr. This sequence was then repeated for the second treatment phase. Plasma samples were assayed for PbOH as described above. Urine samples were assayed for PbOH before and after hydrolysis with β-glucuronidase and 6 *N* HCl acid.

Study II. The protocol for the phenobarbital–valproate study is outlined in Fig. 1. Phenobarbital was infused at 0.55 mg/hr for 9 days. The study was divided into two phases, with each rat serving as its own control. Rats were randomized to receive either valproate (7.5 mg/hr) or saline infusions for Phase I (Days 1–5) and the reverse treatment for

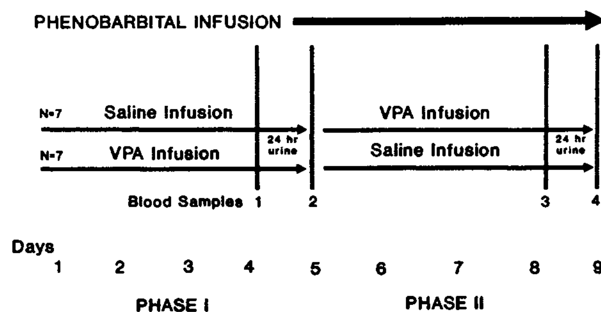

Fig. 1. Protocol for phenobarbital–valproate study.

Phase II (Days 5–9). Fourteen male Sprague–Dawley rats (259 ± 17-g body weight) were cannulated in the right jugular vein under ether anesthesia. Surgeries were performed on the morning of Day 1. At 5 PM on Day 1 an intravenous loading dose of phenobarbital (10 mg) was given, immediately followed by the phenobarbital infusion. On Day 4 (9 AM) the rats were anesthetized with ether and blood was drawn (300 μl) from the tail artery (No. 1). Rats were transferred to metabolic cages and a 24-hr urine collection was obtained. At the end of the first 24-hr urine collection, rats were removed from the metabolic cages and anesthetized with ether, and blood sample No. 2 was obtained. During the Phase II treatment, blood sample No. 3 was obtained at 9 AM on Day 8, at the same time that the second 24-hr urine collection began. Blood sample No. 4 was obtained at the end of this urine collection period. Rats were allowed free access to food and water at all times during the study. All samples were stored at −20°C until assayed. Plasma samples were assayed for phenobarbital, PbOH, and valproate as described above. Urine samples were assayed for phenobarbital, PbOH, and MOHPB before and after hydrolysis with β-glucuronidase.

Isolated Perfused Rat Liver Studies

Liver Perfusion Preparation. Male Sprague–Dawley rats (200- to 225-g weight) were anesthetized with urethane (0.5 g/kg) by intraperitoneal injection. The surgical preparation of the *in situ* rat liver was performed as described previously (9). The hemoglobin-free perfusion medium consisted of a Krebs–Ringer bicarbonate buffer, 1.5% dextran, 1.2% bovine serum albumin, and 0.2% dextrose. Concentrations of phenobarbital and valproate were chosen to be within the clinical therapeutic range. A commercially available perfusion apparatus (Perfuser two/ten, MX International, Aurora, CO) was used for the studies at a perfusion flow rate of 30 ml/min.

Study Design. Measurement of the intrinsic formation clearance of PbOH from phenobarbital requires total accountability of all PbOH formed from phenobarbital. Therefore, in the initial set of studies, perfusions were performed on livers obtained from six Sprague–Dawley rats using PbOH as a substrate. Based on preliminary studies with phenobarbital, PbOH was used at a concentration of 0.1 μg/ml. During the treatment phase, valproate was infused into the perfusion line to achieve an afferent concentration of 55 μg/ml. Each rat liver was used as its own control, with each study consisting of three 40-min phases: Phase I, control

(0–40 min); Phase II, valproate treatment (40–80 min); and Phase III, control (80–120 min). This study design allows the determination of the rate of onset, as well as the reversibility and the extent of the interaction. Efferent perfusate samples were obtained at 5-min intervals for the first 25 min and then at 3-min intervals for the remainder of each phase. Three afferent perfusate samples were collected, one during each phase. Bile samples were collected in 20-min intervals. Efferent perfusate flow was measured directly several times during each phase. As rat bile is composed of 97% water, bile flows were determined by weighing bile tubes before and after bile collection and assuming a specific gravity of 1.00. Perfusate and bile samples were stored at −20°C until assayed as described below. Perfusate and bile samples from the last 20 min of each phase were used in the calculations of intrinsic clearances.

Phenobarbital was perfused as described above at a substrate concentration of 30 μg/ml in 10 rat livers perfused with and without valproate (55 μg/ml).

Protein Binding

The extent of protein binding of phenobarbital and PbOH in perfusate with and without valproate was determined by equilibrium dialysis in plexiglass cells using Spectraphor dialysis membranes. One-milliliter perfusate samples from each of the three phases were dialyzed for 5 hr against Krebs Ringer bicarbonate buffer (pH 7.4) at 37°C. Preliminary studies indicated that equilibrium was reached within this time. All buffer and perfusate samples were stored at −20°C until assayed.

Data Analysis

Study I. Total plasma clearance (Cl_p) of PbOH was determined by the ratio of dose to area under the plasma concentration curve. The renal clearance (Cl_R) of unconjugated PbOH was calculated as the product of Cl_p and the fraction of the dose excreted unchanged in the urine. The formation clearance of PbOH glucuronide was obtained from the product of the CL_p and the fraction of the dose excreted in the urine as PbOH glucuronide.

Study II. The phenobarbital concentrations in blood samples obtained on either side of the two urine collections were averaged to determine a mean steady-state phenobarbital concentration during Phases I and II. Phenobarbital Cl_p was determined as the ratio of the infusion rate to the mean steady-state phenobarbital concentration for both treatment phases. Cl_R of phenobarbital was determined as the product of Cl_P and the fraction of dose excreted unchanged during the 24-hr interval. The formation clearances (Cl_F) of PbOH and MOHPB were determined as the product of Cl_p and the fraction of the dose excreted as the respective metabolite (PbOH or MOHPB) during the 24-hr interval. The remainder clearance of phenobarbital was calculated by subtraction of the phenobarbital Cl_R and CL_F of PbOH and MOHPB from Cl_p. All data are presented as the mean ± standard deviation. Statistical analysis was performed by two-tailed paired *t* test at the 0.05 level of significance.

Isolated Perfused Rat Liver Studies. The theoretical considerations of the liver perfusion model used for data analysis are described in the Appendix.

RESULTS

In Vivo

During both saline and valproate treatment the dose of PbOH could be completely accounted for in urine (97.0 ± 5.5%) as unconjugated PbOH and the glucuronide conjugate. This mass balance of the dose of PbOH allowed an accurate determination of the formation clearance of PbOH after phenobarbital administration. Results of the PbOH–valproate study are described in Table I. Valproate administration was associated with a 43% decrease in PbOH total plasma clearance. Valproate significantly decreased both glucuronidation of PbOH (44%) and renal clearance of the unconjugated PbOH (40%). Due to the equal decrease in both pathways, the fractions excreted as PbOH and PbOH glucuronide were unchanged.

As shown in Table II, valproate treatment in rats receiving a phenobarbital infusion resulted in a 31% decrease in phenobarbital plasma clearance. Measurable PbOH plasma concentrations were found only during the valproate phase of the study (1.6 ± 1.2 μg/ml). There was not a significant effect on the renal clearance of unchanged phenobarbital. Valproate administration was also associated with an increase in urine flow (22.6 ± 6.8 vs 29.3 ± 9.1 ml/day; $P < 0.02$). A weak correlation between phenobarbital Cl_R and urine flow was also found ($r = 0.44$, $P < 0.02$). Interestingly, there was no significant effect on the formation clearance of PbOH. The formation clearance of MOHPB decreased significantly; however, it accounted for less than 2% of the phenobarbital dose. There was no change in the ratio of PbOH glucuronide to total PbOH (PbOH glucuronide + PbOH unconjugated). This finding was consistent with the observations made after PbOH administration. The lack of effect of valproate on PbOH formation was surprising in light of the finding of competitive inhibition in microsomal preparations (6). Therefore, *in vitro* liver perfusion studies were done to isolate the effect of valproate on phenobarbital hepatic metabolism independent of its effect on renal elimination and enterohepatic recycling. Also, it is possible that inhibition observed in microsomes (where enzymes are ex-

Table I. Effect of Valproate on the Clearance of Parahydroxyphenobarbital[a]

	Saline	Valproate
Total PbOH plasma clearance (ml/min/kg)	69.4 ± 26.6	39.8 ± 10.4
	($P < 0.02$)	
PbOH glucuronide/PbOH dose	0.41 ± 0.13	0.38 ± 0.04
	(NS)	
Formation clearance PbOH glucuronide (ml/min/kg)	26.3 ± 8.6	14.8 ± 3.8
	($P < 0.02$)	
PbOH unconjugated PbOH dose	0.57 ± 0.12	0.61 ± 0.06
	(NS)	
Renal clearance unconjugated PbOH (ml/min/kg)	40.6 ± 22.1	24.5 ± 7.3
	($P < 0.05$)	

[a] Mean ± SD; $n = 5$.

Table II. Effect of Valproate on the Clearance of Phenobarbital[a]

	Saline	Valproate
Total PB plasma clearance (ml/hr/kg)	95.4 ± 29.0	65.8 ± 20.2 ($P < 0.001$)
PB renal clearance (ml/hr/kg)	8.8 ± 4.0	10.3 ± 2.7 (NS)*
Formation clearance of PbOH (ml/hr/kg)	24.1 ± 15.2	24.1 ± 9.6 (NS)
Formation clearance of MOHPB (ml/hr/kg)	1.4 ± 0.7	0.64 ± 0.42 ($P < 0.01$)
Remainder clearance (ml/hr/kg)	60.1 ± 24.9	31.7 ± 13.7 ($P < 0.001$)
PbOH glucuronide total PbOH[b]	0.82 ± 0.11	0.78 ± 0.06 (NS)

[a] Mean ± SD; $n = 14$.
[b] Total PbOH = PbOH unconjugated + PbOH glucuronide.
* Not significant, $P < 0.05$.

posed) may not reflect the actual interaction taking place in intact cells.

An example of the perfusate and bile data obtained using the isolated perfusion system is shown in Fig. 2. The free fraction of PbOH in perfusate was determined to be 83.3 ± 5.3% and was unaffected by the presence of valproate. On single-pass perfusion of PbOH, greater than 95% of the unconjugated PbOH was eliminated in the efferent perfusate and greater than 95% of the unconjugated PbOH was found in the bile. The effects of valproate on the hepatic elimination of PbOH are shown in Fig. 3. The total intrinsic clearance of PbOH by the perfused rat liver was decreased by 60% during the valproate treatment phase. The effect of valproate occurred immediately and was rapidly reversible on removal of valproate. The intrinsic formation clearance of PbOH glucuronide was also significantly decreased, from 17.6 to 5.9 ml/min.

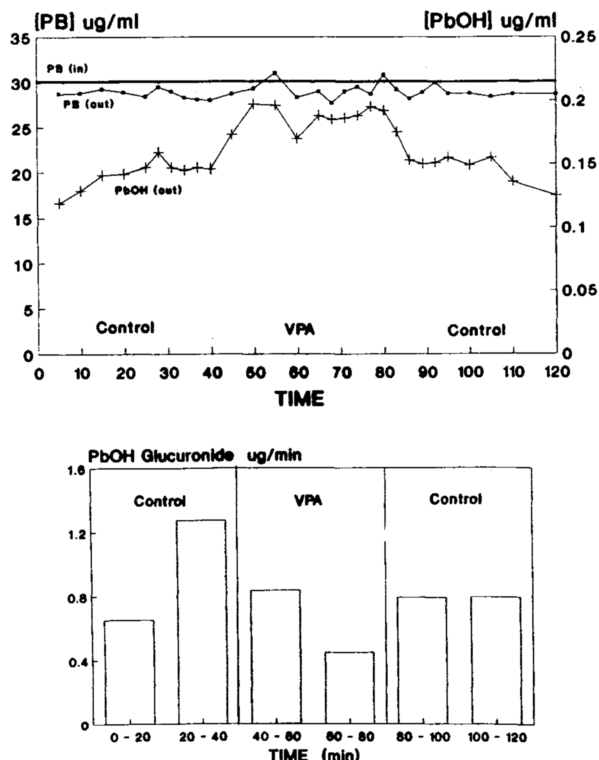

Fig. 2. Example of perfusate and biliary concentration vs time data obtained during the perfusion of phenobarbital with or without valproate. Top: Perfusate concentration data for phenobarbital and unconjugated PbOH with constant perfusate flow (30 ml/min). Bottom: Bile elimination rate for PbOH glucuronide vs time. Biliary concentrations are corrected for the 50% increase in bile flow that occurs during the valproate treatment phase.

The free fraction of phenobarbital in perfusate was 86 ± 2.5% and was unaffected by the presence of valproate or PbOH. On single-pass perfusion of phenobarbital, the efferent perfusate contained more than 95% of the unconjugated PbOH, as well as unmetabolized phenobarbital, while more than 95% of the metabolites of PbOH were found in the bile. Low extractability of phenobarbital by the isolated perfused rat liver did not allow accurate evaluation of the effect of valproate on the extraction ratio or total intrinsic clearance of phenobarbital. However, the formation of PbOH and PbOH metabolites could be measured and the effect of valproate on the individual pathways was determined. The intrinsic formation clearance of PbOH and its glucuronide conjugate for the three phases are shown in Fig. 4. There was a small, but significant decrease (10%) in the intrinsic formation clearance of PbOH. There was a substantial effect of valproate on the intrinsic formation clearance of PbOH glucuronide, which agrees with the observations made when PbOH was perfused itself and suggests that PbOH behaves similarly whether its administered or formed from phenobarbital.

A small amount (<1%) of an unknown acid labile metabolite of phenobarbital was detected in the bile of all rats studied. This metabolite was hydrolyzed by acid but not β-glucuronidase; these characteristics are consistent with the *N*-glucoside metabolite of phenobarbital (9). There was no apparent effect of valproate on the formation clearance of this minor metabolite(s).

Inherent in the assumptions of the "well-stirred" model is that the rate of the sequential metabolism of PbOH to the glucuronide is the same whether phenobarbital or PbOH is administered. There was not a statistically significant difference in the intrinsic formation clearances of PbOH glucuronide obtained during the control phases of the PbOH or phenobarbital perfusions. This finding indicated that the model assumptions were reasonable for the substrates evaluated in this study.

DISCUSSION

The present results demonstrate that valproate affected phenobarbital and its metabolites in multiple ways. Valproate inhibited the renal clearance of unconjugated PbOH. It is interesting that valproate also decreased the renal clearance of the major metabolite of carbamazepine, carbamazepine epoxide, in the rat (12). How valproate affects the renal clearance of these substrates is unclear. However, as renal blood flow in an unanesthetized rat is approximately 51 ml/min/kg (13), the renal clearance of PbOH found in this

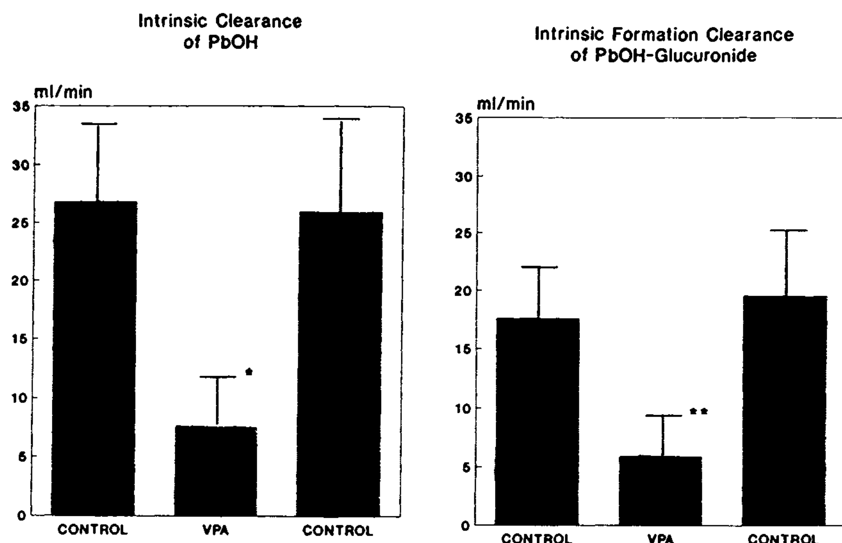

Fig. 3. The effect of valproic acid treatment on the PbOH total intrinsic clearance and intrinsic formation clearance of PbOH glucuronide during PbOH perfusion. Significantly different from control phase: *, $P < 0.002$; **, $P < 0.01$.

study suggested that PbOH is excreted by the kidneys with a high degree of tubular secretion as organic anions. Although only a small fraction of valproate is excreted unchanged by the kidneys (<1%), the ratio of concentrations of valproate to PbOH approximates 100:1. Therefore, a competitive interaction at the site of tubular secretion is possible. It is also possible that one of the renally excreted metabolites of valproate may play a role in this interaction.

Valproate had a pronounced inhibitory effect on the glucuronidation of PbOH *in vivo* and *in vitro*. This observation is similar to the finding of decreased glucuronidation of a new anticonvulsant, lamotrigine, in humans upon coadministrations of valproate. Lamotrigine half-life increased from 24 ± 6 to 30–89 hr with concurrent valproate therapy (14). Mechanistically, valproate has been shown to cause a depletion of the cosubstrate UDP–glucuronic acid in mouse livers, albeit at doses well above the therapeutic range (15). However, this mechanism would be inconsistent with the rapid reversibility of the inhibition observed in these present studies. In addition, valproate has been shown to be a competitive inhibitor of PbOH glucuronide formation in rat liver microsomal preparations (16). The UDP–glucuronyl transferase system has been shown to consist of multiple isozymes. Both PbOH (17) and valproate (18) are substrates for the phenobarbital inducible isozyme of UDP–glucuronyl transferase and a competitive inhibition mechanism could explain the behavior of valproate (i.e., rapidly reversible inhibition). It should be noted that the effect of valproate on the glucuronidation of PbOH cannot be evaluated when the parent compound (phenobarbital) is administered. The ratio

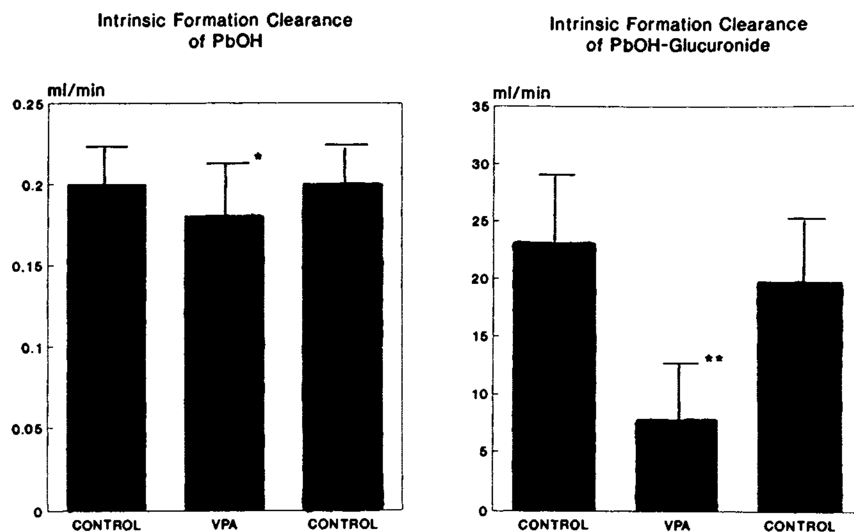

Fig. 4. The effect of valproic acid treatment on the intrinsic formation clearance of PbOH and its glucuronide conjugate during phenobarbital perfusion. Significantly different from control phase: *, $P < 0.02$; **, $P < 0.002$.

of PbOH glucuronide to total PbOH is dependent on three different processes: the formation clearance of PbOH, the formation clearance of PbOH glucuronide, and the renal clearance of unconjugated PbOH. Thus, this ratio is useful only if valproate affects only one of these pathways. This is not the case in humans (5) or rats.

Valproate caused a significant decrease in the total plasma clearance of phenobarbital in the rat. Valproate appeared to have no effect on the formation of PbOH. In perfused rat liver, valproate caused a small but significant inhibition of the oxidative formation of PbOH from phenobarbital. This result agrees with rat liver microsomes results reported by Kapetanovic and Kupferberg (6). The reason for the difference between *in vivo* and *in vitro* results may be associated with the parallel effects of valproate on renal and biliary excretion of PbOH. In the *in vitro* perfusion studies, valproate treatment was associated with a 50% increase in bile flow. This effect occurred immediately after the start of valproate infusion and was rapidly reversible after removal of valproate from the perfusate. The increase in biliary flow in combination with the decrease in the formation of PbOH glucuronide (which undergoes enterohepatic recycling) may explain why urinary PbOH recovery is insensitive as an indicator of PbOH formation.

The small inhibition of the intrinsic formation clearance of PbOH by valproate suggested that the mechanism by which valproate decreases the total plasma clearance of phenobarbital cannot be fully explained by the decreased formation of PbOH. Even though valproate significantly inhibited the formation of MOHPB, this is a minor metabolite. Therefore, the most significant effect of valproate on phenobarbital elimination in the rat is on the unknown fraction of phenobarbital. Since it was recently confirmed by other investigators (20) that phenobarbital *N*-glucoside is not a major metabolite in the rat, inhibition of *N*-glucosidation by valproate cannot explain the decrease in phenobarbital clearance. It is possible to speculate that valproate inhibition of glucuronidation of PbOH *in vivo* caused elevated plasma concentrations of PbOH, followed by subsequent product inhibition of further PbOH formation. This phenomenon has been reported to occur with phenytoin and its major metabolite, parahydroxyphenytoin (21).

In conclusion, valproate significantly decreased the total clearance of phenobarbital, confirming that the rat was a good model for this interaction. In addition to inhibiting two types of metabolic processes (oxidation and glucuronidation), valproate was also shown to have a substantial inhibitory effect on renal elimination. Thus, the mechanism of the interaction between phenobarbital and valproate appears to be more complex than originally hypothesized. A complete understanding of the mechanism will require a complete accounting of the phenobarbital dose in rats or in humans.

APPENDIX: THEORETICAL CONSIDERATIONS

The well-stirred model was used in the data analysis as neither phenobarbital nor PbOH is a highly excreted compound (22). The liver model describing phenobarbital elimination is shown in Fig. 5 and includes the sequential single-pass formation of PbOH to form PbOH glucuronide and elimination of parent drug and metabolites in both emergent fluids (bile and efferent perfusate). Based on this model, the rate of change of the amounts of phenobarbital (PB), PbOH, and PbOH glucuronide (PbOGluc) in the liver are described by Eqs. (1), (2), and (3), respectively.

$$dPB/dt = Q_P \times [PB]_{in} - Cl_T \times f\,[PB_p]_{out} - Q_P \times [PB]_{out} - Q_B \times [PB_B]_{out} \quad (1)$$

$$dPbOH/dt = Cl_{F,1} \times f\,[PB_P]_{out} - Cl_{F,2} \times f\,[PbOH_P]_{out} - Q_P \times [PbOH_P]_{out} - Q_B \times [PbOH_B]_{out} \quad (2)$$

$$dPbOGluc/dt = Cl_{F,2} \times f\,[PbOH_P]_{out} - Q_P \times [PbOGluc_P]_{out} - Q_B \times [PbOGluc_B]_{out} \quad (3)$$

where

Cl_T = total intrinsic clearance of PB

$Cl_{F,1}$ = intrinsic formation clearance of PbOH

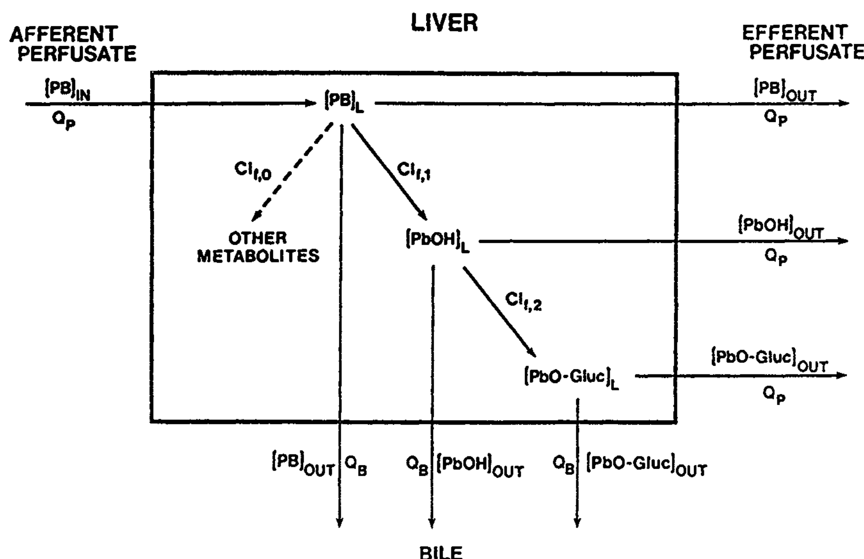

Fig. 5. Scheme of hepatic elimination of phenobarbital during single-pass perfusion.

$Cl_{F,o}$ = intrinsic formation clearances of other PB metabolites

$Cl_{F,2}$ = intrinsic formation clearance of PbOGluc

Q_P = perfusate flow

Q_B = bile flow

[ ] = total concentration

f[ ] = free concentration

and the subscripts are as follows: B, bile; P, perfusate; in, afferent; out, efferent.

The single-pass isolated perfused liver yields steady-state conditions once equilibrium within the liver is attained. At steady state, the rate of change of the amount of any species within the liver is zero, therefore the following parameters can be calculated from the experimentally determined flows and concentrations in the afferent and efferent fluids.

$$Cl_T = \frac{Q_P \times [PB_P]_{in} - Q_P \times [PB_P]_{out} - Q_B \times [PB_B]_{out}}{f\,[PB_P]_{out}} \quad (4)$$

$$Cl_{F,1} = \frac{Q_P \times [PbOH_P + PbOGluc_P]_{out}}{f\,[PB_P]_{out}} + \frac{Q_B \times [PbOH_B + PbOGluc_B]_{out}}{f\,[PB_P]_{out}} \quad (5)$$

$$Cl_{F,2} = \frac{Q_P \times [PboGluc_P]_{out} + Q_B \times [PbOGluc_B]_{out}}{f\,[PbOH_P]_{out}} \quad (6)$$

The total intrinsic clearance of PbOH can be determined by Eq. (7).

$$Cl_T = \frac{Q_P \times [PbOH_P]_{in} - Q_P \times [PbOH_P]_{out} - Q_B \times [PbOH_B]_{out}}{f\,[PbOH_P]_{out}} \quad (7)$$

The intrinsic formation clearance of PbOGluc ($Cl_{F,2}$) after administration of PbOH is calculated as described above after PB administration [Eq. (6)].

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