Biochemical Pharmacology, Vol. 43, No. 4, pp. 775–783, 1992.
Printed in Great Britain.

0006-2952/92 $5.00 + 0.00

INTERACTION OF VALPROIC ACID AND SOME ANALOGUES WITH MICROSOMAL EPOXIDE HYDROLASE

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(*Received* 29 *April* 1991; *accepted* 21 *October* 1991)

Abstract—Valproic acid (VPA) and its analogues valpromide (VPM), valproyl-Coenzyme A (VP-CoA) and valproyl-ethylester (VPE) were examined as potential inhibitors of microsomal epoxide hydrolase (mEH_b) using styrene-7,8-oxide (STO) and benzo(*a*)pyrene-4,5-oxide (BPO) as enzyme substrates. The effect of each potential inhibitor was examined using mEH_b from rat liver, human livers (from a child, woman and man) and from human placenta. Of the compounds tested, only VPM (2 mM) expressed significant inhibition of mEH_b activity with a maximum inhibition of 49%, 48%, 35% and 33% for liver microsomes from the child, woman, man and rat, respectively, using STO (2 mM) as substrate. Human placenta mEH_b was inhibited 59% under the same conditions. The inhibition was found to be competitive, with closely related K_I values of 0.11, 0.16, 0.28, 0.27 and 0.31 mM for mEH_b obtained from rat liver, human placenta, child, female and male liver, respectively. VPA demonstrated only a slight inhibition (maximum 16%) of mEH_b at high concentrations (10 mM), and VP-CoA was found to activate STO hydrolysis slightly at concentrations between 1 and 5 mM. VPE caused a moderate concentration-dependent activation of mEH_b in all microsomal preparations examined. The inhibitory or activating properties of each compound were independent of the substrate and influenced slightly by the pH used in the incubation medium. The lack of inhibition of mEH_b by VPA and its analogues other than VPM shows that neither masking of the carboxyl function of VPA nor the introduction of higher lipophilicity are sufficient to account for the inhibitory properties of VPM for mEH_b. A molecular mechanism for the inhibition of mEH_b by VPM is discussed.

Valproic acid (VPA§) is a major anticonvulsant for the treatment of absence seizures. Since its introduction as an anticonvulsant, VPA has frequently been associated with altered endogenous metabolism and with hepatotoxicity. Much of the toxicity of VPA appears to be associated with alterations in mitochondrial metabolism (for review see Refs 1 and 2). In addition, VPA also affects enzymes within the endoplasmic reticulum. Recent reports indicate that VPA inhibits epoxide hydrolase activity in healthy volunteers [3] and epileptic patients [4]. One group has reported inhibition of mEH_b by VPA *in vitro* using *S*-styrene oxide and carbamazepine-10,11-epoxide as substrates [5]. Previous studies, however, have failed to detect such a direct interaction [6].

Interestingly, valpromide, the amide analogue of VPA, is an inhibitor of mEH_b *in vitro* [5–7] as well as *in vivo* [3, 8]. However, since VPM is rapidly converted to VPA *in vivo* [9], it is unclear how VPM exerts its effect at the active site of the enzyme. Questions still remain with regard to the inhibition of epoxide hydrolase by VPA and how it may be influenced by particular functional groups masking the carboxyl moiety of VPA. As development of future anticonvulsants may entail the synthesis of structural analogues of VPA, it is important to understand how such structural modifications of this antiepileptic drug will affect this critical detoxifying enzyme.

VP-CoA is reported to be a major metabolite of VPA in rats [10]. The role of this metabolite has not been examined with regard to its potential effect on epoxide hydrolase activity, but it is known to be poorly hydrolysed by hepatic medium-chain acyl-CoA hydrolase [11]. Consequently, VP-CoA may accumulate in human liver and interfere with some biochemical processes during VPA administration [12–14]. It is also possible that simple blockage of the carboxyl moiety on VPA will result in a more effective epoxide hydrolase inhibitor. To address this possibility, the ethyl ester of VPA was examined for its potential influence on epoxide hydrolase activity.

MATERIALS AND METHODS

Chemicals. [2,3-^{3}H]2-Phenyloxirane([^{3}H]STO, styrene oxide; 11.7 GBq/mmol) and [G-^{3}H]-benzo(*a*)pyrene-4,5-oxide (BPO; 0.433 GBq/mmol) were synthesized as described in Refs 15 and 16, respectively.

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§ Abbreviations: VPA, valproic acid; STO, styrene-7,8-oxide (2-phenyloxirane); BPO, benzo(*a*)pyrene-4,5-oxide; mEH_b, microsomal epoxide hydrolase; VPM, valpromide; VPE, valproyl-ethylester; VP-CoA, valproyl-Coenzyme A ester; SDS–PAGE, sodium dodecyl sulphate–polyacrylamide gel electrophoresis.

Table 1. Source of microsomal preparations

Microsomal preparation	Source
Human placenta	obtained after full term delivery from 34-year-old woman
Human liver	56-year-old woman: non-neoplastic tissue obtained after surgical removal of hepatic tumor
Human liver	23-year-old man: died in accident
Human liver	2-year-old child died in accident
Rat liver	male Sprague–Dawley rat (starved for 16 hr; 180–220 g body wt)

VPA and VPE were obtained from Abbott-Laboratories (North Chicago, IL 60064). VPM was a generous gift from LABAZ GmbH (München, F.R.G.). VP-CoA was synthesized according to Becker and Harris [10] with minor modifications. The final product was obtained from two consecutive TLC separations on 1000 μm Avicel cellulose plates (Analtech) developed with *n*-butanol/acetic acid/water (5:2:3; by vol.). The R_f values for VP-CoA and CoA, 0.69 and 0.43, respectively, corresponded well to those reported earlier [10]. The purity of VP-CoA was determined according to Becker and Harris [10] by means of UV spectroscopic analysis. The concentration was calculated by using the millimolar extinction coefficient of 15.4 $cm^2/\mu mol$ at 260 nm. Based upon this method, the VP-CoA was estimated to be 84% pure. The lower than expected purity may be in part due to some of the Avicel material remaining in the water extract as the VP-CoA appeared as a single spot on TLC. In addition, ^1H and ^{13}C NMR spectra were run of VP-CoA and compared with the spectra of VPA and CoA to confirm the presence of VP-CoA. The integrated NMR spectra suggested VP-CoA to be 92% pure with a minor degree of CoA contamination. Stability tests demonstrated that VP-CoA and (VPE) did not hydrolyse for more than 4 hr under the assay conditions used to measure mEH_b activity (data not shown). All other chemicals were of analytical grade or the purest grade commercially available.

Preparation of liver and placenta microsomes. Table 1 summarizes the microsomal preparations employed in this investigation and their respective sources.

Fresh human placenta (70 g) obtained after full term delivery was washed with a buffer containing 250 mM sucrose, 20 mM Tris and 0.1 mM EDTA, pH 7.5, until most of the blood was removed. After adjusting the tissue–buffer mixture to a 25% (w/v) ratio, the tissue was homogenized with a tissue grinder (3 × 30 sec on ice). The suspension was re-homogenized using a Teflon homogenizer with an additional three strokes. The suspension was then spun at 10,000 *g* for 20 min and the resulting supernatant spun at 100,000 *g* for 1 hr. The microsomal pellet was resuspended in a buffer containing 1.15% potassium chloride, 20 mM Tris and 0.1 mM EDTA, pH 7.5 (buffer B), homogenized with a Teflon homogenizer (three strokes) and respun at 100,000 *g* for 1 hr. The final pellet was resuspended in 15 mL of buffer B and frozen in 0.5 mL aliquots at −80° until used.

Human liver samples (12–13 g) were homogenized (3 × 30 sec) in 50 mL of 150 mM KCl, 1 mM EDTA, pH 7.4, using an Ultra-Turrax homogenizer. After centrifugation at 10,000 *g* for 15 min the supernatants were spun for 1 hr at 100,000 *g*. Microsomal pellets were resuspended in 0.15 M KCl, 1 mM EDTA, pH 7.5, to a final protein concentration of 7–19 mg/mL and frozen in 1 mL aliquots in liquid nitrogen until used.

Rat liver microsomes were prepared from male Sprague–Dawley rats (180–220 g) previously starved for 16 hr, by homogenization of the livers in 10 mM Tris–HCl, 1 mM EDTA, pH 7.4, containing 250 mM sucrose. The 25% homogenate was centrifuged for 15 min at 10,000 *g* and the resulting supernatant spun for 60 min at 100,000 *g*. The microsomes were washed with homogenizing buffer as described above and resuspended in a volume of homogenization buffer equivalent to the initial liver weight and frozen in liquid nitrogen until used.

Purification of mEH_b. mEH_b was purified from male Sprague–Dawley rat liver as described by Bentley and Oesch [17]. The enzyme preparation appeared homogenous upon SDS–PAGE and had a specific activity of 435 nmol/min × mg with STO as substrate.

The protein content of the microsomal fractions and pure enzyme preparations was determined according to Lowry *et al.* [18] with bovine serum albumin as a standard. The procedure was modified by including a 10% trichloroacetic acid precipitation in a final volume of 220 μL, and adapted to micro scale.

Measurement of mEH_b activity. The assay to test for mEH_b activity was performed in conical 14 × 100 mm stoppered glass reaction tubes using [^3H]STO as substrate according to Oesch *et al.* [15] with the modifications given in Ref. 19. Briefly, the reaction mixture contained in a final volume of 200 μL 0.125 M Tris–HCl buffer, pH 9, or potassium phosphate buffer, pH 7.4, and 0.1 mM EDTA. [^3H]-STO was added in 10 μL of acetonitrile to give final concentrations between 0.084 and 2.2 mM. The inhibitors were either added in 2 μL acetonitrile to allow for final concentrations of 0.5–10 mM for VPA, 0.2–4 mM for VPM and 0.05–5 mM for VPE,

Table 2. Summary of the Michaelis–Menten parameters obtained from all inhibition studies using VPM as inhibitor and [^{3}H]STO as substrate

Tissue	V_{max} (nmol/mg/min)	K_M (mM)	K_I^* (mM)
Purified rat liver mEH_b (pH 9)	353	0.15	0.11
Purified rat liver mEH_b (pH 7.4)	4.6	0.20	0.49
Rat liver microsomes (pH 9)	3.7	0.11	0.18
Rat liver microsomes (pH 7.4)	5.1	0.22	0.47
Human placenta mEH_b (pH 9)	0.13	0.13	0.16
Human liver mEH_b child (pH 9)	9.2	0.35	0.28
Human liver mEH_b child (pH 7.4)	6.6	0.06	0.09
Human liver mEH_b man (pH 9)	20.2	0.27	0.31
Human liver mEH_b woman (pH 9)	13.6	0.24	0.27

* K_I determined by slope versus inhibitor concentration replot.

or in water (VP-CoA; 0.125–5 mM). In order to exclude any inhibitory or activating action of the solvent as such, the corresponding control activities (no inhibitor added) for VPA, VPM and VPE were determined in the presence of 2 μL of acetonitrile. The reaction was started by addition of the microsomal suspension at an average of 0.3 mg protein and allowed to proceed for 10 min at 37° in a waterbath. In the case of placenta microsomes, an incubation time of 30 min was used due to the low activity of placenta mEH_b. Assay validation prior to the inhibition studies ensured linearity of the reaction rates with respect to protein concentration and incubation time under these conditions, and allowed the accurate determination of mEH_b activities at concentration levels below and above the K_M for the substrate STO (Table 2).

The assay to test for mEH_b activity using [^{3}H]-BPO was performed in an analogous fashion [16]. [^{3}H]BPO was added in a volume of 4 μL acetonitrile, and the incubation times were 7 and 30 min for liver mEH_b and placenta mEH_b, respectively. Again, the reaction rates were ensured to be linear with respect to time and protein concentration under these conditions.

The activity of mEH_b was expressed in both cases as nmol of diol formed per mg of microsomal protein per min (nmol/mg/min). For kinetic studies appropriate controls were performed by addition of equivalent amounts of the corresponding solvents. Blanks were carried out to determine non-enzymatic epoxide hydrolysis for each substrate concentration with boiled (5 min at 95°) enzyme or microsomes.

Data analysis. Linear regression was applied to calculate V_{max}, K_{app} and the slope from the individual graphs of the Lineweaver–Burk plots, and to estimate K_I and K_M from the slope and from the K_{app} replots, respectively [20]. Average K_I values obtained from Dixon plots were approximated by eye in order to confirm the nature of mEH_b inhibition by VPM. The extent of inhibition of mEH_b by VPA, VP-CoA, VPE and VPM was expressed by means of per cent mEH_b activity compared to control (no inhibitor added).

RESULTS

Human liver tissues obtained from a man, woman and child were utilized in this study to investigate the mEH_b response to VPA and some of its derivatives. Figure 1 gives representative inhibition and activation curves for mEH_b from rat (A) and human female (B) liver microsomes using VPA, VP-CoA, VPE and VPM as inhibitors and [^{3}H]STO as substrate. Of these substances, VPM was the only VPA analogue that showed any consistent and pronounced inhibition of mEH_b. However, concentrations higher than 2 mM VPM to achieve 100% enzyme inhibition were not effective due to the limited solubility of this compound in water (Fig. 1A), and were therefore omitted in the experiments reported in Fig. 1B. A slight inhibition, not exceeding 16% of control activity, was observed with 10 mM VPA but only when added in acetonitrile (data not shown). VP-CoA slightly activated rat microsomal mEH_b only at concentrations between 1 and 5 mM, while VPE appeared to activate both the rat and human enzyme slightly. The strongest activation by VPE of 50% was observed in rat microsomes with STO as substrate (Fig. 1A).

The microsomal esterase activity was not assessed, and hence the actual rate of VPE hydrolysis to VPA in the assay mixture was not known. However, the results presented in Figs 1 and 2 support the assumption that the majority of VPE added remained unchanged throughout the incubation for mEH_b activity determination.

To determine whether the results were dependent upon the substrate (STO), the inhibition experiments were repeated with the human female liver preparation using BPO as substrate (Fig. 2). The results demonstrated that only VPM inhibited mEH_b.

The inhibition of mEH_b by VPM was assessed by determining the effect of different VPM concentrations at various STO concentrations. The Lineweaver–Burk plots (Fig. 3) show VPM inhibition to be purely competitive for purified rat liver mEH_b (Fig. 3A), and mEH_b activity of human placenta (Fig. 3B) and child liver microsomes (Fig. 3C). Similar results were also obtained for the human female and male liver preparation (data not shown). Further evidence for the inhibition of female liver mEH_b by VPM was obtained by the Eadie–Hofstee plots (data not shown). The competitive nature of mEH_b inhibition by VPM was independent of the assay pH used (Fig. 3C).

Table 2 summarizes the kinetic parameters for all microsomal preparations and purified rat liver mEH_b. The listed K_I values for the VPM inhibition were obtained from the slope versus inhibitor concentration plots (Fig. 3) by means of linear regression. The K_I values determined from the K_{app}

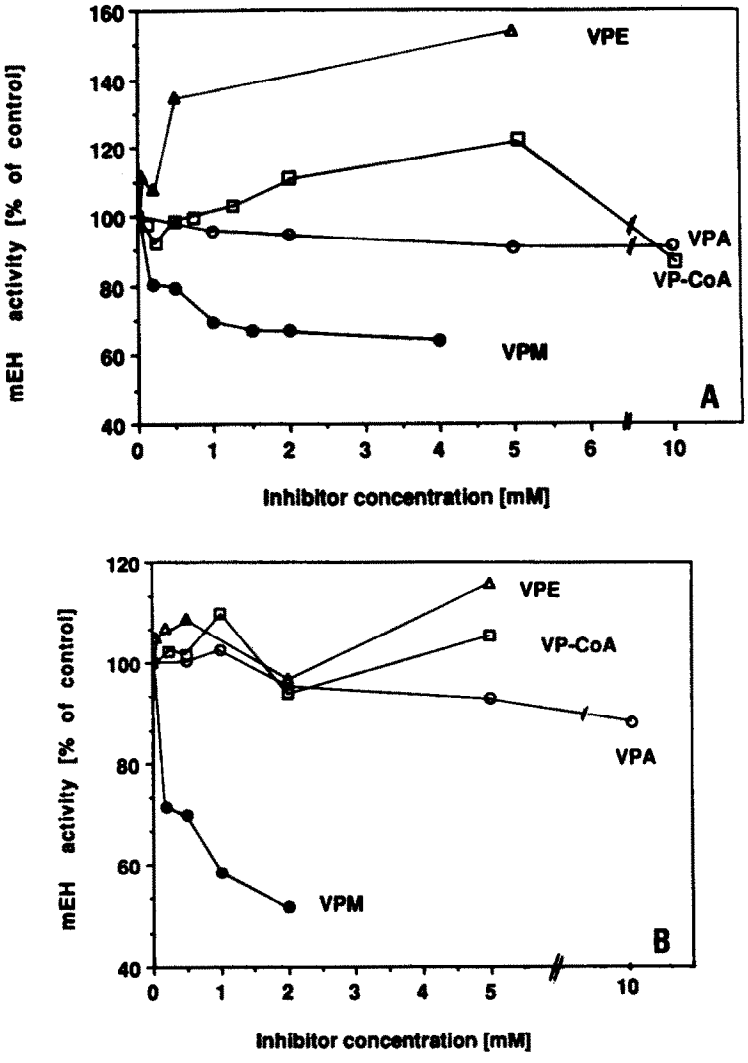

Fig. 1. Effect of increasing concentrations of VPA (○), VPM (●), VPE (△) and VP-CoA (□) on the mEH_b activity of rat (A) and female human (B) liver microsomes. mEH_b activity was determined in 125 mM Tris–HCl buffer, pH 9, in the presence of 2.0 (A) and 2.2 mM (B) STO as described in Materials and Methods. All inhibitors were added in 2 μL acetonitrile, except for VP-CoA, which was added in water. The 100% mEH_b activity corresponded to 3.1 and 12.0 nmol/min/mg for rat and human female liver microsomes, respectively.

versus inhibitor concentration plot and from the individual Dixon plots (rat: 0.15–0.23 mM; placenta: 0.17–0.24 mM; human liver: 0.24–0.44 mM—data not shown) lie within the same range. Except for the low K_M and K_I values obtained at pH 7.4 for child liver microsomes, the kinetic constants for the VPM inhibition of child and rat liver microsomes with STO as substrate at pH 7.4 were in the same order of magnitude as those obtained at pH 9 (Table 2). While the K_M was basically unchanged for rat liver microsomes, the K_M for child liver microsomes dropped from 0.35 mM at pH 9 to about 0.06 mM at pH 7.4. At the same time, the K_I value appeared slightly increased for rat liver microsomes, whereas the K_I value for child liver microsomes dropped from 0.35 to 0.09 mM at pH 7.4.

DISCUSSION

The observation that VPA does not significantly inhibit mEH_b in rat or human tissues confirms the results obtained by Pacifici and Rane [6]. In some of the inhibition studies, a slight decrease in mEH_b activity was observed at VPA concentrations of 5 and 10 mM. The small decrease observed, however, appeared to be due to a change in pH, as this decrease was not observed when VPA was dissolved in 0.5 mM Tris, pH 9.0, prior to its addition to the reaction mixture. Additionally, preincubation of microsomes with the inhibitor also suggested that inhibition of mEH_b by VPA via an uncompetitive or non-competitive mechanism can be excluded (data not shown). VPM was the only analogue that

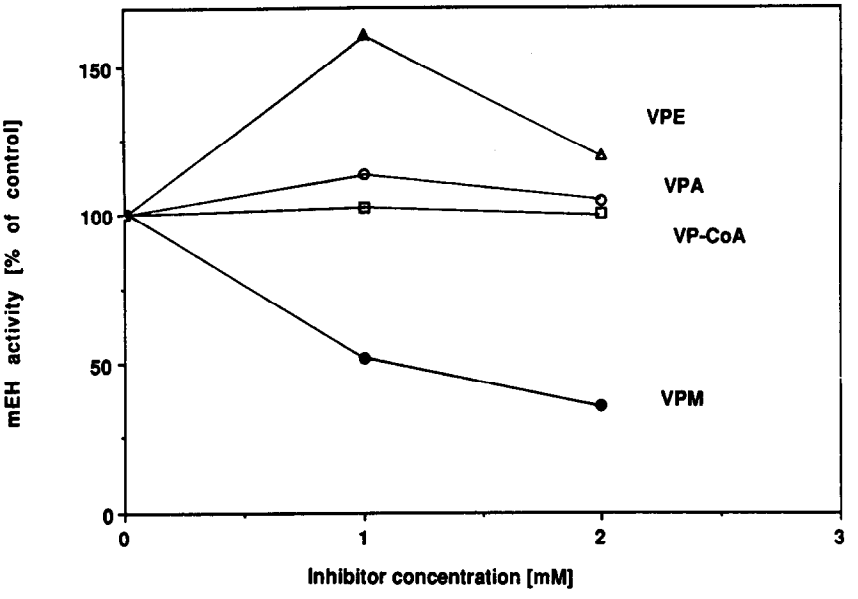

Fig. 2. Effect of increasing concentrations of VPA (○), VPM (●), VPE (△) and VP-CoA (□) on the mEH_b activity of female human liver microsomes. mEH_b activity was determined in 125 mM Tris–HCl buffer, pH 9, in the presence of 0.126 mM BPO as described in Materials and Methods. All inhibitors were added in 2 μL acetonitrile, except for VP-CoA which was added in water. The 100% mEH_b activity corresponded to 8.67 nmol/min/mg for female liver microsomes.

significantly inhibited mEH_b *in vitro*, and its interaction with this enzyme was independent of pH. Thus, artifacts due to the non-physiological pH of 9 used in the assay, the pH optimum for mEH_b catalysis [21], can be excluded. As neither VP-CoA nor the VPE inhibited mEH_b activity, enhanced lipophilicity alone or simple blockage of the acidic group of VPA cannot account for the inhibition observed with VPM. Therefore, it is unlikely that other VPA-esters, such as VPA-carnitine or VPA-glucuronides which are formed during VPA metabolism [22–24], will inhibit mEH_b. The effect on epoxide hydrolase of VPA metabolites formed during phase I metabolism, however, requires further investigation. The mechanism of mEH_b inhibition by VPM appears to be more specific than initially anticipated, and may be unique to lipophilic amides as suggested by Kerr and Levy [5, 25]. Interestingly, the simple ethylester of VPA in the reaction medium enhanced mEH_b activity (Fig. 1A and B). As the activity of mEH_b is reported to be dependent on the assay medium [26], an explanation for this observation may be either the integration of this non-polar compound into the microsomal membrane followed by an enhancement of the lipophilicity of the surrounding of mEH_b or a direct activation of the enzyme by interaction with hydrophobic (allosteric) sites. An experimental evaluation of these hypotheses is currently being conducted.

The key for an understanding of the molecular mechanisms underlying mEH_b inhibition by VPM may have to be sought in the catalytic function of the enzyme and its active site topography. A catalytic mechanism for mEH_b, involving an essential histidine residue ("base catalyst") and an unidentified "acid catalyst", has initially been proposed by Levin *et al.* [27] on the basis of results from chemical modification of the enzyme with 2-bromo-4'-nitroacetophenone. Studies in our laboratory [28] have confirmed the previously postulated histidine residue as a prerequisite for the catalytic activity of mEH_b and provided evidence from chemical modification with acetic anhydride for lysine as the proposed essential "acid catalyst". Hammock and co-workers [29–31] suggested a catalytic site topography model for cytosolic epoxide hydrolase and mEH_b on the basis of results from inhibition studies with a number of chalcone oxides, α,β-epoxyketones and isosteric analogues thereof, as well as with cyclopropyloxiranes, which included, in addition to the postulated acid and base catalyst, two hydrophobic pockets to accommodate the lipophilic side-chains of the enzyme substrates (Fig. 4A). Therefore, it appears very likely that VPM, as well as other unsubstituted lipophilic amides [25], act as mechanism-based substrate analoga by interacting with the two proposed essential catalytic site amino acid residues of mEH_b via hydrogen bonding, and with the hydrophobic enzyme pockets via hydrophobic interaction as depicted in Fig. 4B. This model is also valid to explain why VPA and other investigated analogues thereof may not act as inhibitors of mEH_b. VPA, for example, is a carboxylic acid that provides an excess proton which would prevent the VPA interaction with histidine. VPE, on the other hand, does not provide a hydrogen atom for hydrogen bonding with histidine, while VP-CoA may be too bulky and hydrophilic to enter the catalytic site.

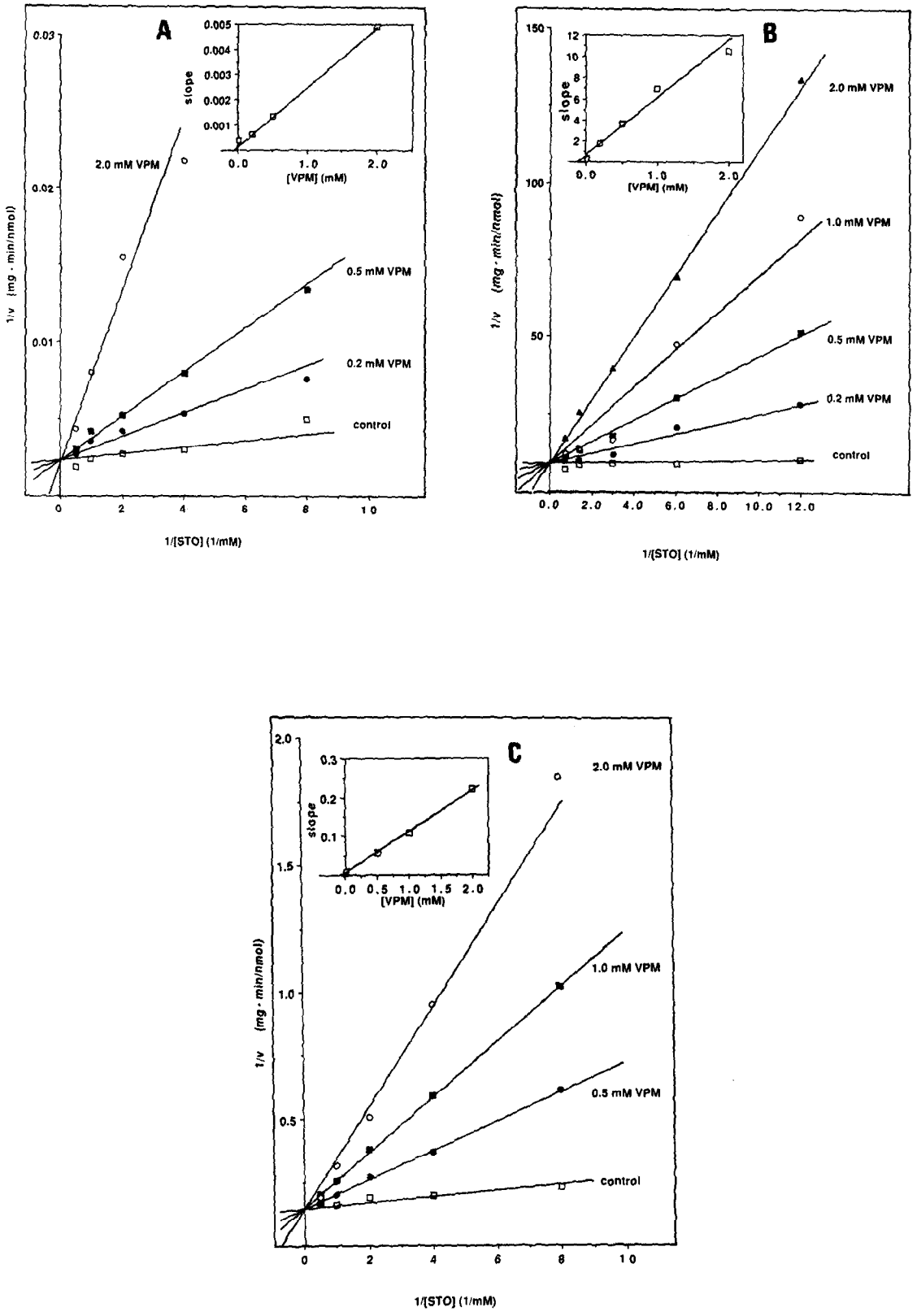

Fig. 3. Double reciprocal plots of $1/v$ versus 1/[STO] at various fixed concentrations of VPM for purified rat liver mEH$_b$ (A), human placenta (B) and child liver (C) microsomes. Enzyme activity was determined at pH 9 (A and B) or pH 7.4 (C) as described in Materials and Methods. Slope plots (inserts) were used to evaluate K_i.

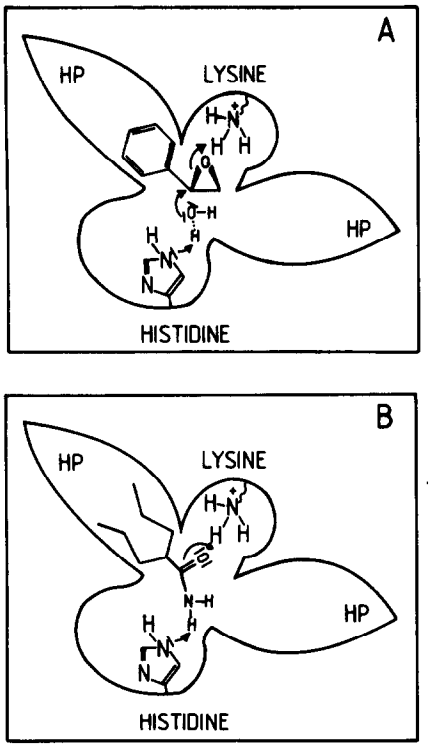

Fig. 4. Catalytic site topography for mEH_b as proposed from the results of inhibition studies and chemical modification (A). Putative interaction of VPM with the catalytic site of mEH_b (B). HB, hydrophobic pocket.

In contrast to these mechanistic considerations, however, the nature of mEH_b inhibition by VPA which has been observed *in vivo* is still unclear.

The VPM mediated inhibition of mEH_b was purely competitive as demonstrated by Lineweaver–Burk plots (Fig. 3). These findings were confirmed by other graphic methods (Dixon plots and Eadie–Hofstee plots) which yielded K_I values in the same range as listed for the slope replot from the Lineweaver–Burk plots (data not shown). Although the K_I values were lower than those reported by Pacifici *et al.* [6, 7], they lie within a similar range. On the other hand, these values are about 150-fold higher than those reported by Kerr and Levy [5, 25]. As the obtained K_I values for VPM inhibition at pH 7.4 and pH 9 were within similar range, the assay pH can be excluded as a reason for these discrepancies.

One possible explanation may reside with the differences in total microsomal protein used by Kerr and Levy [5, 25] and in the present investigation. All estimates of K_M are based on total substrate concentration, yet only unbound substrate is available to the mEH_b enzyme for hydrolysis. If the fraction of substrate which was unbound in the incubation medium is affected significantly by the amount of protein used, then differences in the estimates for K_M would result. Since differences in substrate binding have no effect on the maximal rate of enzyme activity, the rate of STO hydrolysis should be similar in both laboratories. This was in fact observed (6.6 versus 6.6 ± 0.2 nmol/min/mg protein) in incubations carried out at a similar pH.

The initial mEH_b activity did not drop much below 50% in spite of increasing the VPM concentration beyond 2 mM (Fig. 1). This can be attributed to a limited solubility of VPM in the reaction mixture. This was also apparent from the K_{app} plots, which tended to bend when the VPM concentration approached 2 mM.

Other investigators have achieved higher VMP solubility by adding the inhibitor in 4 μL dimethyl sulphoxide [6, 7] or increasing the volume of the assay medium, both of which was avoided in this investigation.

It has been suggested that the inhibition of epoxide hydrolase enhances the teratogenicity of xenobiotics [32, 33]. Thus, the effect of VPA-analogues on mEH_b in human liver and mEH_b from human placenta was of interest. The latter tissue exhibits low but measurable mEH_b activity [34]. The results obtained from the inhibition studies using placenta mEH_b were similar to those of human liver mEH_b, suggesting that placenta mEH_b is likely to be affected by the same compounds which affect liver mEH_b. The K_I for VPM inhibition using the placenta preparation was found to be within the same range as the K_I values obtained for the human liver preparations (Table 2). The values obtained for the maximum activity and K_M in our study correspond well with the results of Pacifici and Rane [34]. However, the biphasic relationship between v and v/S in the substrate range of 0.1 to 2 mM for [^{3}H]-STO and 0.01 to 0.13 mM for [^{3}H]BPO reported earlier [34] was not observed. The somewhat lower K_M obtained for the placenta mEH_b compared to the human liver microsomes may be explained by an enhanced variability of the data due to the low mEH_b activity in placenta microsomes. Additionally, the data obtained from preliminary studies using [^{3}H]BPO did not suggest the presence of different mEH_b enzyme forms in different human tissues. The lower K_M and K_I values obtained from the purified rat liver mEH_b compared to human liver microsomal preparations can be explained by the presence of a similar but structurally different epoxide hydrolase isozyme in this species [35]. However, since the various VPA analogues exhibit analogous behaviour with both forms of mEH_b a very similar inhibition or activation site in both enzyme forms must exist.

In conclusion, VPM was the only VPA analogue exerting any significant inhibition of mEH_b. The nature of this inhibition cannot be attributed solely to an increase in the lipophilicity of VPA by masking its acidic carboxyl moiety as demonstrated by the effect of VPE on this enzyme. However, because of the very low plasma concentration of VPM after oral administration to humans [36], the direct inhibition of mEH_b by this substance is not expected to be of clinical relevance. As it appears that the inhibition of mEH_b may be specific to the amide function of VPM, the question remains, as to how VPA and VPM exert their effects on mEH_b *in vivo*.

Of the numerous compounds designed and/or investigated for their inhibitory properties towards

epoxide hydrolases [19, 29–31, 37, 38], VPM is the first compound that *is not* metabolized by mEH_b *and* acts as a purely competitive and reversible inhibitor on rat and human mEH_b. This characteristic of VPM should make this compound a useful tool for gaining more insight into the structure of the catalytic site and the mechanism of action of mEH_b in the future.

Acknowledgements—The authors wish to thank Prof. K. L. Platt for providing the human liver samples, and Mrs I. Böhm and Miss E. Schweizer for typing the manuscript. This investigation was funded in part by the Epilepsy Foundation of America and the Deutsche Forschungsgemeinschaft (SFB 302).

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