Biochemical Pharmacology, Vol. 35, No. 19, pp. 3309–3316, 1986.
Printed in Great Britain.

0006-2952/86 $3.00 + 0.00
Pergamon Journals Ltd.

DISTRIBUTION AND INDUCIBILITY OF CYTOSOLIC EPOXIDE HYDROLASE IN MALE SPRAGUE–DAWLEY RATS

LUDWIG SCHLADT,* WALTER WÖRNER, FRANS SETIABUDI and FRANZ OESCH
Institut für Toxikologie, Universität Mainz, Obere Zahlbacher Straße 67, D-6500 Mainz, Federal Republic of Germany

(*Received* 6 *December* 1985; *accepted* 10 *April* 1986)

Abstract—Cytosolic epoxide hydrolase (cEH) activity has been determined in liver and various extrahepatic tissues of male Sprague–Dawley rats using *trans*-stilbene oxide (TSO) and *trans*-ethylstyrene oxide (TESO) as substrates. Large interindividual differences in the specific activity of cytosolic epoxide hydrolase in the liver from more than 80 individual rats were observed varying by a factor of 38. In a randomly selected group of five animals liver cEH varied by a factor of 3.9 and kidney cEH by a factor of 2.7, whereas liver microsomal epoxide hydrolase and lactate dehydrogenase showed only very low variations (1.4- and 1.1-fold, respectively). The individual relative activity of kidney cEH was related to that of the liver.

Cytosolic epoxide hydrolase activity was present in all of six extrahepatic rat tissues investigated. Interestingly specific activities were very high in the heart and kidney (higher than in liver), followed by liver > brain > lung > testis > spleen.

TSO and TESO hydrolases in subcellular fractions of rat liver were present at highest specific activities in the cytosolic and the heavy mitochondrial fraction. As indicated by the marker enzymes, catalase, urate oxidase and cytochrome oxidase, this organelle-bound epoxide hydrolase activity may be of peroxisomal and/or mitochondrial origin. In the microsomal fraction, TSO and TESO hydrolase activity is very low, whereas STO hydrolase activity is highest in this fraction and very low in cytosol. In kidney, subcellular distribution is similar to that observed in liver.

None of the commonly used inducers of xenobiotic metabolizing enzymes caused significant changes in the specific activities of rat hepatic cEH (*trans*-stilbene oxide, α-pregnenolone carbonitrile, 3-methylcholanthrene, β-naphthoflavone, isosafrole, butylated hydroxytoluene, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin, dibenzo[*a*,*h*]anthracene, phenobarbitone).

However, clofibrate, a hypolipidemic agent, very strongly induced rat liver cEH (about 5-fold), whereas microsomal epoxide hydrolase activity was not affected. Specific activity of kidney cEH was increased about 2-fold.

Epoxides are common products of cytochrome P-450 catalyzed oxidations of aromatic and olefinic compounds. They can lead to toxic, mutagenic or carcinogenic effects, although these epoxides vary dramatically in their chemical reactivity and in their ability to bind covalently to biological molecules. One possibility for their inactivation is the glutathione transferase-catalyzed nucleophilic attack by glutathione leading to the corresponding conjugates [1]. Epoxides may also be metabolized by epoxide hydrolases, which catalyze the addition of water to the epoxide moiety to yield a diol. The importance of microsomal epoxide hydrolase in the detoxification of toxins, mutagens and carcinogens is well established [2–4].

Using a juvenile hormone analogue Gill *et al.* [5] demonstrated the existence of a cytosolic epoxide hydrolase. The activity of this enzyme was not detected in earlier investigations because its substrate specificity is very different from that of the microsomal enzyme. Although there is no quantitative inverse relationship and an overlap is observed in some cases, substrate specificities of cytosolic and microsomal epoxide hydrolase are complementary to some extent. Substituted styrene oxides can be used as diagnostic substrates: cEH is very active towards *trans*-substituted epoxides, whereas *cis* substituted epoxides including epoxides derived from cyclic systems (e.g. benzo[*a*]pyrene 4,5-oxide) and hydrolyzed almost exclusively by mEH_b [6, 7]. Differences in pH optima [8] in molecular weight [9, 10], in susceptibility to inhibitors [11, 12], in immunological characteristics [13] and in other properties investigated [14] show that mEH_b and cEH are distinct proteins.

Activity of cytosolic epoxide hydrolase is very low in rat liver compared to other species, e.g. rabbit and mouse [15]. For this reason, the mouse has been chosen as the experimental animal for studying properties of cEH. Most work on microsomal epoxide hydrolase has been done in the rat. More infor-

* To whom correspondence and reprint requests should be addressed.

† Abbreviations used: STO, 2-phenyloxirane (styrene 7,8-oxide); TSO, *trans*-2,3-diphenyloxirane (*trans*-stilbene oxide); TESO, *trans*-2-phenyl-3-ethyloxirane (*trans*-β-ethyl styrene oxide); mEH_b, microsomal epoxide hydrolase with broad substrate specificity for various xenobiotic epoxides, specifically including *b*enzo(*a*)pyrene 4,5-oxide; cEH, cytosolic epoxide hydrolase; LDH, lactate dehydrogenase; TCDD, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin.

mation on rat cEH would be desirable to allow a direct comparison of these two enzymes to assess their individual role in the metabolism of epoxides.

In the present study, we have examined the organ and subcellular distribution as well as the inducibility of cytosolic epoxide hydrolase in male Sprague–Dawley rats. These data are compared with those of the microsomal counterpart, mEH_b, within the same species and with cytosolic epoxide hydrolase in other species.

MATERIALS AND METHODS

Materials

[2,3-^{3}H]2-phenyloxirane ([^{3}H]STO, 11.7 GBq/mmol [16]), [2,3-^{3}H]*trans*-2,3-diphenyloxirane ([^{3}H]TSO, 0.41 GBq/mmol [17]), and [2,3-^{3}H]*trans*-2-phenyl-3-ethyloxirane ([^{3}H]TESO, 1.65 GBq/mmol [18]) were synthesized as indicated. For the TESO hydrolase assay the tritiated substrate was diluted with unlabelled TESO (synthesized according to Imuta and Ziffer [19]) to give a final stock solution with a specific radioactivity of 0.105 GBq/mmol. All other chemicals used were of analytical grade or the purest grade commercially available. Male Sprague–Dawley rats (180–220 g) were obtained from the Süddeutsche Versuchstieranstalt, Tuttlingen, F.R.G.; male Fischer F-344 rats (150–160 g) were obtained from Charles River Wiga, Sulzfeld, F.R.G. They were kept at constant temperature, under a constant light–dark cycle and free access to water and a defined diet (Altromin). Purified mEH_b was prepared from rat liver according to Bentley and Oesch [9].

Subcellular fractionation

(a) *Preparation of the cytosolic and microsomal fractions.* Male Sprague–Dawley rats were killed at a constant day time (8.30 a.m.) by cervical dislocation. The livers were perfused with ice-cold homogenization buffer (10 mM Tris–HCl, pH 7.4, containing 0.25 M sucrose) and removed immediately. Homogenization was done in the same buffer using an Ultra-Turrax to give a 25% (w/v) homogenate.

The homogenate was centrifuged for 20 min at 10,000 *g* and the resulting supernatant for 60 min at 100,000 *g* to give the cytosolic supernatant. The 100,000 *g* pellet was resuspended in 20 mM Tris–HCl, pH 8.0, containing 0.5 M KCl, equivalent to the initial volume and recentrifuged for 60 min at 100,000 *g*. The microsomal pellet was resuspended in homogenization buffer equivalent to half of the original buffer.

The preparation of the cytosolic and microsomal fractions of extrahepatic organs was performed as described above, without prior perfusion.

(b) *Preparation of several subcellular fractions.* Animals were fasted for 16 hr and killed by cervical dislocation. After perfusion with ice-cold homogenization buffer livers were taken out and cut into small pieces. Homogenization was done with two strokes of a loose fitting Potter-Elvehjem homogenizer to give a 25% (w/v) homogenate. Kidneys were homogenized with an Ultra-Turrax. Subcellular fractions were obtained by differential centrifugation as described by Gill and Hammock [20]. The homogenate was centrifuged successively at 600 *g* (10 min), 6500 *g* (10 min), 12,000 *g* (10 min) and 100,000 *g* (60 min). The pellets were washed twice with exception of the 100,000 *g* pellet, which was washed once. The supernatants of every centrifugation step were combined with the supernatants from the washing procedure. The pellets were resuspended in homogenization buffer and are referred to as the cell debris and nuclear fraction, the heavy mitochondrial fraction, the light mitochondrial fraction and the microsomal fraction, respectively.

Marker enzyme assays

Subcellular fractions were characterized by measuring the activity of enzymes which have been reported to be specific or selective for the organelles indicated.

Activities of acid phosphatase (lysosomes [21]), catalase (peroxisomes [21]), urate oxidase (peroxisomes [22]), lactate dehydrogenase (cytosol [21]), *o*-nitrophenyl acetate esterase (endoplasmic reticulum [23, 24]), NADPH-cytochrome *c* reductase (endoplasmic reticulum [25]) and cytochrome oxidase (mitochondria [25, 26]) were determined according to published procedures.

Epoxide hydrolase assays

Epoxide hydrolase activity was monitored using either TSO, TESO or STO, as substrates. The enzyme assays were performed under conditions where product formation was linear with incubation time and amount of protein.

Hydrolysis of TSO. The incubation mixture contained in a total volume of 200 μl, 50 μl of 0.5 M Tris–HCl, pH 7.4, 5 nmol [^{3}H]TSO in 1 μl of ethanol and 1 μl of 250 mM 1-chloro-2,4-dinitrobenzene (in ethanol) to reduce substrate depletion due to conjugation with glutathione, by glutathione transferases. The reaction was started by adding the appropriate amount of protein (usually about 0.3–2.0 mg of rat liver cytosolic protein). Following incubation for 5 to 10 min at 37° the reaction was stopped by addition of 3 ml of light petroluem ether and 250 μl of dimethylsulfoxide, shaking the tube for 10 sec on a Vortex mixer and cooling in an ice bath. For extraction of the substrate, the tubes were shaken for 3 min at 40 rpm (Roto Shaker, Kühner, Basel, Switzerland) and centrifuged at 400 *g* for 1 min. The upper phase was removed by aspiration. Substrate extraction was repeated with 3 ml of petroleum ether, then the formed diol was extracted into 1 ml of ethyl acetate* by shaking for 5 min. After phase separation by centrifugation an aliquot of 500 μl of the ethyl acetate phase was added to 6 ml of Rotiszint 1100 (G. Roth, Karlsruhe). The radioactivity was determined using a Packard-Tricarb 300 C scintillation counter. The recovery of the diol was 82%.

Hydrolysis of TESO. A modification of the procedure described by Mullin and Hammock [18] was used. The incubation conditions were the same as

* Because of the hepatotoxicity of ethyl acetate, the extraction of the formed diol should only be used when the conjugation reaction of the substrate with glutathione by glutathione transferases is high.

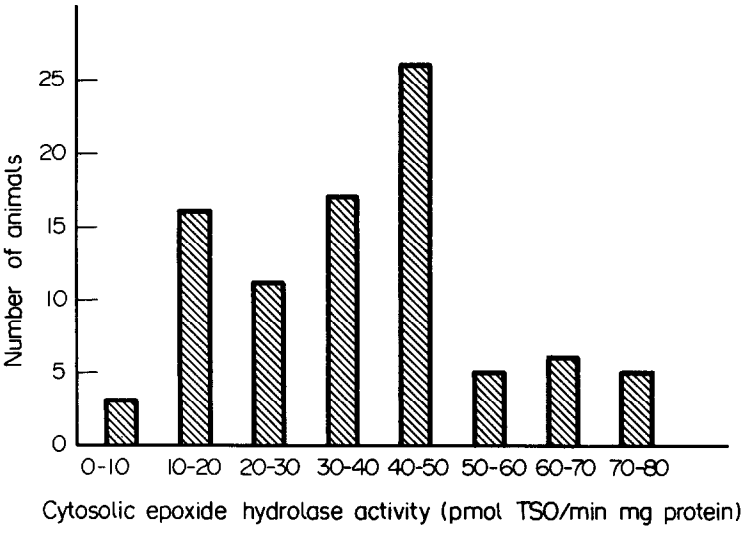

Fig. 1. Interindividual variation of cEH. Specific activity of cEH was determined in the livers of male Sprague–Dawley rats.

described above for TSO, except that [^{3}H]TSO was substituted by 100 nmol [^{3}H]TESO in 10 μl of ethanol. The incubation contained usually about 0.1–1.0 mg of rat liver cytosolic protein. At the end of the incubation the substrate was removed by extraction with isooctane (2 × 0.5 ml, 5 min), and the product was extracted with 1 ml of ethyl acetate* and the radioactivity was determined as described above.

Hydrolysis of STO. The assay for mEH_b was performed as described by Oesch *et al.* [16].

RESULTS

Interindividual variations of cytosolic epoxide hydrolase

The TSO hydrolase activity in liver cytosol of male Sprague–Dawley rats has been determined for more than 80 animals (Fig. 1). Large interindividual differences in the specific activity have been observed varying by a factor of 38.5 from 2 to 77 pmol/mg protein/min, with a median of 38.5 pmol/mg protein/min. Interindividual variations were much lower for mEH_b and the cytosolic lactate dehydrogenase. The specific activities of cEH, mEH_b and lactate dehydrogenase in liver of five randomly selected animals varied by a factor of 3.9, 1.4 and 1.1, respectively (Fig. 2 A–C). In kidney a 2.7-fold variation in cEH activity was observed (Fig. 2D). For all individuals, activity of cEH was higher in kidney than in liver (1.6- to 2.6-fold). When TSO was substituted by TESO to assay cEH activity, qualitatively identical results were obtained regarding interindividual variation. The inter assay variation of specific activity of cEH was lower than 10%. The Sprague–Dawley rat strain used was outbred. With inbred Fischer F-344 rats the variation in the specific activity of liver cEH was much lower. The specific activity of 25 individuals varied only by a factor of two. The mean specific activity is nearly the same in both rat strains (data not shown).

Organ distribution of cytosolic epoxide hydrolase

Activity of cEH using TSO as substrate has been determined in the cytosolic fractions of seven organs. cEH activity was present within all organs tested. Specific activity was highest in kidney and heart followed by liver, brain, lung, testis and spleen (Table 1). The same order of specific activity was observed using TESO as substrate (data not shown).

Distributions of epoxide hydrolase activity in subcellular fractions of liver and kidney

Subcellular distribution of STO, TSO and TESO hydrolase activity in rat liver and kidney is shown in Fig. 3. Cross contamination of the subcellular fractions was determined using marker enzymes selective or specific for the various subcellular compartments and was similar for both tissues, liver and kidney.

Cytochrome oxidase (a mitochondrial marker enzyme) and lactate dehydrogenase (cytosolic marker enzyme) were almost exclusively located in the mitochondrial and cytosolic fractions, respectively. In liver and kidney, the heavy and light mitochondrial fractions were contaminated with microsomes as indicated by the distribution of esterase and NADPH-cytochrome *c* reductase. The distribution of STO hydrolase activity equalled that of the microsomal marker enzymes, indicating that hydrolysis of STO occurred predominantly in the microsomes and is catalyzed by mEH_b. In the cytosol very low STO hydrolase activity was detected.

Peroxisomes (urate oxidase and catalase as marker enzymes) cosedimented mainly with the heavy and light mitochondrial fraction, although significant amounts of catalase are found in the cytosolic fraction. Kidney peroxisomes do not contain urate oxidase [27]. Therefore, this enzyme has not been

* Because of the hepatotoxicity of ethyl acetate, the extraction of the formed diol should only be used when the conjugation reaction of the substrate with glutathione by glutathione transferases is high.

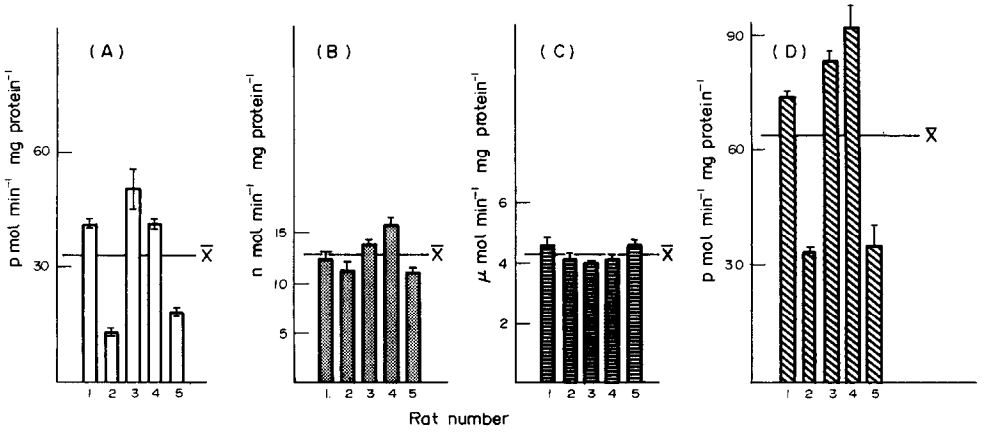

Fig. 2. Interindividual variation of cEH, mEH_b and LDH. Specific activities of cEH (A), mEH_b (B) and LDH (C) in rat liver and of cEH (D) in rat kidney have been determined in five randomly selected individuals.

measured in the subcellular fractions of the kidney. Lysosomes (acid phosphatase as marker enzyme) were found in all subcellular fractions. TSO and TESO hydrolase activities were present at similar specific activities in the heavy mitochondrial and the cytosolic fractions of liver, and at lower activities in the light mitochondrial fraction. In the kidney, the cytosolic fraction accounted for most of TSO and TESO hydrolase activity. In the microsomal fractions of both organs, only very low TSO and TESO hydrolase activity could be detected. This is in agreement with experiments using mEH_b, which was purified according to Bentley and Oesch [9]: TSO and TESO were not measurably or only very slowly hydrolyzed by mEH_b (TSO: <0.5 pmol/min/mg protein; TESO: 2.3 nmol/min/mg protein; STO: 350 nmol/min/mg protein).

Induction of cytosolic epoxide hydrolase

Induction of cEH was investigated with nine inducers of xenobiotic metabolizing enzymes. None of the compounds did affect the specific activity of cEH (Table 2). However, one has to take into account the large interindividual variation of cEH, which makes it difficult to obtain statistical significance for small changes in enzyme activity. For dibenzo[*a*,*h*]anthracene and phenobarbitone, the data for liver and kidney cEH are given in Table 3.

In contrast to the classical inducers, clofibrate, a hypolipidemic drug, caused a significant induction* of cEH. Upon daily administration by i.p. injection of 200 mg/kg clofibrate for three days, specific activity of cEH was increased about 5-fold in liver and about 2-fold in kidney (Table 3).

Similar results were obtained from feeding the animals with a 0.2% clofibrate diet for one week (Table 4). Activity of mEH_b was not significantly affected by treatment with clofibrate, whereas induction of cEH was significant ($\alpha < 0.01$ (liver) and $\alpha \leq 0.05$ (kidney)) using the *U*-test of Wilcoxon, Mann and Whitney. Induction in other tissues (heart, brain, testis, lung, spleen) was very low (≤1.5-fold) or absent (data not shown).

DISCUSSION

Assay of cytosolic epoxide hydrolase

TSO and TESO were used to assay cEH activity; mEH_b has been tested using STO. These substrates can effectively distinguish between the two forms of rat epoxide hydrolase (see results of subcellular localization).

Specific activity of cEH in rat liver was found to be very low as compared to other species, e.g. cEH activity in mice was about 70-fold higher using 1-(4′-ethylphenoxy)-3,7-dimethyl-6,7-epoxy-*trans*-2-octene as substrate [15]. However, because of the low spontaneous hydrolysis of TSO and TESO under the assay conditions described, detection and quantification of cEH in rat liver cytosol is possible. Rat cEH activity was determined in some cases using the TLC method of Jerina *et al.* [28] optimized for [^{3}H]TSO as substrate [29]. Identical results were obtained, with both methods, but the TLC method is more time consuming. The radioactivity of the experimental values was mostly more than 15-fold

Table 1. Organ distribution of cytosolic epoxide hydrolase

Organ	Specific activity (pmol/min × mg protein)	% of liver activity
Kidney	53.9 ± 2.7	156
Heart	49.6 ± 7.6	144
Liver	34.4 ± 1.1	100
Brain	12.7 ± 1.3	37
Lung	6.0 ± 0.1	17
Testis	3.6 ± 0.3	10
Spleen	3.0 ± 0.8	9

Values reported are means ± SD. Using TSO as substrate, activity of cEH was determined in triplicate from pooled organs of 5 male Sprague–Dawley rats. The experiment was repeated once.

* 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.

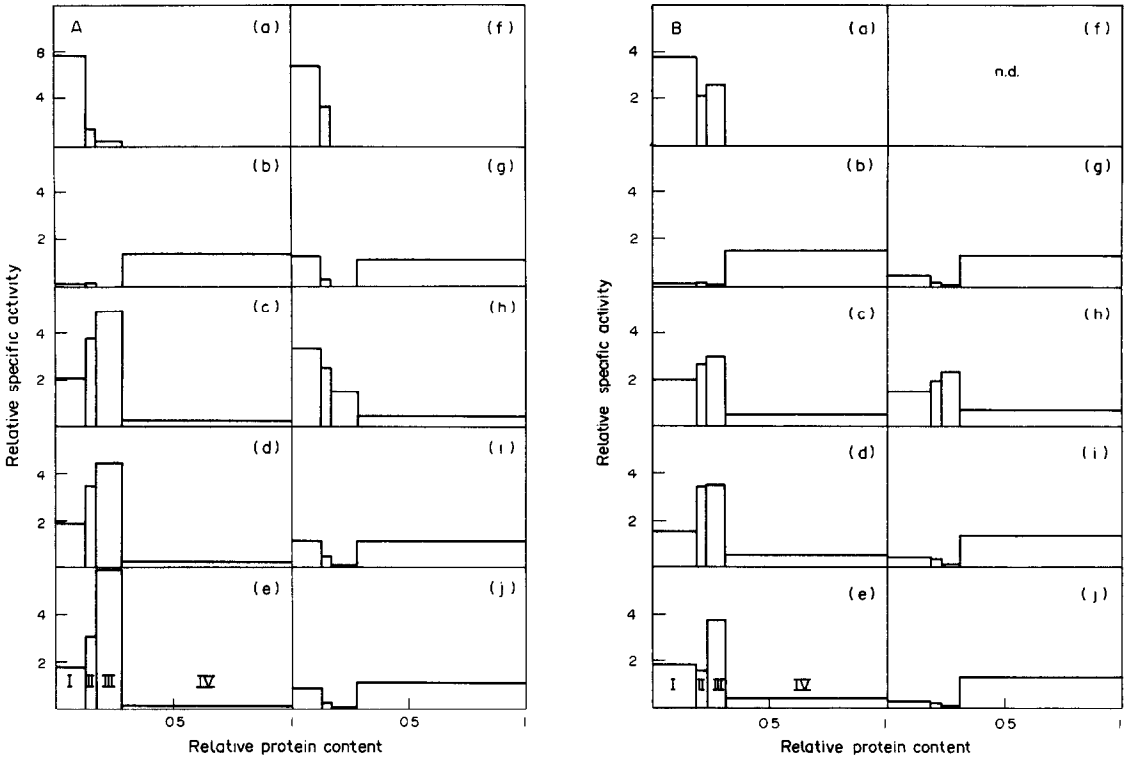

Fig. 3. Subcellular distribution of TSO, TESO and STO hydrolase activities in rat liver and kidney. The histograms show the distribution of STO (e), TSO (j) and TESO hydrolase (i) and of marker enzyme activities: cytochrome oxidase (a), lactate dehydrogenase (b), esterase (c), NADPH-cytochrome *c* reductase (d), urate oxidase (f), catalase (g), acid phosphatase (h) in heavy (I) and light mitochondrial (II), the microsomal (III) and the cytosolic (IV) fractions of rat liver (A) and kidney (B). The 600 *g* pellet contained unbroken cells, cell debris and nuclei. Relative specific enzyme activities of this fraction for liver and kidney (data are given in brackets) were: cytochrome oxidase: 1.50 (1.66); lactate dehydrogenase: 0.16 (0.10); esterase: 0.88 (1.03); NADPH-cytochrome *c* reductase: 0.89 (0.95); urate oxidase 0 (n.d.); catalase: 0.27 (0.52); and phosphatase: 0.85 (1.06); TESO hydrolase: 0.51 (0.41); TSO hydrolase: 0.22 (0.35); STO hydrolase: 0.94 (0.82); protein: 0.46 (0.44). Relative specific activity is defined as the ratio of the percentage of activity from total activity in a certain fraction to the percentage of protein content from total protein content of this fraction. Enzyme assays were performed as described in "Materials and Methods" with the exception that ethanol was substituted for acetonitrile as a solvent for STO in the STO hydrolase assay as cEH is significantly inhibited at 2.5% (v/v) of acetonitrile and a potential STO hydrolase activity of cEH would otherwise escape detection. N.D. means not determined.

higher than that of the background values. Care has to be taken, to maintain the incubation temperature exactly at 37° as activity of cEH is very sensitive to changes in temperature and has an optimum at about 50° (data not shown). This has also been observed for purified mouse cEH [30]. The low activity of cEH necessitated the use of large amount of cytosolic protein. As glutathione *S*-transferases are very effective in catalyzing conjugation of the styrene oxides with glutathione, CDNB had to be included to prevent substrate depletion during the incubation. The formed diol was extracted with ethyl acetate because the glutathione *S*-transferases could not be completely inhibited. Same specific activities were obtained, if rat liver cytosol was dialyzed overnight against two changes of isolation buffer and then assayed for cEH activity in the absence of CDNB. Inclusion of CDNB and diol extraction were not necessary using mouse cytosol [18]. cEH is very sensitive to many solvents used to assay the substrates (data not shown [11]). Solvents other than ethanol or methanol should, therefore, be avoided. Various concentration of ethanol (up to 5%) did not affect enzyme activity.

Interindividual variations of cytosolic epoxide hydrolase

Large interindividual variations have been observed in the activity of cEH in the liver of male Sprague–Dawley rats (outbred rat strain). These fluctuations in activity are neither correlated with mEH_b activity, another epoxide-metabolizing enzyme, nor with LDH activity, another cytosolic protein. In contrast to cEH, both mEH_b and LDH are present at similar levels in the various individuals investigated.

Assay conditions could not be responsible for the large variation as: (1) interassay variation of specific

Table 2. Compounds which were found to be ineffective as inducers of cytosolic epoxide hydrolase in male Sprague–Dawley rat liver

(1) *trans*-Stilbene oxide	(6) Butylated hydroxytoluene
(2) α-Pregnenolone carbonitrile	(7) Dibenzo[*a*,*h*]anthracene
(3) 3-Methylcholanthrene	(8) Phenobarbitone
(4) β-Naphthoflavone	(9) TCDD
(5) Isosafrole	

Animals were treated with the indicated compounds by i.p. injection of 400 mg/kg (1), 50 mg/kg (2), 25 mg/kg (3, 4 and 7), 150 mg/kg (5), 250 mg/kg (6), 80 mg/kg (8) on three consecutive days, whereas (9) was given only once (0.12 mg/kg). After starving overnight, animals were sacrificed on the fourth day.

activity was lower than 10%; (2) all assays were performed as described in the "Materials and Methods" section so that the various incubations contained always the same amount of ethanol; (3) with Fischer rats (inbred rat strain) the variation in the specific activity of cEH was much lower, although the same assay procedure was used.

We had previously observed large interindividual variations of cEH from human liver. The specific activity of cEH from human liver biopsies showed a total 539-fold interindividual variation [29]. Soluble epoxide hydrolase in mononuclear leukocytes from 27 different subjects has been found to vary 5-fold [31].

Such interindividual differences may result from genetic constitution, age, sex, diet, exposure to environmental chemicals or drugs that induce or repress, activate or inhibit the enzymes. As external factors can be controlled in animal experiments and regarding the low interindividual differences of cEH in Fischer F-344 rats, the high variations observed with male Sprague–Dawley rats may result from their genetic constitution. In man, besides a genetically determined "basic variation", drugs, environmental or other factors may increase the variation range.

Table 3. Response of rat liver and kidney cytosolic epoxide hydrolase following treatment with dibenzo[*a*,*h*]anthracene (DBA), phenobarbitone (PB) and clofibrate

Tissue	Individual	Specific activity of cEH pmol TSO/(mg protein × min) Control	DBA	PB	Clofibrate
Liver	1	47.1	16.9	49.4	47.7
	2	30.0	19.8	18.1	110.3
	3	43.9	44.6	47.4	105.5
	4	7.0	39.2	35.2	261.2
	5	24.4	40.7	50.8	197.5
	6	46.3	38.8		188.8
	$\bar{x}$	33.1	33.3	40.2	151.8†
Kidney	1	90.3	47.5	102.7	68.4
	2	84.8	37.8	45.4	163.7
	3	87.5	77.7	88.8	121.5
	4	2.8	91.4	77.3	117.4
	5	36.3	100.1	124.9	121.3
	6	100.9	91.1		106.3
	$\bar{x}$	60.3	74.3	87.8	109.8*

Animals were treated by i.p. injection of 25 mg/kg DBA, 80 mg/kg PB or 200 mg/kg clofibrate on three consecutive days. After starving overnight, animals were sacrificed on the fourth day. *,† significantly different from control animals (*$\alpha = 0.05$ and †$\alpha = 0.01$) according to the *U*-test of Wilcoxon, Mann and Whitney.

Table 4. Response of rat liver cytosolic epoxide hydrolase and microsomal epoxide hydrolase following treatment with clofibrate

Individual	cEH pmol TSO/(min × mg protein) Control	Clofibrate	mEH_b nmol STO/(min × mg protein) Control	Clofibrate
1	34.2	92.6	12.79	10.59
2	18.7	167.8	13.51	10.07
3	45.0	129.5	7.49	12.15
4	18.6	231.7	11.15	11.81
5	58.6	85.0	11.25	10.79
$\bar{x}$	35.0	141.3*	11.24	11.08

Animals were fed for 1 week a pelleted diet containing 0.2% clofibrate (prepared by soaking the diet in the appropriate amount of clofibrate dissolved in acetone).

* Statistically significant from the control animals at $\alpha = 0.01$ using the *U*-test of Wilcoxon, Mann and Whitney.

The interindividual variation of cEH is not restricted to liver but was also observed in kidney (Fig. 2). Although the quotient of kidney/liver activity is not constant for different individuals, there is, however, a relationship between the activities in liver and kidney ($r = 0.863$; estimated from specific activity of liver and kidney cEH from 16 different rats, not shown), i.e. high activity in liver implies high activity in kidney and vice versa.

Organ distribution of cytosolic epoxide hydrolase

From a quantitative point of view most activity of cEH is present in the liver of male Sprague–Dawley rats. However, some extrahepatic organs also contain significant amounts of cEH and specific activities in kidney and heart even exceed that of liver.

In other species studied, liver shows highest specific activity for cEH whereas kidney constitutes 70% (rabbit [32]) and about 50% (mouse [15]) of liver activity. Activity of rat cEH in lung, testis, and spleen is significantly lower than in liver which is consistent with the findings in mouse and rabbit.

Organ distribution of rat cEH also contrasts that of its microsomal counterpart. For mEH_b, specific activity is highest in liver, and with the exception of testis (25%) and kidney (10%) is below 10% of liver activity for the extrahepatic tissues investigated [33].

Subcellular distribution of cytosolic epoxide hydrolase

Specific activity of STO hydrolase activity is highest in rat liver and kidney microsomes, whereas in the cytosol very low STO hydrolase activity could be detected. On the other hand, in the cytosolic

fractions of rat liver and kidney TSO hydrolase activity is much higher (about 15- and 20-fold, respectively) than in microsomes. This agrees with the results obtained with mouse liver [34], whereas in Rhesus monkey liver the microsomal fraction had a more than 2-fold higher activity with TESO as substrate than the cytosol [35]. This indicates that subcellular distribution of EH or substrate specificities of cytosolic or microsomal EHs may be different in various species.

In rat liver about 11% of total TSO and TESO hydrolase activity is located in the heavy mitochondrial fraction with a specific activity similar to that of the cytosolic fraction. As only 1.5% of total LDH activity is present in the heavy mitochondrial fraction, the high TSO and TESO hydrolase activity cannot be accounted for by a cytosolic contamination, nor by the considerable microsomal contamination of the heavy mitochondrial fraction, as TSO and TESO are not measurably or only slowly hydrolyzed by the microsomal fraction or even by purified mEH_b.

As indicated by the marker enzymes catalase, urate oxidase and cytochrome oxidase, this fraction contains peroxisomes and mitochondria, which may both be the source of organelle-associated TSO and TESO epoxide hydrolase activity. Early evidence indicated that the cEH activity in the 6500 *g* and 12,000 *g* fractions of mouse liver is of mitochondrial origin [20]. Immunological studies using double diffusion analysis and immunoprecipitation experiments demonstrated that the epoxide hydrolase of the mitochondrial fractions of mouse liver is similar to cytosolic EH [36]. However, subfractionation of the light mitochondrial fraction by isopycnic gradient centrifugation presented strong evidence that organelle-associated TSO hydrolase activity is localized in peroxisomes [37].

Subcellular distribution in rat kidney is similar to that of liver. Some of the differences observed (i.e. cytochrome oxidase activity in the light mitochondrial and microsomal fraction) may be explained by the more vigorous method of homogenisation of kidney.

Induction of cytosolic epoxide hydrolase

A variety of compounds, which are known to induce xenobiotic metabolizing enzymes, did not alter the specific activity of cEH in rat liver or kidney (Table 2 and 3).

The lack of induction of the hepatic cEH by 3-methylcholanthrene, TSO and butylated hydroxytoluene agrees with the findings of Hammock and Ota [38].

With phenobarbitone no significant change in adult male Sprague–Dawley rat cEH activity was observed in this study, whereas a statistically significant decrease in mouse cEH activity and a slight increase in rat cEH activity was reported [38]. The lack of induction of cEH by the compounds listed in Table 2 demonstrate that cEH regulation is under a different control, than other drug metabolizing enzymes. In contrast to the classical inducers of xenobiotic metabolizing enzymes, clofibrate, a hypolipidemic drug, caused a drastic (about 5-fold) increase in cEH activity. This increase was observed by either oral application (included in the diet) or intraperitoneal injection. Clofibrate did not affect activity of mEH_b in rat liver.

A weaker but significant induction of cEH and mEH_b by hypolipidemic compounds has been described in mice with clofibrate or diethylhexylphthalate [38] and nafenopin [39]. Although dose of clofibrate was higher and duration of treatment was longer, induction of mouse cEH was only about 2-fold.

Thus, cEH seems to be more sensitive to induction by hypolipidemic drugs in rat than in mouse, whereas the reverse is true for mEH_b. Apart from rat liver, where we have observed a 5-fold induction, a 2-fold induction of cEH was also found in rat kidney.

Nafenopin and clofibrate are hypolipidemic drugs. These agents induce striking changes in the liver of rats and mice including a marked increase in the number of peroxisomes [40, 41] and changes in the peroxisomal structure and enzyme composition [42]. As cEH catalyzes the hydrolysis of several epoxides derived from unsaturated fatty acids, such as oleic acid, elaic acid [43] and arachidonic acid [44], it will be interesting to learn whether cEH may be of importance in lipid metabolism and the action of the lipid lowering agents.

Acknowledgements—The authors thank R. Hartmann for excellent technical assistance, and Mrs Böhm for typing the manuscript. The financial support of the Deutsche Forschungsgemeinschaft (SFB 302) is gratefully acknowledged.

REFERENCES

1. L. F. Chasseaud, *Adv. Cancer Res.* **29**, 175 (1979).
2. F. Oesch, *Xenobiotica* **3**, 305 (1973).
3. F. P. Guengerich, in *Reviews in Biochemical Toxicology 4* (Eds. Hodgson, Bend, Philpot), p. 5. Elsevier Biomedical, New York (1982).
4. F. Oesch, in *Developmental Pharmacology* (Ed. S. MacLeod), p. 81. Allan R. Liss, New York (1983).
5. S. S. Gill, B. D. Hammock and J. E. Casida, *J. Agric. Food Chem.* **22**, 386 (1974).
6. B. D. Hammock and L. S. Hasagawa, *Biochem. Pharmac.* **32**, 1155 (1983).
7. F. Oesch and M. Golan, *Cancer Lett.* **9**, 169 (1980).
8. K. Ota and B. D. Hammock, *Science* **207**, 1479 (1980).
9. P. Bentley and F. Oesch, *FEBS Lett.* **59**, 291 (1975).
10. F. Waechter, M. Mertes, F. Bieri, W. Stäubli and P. Bentley, *Eur. J. Biochem.* **125**, 457 (1982).
11. C. A. Mullin and B. D. Hammock, *Archs Biochem. Biophys.* **216**, 423 (1982).
12. F. Oesch, N. Kaubisch, D. M. Jerina and J. Daly, *Biochemistry* **10**, 4858 (1971).
13. T. M. Guenthner, B. D. Hammock, U. Vogel and F. Oesch, *J. biol. Chem.* **256**, 3163 (1981).
14. C. Timms, F. Oesch, L. Schladt and W. Wörner, in *Proceedings of the 9th International Congress of Pharmacology* (Eds. J. F. Mitchell, W. Paton and P. Turner), p. 231. Macmillan Press, London (1984).
15. S. S. Gill and B. D. Hammock, *Biochem. Pharmac.* **29**, 389 (1980).
16. F. Oesch, D. M. Jerina and J. Daly, *Biochim. biophys. Acta* **227** 685 (1971).
17. F. Oesch, A. J. Sparrow and K. L. Platt, *J. Labelled Compd. Radiopharm.* **17**, 93 (1980).
18. C. A. Mullin and B. D. Hammock, *Analyt. Biochem.* **106**, 476 (1980).
19. M. Imuta and H. Ziffer, *J. org. Chem.* **44**, 1351 (1979).

20. S. S. Gill and B. D. Hammock, *Biochem. Pharmac.* **30**, 2111 (1981).
21. H. U. Bergmeyer (Ed.) *Methoden der enzymatischen Analyse*, Vols. 1 and 2. Verlag Chemie, Weinheim (1970).
22. F. Leighton, B. Poole, H. Beaufay, P. Baudhuin, J. W. Coffey, S. Fowler and C. De Duve, *J. Cell Biol.* **37**, 482 (1968).
23. H. Beaufay, A. Amar-Costesec, E. Feytmans, D. Thinès-Sempoux, M. Wibo, M. Rokki and J. Berthet, *J. Cell Biol.* **61**, 188 (1974).
24. A. Amar-Costesec, H. Beaufay, M. Wibo, D. Thinès-Sempoux, E. Feytmans, M. Robbi and J. Berthet, *J. Cell Biol.* **61**, 201 (1974).
25. G. L. Sottocasa, B. Kuylenstierra, L. Ernster and A. Bergstrand, *J. Cell Biol.* **32**, 415 (1967).
26. S. J. Cooperstein and A. Lazarow, *J. biol. Chem.* **189**, 665 (1951).
27. L. De Duve and P. Baudhuin, *Physiol. Rev.* **46**, 323 (1966).
28. D. M. Jerina, P. M. Dansette, A. Y. H. Lu and W. Levin, *Molec. Pharmac.* **13**, 342 (1977).
29. I. Mertes, R. Fleischmann, H. R. Glatt and F. Oesch, *Carcinogenesis* **6**, 219 (1985).
30. J. Meijer and J. W. DePierre, *Eur. J. Biochem.* **150**, 7 (1985).
31. J. Seidegård, J. W. DePierre, and R. W. Pero, *Cancer Res.* **44**, 3654 (1984).
32. B. D. Hammock, S. S. Gill, S. M. Mumby and K. Ota, in *Molecular Basis of Environmental Toxicity* (Ed. R. S. Bhatnagar), p. 229. Ann Arbor, Ann Arbor Science Publishers (1980).
33. F. Oesch, H. R. Glatt and H. U. Schmassmann, *Biochem. Pharmac.* **26**, 603 (1977).
34. S. S. Gill and B. D. Hammock, *Nature, Lond.* **291**, 167 (1981).
35. S. S. Gill, K. Ota, B. Ruebner and B. D. Hammock, *Life Sci.* **32**, 2693 (1983).
36. S. S. Gill, *Biochem. biophys. Res. Comm.* **122**, 1434 (1984).
37. F. Waechter, P. Bentley, F. Bieri, W. Stäubli, A. Völkl and H. D. Fahimi, *FEBS Lett.* **158**, 225 (1983).
38. B. D. Hammock and H. Ota, *Toxic. appl. Pharmac.* **71**, 254 (1983).
39. F. Waechter, F. Bieri, W. Stäubli and P. Bentley, *Biochem. Pharmac.* **33**, 31 (1984).
40. R. Hess, W. Stäubli and W. Riess, *Nature, Lond.* **208**, 856 (1965).
41. S. D. Barnard, J. A. Molello, W. J. Caldwell and J. E. Le Beau, *J. Toxic. Envir. Health* **6**, 547 (1980).
42. D. E. Moody and J. K. Reddy, *Am. J. Pathol.* **90**, 435 (1978).
43. S. S. Gill and B. D. Hammock, *Biochem. biophys. Res. Comm.* **89**, 965 (1979).
44. N. Chacos, J. Capdevilla, J. R. Falck, S. Manna, C. Martin-Wixtrom, S. S. Gill, B. D. Hammock and R. W. Estabrook, *Archs Biochem. Biophys.* **223**, 639 (1983).