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ORGAN DISTRIBUTION OF EPOXIDE HYDROLASES IN CYTOSOLIC AND MICROSOMAL FRACTIONS OF NORMAL AND NAFENOPIN-TREATED MALE DBA/2 MICE

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Abstract—Using *trans*-stilbene oxide and styrene oxide as substrates, epoxide hydrolase activities were measured in cytosolic and microsomal fractions from liver, kidney, heart, lung and testis of male DBA/2 mice. The activities towards these two substrates are remarkably organ specific: *trans*-stilbene oxide was most effectively hydrolyzed in subcellular fractions from liver, kidney and heart, whereas styrene oxide was predominantly hydrolyzed in those from liver, lung and testis.

Immunoblotting experiments were performed with two polyclonal antibodies isolated from goat antisera. Using an anti-mouse liver cytosolic epoxide hydrolase antibody, the corresponding antigen protein was predominantly detected in both cytosolic and microsomal fractions from liver, kidney and heart. An anti-rat liver microsomal epoxide hydrolase antibody proved to be cross-reactive with the mouse enzyme and stained SDS-gels run with microsomal fractions from liver, lung and testis.

The anti-mouse liver cytosolic epoxide hydrolase antibody precipitated cytosolic epoxide hydrolase activities from liver, kidney and heart cytosolic fractions.

Dietary exposure to the hypolipidemic agent nafenopin (2000 ppm/10 days) caused an induction of *trans*-stilbene oxide hydrolase and styrene oxide hydrolase activities in cytosolic and microsomal liver fractions whereas, in the other organs, the same activities were unaffected by this treatment. This finding was in accordance with the increased amounts of antigen protein as detected with the antibodies in liver fractions from treated animals. The anti-mouse liver cytosolic epoxide hydrolase antibody was found to precipitate the whole *trans*-stilbene oxide hydrolase activity also from liver cytosol of nafenopin-treated mice, which indicates the presence of a single cytosolic epoxide hydrolase following induction.

Epoxide hydrolases (EC 3.3.2.3) catalyze the hydrolysis of a wide variety of arene and alkene oxides to their corresponding *trans*-dihydrodiols. The enzyme activities vary with the substrate used and have been found mainly in microsomal and cytosolic fractions of the liver and other organs in different species. In the last few years much attention has been given to liver cytosolic epoxide hydrolase which exhibits markedly different properties from its well characterized microsomal counterpart. Both enzymes catalyze the hydrolysis of mono-substituted epoxides, but the cytosolic enzyme also catalyzes the hydrolysis of *trans*-1,2-disubstituted, tri- and even tetrasubstituted oxiranes, which are poor substrates for the microsomal hydrolase [1–4]. High activities are found in soluble liver fractions from mice and rabbits [2, 5], whereas the activity found in rat liver cytosol is much lower [2, 6–8].

Using TSO† or *trans*-β-ethylstyrene oxide as substrates, more than half of the total mouse liver epoxide hydrolase activity (60%) was found in the cytosolic fraction, whereas the remaining 40% were recovered in different organelle fractions [9, 10]. The organelle-bound TSO hydrolase activity seems to be localized mainly in peroxisomes as revealed by density gradient centrifugation experiments [10–12]. Immunological evidence as well as isoelectric focusing experiments suggest that the cytosolic and organelle-bound enzymes might be very similar if not identical [13, 14].

Of interest in connection with this subcellular localization was the discovery, that the activity of TSO hydrolase as well as *trans*-β-ethylstyrene oxide hydrolysis are inducible in mouse liver cytosolic fractions following administration of compounds known to cause peroxisome proliferation [15–18]. A series of other xenobiotics, known to induce microsomal epoxide hydrolase and other xenobiotic-metabolizing enzyme activities did not induce cytosolic hydrolase activity [15, 18].

In mice, TSO hydrolase activity was also found in cytosolic fractions from several extrahepatic tissues. The greatest specific hydrolysis rate was found in liver followed by kidney, testis and lung [17]. The kidney activity is induced by the peroxisome proliferator clofibrate [17], and appears to be closely related to the liver enzyme [14, 17].

The organ distribution of TSO hydrolase activity and its inducibility have recently also been assessed in rat cytosolic fractions. In this species the specific

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† Abbreviations used: TSO, *trans*-stilbene oxide; SO, styrene oxide; cEH, the enzyme antigen recognized by an antibody against purified mouse liver cytosolic TSO hydrolase; mEH, the enzyme antigen recognized by an antibody against purified rat liver microsomal SO hydrolase; PBS, phosphate buffered saline.

activities were generally much lower [6–8]. Concerning the organ distribution in male Sprague–Dawley rats, partially contradictory results have been reported: activities in decreasing order were heart ≈ kidney > liver ≫ lung > testis [7] or kidney ≈ liver ≈ testis [6]. Following treatment with clofibrate, cytosolic TSO hydrolase activities were found to be induced in liver and kidneys [6, 7]. In male Fischer F-344 rats, the activity was found to be higher in kidneys than in the liver [8]. Known peroxisome proliferators were able to induce liver and kidney activities also in this strain [8].

The aim of the present study was to investigate the organ distribution of TSO hydrolysis in cytosolic and microsomal fractions of male DBA/2 mice following administration of nafenopin, a compound known to cause hepatic peroxisome proliferation and to induce hepatic peroxisomal β-oxidation [19, 20]. A polyclonal antibody against mouse liver cytosolic epoxide hydrolase was raised in goats and used in immunoblotting and immunoprecipitation experiments to characterize further the nature of TSO hydrolase activity in these subcellular fractions. To permit a direct comparison of the distribution with that of mEH, the same organ fractions were also investigated for SO hydrolysis and with a goat anti rat liver microsomal SO hydrolase antibody, which proved to be cross-reactive with the mouse enzyme.

MATERIALS AND METHODS

Chemicals. [8-^{14}C]Styrene oxide (Amersham International, Buckinghamshire, U.K.) was diluted with unlabelled styrene oxide and repurified by extraction and distillation. The final specific radioactivity was 4.44 MBq/mmol. [7-^{14}C]-*trans*-stilbene oxide (13.5 MBq/mmol) was provided by Dr W. Küng (Ciba-Geigy Ltd). Nafenopin (2-methyl-2-[*p* - (1,2,3,4 - tetrahydro - 1 - naphthyl)phenoxy]-propionic acid, SU-13437) and PBS (0.01 M sodium phosphate, pH 7.0, containing 0.145 M sodium chloride) were provided by Ciba-Geigy Ltd. All other reagents were of the highest purity available.

Animals and treatment. Male DBA/2 mice (22–28 g) were obtained from GR Bomholdgard (Ry, Denmark). Control animals had free access to water and a normal diet (Nafag No. 890, Nafag, Gossau, Switzerland). Animals were treated for 10 days with free access to the same diet containing 2000 ppm nafenopin.

Preparation of subcellular fractions. After termination of the treatment, the animals were fasted overnight and then killed by exsanguination. Livers, kidneys, hearts, lungs and testes were removed, immediately frozen in liquid nitrogen and then stored at −60°. For the preparation of subcellular fractions, the tissue samples were thawed to 0° and homogenized in 5 mM 3-[*N*-morpholino]propanesulfonic acid, pH 7.4, containing 0.25 M sucrose, 1 mM EDTA and 0.1% ethanol (0.2 g tissue/ml). Microsomal and cytosolic fractions were prepared by centrifugation of a 10,000 *g* supernatant for 1 hr at 100,000 *g*. The microsomal pellets were resuspended in the same buffer.

Biochemical determinations. Epoxide hydrolase activities with TSO and SO as substrates were measured essentially as described [5, 16] under conditions where diol formation was linear with time and the protein content. Routine assays were performed at 37° and pH 6.8 (TSO hydrolase) or pH 9.0 (SO hydrolase). TSO was added to the incubation mixture (250 μl) in 5 μl ethanol. The protein contents were determined according to the method of Smith *et al.* [21] with serum albumin as the standard.

Preparation of polyclonal antibodies. Liver cytosolic TSO hydrolase (cEH) was purified to homogeneity from nafenopin-treated DBA/2 mice essentially as described for the rabbit liver cytosolic hydrolase [5]. An adult goat received several subcutaneous injections of 800 μg purified enzyme in Freund's complete (first immunization) or incomplete adjuvant, separated by a two month interval. Serum was collected 7 or 10 days after each immunization, immunoglobulin fractions were isolated [22] and the response was tested by means of Ouchterlony double-diffusion and dot-blot analysis with the purified antigen (data not shown). Goat antibodies against rat liver microsomal SO hydrolase (mEH) were prepared as described [23].

Immunoblotting. SDS–polyacrylamide gel electrophoresis (mini gels) was performed as described by Laemmli [24]. Gels were equilibrated in blotting buffer (25 mM Tris, 192 mM glycine, 20% methanol, pH 8.5) for 10–20 min and then transferred onto nitrocellullose paper (1 hr, 100 V). The blots were blocked with 10% horse serum in PBS for 2 hr and washed in PBS. Incubations with the antibodies (5 μg/ml in PBS containing 10% horse serum) were performed overnight followed again by a PBS-wash. The nitrocellulose sheets were then incubated with PBS containing 10% horse serum and 1 : 1000 diluted rabbit anti goat IgG-peroxidase conjugate (Miles Scientific, Cavenago, Italy) for 2 hr. Visualizing was performed with 4-chloro-naphthol/H_2O_2 (47 ml PBS + 3 ml 0.3% 4-chloro-naphthol in methanol + 20 μl 37% H_2O_2). After 15 min incubation the reaction was stopped with water.

Immunoprecipitation of cytosolic TSO hydrolase. Five to twenty microlitres of mouse liver, kidney or heart cytosol were incubated with 0–300 μg goat anti mouse cEH antibody in a final volume of 50 μl PBS. Unspecific goat IgG (Miles Scientific, Cavenago, Italy) was added to all tubes so that the total amount of IgG was 300 μg. After 6 hr incubation at room temperature, 500 μg rabbit anti goat IgG (Miles Scientific, Cavengo, Italy) in PBS were added to give a final volume of 200 μl. The tubes were incubated for 18 hr at 3°, centrifuged and the supernatants were assayed for TSO and eventually also SO hydrolyase activity. Blanks contained the same amounts of antibodies in the absence of cytosol.

RESULTS AND DISCUSSION

TSO and SO hydrolase activities

TSO and SO hydrolase activities were assayed in cytosolic and microsomal fractions from different tissues of untreated DBA/2 mice at pH 6.8 and pH 9.0, respectively (Table 1). In cytosolic fractions, the observed organ distribution was independent of the substrate used. The highest activity with both

Table 1. The effect of nafenopin-treatment upon TSO and SO hydrolase activities in cytosolic and microsomal fractions from different tissues of male DBA/2 mice†

Tissue	Treatment	Specific activity nmol TSO/(mg protein × min) pH 6.8		Specific activity nMol SO/(mg protein × min) pH 9.0	
		Cytosol	Microsomes	Cytosol	Microsomes
Liver	Control	5.5 ± 0.3	1.4 ± 0.1	0.39 ± 0.04	1.7 ± 0.3
	Nafenopin	11.4 ± 1.0**	2.1 ± 0.2**	0.91 ± 0.07**	8.3 ± 0.8**
Kidney	Control	2.8 ± 0.4	0.93 ± 0.16	0.25 ± 0.01	0.13 ± 0.06
	Nafenopin	3.4 ± 0.3*	1.1 ± 0.3	0.30 ± 0.02*	0.17 ± 0.08
Heart	Control	0.90 ± 0.26	0.40 ± 0.11	0.088 ± 0.031	0.041§
	Nafenopin	1.3 ± 0.1	0.29 ± 0.04	0.11 ± 0.02	0.062§
Lung	Control	0.024 ± 0.006	0.057 ± 0.011	N.D.‡	0.46 ± 0.07
	Nafenopin	0.045 ± 0.004**	0.052 ± 0.013	N.D.	0.31 ± 0.10*
Testis	Control	0.033 ± 0.005	0.057 ± 0.007	N.D.	1.0 ± 0.1
	Nafenopin	0.038 ± 0.008	0.074 ± 0.009*	N.D.	0.62 ± 0.07*

* $P < 0.05$, ** $P < 0.01$ (Student's t-test).
‡ Not detectable.
§ Pooled microsomal fractions of four control or four treated animals.
Values are means ± SD of four control or four treated animals.

substrates was found in the liver followed by kidney and heart. Cytosolic fractions from lung and testis had much lower TSO hydrolase activities (less than 0.5% of the hepatic activity) such that SO hydrolysis could not be detected under routine assay conditions. High levels of soluble epoxide hydrolase activity have also been found in livers and kidneys of Swiss-Webster mice [2, 17] as well as in livers of C57Bl/6, Balb C and C3H mice [16]. The mouse heart is obviously a third organ, which exhibits substantial cytosolic epoxide hydrolase activity.

The organ distribution of epoxide hydrolase activity in microsomal fractions was strongly dependent on the substrate used. With TSO, the distribution of microsomal TSO hydrolysis was found substrates in cytosolic fractions: liver > kidney > heart ≫ lung ≈ testis. A comparable organ distribution of microsomal TSO hydrolysis was found when livers, kidneys, lungs and testes from Swiss–Webster mice were investigated [17]. However, the specific activities in DBA/2 mice were considerably higher than those of Swiss–Webster mice. The use of unwashed microsomal fractions in our experiments could at least partially account for this difference [25]. In liver, kidney and heart, the protein-specific TSO hydrolase activities in microsomes were 2–4 times lower than those in the corresponding cytosolic fractions.

When microsomal epoxide hydrolysis was measured with SO at pH 9.0, a different organ distribution emerged. The highest activity was again found in the liver followed by testis > lung > kidney > heart. Using *cis*-stilbene oxide or benzo(*a*)pyrene-4,5-oxide as substrates, a comparable organ distri-

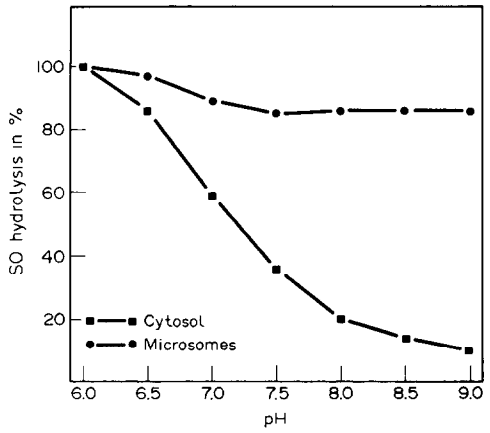


Fig. 1. Liver SO hydrolase activity as a function of pH. Activity measurements were performed in 0.125 M potassium phosphate buffer with cytosolic and microsomal fractions from untreated DBA/2 mice. Values are given in per cent of the maximal activities which were 3.2 nmol/(mg protein × min) for the cytosolic fraction and 2.3 nmol/(mg protein × min) for the microsomal fraction.

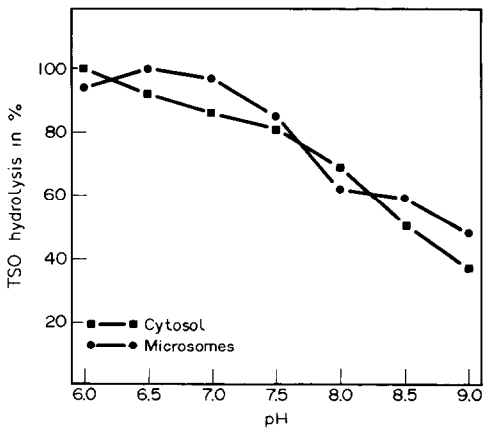
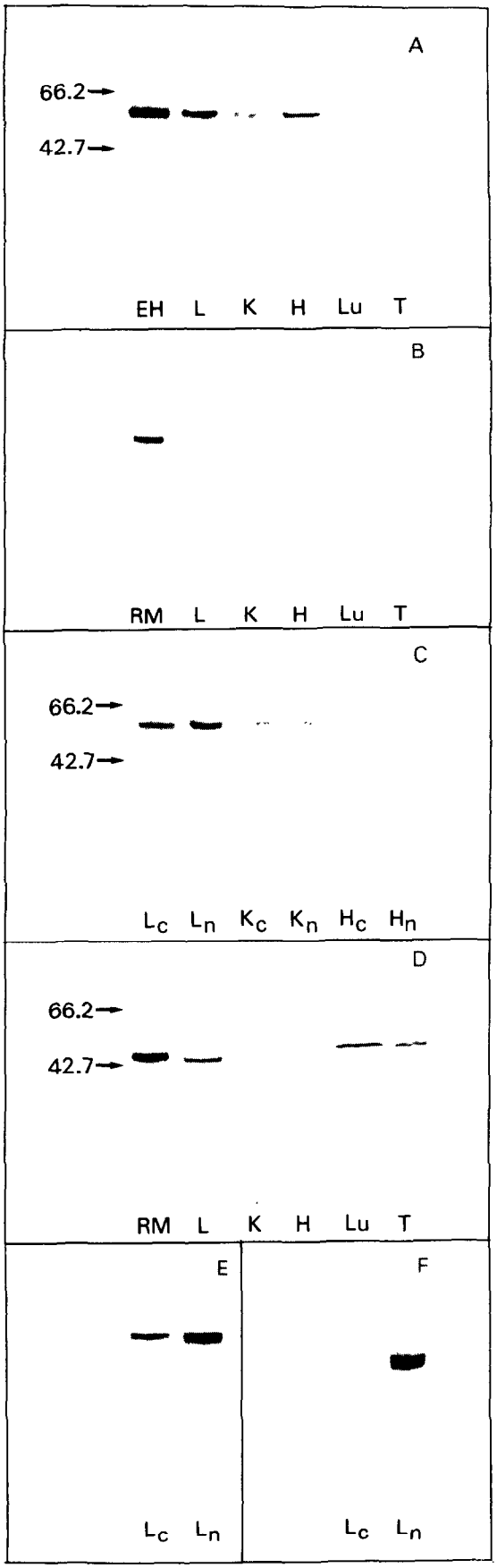


Fig. 2. Liver TSO hydrolase activity as a function of pH. Activity measurements were performed in 0.125 M potassium phosphate buffer with cytosolic and microsomal fractions from untreated DBA/2 mice. Values are given in per cent of the maximal activities which were 4.3 nmol/(mg protein × min) for the cytosolic fraction and 1.3 nmol/(mg protein × min) for the microsomal fraction.



bution (testis > liver > lung ≫ kidney) was found in microsomal fractions of Swiss–Webster mice [17].

The pH dependence of TSO and SO hydrolysis was investigated in liver cytosolic and microsomal fractions (Figs 1 and 2). The activity of microsomal SO hydrolase was fairly constant between pH 6.0 and 9.0 whereas the cytosolic activity decreased by a factor of ten in the same pH range (Fig. 1). Thus, at pH 7.0 microsomal and cytosolic liver fractions had similar specific SO hydrolase activities. In mice, a genetic polymorphism has been described for liver microsomal SO hydrolase [26]. With DBA/2J mice, these authors found a maximal activity around pH 8.7 with a slow decrease towards lower pH values and a sharp decrease above pH 9.0. When TSO was used as substrate, cytosolic and microsomal liver fractions showed a very similar pH dependence, namely a decrease by a factor of 2–2.5 from pH 6.0 to 9.0 (Fig. 2). Very similar pH dependences of liver cytosolic and microsomal TSO hydrolase activities have been observed in C57Bl/6 mice [27].

As expected, feeding DBA/2 mice a diet containing 2000 ppm nafenopin for 10 days caused a marked increase in the absolute liver weights (190% with respect to the controls) whereas the body weights were not affected (data not shown). The effect of nafenopin treatment on TSO and SO hydrolase activities was more or less restricted to cytosolic and microsomal fractions of the liver (Table 1). Activities in the other organs were, if at all, only slightly affected (TSO and SO hydrolysis in cytosolic kidney fractions, TSO hydrolysis in testicular microsomes) and/or remained very low although they were induced following treatment with nafenopin (TSO hydrolysis in cytosolic fractions from lung and testicular microsomes). In this respect it is of interest that the TSO hydrolase activity of testis microsomal fractions was slightly induced, whilst the SO hydrolase activity of these fractions appeared to be reduced by nafenopin treatment.

The inducibility of liver cytosolic and microsomal epoxide hydrolase activities in different mouse strains has also been demonstrated with other peroxisome proliferators such as clofibrate [15, 17], di-(2-ethylhexyl)phthalate [17], 2-ethyl-1-hexanol [17]

Fig. 3. Immunochemical determination of cEH and mEH in cytosolic and microsomal fractions from different organs. Abbreviations: Purified mouse liver cEH (EH), rat liver microsomes (RM), liver (L), kidney (K), heart (H), lung (Lu) and testis (T). The positions of the molecular weight markers are indicated by arrows. (A) Cytosolic fractions from untreated mice and goat anti-mouse liver cEH. Samples of 10 μg (L, K, H) or 50 μg protein (Lu, T) were applied. (B) Cytosolic fractions from untreated mice and goat anti-rat liver mEH. Samples of 15 μg (RM) or 50 μg protein were applied. (C) Microsomal fractions from untreated (c) or nafenopin-treated (n) mice and goat anti-mouse liver cEH. Samples of 30 μg protein were applied. (D) Microsomal fractions from untreated mice and goat anti-rat liver mEH. Samples of 15 μg (RM) or 30 μg protein were applied. (E) Liver cytosolic fractions from untreated (L_c) or nafenopin-treated (L_n) mice and goat anti mouse liver cEH. Samples of 10 μg protein were applied. (F) Liver microsomal fractions from untreated (L_c) or nafenopin-treated (L_n) mice and goat anti-rat liver mEH. Samples of 20 μg protein were applied.

and chlorinated phenoxyacetic acids [18]. Clofibrate, a structural analogue of nafenopin, has been tested for its potency to induce epoxide hydrolase in extrahepatic tissues of Swiss–Webster mice [17]. These authors found an induction of cytosolic TSO hydrolysis in kidney whilst the activities in lung and testis remained unchanged. This is in contrast to our observation that nafenopin treatment induced cytosolic hydrolase in the lung. Whether this dissimilarity reflects a strain difference or is due to different inducing properties of nafenopin and clofibrate is not known. A strong inducing effect of peroxisome proliferators on cytosolic epoxide hydrolase activity has been demonstrated in rat liver and kidney [6–8], whereas the testicular activity remained unchanged in this species [6]. However, it must be borne in mind, that the specific cytosolic epoxide hydrolase activities in untreated rats are much lower (<2%) than those found in mice [6–8].

The nature of epoxide hydrolase activity in cytosolic fractions

Using a goat anti-mouse liver cEH antibody, immunoblots were performed with cytosolic fractions from liver and extrahepatic tissue (Fig. 3a). In agreement with the activity measurements, the antigen was present in liver, kidney and heart. The apparent molecular weight was 57 kD which is similar to the value of 60 kD reported for cEH purified from C57Bl/6 mice [28]. In lung and testis, which have very low cytosolic epoxide hydrolase activities (about 3% of that found in the heart), the antigen could not be detected, most likely because the amount of protein (50 μg compared to 10 μg for liver, kidney and heart) applied on the gel was too low. Immunoblots with the same cytosolic fractions were performed with a goat anti rat mEH antibody (Fig. 3b). As expected, the corresponding antigen was not present in cytosolic fractions, although the antibody was shown to be cross-reactive with the mouse microsomal epoxide hydrolase (Fig. 3d).

The goat antibody against mouse liver cEH quantitatively precipitated TSO hydrolase activity from liver, kidney and heart cytosolic fractions (Fig. 4) and was also able to precipitate SO hydrolase activity from liver cytosol (data not shown).

Taken together, these immunological observations suggest, that one single enzyme is responsible for the hydrolysis of TSO and SO in cytosolic fractions from mouse liver, kidney and heart. This conclusion is also in accordance with the biochemical data since: (i) The ratio of specific activity observed with TSO (assayed at pH 6.8) and SO (assayed at pH 9.0) is very similar (11.8 ± 2.0) in cytosolic fractions from these organs (Table 1). (ii) at pH 7.0, the ratio of specific activity for liver cytosol is 2.3 (Figs 1 and 2), a value which is similar to the ratio of 2.0, which has been reported for purified mouse cEH [4]. However, one has to keep in mind the possibility that the cEH recovered in cytosolic fractions arises from leaky peroxisomes where the enzyme could be localized predominantly in intact cells [10].

The increased liver cytosolic epoxide hydrolase activities observed following treatment with nafenopin (Table 1) are due to the presence of higher amounts of the same enzyme protein, as shown by

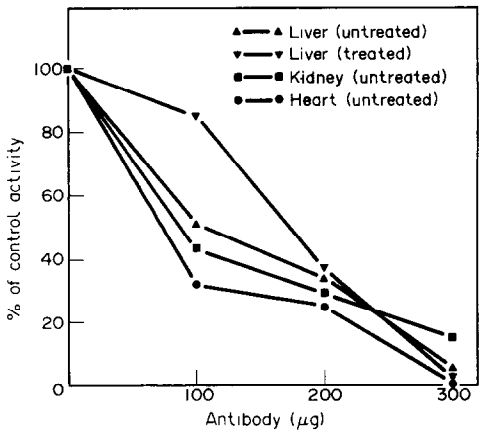


Fig. 4. Immunoprecipitation of cytosolic TSO hydrolase activity. Cytosolic fractions from livers of untreated and nafenopin-treated mice, as well as from kidney and heart of untreated mice were subjected to immunoprecipitation with goat anti mouse liver cEH. When expressed in nmol/(mg protein × min), 100% activity corresponds to 4.8 (liver cytosol from control mice), 7.3 (liver cytosol from nafenopin-treated mice), 4.2 (kidney cytosol) and 0.80 (heart cytosol).

immunoblotting (Fig. 3e) and immunoprecipitation (Fig. 4).

Some data on the immunological characterization of cEH in Swiss–Webster and C57Bl/6 mice have been published. Rabbit serum against purified cEH from Swiss mice stained one band on Western blots, when cytosolic fractions from control liver as well as from livers and kidneys of clofibrate-treated Swiss–Webster mice were investigated [14]. The same antiserum precipitated cytosolic TSO hydrolase from liver of untreated Swiss–Webster mice [13]. Following administration of di- and trichlorophenoxyacetic acid to C57Bl/6 mice, liver cytosolic TSO hydrolase activity was induced, and increased amounts of enzyme protein of the same molecular weight were found on Western blots by means of an antibody against cEH purified from the same mouse strain [18].

The nature of epoxide hydrolase activity in microsomes

Immunoblotting was also performed with the microsomal fractions from the different mouse organs. As in cytosolic fractions, the antibody against mouse liver cEH identified a 57 kD protein in liver, kidney and heart microsomes (Fig. 3c), whereas in lung and testis microsomes cEH could not be detected (data not shown). A different result was obtained with the antibody against rat liver mEH: the respective antigen, exhibiting a molecular weight similar to that of rat liver mEH, was also found in mouse liver, furthermore in lung and testis but not in kidney and heart microsomes (Fig. 3d). Obviously, only microsomal fractions from liver contain significant amounts of both cEH and mEH.

In contrast to cytosolic fractions, the ratios of the specific activity of TSO hydrolysis (measured at pH 6.8) and that of SO hydrolysis (measured at pH 9.0) in microsomal fractions (Table 1) are quite

different in the investigated organs (liver: 0.82, kidney: 7.2, heart: 9.8, lung: 0.12 and testis: 0.057). TSO is a *trans*-1,2-disubstituted epoxide and consequently a very poor substrate for mEH [29]. It is therefore reasonable to assume that at least in microsomal fractions from mouse liver, kidney and heart, TSO is almost exclusively hydrolysed by cEH. Furthermore, there is good evidence, that the cEH found in cytosolic fractions and that found in microsomal fractions are identical proteins. One might therefore conclude from the activity ratios in cytosolic fractions (Table 1), that the contribution of cEH to SO hydrolysis in microsomal fractions is negligible in lung and testis. In the liver, one can expect that this contribution is below 10% at pH 9.0 and around 30% at the more physiological pH 7.0 (Table 1, Fig. 1). However, in kidney and heart microsomes, the respective contributions are expected to be larger than 50% at pH 9.0 and presumably even higher at pH 7.0.

A TSO hydrolase, which is very similar if not identical to cEH, has also been found in crude mitochondrial fractions [14] and purified peroxisomes [10, 11] from mouse liver. Microsomal fractions which consist mainly of membraneous vesicles from the endoplasmic reticulum are also known to contain peroxisomes [30], and substantial amounts of the peroxisomal marker enzyme catalase [31, 32] are recovered in microsomal fractions [10, 30]. The ratio of activity catalase/TSO hydrolase as calculated from our published data [10] is very similar in microsomal fractions and purified peroxisomes. The microsomal TSO hydrolase activity as found in the liver, and perhaps also that found in kidney and heart, is therefore most probably localized in peroxisomes co-isolated with the membranes of the endoplasmic reticulum.

Following treatment with nafenopin, microsomal TSO hydrolase activity is marginally increased in the liver but not in kidney and heart (Table 1). In accordance with this finding, the amount of cEH antigen is slightly increased only in liver microsomes from treated animals (Fig. 3c). The prominent effect of nafenopin on liver microsomal SO hydrolase activity (Table 1) is reflected in the presence of much larger amounts of mEH antigen in these fractions (Fig. 3f).

Possible biological significance of cEH

Among the five organs investigated in DBA/2 mice, significant amounts of both epoxide hydrolases, mEH and cEH, are found only in the liver. The other tissues contain appreciable amounts of either mEH (lung and testis) or cEH (kidney and heart).

Many exogenous compounds are metabolized in a first step by cytochrome P-450 dependent monooxygenases to electrophilic and highly toxic epoxides [33, 34]. With its broad substrate specificity, mEH is thought to play an important role in the inactivation of metabolically formed epoxide intermediates [33–35].

The biological function of cEH is not known. However, some properties of this enzyme, as elaborated within the last few years, open interesting perspectives. Mouse liver peroxisomes, for example, were shown to contain significant amounts of cEH [10, 11]. It has also been shown that murine and rat liver cEH are inducible by peroxisome proliferators [6–8, 15–18], a unique class of compounds, known to stimulate the metabolism of lipids and fatty acids [36]. Moreover, *in vitro* experiments demonstrated the ability of cEH to hydrolyse compounds like *trans*-epoxymethylstearate [37], epoxyeicosatrienoic acids [38] or leukotriene A_4 [39]. It has therefore been speculated that cEH might participate in the metabolism of (poly)unsaturated fatty acids [38, 39]. With respect to the biological significance of cEH, it would be useful to know the cell types in which this enzyme is present, e.g. in liver, kidney and heart. We are therefore currently investigating immunohistochemically the localization of cEH in different organs of the mouse (M. Germer and F. Waechter, manuscript in preparation).

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