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# INDUCTION OF CYTOSOLIC AND MICROSOMAL EPOXIDE HYDROLASES BY THE HYPOLIPIDAEMIC COMPOUND NAFENOPIN IN THE MOUSE LIVER

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**Abstract**—The repeated oral administration of nafenopin, a hypolipidaemic compound, at a dose of 100 mg/kg to male C57BL/6, DBA/2, Balb c and C3H mice caused an increase in the specific activity of liver cytosolic epoxide hydrolase, the activity of microsomal epoxide hydrolase was also increased in all except the C3H mice.

The dose dependence and the specificity of this induction was investigated in male DBA/2 mice. In the range of 10–200 mg/kg nafenopin the induction of the two hydrolase activities was found to increase with increasing doses of the test compound. Two other cytosolic enzyme activities, lactate dehydrogenase and glutathione *S*-transferase, remained essentially unchanged within the dose range investigated.

Epoxide hydrolase (E.C. 3.3.2.3) activity is found in the microsomal and cytosolic fractions of mammalian livers. The microsomal hydrolase has received much attention [1, 2] and has been purified from several species [3–6]. The cytosolic enzyme, although less well studied, has also recently been purified [7, 8]. Comparison of the two enzymes reveals marked differences in structural, catalytic and immunological properties [7–11]. In certain aspects the catalytic properties of the two hydrolases are the antipathy of each other, for example trans-substituted epoxides are well hydrolysed by the cytosolic enzyme, but not by the microsomal enzyme [12, 13], whereas chalcone oxides, potent inhibitors of the cytosolic enzyme [10], have been reported to activate the microsomal enzyme [14].

To our knowledge nothing is known about the inducibility of cytosolic epoxide hydrolase. However, the microsomal enzyme is known to be induced by many compounds, including several hepatocarcinogens such as aflatoxin $B_1$, 2-acetylaminofluorene, 3′-methyl-*N*,*N*-dimethyl-4-aminoazobenzene, phenobarbital or cyproterone acetate [15, 16].

Phenoxyisobutyric acid derivatives are a class of hypolipidaemic agents which induce liver tumors in rodents [17, 18]. Sub-chronic treatment with such compounds induces a unique hepatomegaly which is characterized by an increase in the number and size of hepatic peroxisomes and in the specific activity of several peroxisomal enzymes [19–21]. In the course of further studies of the effect of peroxisome proliferators on liver enzyme activities we have investigated microsomal and cytosolic epoxide hydrolases and report here the induction of both these activities following nafenopin treatment.

## MATERIALS AND METHODS

[7-$^{14}$C]-*Trans*-stilbene oxide was provided by Dr. W. Küng (CIBA-GEIGY Limited). [$^3$H]-Benzo(a)pyrene 4,5-oxide was a gift from Prof. F. Oesch (Mainz, F.R.G.). Male mice of the strains DBA/2, Balb c, C3H and C57BL/6, weighing 20 g, were obtained from the CIBA-GEIGY breeding station, maintained at 22 ± 2° and a relative humidity of 55 ± 5% in rooms exposed to alternating light–dark cycles of 12 hr.

Two different treatment and assay schedules were used. In initial experiments 4 different mouse strains were screened for inducibility. Eight male mice were treated daily with 100 mg/kg nafenopin†, adsorbed to carboxymethylcellulose, by gastric intubation for 5 days. Eight control animals received the vehicle alone. After the last application (day 5), animals were starved overnight and killed by exsanguination. Livers were removed, weighed and homogenized as a pool of 8 livers per group in 10 mM Tris buffer, pH 7.5, containing 0.25 M sucrose. For studies on dose dependence groups of 6 male DBA/2 mice were treated with the appropriate dose of nafenopin (10–200 mg/kg), adsorbed to carboxymethylcellulose, by gastric intubation for 7 consecutive days. Six mice received the vehicle alone and served as controls. Animals were starved and killed as described above, but in this case livers were homogenized separately.

In both cases microsomal and cytosolic fractions were prepared by centrifugation of a 12,000 *g* supernatant for 1 hr at 100,000 *g* as described previously [22]. Microsomal fractions were suspended in 10 mM Tris, pH 7.5, and centrifuged to produce washed microsomal fractions.

Cytosolic epoxide hydrolase activity was measured with [7-$^{14}$C]-*trans*-stilbene oxide (290 μCi/mmol) as substrate essentially as described by Oesch and Golan [23]. The assay was performed in 0.1 M phosphate buffer pH 6.8 with a *trans*-stilbene oxide con-

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† (= 2-methyl-2-[p-(1,2,3,4-tetrahydro-1-naphthyl)-phenoxy] propionic acid).

Table 1. Effect of 5 daily oral doses of 100 mg/kg nafenopin upon microsomal and cytosolic epoxide hydrolase activities in several mouse strains

| | Microsomal epoxide hydrolase | | Cytosolic epoxide hydrolase | | Lactate dehydrogenase | |
|---|---|---|---|---|---|---|
| Mouse strain | Control | Treated | Control | Treated | Control | Treated |
| C57BL/6 | 0.39 | 0.78 | 1.74 | 3.28 | 4210 | 3650 |
| DBA/2 | 0.88 | 1.46 | 1.73 | 3.65 | 4090 | 3680 |
| Balb c | 0.54 | 0.78 | 1.37 | 2.91 | 3430 | 3080 |
| C3H | 0.78 | 0.74 | 1.45 | 2.98 | 2970 | 3410 |

Results shown are specific activities, expressed as nmol product (min.mg protein)$^{-1}$ and represent measurements on a pool of eight mouse livers.

centration of 0.4 mM. Microsomal epoxide hydrolase activity was assayed with generally labelled [$^3$H]-benzo(a)pyrene 4,5-oxide (450 $\mu$Ci/mmol) as substrate (75 $\mu$M) in 0.1 M Tris–HCl buffer pH 9.0 as described by Schmassmann *et al.* [24]. Glutathione *S*-transferase activity was measured spectrophotometrically using 1-chloro-2,4-dinitrobenzene as substrate [25]. Lactate dehydrogenase was assayed as described [26]. Glutathione *S*-transferase and lactate dehydrogenase activities were both determined in the cytosolic fraction. Protein concentrations were estimated by the method of Lowry *et al.* [27] using bovine serum albumin (Armour Pharmaceutical Company, Chicago, Illinois) as standard.

## RESULTS AND DISCUSSION

The effect of nafenopin treatment upon the microsomal and cytosolic epoxide hydrolase activities of 4 different mice strains is shown in Table 1. Treatment resulted in an approximately 2-fold increase in cytosolic hydrolase activity in each of the tested strains. To our knowledge this is the first reported induction of cytosolic epoxide hydrolase and the results indicate that this enzyme is equally sensitive to the inductive effects of nafenopin in all the mouse strains tested. Lactate dehydrogenase activity was essentially unaffected by nafenopin treatment in the tested strains (Table 1). This indicates that the increase in cytosolic hydrolase activity is not an artefact caused by decreases in the cytosolic protein content. Somewhat different results were obtained with the microsomal epoxide hydrolase activity, which was increased about twofold in C57BL/6 mice, about 1.5-fold in DBA/2 and Balb c mice, but not increased at all in C3H mice. The results suggest that in C3H mice cytosolic and microsomal epoxide hydrolases are subject to different control mechanisms.

The effect of nafenopin treatment upon epoxide hydrolase activities was further investigated in DBA/2 mice. As shown in Table 2, nafenopin doses above 50 mg/kg resulted in marked increases in relative liver weight. Electron microscopic examination of liver sections demonstrated that this hepatomegaly was accompanied by the expected increase in the number of hepatic peroxisomes (data not shown). The content of microsomal protein was statistically significantly increased in all dosed groups, whereas the cytosolic protein content remained largely unchanged. Microsomal and cytosolic epoxide hydrolase activities were both increased in a dose related fashion (Fig. 1). However, the microsomal enzyme was induced to a greater extent than the cytosolic enzyme in the two highest dosage groups (4.6-fold compared to 2.9-fold). This finding is somewhat in contrast to the results obtained with only 5 consecutive doses of 100 mg/kg nafenopin (Table 1), in which case the same mouse strain showed a larger increase in cytosolic hydrolase activity (2.1-fold) than in the microsomal activity (1.7-fold). This is most likely the result of different time dependences for the induction of the two enzymes.

The increase in the epoxide hydrolase activities

Table 2. Effects of different daily oral doses of nafenopin on body weight, relative liver weight and liver microsomal and cytosolic protein content of male DBA/2 mice

| | Nafenopin dose (mg/kg) | | | | | |
|---|---|---|---|---|---|---|
| | 0 | 10 | 20 | 50 | 100 | 200 |
| Body weight (g) | 20.5 ± 1.3 | 20.6 ± 1.7 | 20.1 ± 0.7 | 21.2 ± 1.4 | 21.4 ± 1.2 | 21.5 ± 0.8 |
| Relative liver weight in (%) of body weight | 4.0 ± 0.3 | 4.1 ± 0.2 | 4.1 ± 0.4 | 6.1 ± 0.2** | 7.0 ± 0.2** | 7.6 ± 0.4** |
| Microsomal protein (mg/g liver) | 14.9 ± 2.0 | 23.7 ± 1.7** | 19.4 ± 2.4* | 23.7 ± 1.7** | 27.8 ± 2.2** | 24.9 ± 1.8** |
| Cytosolic protein (mg/g liver) | 87.3 ± 10 | 85.8 ± 6.2 | 90.3 ± 9.8 | 92.8 ± 4.2 | 79.1 ± 4.2 | 80.6 ± 6.6 |

Values are means ± S.D. and those marked with asterisks differ significantly (Student's *t*-test) from the corresponding control value: *$P < 0.01$, **$P < 0.001$.

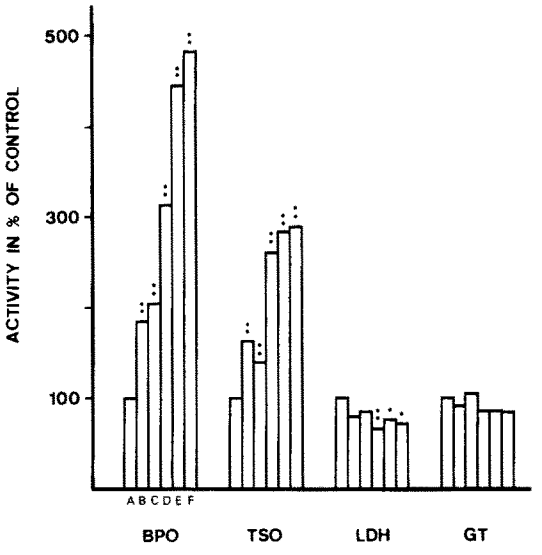


Fig. 1. Benzo(a)pyrene 4,5-oxide activities (BPO), *trans*-stilbene oxide activities (TSO), lactate dehydrogenase activities (LDH) and glutathione *S*-transferase activities (GT) as a function of the administered nafenopin dose. The specific activities are given in percent of the control values. The following specific activities in nMoles (min.mg protein)$^{-1}$ were observed in the control group: 1.36 (BPO), 1.72 (TSO), 5360 (LDH) and 4.96 (GT). Standard deviations within groups were smaller than 15% for all enzyme activities. Those bars marked with asterisks differ significantly (Student's *t*-test) from the corresponding control value ($^{*}P < 0.01$, $^{**}P < 0.001$). Doses administered (mg/kg): 0 (A), 10 (B), 20 (C), 50 (D), 100 (E) and 200 (F).

was not a consequence of reversible enzyme activation or stimulation by nafenopin (or its metabolites) present in the homogenate. Experiments were performed in which cell fractions from control and treated animals were mixed in different proportions and the measured hydrolase activities in these mixed fractions were always identical with those calculated from the individual fractions (results not shown).

Two other cytosolic enzymes, lactate dehydrogenase and glutathione *S*-transferase, were not affected by nafenopin treatment. Although glutathione *S*-transferase activity was assayed with only one substrate, a generalized effect of nafenopin upon cytosolic enzyme activities can be ruled out.

Induction of microsomal and cytosolic epoxide hydrolase activities by nafenopin is of interest and could imply a role for the hydrolases in lipid metabolism since nafenopin treatment is accompanied by a variety of alterations to hepatic lipid metabolism [28]. Several epoxides derived from unsaturated fatty acids are known to be substrates for cytosolic and microsomal epoxide hydrolases [29–31]. Particularly interesting in this respect is the finding that the physiologically important 5,6-epoxyeicosatrienoic acid is metabolised by the cytosolic rather than the microsomal enzyme [31].

An epoxide hydrolase closely related or identical to the cytosolic enzyme is also reported to be associated with the mitochondrial fraction [32]. Clofibrate, another hypolipidaemic compound, has been shown to increase the protein concentration of mitochondrial fractions [33]. However, in the four mouse strains investigated in the present study, nafenopin failed to increase specific rates of *trans*-stilbene oxide hydration by mitochondrial fractions (data not shown).

The effect of several phenoxyisobutyric acid derivatives upon microsomal enzyme activities has been investigated [21, 34, 35]. Generally, only slight changes were observed, although Stäubli et al. reported a doubling of the specific surface area of the smooth endoplasmic reticulum [36]. Recently, Orton and Parker [37] have shown that clofibrate treatment selectively induces $\omega$ and $\omega - 1$ oxidation of fatty acids by microsomal fractions. Thus, it would appear that the metabolic changes induced by hypolipidaemic compounds generally centre around lipid metabolism. In this respect nafenopin treatment provides an opportunity to induce epoxide hydrolase activity without markedly affecting the activities of other enzymes involved in foreign compound metabolism.

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