Sex Hormone-Related Control of Hepatic Epoxide Hydrolase Activities in Mice

Naoto INOUE, Kisa YAMADA, Kimie IMAI, and Tachio AMOTO

Faculty of Pharmaceutical Sciences, Setsunan University, 45-1 Nagaotoge-cho, Hirakata, Osaka 573-01, Japan.

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Sex-related differences in hepatic epoxide hydrolase (EH) activities towards 7-(2',3'-epoxy)propoxycoumarin (7-glycidoxycoumarin, GOC) were investigated, mainly in mice but also in rats.

Hepatic subcellular EH activities in the ddY mouse were higher in microsomes than in the soluble and mitochondrial fractions and sex-related differences were noted in all the subcellular fractions where males had significantly higher activities than females. Sex differences in the hepatic microsomal and soluble activities similar to those in the ddY strain were also observed in two other strains of mice, A/J and C3H/He, and in Wistar rats.

In the ddY strain, castration of the males caused decreases in microsomal and soluble EH activities, while no alteration in the activities in those fractions was found following castration of females. Treatment of the male castrates with testosterone led to recovery of the activities in microsomal and soluble fractions while hormone treatment of female castrates caused a rise only in microsomal activity. Estradiol treatments of castrates of both sexes did not cause any changes in the hepatic subcellular activities.

In intact ddY mice, testosterone treatment did not affect the male microsomal and soluble EH activities, but resulted in stimulation of both subcellular enzyme activities in females. In contrast, estradiol treatment showed a suppressive effect on both subcellular activities in males, but had no effect on female activities.

These results show that hepatic EH activities towards GOC are mainly under androgenic stimulatory control in mice.

Keywords: epoxide hydrolase; mouse liver; sex-related difference; castration; testosterone; estradiol

Epoxides have been implicated as proximate cytotoxins, mutagens, or carcinogens due to their highly electrophilic nature and are transformed non-enzymatically or enzymatically to normally less reactive glutathione conjugates and/or dihydrodiol metabolites within the organism. Two main enzyme systems are responsible for the metabolism of epoxides, i.e., epoxide hydrolase (EH) and glutathione S-transferases. At present, there are at least four different EHs in mammalian tissues. Two are apparently not involved in xenobiotic metabolism and include a microsomal EH specific for \( \Delta^2 \)-steroid epoxides and a cytosolic EH specific for leukotriene \( \Delta_4 \). The other two are another microsomal EH which hydrolyzes preferentially mono- and \( \Delta_1,2 \)-disubstituted epoxides and arene epoxides and another cytosolic EH which acts selectively on \( \Delta^2 \) epoxides. The activities of these latter two enzymes, catalyzing hydration of a large number of xenobiotic epoxides, are reported to vary with species, with strains in the rat, with sex in the mouse, rat and several other species, and with age in the mouse, rat, hamster and rabbit. We previously reported that the EH activity towards 7-(2',3'-epoxy)propoxycoumarin (7-glycidoxycoumarin, GOC) in the hepatic 9000 \( \times g \) supernatant fraction was higher in male than in female mice. However, little is known about the control mechanism of the sex-related differences in enzyme activity.

Therefore, the present experiments were undertaken to investigate further sex-related differences in hepatic EH activities towards xenobiotic epoxides in mice and to clarify the mechanisms by which the enzyme activities were controlled. The activities were determined using a convenient fluorophotometric method developed by Ishikawa et al. and Watabe et al. in which GOC was used as the EH substrate. We report here that the sex-related differences in the enzyme activities towards GOC are mainly under androgenic control.

Materials and Methods

Chemicals: GOC and 7-(2',3'-dihydroxy)propoxycoumarin (DHC) were synthesized in our laboratory according to the method of Watabe et al. Testosterone propionate and estradiol benzoate were purchased from Wako Pure Chemical Ind., Ltd. and Nacalai Tesque Inc., respectively. Panacete 810\( ^{\text{R}} \) was obtained from Nippon Oils & Fats Co., Ltd. All other chemicals were of reagent grade.

Animals and Treatments: Male and female mice of ddY, A/J and C3H/He strains and Wistar rats of both sexes were obtained from Japan SLC, Inc. In the study for development of EH activities, neonates, juveniles and adults of ddY mice, which were bred in our animal facility, were used. The animals were kept at a temperature of 22-23°C under a 12-h light-dark cycle (lights on 7:00 a.m.) with free access to tap water and laboratory chow (MF; Oriental Yeast Co., Ltd.). In the gonadal steroid replacement studies, four groups of ddY mice (7-8 weeks of age) of each sex were used. Three groups of them were castrated under pentobarbital anesthesia, while the rest were sham-operated. All animals were allowed to rest for 1 month prior to steroid administration. Two groups of castrates of each sex were then injected subcutaneously with either testosterone propionate (50 mg/mg Panacete 810\( ^{\text{R}} \)) or estradiol benzoate (2.5 mg/ml Panacete 810\( ^{\text{R}} \)) daily for 10 d. The other two groups, sham-operated animals and castrated control animals, were treated with an equivalent volume of vehicle alone.

In the other experiments, intact ddY mice (11-12 weeks of age) of both sexes were treated with either the androgen or estrogen in the same manner as described above. Control mice of both sexes received the vehicle alone. The animals were killed 24 h after the last treatment.

Preparation of Hepatic Subcellular Fractions: 1) Microsomal and Soluble Fractions: All animals were fasted overnight but allowed free access to water prior to sacrifice. They were decapitated between 9:00 and 10:00 a.m., and their livers were perfused in situ with ice-cold 1.15% KCl, quickly excised, minced and then homogenized in 2 volumes of ice-cold 1.15% KCl-50 mM potassium phosphate buffer (pH 7.4) using a Potter-Elvehjem type glass-Teflon homogenizer. The homogenate was centrifuged at 9000 \( \times g \) for 20 min and the resultant supernatant fraction was centrifuged at 105000 \( \times g \) for 1 h. The resulting pellet was suspended in the same buffer and used as the microsomal fraction, and the supernatant was used as the soluble (cytosolic) fraction.

2) Mitochondrial Fraction: Livers from ddY mice, treated as in 1), were quickly minced and homogenized with 9 volumes of ice-cold 0.25 M sucrose-5 mM Tris–HCl buffer (pH 7.4) to make a 10% homogenate.

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Isolation of mitochondria from the homogenate was carried out by the method of Utsumi.24

**Determination of Protein** The protein content of subcellular fractions was assayed by the method of Lowry et al.25

**Assay of EH Activity** 1) Microsomal Activity: Reaction mixture, consisting of 50 μM GOC, 100 μg microsomal protein and 160 mM Tris-HCl buffer (pH 9.0) in a total volume of 1.0 ml, was incubated at 37°C for 10 min. The reaction was stopped by cooling the incubate at 0°C and DHC formed was assayed as described by Ishikawa et al.26 and Watabe et al.23

2) Activities in Soluble and Mitochondrial Fractions: The reaction mixture consisted of 50 μM GOC, 100 μg protein of soluble fraction or 300 μg protein of mitochondria, and 160 mM potassium phosphate buffer (pH 8.0) in a total volume of 1.0 ml. Incubation conditions and determination of DHC were the same as those for microsomal EH.

**Statistical Analysis** The data were analyzed by Student's t test except the data obtained from the steroid replacement studies; these were assessed by analysis of variance procedures, and p values of less than 0.05 were considered to be significant.

**Results**

The EH activities towards GOC in the hepatic subcellular fractions of male and female ddY mice, 8 weeks of age, are shown in Fig. 1. The enzyme activities in microsomes of both sexes were much higher than those in the soluble and mitochondrial fractions. Sex-related differences in the activities were observed in all subcellular fractions examined with male activities being significantly greater than female activities. In particular, the microsomal activities in males were about 80% higher than those in females.

**Pattern of microsomal EH activities developing with age**

The EH activities of both sexes were observed by as early as 2 weeks after birth, but these were quite low. The activities of both males and females increased sharply after 3 weeks, reaching their maximum levels at 8 and 5 weeks of age, respectively, and then declined. After 13 weeks, further gradual decline of the male activity was observed.

![Fig. 1. Epoxide Hydrolase Activities in Hepatic Subcellular Fractions of ddY Mice](image1)

*M, male; F, female. Data are expressed as the mean with S.E. (vertical bars) of 6 mice. a) Significantly different from the corresponding males (p<0.05).*

![Fig. 2. Age- and Sex-Related Changes in Hepatic Microsomal Epoxide Hydrolase Activities of ddY Mice](image2)

*Each point represents the mean with S.E. (vertical bars) of 4-6 mice. a) Significantly different from the corresponding male groups (p<0.05).*

![Fig. 3. Epoxide Hydrolase Activities in Hepatic Subcellular Fractions from Three Different Strains of Mouse](image3)

*M, male; F, female; Mm, microsomes; SF, soluble fraction. Data are expressed as the mean with S.E. (vertical bars) of 6 mice. a) Significantly different from the corresponding males (p<0.05).*
although a steady level of enzyme activity was maintained in females. Males had significantly higher activities than females after 4 weeks and this sex-related difference was statistically significant through 26 weeks of age.

EH activities in hepatic microsomal and soluble fractions were determined in the other two strains, A/J and C3H/He, of 8-week-old mice as well as in the ddY strain. The results are given in Fig. 3. Sex-dependent differences in the enzyme activities, with males having higher activities than females, were also observed in each hepatic subcellular fraction from the A/J and C3H/He mice, as found in the ddY mice. The microsomal activities in the C3H/He mice of both sexes were somewhat lower than the corresponding activities in the ddY and A/J mice of both sexes (p < 0.05).

Enzyme activities in the Wistar rats of both sexes were also examined at ages of 4, 8, 11 and 13 weeks (Table I). Sex-related differences in the enzyme activities were noted in microsomal (13 weeks old) and soluble (11 and 13 weeks old) fractions where the activities were significantly higher in males than in females. The microsomal activities in rats of both sexes were comparable to or higher than those of mice shown in Fig. 2. In particular, the neonatal (4 weeks old) activities in microsomes of each sex were much greater than the corresponding values in mice of the same age (Fig. 2). On the other hand, the soluble EH in rats showed generally lower activities compared with those in mice (Fig. 1).

The effects of castration and replacement with gonadal steroid hormones on hepatic microsomal and soluble EH activities were investigated in the ddY mice of both sexes. As shown in Fig. 4, in microsomes, orchietomy resulted in a significant 34% decrease in enzyme activity to reach that in sham-operated (control) females, while ovariectomy did not cause any changes in enzyme activity. Testosterone replacement in castrated males brought the activity up to that in sham-operated (control) males that received only

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**Table 1. Epoxide Hydrolase Activities in Hepatic Subcellular Fractions of Wistar Rats**

<table>
<thead>
<tr>
<th>Age (wk)</th>
<th>Sex</th>
<th>Microsomes</th>
<th>Soluble fraction</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>Male</td>
<td>103.3 ± 4.2</td>
<td>3.4 ± 0.3</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>91.8 ± 4.6</td>
<td>2.5 ± 0.2</td>
</tr>
<tr>
<td>8</td>
<td>Male</td>
<td>75.0 ± 3.8</td>
<td>1.9 ± 0.1</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>73.6 ± 3.7</td>
<td>2.4 ± 0.4</td>
</tr>
<tr>
<td>11</td>
<td>Male</td>
<td>75.1 ± 3.7</td>
<td>6.1 ± 0.7</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>73.4 ± 4.9</td>
<td>3.1 ± 0.3b</td>
</tr>
<tr>
<td>13</td>
<td>Male</td>
<td>72.5 ± 2.0</td>
<td>4.3 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>57.7 ± 2.2b</td>
<td>1.8 ± 0.2b</td>
</tr>
</tbody>
</table>

The results are expressed as the mean ± S.E. of 6 rats. a) nmol DHC formed/mg protein/10 min. b) Significantly different from the corresponding males (p < 0.05).
vehicle. On the other hand, the treatment of castrated females with the hormone increased the enzyme activity by about 50% above that of control and castrated females and the activity reached same level as that in control males. In contrast to the testosterone treatment, no alteration in EH activities was detected following treatment of both male and female castrates with estradiol. Results similar to those microsomal activities were obtained in soluble activities following castration and steroid replacement of males, while such treatments did not cause any changes in the activities in females.

Effects of the androgen and estrogen administration on hepatic EH activities were also investigated in intact ddY mice of both sexes (Fig. 5). In microsomes, testosterone treatment caused no alteration in male activity but raised female activity to 35% above that of control females, whereas estradiol treatment suppressed significantly the male activity (57% of the control) but showed no effect on female activity. Results in the soluble fractions of both sexes similar to those in microsomes were also obtained after treatment with the steroid hormones.

Discussion

The present study has clearly shown that there are sex-dependent differences in EH activities towards GOC in three different subcellular fractions (mitochondria, microsomes and cytosol) of ddY mouse livers with the activities of males being significantly higher than females. These results were similar to the finding by Gill and Hammock in which cytosolic EH activity towards the substrate, 1-(4'-ethylphenoxype)3,7-dimethyl-6,7-epoxy-trans-2-ocetone, in the livers of Swiss-Webster mice was 2.3 times greater in males than in females.

A strain-related difference in EH activity, although not large, was observed only in the hepatic microsomes among three different strains, ddY, A/J and C3H/He, of mice of both sexes. This result disagrees with the results reported by Walker et al. who failed to find any strain-related differences in the hepatic microsomal EH activities with reference to three different substrates, styrene oxide, benzo[a]pyrene, 4,5-oxide and 1,2,3,4,9,9-hexachloro-6,7-epoxy-1,4,4a,5,6,7,8,8a-octahydro-1,4-methanonaphthalene, in male mice of four different strains including the A/J strain used in the present study. Thus, interstrain differences in hepatic EH activity seem to be small or absent in mice.

As for rat EH, similar sex-related differences were also noted in hepatic microsomal and cytosolic activities, but the differences were smaller than those observed in mice. A significant difference was observed as late as 11 (cytosol) and 13 (microsomes and cytosol) weeks after birth, in agreement with the result of Chengelis where it was not until 11 weeks that the hepatic microsomal activities, with respect to styrene oxide, of males became significantly higher than those of females.

In mice, the microsomal EH level of males, higher than that of females during young period, declined gradually to the female level with age and was no longer significantly different from that of females at 37 weeks. This seems to be related to feminization of male gonadal function, resulting in a decreased secretory activity of androgen from the testes. Thus, an androgen such as testosterone, a predominant gonadal steroid hormone secreted by male mice, seems to be involved in the control of EH activity.

Orchietomy reduced hepatic EH activities to the same levels as those of intact females and treatment of male castrates with testosterone raised the activities to those of intact male levels. Treatment of ovariectomized females with the hormone resulted in a stimulation of the enzyme activities surpassing control levels. Thus, the androgen plays an important role in the maintenance and/or stimulation of the enzyme activities. On the other hand, estradiol, an estrogen, seems to have a suppressive action on the enzyme by antagonizing the action of testosterone, since exogenous testosterone caused only a moderate stimulation in intact females compared with a marked stimulation in female castrates. Thus, hepatic EH activities of mice are under androgenic stimulatory control.

References

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