Involvement of Pituitary Hormone in the Sex-Related Regulation of Hepatic Epoxide Hydrolase Activity in Mice

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The involvement of the pituitary gland in the expression of sex-related differences in the activities of epoxide hydrolase (EH) in liver was examined in hypophysectomized and neonatally monosodium L-glutamate (MSG)-treated male and female mice.

In hypophysectomized mice, the hepatic microsomal EH activity of males and females increased considerably above those of the respective sham-operated controls, and the female activity exceeded that of the sham-operated males. The hypophysectomy caused a decrease in soluble EH activity in males, while the operation led to an elevation of the activity in females. Neonatal treatment with MSG resulted in marked increases of microsomal EH activity in males and females at adulthood and the activity was about 2 times that of the male control mice. Treatment of hypophysectomized males and females with growth hormone (GH) resulted in a significant decrease in microsomal EH activity below those of the corresponding hypophysectomized controls, while the hormone treatment had no effect on the soluble enzyme activities.

These results suggest that murine hepatic microsomal EH activity is principally under the suppressive control of the pituitary and GH is a causal hormone involved in the expression of sexual dimorphism in the enzyme activity.

Key words epoxide hydrolase; mouse liver; sex-related difference; hypophysectomy; growth hormone

It is well known that xenobiotic epoxides are non-enzymatically hydrated or enzymatically metabolized by epoxide hydrolases (EH) and glutathione S-transferases in organisms. In mammalian livers there exist two such EHs, a microsomal EH, hydrolyzing preferentially monosubstituted and *cis*-1,2-disubstituted epoxides and arene epoxides, and a cytosolic EH which acts selectively on *trans* epoxides.^{1,2)}

In our previous report, we have shown that hepatic subcellular EH activities towards 7-(2',3'-epoxy)propoxycoumarin (7-glycidoxycoumarin, GOC) are much higher in microsomes than in cytosol in mice of both sexes and that a sexual dimorphism in the activities, higher in males than in females, is observed and expressed mainly through an androgenic stimulation.³⁾

Several reports have demonstrated that the activities of murine hepatic monooxygenases are generally higher in females than in males,⁴⁻⁷⁾ and the growth hormone (GH) is shown to be involved in a suppressive regulation of the activities of monooxygenases.⁸⁾ Furthermore, well-known sexual dimorphisms observed in the rat hepatic monooxygenase system are under an indirect control by gonadal steroid hormones through a mediation of the hypothalamo-pituitary axis.⁹⁻¹¹⁾

Multiple treatment of neonates of mice and rats with relatively high doses of monosodium L-glutamate (MSG) has resulted in a well-defined syndrome of retarded growth, obesity, and reduced organ weights, accompanied by a selective secretory deficiency of GH from the pituitary.^{7,12-14)}

It is, therefore, of interest to examine whether the pituitary and GH contribute to the expression of sex-related differences in hepatic EH activities. The present experiments were carried out using hypophysectomized and neonatally MSG-treated mice of both sexes.

MATERIALS AND METHODS

Chemicals GOC and 7-(2',3'-dihydroxy)propoxycoumarin were synthesized in our laboratory according to the method of Watabe *et al.*¹⁵⁾ MSG and estradiol benzoate were purchased from Nacalai Tesque, Inc. Testosterone propionate was obtained from Wako Pure Chemical Ind., Ltd. and Panacete 810® was from Nippon Oils & Fats Co., Ltd. Human GH (Genotropin, genetical recombination) was obtained from Sumitomo Pharmaceuticals Co., Ltd. Other reagents were from commercial sources and were of the highest grade available.

Animals and Treatment Male and female ddY mice were purchased from Japan SLC, Inc. The animals were maintained under a standardized condition of light (7:00—19:00) and temperature (22—23 °C), and fed on laboratory chow (MF, Oriental Yeast Co., Ltd.) and tap water, which were both available *ad lib*. Mice of both sexes (7—8 weeks of age) were hypophysectomized or sham-operated under pentobarbital anesthesia by the transaural route, and were left to recover for at least a week. Thorough hypophysectomy was ascertained by inspection of the sella under a light microscope at the time of sacrifice. Hypophysectomized mice received a subcutaneous injection of either testosterone propionate (50 mg/ml Panacete 810®/kg) or estradiol benzoate (2.5 mg/ml Panacete 810®/kg) once daily for 10 d. Control (sham-operated) mice were treated with an equivalent volume of vehicle alone. The animals were killed 11 d after the initial injection. In another experiment, hypophysectomized and control mice were administered either GH (0.5 I.U./μl 2.4% glycine/kg) or an equivalent volume of 2.4% glycine twice a day for 7 d. The animals were killed 8 d after the initial injection.

In other studies, neonatal mice, bred in our animal facility, were treated with an intraperitoneal injection of MSG (4 g/10 ml 0.9% NaCl/kg) or 10% NaCl as an

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isosmotic control on 1, 3, 5, 7 and 9 d of age. The pups were weaned and sexed at the age of 21 d, and group-housed until 49 d of age.

Preparation of Liver Microsomal and Soluble Fractions

All animals were fasted overnight but allowed free access to tap water prior to sacrifice. They were decapitated between 9:00 and 10:00, and their hepatic microsomal and soluble fractions were prepared by differential centrifugation as previously reported.³⁾

Determination of Protein The protein content in the microsomal and soluble fractions was determined according to the method of Lowry *et al.*,¹⁶⁾ using bovine serum albumin as a standard.

Assay of EH Activity Microsomal and cytosolic hydroxylation of GOC were measured as reported previously.³⁾

Statistical Analysis Statistical analysis was done using analysis of variance procedures, and *p* values of less than 0.05 were considered to be significant.

RESULTS AND DISCUSSION

The EH activities towards GOC in hepatic microsomal and soluble fractions of hypophysectomized male and female mice are shown in Fig. 1. The activities of both subcellular fractions in the controls showed a sexual dimorphism, with higher activities in males than in females, in agreement with the results reported previously.³⁾

Hypophysectomy of males and females resulted in 46% and 77% increases in the enzyme activities of their hepatic microsomes, respectively. The gender dimorphism in the fraction disappeared with hypophysectomy. These results suggest that hepatic microsomal EH is under suppressive regulation by the pituitary, and the gland also contributes to the expression of the gender dimorphism observed in the EH activity. In relation to these results, Lee *et al.* have demonstrated a reverse effect of hypophysectomy on the testicular microsomal EH of rats: the operation caused a marked reduction in the EH activity towards [³H]-benzo[*a*]pyrene-4,5-oxide as compared to the sham-operated controls.¹⁷⁾ Therefore, it is thought that the pituitary might differently regulate microsomal EHs depending on tissues in which the enzymes are present. In the soluble fraction, male activity decreased, while female activity increased following the operation, but no significant difference in the activities was observed between sexes.

MSG treatment of the neonates of both sexes resulted in a suppression in body weight gain and pituitary growth, as compared with control animals treated with 10% saline only (data not shown), consistent with the reported results obtained with mouse^{7,12)} and rat.^{13,14)} The treatment caused marked increases in hepatic microsomal EH activity in male and female mice at the age of 49 d (Fig. 2). These results further support that the pituitary is a suppressor of hepatic microsomal EH. Neonatal MSG

Fig. 1. Effect of Hypophysectomy on EH Activities of Hepatic Subcellular Fractions

DHC, 7-(2',3'-dihydroxy)propoxycoumarin; Cont, control; Hx, hypophysectomized. Data are expressed as the mean with S.E. (vertical bars) of 10 mice. Data connected with horizontal lines are significantly different from each other (*p* < 0.05).

Fig. 2. Effect of Neonatal MSG Treatment on EH Activities of Hepatic Subcellular Fractions

Cont, 10% NaCl treatment; MSG, MSG (4 g/kg) treatment. Data are expressed as the mean with S.E. (vertical bars) of 6–10 mice. Data connected with horizontal lines are significantly different from each other (*p* < 0.05).

treatment selectively causes a markedly reduced secretion of GH from the pituitary, resulting in a low serum level of the hormone, without affecting the circulating levels of other hypophyseal hormones.^{13,14} Thus, GH seems to be a causal hypophyseal factor involved in the control of EH activity. In soluble fraction, male activity was suppressed by the treatment and was the same level as that of females, while no alteration in female activity was observed with the neonatal MSG treatment. The finding that a reduction of male soluble EH activity was observed after either hypophysectomy or neonatal MSG treatment was the reverse of the results obtained from microsomes. Thus, another mechanism, different from that for the control of microsomal EH, seems to be present in the control of cytosolic EH in males.

The effect of testosterone and estradiol, gonadal steroid hormones, on hepatic EH activities was investigated in hypophysectomized mice of both sexes. As shown in Fig. 3, neither male nor female microsomal EH responded to the testosterone treatment in hypophysectomized animals, indicating that the pituitary was necessary for the androgen action reported previously.³ On the other hand, estradiol caused a significant decrease in the microsomal activity of operated males, but the activity of females was not affected by the estrogen administration. Furthermore, no difference was observed between the activities of the estrogen-treated hypophysectomized females and the sham-operated controls. These results suggest that the estrogen has a direct suppressive action on microsomal EH, in addition to an indirect suppressive action *via* a mediation of the hypophysis. In hepatic soluble fraction, EH activities of hypophysectomized male and female mice significantly increased following treatment with testosterone, suggesting that the androgen has a direct stimulatory action on cytosolic EH, in addition to the stimulatory action dependent on the pituitary as described before. No alteration was noticed following the estradiol treatment of the operated mice of both sexes.

The effect of GH on the EH activities in hepatic subcellular fractions of hypophysectomized mice are shown in Fig. 4. The GH decreased the enzyme activity in microsomes by 51% and 78% in the hypophysectomized males and females, respectively, as compared with those

in the vehicle-treated hypophysectomized controls, suggesting again that GH is a possible hypophyseal factor involved in the control of EH activity. However, involvement of other pituitary hormone(s) in the control of the hepatic EH activity cannot be excluded, since thyroid hormone is also reported to contribute to the hormonal regulation of hepatic P450 isozymes in rats.^{18,19} On the other hand, no alteration was observed in the activities of the soluble fractions of the operated mice of both sexes.

The present results indicate that the pituitary seems

Fig. 3. Effect of Testosterone and Estradiol Treatment on EH Activities of Hepatic Subcellular Fractions in Hypophysectomized Mice

Cont, control; Hx, hypophysectomized; T, testosterone propionate (50 mg/kg) treatment; E, estradiol benzoate (2.5 mg/kg) treatment. Data are expressed as the mean with S.E. (vertical bars) of 5–8 mice. Data connected with horizontal lines are significantly different from each other ($p < 0.05$).

Fig. 4. Effect of GH Treatment on EH Activities of Hepatic Subcellular Fractions in Hypophysectomized Mice

Cont, control; Hx, hypophysectomized; GH, human GH (0.5 I.U./kg) treatment. Data are expressed as the mean with S.E. (vertical bars) of 4–10 mice. Data connected with horizontal lines are significantly different from each other ($p < 0.05$).

to exert a repressive control on murine hepatic EH activity through a mediation of GH, a possible hypothyseal hormone involved in the expression of a sexual dimorphism in the enzyme activities. In rats, it is well known that the secretion patterns of GH are different between males and females,²⁰⁾ and the difference is related to the expression of sex differences in the activities and quantities of hepatic P450 isozymes.²¹⁾ In mice, different patterns of GH secretion are also reported to be present between sexes,²²⁾ although the patterns are somewhat different from those in rats. Thus, the GH secretion pattern specific for sexes is thought to be involved in the expression of the sexual dimorphism in hepatic EH activities in mice, as in rats. However, this remains to be elucidated.

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