Biochemical Pharmacology, Vol. 38, No. 4, pp. 603–610, 1989.
Printed in Great Britain.

0006-2952/89 $3.00 + 0.00

HORMONAL REGULATION OF THE DEVELOPMENTAL PATTERN OF EPOXIDE HYDROLASES

STUDIES IN RAT LIVER

CHERYL L. DENLINGER and ELLIOT S. VESELL*
Department of Pharmacology, Pennsylvania State University College of Medicine, Hershey, PA 17033, U.S.A.

(*Received* 19 *May* 1988; *accepted* 18 *July* 1988)

Abstract—Hormonal influences on the developmental pattern of epoxide hydrolases (EH) were investigated in livers from male and female Fischer F-344 rats. During ontogeny, activities of rat liver microsomal and cytosolic epoxide hydrolases (cEH and mEH) increased gradually until puberty when activities in males rose rapidly to become 1.5-fold to 2.0-fold higher than those in females. These sex differences were not observed in the adult rat if males were castrated 24 hr after birth. In castrated males, as well as in females, testosterone propionate (TP, 0.5 mg s.c. in 50 μl peanut oil) injected on days 1, 3 and 5 postpartum increased mEH and cEH activities at adulthood compared to peanut oil-treated controls. In another study to determine effects on adults of neonatal exposure to a prototype-inducing agent, phenobarbital (PB, 30 mg/kg s.c., qd, in 0.9% saline) was injected in male and female neonates on days 1 through 5 postpartum. Although no long-term effect on mEH activities appeared on days 28, 45 and 60, hepatic mEH activities increased in all treatment groups on days 90 and 120. Collectively, these studies indicate that neonatal exposure to testosterone and PB alters the developmental pattern of EH activities, including final adult levels. Thus, full adult expression of these activities depends on hormonal influences exerted neonatally.

Epoxides are of interest in toxicology and chemical carcinogenesis because of their reactivity with cellular nucleophiles. In addition to reactions with proteins and nucleic acids, epoxides have several other potential fates: hydration to form *trans*-1,2-dihydrodiols [1, 2], catalyzed by the enzyme epoxide hydrolase (EH), located both on the endoplasmic reticulum and in the cytosol. This hydration has toxicological consequences since diols possess much less electrophilic reactivity than their corresponding epoxides. Many epoxides can exert toxic, mutagenic, and carcinogenic effects so that the ability of EH to convert epoxides to dihydrodiols constitutes a principal cellular defense mechanism in the detoxification of these reactive intermediates [1, 2]. Rarely, EH can act on a substrate, such as benzo[*a*]pyrene 7,8-oxide, to enhance, rather than diminish, its toxicity [3, 4]. Since many enzymes, including EH, exhibit low activity during the neonatal period, young animals may be particularly vulnerable to toxicity from reactive intermediates, and perturbations affecting EH activity in young animals could be of special toxicological significance.

In adult rats, sex-dependent differences occur in the hepatic oxidative metabolism of many substrates, including aminopyrine, ethylmorphine, hexobarbital [5–7], and endogenous steroids [8, 9]. Some aspects of these well documented differences are modulated by testosterone during and after sexual maturation. Adult castration of males generally decreases and testosterone treatment of females usually enhances hepatic drug-metabolizing enzyme activities [5–7].

A related aspect of sex-dependent differences in hepatic drug metabolism concerns the so-called "imprinting" influence of androgens that occurs during early postnatal life [10]. Imprinting refers to hormonal manipulations during the neonatal period, the effects of which are manifested much later in life. Both Gustafsson *et al.* [9] and later McEwen [11] proposed mechanisms whereby a fetal pattern of female metabolism is "masculinized" through release of androgens during the neonatal organizational period; this transformation is apparently mediated through hypothalamic regulation of pituitary secretion of growth hormone [9–13]. During the neonatal period critical for imprinting, exogenous chemicals (xenobiotics) can also affect maturation of the hypothalamic–pituitary–gonadal axis and, therefore, function as imprinting agents. For example, phenobarbital (PB), a classical barbiturate inducer of the monooxygenase system, imprints adult levels of several forms of hepatic cytochrome P-450, including those designated arylhydrocarbon hydroxylase [14] and ethylmorphine *N*-demethylase [15, 16].

Although EH is ubiquitous throughout mammalian tissues, knowledge of the hormonal control of the developmental pattern is still incomplete. Oesch [17] reported an approximately 4-fold increase from the 5-day male rat to adult levels of mEH. Adult males exhibited 2.5 to 3 times higher mEH activity than adult females, whereas neonates 5 days of age

* Corresponding author: Elliot S. Vesell, M.D., Evan Pugh Professor and Chairman, Department of Pharmacology, The Pennsylvania State University College of Medicine, P.O. Box 850, Hershey, PA 17033.

exhibited no sex difference in mEH. Gill and Hammock [18] observed an approximately 5-fold increase from 3-week-old male mice to adult levels of cEH, whereas females increased only 2-fold. A precise description of the regulation of these developmental patterns remains to be provided and constitutes main objectives of the present study, which also explores effects of various hormonal and environmental manipulations on this developmental pattern. Specifically, we examined and report effects of neonatally administered testosterone and a neonatally administered xenobiotic, PB, on the developmental pattern of EH activities.

MATERIALS AND METHODS

Animals and treatments. Adult male and female Fischer F-344 rats, weighing approximately 250 g and 175 g, respectively, were obtained from Charles River Laboratories (Wilmington, MA) for use as breeders. Rats were housed six per cage in stainless-steel, wire-bottomed cages without bedding in a temperature-controlled environment (12 hr light–dark cycle: 7:00 a.m. lights on) and were fed Purina Rodent Lab Chow and tap water *ad lib.*

Two females were mated with a randomly chosen male until the appearance of a vaginal sperm plug, which was considered day 1 of pregnancy. A week before delivery, females were housed separately in solid-bottom, stainless-steel cages with corn-cob bedding. Pups were born on days 23 or 24 and remained with their mothers until weaned at 24 days of age.

Studies involving neonatal castration and testosterone replacement were performed as follows. At birth, litters were divided into males and females. The males were then divided into two groups. Half of the males were castrated within 24 hr using the hypothermic technique of Pfeiffer [19], while others were sham-operated. The neonatally castrated group was further subdivided. Half of the castrates received testosterone propionate (TP, Sigma, 0.5 mg in 50 μl peanut oil) given s.c. on days 1, 3 and 5. The other neonatally castrated males received the vehicle (50 μl peanut oil) s.c. on days 1, 3 and 5.

Females were also divided into two groups. Half of the females received TP (0.5 mg in 50 μl peanut oil) s.c. on days 1, 3 and 5. The remaining females received the vehicle (50 μl peanut oil) s.c. on days 1, 3 and 5.

One sham-operated male, one castrated male given peanut oil or TP, and one female given peanut oil or TP from each of eight litters were used to assay for cEH and mEH activities at 14, 28, 45, 60, and 90 days of age.

Studies involving neonatal PB administration were performed as follows. Litters were randomly assigned at birth to receive either 30 mg/kg PB [sodium pentobarbital (PB), Merck, Sharp & Dohme] dissolved in 0.9% saline or 0.9% saline alone. Neonates were injected s.c. once each day on days 1 through 5 postpartum. Both PB- and saline-treated males were subdivided into either neonatally castrated or sham-operated groups. PB- and saline-treated females were subdivided into groups receiving either TP (0.5 mg in 50 μl peanut oil) or 50 μl peanut oil on days 1, 3 and 5. One male and one female from each treatment group (six animals per treatment group) were used to assay hepatic mEH activity at 14, 28, 45, 60, 90 and 120 days of age.

Preparation of microsomal and cytosolic fractions. Animals were decapitated and bled, and the livers were rapidly removed and placed on ice. Liver microsomal and cytosolic fractions were prepared as previously published [20]. Protein concentrations of both fractions were estimated by the Biuret method [21].

EH assays. EH activity in hepatic microsomes was determined by the radiometric method of Schmassmann *et al.* [22] using [^{3}H]benzo[*a*]pyrene-4,5-oxide (BPO) as the substrate. The BPO was purchased from the Chemical Carcinogen Reference Standard Repository, NCI, NIH, Bethesda, MD. The incubation contained the following: 25 nmol BPO (4.0×10^4 dpm), 0.05 mg enzyme suspension, and 0.5 M Tris–HCl buffer, pH 9.0, at a final incubation volume of 0.5 ml. The incubation was carried out at 37° for 10 min.

EH activity in the hepatic cytosolic fraction was determined by the radiometric method of Oesch and Golan [23] using [^{3}H]*trans*-stilbene oxide (TSO) as the substrate. The TSO was custom synthesized by the Amersham Corp., Arlington Heights, IL. The incubation contained the following: 10 nmol TSO (4.0×10^5 dpm), 1.0 mg cytosolic protein, and 0.5 M Tris–HCl buffer, pH 7.4, at a final incubation volume of 0.2 ml. The incubation was carried out at 37° for 10 min.

Both substrates were removed by solvent extraction, and the remaining diol products were quantitated by liquid scintillation spectrometry. Under conditions employed, reaction rates were linear with respect to time and protein concentration. Incubation blanks contained either boiled microsomes, boiled cytosol or buffer in place of active microsomes and cytosol.

Immunoblot analysis. Immunochemical quantitation of hepatic mEH and cEH protein was accomplished through the use of antibodies against rat microsomal and mouse cytosolic EH in the "Western" blot procedure. These antibodies were provided by Dr. Peter Guengerich (anti-rat mEH antibody) and Dr. Bruce Hammock (anti-mouse cEH antibody). Microsomal or cytosolic protein samples (100 μg each) from livers of 120-day-old sham-operated males, females and neonatally castrated male rats were resolved by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) according to Laemmli [24]. After electrophoresis, the resolved proteins were transferred electrophoretically to nitrocellulose sheets as described previously [25]. The protein bands were probed first with anti-mEH and anti-cEH antibodies and then with goat anti-rabbit IgG conjugated with alkaline phosphatase. The bands containing cEH and mEH protein were visualized as described by Blake *et al.* [26] and quantitated by densitometry.

Determination of K_m *and* V_{max}. Determination of the kinetic constants for both mEH and cEH was performed as follows. The kinetics of BPO hydration were determined in an incubation mixture (0.5 ml) that contained 0.5 M Tris–HCl (pH 9.0) and micro-

somal protein (0.05 and 0.10 mg) from adult males, adult females or neonatally castrated males. Each microsomal preparation was incubated with five substrate concentrations over a range of 6.25 to 150 μM BPO.

The kinetics of TSO hydration were determined in an incubation mixture (0.2 ml) that contained 0.5 M Tris–HCl (pH 7.4) and cytosolic protein (0.5 and 1.0 mg) from adult males, adult females or neonatally castrated males. Each cytosolic preparation was incubated with five substrate concentrations over a range of 4.0 to 80.0 μM TSO.

BPO and TSO assays were performed as described above. The apparent K_m and V_{max} values were obtained from Lineweaver–Burk plots from the average of three independent determinations.

Statistical analysis. Analysis of variance procedures was used to assess the significance of sex effects, treatment effects and sex/treatment interactions.

Significant F-ratios were evaluated by the studentized range statistic (Newman–Keuls *t*-statistic). For all statistical measures, $P \leq 0.05$ was considered significant. Bar graphs represent the mean value for each treatment group ± the standard deviation.

RESULTS

Effects of neonatal castration and testosterone replacement on hepatic mEH and cEH activities. On days 14 and 28, no differences in mEH activity occurred between males and females for any treatment group (Fig. 1). However, on day 45 when sexual differentiation had taken place, mEH activity in the sham-operated control males was twice that of control females, whereas mEH activity in neonatally castrated males remained at female levels. Moreover, on day 45, rats that had received testosterone propionate during the neonatal period (females and castrated males) showed an increase in mEH activity over controls that received the vehicle only. This pattern was also observed on days 60 and 90. By day 60, females and castrated males that had received neonatal testosterone attained the same level of mEH activity as sham-operated males.

Figure 2 shows effects of neonatal castration and testosterone replacement on developmental patterns of cEH activity in both males and females. As observed with mEH, no differences in cEH activities occurred among any treatment group on either day 14 or day 28. On day 45, cEH activity in sham-operated males increased over that of control females, whereas neonatally castrated males remained at female levels. Also, those groups receiving testosterone neonatally increased their cEH activity over controls receiving peanut oil. This same pattern also occurred on day 60 and day 90. The overall effect was that neonatal castration abolished the sex differences in mEH and cEH activities observed in normal adult rats. Testosterone administration during days 1, 3 and 5 postpartum had no effect on enzyme levels at 14 or 28 days, but increased adult mEH and cEH activities beginning 40 days after completion of TP treatment (Figs 1 and 2).

Effect of neonatal PB administration on the developmental pattern of hepatic mEH activity. Table 1 shows effects of neonatal PB administration on mEH activity in 28-, 45-, 60-, 90-, and 120-day-old rats. Sham-operated, saline-treated (control) males, neonatally castrated, saline-treated (control) castrates, peanut oil, saline-treated (control) females and testosterone, saline-treated (TP control) females all exhibited the same developmental pattern as described in Fig. 1, thus confirming the conclusions of the first study.

Neonatal PB administration did not affect the mEH activity of any group compared to saline-treated controls through 60 days of age. However,

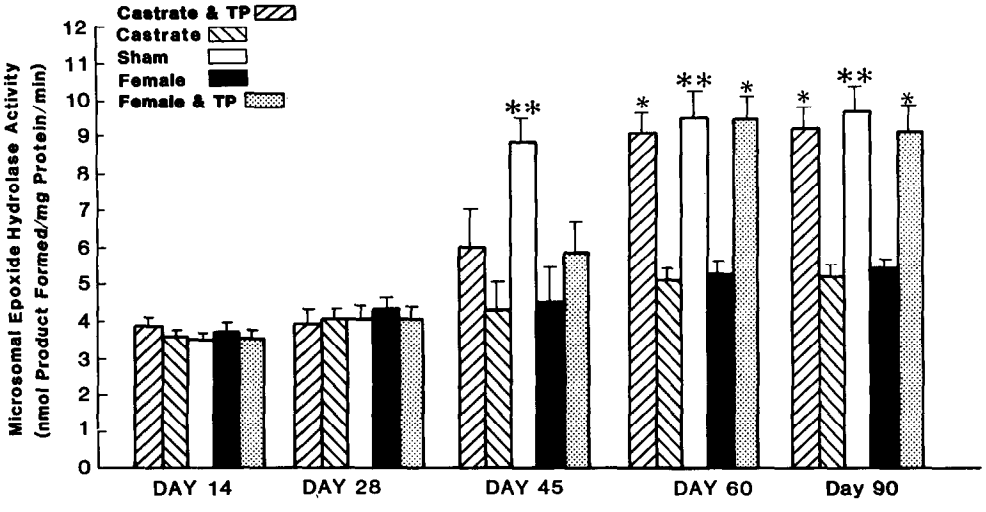

Fig. 1. Effects of castration and testosterone replacement during the neonatal period on the developmental pattern of rat liver mEH activity. Results are expressed as means ± SD, N = 8. The single asterisk (*) indicates that both castrates and females given TP neonatally differed from control castrates and females given peanut oil for the same time point ($P < 0.05$), one-way ANOVA, Newman–Keuls. The double asterisk (**) indicates that sham-operated males differed from control castrates and females for the same time point ($P < 0.05$), one-way ANOVA, Newman–Keuls.

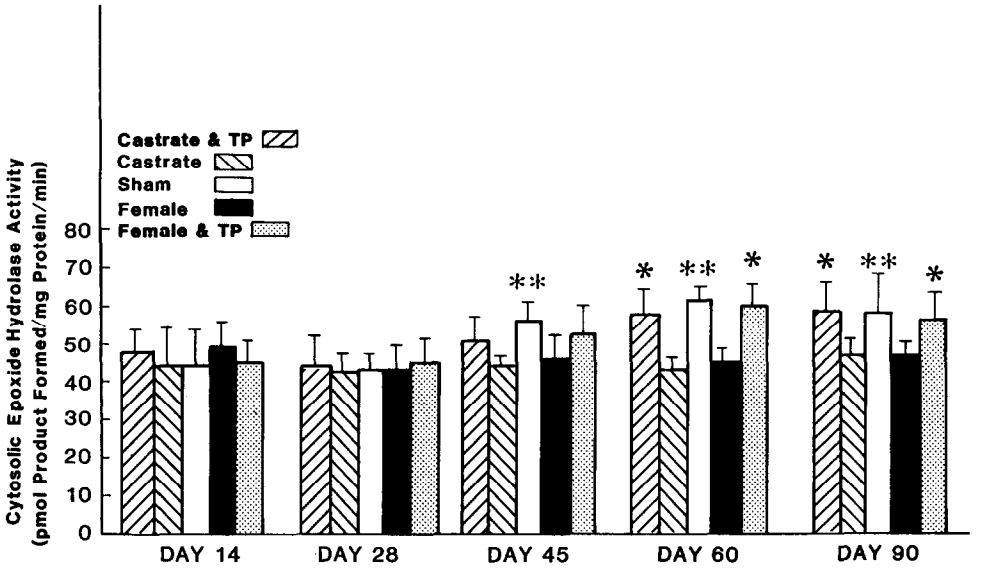

Fig. 2. Effects of castration and testosterone replacement during the neonatal period on the developmental pattern of rat liver cEH activity. Results are expressed as means ± SD, N = 8. The single asterisk (*) indicates that both castrates and females given TP neonatally differed from control castrates and females given peanut oil for the same time point ($P < 0.05$), one-way ANOVA, Newman–Keuls. The double asterisk (**) indicates that sham-operated males differed from control castrates and females for the same time point ($P < 0.05$), one-way ANOVA, Newman–Keuls.

in 90-day-old rats, neonatal PB administration increased mEH activity in both castrated and sham-operated males and in females given either testosterone or peanut oil compared to saline-treated controls (Table 1). Groups with low concentrations of neonatal testosterone (castrated males and peanut oil females) responded to PB by an increase that was higher than those exposed to higher concentrations of neonatal testosterone (sham-operated males and TP-treated females). In 120-day-old rats, this same pattern of increased mEH activity occurred in the animals given PB during the neonatal period. Thus, in all groups, neonatal PB administration increased adult mEH activity, an effect observable beginning at day 90 and also persisting on day 120.

Data in Table 2 illustrate effects of various neonatal treatments on body and liver weights at 90 and 120 days of age. Castration of male neonates

Table 1. Effect of phenobarbital (PB) administration neonatally on hepatic microsomal epoxide hydrolase activity in 28-, 45-, 60-, 90- and 120-day-old rats*

Group	Drug		Microsomal epoxide hydrolase activity (nmol product/mg protein/min) 28	45	60	90	120
Males							
Castrated	Saline	A	4.27 ± 0.3	4.01 ± 0.7[a]	5.78 ± 0.8[a]	5.77 ± 1.5	6.46 ± 1.1
	PB	B	4.24 ± 0.4	4.11 ± 0.5[a]	5.67 ± 0.6[a]	10.30 ± 1.2[c] (178%)†	10.88 ± 3.3[c] (168%)
Sham-operated	Saline	C	4.02 ± 0.2	8.08 ± 1.1	10.23 ± 1.4	9.53 ± 1.0	9.56 ± 1.6
	PB	D	4.25 ± 0.3	8.46 ± 1.6	9.69 ± 1.0	12.08 ± 0.8[d] (127%)	12.20 ± 2.6[d] (127%)
Females							
Peanut oil-treated	Saline	E	4.42 ± 0.2	3.93 ± 0.8	6.20 ± 0.6	6.25 ± 0.5	6.72 ± 1.0
	PB	F	4.47 ± 0.5	3.94 ± 0.3	6.06 ± 0.6	9.95 ± 0.4[e] (159%)	10.32 ± 1.4[e] (154%)
Testosterone-treated	Saline	G	4.03 ± 0.4	5.80 ± 0.7[b]	10.24 ± 1.6[b]	9.31 ± 1.0	9.6 ± 1.8
	PB	H	4.11 ± 0.5	5.49 ± 0.3[b]	10.86 ± 1.8[b]	11.40 ± 2.0[f] (122%)	11.73 ± 1.3[f] (122%)

* Animals were injected s.c. with either 30 mg/kg PB or 0.9% saline on days 1–5 postpartum. Each value is the mean ± SD, N = 6.

† Numbers in parentheses are PB animals as percentage of saline controls.

[a] Significantly different from C and D, $P < 0.01$.

[b] Significantly different from E and F, $P < 0.01$.

[c] Significantly different from A, $P < 0.01$.

[d] Significantly different from C, $P < 0.05$.

[e] Significantly different from E, $P < 0.01$.

[f] Significantly different from G, $P < 0.05$.

Table 2. Effect of PB treatment neonatally on growth variables in 90- and 120-day-old rats*

Group	Drug	Growth variables—90 day			Growth variables—120 day		
		Body wt (g) (BW)	Liver wt (g) (LW)	LW/BW × 100	Body wt (g) (BW)	Liver wt (g) (LW)	LW/BW × 100
Males							
Sham-operated	Saline	193 ± 17.2	7.4 ± 1.4	3.5 ± 0.1	237 ± 25.6	8.1 ± 0.8	3.3 ± 0.2
	PB	213 ± 40.1	7.7 ± 0.5	3.6 ± 0.2	230 ± 20.9	7.7 ± 1.0	3.3 ± 0.2
Castrated	Saline	149 ± 27.6†	4.9 ± 1.0†	3.3 ± 0.2	176 ± 6.5†	5.3 ± 0.2†	3.2 ± 0.2
	PB	148 ± 16.5†	5.0 ± 0.8†	3.4 ± 0.2	170 ± 12.9†	5.2 ± 0.6†	3.2 ± 0.1
Females							
Peanut oil-treated	Saline	143 ± 23.5	5.2 ± 0.9	3.6 ± 0.2	152 ± 6.3	5.0 ± 0.3	3.3 ± 0.2
	PB	138 ± 5.5	4.8 ± 0.2	3.5 ± 0.4	152 ± 11.5	5.1 ± 0.7	3.3 ± 0.3
Testosterone-treated	Saline	167 ± 7.2†	5.8 ± 0.8†	3.4 ± 0.2	183 ± 10.8†	6.3 ± 0.8†	3.4 ± 0.2
	PB	159 ± 9.1†	5.3 ± 0.4	3.3 ± 0.4	182 ± 12.2†	6.5 ± 1.0†	3.4 ± 0.3

* Animals were injected s.c. with either 30 mg/kg PB or 0.90% saline on days 1–5 postpartum. Each value is the mean ± SD, N = 6.
† Significantly different from control ($P < 0.01$), within each sex.

decreased body and liver weights, as compared to sham-operated male controls. However, no alteration occurred in liver weight as a percentage of body weight, indicating that reduced liver weight was associated with reduced body weight (Table 2). Neonatal testosterone treatment increased female body and liver weights. Again liver weight as a percentage of body weight was unaltered. PB administered neonatally had no effect on either variable at 90 or 120 days of age, suggesting that the nutritional status of the neonates had not been altered grossly by PB.

Immunoblot analysis. Analysis of hepatic microsomal and cytosolic protein content from 120-day-old males, females and neonatally castrated males was performed by SDS–PAGE and Western blotting. Polypeptide bands migrated to the same position as the corresponding purified mouse cEH (58 kDa) and purified rat mEH (50 kDa), respectively. Densitometric analysis indicated an approximate 2.0-fold increase in immunostaining for both 50 and 58 kDa bands following transfer to nitrocellulose in the 120-day-old males compared to females and neonatally castrated males (Figs 3 and 4). Thus, changes in both the microsomal and cytosolic protein content of EH corresponded to similar changes in enzyme activity of males and females.

Determination of K_m *and* V_{max}. Determination of the kinetic constants for both BPO hydration and TSO hydration showed sex-dependent differences (Table 3). Specifically, for both BPO and TSO hydration, the V_{max} was higher in the adult male compared to the adult female. Sex differences in V_{max} were abolished by neonatal castration. Some changes, extending up to 2-fold, also occurred in the K_m values for both substrates (Table 3), raising the possibility of qualitative differences between males and females in microsomal and cytosolic EH.

DISCUSSION

These studies provide the first detailed description of hormonal regulation of the developmental patterns of mEH and cEH activities in rat liver. Previously, Oesch [17] and Gill and Hammock [18] showed that, during the neonatal period, EH activities are lower than those of adults and fail to exhibit the sex differences characteristic of the adult. Our establishment of these developmental patterns and their hormonal regulation permits future investigations of how they can be altered by a variety of environmental manipulations. In this connection, our results agree with the ontogeny of certain other detoxification systems residing in the endoplasmic reticulum such as some enzymes of the monooxygenase system [17, 27]. Depending on the particular cytochrome P-450 isozyme and substrate studied, the ontogeny can differ appreciably. Our pattern, characterized by similar activities at 14 and 28 days, followed by a rapid rise to adult levels at 45 days where it remained stationary for up to 90 days, conforms most to patterns described for *N*-demethylation of aminopyrine [28]. By contrast, female rats after puberty exhibit reductions with age for ethylmorphine *N*-methylase, whereas males follow the pattern shown in Fig. 1 [29].

Table 3. Sex differences in the kinetic constants for benzo[*a*]pyrene-4,5-oxide (BPO) and *trans*-stilbene oxide (TSO) hydration

Group	BPO		TSO	
	V_{max} [nmol·(mg microsomal protein)$^{-1}$·min^{-1}]	K_m (μM)	V_{max} [pmol·(mg cytosolic protein)$^{-1}$·min^{-1}]	K_m (μM)
Intact adult male	9.95 ± 0.2	7.3 ± 0.8	62.5 ± 6.2	36.8 ± 4.2
Intact adult female	4.92 ± 0.4*	12.5 ± 0.9*	45.7 ± 3.1*	20.8 ± 3.8*
Neonatally castrated male	5.27 ± 0.6*	14.7 ± 1.1*	42.1 ± 3.6*	19.2 ± 3.1*

* Significantly different from control male ($P < 0.01$).

Since EH activities significantly influence the detoxification capability of the adult individual, the developmental pattern can serve to test effects of various environmental manipulations applied neonatally on toxicological defenses and susceptibilities of adult animals. Accordingly, the present studies explored the influence on this normal male and female developmental pattern of castration, testosterone administration, and PB administration, all during the neonatal period (Table 1; Figs 1 and 2). The results indicated that neonatal exposure to testosterone was required to develop normal adult male EH activities. Moreover, PB administration neonatally enhanced not only adult male but also adult female EH activities. Collectively, our kinetic and immunochemical studies suggest that the sex differences in EH activity are associated with an increased amount of enzyme in the male (Figs 3 and 4) and possibly even a qualitatively different protein. These observations indicate the critical role that neonatal exposure to hormones and environmental chemicals can play in regulating adult levels of an enzyme critical in detoxification reactions.

Many environmental changes, in addition to testosterone and PB, also can play a role in determining adult levels of this enzyme, thereby modulating the developmental pattern. One gross nutritional parameter will be cited here as an example: starvation.

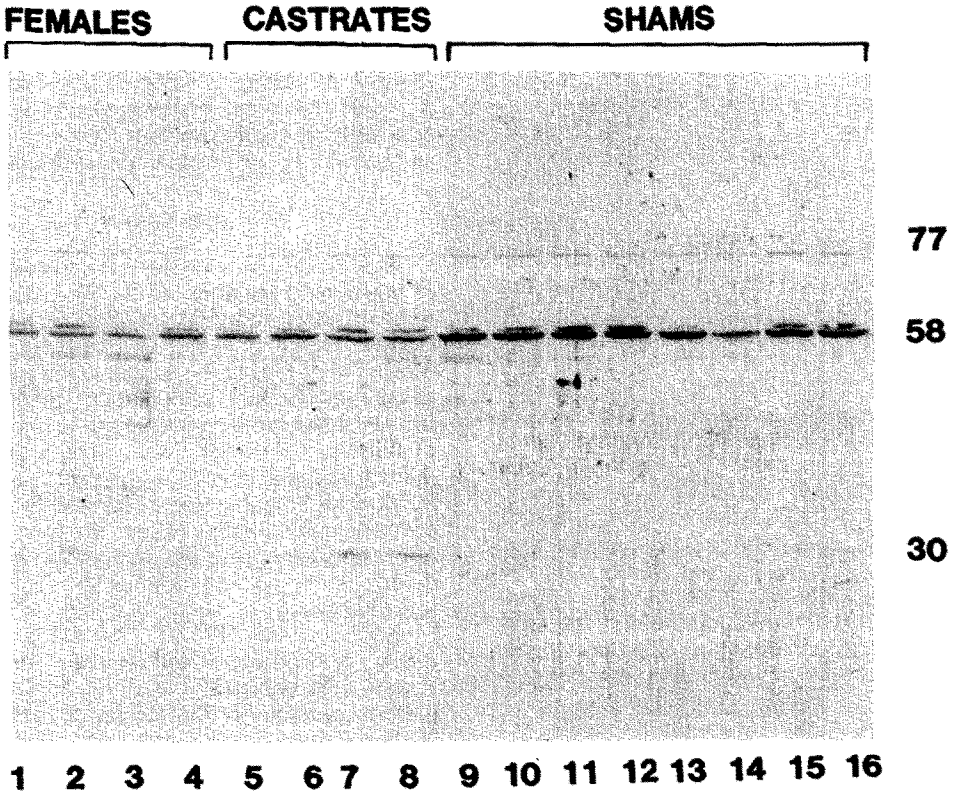

Fig. 3. Cytosolic epoxide hydrolase (cEH) proteins in rat liver cytosol. Cytosolic fractions (100 μg each) were subjected to SDS–PAGE and blotted. The blot was probed with anti-cEH antibodies and detected using NBT/BCIP chromogens. Lanes 1–4: females (day 120); lanes 5–8: neonatally castrated males (day 120); lanes 9–16: sham-operated males (day 120). The doublet band at 58 kDa corresponds to cEH.

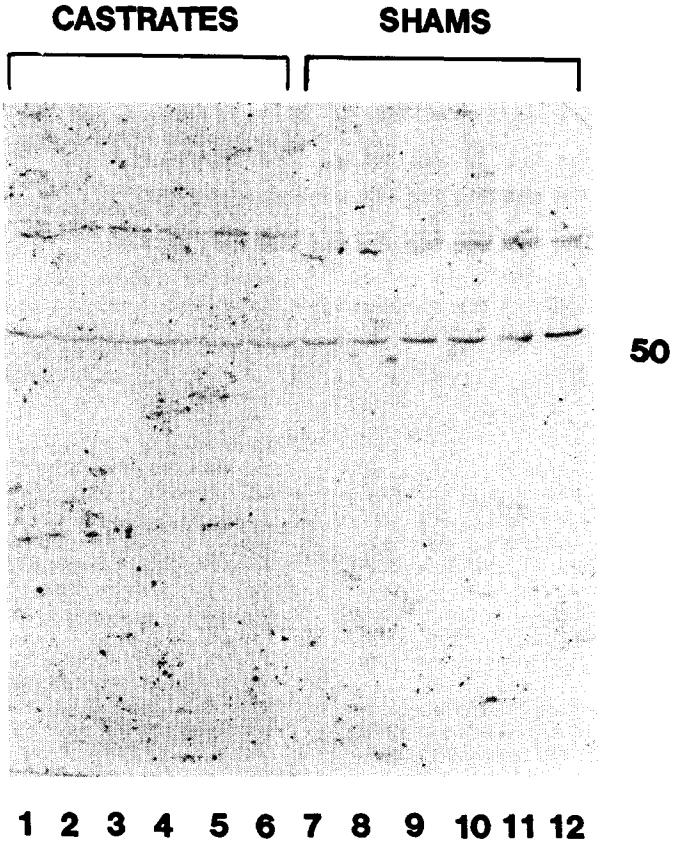

Fig. 4. Microsomal epoxide hydrolase (mEH) proteins in rat hepatic microsomes. Microsomal fractions (100 μg each) were subjected to SDS–PAGE and blotted. The blot was probed with anti-mEH antibodies and detected using NBT/BCIP chromogens. Lanes 1–6: neonatally castrated males (day 120); lanes 7–12: sham-operated males (day 120). The band at 50 kDa corresponds to mEH.

Overnight starvation stimulated EH activities in both males and females, enhancing them by 20%.

To relate our results to previous work, it should be mentioned that the following additional host factors have been implicated. Genetic factors, such as species, strain and other genetically-mediated inter-individual variations, affect EH activity [30–35]. Large interindividual differences, exceeding 38-fold, occurred in the specific activity of cEH in livers from more than eighty adult Sprague–Dawley rats [33]. By contrast, differences in hepatic mEH activity among twenty-two rat strains were only 3-fold [34]. However, in combination with interindividual variations, the factors of sex differences and induction produced 20-fold interindividual variations in hepatic mEH activities [34]. Moreover, interindividual variations of 539-fold in mEH activities have been reported from human liver biopsies [35]. Mean basal cEH activities using TSO as substrate exhibited 2-fold variations among twelve strains of mice [32].

Thus, interindividual differences in EH activity may result from multiple causes, including genetic constitution, developmental history, sex, diet, exposure to environmental chemicals or drugs that induce or inhibit these enzymes, or various combinations of these factors. Dynamic, fluctuating interactions among these and other factors that determine the EH activity of a subject can influence that subject's susceptibility to an environmental chemical or toxin at any given time in the life cycle. Since the developmental stage is so critical in this regard, our study was designed to establish precise developmental patterns of cEH and mEH activities and influences exerted on these patterns by early hormonal and xenobiotic interventions.

Acknowledgements—We gratefully acknowledge Dr. Bruce Hammock (Department of Entomology and Environmental Toxicology, University of California, Davis) for the gifts of purified mouse cEH and cEH antibody, Dr. F. Peter Guengerich (Department of Biochemistry, Vanderbilt University School of Medicine, Nashville) for the gifts of purified rat mEH and mEH antibody, and Dr. Melvin Billingsley (Department of Pharmacology, Pennsylvania State University College of Medicine) for his help with the immunoblots and their interpretation.

REFERENCES

1. Oesch F, Mammalian epoxide hydrolases: Inducible enzymes catalyzing the inactivation of carcinogenic and cytotoxic metabolites derived from aromatic and olefinic compounds. *Xenobiotica* **3**: 305–340, 1973.
2. Lu AYH and Miwa GT, Molecular properties and biological functions of microsomal epoxide hydrase. *Annu Rev Pharmacol Toxicol* **20**: 513–531, 1980.

3. Wood AW, Levin W, Lu AYH, Yagi H, Hernandez O, Jerina DM and Conney AH, Metabolism of benzo[*a*]pyrine and benzo[*a*]pyrene derivatives to mutagenic products highly purified hepatic microsomal enzymes. *J Biol Chem* **251**: 4882–4890, 1976.
4. Kapitulnik J, Wislocki PG, Levin W, Yagi H, Jerina DM and Conney AH, Tumorigenicity studies with diol-epoxides of benzo(*a*)pyrene which indicate that (±)-*trans*-7β,8α-dihydroxy-9α,10α-epoxy-7,8,9,10-tetrahydrobenzo(*a*)pyrene is an ultimate carcinogen in newborn mice. *Cancer Res* **38**: 354–358, 1978.
5. Quinn GP, Axelrod J and Brodie BB, Species, strain, and sex differences in metabolism of hexobarbitone, aminopyrine, antipyrine, and aniline. *Biochem Pharmacol* **1**: 152–159, 1958.
6. Kato R and Gillette JR, Effect of starvation on NADPH-dependent enzymes in liver microsomes of male and female rats. *J Pharmacol Exp Ther* **150**: 279–284, 1965.
7. Schenkman JB, Frey J, Remmer H and Estabrook RW, Sex differences in drug metabolism by rat liver microsomes. *Mol Pharmacol* **3**: 516–521, 1967.
8. Denef C, Differentiation of steroid metabolism in the rat and mechanisms of neonatal androgen action. *Enzyme* **15**: 254–271, 1973.
9. Gustafsson J-A, Eneroth P, Pousett A, Skett P, Sonnenschein C, Stenberg A and Ahlen A, Programming and differentiation of rat liver enzymes. *J Steroid Biochem* **8**: 429–435, 1977.
10. Gustafsson J-A and Stenberg A, Irreversible androgenic programming at birth of microsomal and soluble rat liver enzymes active on 4-androstene-3,17-dione and 5α-androstane-3α,17β-diol. *J biol Chem* **249**: 711–718, 1974.
11. McEwen BS, Sexual differentiation of gonads and reproductive tract in mammals. In: *Sexual Differentiation of the Brain* (Eds. Ferrendalli JA, McEwen BS and Snyder SH), pp. 83–89. MIT Press, Cambridge, MA, 1976.
12. Gustafsson J-A, Mode A, Norstedt G, Hokfelt T, Sonnenschein C, Eneroth P and Skett P, The hypothalamo–pituitary–liver axis, a new hormonal system in control of hepatic steroid and drug metabolism. In: *Biochemical Action of Hormones* (Ed. Litwack G), pp. 47–89. Academic Press, New York, 1980.
13. Mode A, Gustafsson JA, Jansson J-O, Eden S and Isaksson O, Association between the plasma level of growth hormone and sex differentiation of hepatic steroid metabolism in the rat. *Endocrinology* **111**: 1692–1697, 1982.
14. Simpson VJ and Chung LWK, Programming of rat hepatic arylhydrocarbon hydroxylase activity during the late neonatal period. *Proc West Pharmacol Soc* **25**: 257–261, 1982.
15. Faris RA and Campbell TC, Long-term effects of neonatal phenobarbital exposure on aflatoxin β_1 disposition in adult rats. *Cancer Res* **43**: 2576–2583, 1983.
16. Bagley DM and Hayes JR, Xenobiotic imprinting of the hepatic monooxygenase system. Effects of neonatal phenobarbital administration. *Biochem Pharmacol* **34**: 1007–1014, 1985.
17. Oesch F, Differential control of rat microsomal "aryl hydrocarbon" monooxygenase and epoxide hydrolase. *J Biol Chem* **251**: 79–87, 1976.
18. Gill SS and Hammock BD, Distribution and properties of a mammalian soluble epoxide hydrase. *Biochem Pharmacol* **29**: 389–395, 1980.
19. Pfeiffer CA, Sexual differentiation of the hypophyses. *Am J Anat* **58**: 195–221, 1936.
20. Vesell ES, Passananti GT and Hepner GH, Interaction between antipyrine and aminopyrine. *Clin Pharmacol Ther* **20**: 661–669, 1976.
21. Gornall AG, Bardawill CJ and David MM, Determination of serum proteins by means of the Biuret reaction. *J Biol Chem* **177**: 751–766, 1949.
22. Schmassmann HU, Glatt HR and Oesch F, A rapid assay for epoxide hydratase activity with benzo(a)-pyrene 4,5-(K-region-)oxide as substrate. *Anal Biochem* **74**: 94–104, 1976.
23. Oesch F and Golan M, Specificity of mouse liver cytosolic epoxide hydrolase for K-region epoxides derived from polycyclic aromatic hydrocarbons. *Cancer Lett* **9**: 169–175, 1980.
24. Laemmli UK, Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* **227**: 680–685, 1970.
25. Towbin H, Straehelin T and Gordon J, Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets. Procedure and some applications. *Proc Natl Acad Sci USA* **76**: 4350–4354, 1979.
26. Blake MS, Johnston KH, Russel-Jones GJ and Gotschlich EC, A rapid, sensitive method for detection of alkaline phosphatase-conjugated anti-antibody on Western blots. *Anal Biochem* **136**: 175–179, 1984.
27. Short CR, Kinden DA and Smith R, Fetal and neonatal development of the microsomal monooxygenase system. *Drug Metab Rev* **5**: 1–42, 1976.
28. MacLeod SM, Renton KW and Eade NR, Development of hepatic drug-metabolizing enzymes in immature male and female rats. *J. Pharmacol Exp Ther* **183**: 489–498, 1972.
29. Cohen GM and Mannering GJ, Sex-dependent differences in drug metabolism in the rat. III. Temporal changes in Type I binding and NADPH–cytochrome P-450 reductase during sexual maturation. *Drug Metab Dispos* **2**: 285–292, 1974.
30. Oesch F, Morris N, Daly J, Gielen JE and Nebert DW, Genetic expression of the induction of epoxide hydrase and aryl hydrocarbon hydroxylase activities in the mouse by phenobarbital or 3-methylcholanthrene. *Mol Pharmacol* **9**: 692–696, 1973.
31. Lyman SD, Poland A and Taylor BA, Genetic polymorphism of microsomal epoxide hydrolase activity in the mouse. *J Biol Chem* **255**: 8650–8654, 1980.
32. Gill SS and Kaur S, Hepatic epoxide hydrolase activities and their induction by clofibrate and diethylhexylphthalate in various strains of mice. *Biochem Pharmacol* **36**: 4221–4227, 1987.
33. Schladt L, Worner W, Setiabudi F and Oesch F, Distribution and inducibility of cytosolic epoxide hydrolase in male Sprague–Dawley rats. *Biochem Pharmacol* **35**: 3309–3316, 1986.
34. Oesch F, Zimmer A and Glatt HR, Microsomal epoxide hydrolase in different rat strains. *Biochem Pharmacol* **32**: 1783–1788, 1983.
35. Mertes I, Fleischman R, Glatt HR and Oesch F, Interindividual variations in the activities of cytosolic and microsomal epoxide hydrolase in human liver. *Carcinogenesis* **6**: 219–223, 1985.