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SHORT COMMUNICATIONS

Studies on the developmental and adrenal regulation of cytosolic and microsomal epoxide hydrolase activities in rat liver

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Abstract—The present study was undertaken to ascertain developmental profiles of microsomal epoxide hydrolase (mEH) and cytosolic epoxide hydrolase (cEH) enzyme activities in rat liver. During development, mEH activity reached an optimum by 6 weeks of age (63 nmol/min/mg protein). Activities decreased thereafter in both sexes although in adult male liver the activity was twice that measured in adult female liver. Thus, the importance of pituitary maturation was suggested from these findings. Since glucocorticoids have been implicated in the regulation of mEH gene expression the effect of adrenalectomy on mEH activity was investigated in adult male rat liver. The procedure increased mEH activity almost two-fold and the increase was reversed by dexamethasone, but not deoxycorticosterone, administration. With respect to hepatic cEH activity, the developmental profiles indicated that enzyme activity was greatest in rats at 1 week of age (12–15 nmol/min/mg protein) and very little activity was detected beyond 4 weeks of age (~5 nmol/min/mg protein); sex differences in cEH activity were not apparent at any age. Thus, the pituitary appears to be important in the developmental induction of mEH but not cEH. Glucocorticoids appear to provide the major hormonal influence on mEH expression. Thus, the hypothalamus–pituitary–adrenal axis is involved in the regulation of mEH but the regulation of the cEH enzyme remains unclear.

Epoxide hydrolases (EH*) catalyse the formation of dihydrodiols and other glycols from biologically reactive epoxides. There are several forms of EH in rat liver including the major microsomal EH (mEH), which hydrates a range of substrates such as epoxides of cyclic systems, styrene oxide, benzo[*a*]pyrene 4,5-oxide and *cis*-stilbene oxide [1, 2]. The cytosolic EH (cEH) hydrates many epoxide substrates, especially aliphatic 1,2-disubstituted *trans*-epoxides; *trans*-stilbene oxide is perhaps the most commonly used substrate [1–4]. Apart from substrate specificity, the cEH and mEH are also immunochemically distinct proteins [5].

In rat liver, using styrene oxide as substrate, Oesch [6] reported a developmental increase in mEH activity; activity was also 2.5–3-fold greater in adult male than in adult female liver. From northern analysis it was noted that amounts of the mRNA corresponding to mEH increased in liver after birth [7]. A role for glucocorticoids in the regulation of mEH has also been suggested from studies at the molecular level [8]. In the case of the cytosolic enzyme, little difference has been reported in activity between male and female rat livers from 2 weeks of age [4], although the activity of this enzyme in rat liver is low [9]. Little information is currently available regarding the regulation of the cEH enzyme. The present study was undertaken to compare the developmental profiles of mEH and cEH activities and to determine the relative importance of glucocorticoids in the *in vivo* regulation of these enzymes.

Materials and Methods

Animals. Male and female Wistar rats (1–15 weeks of age) were obtained from the Westmead Hospital animal holding facility and housed under conditions of constant temperature, humidity and lighting (12 hr light:12 hr dark cycle).

Animals were exsanguinated under ether anaesthesia, livers removed and perfused with cold saline. Hepatic microsomal and cytosolic fractions were isolated by standard procedures of ultracentrifugation as described elsewhere [10] and stored at −70° until used in experiments; storage did not influence the activity of either EH. Protein was determined by the method of Lowry *et al.* [11] with bovine serum albumin as standard.

Animal treatments. Developmental profiles of mEH and cEH activities were determined in rats of both sexes. The rats were permitted free access to water and rat chow for varying periods after weaning and were killed at 4, 6, 10 and 15 weeks of age. In the case of early time points (1 and 2 weeks of age) sucklings were removed from the nest. For the 1-week-old time point six livers of each sex were pooled separately, and assays were performed on each pool in triplicate.

Adrenalectomy of male rats was performed under ether anaesthesia. Sham-controls underwent the same operation without the removal of the adrenals. All animals had free access to standard rat chow and 0.9% (w/v) saline and were killed 2 weeks after operation. Adrenalectomized male rats were administered either dexamethasone (DEX; 60 μg/100 μL corn oil) or deoxycorticosterone (DOC; 600 μg/100 μL corn oil) for 6 days [12]; control groups were either sham-operated or adrenalectomized animals that received corn oil. Animals were killed 2 hr after final injection.

cEH assay. The cEH assay was performed based on Wixtrom and Hammock [3]. The 1 mL assay system consisted of 150 μg cytosolic protein, 100 mM potassium phosphate buffer (pH 7.0), 0.1 mM *trans*-1,2-epoxy-1-(2′-quinolyl)-pentane (EQU 5) and 0.01% bovine serum albumin. Substrate utilization at 37° was monitored at 320 nm. Activities were calculated using the extinction coefficient $E_{320} = -3.7\ mM^{-1}\ cm^{-1}$.

mEH assay. This assay was based upon the method of Lu and Levin [13], using [^{14}C]styrene oxide instead of [^{3}H]-styrene oxide. The assay system consisted of 100 μg microsomal protein and 1.46 mM [^{14}C]styrene oxide (2–3 × 10^4 dpm per assay) in 0.5 M Tris (pH 9.0 at 25°). After 10 min incubation at 37° the reaction was stopped by the addition of 5 mL hexane, mixed, and centrifuged at ~750 *g* for 15 min. The organic phase was removed and the

* Abbreviations: EH, epoxide hydrolase; EQU 5, *trans*-1,2-epoxy-1-(2′-quinolyl)-pentane; cEH, cytosolic epoxide hydrolase; mEH, microsomal epoxide hydrolase; DOC, deoxycorticosterone; DEX, dexamethasone.

procedure was repeated. Ethyl acetate (2 mL) was added to the aqueous phase, mixed and 30 min later, 0.3 mL of the upper phase (containing the ^{14}C-labelled styrene glycol) was mixed with 5 mL Aquasol scintillant (NEN-Du Pont, Sydney, Australia) for scintillation spectrometry.

Chemicals and biochemicals. EQU 5 was the generous gift of Dr B. D. Hammock, University of California, Davis, CA, U.S.A. [^{14}C]Styrene oxide was purchased from Amersham Australia, Sydney, and the unlabelled styrene oxide was from the Aldrich Chemical Co. (Milwaukee, WI, U.S.A.). DEX and DOC were purchased from the Sigma Chemical Co. (St Louis, MO, U.S.A.) All other chemicals used were at least analytical reagent grade.

Statistics. Age-related differences between mean values within the same sex were detected by single-factor analysis of variance in combination with the Scheffé statistic. Comparisons between sexes at each time point were made with the Student's *t*-test.

Results and Discussion

Developmental profiles of EH activity in rat liver. mEH activity exhibited an age-related increase in rat liver of both sexes (Fig. 1). At 1 week of age, mEH activity in both sexes was ~5 nmol styrene glycol produced/min/mg protein and attained optima around 6 weeks of age (~63 nmol styrene glycol produced/min/mg protein). By 10 and 15 weeks, mEH activity decreased in liver of both sexes and a sexual dimorphism was apparent (female 17 ± 4 and male 41 ± 6 nmol/min/mg protein).

Figure 2 shows the development related alterations in cEH activity. Activities were optimal in liver from rats of 1 week of age (female 15 and male 12 nmol EQU 5 hydrolysed/min/mg protein). Thereafter progressive decreases to 4.9 ± 1.1 (female) and 2.9 ± 0.7 (male) nmol EQU 5 hydrolysed/min/mg protein at 15 weeks of age were apparent. In contrast to mEH, sex-related differences in cEH activity were not detected.

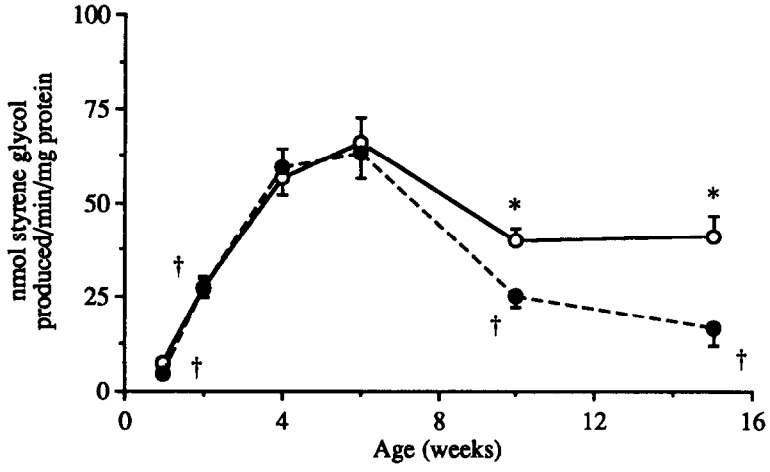

Fig. 1. Age profiles of mEH activity. Key: (●) female, (○) male; *$P < 0.001$ comparing male and female at same ages, †$P < 0.001$ comparing activities at 4 weeks old to activities at the different ages in the same sex. Activities are expressed as means ± SD using 4–8 animals in each group, with the exception of the 1 week groups which are means of triplicate estimates obtained from pooled samples of 6 livers.

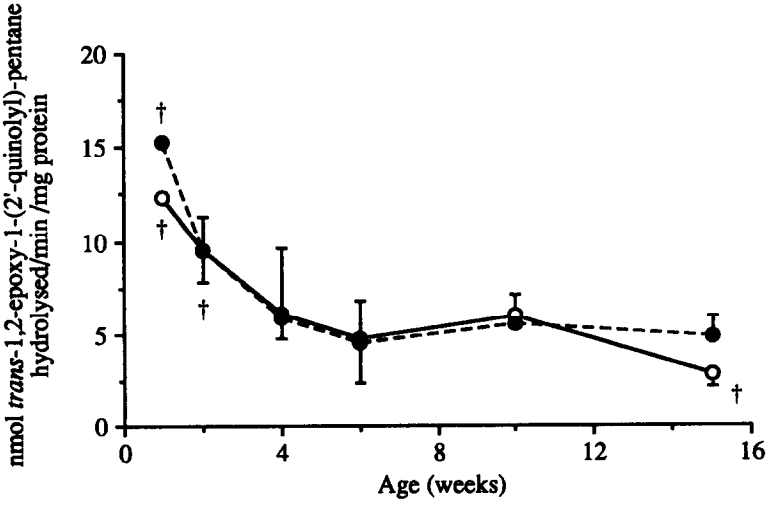

Fig. 2. Age profiles of cEH activity. Key: (●) female, (○) male; †$P < 0.05$ comparing enzyme activities in liver from the same sex to the activity at 4 weeks of age; no significant differences between the sexes at corresponding age points. Activities are expressed as mean ± SD using 4–8 animals in each group, with the exception of the 1 week groups which are means of triplicate estimates obtained from pooled samples of 6 livers.

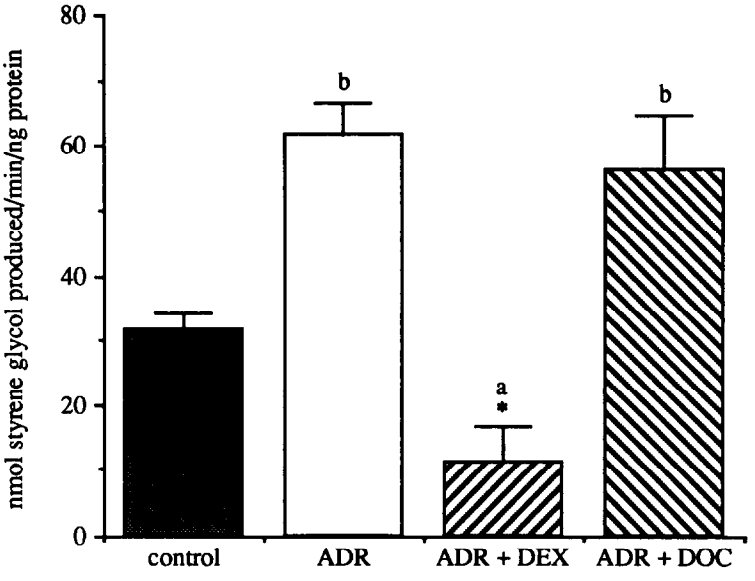

Fig. 3. Hepatic mEH activity in adrenalectomized male rats. Key: control = sham-operated animals; ADR = adrenalectomy; ADR + DEX = adrenalectomy plus dexamethasone; ADR + DOC = adrenalectomy plus deoxycorticosterone; $^{a}P < 0.01$ and $^{b}P < 0.001$ comparing with sham-operated controls; $^{*}P < 0.001$ compared to adrenalectomy alone. Activity expressed as means ± SD using groups of 4–5 animals per treatment.

The developmental profiles strongly suggest that the regulatory mechanisms for the two epoxide hydrolases are distinct. The mEH exhibits age-dependent increases and a post-pubertal sex dimorphism somewhat similar to that established for the hepatic microsomal P450 steroid 6β- and 2α-/16α-hydroxylases [14]. This pattern is consistent with the findings of Oesch [6] and Denlinger and Vesell [4]. However, cEH activity appears optimal in neonatal tissue and exhibits an age-related decrease; no sexual dimorphism was observed. This observation strongly suggests that cEH is only expressed at low level in adult rat livers but, to our knowledge, the present report is the first to recognize the high activity present in neonatal rat liver. These results suggest that cEH is preferentially expressed in neonatal liver and, indeed, that cEH is the dominant EH enzyme in these animals.

Effect of hormonal manipulation on EH activities in rat liver. The possible involvement of pituitary hormones in the regulation or maintenance of mEH was suggested from the developmental profiles. Previous molecular studies have implicated glucocorticoids in the regulation of mEH gene transcription [8]. Accordingly, the potential regulatory role of adrenal hormones was investigated in the present study. From Fig. 3, adrenalectomy of adult male rats led to a 95% increase in hepatic mEH activity over sham-operated controls ($P < 0.001$). Supplementation of these rats with the glucocorticoid DEX led to significant suppression of hepatic mEH activity compared with that in sham-operated control liver (11.0 ± 5.6 vs 31.7 ± 4.9 nmol styrene glycol produced/min/mg protein; $P < 0.01$) and hepatic microsomes from adrenalectomized animals (61.7 ± 5.0; $P < 0.001$). Mineralocorticoid (DOC) administration did not modify the effect of adrenalectomy on apparent hepatic mEH activity. Therefore, these studies of mEH activity are consistent with the findings of Bell *et al.* [8] who have demonstrated an interaction between the glucocorticoid hormone–receptor complex and the promoter region of the hepatic mEH gene. Glucocorticoids appear to have a suppressive effect upon mEH expression.

In contrast to the situation with mEH, adrenal factors do not appear to be involved in either regulation or maintenance of cEH in adult male rat liver. Adrenalectomy did not modulate the rate of EQU 5 hydrolysis measured in hepatic cytosols (sham-operated controls 1.9 ± 0.6 and adrenalectomy 2.2 ± 0.9 nmol EQU 5 hydrolysed/min/mg protein).

Conclusion. Microsomal EH is a rat hepatic enzyme that, as is evident from catalytic studies, exhibits a sexually dimorphic pattern of expression. The developmental profiles measured in both sexes indicate that pituitary maturation is an important factor in the development of the sexual dimorphism. Glucocorticoids and not mineralocorticoids seem important for the correct maintenance of this enzyme during adulthood. However, it is unlikely that glucocorticoid could be responsible for dimorphic mEH activity.

Sex differences in cEH activity were not apparent. A finding that has emerged from the developmental profiles in the present study is that the enzyme is expressed preferentially in neonatal rat liver. The decrease to adult levels by 4 weeks of age suggests that pituitary maturation is associated with the release of an as yet unidentified repressive factor, but adrenal factors are clearly not involved in the regulation of cEH.

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REFERENCES

1. Hammock BD and Hasegawa LS, Differential substrate selectivity of murine hepatic cytosolic and microsomal epoxide hydrolases. *Biochem Pharmacol* **32**: 1155–1164, 1983.
2. Wixtrom RN and Hammock BD, Membrane-bound and soluble-fraction epoxide hydrolases: Methodological Aspects. In: *Biochemical Pharmacology and Toxicology, Volume 1: Methodological Aspects of Drug Metabolizing Enzymes* (Eds. Zakim D and Vessey DA), pp. 1–93. John Wiley & Sons, New York, 1985.
3. Wixtrom RN and Hammock BD, Continuous spectrophotometric assays for cytosolic epoxide hydrolase. *Anal Biochem* **174**: 291–299, 1988.
4. Denlinger CL and Vesell ES, Hormonall regulation of the developmental pattern of epoxide hydrolases: studies in rat liver. *Biochem Pharmacol* **38**: 603–610, 1989.
5. Guenthner TM, Hammock BD, Vogel U and Oesch F, Cytosolic and microsomal epoxides are immunologically distinguishable from each other in the rat and mouse. *J Biol Chem* **256**: 3163–3166, 1981.
6. Oesch F, Differential control of rat microsomal "aryl hydrocarbon" monooxygenase and epoxide hydratase. *J Biol Chem* **251**: 79–87, 1976.
7. Simmons DL and Kasper CB, Quantitation of mRNAs specific for the mixed-function oxidase system in rat liver and extrahepatic tissues during development. *Arch Biochem Biophys* **271**: 10–20, 1989.
8. Bell PA, Falany CN, McQuiddy P and Kasper CB, Glucocorticoid repression and basal regulation of the epoxide hydrolase promoter. *Arch Biochem Biophys* **279**: 363–369, 1990.
9. Gill SS and Hammock BD, Distribution and properties of a mammalian soluble epoxide hydrolase. *Biochem Pharmacol* **29**: 389–395, 1980.
10. Murray M, Cantrill E and Farrell GC, Drug metabolism in cirrhosis: selective changes in cytochrome P-450 isoenzymes in the choline deficient rat model. *Biochem Pharmacol* **35**: 1817–1824, 1986.
11. Lowry OH, Rosebrough NJ, Farr AL and Randall RJ, Protein measurement with the Folin phenol reagent. *J Biol Chem* **193**: 265–275, 1951.
12. Khalid BA, Morat P and Merican Z, The effects of naloxone, dexamethasone, deoxycorticosterone and 17-hydroxyprogesterone on blood pressure responses of normal and adrenalectomized rats during hypovolaemic shock. *Clin Exp Pharmacol Physiol* **14**: 111–117, 1987.
13. Lu AYH and Levin W, Purification and assay of liver microsomal epoxide hydrase. *Methods Enzymol* **52**: 193–200, 1978.
14. Waxman DJ, Dannan GA and Guengerich FP, Regulation of rat hepatic cytochrome P-450: age-dependent expression, hormonal imprinting and xenobiotic inducibility of sex-specific isoenzymes. *Biochemistry* **24**: 4409–4417, 1985.