Biochemical Pharmacology, Vol. 29, pp. 3245–3251.

0006-2952/80/1215-3245 $02.00/0

SEX AND AGE DEPENDENCE OF THE "SELECTIVE" INDUCTION OF RAT HEPATIC MICROSOMAL EPOXIDE HYDRATASE FOLLOWING *TRANS*-STILBENE OXIDE, *l*-α-ACETYLMETHADOL, OR PHENOBARBITAL TREATMENT

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(*Received* 12 *February* 1980; *accepted* 10 *June* 1980)

Abstract—Conflicting reports exist regarding the "selective" induction of rat hepatic microsomal epoxide hydratase (EH) by *trans*-stilbene oxide (*t*-SO). We reasoned that rats of differing age and sex used in previous studies could be the source of the apparent discrepancy. To clarify the effect of *t*-SO on the microsomal enzymes, immature male, adult male and adult female rats were used. The rats were treated with *t*-SO, phenobarbital, or *l*-α-acetylmethadol (LAAM), all inducers of EH, and the effects of age and sex on the responses of selected microsomal enzymes were determined. *t*-SO was found to increase benzo[*a*]pyrene hydroxylase (AHH) activity in the adult females and immature males. However, it did not increase AHH activity in the adult male. *t*-SO increased aminopyrene *N*-demethylase activity in adult males and adult females, and it elevated EH activity in all rats studied. The effects of *t*-SO were similar to those of phenobarbital. LAAM was found to increase EH in adult male and adult female rats; it increased AHH in the females, but not in the males. In castrated male rats, *t*-SO and phenobarbital affected AHH in a fashion equivalent to that observed in normal adult males. In adult males, each of the agents studied raised EH activity without a simultaneous increase in AHH activity. This "selectivity", however, did not hold for other cytochrome P-450-dependent monooxygenases.

An abundance of evidence indicates that reactive metabolites are responsible for certain toxicities produced by numerous chemicals, including therapeutic agents, insecticides, and carcinogens [1, 2]. Labile epoxide intermediates have been implicated in the causation of polycyclic aromatic hydrocarbon, aflatoxin B_1, and trichloroethylene carcinogenicity [3–5], of bromobenzene and furosemide hepatotoxicity [6, 7], and of diphenylhydantoin teratogenicity [8]. Epoxides are generated by cytochrome P-450-dependent oxygenation of olefinic or aromatic moieties in the parent molecule [9]. The enzyme epoxide hydratase (EH) catalyzes the addition of water to a structurally diverse range of epoxides, yielding chemically nonreactive dihydroxylated products (dihydrodiols) [10]. Consequently, the steady-state level of epoxides and, thus, the degree of toxicity will depend, in part, on the rates of formation and further biotransformation of these intermediates. Compounds that could selectively increase EH activity without a concomitant increase in cytochrome P-450-dependent mixed function oxygenase activities (MFO) would be useful tools for studying the role of EH in the disposition and toxicity of these compounds.

Trans-stilbene oxide (*t*-SO) treatment has been found to increase rat hepatic microsomal EH activity, but conflicting reports exist regarding its effects on MFO. Schmassmann and Oesch [11] demonstrated an increased EH activity without a simultaneous augmentation of cytochrome P-450, benzo[*a*]pyrene hydroxylase (AHH), or aminopyrine *N*-demethylase activities and, therefore, suggested that *t*-SO was a "selective" inducer of EH. In a subsequent report, Bucker *et al.* [12] again demonstrated that AHH was unaffected by *t*-SO. Mukhtar *et al.* [13], however, found that EH activity and cytochrome P-450, aminopyrine *N*-demethylase, and AHH activities were all increased; they concluded that *t*-SO was an inducer of both EH and MFO.

It is well known that the basal activities of various rat hepatic cytochrome P-450-dependent enzymes are greater in adult males than in adult females or immature males [14, 15]. Further, the inducibility of these enzymes is influenced by both the age and sex of the rats employed [14]. We have reported previously that methadone treatment increased hepatic microsomal EH activity without altering AHH activity in adult male rats. In adult female rats, however, AHH activity was increased by methadone [16]. Therefore, we hypothesized that the apparent discrepancy in the *t*-SO studies could have been the result of using different groups of rats. Schmassmann and Oesch employed adult male rats, whereas Mukhtar and coworkers carried out their experiments in immature males and females.

In the present study, we reinvestigated the effects of *t*-SO in immature male, adult male, and adult female rats. We also compared the effects of *t*-SO with those of two other inducers of rat hepatic EH: *l*-α-acetylmethadol (LAAM), a longer acting congener of methadone [17], and phenobarbital [18]. We found that all these compounds increased hepatic

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microsomal EH activity, as well as that of selected cytochrome P-450-dependent monooxygenases. In addition, we demonstrated that these compounds exert varying effects on AHH activity depending on the age and sex of the animals.

MATERIALS AND METHODS

Animals and treatments. Wistar rats were obtained from Canadian Breeding Farms Ltd., La Prairie, Quebec. They were raised under controlled conditions (22°, lights on 6:00 a.m. to 8:00 p.m.) on corncob bedding (Lobund grade, Paxton Processing Ltd., Paxton, IL). Purina lab chow and water were available *ad lib.* Animals were allowed to equilibrate for at least 1 week before use. The adult male, adult female, and immature male rats at the time of killing weighed 200–300, 200–250 and 80–100 g respectively. *t*-SO in corn oil was administered i.p. once daily for 3 consecutive days at 100, 200 or 400 mg/kg. Phenobarbital was administered at 80 $mg \cdot kg^{-1} \cdot day^{-1}$ i.p. in saline for 4 consecutive days. 3-Methylcholanthrene in corn oil was administered i.p. once daily at 20 mg/kg for 2 days. Control animals received the appropriate vehicle. All animals were killed 24 hr after the last injection. LAAM was administered in the drinking water for 2 weeks. The average daily dose of LAAM ranged from 0.3 to 25 $mg \cdot kg^{-1} \cdot day^{-1}$. The animals were maintained on LAAM until the time of killing. The castrated rats were gonadectomized at 3–4 weeks of age.

Chemicals. *l*-α-Acetylmethadol·HCl (Mallinckrodt, Pointe Claire, P.Q.) was obtained through the Canadian Department of Health and Welfare. 3-Methylcholanthrene was supplied by the Eastman Organic Chemical Co. (Rochester, NY). Benzo[*a*]pyrene, NADP, NADPH, NADH, glucose 6-phosphate, and glucose 6-phosphate dehydrogenase were obtained from the Sigma Chemical Co. (St. Louis, MO). *trans*-Stilbene oxide was purchased from the Aldrich Chemical Co., Inc. (Milwaukee, WI). Phenobarbitone sodium was obtained from the British Drug House (Toronto, Ont.). All other reagents and chemicals employed were the best commercial grades available.

Preparation of microsomes. Animals were stunned by a blow on the head, decapitated and bled. The livers were perfused with 1.15% KCl, excised, and placed in ice-cold 1.15% KCl. All procedures were subsequently carried out at 4°. The livers were blotted, minced, and homogenized 1:4 (w/v) in 1.15% KCl. The homogenates were centrifuged for 10 min at 10,000 *g*, and the resulting supernatant fluid was centrifuged at 100,000 *g* for 1 hr. The pellets were washed and resuspended in the appropriate buffer.

Enzyme assays. AHH activity was determined according to the fluorometric assay of Nebert and Gelboin [19]. The microsomal pellets were resuspended in 50 mM Tris buffer, 3 mM $MgCl_2$, pH 7.5. The incubation was carried out in a final volume of 1 ml containing: 80 nmoles benzo[*a*]pyrene added in 40 μl acetone, 0.31 ml H_2O, 0.5 ml 50mM Tris buffer, (pH 7.5), 0.37 μmole NADH, 0.38 μmole NADPH, 0.6 mg bovine serum albumin, 3.45 μmoles $MgCl_2$, and 0.15 ml microsomal suspension (about 0.05 mg protein). The incubation was carried out at 37° for 5 min. EH activity was determined by the method of Oesch *et al.* [18]. The microsomal pellets were resuspended in 0.5 M Tris buffer, 0.1% Tween 80, pH 9.0. The incubation was carried out in a total volume of 0.4 ml containing: 0.81 μmole [^{3}H]styrene oxide (1000 becquerel) in 20 μl acetonitrile; 0.10 ml 0.5 M Tris buffer, 0.1% Tween 80, pH 9.0; 0.08 ml H_2O; and 0.2 ml enzyme suspension (about 2 mg protein). The incubation was carried out at 37° for 10 min. Aminopyrine *N*-demethylase activity was estimated by formaldehyde formation according to the methods of El Defrawy El Masry *et al.* [15] and Nash [20]. The microsomal pellets were resuspended in 0.1 M phosphate buffer, pH 7.2. The incubation was carried out in a final volume of 1.5 ml containing: 20 μmoles aminopyrine in 100 μl H_2O, 600 μl of 64.5 mM Tris buffer (pH 7.5), 100 μl H_2O, 6.3 μmoles $MgCl_2$, 11.2 μmoles semicarbazide, 5 units glucose-6-phosphate dehydrogenase, 10 μmoles glucose-6-phosphate, 1 μmole NADP, 0.5 ml 50 mM Tris buffer (pH 7.5), and 0.2 ml enzyme suspension (about 2 mg protein). The incubation was carried out at 37° for 10 min. The formation of product was linear with incubation time and protein content for all enzyme assays employed. In addition, the substrate and cofactor concentrations that were used yielded optimal enzyme activity. Proteins were estimated by the method of Sutherland *et al.* [21] as modified by Robson *et al.* [22]. Student's *t*-test was employed for all statistical analyses. Significant differences were assumed when $P < 0.05$ (unpaired sample means, two-tailed).

RESULTS

t-*SO treatment.* *t*-SO increased EH activity in the immature male, adult female, and adult male rats. AHH activity, however, was increased only in the adult females and immature males. These results are shown in Fig. 1. At 200 $mg \cdot kg^{-1} \cdot day^{-1}$ for 3 days, AHH activity was increased in adult female rats by 110 per cent over control. At 400 $mg \cdot kg^{-1} \cdot day^{-1}$ for 3 days, AHH activity was increased in immature male rats by 85 per cent. In contrast, AHH activity was decreased to 65 and 50 per cent of control in adult males following *t*-SO at 200 and 400 $mg \cdot kg^{-1} \cdot day^{-1}$ respectively. These doses of *t*-SO increased EH activity by 3- to 4-fold over control in all animals studied. The weight gain in the adult male rats receiving 400 mg $t\text{-SO} \cdot kg^{-1} \cdot day^{-1}$ for 3 days was significantly less than control. Control and drug-treated groups were matched for weight. The adult male rats treated with 400 mg $t\text{-SO} \cdot kg^{-1} \cdot day^{-1}$ averaged 25 g less than controls at the time of killing. In addition, three out of twenty-four adult male rats that received this dose had obvious liver pathology (the enzyme activities in these livers were many-fold lower than the mean and were not included in the results). These livers were indurated and exhibited patchy white areas. Weight gain was normal and no overt signs of liver damage were observed in all other rats treated with *t*-SO. Aminopyrine *N*-demethylase activities were also determined in adult male and adult female rats (Table 1). At 200 mg $t\text{-SO} \cdot kg^{-1} \cdot day^{-1}$ for 3 days, a dose which

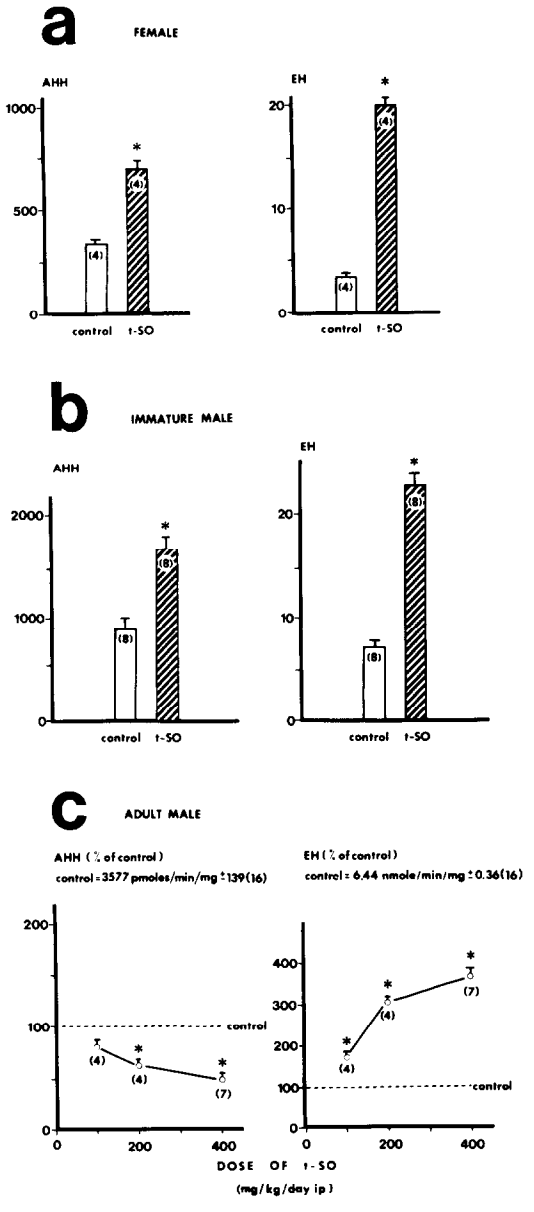

Fig. 1. Effects of *trans*-stilbene oxide (*t*-SO) treatment (3 consecutive days, i.p.) on hepatic microsomal benzo[*a*]pyrene hydroxylase (AHH) and epoxide hydratase (EH) activities in the rat. Adult female rats received 200 mg *t*-SO·kg^{-1}·day^{-1} (panel a). Immature male rats received 400 mg *t*-SO·kg^{-1}·day^{-1} (panel b). AHH activity is expressed as pmoles 3-hydroxybenzo[*a*]pyrene formed·min^{-1}·(mg protein)$^{-1}$ ± S.E.M. (N), and EH activity as nmoles styrene glycol formed·min^{-1}·(mg protein)$^{-1}$ ± S.E.M. (N), except in panel c where enzyme activities are expressed as per cent of control. The asterisk indicates significant difference from control, $P < 0.05$.

decreased AHH by 35 per cent in the adult males, aminopyrine *N*-demethylase activity was increased by 45 per cent. In the adult female, *t*-SO at 200 mg·kg^{-1}·day^{-1} elevated aminopyrine *N*-demethylase activity by 110 per cent.

Phenobarbital treatment. The effects of phenobarbital (80 mg·kg^{-1}·day^{-1} for 4 days, i.p.) on AHH, aminopyrine *N*-demethylase, and EH activities in adult female, immature male, and mature male rats are given in Fig. 2. AHH activity was increased by 340 per cent in the adult female and by 135 per cent in the immature male. In the adult male rat, however, AHH activity was unaffected. In contrast, aminopyrine *N*-demethylase activity was increased in the adult male rat, as well as in the adult female and immature male. EH activity was increased by 2- to 3-fold in all the animals studied.

Table 1. Effects of *trans*-stilbene oxide (*t*-SO) treatment (3 consecutive days, i.p.) on hepatic microsomal aminopyrine *N*-demethylase activity in adult male and adult female rats

Sex	Treatment (mg·kg^{-1}·day^{-1})	Aminopyrine *N*-demethylase activity*
Male	Control	2.47 ± 0.16 (8)
	t-SO (200)	3.52 ± 0.10† (8)
Male	Control	1.33 ± 0.07 (7)
	t-SO (400)	1.78 ± 0.10† (6)
Female	Control	0.62 ± 0.03 (8)
	t-SO (200)	1.30 ± 0.03† (8)

* Aminopyrine *N*-demethylase activities are expressed as nmoles formaldehyde formed·min^{-1}·(mg protein)$^{-1}$ ± S.E.M. (N).

† Indicates significant difference from control, $P < 0.05$.

LAAM treatment. The effects of LAAM in the drinking water for 2 weeks on AHH and EH activities in adult male and adult female rats are illustrated in Fig. 3. LAAM was found to increase EH activity in both male and female rats. In the adult female, AHH activity was raised to about 300 per cent of control. The response of AHH in the adult male was variable. LAAM either had no effect or produced a slight but significant decrease in AHH activity.

3-Methylcholanthrene treatment. Pretreatments of adult male rats with 3-methylcholanthrene (20 mg·kg^{-1}·day^{-1} for 2 days, i.p.) produced a 2-fold increase in AHH activity, but failed to alter EH or aminopyrine *N*-demethylase activities (Table 2). Increases in AHH activity of 950 and 1250 per cent were seen in immature male and adult female rats respectively. It should be noted that the absolute increases in AHH activity among the three groups of animals were approximately the same (5000–7000 pmoles 3-hydroxybenzo[*a*]pyrene formed min^{-1}·(mg protein)$^{-1}$.

Castrated animals. Rats were castrated at 3–4 weeks of age and used when they weighed 200–250 g (Table 3). The basal level of AHH in these rats was about 40 per cent of that observed in the normal adult male. In these rats 80 mg phenobarbital·kg^{-1}·day^{-1} for 4 days had no effect on AHH activity. In the normal adult male, the same dose also had no effect on AHH activity. In addition, 400 mg *t*-SO·kg^{-1}·day^{-1} for 3 days was found to decrease the AHH activity in the castrated males by 35 per cent. At the same dose, *t*-SO was found to decrease AHH activity in the normal male rat by 50 per cent.

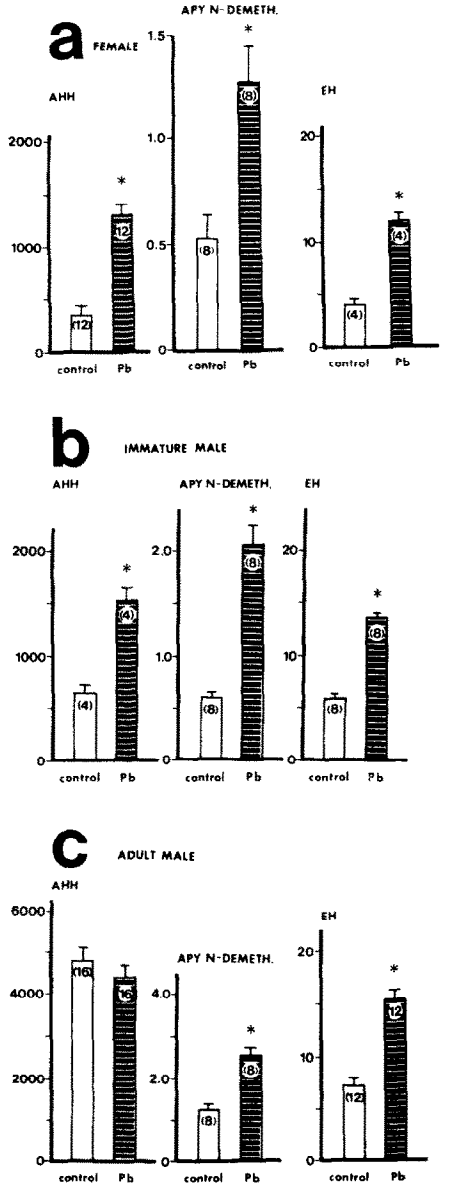

Fig. 2. Effects of phenobarbital (Pb) treatment (80 mg·kg^{-1}·day^{-1} for 4 days, i.p.) on hepatic microsomal benzo[*a*]pyrene hydroxylase (AHH), aminopyrine *N*-demethylase (APY *N*-DEMETH) and epoxide hydratase (EH) activities in adult female (a), immature male (b) and adult male (c) rats. AHH activity is expressed as pmoles 3-hydroxybenzo[*a*]pyrene formed·min^{-1}·(mg protein)$^{-1}$ ± S.E.M. (N), APY *N*-DEMETH activity as nmoles formaldehyde formed·min^{-1}·(mg protein)$^{-1}$ ± S.E.M. (N), and EH activity as nmoles styrene glycol formed·min^{-1}·(mg protein)$^{-1}$ ± S.E.M. (N). The asterisk indicates significant difference from control, $P < 0.05$.

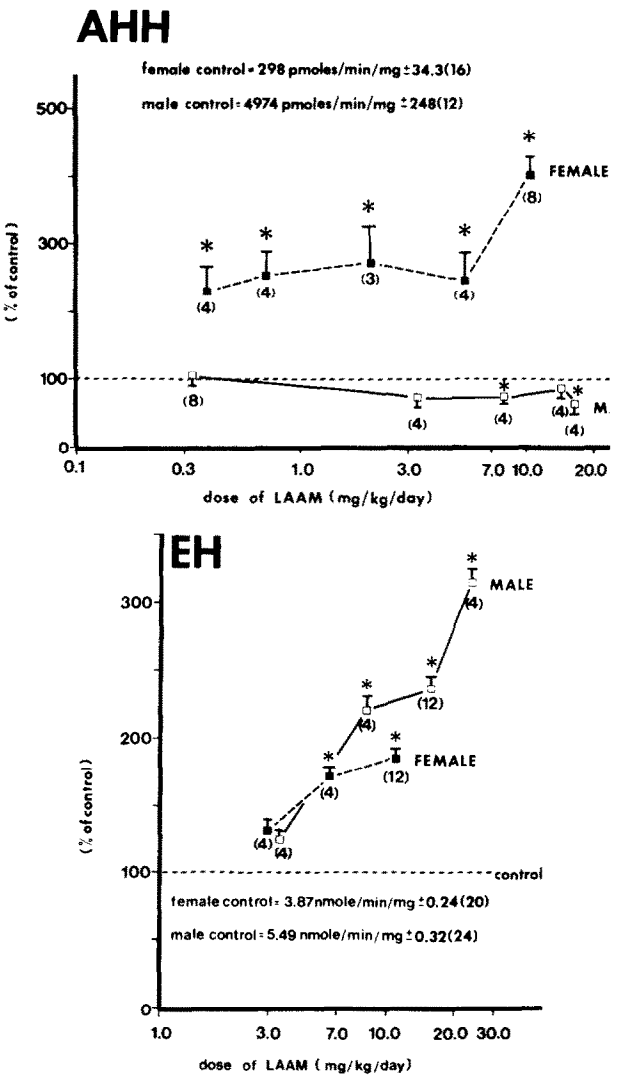

Fig. 3. Effects of *l*-α-acetylmethadol (LAAM) treatment (in the drinking water for 2 weeks) on hepatic microsomal benzo[*a*]pyrene hydroxylase (AHH) and epoxide hydratase (EH) activities in adult male and adult female rats. The dose of LAAM represents the average daily intake per animal over 2 weeks. AHH and EH activities were expressed as per cent of control. The control levels of AHH and EH were represented as pmoles 3-hydroxybenzo[*a*]pyrene formed·min^{-1}·(mg protein)$^{-1}$ ± S.E.M. (N) and nmoles styrene glycol formed·min^{-1}·(mg protein)$^{-1}$ ± S.E.M. (N). The asterisk indicates significant difference from control, $P < 0.05$.

Table 2. Effects of 3-methylcholanthrene (3-MC) treatment (20 mg·kg^{-1}·day^{-1} for 2 consecutive days, i.p.) on benzo[*a*]pyrene hydroxylase (AHH), aminopyrine *N*-demethylase (APY *N*-DEMETH) and epoxide hydratase (EH) activities in the rat

Sex and age	Treatment	AHH activity*	EH activity†	APY *N*-DEMETH activity‡
Adult Male	Corn oil	4723 ± 269 (9)	6.76 ± 0.7 (8)	1.0 ± 0.1 (4)
	3-MC	9407 ± 606§ (9)	8.05 ± 0.4 (8)	1.1 ± 0.03 (4)
Adult Female	Corn oil	581 ± 26 (8)	ND‖	ND
	3-MC	7764 ± 104§ (4)		
Immature Male	Corn oil	677 ± 34.8 (12)	ND	ND
	3-MC	7194 ± 555§ (8)		

* AHH activity is expressed as pmoles 3-hydroxybenzo[*a*]pyrene formed·min^{-1}·(mg protein)$^{-1}$ ± S.E.M. (N).

† EH activity is expressed as nmoles styrene glycol formed·min^{-1}·(mg protein)$^{-1}$ ± S.E.M. (N).

‡ APY *N*-DEMETH activity is expressed as nmoles formaldehyde formed·min^{-1}·(mg protein)$^{-1}$ ± S.E.M. (N).

§ Significantly different from its own control, $P < 0.05$.

‖ Not determined.

Table 3. Effects of *trans*-stilbene oxide (*t*-SO) and phenobarbital treatment on hepatic microsomal benzo[*a*]pyrene hydroxylase (AHH) activity in castrated male rats*

Treatment	AHH activity†
Corn oil control	1544 ± 244 (8)
t-SO (400 mg·kg^{-1}·day^{-1} for 3 days, i.p.)	988 ± 127 (8)‡
Saline control	1657 ± 318 (8)
Phenobarbital (80 mg·kg^{-1}·day^{-1} for 4 days, i.p.)	1844 ± 242 (8)

* Male rats were castrated between 21 and 24 days of age. They were treated and killed when they weighed 200–250 g.

† AHH activity is expressed as pmoles 3-hydroxybenzo[*a*]pyrene formed·min^{-1}·(mg protein)$^{-1}$ ± S.E.M. (N).

‡ Indicates significant difference from control, $P < 0.05$.

DISCUSSION

The results presented in this paper confirm that *t*-SO is an inducer of the cytochrome P-450-dependent monooxygenases, as well as of EH. We have also demonstrated that the response of AHH to *t*-SO, phenobarbital and LAAM, as to methadone [16] is dependent on the age and sex of the rats used. *t*-SO, like phenobarbital, LAAM and methadone [16] (other inducers of EH), increased AHH activity in the adult female rat. *t*-SO and phenobarbital were also found to increase AHH activity in the immature male. In the adult male rat, however, these four agents increased EH activity without producing a simultaneous increase in AHH activity. When aminopyrine *N*-demethylase was determined, its activity was found to be increased by treatment with *t*-SO or phenobarbital in both adult males and adult females. The finding that *t*-SO increased aminopyrine *N*-demethylase activity in the adult male does not support the results of Schmassmann and Oesch [11] who reported that this activity was unaltered. They also reported that cytochrome P-450 levels were unaltered in these rats. Recently, Seidegard *et al.* [23] showed that cytochrome P-450 levels in the adult male were elevated more than 2-fold following *t*-SO. The effects of *t*-SO on AHH and aminopyrine *N*-demethylase activities in the adult female and immature male (Fig. 1 and Table 1) are in accord with the previous work of Mukhtar *et al.* [13]. In contrast with the findings of both the present study and that previously stated [13], Oesch and Schmassmann [24] found that AHH activity in the female rat was not altered by *t*-SO.

Inducers of the microsomal drug-metabolizing enzymes have been classically divided into two groups: phenobarbital (P-450)-type or 3-methylcholanthrene (P-448)-type inducers [25]. In the present experiments, the following responses were observed with the prototypes. As expected, 3-methylcholanthrene did not increase EH or aminopyrine *N*-demethylase activities [14, 26]. In addition, 3-methylcholanthrene increased AHH activity in the immature male, adult female, and adult male

rat. In contrast, phenobarbital produced markedly smaller increases in AHH activity in the adult female and immature male, and failed to increase AHH activity in the adult male. Phenobarbital was found, however, to increase EH activity, as well as aminopyrine *N*-demethylase activity, in all rats studied. *t*-SO is a phenobarbital (P-450)-type inducer of microsomal enzymes in that, like phenobarbital, *t*-SO does not increase AHH activity in the adult male; it increases AHH activity in the adult female and immature male, and it increases EH and aminopyrine *N*-demethylase activities in adult males and adult females. In support of this suggestion, Seidegard *et al.* [23] have shown that *t*-SO increases cytochrome P-450 and does not cause the formation of the spectrally distinct cytochrome P-448, a major characteristic of 3-methylcholanthrene (P-488)-type inducers [25].

In the adult male, *t*-SO actually produced a decrease in AHH activity. The dose of *t*-SO employed was quite high, so direct inhibition of the enzyme by *t*-SO present in the microsomes could be a factor. Also, we noted a decreased weight gain in these animals, as well as signs of liver injury (three out of twenty-four with gross liver necrosis). Therefore, hepatic toxicity could be an important factor in the decreased AHH levels. Furthermore, the increases in the other enzyme activities as well as the increases in AHH seen in immature males and adult females could have been affected by these factors. Indeed, enzyme inhibition or toxicity could have masked what may actually be greater increases by *t*-SO.

There is no sex difference in AHH activity in the immature rat [27]. Upon maturity, however, the levels of AHH activity increase dramatically in males. This effect is attributed to the increased production of testosterone, an endogenous inducer. Testosterone administration to immature females or castrated males results in the AHH levels observed in adult males [28]. Castration at 3–4 weeks of age, however, does not decrease AHH activity from the adult male to the adult female level [28]. We have observed this previously [29, 30], and again in the present study. Such a castrated male is not a "female" or "immature male" with regard to AHH activity. First, the basal level of AHH in the castrated male is many-fold higher than in the adult female. Also, phenobarbital and *t*-SO treatments affect AHH in the castrated male in the same manner as in the normal adult male. Whether this is due to the castration failing to reduce testosterone levels to that of the adult female or to testosterone influencing this enzyme irreversibly with respect to its inducibility by the time castration occurred is not known at present. Once AHH activity has increased to its normal level in the adult male, however, this enzyme has not been shown to be increased further by phenobarbital (P-450)-type inducers. 3-Methylcholanthrene (P-448)-type inducers increase AHH to the same extent in all rats. This induction appears not to be influenced by age or sex. The induction of aminopyrine *N*-demethylase by phenobarbital is also independent of age and sex.

As a result of the non-inducibility of AHH by phenobarbital (P-450)-type inducers in the adult male rat, compounds of this type which increase EH activity could be used as selective inducers of this enzyme without producing an effect on benzo[*a*]pyrene monooxygenation. Such compounds, however, are not selective inducers of EH with respect to other cytochrome P-450-dependent monooxygenases [31, 32], nor are they selective inducers in adult female or immature male rats.

Acknowledgements—The present study was supported by the Medical Research Council of Canada. We are grateful to Malcolm Finlayson and Ming Li for their contributions to this paper.

REFERENCES

1. J. R. Gillette, *Biochem. Pharmac.* **23**, 2785 (1974).
2. J. A. Miller, *Cancer Res.* **30**, 559 (1970).
3. P. Sims, P. L. Grover, A. Swaisland, K. Pal and A. Hewer, *Nature, Lond.* **252**, 326 (1974).
4. K. Alexandrov and C. Frayssinet, *Cancer Res.* **34**, 3289 (1974).
5. B. L. Van Duuren and S. Banergee, *Cancer Res.* **36**, 2419 (1976).
6. N. Zampaglione, D. J. Jollow, J. R. Mitchell, B. Stripp, M. Hamrick and J. R. Gillette, *J. Pharmac. exp. Ther.* **187**, 218 (1973).
7. J. R. Mitchell, W. L. Nelson, W. Z. Potter, H. A. Sasame and D. J. Jollow, *J. Pharmac. exp. Ther.* **199**, 41 (1976).
8. F. Martz, C. Failinger III and D. A. Blake, *J. Pharmac. exp. Ther.* **203**, 231 (1977).
9. C. Heidelberger, *A. Rev. Biochem.* **44**, 79 (1975).
10. F. Oesch, *Xenobiotica* **3**, 305 (1973).
11. H. Schmassmann and F. Oesch, *Molec. Pharmac.* **14**, 834 (1978).
12. M. Bucker, M. Golan, H. U. Schmassmann, H. R. Glatt, P. Stasiecki and F. Oesch, *Molec. Pharmac.* **16**, 656 (1979).
13. H. Mukhtar, T. H. Elmamlouk and J. R. Bend, *Chem. Biol. Interact.* **22**, 125 (1978).
14. A. H. Conney, *Pharmac. Rev.* **19**, 317 (1967).
15. S. El Defrawy El Masry, G. M. Cohen and G. J. Mannering, *Drug Metab. Dispos.* **2**, 267 (1974).
16. G. D. Bellward, L. S. Gontovnick and M. Otten, *Drug Metab. Dispos.* **5**, 211 (1977).
17. G. D. Bellward, M. Li, G. D. Lopaschuk and P. Warren, *Res. Commun. Chem. Path. Pharmac.* **18**, 415 (1977).
18. F. Oesch, D. M. Jerina and J. Daly, *Biochim. biophys. Acta* **227**, 685 (1971).
19. D. W. Nebert and H. V. Gelboin, *J. biol. Chem.* **243**, 6242 (1968).
20. T. Nash, *Biochem. J.* **55**, 416 (1953).
21. E. W. Sutherland, C. F. Cori, R. Haynes and N. S. Olson, *J. biol. Chem.* **180**, 825 (1949).
22. R. M. Robson, D. E. Gall and M. J. Temple, *Analyt. Biochem.* **24**, 339 (1968).
23. J. Seidgard, R. Morgenstern, J. W. DePierre and L. Ernster, *Biochim. biophys. Acta* **586**, 10 (1979).
24. F. Oesch and H. Schmassmann, *Biochem. Pharmac.* **28**, 171 (1979).
25. G. J. Mannering, in *Selected Pharmacological Testing Methods* (Ed. A. Burger), pp. 51–119. Marcel Dekker, New York (1968).
26. E. Bresnick, H. Mukhtar, T. A. Stoming, P. M. Dansette and D. M. Jerina, *Biochem. Pharmac.* **26**, 891 (1977).
27. F. Oesch, *J. biol. Chem.* **251**, 79 (1976).
28. H. L. Gurtoo and N. B. Parker, *Drug Metab. Dispos.* **5**, 474 (1977).

29. P. M. Warren and G. D. Bellward, *Biochem. Pharmac.* **27**, 2537 (1978).
30. L. S. Gontovnick, L. Roelofs and G. D. Bellward, *Can. J. Physiol. Pharmac.* **57**, 286 (1978).
31. T. C. Spaulding, L. Minium, A. N. Kotake and A. E. Takemori, *Drug Metab. Dispos.* **2**, 458 (1974).
32. G. A. Lesher and M. McCarren, *Pharmacologist* **21**, 321 (1979).