Biochemical Pharmacology, Vol. 34, No. 19, pp. 3533–3536, 1985.
Printed in Great Britain.

0006-2952/85 $3.00 + 0.00

SEX DIFFERENCES IN THE EFFECTS OF MICROSOMAL ENZYME INDUCERS ON HEPATIC PHASE I DRUG METABOLISM IN THE RAT

PAUL SKETT and PAULINE PATERSON*
Department of Pharmacology, The University, Glasgow G12 8QQ, U.K.

(*Received* 16 *February* 1985; *accepted* 13 *May* 1985)

Abstract—This study investigates the sex-dependence of the effects of microsomal enzyme inducers (phenobarbitone, isosafrole and ethanol) on the hepatic phase I metabolism of lignocaine and imipramine. It is shown that all of the inducers exert sex-dependent effects on the enzymes activities known to be sex related in the rat, e.g. lignocaine *N*-deethylase activity is decreased by phenobarbitone pretreatment in the male but increased by the same treatment in the female. The inducers tend to decrease the sex differences seen in untreated animals. Ethanol may give this effect by its action of decreasing serum testosterone levels but the mechanism of action of the other compounds is uncertain. It is possible that the sex-dependent cytochrome P-450 species are selectively sensitive to the action of the compounds in terms of induction, repression or inhibition. It is clear, however, that the effects of the pretreatments are related to the sex differences in phase I metabolism in the rat.

Many compounds are known to induce the hepatic microsomal metabolism of drugs, xenobiotics and endogenous compounds [1]. These compounds were originally classified into two types, namely phenobarbitone-type and 3-methylcholanthrene(3MC)- or polycyclic hydrocarbon-type on the basis of their ability to preferentially induce different pathways of metabolism. The phenobarbitone-type was said to induce the metabolism of most lipid-soluble compounds [2] whereas the 3MC-type was more specific in inducing aryl hydrocarbon hydroxylase [3]. More recently additional classes of inducers have appeared such as the isosafrole-type [4] and the ethanol-type [5–7], each with their own induction profile. The differential induction has been linked to the ability of the inducers to stimulate the production of one or more isoenzymes of cytochrome P-450 [4].

In most of these studies only one sex of animal has been investigated (usually the male). This is rather unsatisfactory owing to the known sex differences in hepatic microsomal metabolism of drugs seen particularly in the rat [8–10]. Indeed, when male and female animals have been investigated in induction experiments sex differences have been observed. For instance, induction of hepatic metabolism by polychlorinated biphenyls [11], flumecinol [12], toluene [13], acetylmethadol [14] and mirex [15] has been shown to be sex related.

This study was designed to investigate the sex differences in induction caused by three different types of compounds, phenobarbitone, isosafrole and ethanol and to attempt to relate the differences to the known sex-dependence of the enzymes under study.

* Present address: Department of Biochemistry, University of Birmingham, P.O. Box 363, Birmingham B15 2TT.

MATERIALS AND METHODS

Animals. Wistar rats, bred in the department, were used throughout the experiment. Males were 250–300 g and females 225–250 g body weight at the start of the experiment. Animals were kept in a light- and temperature-controlled room (lights on 08:00 – 20:00; 19 ± 1°) in wire-bottomed cages (except during ethanol induction—see below). Animals were allowed free access to food (CRM Nuts, Labsure Diets, Croydon) and tap water during the experiment.

Chemicals. Ethanol (96%, containing no denaturants) was obtained from James Burroughs Ltd. (London) and isosafrole (1,2-(methylenedioxy)-4-propenylbenzene) from K & K Laboratories Inc. (Plainview, New York). Phenobarbitone, sodium salt and sodium isocitrate were purchased from Sigma Chemical Co. (Poole). $NADP^+$ and isocitric dehydrogenase (EC 1.1.1.42) were obtained from Boehringer Corporation Ltd. (London). Carbonyl-^{14}C lignocaine hydrochloride was purchased from New England Nuclear Ltd. (Southampton) and 2-^{14}C imipramine from Amersham International p.l.c. (Amersham). Unlabelled lignocaine and imipramine were the kind gifts of Astra läkemedel AB (Södertälje, Sweden) and Ciba-Geigy AG. (Basel, Switzerland) respectively. All other chemicals were of at least reagent grade.

Animal treatments. Male and female animals (five in each group) were treated in one of four ways: once daily for three days with 90 mg/kg phenobarbitone (in distilled water) by i.p. injection; once daily for three days with 450 mg/kg isosafrole (dissolved in corn oil) by i.p. injection; for ten days with ethanol using the vapour inhalation technique of Ferko and Bobyock [16] described below; untreated as controls.

Animals undergoing ethanol induction were

housed in a Perspex box (1 m × 1.5 m × 0.3 m) through which air was pumped at a rate of 8 l/min. Before entry into the box, the air passed through a heated flask into which ethanol was dripped at a starting rate of 80 mg/min increasing to 160 mg/min over three days. The latter rate of ethanol was maintained for a further seven days.

Animals were killed by CO_2-asphyxiation and cervical dislocation 24 hr after the last dose of phenobarbitone and isosafrole or directly after removal from the ethanol vapour box. The livers were quickly removed and cooled in ice-cold buffer (0.25 M sucrose/0.1 M Tris pH 7.4). Microsomal fractions were prepared by the Ca^{2+}- precipitation technique of Cinti *et al.* [17] and resuspended in the same buffer at a concentration of 10 mg protein/ml.

Assays. Microsomes (1 ml) were incubated with either lignocaine or imipramine in the presence of an NADPH-regenerating system as described previously [18].

Protein content was measured by the method of Lowry *et al.* [19] using bovine serum albumin as standard and cytochrome P-450 content by the method of Omura and Sato (20) using a dual beam Shimadzu UV-240 spectrophotometer.

Results were expressed as nmoles product/min/mg protein and as nmoles product/min/nmole cytochrome P-450. Means, standard deviations and variances were calculated for each group and statistical analyses performed using Duncan's multiple range test using the relevant control group for comparison. The level of significance was set at $P < 0.05$.

Table 1. Effects of phenobarbitone (PB), ethanol (EtOH) and isosafrole (Iso) on the metabolism of lignocaine in the rat liver

Treatment	*N*-deethylase	3-OHase
Male PB	63 ± 7*	222 ± 42‡
Male EtOH	21 ± 3†	62 ± 24*
Male Iso	94 ± 50	437 ± 156†
Female PB	207 ± 14‡	323 ± 46‡
Female EtOH	78 ± 25	84 ± 20
Female Iso	155 ± 28*	236 ± 48†
(Above results expressed as percentage of control calculated from nmoles product/min/mg protein.)		
100% values:		
Male	0.23 ± 0.08	0.04 ± 0.01
Female	0.08 ± 0.01	0.04 ± 0.01
Male PB	26 ± 3†	94 ± 10
Male EtOH	15 ± 4‡	36 ± 23†
Male Iso	71 ± 39	318 ± 132†
Female PB	73 ± 20	132 ± 33
Female EtOH	95 ± 22	81 ± 11
Female Iso	126 ± 54	184 ± 67
(Above results expressed as percentage of control derived from nmoles product/min/nmole cyt.P-450.)		
100% values:		
Male	1.31 ± 0.39	0.20 ± 0.05
Female	0.64 ± 0.08	0.25 ± 0.13

* $P < 0.05$; † $P < 0.01$; ‡ $P < 0.001$.

RESULTS

The effects of pretreatment on liver weight, protein and cytochrome P-450 content of the microsomes are as outlined below. It is seen that liver weight expressed as a percentage of body weight was unaffected by any treatment (control values, 4.8% and 4.4% for male and female animals respectively) but that protein content was significantly increased by phenobarbitone in the male (28%) and by phenobarbitone and ethanol in the female (62% and 42% respectively; control values being 30.0 and 31.3 mg/g wet weight liver for male and female animals respectively). Increase in protein content was always more pronounced in the females. Induction of cytochrome P-450 was only seen for phenobarbitone pretreatment and was unaffected by the sex of the animal (137% and 138% in the male and female respectively; control values being 0.20 and 0.21 nmoles/mg protein in male and female animals respectively). Changes in the absorption maximum of the CO-induced spectral change of cytochrome P-450 were noticed. Isosafrole caused a blue shift to 447–448 nm whereas ethanol caused a red shift to 451–452 nm in both sexes. Phenobarbitone did not affect the absorption maximum (results not shown).

Table 1 shows the effect of the inducing agents on the metabolism of lignocaine. It is seen that, when activity is expressed per mg protein, phenobarbitone caused a marked reduction in *N*-deethylation (37%) and a marked induction of 3-hydroxylation (122%) in the male while causing induction of both activities in the female (107% and 223% for the *N*-deethylase and 3-hydroxylase respectively). Isosafrole induced 3-hydroxylation in the male (337%) but *N*-deethylation (55%) and 3-hydroxlyation (136%) in the female. Induction of 3-hydroxylation was more pronounced in the male with isosafrole and with phenobarbitone in the female. Ethanol pretreatment caused a marked reduction in the metabolism of lignocaine by both pathways in the male (79% for the *N*-deethylase and 38% for the 3-hydroxylase) but was without any significant effect in the female.

If the metabolism of lignocaine is expressed per nmole P-450 then no significant effects are seen in the female but the male shows selective induction of 3-hydroxylation (218%) after isosafrole pretreatment. Decreases in both activities are seen after ethanol (85% for the *N*-deethylase and 64% for the 3-hydroxylase) and a decrease in *N*-deethylation after phenobarbitone (74%) in the male. Ethanol would appear to be more powerful in this respect than phenobarbitone in the male.

Table 2 shows the effect of inducing agents on the metabolism of imipramine. It is seen that, when expressed as enzyme activity per mg protein, the inducing agents had little effect on the metabolism of imipramine in the male except that phenobarbitone decreased *N*-demethylation (32%). In the female, phenobarbitone caused a decrease in 2-hydroxylation (35%) but an increase in *N*-demethylation (118%). Ethanol caused a decrease in 2-hydroxylation and *N*-oxidation (40% and 64% respectively) and isosafrole caused a significant decrease in all

Table 2. Effects of phenobarbitone (PB), ethanol (EtOH) and isosafrole (Iso) on imipramine metabolism in rat liver

Treatment	2-OHase	*N*-demethylase	*N*-oxidase
Male PB	111 ± 28	68 ± 9‡	91 ± 12
Male EtOH	91 ± 21	82 ± 11	80 ± 15
Male Iso	74 ± 25	84 ± 31	124 ± 64
Female PB	65 ± 19*	218 ± 40†	71 ± 7
Female EtOH	60 ± 11‡	72 ± 16	32 ± 5*
Female Iso	49 ± 24†	62 ± 20*	27 ± 11*
(Above results expressed as percentage of control (mean ± S.D. of 5 animals) calculated as nmoles product/min/mg protein.)			
100% values:			
Male	0.07 ± 0.01	0.18 ± 0.02	0.03 ± 0.01
Female	0.13 ± 0.03	0.10 ± 0.02	0.03 ± 0.01
Male PB	42 ± 7‡	26 ± 4‡	32 ± 3‡
Male EtOH	55 ± 20†	64 ± 22	67 ± 5
Male Iso	56 ± 25*	63 ± 24	77 ± 32
Female PB	28 ± 12†	56 ± 5*	33 ± 10
Female EtOH	61 ± 18	71 ± 13	55 ± 17
Female Iso	34 ± 16†	53 ± 13*	28 ± 13*
(Above results expressed as percentage of control (mean ± S.D of 5 animals) calculated as nmoles product/min/nmole cyt.P-450.)			
100% values:			
Male	0.44 ± 0.11	0.95 ± 0.31	0.22 ± 0.04
Female	0.83 ± 0.26	0.41 ± 0.12	0.17 ± 0.10

* $P < 0.05$; † $P < 0.01$; ‡ $P < 0.001$.

activities (51%, 38% and 73% for the 2-hydroxylase, *N*-demethylase and *N*-oxidase respectively).

If imipramine metabolism is expressed per nmole cytochrome P-450 then phenobarbitone was seen to cause a marked reduction in all activities in the male (58%, 74% and 68% for the 2-hydroxylase, *N*-demethylase and *N*-oxidase respectively) and both ethanol and isosafrole caused a decrease in 2-hydroxylase activity (45% and 44% respectively). In the female phenobarbitone caused a reduction in 2-hydroxylase and *N*-demethylase activity (72% and 44% respectively) while isosafrole pretreatment led to a decrease in all activities (66%, 47% and 72% for the 2-hydroxylase, *N*-demethylase and *N*-oxidase respectively). Ethanol was without effect in the female. No specific induction is seen for imipramine metabolism.

DISCUSSION

Induction of hepatic microsomal enzymes by phenobarbitone, isosafrole and ethanol are well documented. Phenobarbitone has been shown to be a relatively non-specific inducer [2] causing marked increases in cytochrome P-450 content without any change in absorption maximum for the CO-induced spectrum of the cytochrome [21]. Isosafrole induces a form of cytochrome P-450 different from that induced by phenobarbitone and having an absorption maximum of the CO-induced spectrum below 450 nm [8]. Ethanol treatment leads to the appearance of a unique isoenzyme of cytochrome P-450 with a red shift in its CO-induced difference spectrum [7]. The results obtained in this study are consistent with the earlier data and indicate that the treatment schedules used have produced the desired induction. The effect of the inducers on liver weight and protein content are similar to earlier data also [22, 23].

The effects of phenobarbitone in the male can be summarized as induction of 3-hydroxylation of lignocaine and reduction of *N*-dealkylation of both lignocaine and imipramine. In the female, induction of *N*-dealkylation of both substrates and 3-hydroxylation of lignocaine is seen but reduction in imipramine 2-hydroxylase activity. There is, thus, a clear sex difference in the effect of phenobarbitone. This effect has been noted earlier for the induction of aryl hydrocarbon hydroxylase [24] where an effect was seen in the female but not in the male. Such sex differences in induction by phenobarbitone are also seen in the rainbow trout [25]. Phenobarbitone caused no increase in activity when expressed as a turnover number (i.e. nmoles product/nmole P-450), indeed significant decreases in turnover number were observed. Thus, although the activity of the *N*-dealkylase is increased in the female, it is not *specifically* induced. Phenobarbitone is, therefore, not a specific inducer for any of the enzymes studied.

Isosafrole induced the activity of the lignocaine 3-hydroxylase in both sexes but the *N*-deethylase only in the female. In the metabolism of imipramine, isosafrole decreased the activity of all the enzymes. Isosafrole is unique in this study in selectively inducing one of the enzymes studied, namely the lignocaine 3-hydroxylase. The lignocaine 3-hydroxylase has been proposed to be polycyclic hydrocarbon-

inducible [26] but the above data would suggest it is also isosafrole-inducible. These two statements may be compatible, however, as isosafrole has been reported to also induce a 3MC-like form of cytochrome P-450 [27]. The marked sex difference in effect on the *N*-dealkylation is again seen. One disadvantage with isosafrole as an inducer is that, following induction, up to 30% of the cytochrome P-450 is complexed with a metabolite of isosafrole which is not easily removed [4]. This bound metabolite will interfere with the measured activity of the microsomal preparation and may be the reason for the inhibition of some of the enzyme activities in this study.

Ethanol caused a general decrease in enzyme activity and in no case was any induction seen. This contrasts with earlier data showing that ethanol induces aniline 4-hydroxylation [15]. The substrates chosen in this study apparently are not metabolized by the ethanol-induced cytochrome P-450. The decrease in enzyme activity seen could be due to residual ethanol being present in the microsomal preparation as ethanol is a known drug metabolism inhibitor [28]. Another possible reason for the inhibition of drug metabolism is discussed below. The data available do not allow discrimination between inhibition of formed enzymes and repression of the formation of new enzyme and, thus, either effect could account for the decrease in metabolism seen for some activities.

Sex differences exist in the metabolism of the substrates under study [18] and it is interesting to note that the marked sex differences in effect of the inducers are related to the sex-dependent enzymes. *N*-dealkylation of both substrates is higher in the male than in the female and it is seen that phenobarbitone decreases these activities in the male and induces them in the female, indeed the activities of the *N*-dealkylases are very similar in the male and female after phenobarbitone induction. It, thus, seems likely that the differences in effects of the inducers in the male and female are related to the sex differences in drug metabolism noted previously. It would appear that the sex-dependent cytochrome P-450 species, recently described by Kamazaki *et al.* [29], have different susceptibilities to induction, repression and/or inhibition by the compounds under study, e.g. the *N*-dealkylase in the male is not inducible by phenobarbitone whereas that in the female is.

In the case of ethanol, another possible interaction must also be taken into account. Ethanol treatment has been shown to reduce serum testosterone levels [30] and this can lead to changes in drug metabolism as some enzyme activities are correlated to serum testosterone [18]. This could explain the sex dependence of the effect of ethanol on lignocaine *N*-deethylase.

In conclusion, sex differences exist in the effect of phenobarbitone, isosafrole and ethanol on hepatic phase I metabolism of drugs. These differences are related to the sex-dependence of hepatic drug metabolism in the rat. The effects seen may be related to sex specific induction, inhibition or repression of the enzymes involved and, in the case of ethanol, may be related to an effect of the inducer on the hormonal status of the animal. It is, thus, important to consider not only the compound being used but also the sex of the animals when induction studies are carried out in rats.

Acknowledgements—This work was supported by the University of Glasgow Medical Research Fund.

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