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Similarities and Differences in the Glucuronidation of Estradiol and Estrone by UDP-Glucuronosyltransferase in Liver Microsomes from Male and Female Rats

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ABSTRACT. In this study, we evaluated the effects of pH, *in vitro* enzyme inhibitors, *in vivo* enzyme inducers, age, and sex on the glucuronidation of estradiol and estrone by rat liver microsomes. Although the pH dependence curves for the glucuronidation of estradiol and estrone were similar, the pH dependence curves for these estrogens by liver microsomes from adult male rats were very different from those by liver microsomes from adult female rats. These results suggest that liver microsomes from adult male and female rats have different estrogen glucuronosyltransferases. Liver microsomes from immature or adult female rats catalyzed the glucuronidation of estrone and estradiol more rapidly than liver microsomes from age-matched male rats. Intraperitoneal injection of sodium phenobarbital (75 mg/kg/day) or dexamethasone (75 mg/kg/day) into immature or adult male or female rats for 3–4 days resulted in a 33–58% increase in liver microsomal glucuronosyltransferase activity for estradiol, but there was little or no stimulatory effect on glucuronosyltransferase activity for estrone. Treatment of immature or adult male or female rats with 3-methylcholanthrene (25 mg/kg/day) for 3–4 days did not stimulate liver microsomal glucuronosyltransferase activity for estradiol or estrone, but the glucuronidation of 4-nitrophenol was stimulated several-fold. The *in vitro* addition of testosterone had a strong inhibitory effect on the glucuronidation of estradiol and estrone by liver microsomes from both adult male and female rats, whereas the *in vitro* addition of 4-nitrophenol had a slightly greater inhibitory effect on the glucuronidation of estradiol and estrone by adult male liver microsomes than by adult female liver microsomes. In conclusion, our results suggest that male and female rat livers have different estrogen glucuronosyltransferases and that the glucuronidation of estradiol, estrone, and 4-nitrophenol is catalyzed by different glucuronosyltransferases that are under different regulatory control. BIOCHEM PHARMACOL 51;9:1195–1202, 1996.

KEY WORDS. glucuronosyltransferase; microsomal enzymes; enzyme induction; glucuronidation; estradiol; estrone

Estrogens are female sex hormones that mediate a wide spectrum of biological effects including a superpotent mitogenic action in target tissues (e.g. the uterus and breast). Exposure to excessive estrogenic hormonal stimulation has also been implicated in the etiology of various estrogen-associated tumors in animal models [1–3] as well as in humans [4–11]. In experimental animals and humans, estrogens are efficiently disposed of by metabolic inactivation, which includes both oxidative metabolism (catalyzed by cytochrome P450 enzymes) and conjugative metabolism (glucuronidation and sulfonation catalyzed by glucuronosyltransferase and sulfotransferase, respectively). Previous studies have shown that alterations in the rate of metabolic inactivation of estrogens may alter significantly their hormonal as well as their carcinogenic activity. For instance, the use of certain inducers of drug-metabolizing enzymes which increase the oxidative metabolism of endogenous and synthetic estrogens decreases the uterotropic activity of these hormones *in vivo* [12–17] and inhibits the formation of spontaneous mammary tumors in C3H/OuJ mice [18]. It should be pointed out that some of these enzyme inducers not only increase the metabolic conversion of estrogenic hormones to inactive metabolites but may also increase their conversion to estrogenic and potentially genotoxic metabolites such as 4-hydroxyestradiol and 16α-hydroxyestrone [17, 19–23]. In comparison with inducers of monooxygenases, monofunctional inducers of estrogen conjugation may lower estrogenic hormonal activity solely by increasing the levels of enzymes for conjugation to hormonally inactive or less active metabolites that are excreted rapidly in the urine. Moreover, the induction of conjuga-

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tion enzymes will also accelerate the conjugation of estrogenic or potentially genotoxic oxidative metabolites of estrogens and thus enhance their urinary excretion.

The UDPGA†-dependent biotransformation of estrogens and their hydroxylated metabolites to water-soluble glucuronides is one of the major conjugation reactions in the body [24, 25], and this reaction is catalyzed by cellular UDP-glucuronosyltransferases [26]. Previous studies have shown that the glucuronosyltransferase activity for some xenobiotics and/or for estradiol is inducible by treatment with sodium phenobarbital and 3-methylcholanthrene [27–31] and that the rate of glucuronidation of some substrates is dependent on the sex and age of the animals [30, 32–34]. In this study, we evaluated some factors that influence the microsomal glucuronidation of estradiol and estrone by liver microsomes from Long-Evans rats. Specifically, we examined: (i) the effect of pH on the rate of glucuronidation of estradiol and estrone by liver microsomes from male and female rats; (ii) the effects of sex and age on the rate of microsomal glucuronidation of estradiol and estrone; (iii) the effects of alternative substrates of microsomal glucuronosyltransferases (e.g. 4-nitrophenol and testosterone) on the glucuronidation of estradiol and estrone; and (iv) the effect of treatment of rats with sodium phenobarbital, 3-methylcholanthrene, and dexamethasone, which are known to stimulate the liver microsomal hydroxylation of estradiol, on microsomal glucuronosyltransferase activities for estradiol and estrone.

MATERIALS AND METHODS

Chemicals

Estrone, estradiol, testosterone, 3-methylcholanthrene, dexamethasone, UDPGA triammonium salt, and 4-nitrophenol were obtained from the Sigma Chemical Co. (St. Louis, MO). Sodium phenobarbital was purchased from Eli Lilly Laboratories of Eli Lilly & Company (Indianapolis, IN). [6,7-^{3}H]Estradiol and [6,7-^{3}H]estrone (specific activities of 55 and 57 Ci/mmol, respectively) were obtained from the Du Pont Co., New England Nuclear Research Products (Boston, MA). All solvents (HPLC grade) were obtained from the Fisher Scientific Co. (Springfield, NJ).

Treatment of Rats with Inducers of Cytochrome P450 and Preparation of Liver Microsomes

Female and male immature (3 weeks old) and adult (8 weeks old) Long-Evans rats were obtained from Harlan Sprague–Dawley (Indianapolis, IN). Animals were kept on a 12-hr light–12-hr dark cycle and had free access to Purina Laboratory Chow-5012 (Ralston Purina Co., St. Louis, MO) and water throughout the experiment. Animals were allowed to acclimatize to their environment for 4 days prior to treatment. Animals were then treated intraperitoneally with sodium phenobarbital (75 mg/kg/day in saline), 3-methylcholanthrene (25 mg/kg/day in corn oil), dexamethasone (75 mg/kg/day in corn oil), or vehicle (saline or corn oil). Immature animals were treated for 3 days and adult animals were treated for 4 days. Animals were killed for preparation of liver microsomes 24 hr after the last injection. Livers from 4–5 rats in each treatment group were pooled, and microsomes were prepared by differential centrifugation as previously described [35]. Aliquots of the liver microsomes (approximately 15–20 mg protein/mL) were stored as a suspension in 0.25 M sucrose at −80°. Microsomal enzymes were stable for up to 2 years when stored at this temperature. The content of cytochrome P450 in microsomes was determined by the method of Omura and Sato [36], and the content of protein was determined by the method of Lowry *et al.* [37] with bovine serum albumin as a standard.

All induced liver microsomes used in this study had markedly enhanced activities toward probe substrates. Treatment with phenobarbital increased cytochrome P450 2B1/2B2-dependent activity (determined by O-debenzylation of 7-benzyloxyresorufin [38]) in male and female rat liver microsomes by 150- to 290-fold (data not presented). Treatment with 3-methylcholanthrene increased cytochrome P450 1A1/1A2-dependent activity (determined by O-deethylation of 7-ethoxyresorufin [38]) in male and female rats by 35- to 45-fold (data not presented). Treatment with dexamethasone increased cytochrome P450 3A-dependent activity (determined by 6β-hydroxylation of testosterone [39]) in male and female rats by 4-fold and 18- to 61-fold, respectively (data not presented).

Measurement of Microsomal Glucuronosyltransferase Activity for Estrogens and 4-Nitrophenol

The glucuronidation of 4-nitrophenol was done as described previously [28]. The glucuronidation of estradiol and estrone was carried out according to a previously described procedure with minor modifications [27]. Briefly, a 1-mL reaction mixture, unless otherwise indicated, consisted of 1.0 mg of microsomal protein, 2 mM UDPGA, 5 mM $MgCl_2$, and 100 μM estradiol or estrone (containing approximately 0.20 μCi ^{3}H-labeled estrogen) in Tris–HCl buffer (50 mM, pH 8.75). The reaction was initiated by the addition of microsomal protein. Incubations were carried out at 37° for 15 min, and reactions were arrested by immediately placing the test tube on ice and adding 0.5 mL of ice-cold 50 mM Tris–HCl solution (pH 8.75). The reaction mixture was then extracted twice with 7 mL toluene to separate unconjugated estrogen from water-soluble estrogen glucuronides. As determined in this study, two extractions with 7 mL toluene removed approximately 98 to 99.5% of unconjugated ^{3}H-labeled estrogen from the reaction mixture. The toluene-phase, which contains the unconjugated estrogen, was decanted. Portions (200 μL) of the aqueous phase, which contains glucuronidated estrogen metabolites,

† *Abbreviation:* UDPGA, uridine diphosphate glucuronic acid.

were precisely removed and measured for radioactivity content with a Beckman liquid scintillation spectrometer (model-LS 1701). Blank values, obtained with incubations in the absence of UDPGA, were determined in each individual assay and subtracted. In all studies, background radioactivity in the aqueous phase obtained after incubations in the absence of UDPGA was less than 30% of the radioactivity obtained from incubations of estrogen substrates (20–200 μM) in the presence of UDPGA. The average intra-assay variations, as determined with pooled control or induced liver microsomes, were within 15%.

Under the above incubation conditions, the formation of water-soluble estrogen metabolites was entirely UDPGA dependent, and incubations in the absence of UDPGA did not result in the formation of water-soluble metabolites. In addition, we did not see the formation of 2-hydroxylated estrogen metabolites or other estrogen metabolites (determined by HPLC analysis as described in Ref. 40) during incubation of [^{14}C]estradiol with liver microsomes alone (without cofactor). In our studies on the pH-dependent glucuronidation of [^{3}H]estradiol and [^{3}H]estrone, we also compared the effects of two different pH conditions (pH 5.5 and pH 11) on the blank values of radioactivity left in the microsomal reaction mixture after two extractions with toluene. We found that under these two different pH conditions there was no observable difference in the extraction of estradiol and estrone or in the low blank levels of water-soluble radioactivity.

In additional preliminary studies to optimize the reaction conditions, we evaluated the effects of incubation time and microsomal protein concentration on the rate of glucuronidation of estradiol and estrone. At a 100 μM substrate concentration, rates of glucuronidation of estradiol and estrone by liver microsomes (1 mg protein/mL) from adult male rats were constant for 40–50 min; at a 10 μM substrate concentration, rates of estrogen glucuronide formation were constant only for 20 min. The rates of glucuronidation of estradiol and estrone by liver microsomes from adult male and female rats were dependent on microsomal protein concentration (assayed from 0.3 to 3 mg/mL), but a linear relationship was only seen at microsomal protein concentrations that were less than 1 mg/mL. In this study, therefore, an incubation time of 15 min and a final microsomal protein concentration of 1 mg/mL were used for measuring glucuronosyltransferase activity for estradiol and estrone.

RESULTS

Effect of pH on the Rate of Glucuronidation of Estradiol and Estrone by Liver Microsomes from Male and Female Rats

The glucuronidation of 100 μM estradiol and estrone by liver microsomes from control adult male rats was dependent on the pH of the reaction mixture, and maximal rates of glucuronidation occurred at pH values of 8–9 (Fig. 1). The pH-optimum curves for both estradiol and estrone metabolism by liver microsomes from adult male rats were very similar (Fig. 1, left panel). The glucuronidation of 100 μM estradiol and estrone by liver microsomes from control adult female rats had a very different pH dependency pattern from that observed with liver microsomes from adult male rats (Fig. 1, right panel). The rate of glucuronidation of estradiol and estrone by female liver microsomes increased markedly from pH 6.5 to 8.75, and then stayed at high rates up to pH 10.5, the most alkaline condition tested. At pH 7.4 and at optimum pH values, estradiol was metabolized more extensively than estrone by both male and female liver microsomes. The optimum pH range for the microsome-mediated glucuronidation of estrogens as observed in this study was very similar to a previous report with liver microsomes from uninduced Wistar rats, in which the maximal rates were attained between pH 8.4 and 9.6 [30].

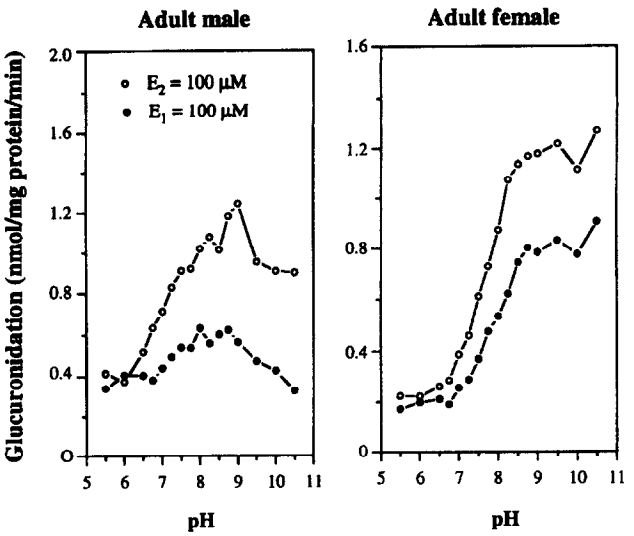

FIG. 1. Effect of pH on the rate of glucuronidation of estradiol and estrone by liver microsomes from male and female rats. Microsome-mediated glucuronidation of estradiol (E_2) and estrone (E_1) and the assay of estrogen glucuronides were carried out as described in Materials and Methods. Each value is the mean of triplicate determinations of the same microsomal preparation.

Comparison of the Rate of Glucuronidation of Estradiol and Estrone by Microsomes from Immature and Adult Male and Female Rats

We evaluated the effect of different substrate concentrations on the rate of glucuronidation of estrogens by liver microsomes from male and female rats (immature and adult). When different concentrations of estrone substrate were assayed, the glucuronidation by liver microsomes from male and female rats (immature or adult) all exhibited hyperbolic curve patterns, yielding straight lines when the data were examined using double-reciprocal plots (Fig. 2). The apparent K_m and V_{max} values for the glucuronidation of estrone by these liver microsomes are summarized in Table 1. The glucuronidation of estrone by liver micro-

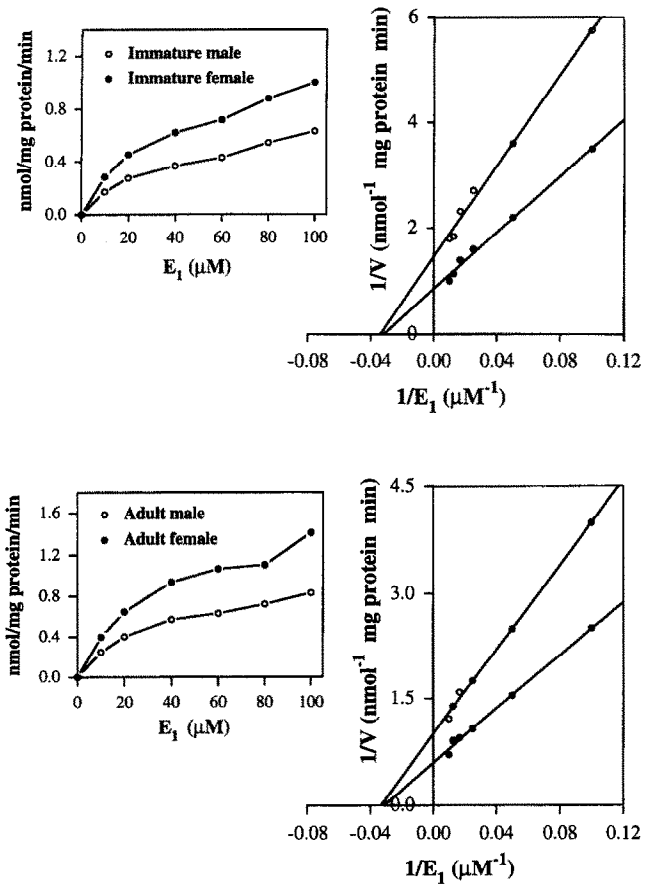

FIG. 2. Double-reciprocal plots of the glucuronidation of estrone (E_1) by liver microsomes from male and female rats. The left inserts represent the rates of microsome-mediated glucuronidation of estrone as a function of the substrate concentration. The microsomal reaction and the assay of estrone glucuronide formed were carried out as described in Materials and Methods. Each value is the mean from replicate determinations of the same microsomal preparation.

somes from immature and adult female rats proceeded at faster rates than that by liver microsomes from age-matched male rats, although their apparent K_m values were not markedly different from each other (Table 1). Our kinetic values for the glucuronidation of estrone by adult female liver microsomes (K_m = 32.2 μM and V_{max} = 1.66 nmol/mg protein/min) were similar to a previous observation with female Wistar rat liver microsomes (15.5 μM and 1.57 nmol/mg protein/min, respectively) [30].

When different concentrations of estradiol were studied, the rate of glucuronidation by liver microsomes from adult female rats was slightly higher than that by liver microsomes from adult male rats (Table 1). The apparent K_m and V_{max} values for the glucuronidation of estradiol by these liver microsomes were calculated from double-reciprocal plots and are summarized in Table 1. The glucuronidation of increasing concentrations of estradiol (25–200 μM) by liver microsomes from immature male rats was similar to that by liver microsomes from immature female rats (data not shown). Kinetic parameters could not be assigned to the glucuronidation of estradiol by these liver microsomes because the enzymes were not saturated at the highest soluble substrate concentration (200 μM) (data not shown).

Effect of the in vitro *Addition of Testosterone and 4-Nitrophenol on the Microsomal Glucuronidation of Estradiol and Estrone*

We examined the effects of possible alternative substrates for microsome-mediated glucuronidation of estradiol and estrone. When liver microsomes from control adult male and female rats were studied, testosterone markedly inhibited the glucuronidation of 30 μM estradiol and estrone in a concentration-dependent manner (Fig. 3, upper left and lower left panels). The IC_{50} values of testosterone for inhibition of estradiol and estrone glucuronidation catalyzed by adult male or female liver microsomes were similar (approximately 60 μM). 4-Nitrophenol also inhibited the glucuronidation of estradiol and estrone by liver microsomes from control female or male rats, but with a slightly weaker potency than testosterone (Fig. 3, upper right and lower right panels). 4-Nitrophenol exerted a slightly more potent inhibitory effect on the glucuronidation of estrogens

TABLE 1. Kinetic parameters (apparent K_m and V_{max} values) for the glucuronidation of estradiol and estrone by microsomes from male and female rats

	Estrone as the substrate		Estradiol as the substrate	
Animal	K_m (μM)	V_{max} (nmol/mg protein/min)	K_m* (μM)	V_{max}* (nmol/mg protein/min)
Adult male	29.3	1.01	50.1	1.87
Immature male	29.8	0.82		
Adult female	32.2	1.66	53.8	2.61
Immature female	31.5	1.18		

Incubation conditions were described in detail in Materials and Methods. The apparent K_m and V_{max} values were calculated from double-reciprocal plots.

* Proper kinetic parameters could not be assigned to the glucuronidation of estradiol by liver microsomes from immature male and female rats because the rate of conjugation was still increasing at the highest soluble substrate concentration tested (200 μM).

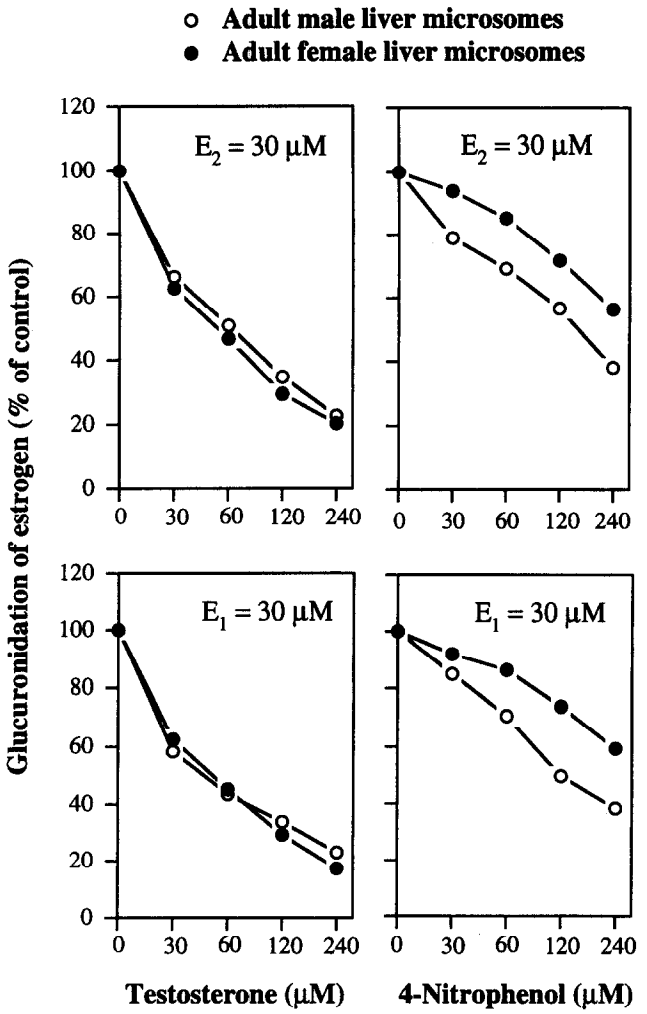

FIG. 3. Inhibitory effects of testosterone and 4-nitrophenol on the glucuronidation of estradiol (E_2) and estrone (E_1) by liver microsomes from adult male and female rats. The concentrations of estradiol and estrone were 30 μM, and the inhibitor concentrations were 30, 60, 120, or 240 μM, as indicated. The microsomal reaction and the assay of estrone glucuronide formed were carried out as described in Materials and Methods. In the absence of inhibitor, the rates of glucuronidation of 30 μM estradiol by adult male and female liver microsomes were 0.71 and 0.92 nmol/mg microsomal protein/min, respectively, and the corresponding rates for 30 μM estrone were 0.52 and 0.81 nmol/mg microsomal protein/min, respectively. Each value is the mean of replicate determinations of the same microsomal preparation.

by male liver microsomes (IC_{50} values approximately 120 μM) than by female liver microsomes (IC_{50} values greater than 240 μM).

Effect of Treatment of Rats with Sodium Phenobarbital, Dexamethasone, and 3-Methylcholanthrene on the Glucuronidation of Estradiol and Estrone

Treatment of immature or adult female rats with sodium phenobarbital for 3–4 days increased the liver microsomal glucuronidation of estradiol by 40–60%, but did not increase the glucuronidation of estrone (Table 2). Treatment with 3-methylcholanthrene for 3–4 days had little or no stimulatory effect on glucuronosyltransferase activity for estradiol or estrone in liver microsomes from immature and adult female rats (Table 2). In contrast to these results, treatment of immature or adult rats with 3-methylcholanthrene increased liver microsomal glucuronosyltransferase activity for 4-nitrophenol by 10- to 14-fold (Table 2). Treatment of immature or adult female rats with dexamethasone for 3–4 days increased glucuronosyltransferase activity for estradiol by approximately 60% but had little or no effect on glucuronosyltransferase activity for estrone (Table 2). Treatment of immature female rats with dexamethasone markedly stimulated the enzyme activity for glucuronidation of 4-nitrophenol, but this activity was not increased in adult female rats.

Treatment of immature or adult male rats with sodium phenobarbital or dexamethasone for 3–4 days increased microsomal glucuronosyltransferase activity for estradiol by approximately 40% but did not increase significantly the glucuronidation of estrone (Table 3). Treatment with 3-methylcholanthrene for 3–4 days did not increase the microsomal glucuronosyltransferase activity for estradiol and estrone, although it increased glucuronosyltransferase activity for 4-nitrophenol by 2- to 12-fold. Treatment with dexamethasone for 3–4 days slightly increased glucuronosyltransferase activity for estradiol in liver microsomes from immature and adult male rats, but it did not increase significantly the glucuronidation of estrone (Table 3).

DISCUSSION

In the present study with Long-Evans rats, we showed that estradiol and estrone were glucuronidated more rapidly by female liver microsomes than by male liver microsomes (Table 1), whereas there was an opposite tendency for the glucuronidation of 4-nitrophenol by these same microsomes (Tables 2 and 3). The sex-dependent differences described above for the glucuronidation of estradiol and estrone by liver microsomes were similar to previous observations with Wistar rats [30]. Our results also showed that the glucuronidation of estradiol and estrone by liver microsomes from female and male rats exhibited sex-related differences in pH dependence (Fig. 1), suggesting the presence of different profiles of glucuronosyltransferase(s) in male and female rat liver.

The results of our studies are consistent with earlier studies, indicating that estrogenic hormones play an important role in regulating hepatic microsomal glucuronosyltransferase activities for estrogens [30]. Estrone glucuronosyltransferase activity in liver microsomes of rats was decreased by 43% at 19–30 days after ovariectomy and was increased by 160% after treatment of these ovariectomized rats with estradiol (i.m., 200 μg/kg/day) for 2 weeks [30]. However, estrone glucuronosyltransferase activity in liver microsomes from non-ovariectomized rats did not respond to estradiol

TABLE 2. UDP-glucuronosyltransferase activity for estradiol, estrone, and 4-nitrophenol in liver microsomes of immature and adult female Long-Evans rats treated with sodium phenobarbital, 3-methylcholanthrene, or dexamethasone

Treatment	Glucuronidation (pmol/mg protein/min)		
	Estradiol	Estrone	4-Nitrophenol
Immature female rats			
Control (saline)	1250 ± 59 (100)*	898 ± 109 (100)	850 (100)
Phenobarbital	1771 ± 130† (142)	564 ± 102‡ (63)	797 (94)
Control (corn oil)	1102 ± 84 (100)	829 ± 58 (100)	902 (100)
3-Methylcholanthrene	1028 ± 50 (88)	617 ± 162‡ (74)	9294 (1030)
Dexamethasone	1813 ± 27† (156)	977 ± 207 (118)	4021 (446)
Adult female rats			
Control (saline)	1214 ± 61 (100)	921 ± 78 (100)	703 (100)
Phenobarbital	1890 ± 65† (156)	1064 ± 108 (115)	1469 (209)
Control (corn oil)	1174 ± 36 (100)	856 ± 43 (100)	678 (100)
3-Methylcholanthrene	1366 ± 48‡ (116)	900 ± 155 (105)	9801 (1446)
Dexamethasone	1859 ± 96† (158)	825 ± 169 (96)	486 (72)

Rats were treated i.p. with sodium phenobarbital (75 mg/kg/day in saline), 3-methylcholanthrene (25 mg/kg/day in corn oil), dexamethasone (75 mg/kg/day in corn oil), or vehicle (saline or corn oil). Immature animals were treated for 3 days and adult animals were treated for 4 days. Livers from 4–5 animals per group were pooled, and microsomes were prepared 24 hr after the last injection. The liver microsomal glucuronidation of estradiol (100 μM), estrone (100 μM), and 4-nitrophenol (200 μM) was as described in Materials and Methods. Incubations of estrogen or 4-nitrophenol with liver microsomes from control and induced immature female rats were carried out side-by-side on the same day and incubations of estrogen and 4-nitrophenol with liver microsomes from control and induced adult female rats were done side-by-side on another day. Data for 4-nitrophenol glucuronidation are also described in Ref. 28. Means ± SD of 3–4 determinations from the pooled liver microsomal preparations are indicated.

* Values in parentheses represent the percentage of the corresponding control value.

† $P < 0.01$ as compared with the corresponding control (Student's *t*-test).

‡ $P < 0.05$ as compared with the corresponding control (Student's *t*-test).

treatment, and fluctuations in estrogen concentration during the estrus cycle did not influence hepatic estrone glucuronosyltransferase activity [30]. Our observation that female rat liver microsomes contained higher estrogen glucuronosyltransferase activity for estradiol and estrone metabolism than male rat liver microsomes is consistent with a stimulatory effect of estrogenic hormones on these microsomal enzyme activities.

TABLE 3. UDP-glucuronosyltransferase activity for estradiol, estrone, and 4-nitrophenol in liver microsomes of immature and adult male rats treated with sodium phenobarbital, 3-methylcholanthrene, or dexamethasone

Treatment	Glucuronidation (pmol/mg protein/min)		
	Estradiol	Estrone	4-Nitrophenol
Immature male rats			
Control (saline)	1145 ± 54 (100)*	584 ± 24 (100)	1255 (100)
Phenobarbital	1553 ± 80† (136)	615 ± 12 (105)	1260 (100)
Control (corn oil)	1187 ± 41 (100)	602 ± 28 (100)	1211 (100)
3-Methylcholanthrene	1085 ± 171 (91)	479 ± 21‡ (80)	2594 (207)
Dexamethasone	1585 ± 20† (133)	668 ± 22 (111)	315 (26)
Adult male rats			
Control (saline)	1212 ± 51 (100)	694 ± 9 (100)	1307 (100)
Phenobarbital	1688 ± 19† (139)	609 ± 26 (88)	2078 (159)
Control (corn oil)	1107 ± 43 (100)	732 ± 24 (100)	1208 (100)
3-Methylcholanthrene	1072 ± 47 (97)	710 ± 8 (97)	14827 (1227)
Dexamethasone	1477 ± 22‡ (133)	624 ± 40‡ (85)	902 (75)

Treatment of rats and incubation of liver microsomes were as described under Table 2 and in Materials and Methods. Incubations of estrogen or 4-nitrophenol with liver microsomes from control and induced immature rats were carried out side-by-side on the same day, and incubations of estrogen or 4-nitrophenol with liver microsomes from control and induced adult male rats were done side-by-side on another day. Data for 4-nitrophenol glucuronidation are also described in Ref. 28. Means ± SD of 3–4 determinations from pooled liver microsomal preparations are indicated.

* Values in parentheses represent the percentage of the corresponding control value.

† $P < 0.01$ as compared with the corresponding control (Student's *t*-test).

‡ $P < 0.05$ as compared with the corresponding control (Student's *t*-test).

The results of this study showed that the stimulatory effect of certain microsomal enzyme inducers on glucuronosyltransferase activity for estrogens was much smaller than that for 4-nitrophenol (Tables 2 and 3). Treatment of rats with 3-methylcholanthrene stimulated glucuronosyltransferase activity for 4-nitrophenol by up to 14-fold but had little or no stimulatory effect on the glucuronidation of estradiol or estrone. Similarly, a previous study showed that 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) increased the glucuronosyltransferase activity for 4-nitrophenol by 5- to 6-fold, but had little or no effect on the glucuronidation of estrone [41]. In another study, liver microsomal glucuronosyltransferase activity for the glucuronidation of 4-nitrophenol was found to be chromatographically separable from the activity for the glucuronidation of estrone [42]. Taken together, these results suggest that the glucuronosyltransferases for estrogens and those for 4-nitrophenol are under different regulatory control. The very weak effect or the lack of an inducing effect of sodium phenobarbital or 3-methylcholanthrene on estradiol and estrone glucuronosyltransferase activity per mg liver microsomal protein (<60% increase in activity) is also consistent with several previous reports [27, 30, 43]. Intraperitoneal injection of sodium phenobarbital (50 or 100 mg/kg/day for 1 day or 100 mg/kg/day for 5–8 days) into female Wistar rats did not increase estrone glucuronosyltransferase activity in liver microsomes, and treatment of these rats with 3-methylcholanthrene only resulted in a small increase (up to 35%) in estrone glucuronosyltransferase activity [30]. In another study, treatment of Long-Evans rats with sodium phenobarbital intraperitoneally (75 mg/kg/day for 3–4 days) or oral administration of 0.1% sodium phenobarbital in the drinking water (about 150 mg/kg/day) for 20 days resulted in only small increases in glucuronosyltransferase activity for estradiol and estrone [27]. Our data together with the data of others suggest that microsomal glucuronosyltransferase activity for the endogenous estrogenic hormones is more difficult to induce by some commonly utilized monooxygenase inducers (e.g. 3-methylcholanthrene and TCDD) than enzyme activity for the glucuronidation of exogenous compounds (e.g. 4-nitrophenol). Differences in the inducibility of glucuronosyltransferase activities for estrogenic hormones and foreign compounds like 4-nitrophenol are consistent with the physiological needs of the body to maintain homeostasis for endogenous sex hormones and to effectively dispose of exogenously administered foreign chemicals.

The results of our studies with microsomal enzyme inducers indicate a small and selective stimulatory effect of sodium phenobarbital and dexamethasone administration on the glucuronidation of estradiol but not on the glucuronidation of estrone (Tables 2 and 3). The lack of a stimulatory effect of sodium phenobarbital and 3-methylcholanthrene on estrone glucuronosyltransferase activity was also reported previously [44]. It is known that there are two rat glucuronosyltransferases (UDP-$GT_{r\text{-}2}$ and UDP-$GT_{r\text{-}3}$) that are active towards estradiol but not towards estrone [45]. The UDP-$GT_{r\text{-}2}$ isozyme is inducible by treatment with phenobarbital [45], which is consistent with our observation that phenobarbital selectively stimulated the glucuronosyltransferase activity for estradiol but not for estrone (Tables 2 and 3). Our present results and those reported earlier indicate that certain glucuronosyltransferase isozymes selectively catalyze the glucuronidation of estradiol but not estrone. Our data also indicate that the glucuronidations of estradiol and estrone have very similar pH optimum curves, similar K_m values, and are similarly inhibited by testosterone and 4-nitrophenol. In previous studies by others, estradiol was found to inhibit the glucuronidation of estrone by a predominantly competitive mechanism [30]. Taken together, these observations suggest that a portion of the glucuronosyltransferase activity in rat liver microsomes may catalyze the glucuronidation of both estradiol and estrone.

In summary, we have described similarities and differences in the regulation of the glucuronidation of estradiol, estrone, and 4-nitrophenol by rat liver microsomes. The results indicate that the glucuronidation of these substrates is catalyzed by multiple glucuronosyltransferases that are under different regulatory control.

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