Developmental alteration of hepatic UDP-glucuronosyltransferase and sulphotransferase towards androsterone and 4-nitrophenol in Wistar rats

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Postnatal development of hepatic UDP-glucuronosyltransferase and sulphotransferase activities towards androsterone and 4-nitrophenol as well as cytochrome *P*-450 contents was studied in male and female Wistar rats. The rats with high and low UDP-glucuronosyltransferase activity towards androsterone were classified by the genotype of the parent animals. UDP-glucuronosyltransferase activity towards androsterone began rapidly to enhance after 30 days of age in the high-activity group, whereas the transferase activity remained low throughout in the low-activity group. Such a striking difference was not observed in UDP-glucuronosyltransferase activity towards 4-nitrophenol, sulphotransferase activity towards androsterone and 4-nitrophenol, and cytochrome *P*-450 contents. Sex-based difference in the sulphotransferase activity was marked after 30 days of age. Sulphotransferase activity towards androsterone was much higher in adult females than in adult males, whereas higher sulphation activity towards 4-nitrophenol was found in adult males. The results also indicate that the low level of the UDP-glucuronosyltransferase activity did not lead to compensatory stimulation of the sulphotransferase activity.

Glucuronidation and sulphation play an important role in the detoxification of endogenous and exogenous compounds. Hepatic microsomal UDP-glucuronosyltransferase (EC 2.4.1.17) catalyses the transfer of glucuronic acid from UDP-glucuronic acid to various compounds, and hepatic cytosolic aryl (EC 2.8.2.1) and hydroxy steroid (EC 2.8.2.2) sulphotransferase transfer H_2SO_4 from adenosine 3'-phosphate 5'-sulphatophosphate to various substrates. Recent studies *in vitro* revealed the discontinuous variation in hepatic UDP-glucuronosyltransferase activity towards androsterone but not towards testosterone, bilirubin, 4-nitrophenol and phenolphthalein in Wistar rats (Matsui & Hakozaiki, 1977; Matsui & Aoyagi, 1979). About half of the rats excreted large amounts of steroid monoglucuronides into bile, whereas the remaining rats excreted mainly steroid mono- and di-sulphates into bile. Subsequent studies *in vitro* revealed the discontinuous variation in hepatic UDP-glucuronosyltransferase activity towards androsterone but not towards testosterone, bilirubin, 4-nitrophenol and phenolphthalein in Wistar rats (Matsui & Hakozaiki, 1979; Matsui *et al.*, 1979). The genetic expression of high UDP-glucuronosyltransferase activity towards androsterone is inherited as a single autosomal dominant trait (Matsui & Watanabe, 1982).

It is known that activities of hepatic UDP-

glucuronosyltransferases and sulphotransferases change through perinatal development (Dutton & Burchell, 1977; Dutton, 1980; Jakoby *et al.*, 1980). Lucier & McDaniel (1977) and Wishart (1978) classified the activity of UDP-glucuronosyltransferases into a late-foetal or non-steroid group (with 4-nitrophenol, 2-aminophenol, 4-methylumbelliferone etc. as substrate) and a neonatal or steroid group (with bilirubin, testosterone, oestradiol, morphine etc. as substrate) on the basis of the developmental period of the enzyme activity from low to high values. There is also accumulating evidence that sulphotransferase activities vary with age and sex, much higher transferase activities towards phenols and hydroxy steroids being found in adult male and female rats respectively (DeBaun *et al.*, 1970; Singer *et al.*, 1976; Jakoby *et al.*, 1980).

To study the regulatory mechanism of androsterone metabolism, we performed a comparative study on the postnatal development of UDP-glucuronosyltransferase and sulphotransferase activities towards androsterone. For purposes of comparison, UDP-glucuronosyltransferase and sulphotransferase activities towards 4-nitrophenol as well as cytochrome *P*-450 contents were also determined.

Materials and methods

Materials

[1,2-³H]Androsterone (sp. radioactivity 44.5 Ci/mmol) was obtained from New England Nuclear Corp., Boston, MA, U.S.A. Androsterone and UDP-glucuronic acid (disodium salt) were purchased from Sigma Chemical Co., St. Louis, MO, U.S.A., and Boehringer, Mannheim, Germany, respectively. Octaethylene glycol mono-n-dodecyl ether and 4-nitrophenol were obtained from Tokyo Kasei Co., Tokyo, Japan. Adenosine 3'-phosphate 5'-sulphatophosphate was prepared by the method of Singer (1979). All other reagents were of analytical grade.

Animals, partial hepatectomy and breeding experiments

Male and female rats of the Wistar strain weighing 120–200 g were purchased from Sankyo Laboratory Service Co., Tokyo, Japan. To classify the rats with high and low hepatic microsomal UDP-glucuronosyltransferase activity towards androsterone, partial hepatectomy (liver weight 0.8–2.4 g) was performed under diethyl ether anaesthesia by the method of Higgins & Anderson (1931). Breeding experiments were carried out between the classified high-activity rats or between the classified low-activity rats as described previously (Matsui & Watanabe, 1982).

Preparation of cytosolic and microsomal fractions

The animals had free access to food and water, were decapitated or hepatectomized and a 20% (w/v) liver homogenate was prepared in ice-cold 0.25 M-sucrose in 0.1 M-Tris/HCl buffer, pH 7.2, with a Teflon/glass homogenizer. Cytosolic and microsomal fractions were obtained by differential centrifugation (2000 g for 10 min, 16 000 g for 45 min and 105 000 g for 60 min). The microsomal pellets were resuspended in 0.25 M-sucrose in 0.1 M-Tris/HCl buffer, pH 7.2. The microsomal fractions obtained from rats at 1–10 days of age contained lower protein concentrations (approx. 2 mg/ml) than those obtained from older rats (2.5–4 mg/ml). The cytosolic and microsomal proteins were determined by the method of Lowry *et al.* (1951) with bovine serum albumin as standard.

Enzyme assays

UDP-glucuronosyltransferase activity towards androsterone and 4-nitrophenol was assayed by a slight modification of the method described previously (Matsui *et al.*, 1979). The ethanolic solution of the substrate (androsterone and 4-nitrophenol) was evaporated *in vacuo* and the residue was dissolved in one drop of propylene glycol. The standard incubation medium contained microsomal

fraction (0.6–1.2 mg of protein), 0.1 M-Tris/HCl buffer, pH 7.2, 20 μ M-EDTA, 10 mM-MgCl₂, 2 mM-UDP-glucuronic acid and 172 μ M-[³H]androsterone (0.023 μ Ci) or 360 μ M-4-nitrophenol in a total volume of 1.0 ml. For the assay of the activated UDP-glucuronosyltransferase activity, 0.01% (for 4-nitrophenol) and 0.02% (for androsterone) (w/v) octaethylene glycol mono-n-dodecyl ether was added to the incubation medium (Matsui & Watanabe, 1982). The incubation was performed at 37°C for 20 min and terminated by heating in a boiling-water bath for 2 min, followed by cooling in ice/water. The blank values were obtained from control incubations in which UDP-glucuronic acid was omitted. In assay for the transferase activity towards 4-nitrophenol, 7.0 ml of 0.2 M-glycine buffer, pH 10.4, was subsequently added to the reaction mixture. After centrifugation at 3500 rev./min for 20 min, substrate disappearance was measured at 400 nm. In assay for the transferase activity towards androsterone, the reaction mixture was successively made up to 5.0 ml with methanol, centrifuged at 3500 rev./min for 20 min, and a portion (0.5 ml) of the supernatant was submitted for measurement of the total radioactivity. Subsequently the methanolic supernatant (2.0 ml) was evaporated *in vacuo* to dryness. The residue was dissolved in 2.0 ml of water, extracted with 10.0 ml of water-saturated dichloromethane and centrifuged at 3500 rev./min for 20 min. A portion (0.5 ml) of the resultant aqueous phase was submitted for measurement of the radioactivity of the enzymically formed glucuronide.

The standard incubation medium for sulpho-transferase contained cytosolic fraction (2–7 mg of protein), 0.1 M-Tris/HCl buffer, pH 7.2, 20 μ M-EDTA, 10 mM-MgCl₂, 100 μ M-adenosine 3'-phosphate 5'-sulphatophosphate and 86 μ M-[³H]androsterone (0.012 μ Ci) or 63 μ M-4-nitrophenol in a total volume of 1.0 ml. The incubation was performed for 10 (for 4-nitrophenol) and 15 (for androsterone) min at 37°C. The blank values were obtained from control incubations in which adenosine 3'-phosphate 5'-sulphatophosphate was omitted. The reaction was terminated by heating in a boiling-water bath for 1 min, followed by cooling in ice/water. In assay for the transferase activity towards 4-nitrophenol, 2.0 ml of 0.2 M-glycine buffer, pH 10.4, was subsequently added to the reaction mixture, centrifuged and substrate disappearance was measured at 400 nm. In assay for the transferase activity towards androsterone, the reaction mixture was made up to 5.0 ml with methanol and treated as described in the assay for UDP-glucuronosyltransferase activity towards androsterone.

The optimal incubation conditions described above were determined in rats at 40–60 days of age and were used for the assay of the enzyme activities at different ages.

Hepatic microsomal cytochrome *P*-450 contents were determined by the method of Omura & Sato (1964).

Radioactivity measurements

Radioactivity was counted in a Tracor Analytic Mark III scintillation spectrometer, which allowed quenching correction by external standardization. The sample (0.5 ml) was dissolved in 5 ml of the scintillation mixture consisting of 5.5 g of 2,5-diphenyloxazole, 100 mg of 1,4-bis-(5-phenyloxazol-2-yl)benzene, 333 ml of Triton X-100 and 667 ml of toluene. Efficiencies for ^3H counting were about 36%. Results are expressed in d.p.m.

Results

Hepatic UDP-glucuronosyltransferase and sulphotransferase activities towards androsterone and 4-nitrophenol in adult Wistar rats

Hepatic UDP-glucuronosyltransferase and sulphotransferase activities towards androsterone and 4-nitrophenol as well as cytochrome *P*-450 contents were determined in male and female Wistar rats at 80 days of age (Table 1). There was discontinuous variation in UDP-glucuronosyltransferase activity towards androsterone, but not towards 4-nitrophenol, irrespective of sex. Wistar rats could be divided into two groups in terms of the transferase activity towards androsterone. The high-activity group showed 7–10- or 16–20-fold higher transferase activity than did the low-activity group in fresh or the detergent-activated microsomes respectively. The detergent amplified the diversity of androsterone glucuronidation between two groups. These results are consistent with our previous findings (Matsui & Hakozaki, 1979; Matsui *et al.*,

1979). Such a marked difference was found neither in the sulphotransferase activity towards androsterone and 4-nitrophenol nor in cytochrome *P*-450 contents between two groups. However, prominent sex-based difference was characteristic of the sulphotransferase activity. Adult female rats sulphurated androsterone approx. 15-fold more efficiently than adult males. In contrast, males showed 2–3-fold as much sulphotransferase activity towards 4-nitrophenol as females.

Development of hepatic microsomal UDP-glucuronosyltransferase activity towards androsterone and 4-nitrophenol

The high UDP-glucuronosyltransferase activity towards androsterone is inherited in an autosomal dominant fashion (Matsui & Watanabe, 1982). To study the development of UDP-glucuronosyltransferase towards androsterone, the rats with high-activity (genotype HH and Hh) and low-activity (hh) were classified by partial hepatectomy and assay of the hepatic transferase activity towards androsterone. Subsequently homozygotes (HH) and heterozygotes (Hh) were classified from matings of the high-activity (HH and Hh) rats with the low-activity (hh) rats, followed by determination of the high-activity and low-activity population of the offspring as described previously (Matsui & Watanabe, 1982). In this study, the offspring (HH and Hh) from crosses between homozygous (HH) males and heterozygous (Hh) females were used as the high-activity group, whereas the offspring (hh) from matings of the low-activity (hh) rats were used as the low-activity group. Hepatic microsomal UDP-glucuronosyltransferase activity towards androsterone was determined at 1, 10, 20, 30, 40, 60 and 80 days of age (Fig. 1). The transferase activity

Table 1. Comparison of hepatic UDP-glucuronosyltransferase and sulphotransferase activities and cytochrome *P*-450 contents in Wistar rats at 80 days of age

Wistar rats were classified into high-activity and low-activity groups in terms of hepatic UDP-glucuronosyltransferase activity towards androsterone. UDP-glucuronosyltransferase activity towards androsterone and 4-nitrophenol in fresh (F) and octaethylene glycol mono-*n*-dodecyl ether-activated (A) microsomal fractions, cytosolic sulphotransferase activity towards androsterone and 4-nitrophenol, and microsomal cytochrome *P*-450 contents were determined as described in the Materials and methods section. Each value represents the mean $\pm$ s.d. for six animals.

Enzyme	Substrate		Male		Female	
			High-activity	Low-activity	High-activity	Low-activity
UDP-glucuronosyltransferase (nmol/min per mg of protein)	Androsterone	F	0.31 $\pm$ 0.11	0.04 $\pm$ 0.03	0.61 $\pm$ 0.13	0.06 $\pm$ 0.02
		A	3.67 $\pm$ 0.79	0.23 $\pm$ 0.03	3.60 $\pm$ 1.31	0.18 $\pm$ 0.07
	4-Nitrophenol	F	0.80 $\pm$ 0.14	0.90 $\pm$ 0.18	1.22 $\pm$ 0.43	1.32 $\pm$ 0.49
		A	13.70 $\pm$ 1.60	12.00 $\pm$ 1.00	10.40 $\pm$ 1.70	9.60 $\pm$ 1.70
Sulphotransferase (nmol/min per mg of protein)	Androsterone		0.02 $\pm$ 0.01	0.02 $\pm$ 0.01	0.34 $\pm$ 0.06	0.34 $\pm$ 0.07
	4-Nitrophenol		1.81 $\pm$ 0.30	1.30 $\pm$ 0.15	0.57 $\pm$ 0.14	0.57 $\pm$ 0.13
Cytochrome <i>P</i> -450 (nmol/mg of protein)			0.43 $\pm$ 0.09	0.50 $\pm$ 0.06	0.46 $\pm$ 0.08	0.35 $\pm$ 0.08

Fig. 1. Development of hepatic microsomal UDP-glucuronosyltransferase activity towards androsterone. Male (—) and female (---) Wistar rats were classified into high-activity (○ and ●) and low-activity (△ and ▲) groups in terms of UDP-glucuronosyltransferase activity towards androsterone by the genotype of the parent animals. The transferase activity after treatment with octaethylene glycol mono-*n*-dodecyl ether was determined as described in the Materials and methods section. The transferase activity is expressed as nmol of glucuronide/min per mg of protein. Data are the means of three to ten (average six) animals. The vertical lines represent one S.D. in animals at 20–80 days of age. The data from animals at 1 and 10 days of age were obtained from the pooled male and female livers.

was low in both groups up to 20 days of age. After 30 days of age, the transferase activity began rapidly to enhance in the high-activity group and adult enzyme activity was attained at around 60 days of age, indicating the neonatal nature of this enzyme activity. Females showed slower development than males. On the other hand, the transferase activity remained low throughout in the low-activity group. The developmental patterns of the transferase activity towards 4-nitrophenol were not significantly different between the high-activity and low-activity groups (Fig. 2). The transferase activity was already high at 1 day of age, confirming the late-foetal nature of this enzyme activity. However, the lack of detailed data during the first 15 days after birth obscured the typical late-foetal pattern. The highest transferase activity was attained at about 30 days of age and afterwards the enzyme activity began slowly to decline.

Fig. 2. Development of hepatic microsomal UDP-glucuronosyltransferase activity towards 4-nitrophenol. Male (—) and female (---) Wistar rats were classified into high-activity (○ and ●) and low-activity (△ and ▲) groups in terms of UDP-glucuronosyltransferase activity towards androsterone by the genotype of the parent animals. The transferase activity after treatment with octaethylene glycol mono-*n*-dodecyl ether was determined as described in the Materials and methods section. The transferase activity is expressed as nmol of glucuronide/min per mg of protein. Data are means of three to ten (average six) animals. The vertical lines represent one S.D. in animals at 20–80 days of age. Data from animals at 1 and 10 days of age were obtained from the pooled male and female livers.

Development of hepatic cytosolic sulphotransferase activity towards androsterone and 4-nitrophenol

Developmental patterns of the hepatic sulphotransferase activity towards androsterone were similar between the high-activity and low-activity groups, but were sex-dependent (Fig. 3). The transferase activity developed in parallel in both sexes until 30 days of age and the highest activity was attained at about 20 days of age. After that the transferase activity rapidly diminished in male rats. Female rats reduced the enzyme activity by 20–30% at 30 days of age; however, the enzyme activity began slowly to increase after 40 days of age. Adult enzyme activity was attained at about 60 days of age in both sexes. The sulphotransferase activity was slightly higher in the low-activity group than in the high-activity group at 10–30 days of age, but the transferase activity was quite similar in the two groups after 60 days of age.

Fig. 4 shows the developmental patterns of sulphotransferase activity towards 4-nitrophenol. No substantial difference in the enzyme activity was found between the high-activity and low-activity

Fig. 3. Development of hepatic cytosolic sulphotransferase activity towards androsterone

Male (—) and female (---) Wistar rats were classified into high-activity (○ and ●) and low-activity (△ and ▲) groups in terms of UDP-glucuronosyltransferase activity towards androsterone. The transferase activity was determined as described in the Materials and methods section and is expressed as nmol of sulphate/min per mg of protein. Data are means of three to ten (average six) animals. The vertical lines represent one S.D. in animals at 20–80 days of age. Data from animals at 1 and 10 days of age were obtained from the pooled male and female livers.

Fig. 4. Development of hepatic cytosolic sulphotransferase activity towards 4-nitrophenol

Male (—) and female (---) Wistar rats were classified into high-activity (○ and ●) and low-activity (△ and ▲) groups in terms of UDP-glucuronosyltransferase activity towards androsterone. The transferase activity was determined as described in the Materials and methods section and is expressed as nmol of sulphate/min per mg of protein. Data are means of three to ten (average six) animals. The vertical lines represent one S.D. in animals at 20–80 days of age. Data from animals at 1 and 10 days of age were obtained from the pooled male and female livers.

groups. However, there was a marked sex-based difference. The transferase activity developed up to about 30 days of age in both sexes. Subsequently the transferase activity rapidly increased in males and adult values were attained at about 40 days of age. In contrast, females did not show such a surge in the enzyme activity.

Development of hepatic microsomal cytochrome P-450

Cytochrome P-450 developed from very low to adult values within 30 days of age (Fig. 5). There was no substantial difference in cytochrome P-450 contents between the high-activity and low-activity groups.

Discussion

A striking feature of androsterone metabolism is the discontinuous variation in hepatic UDP-glucuronosyltransferase activity in adult male and female rats. Wistar rats could be classified into the high-activity and low-activity groups in terms of the transferase activity towards androsterone. Such a pronounced difference was not found in the hepatic

UDP-glucuronosyltransferase activity towards 4-nitrophenol, sulphotransferase activity towards androsterone and 4-nitrophenol, and cytochrome P-450 contents. However, a marked sex-based difference in the sulphotransferase activity towards androsterone and 4-nitrophenol was observed in adult male and female rats.

We have established the classification of Wistar rats with high and low hepatic UDP-glucuronosyltransferase activity towards androsterone by partial hepatectomy and breedings of the hepatectomized rats (Matsui & Watanabe, 1982), which enabled us to study the development of UDP-glucuronosyltransferase activity towards androsterone. The transferase activity was markedly enhanced after 30 days of age in the high-activity group, whereas the transferase activity remained low throughout in the low-activity group. UDP-glucuronosyltransferase activity is classified into late-foetal (or non-steroid) and neonatal (or steroid) groups (Lucier & McDaniel, 1977; Wishart, 1978). Thus the high transferase activity towards androsterone may belong to the neonatal group, though the

Fig. 5. Development of hepatic microsomal cytochrome P-450

Male (—) and female (---) Wistar rats were classified into high-activity (○ and ●) and low-activity (△ and ▲) groups in terms of UDP-glucuronosyltransferase activity towards androsterone. Cytochrome P-450 contents were determined by the method of Omura & Sato (1964) and are expressed as nmol/mg of protein. Data are means of three to ten (average six) animals. The vertical lines represent one S.D. in animals at 20–80 days of age. Data from animals at 1 and 10 days of age were obtained from the pooled male and female livers.

transferase activity rapidly surged after weaning. Fuchs *et al.* (1977) reported the other examples of late development of UDP-glucuronosyltransferase activity, in which the transferase activity towards testosterone and pregnanediol rapidly increased at around 30 and 60 days of age in female rats respectively. Our preliminary purification experiments on DEAE-cellulose suggest the existence of two peaks of UDP-glucuronosyltransferase active on androsterone (M. Matsui & F. Nagai, unpublished work). Peak I showed low transferase activity and was found in both high-activity and low-activity groups, whereas peak II had high transferase activity and was present only in the high-activity group. Further study under progress may clarify this apparent multiplicity. In contrast with androsterone glucuronidation, the transferase activity towards 4-nitrophenol did not give such variation and was found to be of the late-foetal nature as reported previously (Lucier & McDaniel, 1977; Wishart, 1978).

A pronounced feature of the sulphotransferase activity was a sex-based difference and this difference was marked after 30 days of age. The transferase towards androsterone showed about

15-fold greater activity in adult females than in adult males, whereas 2–3-fold higher sulphation activity towards 4-nitrophenol was found in adult males. The highest transferase activity towards androsterone was attained at about 20 days of age in both sexes. Subsequently the transferase activity markedly decreased in males. In females, the transferase activity began to decrease likewise after weaning, but increased again to a high level after 40 days of age. These results suggest a biphasic pattern of development of sulphotransferase activity towards androsterone in female rats. It is known that sulphotransferases are under hormonal regulation (Jakoby *et al.*, 1980). Sulphotransferase activity towards cortisol is regulated by gonadal (Singer & Sylvester, 1976) and adrenal (Singer, 1978) hormones. The transferase is increased by treatment with oestrogens, decreased by androgens and reduced by adrenalectomy in both sexes. Another interesting aspect is the multiplicity of sulphotransferases. Recently three hydroxy steroid sulphotransferases have been separated and purified from female rat livers (Singer *et al.*, 1976; Jakoby *et al.*, 1980). Thus, further study is required to clarify the regulatory factors, property and multiplicity of our sulphotransferase(s) active on androsterone. In contrast with androsterone sulphation, males developed high sulphotransferase activity towards 4-nitrophenol at 30–40 days of age, whereas females did not show such a surge in the enzyme activity. Recently, four aryl sulphotransferases have been separated and purified from male rat livers (Wu & Straub, 1976; Sekura & Jakoby, 1979, 1981). However, all the purified transferases sulphurylated 4-nitrophenol and had broad substrate specificity.

Glucuronidation and sulphation are competing conjugation reactions. The present study shows that the low level of the UDP-glucuronosyltransferase activity did not lead to compensatory stimulation of the sulphotransferase activity. This developmental study demonstrates that Wistar rats with several combinations of high and low activities of hepatic UDP-glucuronosyltransferase and sulphotransferase towards androsterone are available as a function of age and sex. Further study with this animal model is required to obtain insight into the regulatory mechanism of drug metabolism.

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