Strain Differences in Rat Liver UDP-Glucuronyltransferase Activity towards Androsterone

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Male Donryu, Wistar and Wistar King rats showed discontinuous variations in hepatic microsomal UDP-glucuronyltransferase activities towards androsterone, but not towards testosterone, bilirubin, phenolphthalein and 4-nitrophenol. Fresh microsomal fraction with a low transferase activity towards androsterone formed 0.049–0.080 nmol of glucuronide/min per mg of protein, whereas fresh microsomal fraction with a high transferase activity towards androsterone formed 0.335–0.557 nmol of glucuronide/min per mg of protein. The microsomal fraction with low enzyme activity towards androsterone was not stimulated by treatment with Triton X-100 or freezing and thawing. In contrast, male Long Evans and Sprague–Dawley rats did not exhibit such diversity.

Metabolism of androsterone in female and male Wistar rats is characterized by the discontinuous variations in biliary metabolites (Matsui & Hakozaiki, 1977; Matsui & Aoyagi, 1979) and hepatic microsomal UDP-glucuronyltransferase (EC 2.4.1.17) activity (Matsui & Hakozaiki, 1979). Metabolism *in vivo* of androsterone in male Wistar rats suggests the existence of variance of UDP-glucuronyltransferase activity towards the oxygenated metabolites of androsterone as well (Matsui & Aoyagi, 1979). With testosterone as substrate, UDP-glucuronyltransferase activity was not so variable. Sprague–Dawley rats, Hartley guinea pigs and CF 1 mice did not exhibit marked diversity towards these substrates. To obtain further information on the properties, substrate specificity and strain differences of UDP-glucuronyltransferase activity, we compared the glucuronidation of androsterone, testosterone, bilirubin, phenolphthalein and 4-nitrophenol by fresh, frozen and Triton X-100-treated microsomal fractions from male Wistar and other strains of rat.

Experimental

Materials

[1,2-³H]Androsterone (sp. radioactivity 44.5 Ci/mmol) and [4-¹⁴C]testosterone (sp. radioactivity 20.2 mCi/mmol) were purchased from New England Nuclear Corp., Boston, MA, U.S.A. The radiochemical purities of these radioactively labelled steroids were confirmed by t.l.c. on silica gel GF (Merck, Darmstadt, Germany) with the solvent system chloroform/acetone (29:1, v/v) and were found to be >98%. Androsterone, testosterone and bilirubin were obtained from Sigma Chemical Co.,

St. Louis, MO, U.S.A. UDP-glucuronic acid (disodium salt) was purchased from Boehringer, Mannheim, Germany. All other reagents were of analytical grade.

Animals and preparation of microsomal fractions

Male rats of the Donryu (150–250 g), Long Evans (150–190 g), Sprague–Dawley (215–230 g), Wistar (150–330 g) and Wistar King (150–190 g) strains were purchased from Matsumoto Experimental Animal Laboratory, Tokyo, Japan. Animals were allowed free access to food and water before being killed. Animals were decapitated 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. Microsomal fractions were collected by differential centrifugation (2000g for 10 min, 16000g for 45 min and 105000g for 60 min) in a Hitachi 65P ultracentrifuge. The microsomal pellets were resuspended in 0.25 M-sucrose in 0.1 M-Tris/HCl buffer, pH 7.2, and used as fresh microsomal fractions. The remaining microsomal fractions were stored frozen at –20°C until required for analysis. The microsomal protein was determined by the method of Lowry *et al.* (1951), with bovine serum albumin as protein standard.

Enzyme assays

UDP-glucuronyltransferase activities towards androsterone and testosterone were assayed by a modification of the method of Rao *et al.* (1976) as described previously (Matsui & Hakozaiki, 1979). The standard incubation medium contained microsomal fraction (1.1–2.5 mg of protein), 0.1 M-Tris/HCl buffer, pH 7.2, containing 20 μM-EDTA, 10 mM-

MgCl₂, 2mM-UDP-glucuronic acid and 172 μ M-[³H]androsterone (0.091 μ Ci) or 692 μ M-[¹⁴C]testosterone (0.045 μ Ci) in a total volume of 2.0ml. UDP-glucuronyltransferase activities towards phenolphthalein and 4-nitrophenol were assayed by a slight modification of the method described by Lucier & McDaniel (1977). The standard incubation medium contained microsomal fraction (0.4–0.8 mg of protein), 0.1 M-Tris/HCl buffer, pH 7.2, containing 20 μ M-EDTA, 10mM-MgCl₂, 2mM-UDP-glucuronic acid, and 120 μ M-phenolphthalein or 360 μ M-4-nitrophenol. Bilirubin glucuronyltransferase activity was assayed by the method of Van Roy & Heirwegh (1968). For the assay of the detergent-treated transferase activity, 0.05% (w/v) Triton X-100 was added to the incubation medium and treated as described previously (Matsui & Hakozaiki, 1979). In these incubation conditions, glucuronide formation was linear with time and protein concentration.

Radioactivity measurements

Radioactivity was counted in an Aloka LSC-502 liquid-scintillation spectrometer in a toluene medium as described previously (Matsui *et al.*, 1974). Efficiencies of ³H and ¹⁴C counting were about 40 and 80% respectively.

Results

UDP-glucuronyltransferase activities towards five substrates in male Wistar rat liver microsomal fraction

The specific activities of UDP-glucuronyltransferase towards androsterone, testosterone, bilirubin, phenolphthalein and 4-nitrophenol in fresh, frozen (for 4 weeks) and Triton X-100-treated microsomal

fractions from male Wistar rats are shown in Table 1. A distinctive feature is the existence of a discontinuous variation in the transferase activity towards androsterone, but not towards the other substrates. Wistar rats can be divided into two groups based on the specific activity. The rats with high transferase activity towards androsterone and those with low transferase activity towards androsterone were found to be in the ratio 1:1. The high- to low-specific-activity ratios were approx. 9.4 and 9.1 for fresh and frozen microsomal fractions respectively. The corresponding values for the detergent-treated microsomal fractions were 19.8 and 15.3 respectively, indicating that treatment with the detergent amplified the diversity between the two groups. In contrast, no appreciable variation was observed in the rate of glucuronidation with testosterone, bilirubin, phenolphthalein and 4-nitrophenol between the high- and low-activity groups. UDP-glucuronyltransferase activities towards androsterone and other substrates were stimulated from 3- to 9-fold by treatment of fresh microsomal fraction with Triton X-100 or by freezing microsomal fraction for 4 weeks, whereas treatment of the frozen microsomal fraction with the detergent stimulated glucuronidation only by 1–4-fold. When microsomal fraction was frozen for 2 weeks, the transferase activity was about 82% (range 71–98%) of that of the microsomal fraction frozen for 4 weeks. Fig. 1 shows the effects of repeated freezing and thawing at intervals of 1 week on the transferase activity towards androsterone and testosterone. High- and low-activity microsomal fractions provided marked differences in the enzyme activity towards androsterone, but not towards testosterone. In general, a great increase in the

Table 1. Comparison of UDP-glucuronyltransferase activities of fresh and frozen microsomal fractions in male Wistar rats. Fresh and frozen (for 4 weeks) microsomal fractions with high and low activities of UDP-glucuronyltransferase towards androsterone were incubated with androsterone, testosterone, bilirubin, phenolphthalein and 4-nitrophenol, as described in the Experimental section. Each value represents the mean $\pm$ S.D. for three animals. The values obtained with 0.05% (w/v) Triton X-100 treatment are means $\pm$ S.D. for three animals or means for two animals and are indicated in parentheses.

Substrate	UDP-glucuronyltransferase activity (nmol/min per mg of protein)			
	High-activity microsomal fraction		Low-activity microsomal fraction	
	Fresh	Frozen	Fresh	Frozen
Androsterone	0.500 $\pm$ 0.115 (3.38 $\pm$ 0.44)	2.46 $\pm$ 0.84 (1.91 $\pm$ 0.99)	0.053 $\pm$ 0.013 (0.171 $\pm$ 0.021)	0.271 $\pm$ 0.023 (0.125 $\pm$ 0.006)
Testosterone	0.808 $\pm$ 0.026 (5.19 $\pm$ 0.30)	5.48 $\pm$ 0.56 (3.38 $\pm$ 0.71)	0.659 $\pm$ 0.102 (4.77 $\pm$ 0.48)	5.23 $\pm$ 0.75 (2.86 $\pm$ 0.21)
Bilirubin	0.112 $\pm$ 0.006 (0.606)	0.454 $\pm$ 0.115 (0.421)	0.100 $\pm$ 0.009 (0.864)	0.474 $\pm$ 0.092 (0.358)
Phenolphthalein	0.985 $\pm$ 0.030 (3.90)	2.90 $\pm$ 0.76 (1.08)	1.06 $\pm$ 0.39 (2.76)	3.14 $\pm$ 0.22 (1.45)
4-Nitrophenol	3.94 $\pm$ 0.76 (19.1)	17.4 $\pm$ 1.4 (10.1)	3.31 $\pm$ 0.63 (17.0)	15.0 $\pm$ 2.4 (8.66)

Fig. 1. Effects of repeated freezing and thawing on UDP-glucuronyltransferase activities towards androsterone and testosterone in male Wistar rats

Fresh (0 week) and frozen (repeated freezing and thawing at intervals of 1 week for 1–4 weeks) microsomal fractions, which were the same as those used in Fig. 2, with high (open symbols) and low (filled symbols) activities of UDP-glucuronyltransferase towards androsterone were incubated with androsterone ($\circ$, $\bullet$) and testosterone (Δ , $\blacktriangle$), as described in the Experimental section. The broken line represents 0.05% (w/v) Triton X-100-treated microsomal fraction and the solid line represents untreated microsomal fraction.

Fig. 2. Effects of repeated freezing and thawing on UDP-glucuronyltransferase activities towards bilirubin, phenolphthalein and 4-nitrophenol in male Wistar rats

Fresh (0 week) and frozen (repeated freezing and thawing at intervals of 1 week for 1–4 weeks) microsomal fractions, which were the same as those used in Fig. 1, with high (open symbols) and low (filled symbols) activities of UDP-glucuronyltransferase towards androsterone were incubated with bilirubin ($\square$, $\blacksquare$), phenolphthalein (Δ , $\blacktriangle$) and 4-nitrophenol ($\circ$, $\bullet$), as described in the Experimental section.

transferase activity was observed up to 2 weeks (second freezing and thawing preparations), followed by a gradual decrease in activity, whereas treatment of the frozen microsomal fraction with the detergent afforded the maximal activity at 1 week, which was comparable with that of the detergent-treated fresh microsomal fraction. Fig. 2 shows the effects of repeated freezing and thawing on the transferase activities towards bilirubin, phenolphthalein and 4-nitrophenol. In these cases, maximal transferase activities were found around 2–3 weeks. However, no significant difference was observed between high- and low-activity groups.

UDP-glucuronyltransferase activities towards five substrates in male Donryu, Long Evans, Sprague-Dawley and Wistar King rats

To examine strain difference, the specific activities of the transferase towards androsterone, testosterone,

bilirubin, phenolphthalein and 4-nitrophenol were measured by incubation with microsomal fractions from male Donryu, Long Evans, Sprague-Dawley and Wistar King rats (Table 2). In this study, androsterone and testosterone were incubated with fresh and detergent-treated fresh microsomal fractions, whereas bilirubin, phenolphthalein and 4-nitrophenol were incubated with microsomal fractions frozen for 4 weeks. Of the four rat strains, Donryu and Wistar King rats provided discontinuous variations in their transferase activities towards androsterone, but not towards other substrates. Donryu rats were divided into five high-activity and three low-activity rats. The high- to low-specific-activity ratios were about 7.0 and 14.3 for fresh and detergent-treated fresh microsomal fractions respectively. One Donryu rat showed an intermediate activity. Wistar King rats afforded one high-activity and four low-activity rats. The high- to low-specific-activity ratios were 6.8 and 18.3 for fresh and detergent-treated fresh microsomal fractions respectively. These high- to low-specific-activity ratios were similar

Table 2. Comparison of UDP-glucuronyltransferase activities in male Donryu, Long Evans, Sprague-Dawley and Wistar King rats

Fresh and 0.05% (w/v) Triton X-100-treated fresh microsomal fractions with high, intermediate or low UDP-glucuronyltransferase activities towards androsterone and testosterone, whereas frozen (for 4 weeks) high-, intermediate- or low-activity microsomal fractions were incubated with bilirubin, phenolphthalein and 4-nitrophenol, as described in the Experimental section. Values represent means $\pm$ s.d. (or the results for one animal).

Strain	Microsomal fraction activity	No. of animals	Treatment ..	UDP-glucuronyltransferase activity (nmol/min per mg of protein)									
				Androsterone		Testosterone		Bilirubin	Phenolphthalein		4-Nitrophenol		
				Fresh	Fresh + Triton	Fresh	Fresh + Triton		Frozen	Frozen	Frozen	Frozen	
Donryu	High	5	..	0.557 ± 0.115	1.92 ± 0.58	1.07 ± 0.13	3.63 ± 1.08	0.492 ± 0.104	3.75 ± 1.21	14.9 ± 6.8			
	Intermediate	1		0.254	0.707	0.968	2.71	0.476	4.57	34.7			
	Low	3		0.080 ± 0.013	0.134 ± 0.017	0.918 ± 0.047	3.97 ± 0.88	0.568 ± 0.199	3.31 ± 0.80	23.7 ± 10.8			
Long Evans	High	5		0.655 ± 0.142	2.68 ± 0.54	0.768 ± 0.141	3.08 ± 0.51	0.579 ± 0.078	5.00 ± 0.37	27.8 ± 3.1			
	High	4		0.860 ± 0.188	5.05 ± 1.40	0.938 ± 0.053	5.71 ± 0.73	0.582 ± 0.189	4.72 ± 1.11	23.0 ± 6.1			
	High	1		0.335	1.61	1.25	3.12	0.730	3.04	8.93			
Sprague-Dawley	High	1		0.049 ± 0.007	0.088 ± 0.013	0.609 ± 0.029	2.79 ± 0.28	0.749 ± 0.056	2.95 ± 0.75	13.4 ± 1.6			
	Low	4											

to those of Wistar rats described above. In contrast, Long Evans and Sprague-Dawley rats did not give such a variance.

Discussion

Previous studies from this laboratory showed that male and female Wistar rats gave wide individual differences in hepatic microsomal UDP-glucuronyltransferase activities towards androsterone, but not towards testosterone (Matsui & Hakozaiki, 1979). The rats with high transferase activity and the rats with low transferase activity were found to be approximately in the ratio 5:4 and the high- to low-specific-activity ratios of fresh and detergent-treated microsomal fractions were about 9 and 16 respectively. Less than 10% of the microsomal fraction showed an intermediate transferase activity. No significant sex-based difference was observed in the kinetic data, except that females gave greater enzyme activity than males. The present study extended our previous findings and demonstrated that Wistar rats did not afford marked variance in the transferase activity towards bilirubin, phenolphthalein and 4-nitrophenol. UDP-glucuronyltransferase is latent, probably buried inside the microsomal vesicle, and can be activated by physical, chemical or enzymic perturbation of the microsomal membrane structure (Zakim & Vessey, 1974; Dutton & Burchell, 1977). Freezing the microsomal fraction for 4 weeks activated the transferase towards androsterone and other substrates to an extent comparable with that of the fresh microsomal fraction treated with Triton X-100, whereas the transferase activity of the frozen microsomal fraction was inhibited by the subsequent treatment with the detergent. These results should indicate the complicated mechanism for the enzyme activation. The low activity of the transferase towards androsterone could not be activated by treatment with the detergent or freezing and thawing.

Variability of UDP-glucuronyltransferase activity towards androsterone was found in Donryu and Wistar King rats, but not in Long Evans and Sprague-Dawley rats. When testosterone, bilirubin, phenolphthalein and 4-nitrophenol were used as substrates, no significant differences were observed in the transferase activities in all the strains examined. Though Donryu and Wistar King rats afforded similar high and low specific activities to those of Wistar rats, the proportions of high- to low-activity rats were somewhat different. It is noteworthy that wide individual differences exist in the transferase activity towards an endogenous substrate, androsterone, in several normal rat strains.

Recently evidence for the heterogeneity of UDP-glucuronyltransferase has accumulated on the basis

of inducibility (Dutton & Burchell, 1977), age-dependent development (Fuchs *et al.*, 1977; Lucier & McDaniel, 1977; Wishart, 1978), chromatographic separation (Bock *et al.*, 1977; Tukey *et al.*, 1978) and substrate specificity of the purified transferase (Burchell, 1978), though androsterone was not among the substrates examined. The purified transferase was phospholipid- or detergent-dependent (Burchell & Hallinan, 1978) and its deficiency towards 2-aminophenol in the Gunn strain of rat could be repaired by addition of diethylnitrosamine (Weatherill & Burchell, 1978), suggesting an interaction with the enzyme protein itself or with its linkage to detergent or phospholipid (Weatherill & Burchell, 1978). Thus, the observed low transferase activity towards androsterone in our low-activity microsomal fraction may be due to a defective enzyme and/or abnormal microsomal membrane microenvironment. However, we cannot distinguish between these mechanisms from the present study. Purification of the transferase must be carried out before the mechanism responsible for its regulation can be established.

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