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Classification and genetic expression of Wistar rats with high and low hepatic microsomal UDP-glucuronosyltransferase activity towards androsterone

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Male and female Wistar rats with high and low hepatic microsomal UDP-glucuronosyltransferase activity towards androsterone were classified by partial hepatectomy. The breeding experiments between the classified high-activity and low-activity rats show that the genetic expression of the high transferase activity is inherited in an autosomal dominant fashion.

Metabolism of androsterone in male and female Wistar rats is remarkable for the discontinuous variations in metabolites in bile (Matsui & Hakozaki, 1977; Matsui & Aoyagi, 1979) and hepatic microsomal UDP-glucuronosyltransferase (EC 2.4.1.17) activity (Matsui & Hakozaki, 1979). Variability of UDP-glucuronosyltransferase activity towards androsterone was found in Donryu and Wistar King rats, but not in Long-Evans and Sprague–Dawley rats (Matsui *et al.*, 1979). With testosterone, bilirubin, 4-nitrophenol and phenolphthalein as substrates, the transferase activities were not significantly different (Matsui *et al.*, 1979).

The hereditary deficiency of UDP-glucuronosyltransferase towards bilirubin is well known in Gunn rats, the mutant strain of Wistar rats (Szórády, 1973; Dutton & Burchell, 1977). Our colony of Wistar rats seemed to be another example of UDP-glucuronosyltransferase insufficiency of genetic origin. The present study was initiated to classify Wistar rats into high-activity and low-activity groups in tems of hepatic UDP-glucuronosyltransferase activity towards androsterone by partial hepatectomy and to determine whether these differences in the transferase activity were under genetic regulation in the offspring derived from the classified high-activity and low-activity rats.

Materials and methods

Materials

[1,2-^{3}H]Androsterone (sp. radioactivity 44.5 Ci/mmol) was obtained from New England Nuclear Corp., Boston, MA, U.S.A. UDP-glucuronic acid (disodium salt) and UDP-*N*-acetylglucosamine were purchased from Boehringer, Mannheim, Germany. Androsterone was obtained from Sigma Chemical Co., St. Louis, MO, U.S.A. 4-Nitrophenol and octaethylene glycol mono-n-dodecyl ether were obtained from Tokyo Kasei Co., Tokyo, Japan. 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. Partial hepatectomy (liver weight 0.8–2.5 g) was performed under ether anaesthesia by the technique of Higgins & Anderson (1931). Breeding to obtain offspring from the cross between the hepatectomized rats was done at least 4 weeks after operation.

Preparation of microsomal fractions and assay of UDP-glucuronosyltransferase activity

Animals were allowed free access to food and water before being decapitated or hepatectomized. Preparation of microsomal fractions from rat livers was done as described previously (Matsui *et al.*, 1979). The microsomal protein was determined by the method of Lowry *et al.* (1951), with bovine serum albumin as standard.

UDP-glucuronosyltransferase activities towards androsterone and 4-nitrophenol were assayed essentially as described previously (Matsui *et al.*, 1979), except that unchanged androsterone was extracted with methylene dichloride (Rao *et al.*, 1976). The standard incubation medium contained microsomal fraction (0.7–1.2 mg of protein), 0.1 M-Tris/HCl buffer, pH 7.2, 20 μM-EDTA, 10 mM-$MgCl_2$, 2 mM-UDP-glucuronic acid and 172 μM-[^{3}H]androsterone (0.023 μCi) or 360 μM-4-nitrophenol in a total volume of 1.0 ml. To remove the latency of UDP-

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glucuronosyltransferase, 10mM-diethylnitrosamine (Stevenson *et al.*, 1968), 2mM-UDP-*N*-acetylglucosamine (Vessey *et al.*, 1973), 10mM-pentan-2-one (Lalani & Burchell, 1979), 0.05% (w/v) Triton X-100 (Bock & White, 1974) and 0.02% (w/v, for androsterone) or 0.01% (w/v, for 4-nitrophenol) octaethylene glycol mono-n-dodecyl ether were added to the incubation medium and were treated as described previously (Matsui & Hakozaki, 1979).

Results and discussion

UDP-glucuronosyltransferase is latent, probably buried inside the microsomal vesicle, and can be activated by physical, chemical or enzymic perturbation of microsomal membrane structure (Dutton & Burchell, 1977). Table 1 shows the effect of five chemical activators on the transferase activities. Detergents, Triton X-100 and octaethylene glycol mono-n-dodecyl ether markedly stimulated the glucuronidation of androsterone and 4-nitrophenol. Octaethylene glycol mono-n-dodecyl ether optimally stimulated the transferase activity 20–30% more than did Triton X-100 and was employed as the activator in this study. An endogenous activator, UDP-*N*-acetylglucosamine, gave approximately 2-fold activation of the transferase activity towards androsterone and 4-nitrophenol. UDP-glucurono-

Table 1. *Activation of hepatic microsomal UDP-glucuronosyltransferase activity*

Microsomal fractions were obtained after decapitation of male Wistar rats and incubated with androsterone and 4-nitrophenol in the presence or in the absence of activators as described in the Materials and methods section. Microsomal fractions were classified into high-activity and low-activity groups in terms of UDP-glucuronosyltransferase activity towards androsterone. Each value represents the mean ± S.D. for four animals. Abbreviation: OGDE, octaethylene glycol mono-n-dodecyl ether.

	UDP-glucuronosyltransferase (nmol/min per mg of protein)			
	Androsterone		4-Nitrophenol	
Activator	High-activity	Low-activity	High-activity	Low-activity
None	0.37 ± 0.04	0.06 ± 0.01	2.8 ± 1.0	2.1 ± 0.7
0.05% Triton X-100	2.05 ± 0.40	0.25 ± 0.09	13.4 ± 1.8	12.9 ± 1.5
0.01% OGDE	2.03 ± 0.70	0.22 ± 0.04	16.5 ± 2.0	15.9 ± 2.2
0.02% OGDE	2.78 ± 0.66	0.30 ± 0.03	14.2 ± 2.3	13.5 ± 3.0
2mM-UDP-*N*-acetylglucosamine	0.70 ± 0.15	0.11 ± 0.02	4.7 ± 0.9	4.2 ± 0.4
10mM-Diethylnitrosamine	0.30 ± 0.03	0.04 ± 0.01	3.5 ± 0.7	2.8 ± 0.4
10mM-Pentan-2-one	0.34 ± 0.05	0.05 ± 0.01	2.5 ± 0.7	2.0 ± 0.3

Table 2. *Hepatic microsomal UDP-glucuronosyltransferase activities obtained by hepatectomy and by decapitation at 4 weeks after hepatectomy*

Microsomal fractions were obtained after hepatectomy and after decapitation at 4 weeks after operation. Fresh (F) and octaethylene glycol mono-n-dodecyl ether-activated (A) microsomal fractions were incubated with androsterone and 4-nitrophenol as described in the Materials and methods section. Microsomal fractions were classified into the high-activity and low-activity groups in terms of UDP-glucuronosyltransferase activity towards androsterone. Parentheses indicate numbers of animals. Each value represents the mean ± S.D.

			UDP-glucuronosyltransferase activity (nmol/min per mg of protein)			
			Hepatectomy		Decapitation	
Sex	Microsomal fraction		Androsterone	4-Nitrophenol	Androsterone	4-Nitrophenol
Male	High	F	0.59 ± 0.28	1.2 ± 0.9	0.55 ± 0.30	1.1 ± 0.5
	(7)	A	3.94 ± 1.25	10.8 ± 4.5	3.90 ± 1.49	8.1 ± 1.6
	Low	F	0.05 ± 0.03	1.7 ± 0.7	0.04 ± 0.02	0.6 ± 0.3
	(4)	A	0.25 ± 0.04	13.7 ± 2.2	0.27 ± 0.05	12.1 ± 2.6
Female	High	F	0.51 ± 0.19	1.5 ± 0.8	0.65 ± 0.15	1.6 ± 1.0
	(7)	A	2.34 ± 1.10	6.7 ± 2.7	3.24 ± 1.24	6.4 ± 1.7
	Low	F	0.04 ± 0.03	0.7 ± 0.6	0.08 ± 0.04	0.8 ± 0.2
	(3)	A	0.10 ± 0.08	3.4 ± 2.4	0.16 ± 0.03	3.0 ± 1.2

Table 3. *Genetic expression of hepatic microsomal UDP-glucuronosyltransferase activity towards androsterone in Wistar rats*

Breeding experiments were performed between the hepatectomized rats whose hepatic UDP-glucuronosyltransferase activity towards androsterone had been determined. The matings were done between male (*) and female (*) rats and between the remaining couple in the same genotype group. The offspring were usually decapitated or hepatectomized at 40–60 days of age, and the transferase activity after treatment with octaethylene glycol mono-n-dodecyl ether was determined. The transferase activity (UDPGase) is expressed as nmol/min per mg of protein. Each value represents the mean ± S.D. The offspring were divided into the high- and low-transferase-activity groups and classified into types I–VI according to the following Mendelian segregation ratios:

	Type I	Type II	Type III	Type IV	Type V	Type VI
Genotype P	*HH HH*	*HH Hh*	*Hh Hh*	*HH hh*	*Hh hh*	*hh hh*
F_1	*HH*	*HH Hh*	*HH 2Hh hh*	*Hh*	*Hh hh*	*hh*
Phenotype	High: 100%	High: 100%	High: 75% Low: 25%	High: 100%	High: 50% Low: 50%	Low: 100%

			Offspring				
	Parent		High transferase activity		Low transferase activity		
Sex	Animal	UDPGase	No. of animals	UDPGase	No. of animals	UDPGase	Genotype
Male	M-4*	3.00	17	3.82 ± 1.50	0		
	M_2-7	5.24					
Female	F-12*	2.18	8	2.54 ± 1.30	0		
	F_2-11	4.57					
			Total 25 (100%)		0 (0%)		I or II
Male	M-4*	3.00	10	3.29 ± 1.96	3	0.18 ± 0.06	
	M-6	2.18					
Female	F-6	2.93	9	3.13 ± 1.73	4	0.25 ± 0.11	
	F_2-9*	2.79					
			Total 19 (73%)		7 (27%)		III
Male	M-5*	2.40	11	3.15 ± 1.05	0		
	M_2-7	5.24					
Female	F-18*	0.31	14	2.61 ± 1.63	0		
	F_2-44	0.19					
			Total 25 (100%)		0 (0%)		IV
Male	M-1*	0.33	8	2.64 ± 0.67	10	0.22 ± 0.14	
	M-2	0.23					
Female	F-19*	1.71					
	F_2-11*	4.57	12	2.07 ± 0.84	8	0.24 ± 0.19	
	F_2-19	2.36					
			Total 20 (53%)		18 (47%)		V
Male	M-1*	0.33	0		16	0.24 ± 0.07	
	M-2	0.23					
Female	F-7*	0.10					
	F-11*	0.13	0		13	0.25 ± 0.04	
	F-21	0.13					
	F-32*	0.21					
			Total 0 (0%)		29 (100%)		VI

syltransferase is phospholipid- or detergent-dependent (Burchell & Hallinan, 1978), and its apparent deficiency towards 2-aminophenol in Gunn rats could be repaired by addition of diethylnitrosamine (Weatherill & Burchell, 1978) or alkyl ketones (Lalani & Burchell, 1979), suggesting an interaction with the enzyme protein itself or with its linkage to phospholipid or detergent. However, diethylnitrosamine and pentan-2-one showed little effect on our enzyme activity and were unable to abolish the deficiency of the transferase activity towards androsterone. 'Androsterone UDP-glucuronosyltransferase' may be defective in the low-activity rats, or the effective activator has not yet been discovered.

UDP-glucuronosyltransferase activities in fresh and detergent-activated microsomal fractions obtained by partial hepatectomy showed a discontinuous variation in the transferase activity towards androsterone, but not towards 4-nitrophenol (Table 2). The high-activity/low-activity specific-activity ratios were approximately 12:1 and 19:1 for fresh and detergent-activated microsomal fractions respectively. The detergent amplified the marked diversity of androsterone glucuronidation. The hepatectomized rats were decapitated at 4 weeks after operation and the hepatic transferase activities were determined. The results were in good accord with those obtained by partial hepatectomy. Thus partial hepatectomy provides a good means for unequivocal classification of the high- and low-transferase-activity rats.

In order to study the possible role of inheritance in the regulation of UDP-glucuronosyltransferase activity towards androsterone, breeding experiments were performed between the classified high-activity and low-activity rats. The offspring were usually decapitated or hepatectomized at 40–60 days of age and the hepatic transferase activity was determined (Table 3). The similarity of the data for male and female offspring makes sex-linked inheritance of UDP-glucuronosyltransferase unlikely. Therefore the results for male and female offspring were pooled for the purposes of discussion. All (type I or II) or 73% (type III) of the offspring from matings of the high-activity rats showed high enzyme activity. If a trait is inherited in an autosomal dominant fashion, the expected high-activity population of the offspring derived from crosses between homozygotes (genotype *HH*), between homozygotes (*HH*) and heterozygotes (*Hh*) or between heterozygotes (*Hh*) is 100, 100 or 75% respectively. Thus the data are compatible with the Mendelian inheritance and indicate dominance of the high-activity phenotype. All (type IV) or 53% (type V) of the offspring from the cross between the high-activity and low-activity rats fell into the high-activity group. On the basis of the expected ratios for the offspring, the parent with high transferase activity (type IV) should be normal homozygote (*HH*), whereas the parent with high transferase activity (type V) must be heterozygote (*Hh*). All the offspring (type VI) from matings of the low-activity rats showed low enzyme activity. Overall, these results are compatible with an autosomal recessive inheritance of the low-activity phenotype. In addition, the following genotype assignment can be reasonably made in the hepatectomized rats with the high activity: M-5, M_2-7 and F-12 must be normal homozygotes, whereas M-4, M-6, F-6, F-19, F_2-9, F_2-11 and F_2-19 should be heterozygotes. In conclusion, the present study provides evidence that the genetic expression of the high UDP-glucuronosyltransferase activity towards androsterone is inherited as a single autosomal dominant trait.

Deficiency of UDP-glucuronosyltransferase towards bilirubin is known in humans (Crigler–Najjar syndrome and Gilbert's disease) as well as in Gunn rats, and is characterized as autosomal recessive diseases (Szórády, 1973). In mice, induction of hepatic 4-methylumbelliferone UDP-glucuronosyltransferase by polycyclic aromatic hydrocarbons is genetically regulated in an additive fashion and is probably under the control of the *Ah* locus (Owens, 1977). Although the molecular mechanisms of the regulation of gene expression are yet to be elucidated, the observed low transferase activity towards androsterone may be due to a defective enzyme or abnormal membrane microenvironment that is under genetic control. To distinguish between these mechanisms, purification of the transferase must be established. It is hoped that this animal system will provide useful information on the regulatory mechanism and heterogeneity of UDP-glucuronosyltransferase.

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