Effects of steroid hormones and xenobiotics on the pubertal development of UDP-glucuronosyltransferase activities towards androsterone and 4-nitrophenol in Wistar rats

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(Received 13 February 1984/Accepted 9 May 1984)

Oestradiol benzoate, testosterone propionate, progesterone, corticosterone, 3-methylcholanthrene and phenobarbital were administered to Wistar rats at the pubertal period, and their effects on hepatic UDP-glucuronosyltransferase activities were determined. Pretreatment with oestradiol benzoate had a temporary suppressive effect on androsterone UDP-glucuronosyltransferase activity in rats with the high-activity phenotype of androsterone glucuronidation. The effect was marked in 40-day-old rats, but was not found in older rats. Androsterone UDP-glucuronosyltransferase activity was induced by phenobarbital in rats with the high-activity phenotype, but not in rats with the low-activity phenotype. Foster-feeding experiments showed that breast milk did not alter the genetically determined expression of androsterone UDP-glucuronosyltransferase activity in Wistar rats. In contrast, 4-nitrophenol UDP-glucuronosyltransferase activity was not affected by steroid hormones, but was highly induced by 3-methylcholanthrene.

Glucuronidation is an important pathway in the detoxification of endogenous and exogenous compounds. Hepatic microsomal GT catalyses the transfer of glucuronic acid from UDP-glucuronic acid to various compounds. GT activities are negligible in early-foetal liver, develop perinatally, and are affected by endogenous and exogenous compounds (Dutton, 1980; Dutton & Leakey 1981). In rat liver, GT activities are classified into late-foetal and neonatal groups on the basis of their developmental period from low to high values (Lucier & McDaniel, 1977; Wishart, 1978). The activities are also classified into two groups on the basis of their inducibility by 3-methylcholanthrene (GT₁) and phenobarbital (GT₂) (Bock *et al.*, 1980; Lilienblum *et al.*, 1982); however, there is a remarkable similarity between late-foetal and GT₁ substrates (2-aminophenol, 4-nitrophenol, 4-methylumbelliferone etc.) and between neonatal and GT₂ substrates (morphine, testosterone, phenolphthalein etc.). Prenatal induction of GT activity towards late-foetal substrates seems to be brought

Abbreviations used: GT, UDP-glucuronosyltransferase (EC 2.4.1.17); GT₁, UDP-glucuronosyltransferase activity inducible by 3-methylcholanthrene; GT₂, UDP-glucuronosyltransferase activity inducible by phenobarbital.

about by glucocorticoids, whereas postnatal onset of GT activity towards neonatal substrates presumably needs glucocorticoids and some unknown birth-dependent factor(s) (Dutton, 1980; Dutton & Leakey, 1981). Differences in GT activity between the sexes appears to depend on the substrate (Dutton, 1980).

Previous studies from our laboratory showed discontinuous variation in hepatic GT activity towards androsterone in Wistar rats, the high-activity to low-activity ratios being approx. 16:1 (Matsui & Hakozaiki, 1979; Matsui *et al.*, 1979). Classification and breeding experiments revealed dominance of the high-activity phenotype (Matsui & Watanabe, 1982a). Developmental study demonstrated that androsterone GT activity surged rapidly after 30 days of age in rats with the high-activity phenotype (Matsui & Watanabe, 1982b). Wistar rats with the low-activity phenotype did not show any pubertal surge of the enzyme activity. In contrast, such a striking individual difference was not found in 4-nitrophenol GT activity. There was no obvious sex difference in GT activities towards androsterone and 4-nitrophenol.

The present experiments were primarily designed to elucidate the effects of oestradiol benzo-

ate, testosterone propionate, progesterone, corticosterone, 3-methylcholanthrene and phenobarbital on the pubertal development of GT activities towards androsterone and 4-nitrophenol in Wistar rats. The study includes age-dependent effects of oestradiol benzoate and phenobarbital, and effects of breast milk, on GT activities.

Materials and methods

Materials

[1,2-³H]Androsterone (sp. radioactivity 40.8 Ci/mmol) was purchased from New England Nuclear, Boston, MA, U.S.A. Androsterone, oestradiol, oestradiol benzoate, testosterone propionate, progesterone and corticosterone were obtained from Sigma Chemical Co., St. Louis, MO, U.S.A. UDP-glucuronic acid (disodium salt) and phenobarbital sodium salt were provided by Boehringer, Mannheim, Germany, and Sankyo Pharmaceutical Co., Tokyo, Japan, respectively. The detergent octaethylene glycol mono-n-dodecyl ether, 4-nitrophenol and 3-methylcholanthrene were purchased from Tokyo Kasei Co., Tokyo, Japan. All other reagents were of analytical grade.

Treatment of animals

Male and female Wistar rats were classified into homozygous high-activity (genotype HH) and low-activity (hh) groups in terms of hepatic GT activity towards androsterone, as described previously (Matsui & Watanabe, 1982a,b). In this study, the offspring (HH) from crosses between homozygous dominant male and female rats were used as rats with the high-activity phenotype, and the offspring (hh) from matings of homozygous recessive male and female rats were used as rats with the low-activity phenotype. The present experiments were principally concerned with the high-activity phenotype. The animals were fed on commercial diets, given water *ad libitum* and were housed in air-conditioned (23°C) and light-controlled (12h-light/12h-dark cycle) animal rooms. The animals were weaned at 25 days of age. Oestradiol benzoate (2.5 mg/kg body wt.), testosterone propionate (12 mg/kg), progesterone (80 mg/kg) or corticosterone (30 mg/kg) was injected subcutaneously in 0.2 ml of peanut oil, alternating between right and left fore-legs on days 30, 32, 34, 36 and 38 after birth. Intraperitoneal injection of phenobarbital sodium salt (100 mg/kg) in 0.2 ml of saline (0.9% NaCl) was given on days 36, 37, 38 and 39. 3-Methylcholanthrene (40 mg/kg) was injected intraperitoneally in 0.2 ml of peanut oil on day 36. Control animals received vehicle only under the same conditions. These animals were killed on day 40, and their hepatic GT activities were assayed. For age-dependent experiments, oestradiol benzo-

ate and phenobarbital were administered on days 40–48 or 50–58 and on days 46–49 or 56–59 respectively in a similar way to that described above, and the animals were killed on day 50 or 60 respectively.

To examine the effect of maternal milk on GT activity, newborn animals from parents with the low-activity or high-activity phenotype were suckled from 1 day after birth by foster-mothers with the high-activity or low-activity phenotype respectively. After weaning, the animals were given commercial diets and water *ad libitum* and killed at 65–66 days of age, and their GT activities were determined.

Preparation of microsomal fractions

The 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 obtained by differential centrifugation (2000g for 10 min, 16000g for 45 min and 105000g 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 protein was determined by the method of Lowry *et al.* (1951), with bovine serum albumin as standard.

Enzyme assay

GT activities towards androsterone and 4-nitrophenol were assayed as described previously (Matsui & Watanabe, 1982b). Unactivated or detergent [0.01% (w/v) for 4-nitrophenol and 0.02% (w/v) for androsterone]-activated microsomal fractions (0.5–1.0 mg of protein) were incubated at 37°C for 20 min with 0.17 mM-[³H]androsterone (0.023 µCi) or 0.36 mM-4-nitrophenol, 2 mM-UDP-glucuronic acid, 10 mM-MgCl₂ and 20 µM-EDTA in a total volume of 1.0 ml of 0.1 M-Tris/HCl buffer, pH 7.2. Concentrations of the detergent described above provided maximally activated GT activities.

Statistical significance of difference between the control and treated groups was estimated by Student's *t* test.

Results

Effects of administration of steroid hormones and xenobiotics on liver weight, liver-weight/body-weight ratio and androsterone UDP-glucuronosyltransferase activities expressed in different units

After administration of four steroid hormones and two xenobiotics to male rats with the high-activity phenotype in terms of androsterone glucuronidation, their effects on liver weight, liver-weight/body-weight ratio and androsterone GT activities expressed in different units were exam-

ined at 40 days of age (Table 1). Pretreatment with phenobarbital significantly increased liver weight and liver-weight/body-weight ratio, and treatment with 3-methylcholanthrene increased the latter ratio. The data appear to indicate protein synthesis *de novo* in rat liver induced by the xenobiotics. When steroid hormones were injected into male rats, no significant difference was found in these parameters. The data were similar whether androsterone GT activities were expressed per whole liver, per g of liver or per mg of microsomal protein. The detergent-activated androsterone GT activity was significantly increased by administration of phenobarbital, whereas the activity was decreased by oestradiol benzoate. On the basis of these results, GT activities are expressed in terms of microsomal protein throughout this paper.

Effects of administration of steroid hormones and xenobiotics on the pubertal development of UDP-glucuronosyltransferase activities towards androsterone and 4-nitrophenol

Table 2 shows the effects of pretreatment with steroid hormones and xenobiotics on GT activities in 40-day-old rats with the high-activity phenotype. Treatment of microsomal fractions with the detergent equally activated androsterone GT activity by 5–6-fold, except in the male-liver microsomal fraction pretreated with phenobarbital, which showed approx. 13-fold activation. In control animals, males showed about 2-fold higher androsterone GT activity than did females. By pretreatment with oestradiol benzoate, the activity was decreased to 20–30% of the control in both sexes. The other steroid hormones did not significantly affect the activity. With 4-nitrophenol as substrate, rats showed neither sex difference in

activity nor alteration of the enzyme activity by administration of steroid hormones. Treatment of microsomes with the detergent increased 4-nitrophenol GT activity by 9–11-fold, except in the male-liver microsomal fraction treated with phenobarbital. Pretreatment with phenobarbital did not significantly increase unactivated GT activities towards androsterone and 4-nitrophenol; however, perturbation of microsomal fractions with the detergent disclosed significant induction of the activities (approx. 1.5–2-fold higher than the control) in male rats and, to a smaller extent, in females. Thus treatment with phenobarbital increased the GT activities in the latent form. On the other hand, pretreatment with 3-methylcholanthrene did not alter unactivated and activated androsterone GT activities. 3-Methylcholanthrene induced both unactivated and activated 4-nitrophenol GT activities by approx. 4-fold in both sexes. Much higher induction by 3-methylcholanthrene confirms the GT₁ nature of 4-nitrophenol GT activity. In contrast with phenobarbital, induction by 3-methylcholanthrene does not appear to increase the latency of 4-nitrophenol GT activity.

Age-dependent effects of oestradiol benzoate and phenobarbital on UDP-glucuronosyltransferase activities towards androsterone and 4-nitrophenol

The preceding data show that pretreatment with oestradiol benzoate markedly decreased androsterone GT activity in 40-day-old rats. However, we found no sex difference in androsterone GT activity in adult Wistar rats (Matsui & Watanabe, 1982b). It is well known that the response of drug-metabolizing enzymes to steroid hormones and xenobiotics is age-dependent (Dutton & Leakey,

Table 1. *Effects of administration of steroid hormones and xenobiotics on liver weight, liver-weight/body-weight ratio and hepatic androsterone UDP-glucuronosyltransferase activities in Wistar rats*

Microsomal fractions were obtained from 40-day-old male rats with the high-activity phenotype in terms of androsterone glucuronidation and incubated with androsterone in the presence of the detergent as described in the Materials and methods section. Control values were not significantly different in rats treated with peanut oil and saline, so values from peanut-oil controls are listed in the Table. Each value represents the mean $\pm$ S.D. for four animals. Statistically significant differences from the control: * $P < 0.05$, ** $P < 0.01$.

Treatment	Liver weight (g)	Liver weight/body weight (%)	UDP-glucuronosyltransferase		
			(nmol/min per whole liver)	(nmol/min per g of liver)	(nmol/min per mg of protein)
Control	6.7 $\pm$ 0.8	4.8 $\pm$ 0.2	123 $\pm$ 29	18.4 $\pm$ 4.2	1.89 $\pm$ 0.36
Oestradiol benzoate	6.0 $\pm$ 0.5	5.1 $\pm$ 0.5	46 $\pm$ 10**	7.7 $\pm$ 3.0**	0.82 $\pm$ 0.14**
Testosterone propionate	5.9 $\pm$ 0.6	4.3 $\pm$ 0.2	124 $\pm$ 42	21.0 $\pm$ 7.5	2.03 $\pm$ 0.75
Progesterone	7.6 $\pm$ 0.5	4.8 $\pm$ 0.3	168 $\pm$ 68	22.1 $\pm$ 10.3	2.58 $\pm$ 0.77
Corticosterone	7.6 $\pm$ 1.0	4.6 $\pm$ 0.4	141 $\pm$ 49	18.7 $\pm$ 5.1	2.13 $\pm$ 0.62
3-Methylcholanthrene	7.2 $\pm$ 0.4	5.6 $\pm$ 0.1*	121 $\pm$ 41	16.8 $\pm$ 8.1	1.71 $\pm$ 0.66
Phenobarbital	8.2 $\pm$ 0.5*	5.5 $\pm$ 0.3*	380 $\pm$ 171*	46.3 $\pm$ 14.9*	4.42 $\pm$ 0.93**

Table 2. *Effects of administration of steroid hormones and xenobiotics on hepatic UDP-glucuronosyltransferase activities towards androsterone and 4-nitrophenol in Wistar rats*

Microsomal fractions were obtained from 40-day-old male and female rats with the high-activity phenotype in terms of androsterone glucuronidation and incubated with androsterone and 4-nitrophenol in the presence or in the absence of the detergent as described in the Materials and methods section. Enzyme activity is expressed as nmol of glucuronide/min per mg of protein. Each value represents the mean $\pm$ S.D. for the numbers of animals shown. Statistically significant differences from the control: * $P < 0.05$, ** $P < 0.01$.

Sex	Treatment	No. of animals	Androsterone		4-Nitrophenol	
			Unactivated	Activated	Unactivated	Activated
Male	Control	10	0.45 $\pm$ 0.15	2.24 $\pm$ 0.57	1.11 $\pm$ 0.87	14.1 $\pm$ 2.6
	Oestradiol benzoate	7	0.10 $\pm$ 0.06**	0.69 $\pm$ 0.22**	1.40 $\pm$ 0.96	12.0 $\pm$ 0.9
	Testosterone propionate	4	0.31 $\pm$ 0.10	2.13 $\pm$ 0.70	1.61 $\pm$ 0.74	13.4 $\pm$ 0.9
	Progesterone	6	0.43 $\pm$ 0.17	2.22 $\pm$ 0.85	1.45 $\pm$ 0.74	14.8 $\pm$ 3.4
	Corticosterone	4	0.45 $\pm$ 0.15	2.10 $\pm$ 0.60	1.27 $\pm$ 0.12	14.3 $\pm$ 0.6
	Control	9	0.31 $\pm$ 0.12	1.91 $\pm$ 0.44	0.98 $\pm$ 0.46	15.6 $\pm$ 2.2
	3-Methylcholanthrene	8	0.26 $\pm$ 0.09	1.82 $\pm$ 0.63	5.76 $\pm$ 0.27**	62.3 $\pm$ 23.1**
	Phenobarbital	4	0.31 $\pm$ 0.03	4.06 $\pm$ 0.71**	1.14 $\pm$ 0.29	23.5 $\pm$ 3.8**
	Control	8	0.26 $\pm$ 0.15	1.02 $\pm$ 0.23	1.10 $\pm$ 0.20	12.0 $\pm$ 2.9
Female	Oestradiol benzoate	7	0.08 $\pm$ 0.04*	0.30 $\pm$ 0.18**	1.17 $\pm$ 0.41	15.5 $\pm$ 3.9
	Testosterone propionate	7	0.23 $\pm$ 0.14	1.29 $\pm$ 0.70	1.21 $\pm$ 0.52	12.9 $\pm$ 2.3
	Progesterone	6	0.21 $\pm$ 0.09	1.07 $\pm$ 0.68	1.04 $\pm$ 0.24	12.5 $\pm$ 1.5
	Corticosterone	4	0.26 $\pm$ 0.10	1.57 $\pm$ 0.59	1.10 $\pm$ 0.90	12.4 $\pm$ 2.0
	Control	10	0.15 $\pm$ 0.04	0.70 $\pm$ 0.21	1.74 $\pm$ 0.54	14.5 $\pm$ 1.0
	3-Methylcholanthrene	6	0.14 $\pm$ 0.06	0.74 $\pm$ 0.50	5.48 $\pm$ 2.77**	60.0 $\pm$ 19.3**
	Phenobarbital	4	0.18 $\pm$ 0.06	1.10 $\pm$ 0.15*	2.53 $\pm$ 0.55	21.3 $\pm$ 2.5**

Table 3. *Age-dependent effects of administration of oestradiol benzoate and phenobarbital on UDP-glucuronosyltransferase activities towards androsterone and 4-nitrophenol in Wistar rats*

Microsomal fractions were obtained from male rats with the high-activity phenotype in terms of androsterone glucuronidation and incubated with androsterone and 4-nitrophenol in the presence or in the absence of the detergent as described in the Materials and methods section. Enzyme activity is expressed as nmol of glucuronide/min per mg of protein. Each value represents the mean $\pm$ S.D. for the numbers of animals shown, or the mean of two. Statistically significant differences from the control: * $P < 0.05$, ** $P < 0.01$.

Treatment	No.	Injection period (days)	Time of assay (days)	Androsterone		4-Nitrophenol	
				Unactivated	Activated	Unactivated	Activated
Control	5	30-38	40	0.42 $\pm$ 0.20	2.12 $\pm$ 0.60	1.16 $\pm$ 0.41	13.4 $\pm$ 2.4
Oestradiol benzoate	5	30-38	40	0.11 $\pm$ 0.04**	0.59 $\pm$ 0.18**	1.61 $\pm$ 0.98	11.9 $\pm$ 1.3
Control	3	40-48	50	0.40 $\pm$ 0.07	3.54 $\pm$ 0.73	0.51 $\pm$ 0.33	13.2 $\pm$ 1.4
Oestradiol benzoate	4	40-48	50	0.59 $\pm$ 0.06	4.07 $\pm$ 0.92	0.75 $\pm$ 0.15	11.0 $\pm$ 1.2
Control	5	50-58	60	0.57 $\pm$ 0.09	3.95 $\pm$ 0.39	1.22 $\pm$ 0.52	11.0 $\pm$ 1.3
Oestradiol benzoate	5	50-58	60	0.63 $\pm$ 0.01	3.82 $\pm$ 0.50	1.10 $\pm$ 0.22	9.4 $\pm$ 1.6
Oestradiol benzoate	2	30-38	60	0.67 (0.73, 0.61)	4.20 (4.83, 3.56)	1.56 (1.92, 1.19)	13.4 (16.0, 10.8)
Control	3	36-39	40	0.32 $\pm$ 0.03	1.96 $\pm$ 0.18	0.88 $\pm$ 0.30	17.3 $\pm$ 1.6
Phenobarbital	3	36-39	40	0.31 $\pm$ 0.03	3.96 $\pm$ 0.84**	1.11 $\pm$ 0.35	24.3 $\pm$ 4.2*
Control	3	46-49	50	0.61 $\pm$ 0.11	5.44 $\pm$ 0.49	0.63 $\pm$ 0.19	14.3 $\pm$ 1.7
Phenobarbital	3	46-49	50	0.73 $\pm$ 0.15	7.42 $\pm$ 0.45**	1.02 $\pm$ 0.21	18.4 $\pm$ 0.7*
Control	3	56-59	60	0.49 $\pm$ 0.11	4.15 $\pm$ 0.18	0.91 $\pm$ 0.47	11.4 $\pm$ 1.8
Phenobarbital	3	56-59	60	0.47 $\pm$ 0.04	5.17 $\pm$ 0.56*	1.03 $\pm$ 0.94	17.6 $\pm$ 0.9**

1981). To elucidate the age-dependence phenomenon, male rats with the high-activity phenotype were pretreated with oestradiol benzoate and phenobarbital, and their GT activities were determined subsequently at 40, 50 and 60 days of age (Table 3). In control animals, androsterone GT activity was relatively low at 40 days of age, and

surged to adult value around at 60 days of age, whereas 4-nitrophenol GT activity was already high at 40 days of age. The results are in good accord with our previous study (Matsui & Watanabe, 1982b). The suppressive effect of oestradiol benzoate on androsterone GT activity was pronounced in 40-day-old males, but completely dis-

Table 4. *Effects of phenobarbital treatment and breast milk on androsterone UDP-glucuronosyltransferase activities in Wistar rats*

Microsomal fractions were obtained from 40-day-old (phenobarbital treatment) or 65–66-day-old (foster-feeding) male and female rats with the low-activity and high-activity phenotypes in terms of androsterone glucuronidation and incubated with androsterone in the presence or in the absence of the detergent as described in the Materials and methods section. Enzyme activity is expressed as nmol of glucuronide/min per mg of protein. Each value represents the mean $\pm$ S.D. for three animals or the mean of two.

Treatment		Sex	Genotype	Phenotype	Unactivated	Activated
Phenobarbital administration	Control	Male	hh	Low	0.04 ± 0.01	0.24 ± 0.06
		Female	hh	Low	0.09 ± 0.02	0.21 ± 0.04
	Phenobarbital	Male	hh	Low	0.05 ± 0.04	0.35 ± 0.04
		Female	hh	Low	0.06 ± 0.03	0.31 ± 0.07
Foster-feeding	Foster-mother Offspring	Female	HH	High		
		Male	hh	Low	0.01 ± 0.01	0.18 ± 0.01
		Female	hh	Low	0.02 ± 0.01	0.15 ± 0.04
	Foster-mother Offspring	Female	hh	Low		
		Male	HH	High	0.65 (0.58, 0.72)	4.86 (4.80, 4.94)
		Female	HH	High	0.72 ± 0.06	3.42 ± 0.59

appeared in 50- and 60-day-old males. When the animals were killed at day 60, after injection of the oestrogen on days 30–38 after birth, their androsterone GT activity was restored to normal values. Addition of oestradiol (50 μ M) or oestradiol benzoate (50 μ M) did not show any inhibitory effect on androsterone GT activity. This temporary suppression of androsterone GT activity by oestrogen is compatible with our previous developmental study (Matsui & Watanabe, 1982b). Phenobarbital induction of the detergent-activated androsterone GT activity was most remarkable in 40-day-old rats (twice the control values), but less effective in 50-day-old (1.4-fold) and 60-day-old (1.2-fold) rats, though the overall GT activity was higher in older rats. Such age-dependent phenomena were not obvious in 4-nitrophenol GT activity.

Effects of phenobarbital treatment and breast milk on androsterone UDP-glucuronosyltransferase activity in Wistar rats with the low-activity phenotype

Though phenobarbital pretreatment increased the detergent-activated androsterone GT activity up to 2-fold in 40-day-old rats with the high-activity phenotype (Table 3), the same pretreatment did not significantly increase androsterone GT activity in rats with the low-activity phenotype (Table 4).

To examine the effect of maternal milk on androsterone GT activity, newborn rats from parents with the low-activity phenotype were suckled by foster-mothers with the high-activity phenotype. For purpose of comparison, newborn rats from parents with the high-activity phenotype were nursed by foster-mothers with the low-activity phenotype. After weaning, the animals were fed on commercial diets and water and killed at 65–66 days of age, and their androsterone GT

activity was determined (Table 4). The results indicate that mother's milk did not alter the genetically determined low-activity and high-activity phenotypes.

Discussion

An interesting aspect of androsterone GT activity is its temporary suppression by pretreatment with oestradiol benzoate. This suppression was marked in 40-day-old rats with the high-activity phenotype, but disappeared when the enzyme activity was measured at 3 weeks after the oestrogen treatment or when the oestrogen was administered to older rats. Bock *et al.* (1980) classified GT activities into two groups on the basis of their inducibility by 3-methylcholanthrene (GT₁) and phenobarbital (GT₂). The temporary suppression of GT activity by oestrogen may designate another type of functional heterogeneity of GT activity. In a previous paper (Matsui & Watanabe, 1982b), we reported that androsterone GT activity began to surge rapidly after 30 days of age in rats with the high-activity phenotype, the adult enzyme activity being attained at about 60 days of age. Females showed slower development than males, but reached the high male activities at around 60 days of age. It is known that gonadal hormones normally affect hepatic enzymes after around 30 days of age (puberty) in rats (Skett & Gustafsson, 1979). Thus the slower development of female androsterone GT activity appears to be due to temporary suppression by physiological oestrogens. Moreover, it is of interest to speculate that the comparatively slow development of androsterone GT activity, as compared with other activities in the neonatal cluster (Dutton, 1980; Dutton & Leakey, 1981), might arise from an

inhibitory effect of oestrogens present in breast milk. Lamartiniere *et al.* (1979) reported that neonatal administration of testosterone propionate and diethylstilboestrol irreversibly suppressed post-pubertal development of 4-nitrophenol GT activity in male rats. The high-activity and low-activity phenotypes in Wistar rats can be imprinted prenatally or postnatally. To determine whether rat's breast milk had any imprinting effect on androsterone GT activity, newborn rats from parents with the low-activity or high-activity phenotype were nursed by foster-mothers with the opposite phenotype. The results indicate that breast milk did not alter the genetically determined expression of androsterone GT activity.

Another age-dependent effect on androsterone GT activity was found by phenobarbital treatment in rats with the high-activity phenotype. The induction rate of androsterone GT activity was the highest in 40-day-old rats. Enhancement of the enzyme activity by phenobarbital was detected only in the detergent-activated microsomal fractions. This increased latency may be due to hypertrophy of liver endoplasmic reticulum, by which GT enzymes are buried presumably deeper inside the membrane (Dutton, 1980). Inducibility by phenobarbital and non-inducibility by 3-methylcholanthrene, together with the postnatal development of the enzyme activity (Matsui & Watanabe, 1982b), establish the GT₂ nature of androsterone GT activity. Interestingly, androsterone GT activity was not induced by phenobarbital pretreatment in rats with the low-activity phenotype.

DEAE-cellulose column chromatography of solubilized androsterone GT demonstrated the existence of two peaks of GT active on androsterone (M. Matsui & F. Nagai, unpublished work). Peak-I GT was found in rats with the high-activity and low-activity phenotypes and corresponded to low androsterone GT activity, whereas peak-II GT was found only in rats with the high-activity phenotype and had high androsterone GT activity. Falany & Tephly (1983) purified rat liver androsterone GT isoenzyme to apparent homogeneity by using chromatofocusing and affinity chromatography. The GT isoenzyme reacted with androsterone and etiocholanolone, but showed no activities towards 4-nitrophenol, 1-naphthol, 4-methylumbelliferone, oestrone, oestradiol, testosterone and morphine. Interestingly, our colony of Wistar rats with the low-activity phenotype showed markedly decreased GT activity towards androsterone, but not towards 4-nitrophenol, phenol-

phthalein, bilirubin and testosterone (Matsui *et al.*, 1979). Furthermore, six to seven GT isoenzymes have been separated from rat livers of Gunn and Wistar strains, though some of these isoenzymes appear to have partially overlapping substrate specificity (Chowdhury *et al.*, 1984). Important consequences of these findings may be that androsterone GT isoenzyme should be deficient in Wistar rats with the low-activity phenotype, and that androsterone glucuronidation in consequence should be catalysed poorly by other GT isoenzyme(s) in these rats. The deficiency of bilirubin GT isoenzyme is known in Gunn rats (Burchell, 1981; Chowdhury *et al.*, 1984). Our colony of Wistar rats appears to be another example of GT isoenzyme deficiency. It is hoped that this animal model will provide useful information on the heterogeneity and regulation of GT isoenzymes.

This work was supported in part by a grant from The Science Research Promotion Fund of Japan Private School Promotion Foundation.

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