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ALTERATION OF HEPATIC DRUG METABOLIZING ACTIVITIES AND CONTENTS OF CYTOCHROME P-450 ISOZYMES BY NEONATAL MONOSODIUM GLUTAMATE TREATMENT*

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Abstract—By the treatment of newborn male rats with monosodium glutamate (MSG), microsomal benzo[*a*]pyrene hydroxylation, propoxycoumarin *O*-depropylation, and testosterone (T) 6β- and 2β-hydroxylations in the adult rats were decreased significantly, while microsomal aniline and T 7α-hydroxylations were increased. However, the treatment of newborn female rats did not significantly alter any of the drug-metabolizing activities examined, except that T 6β-hydroxylation and androstenedione formation were slightly increased. The hepatic contents of male-specific cyt. P-450, P-450-male and P-450$_{6\beta}$, which show high catalytic activities on respective T 16α/2α-, and T 6β/2β-hydroxylations, decreased in MSG-treated male rats. The level of the female specific enzyme, P-450-female, slightly decreased in the MSG-treated female rats, whereas higher phenobarbital (PB)-induction of PB-inducible isozymes, P-450b and P-450e, was observed in MSG-treated than in control female rats. These results are consistent with the idea that disruption of a pulsatile secretion of growth hormone, which is induced by the neonatal MSG treatment, leads to changes in drug metabolizing activities through the alteration of the levels of sex-specific cyt. P-450s, but also indicate that MSG-treated rats are not an animal model equivalent to hypophysectomized rats.

Sex-related differences are often observed in drug metabolisms in rats and other experimental animals, and are considered to be a factor of the observed sex-related difference in the drug-induced toxicity and carcinogenicity [1, 2]. We first indicated that major constitutive forms of cytochrome P-450, P-450-male and P-450-female, exist sex-specifically in livers of male and female rats, and showed that higher drug-metabolizing activities in male rather than in female rats can be attributed mainly to broad substrate specificities and high catalytic activities of male-specific forms of cytochrome P-450 including P-450-male [3]. More than five forms of sex-specific forms of cytochrome P-450 are now known to exist in rat livers [3–7]. Diverse changes in the content of these forms could be reflected in the complex pattern of sex-related difference in various drug metabolisms in rats.

On the regulational aspect of sex-associated cytochrome P-450, recent studies indicate that sex-steroids and pituitary hormones are involved in the expression and maintenance of P-450-male [3, 8–10], P-450-female [3, 8, 9, 11], and P-450$_{6\beta}$ [7], P-450b [12] and P-450e [12] in rat livers. In these studies, growth hormone is shown to act as both a stimulative and suppressive factor on the regulation of these enzymes.

Administration of MSG‡ in neonatal rats is known to produce a low serum growth hormone level in adult rats without affecting strikingly the levels of other pituitary hormones [13–15]. Therefore, MSG-treated rats would be expected to provide a useful animal model for growth hormone-deficient states similar to hypophysectomized rats. Thus, the changes in both hepatic drug- and steroid-metabolizing activities and contents of five different forms of cytochrome P-450 in MSG-treated rats were determined to assess the role of growth hormone on the regulation of these enzymes.

MATERIALS AND METHODS

Animal treatment. Pregnant Sprague–Dawley strain rats were obtained from Clea Japan (Tokyo). The male and female pups were treated i.p. with MSG (4 mg/g body weight) at 1, 3, 5, 7 and 9 days after birth as previously described [13]. MSG-treated and control animals were weaned at 22 days and housed in a room on a 12-hr light/12-hr dark cycle until 9 weeks of age. To some female rats, phenobarbital sodium (80 mg/kg, dissolved in saline) was also administered once daily for three successive days. The animals were killed at 18 hr after the last injection. Hepatic microsomes were prepared as previously described [8].

Quantitative determination. Microsomal hydroxylations of benzo[*a*]pyrene [16] and aniline [17], and

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‡ Abbreviations used: MSG, monosodium glutamate.

N-demethylations of aminopyrine and ethylmorphine [18] were measured by fluorometric or colorimetric methods. The typical incubation mixture (1 ml) consisted of 0.8 mM NADP, 8.0 mM glucose 6-phosphate, 1 unit of glucose-6-phosphate dehydrogenase, 6 mM magnesium chloride, 100 mM sodium, potassium phosphate (pH 7.4), 0.1 mM disodium ethylenediaminetetraacetic acid, liver microsomes (1 mg, except for 0.25 mg for benzo[*a*]pyrene hydroxylation) and 0.1 mM benzo[*a*]pyrene or 5 mM of another substrate. The reaction was started by the addition of an NADPH generating system (NADP, glucose 6-phosphate, glucose-6-phosphate dehydrogenase and magnesium chloride) and was incubated at 37° for 15 min. Propoxycoumarin depropylation was quantified as previously described [19]. Testosterone hydroxylation and *O*-ethyl- and *O*-*n*-pentylresorufin dealkylations were determined by HPLC [12]. Immunoblot analyses were performed essentially following the method of Towbin *et al.* [20]. Characteristics of purified cytochrome P-450, P-450-male [3], P-450-female [3], P-450b [12] and P-450e [12] and of rabbit antibodies raised against these forms and P-450 human-1 (human testosterone 6β hydroxylase) [21] were reported elsewhere. Judging from the spectral and catalytic properties and *N*-terminal amino acid sequence, P-450-male probably corresponds to P-450h [5], P-450c/UT-A [6], P-450 RLM5 [4]. P-450-female also corresponds to P-450i [5], P-450d/UT-I [6] and P-450$_{15\beta}$ [11]. P-450b, P-450e, P-448-H and P-448-L correspond to P-450bH, P-450e, P-450d and P-450c [5], respectively. P-450-male [3], P-450-female [3], P-450b [12], P-450e [12], P-448-H [22] or P-448-L [22] were not recognized by anti-P-450 human-1 in the immunoblotting. In our previous study [7], antibodies raised against a rat cytochrome P-450, PB-IIb, which corresponds to P-450$_{6\beta}$ (53 kdalton) or a closely related enzyme in rats, were used for the immunoblotting. However, anti-P-450 human-1 showed higher selectivities to the rat enzyme than did anti-PB-IIb. Thus anti-P-450 human-1 was used for immunoblotting of P-450$_{6\beta}$, although a band (51 kdalton) was also immunostained in hepatic microsomes of male rats. Contents of microsomal cytochromes P-450 and b_5 and of protein were determined by the method of Omura and Sato [23] and Lowry *et al.* [24]. The statistical significance was examined using Student's *t*-test.

RESULTS

Effects of MSG treatment on body and pituitary weights, and contents of hepatic cytochromes P-450 and b_5

Neonatal treatment of MSG retarded the growth of male rats (Table 1). The body weight was significantly lower in MSG-treated than in control male rats. However, the treatment had no significant effect on the body weight of female rats. Total pituitary contents of male and female rats given MSG neonatally were decreased to 39% and 49% levels of the respective controls. These results are consistent with

Table 1. Body and pituitary weights and contents of cytochromes P-450 and b_5 in MSG-treated and control rats

Treatment		Body weight (g)	Total pituitary (mg)	Cyt. P-450 (nmol/mg protein)	Cyt. b_5
Male					
Control	(4)[a]	331.5 ± 18.5	12.15 ± 0.71	0.92 ± 0.10	0.35 ± 0.02
MSG	(4)	261.5 ± 21.5*	4.75 ± 0.73*	0.86 ± 0.14	0.35 ± 0.02
Female					
Control	(4)	201.0 ± 17.2	11.90 ± 1.44	0.64 ± 0.03	0.32 ± 0.02
MSG	(5)	221.8 ± 11.7	5.80 ± 1.06*	0.57 ± 0.02	0.30 ± 0.02

Hepatic microsomal contents of total cytochrome (Cyt.) P-450 and b_5 were determined as described in Materials and Methods.

[a] Number of animals used are indicated in the parentheses.

* Significant difference from the respective control rats (<0.05).

Table 2. Microsomal drug metabolizing activities in MSG-treated male and female rats

Substrate	Male		Female	
	Control	MSG	Control	MSG
Benzo[*a*]pyrene[a]	187 ± 23	105 ± 44*	37 ± 6.0	36 ± 3
Propoxycoumarin[a]	1310 ± 48	579 ± 311*	95 ± 12	106 ± 13
Ethylmorphine[b]	10.53 ± 0.62	6.90 ± 3.51	2.26 ± 0.41	2.12 ± 0.30
Aminopyrine[b]	7.89 ± 0.78	6.64 ± 1.81	4.31 ± 0.45	3.80 ± 0.55
Aniline[b]	0.69 ± 0.02	0.76 ± 0.05*	0.50 ± 0.07	0.51 ± 0.01
O-Ethylresorufin[a]	90.7 ± 12.8	103.5 ± 16.6	70.7 ± 10.4	111.4 ± 36.0
O-Pentylresorufin[a]	16.8 ± 2.7	14.9 ± 2.5	8.8 ± 2.4	10.2 ± 1.7

[a,b] Activities are expressed as pmoles and nmoles/mg protein per min, respectively.

* Significant difference from the respective controls (<0.05).

Data are shown as the mean ± SD of 4–5 different determinations.

Other experimental details are described in Materials and Methods.

Table 3. Formation of testosterone metabolites by hepatic microsomes of MSG-treated and control rats

Site of hydroxylation	Male		Female	
	Control	MSG	Control	MSG
2α	1.22 ± 0.33	0.73 ± 0.63	<0.02	<0.02
2β	0.30 ± 0.08	0.13 ± 0.06*	<0.02	<0.02
6β	3.24 ± 0.82	1.47 ± 0.76*	0.16 ± 0.05	0.23 ± 0.02*
7α	0.06 ± 0.03	0.33 ± 0.12*	0.35 ± 0.04	0.27 ± 0.13
15β	0.10 ± 0.02	0.09 ± 0.03	0.02 ± 0.01	0.04 ± 0.01
16α	1.88 ± 0.57	1.36 ± 1.04	0.07 ± 0.01	0.05 ± 0.02
16β	0.18 ± 0.08	0.20 ± 0.08	0.06 ± 0.02	0.08 ± 0.02
Androstenedione formation	0.97 ± 0.26	1.05 ± 0.49	0.29 ± 0.08	0.68 ± 0.08*

Activities are expressed as nmol/mg protein per min.
Data represented as the mean ± SD of 4–5 different determinations.
Other experimental details are the same as described in Table 2.
* Significant difference from the respective controls (<0.05).

the results reported previously on the effect of neonatal MSG treatment [13–15]. Microsomal contents of total cytochrome P-450 and b_5 were also measured, but no significant difference was observed in the contents between the MSG-treated and control groups.

Effect of MSG treatment on microsomal drug metabolizing activities

As described in Table 2, microsomal benzo[*a*]pyrene hydroxylation and propoxycoumarin depropylation, which are known to be higher in the livers of male rather than female rats [8, 19, 25], were significantly decreased by neonatal MSG treatment of male rats. Similar tendencies were also observed on microsomal *N*-demethylations of aminopyrine and ethylmorphine, but the differences between the two groups were not significant. Interestingly, the rate of aniline hydroxylation, which is known to be insensitive to hormonal treatment [8, 26], was slightly increased by MSG treatment of male rats. Microsomal dealkylations of *O*-ethyl- and *O*-*n*-pentylresorufin were also measured as the probe for 3-methylcholanthrene-type [27] and phenobarbital-type [28] inductions, but their activities did not differ significantly between the two groups. In female rats, neonatal treatment of MSG showed little change on all the microsomal drug metabolizing activities examined, although suppression of growth hormone level in female rats by hypophysectomy is known to express male-type activities and male-specific cytochrome P-450 in livers [8–10, 29–31].

Effect of MSG treatment on testosterone hydroxylation

Recent studies using purified forms of cytochrome P-450 indicate that each cytochrome P-450 shows fairly rigid regio- and stereo-selectivities on the hydroxylation of steroids [32, 33]. Thus, to understand the changes in cytochrome P-450 isozyme pattern in MSG-treated rat livers, the rate of hydroxylation of testosterone was measured with hepatic microsomes of MSG-treated and control rats. Neonatal MSG treatment increased 5- to 6-fold the rate of testosterone 7α-hydroxylation in male rats, while the treatment had no effect on the rate of female rats (Table 3). In addition, both rates of testosterone 6β- and 2β-hydroxylations were significantly decreased in MSG-treated male rats. In female rats testosterone 6β-hydroxylation was rather higher in MSG-treated than in control rats, although the specific rate of the hydroxylation was still more

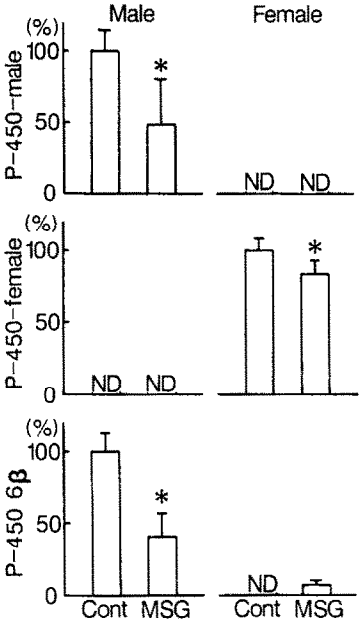

Fig. 1. Specific P-450-male, P-450-female and P-$450_{6\beta}$ contents in MSG-treated rats. The data are shown as the relative values to the respective controls (P-450-male and P-$450_{6\beta}$; control male rats, and P-450-female; control female rats). The column and vertical bar represent the mean and SD of at least 4 individual determinations. * Significant difference to the respective controls (<0.05). N.D.: not detected (less than 0.01 nmoles for P-450-male and P-450-female and less than 3% level of control male rats for P-$450_{6\beta}$).

Table 4. Effect of neonatal MSG treatment on the phenobarbital induction of cytochrome P-450 and drug metabolizing activities in female rats

Treatment		P-450b	P-450e	Ethylmorphine *N*-Demethylation	*O*-Pentylresorufin *O*-pentylation	*O*-Ethylresorufin *O*-ethylation
MSG	PB	(pmol/mg protein)		(nmol/mg/min)	(pmol/mg/min)	
−	−	ND	7.7 ± 2.9	2.26 ± 0.41	8.8 ± 2.4	70.7 ± 10.4
−	+	191.3 ± 25.6*	92.3 ± 10.4*	6.35 ± 1.35*	58.3 ± 16.0*	201.3 ± 22.2*
+	−	ND	17.3 ± 10.6	2.12 ± 0.30	10.2 ± 1.7	111.4 ± 36.0
+	+	446.1 ± 32.4†	201.9 ± 44.1†	11.42 ± 0.55†	160.3 ± 25.5†	407.6 ± 14.3†

ND: not detected (less than 1 pmol per mg protein).
Data are shown as the mean ± SD of at least 3 different determinations.
* Significant difference from control female rats (<0.05).
† Significant difference from phenobarbital (PB)-treated control rats (<0.05).

than 6-fold higher in the livers of MSG-treated male than female rats.

Microsomal contents of cytochrome P-450 isozymes

As described in Fig. 1, contents of a male-specific isozyme, P-450-male, which exhibits high catalytic activities on benzo[*a*]pyrene and testosterone 16α- and 2α-hydroxylations, decreased by the MSG-treatment to a level half that of the control male rats. The level of another male-specific isozyme, P-450$_{6\beta}$, was also quantitated by the use of antibodies raised against human testosterone 6β-hydroxylase, P-450 human-1 [21]. Amounts of immunoreactive P-450$_{6\beta}$ were significantly lower in the livers of MSG-treated than in control male rats, which is consistent with the decreased levels of testosterone 6β-hydroxylation and propoxycoumarin depropylation found in MSG-treated rats (Tables 1 and 2). In control female rats, neither of the male-specific forms of cytochrome P-450 were detected, but trace amounts of P-450$_{6\beta}$ were detected in the MSG-treated rats. The content of a female-specific isozyme, P-450-female, which was detected as a major cytochrome P-450 in the livers of female rats, was slightly decreased in MSG-treated female rats.

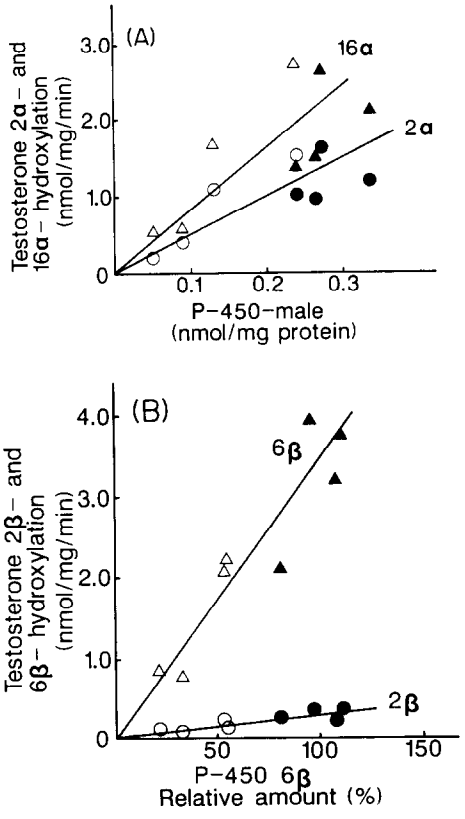

Fig. 2. Correlation between amounts of male specific cytochrome P-450 and rates of microsomal testosterone hydroxylations. Data obtained from individual male MSG-treated and control rats were plotted. (A) Circle and triangle indicate testosterone 2α- and 16α-hydroxylations, respectively. (B) Circle and triangle indicate testosterone 2β- and 6β-hydroxylations, respectively. Open symbol: MSG-treated rats, closed symbol: control rats.

Recently, phenobarbital inducible forms of cytochrome P-450, P-450b and P-450e, were shown to be regulated suppressively by pituitary growth hormone in rats [12]. Thus, the effect of neonatal MSG-treatment on phenobarbital-induction of cytochrome P-450 was examined with female rats. As described in Table 4, amounts of P-450b and P-450e in male and female rats were very low and did not differ significantly between MSG-treated and control groups. Treatment of MSG-treated and control female rats with phenobarbital increased hepatic contents of P-450b and P-450e. Both the hemoprotein contents were 3-fold higher in the MSG-treated rats than in the control rats. In agreement with this result, microsomal activities of ethylmorphine *N*-demethylation, *O*-pentyl- and *O*-ethylresorufin dealkylations were 2–3 times higher in the livers of MSG-treated rats than in control rats after phenobarbital treatment.

DISCUSSION

In the present study, neonatal treatment of rats with MSG significantly changed the various microsomal drug metabolizing activities, although Shapiro *et al.* [34] recently reported that neonatal MSG treatment did not affect the microsomal hexobarbital-metabolizing and UDPG-glucuronyltransferase activities in male rats. As described in

Table 1, the rates of metabolism of most drugs, which are known to show sex-related differences in their metabolisms in rats, were decreased or tend to be diminished by neonatal MSG treatment of male rats. In our previous studies, male-specific forms of cytochrome P-450, P-450-male and P-450$_{6\beta}$, are shown to catalyze the metabolism of a variety of chemicals [3, 7]. The decreases in the microsomal drug metabolizing activities are correlated with the reduced contents of P-450-male and P-450$_{6\beta}$ in MSG-treated male rats (Fig. 2). Significant correlations were observed between content of P-450-male and testosterone 2α-($\gamma = 0.790$) or 16α-hydroxylation ($\gamma = 0.788$), and between content of P-450$_{6\beta}$ and testosterone 2β- ($\gamma = 0.836$) or 6β-hydroxylation ($\gamma = 0.926$) in livers of male rats. An MSG-treated male rat showed no clear decrease in the content of P-450-male and rates of testosterone 2α- and 16α-hydroxylations, in spite of the significant reduction in pituitary and body weights as observed in other MSG-treated rats. The reason for the individual difference is unclear, but experiments with hypophysectomized animals [8–10] indicate that pulsatile secretion with high amplitude of burst and low trough of serum growth hormone is necessary for the maximal expression of P-450-male in rat livers. The decrease in P-450-male level in MSG-treated rats is likely to be a consequence of depressed growth hormone pulse and prolonged trough periods in MSG-treated male rats [14]. The level of P-450$_{6\beta}$ was, in contrast to the enhanced level in hypophysectomized rats [7], decreased by MSG treatment, although the trace level appeared in female rats by neonatal MSG treatment. Hepatic P-450$_{6\beta}$ level is suppressively regulated by serum growth hormone and is reduced by the intermittent injection or continuous infusion of human growth hormone in hypophysectomized rats [7]. These results suggest that the disruption of regular pulsatile secretion by neonatal MSG treatment hampered the full expression of P-450$_{6\beta}$ in male rats and the trough level of serum growth hormone is not decreased sufficiently for the appearance of P-450$_{6\beta}$ in neonatal MSG-treated female rats. The level of P-450-female in female rat livers was decreased, although no obvious change was observed in the drug metabolizing activities in MSG-treated female rats. In addition, phenobarbital caused higher induction of P-450b and P-450e in MSG-treated than control female rats. These results are consistent with the expected low growth hormone level in MSG-treated female rats and also indicate the possibility that combined treatment of animals with MSG and other drug evokes a significant alteration of drug metabolizing capacities, even though a change in serum growth hormone levels induced by MSG treatment in itself is not enough to alter the level of the drug metabolizing enzyme. In conclusion, the present study indicates that neonatal MSG-treatment produces a useful animal model for studies on the growth hormone regulation of drug metabolizing enzymes, but also showed that the animals are not equivalent to hypophysectomized rats in the profile of cytochrome P-450 isozymes.

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