Biochemical Pharmacology, Vol. 53, 1099–1105, 1997.

ISSN 0006-2952/97/$17.00 + 0.00
PII S0006-2952(97)00088-9

Involvement of Growth Hormone as a Regulating Factor in Sex Differences of Mouse Hepatic Aldehyde Oxidase

Shin'ichi Yoshihara and Kiyoshi Tatsumi*
INSTITUTE OF PHARMACEUTICAL SCIENCES, HIROSHIMA UNIVERSITY SCHOOL OF MEDICINE, HIROSHIMA 734, JAPAN

ABSTRACT. The participation of circulating growth hormone (GH) as a regulator of sex differences in hepatic aldehyde oxidase (AO) activity in ddy mice was examined. The 2- to 3-fold higher activities in adult male mice compared with adult female mice were decreased to the female levels by neonatal pretreatment with monosodium glutamate (MSG) or monosodium aspartate (MSA), either of which is known to reduce circulating GH levels. A decline of the activities in the MSG-treated male mice was restored nearly to the male control levels by subsequent injections of human GH every 12 hr for 7 days. These changes in AO activities in male mice caused by the excitotoxic amino acids were not observed in females. Hypophysectomy markedly decreased hepatic AO activities in male mice and partially in female mice. The activities in hypophysectomized male mice were restored again to levels similar to the control males by intermittent injections of human GH. Administration of testosterone propionate (TP) significantly increased the activities of hepatic AO in intact female mice, but not in MSA-treated or hypophysectomized females. On the other hand, the AO activities in adult male mice were decreased partially by the administration of estradiol benzoate. These results indicate that the pituitary GH is involved as one of the major regulatory factors of sex differences in the activities of hepatic AO in mice and TP also contributes to maintaining the higher activity in male mice mainly through the hypothalamus-pituitary system. BIOCHEM PHARMACOL 53;8:1099–1105, 1997. © 1997 Elsevier Science Inc.

KEY WORDS. aldehyde oxidase; xanthine oxidase; mouse liver; sex differences; growth hormone; monosodium glutamate; monosodium aspartate; hypophysectomy; testosterone propionate

AO† (EC 1.2.3.1) and XO (EC 1.2.3.2) are two of the major cytosolic molybdenum hydroxylases that show closely related molecular properties in terms of molecular size (a homodimer of 150 kDa subunit) and the prosthetic group composition (FAD, molybdenum, and iron–sulfur cluster) [1–4]. Both enzymes catalyze the nucleophilic oxidation of a wide variety of endogenous and exogenous *N*-heterocycles as well as aldehydes with somewhat different substrate specificity [5, 6]. Nevertheless, the physiological function(s) of AO has not been determined conclusively, while XO is regarded as an obligatory enzyme in purine metabolism, i.e. conversion of xanthine to uric acid. In recent years, several investigators have demonstrated that AO is involved as a predominant enzyme either in the metabolic activation of prodrugs of antitumor [7] and antiviral [8, 9] agents or in the inactivation of antiviral [10] and antitumor [11] agents. In addition, AO can mediate the anaerobic reduction of a variety of compounds such as sulfoxides [2, 12, 13], *N*-Oxides [14], hydroxamic acids [15, 16], azo dyzes [17, 18], nitropolycyclic aromatic hydrocarbons [19, 20] and arene oxides [21] in the presence of its electron donors. Therefore, these molybdenum hydroxylases, especially AO, have become of great interest as another member of the drug-metabolizing enzymes, showing a complementary substrate specificity to the microsomal monooxygenases such as P450-dependent and flavin-containing monooxygenases [1, 22].

Hepatic AO activity is vastly different not only among animal species [23] but also in strains of the same animal species [24, 25]. Furthermore, in adult mice there exist consistent sex differences that exhibit 2- to 4-fold higher activities in male mice than in female mice [26, 27]. To date, however, there are only a few studies concerning the regulatory factor of sex differences in hepatic AO activity, except for the earlier studies proposing an involvement of sex hormones such as TP [26, 27] and EB [27]. We recently demonstrated that the higher activities in male mice were decreased to female levels by various pretreatments with compounds such as alloxan, dexamethasone, and MSG in

* Corresponding author: Shin'ichi Yoshihara, Ph.D., Institute of Pharmaceutical Sciences, Hiroshima University School of Medicine, 1-2-3, Kasumi, Minami-ku, Hiroshima 734, Japan. Tel. (82) 257-5326; FAX (82) 257-5329; E-mail: yosihara@pharm.hiroshima-u.ac.jp

† *Abbreviations:* AO, aldehyde oxidase; XO, xanthine oxidase; GH, growth hormone; MSG, monosodium glutamate; MSA, monosodium aspartate; HX, hypophysectomy; TP, testosterone propionate; EB, estradiol benzoate; P450, cytochrome P450; and NMN, N^1-methyl nicotinamide.

Received 30 July 1996; accepted 6 November 1996.

neonates.* All of these treatments are known to modulate hypothalamus-pituitary function and then to reduce the circulating GH levels in rats [28–30]. Recently, Pampori and Shapiro [31] found that pulsatile secretion of GH also disappears in both sexes of adult mice treated neonatally with MSG. In fact, the sex differences of P450-mediated drug metabolism are modified markedly not only in rats [32–34] but also in mice [31, 35] by these GH secretion-modulating manipulations as well as by HX [32, 33, 35]. Accordingly, we have suggested that the sex difference in hepatic AO activity in mice may be relevant to circulating GH levels.* Thus, the purpose of this study was to elucidate an involvement of GH as a main regulatory factor of sex differences in hepatic AO activity in mice on the basis of more direct evidence.

MATERIALS AND METHODS

Chemicals

The sources of materials used were as follows: MSG from the Kanto Chemical Co., Inc. (Tokyo); MSA, TP, and EB from Wako Pure Chemical Ind., Ltd., (Osaka); benzaldehyde, menadione, and xanthine from Nakarai Chemicals, Ltd. (Kyoto); and somatotropin (GH) from human pituitaries from the Sigma Chemical Co., (St. Louis MO, U.S.A.). Unless noted otherwise, chemicals used were of the highest quality commercially available.

Animal Treatment

Pregnant Std:ddY mice were obtained from Japan SLC, Inc. (Hamamatsu) and housed in plastic cages containing wood bedding in an air-conditioned room with a photo period of 12 hr light/12 hr dark cycle. The pups were treated i.p. with 4 mg/g body weight of MSG or MSA in saline on days 1, 3, 5, 7, and 9 after birth. The animals were weaned at 3 weeks and maintained on laboratory chow and water *ad lib.* until killed. Some mice (6 weeks old) that were treated neonatally with MSG were treated s.c. with 0.005 IU/10 g body weight of human GH in a solution of 0.03 M $NaHCO_3$ and 0.15 M NaCl (pH 8.25) at an interval of 12 hr for 7 consecutive days. Hypophysectomized and sham-operated mice at 5 weeks of age were shipped to our facility 1 week later from Japan SLC, Inc. After a 1-week acclimation period, some hypophysectomized mice were treated with human GH as described above. EB (2.5 μg/g body weight/day) in propylene glycol was injected s.c. into male mice (7 weeks old) for 10 consecutive days. TP (50 μg/g body weight/day) in propylene glycol was given s.c. to intact and MSA-treated or hypophysectomized female mice at 7 weeks of age for 10 consecutive days. Control animals in all experiments were treated with an equivalent volume of the corresponding vehicle alone. All the animals were killed 24 hr after the last injection of respective hormone for enzyme preparation.

Enzyme Preparation and Enzyme Assay

The livers were perfused with ice-cold saline and homogenized with 4 vol. of 100 mM potassium phosphate buffer (pH 7.4) containing 0.1 mM EDTA. The clear supernatant obtained at 105,000 *g* by differential centifugation was used as the enzyme source of hepatic AO and XO. AO activity was assayed by the spectrophotometric determination of benzaldehyde oxidation sensitive to menadione, a potent inhibitor of AO. The decrease in absorbance at 247 nm consequent upon the oxidation of 0.05 mM benzaldehyde (E = 17.54 cm^{-1} mM^{-1}) to benzoic acid was monitored at 25°. To obtain kinetic parameters by Lineweaver-Burk plots, four different concentrations of benzaldehyde ranging from 0.0075 to 0.05 mM were used. The activity of XO was assayed by the determination of uric acid formed using 0.1 mM xanthine as substrate according to the method of Stirpe and Corte [36]. Cytosolic protein was determined by the method of Lowry *et al.* [37], using bovine serum albumin as a standard.

Statistical Analysis

Student's *t*-test was used for the statistical analysis. Values were considered to be significantly different when the P value was less than 0.05.

RESULTS

Effects of Neonatal Treatment with MSG and Supplement of GH on Hepatic AO Activity in Mice

The activities of hepatic AO, assayed as menadione-sensitive benzaldehyde oxidation in this study, were 2- to 3-fold higher in adult male ddy mice than in female mice. Neonatal treatment with MSG (4 mg/g body weight/day) on alternate days for the first 9 days after birth caused stunted growth in both sexes until the animals were killed (data not shown). These lowered body weight gains were partially rescued by an intermittent injection of human GH at a dose of 0.005 IU/10 g body weight twice a day for 7 consecutive days. As shown in Fig. 1A, only the activities of males neonatally treated with MSG were decreased to the female levels at adulthood (7 weeks old), whereas no effect was observed on the activities of females. Interestingly, this decline of AO activities in MSG-treated male mice was associated with a change of K_m values to that of the female types, showing about two times lower affinity than the male types (Table 1).The intermittent treatment with human GH restored both the activities and the K_m values of MSG-treated male mice to the corresponding control levels (Fig. 1A and Table 1). In MSG-treated female mice, however, the same GH treatment caused a change of K_m values to that of the male type without any significant increase of the activity. On the other hand, the

* Yoshihara S and Tatsumi K, Manuscript submitted for publication.

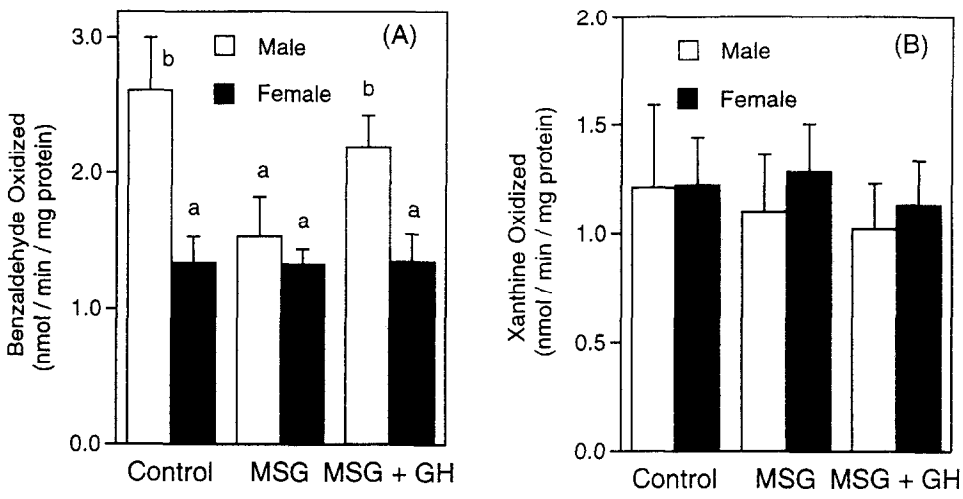

FIG. 1. Effects of neonatal MSG treatment and intermittent GH injection on activities of hepatic AO (A) and XO (B) in mice. Pups were injected i.p. with 4 mg/g body weight/day of MSG or saline on alternate days for the first 9 days after birth. At 6 weeks, mice were injected s.c. with 0.005 IU/10 g body weight of human GH or diluent every 12 hr for 7 consecutive days and were killed 24 hr after the last injection for enzyme assays. The activities of hepatic AO and XO were assayed as described in Materials and Methods. Values are means ± SD of 3–4 mice per group. Key: (a) significantly different from male control ($P < 0.05$); and (b) significantly different from female control ($P < 0.05$).

activities of hepatic XO in both sexes, another cytosolic molybdenum hydroxylase showing no sex difference, were unaffected by treatment with either MSG or GH (Fig. 1B).

Effects of Neonatal Treatment with MSA and Sex Hormone Treatment on Hepatic AO Activity in Mice

As with MSG, neonatal treatment with MSA causes a reduced circulating GH resulting in modification of the sex-specific metabolic profiles of drugs [38]. Thus, to further confirm the results obtained with MSG treatment, newborn mice were treated neonatally with MSA (4 mg/g body weight/day) for the first 9 days as was the case with MSG. As shown in Fig. 2A, neonatal treatment with MSA again decreased the activities of AO in male mice but not in females. Treatment of adult male mice with EB partially decreased the hepatic AO activities. On the contrary, TP treatment of intact adult female mice increased the activities to levels almost similar to those of the male controls. However, this striking increase in the activity of female mice by TP was not realized with the female mice neonatally treated with MSA. XO activity in either sex was not affected by all of these treatments (Fig. 2B).

TABLE 1. Effects of neonatal MSG treatment and intermittent GH injection on kinetic properties of hepatic AO in mice

Gender	Treatment	K_m (10^{-6} M)	V_{max} (nmol/min/mg protein)
Male	Control	3.12 ± 0.10*	3.22 ± 0.13*
	MSG	6.84 ± 0.32†	1.84 ± 0.14†
	MSG + GH	3.33 ± 0.24*	2.63 ± 0.25*
Female	Control	6.34 ± 0.40†	1.61 ± 0.28†
	MSG	7.04 ± 0.11†	1.60 ± 0.61†
	MSG + GH	3.51 ± 0.36*	1.62 ± 0.13†

Neonatal MSG treatment and the subsequent treatment with human GH were performed as described in the legend of Fig. 1. Values are means ± SD of 3–4 mice per group.

* Significantly different from female control ($P < 0.05$).

† Significantly different from male control ($P < 0.05$).

Effects of HX and Supplement of GH or TP on Hepatic AO Activity in Mice

To obtain more direct evidence concerning the circulating GH level as a regulatory factor of the sex differences, the effects of HX on hepatic AO activity in mice were examined (Fig. 3 and Table 2). HX of male mice markedly decreased the activities of hepatic AO to the levels of sham-operated female controls (Fig. 3A). On the other hand, the activities of female mice were also decreased but to a somewhat lesser extent than in the males. Consistent with the profile obtained by MSG treatment (Fig 1A), the intermittent s.c. injections of human GH to hypophysectomized mice caused a marked increase of the activity in the males but a small increase in the females. The K_m values of AO in hypophysectomized male mice were also increased to values similar to those in intact females, which were altered insignificantly by HX (Table 2). As expected, no effect on the activities of hepatic XO was observed by any of these manipulations (Fig. 3B). TP treatment produced a remarkable increase of AO activity in intact female mice; in hypophysectomized females the increase was not as great (Fig. 4). Interestingly, however, TP treatment of female mice caused a shift of K_m values of hepatic AO to those of the male type whether hypophysectomized or not (Table 2).

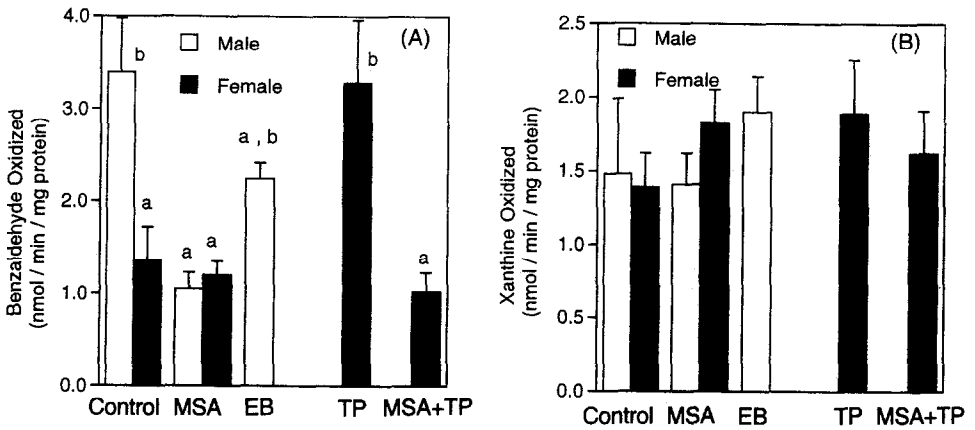

FIG. 2. Effects of neonatal MSA treatment and sex hormone treatment on activities of hepatic AO (A) and XO (B) in mice. Pups were injected i.p. with 4 mg/g body weight/day of MSA or saline on alternate days for the first 9 days after birth. EB (2.5 μg/g body weight/day) or TP (50 μg/g body weight/day) was injected s.c. into the mice for 10 consecutive days at 7 weeks of age. The animals were killed 24 hr after the last injection for enzyme assays. Hepatic AO and XO activities were assayed as described in Materials and Methods. Values are means ± SD of 3–4 mice per group. Key: (a) significantly different from male control ($P < 0.05$); and (b) significantly different from female control ($P < 0.05$).

DISCUSSION

Almost 30 years ago, two groups of investigators [26, 27] found, independently, that the activity of hepatic NMN oxidase, namely AO, in adult male mice is higher than that in females and that the activity of female mice is increased by TP treatment. Huff and Chaykin [26] further demonstrated that the activities of both sexes are not different from each other in neonates but the activity of males is increased at puberty. Based on these lines of evidence, both groups of investigators proposed that TP might be an important factor for maintaining the higher activity in male mice. However, the details of the mechanisms underlying the sex differences of hepatic AO activities in mice have not been elucidated yet. In previous study, we demonstrated that the activity of hepatic AO, but not XO, in male mice is decreased to the female level by manipulations that cause modulation of circulating GH levels, such as alloxan diabetes and treatment with dexamethasone or MSG at the neonatal period.* MacLeod *et al.* [39] have shown that

* Yoshihara S and Tatsumi K, Manuscript submitted for publication.

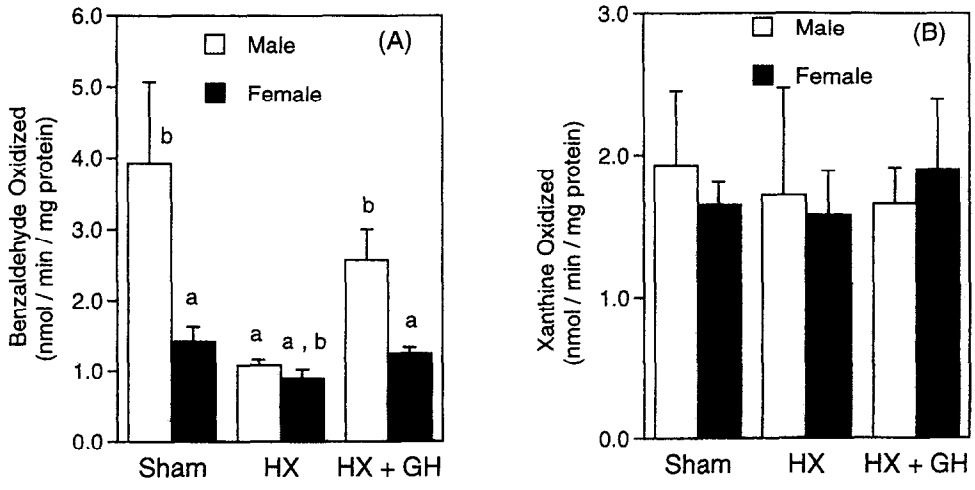

FIG. 3. Effects of HX and intermittent GH injection on activities of hepatic AO (A) and XO (B) in mice. Mice were hypophysectomized or sham-operated at 5 weeks old, and when they were 7 weeks old, they started to receive s.c. injections with 0.005 IU/10 g body weight of human GH or diluent every 12 hr for 7 consecutive days. The animals were killed 24 hr after the last injection for enzyme assays. The activities of hepatic AO and XO were assayed as described in Materials and Methods. Values are means ± SD of 3–4 mice per group. Key: (a) significantly different from sham-operated male control ($P < 0.05$); and (b) significantly different from sham-operated female control ($P < 0.05$).

TABLE 2. Effects of HX and TP treatment on kinetic properties of hepatic AO in mice

Gender	Treatment	K_m (10^{-6} M)	V_{max} (nmol/min/mg protein)
Male	Sham	2.79 ± 0.11*	2.91 ± 0.33
	HX	11.38 ± 3.45†	1.09 ± 0.15*†
Female	Sham	9.65 ± 1.60†	2.02 ± 0.60
	HX	8.76 ± 1.24†	0.87 ± 0.21†
	Sham+ TP	2.70 ± 0.05*	3.12 ± 0.66
	HX+ TP	3.32 ± 0.66*	1.44 ± 0.05

HX or sham-operation and the TP treatment that followed were performed as described in the legend of Fig. 4. Values represent means ± SD of 3–4 mice per group.
* Significantly different form sham-operated female control ($P < 0.05$).
† Significantly different from sham-operated male control ($P < 0.05$).

circulating GH profiles of mice are similar to, but somewhat different from those of rats as follows: (1) GH peaks in males of both species appear less frequently than they do in females, i.e. a pulsatile pattern with an interval of 1.5–2.5 hr; and (2) the duration, height, and area of GH peaks, as well as the average concentration between peaks in mice, are comparable in both sexes, while in rats peak heights are two to four times higher in males and concentrations of trough levels are clearly higher in females. In addition, it is also reported that the orientation of sex differences in certain hepatic P450-dependent drug-metabolizing activities is reversed between rats and mice [35].

In the present study, the lowered activity caused by neonatal MSG treatment of male mice was restored nearly to the control level by the intermittent injections of human GH, mimicking the male secretory pattern [39]. These results were similar to the observations with hypophysectomized male mice, in which hepatic AO activities declined to the levels of sham-operated females but recovered by intermittent injection of human GH. In contrast to these observations with mouse AO in the present study, it is interesting to note that the responsiveness of expression of CYP 2C11, a major drug and steroid-metabolizing enzyme in rat liver microsomes, to GH replacement with the masculine-type secretion pattern differs between HX and MSG-treated rats; the former is effective but the latter is not [40]. On the other hand, the activities of hepatic AO in female mice were decreased modestly by HX, but those of XO in both sexes were almost unaffected by any of these treatments. Accordingly, these findings clearly indicate that the male-type secretion pattern of pituitary GH directly contributes to maintaining the higher activity of hepatic AO in adult male mice. It is interesting, however, that despite the intermittent injection of GH, the AO activity in hypophysectomized female mice was influenced only a little. This may suggest that hepatic AO in either sex differs from each other not only quantitatively but also qualitatively. Supporting this, although the K_m values for benzaldehyde of both sexes were only a little different, this difference was significant. This observation was further confirmed with the highly purified AOs in both sexes of mice [41]. In addition, the K_m value of male mice was shifted to that of the female by neonatal treatment with either MSG or HX. And this change in K_m value of male mice to that of the female was returned to the normal male type by GH supplement with a circulation pattern of the masculine type. This observation, exhibiting different K_m values in each sex of ddy mice, was not consistent with the results reported by Huff and Chaykin [26] who found very similar K_m values for NMN in both sexes of C57BL and DBA mice. Although both the substrate and strain used in each experiment were different, the reason for this discrepancy is unclear at present. Another unexplainable result obtained in this experiment was a shift of the female type K_m value to that of the male type by the intermittent injections of GH into MSG-treated females and TP treatment of hypophysectomized females, both of which did not cause any appreciable increase in the activities of hepatic AO.

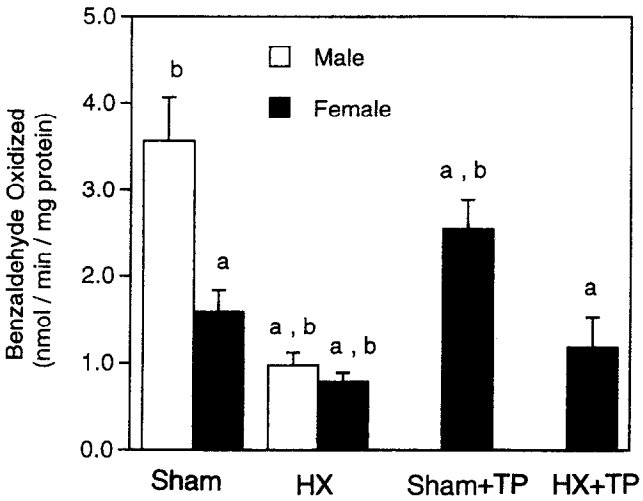

FIG. 4. Effects of HX and testosterone treatment on hepatic AO activity in mice. HX and the sham operation were performed as described in the legend of Fig. 3. TP (50 μg/g body weight/day) was injected s.c. into 7-week-old mice for 10 consecutive days. The animals were killed 24 hr after the last injection for enzyme assays. The activity of hepatic AO was assayed as described in Materials and Methods. Values are means ± SD of 3–4 mice per group. Key: (a) significantly different from sham-operated male control ($P < 0.05$); and (b) significantly different from sham-operated female control ($P < 0.05$).

In the present study, the role of androgen and estrogen on the activity of hepatic AO was also confirmed as previously reported [26, 27]. Administration of TP to intact females caused an increase in the activity to that of the male as well as a shift of K_m value to that of the male type. However, this masculinization of AO in female mice by TP was not exhibited with MSA-treated or hypophysectomized female mice. Therefore, TP likely exerts its effects on hepatic AO activity mainly through the hypothalamus-pituitary system, whereas androgen affects the secretion pattern of GH [42].

In conclusion, the present study clearly demonstrates the

important role of the pituitary hormones, especially GH, on the maintenance of higher activity of hepatic AO in male mice, although an additional factor(s) is also suggested. Considering a lack in the responsiveness to HX and neonatal treatment with MSG or MSA, hepatic AO in female mice and XO in either sex may be regulated by factors other than the circulating GH level.

References

1. Beedham C, Molybdenum hydroxylases as drug-metabolizing enzymes. *Drug Metab Rev* **16:** 119–156, 1985.
2. Yoshihara S and Tatsumi K, Guinea pig liver aldehyde oxidase as a sulfoxide reductase: its purification and characterization. *Arch Biochem Biophys* **242:** 213–224, 1985.
3. Turner NA, Doyle WA, Ventom AM and Bray RC, Properties of rabbit liver aldehyde oxidase and the relationship of the enzyme to xanthine oxidase and dehydrogenase. *Eur J Biochem* **232:** 646–657, 1995.
4. Calzi ML, Raviolo C, Ghibaudi E, Gioia LD, Salmona M, Cazzaniga G, Kurosaki M, Terao M and Garattini E, Purification, cDNA cloning, and tissue distribution of bovine liver aldehyde oxidase. *J Biol Chem* **270:** 31037–31045, 1995.
5. Rajagopalan KV, Xanthine oxidase and aldehyde oxidase. In: *Enzymatic Basis of Detoxification* (Ed. Jakoby WB), Vol. 1, pp. 295–310. Academic Press, New York, 1980.
6. Krenitsky TA, Neil SM, Elion GB and Hitchings GH, A comparison of the specificities of xanthine oxidase and aldehyde oxidase. *Arch Biochem Biophys* **150:** 585–599, 1972.
7. Guo X, Lerner-Tung M, Chen HX, Chang CN, Zhu JL, Chang CP, Pizzorno G, Lin TP and Cheng YC, 5-Fluoro-2-pyrimidinone, a liver aldehyde oxidase-activated prodrug of 5-fluorouracil. *Biochem Pharmacol* **49:** 1111–1116, 1995.
8. Chang CN, Doong SL and Cheng YC, Conversion of 5-iodo-2-pyrimidinone-2′-deoxyribose to 5-iodo-deoxyuridine by aldehyde oxidase. Implication in hepatotropic drug design. *Biochem Pharmacol* **43:** 2269–2273, 1992.
9. Clarke SE, Harrell AW and Chenery RJ, Role of aldehyde oxidase in the *in vitro* conversion of famciclovir to penciclovir in human liver. *Drug Metab Dispos* **23:** 251–254, 1995.
10. Miranda PD and Good SS, Species differences in the metabolism and disposition of antiviral nucleoside analogues. 1. Acyclovir. *Antiviral Chem Chemother* **18:** 1–8, 1992.
11. Robertson IGC and Bland TJ, Inhibition by SKF-525A of the aldehyde oxidase-mediated metabolism of the experimental antitumour agent acridine carboxamide. *Biochem Pharmacol* **45:** 2159–2162, 1993.
12. Yoshihara S and Tatsumi K, Metabolism of diphenyl sulfoxide in perfused guinea pig liver: Involvement of aldehyde oxidase as a sulfoxide reductase. *Drug Metab Dispos* **18:** 876–881, 1990.
13. Lee SC and Renwick AG, Sulphoxide reduction by rat and rabbit tissues *in vitro*. *Biochem Pharmacol* **49:** 1557–1565, 1995.
14. Kitamura S and Tatsumi K, Involvement of liver aldehyde oxidase in the reduction of nicotinamide *N*-oxide. *Biochem Biophys Res Commun* **120:** 602–606, 1984.
15. Sugihara K, Kitamura S and Tatsumi K, Involvement of liver aldehyde oxidase in conversion of *N*-hydroxyurethane to urethane. *J Pharmacobiodyn* **6:** 677–683, 1983.
16. Kitamura S, Sugihara K and Tatsumi K, Reductase activity of aldehyde oxidase toward the carcinogen *N*-hydroxy-2-acetylaminofluorene and the related hydroxamic acids. *Biochem Mol Biol Int* **34:** 1197–1203, 1994.
17. Kitamura S and Tatsumi K, Azoreductase activity of liver aldehyde oxidase. *Chem Pharm Bull (Tokyo)* **31:** 3334–3337, 1983.
18. Stoddart AM and Levine WG, Azoreductase activity by purified rabbit liver aldehyde oxidase. *Biochem Pharmacol* **43:** 2227–2235, 1992.
19. Kitamura S, Narai N and Tatsumi K, Rabbit liver enzymes responsible for reduction of nitropolycyclic aromatic hydrocarbons. *Chem Pharm Bull (Tokyo)* **31:** 776–779, 1983.
20. Bauer SL and Howard PC, Kinetics and cofactor requirements for the nitroreductive metabolism of 1-nitropyrene and 3-nitrofluoranthene by rabbit liver aldehyde oxidase. *Carcinogenesis* **12:** 1545–1549, 1991.
21. Hirao Y, Kitamura S and Tatsumi K, Epoxide reductase activity of mammalian liver cytosols and aldehyde oxidase. *Carcinogenesis* **15:** 739–743, 1994.
22. Rodrigues AD, Comparison of levels of aldehyde oxidase with cytochrome P450 activities in human liver *in vitro*. *Biochem Pharmacol* **48:** 197–200, 1994.
23. Krenitsky TA, Tuttle JV, Cattau EL Jr and Wang P, A comparison of the distribution and electron acceptor specificities of xanthine oxidase and aldehyde oxidase. *Comp Biochem Physiol* **49B:** 687–703, 1974.
24. Watson JG, Higgins TJ, Collins PB and Chaykin S, The mouse liver aldehyde oxidase locus (*Aox*). *Biochem Genet* **6:** 195–204, 1972.
25. Sugihara K, Kitamura S and Tatsumi K, Strain differences of liver aldehyde oxidase activity in rats. *Biochem Mol Biol Int* **37:** 861–869, 1995.
26. Huff SD and Chaykin S, Genetic and androgenic control of N^1-methylnicotinamide oxidase activity in mice. *J Biol Chem* **242:** 1265–1270, 1967.
27. Gluecksohn-Waelsch S, Greengard P, Quinn GP and Teicher LS, Genetic variations of an oxidase in mammals. *J Biol Chem* **242:** 1271–1273, 1967.
28. Tannenbaum GS, Growth hormone secretory dynamics in streptozotocin diabetes: evidence of a role for endogenous circulating somatostatin. *Endocrinology* **108:** 76–82, 1981.
29. Wehrenberg WB, Janowski BA, Piering AW, Culler F and Jones KL, Glucocorticoids: Potent inhibitors and stimulators of growth hormone secretion. *Endocrinology* **126:** 3200–3203, 1990.
30. Millard WJ, Martin JB Jr, Audet J, Sagar SM and Martin JB, Evidence that reduced growth hormone secretion observed in monosodium glutamate-treated rats is the result of a deficiency in growth hormone-releasing factor. *Endocrinology* **110:** 540–550, 1982.
31. Pampori NA and Shapiro BH, Effects of neonatally administered monosodium glutamate on the sexually dimorphic profiles of circulating growth hormone regulating murine hepatic monooxygenases. *Biochem Pharmacol* **47:** 1221–1229, 1994.
32. Yamazoe Y, Shimada M, Murayama N, Nagata K and Kato R, Hormonal regulation of hepatic cytochrome P-450. In: *Xenobiotic Metabolism and Disposition* (Eds. Kato R, Estabrook RW and Cayen MN), pp. 37–44. Taylor & Francis, London, 1988.
33. Yamazoe Y, Shimada M, Murayama N, Yamauchi K and Kato R, Alteration of hepatic drug metabolizing activities and contents of cytochrome P-450 isozymes by neonatal monosodium glutamate treatment. *Biochem Pharmacol* **37:** 1687–1691, 1988.
34. Pampori NA, Agrawal AK, Waxman DJ and Shapiro BH, Differential effects of neonatally administered glutamate on the ultradian pattern of circulating growth hormone regulating expression of sex-dependent forms of cytochrome P450. *Biochem Pharmacol* **41:** 1299–1309, 1991.
35. MacLeod JN and Shapiro BH, Growth hormone regulation of hepatic drug-metabolizing enzymes in the mouse. *Biochem Pharmacol* **38:** 1673–1677, 1989.
36. Stirpe F and Corte ED, The regulation of rat liver xanthine oxidase: Conversion *in vitro* of the enzyme activity from de-

hydrogenase (type D) to oxidase (type O). *J Biol Chem* **244:** 3855–3863, 1969.
37. Lowry OH, Rosebrough NJ, Farr AL and Randall RJ, Protein measurement with the Folin phenol reagent. *J Biol Chem* **193:** 265–275, 1951.
38. Agrawal AK, Pampori NA and Shapiro BH, Sex- and dose-dependent effects of neonatally administered aspartate on the ultradian patterns of circulating growth hormone regulating hexobarbital metabolism and action. *Toxicol Appl Pharmacol* **108:** 96–106, 1991.
39. MacLeod JN, Pampori NA and Shapiro BH, Sex differences in the ultradian pattern of plasma growth hormone concentrations in mice. *J Endocrinol* **131:** 395–399, 1991.
40. Shapiro BH, Pampori NA, Ram PA and Waxman DJ, Irreversible suppression of growth hormone-dependent cytochrome P450 2C11 in adult rats neonatally treated with monosodium glutamate. *J Pharmacol Exp Ther* **265:** 979–984, 1993.
41. Yoshihara S and Tatsumi K, Purification and characterization of hepatic aldehyde oxidase in male and female mice. *Arch Biochem Biophys*, in press.
42. Jansson JO, Ekberg S, Isaksson O, Mode A and Gustafsson JA, Imprinting of growth hormone secretion, body growth, and hepatic steroid metabolism by neonatal testosterone. *Endocrinology* **117:** 1881–1889, 1985.