Biochemical Pharmacology, Vol. 38, No. 10, pp. 1673–1677, 1989.
Printed in Great Britain.

0006-2952/89 $3.00 + 0.00

GROWTH HORMONE REGULATION OF HEPATIC DRUG-METABOLIZING ENZYMES IN THE MOUSE

JAMES N. MACLEOD* and BERNARD H. SHAPIRO
Laboratories of Biochemistry, Department of Animal Biology, School of Veterinary Medicine, University of Pennsylvania, Philadelphia, PA 19104-6048, U.S.A.

(*Received* 11 *July* 1988; *accepted* 7 *October* 1988)

Abstract—Hepatic microsomal hexobarbital hydroxylase and aminopyrine *N*-demethylase activities increased in adult male mice following hypophysectomy to female-like levels, eliminating the normal sexually dimorphic pattern of these enzymes. Exogenous growth hormone replacement (0.08 I.U./100 g body weight/day) re-established the lower masculine activities only when administered subcutaneously once every 12 hr. Enzyme activities remained elevated at female-like levels when the same total dose of growth hormone was infused continuously using osmotic pumps or was injected once every 6 hr. These data suggest that, despite the reversed orientation of sex differences in hepatic drug-metabolizing enzymes between rats and mice (i.e. higher enzyme activities in female mice and male rats), the basic hormonal regulatory axis is similar in the two species. Cyclic fluctuations of systemic growth hormone concentrations masculinize kinetic parameters of hepatic hexobarbital hydroxylase and aminopyrine *N*-demethylase in both species. Rats and mice differ in that these similar hormonal signals lower the apparent V_{max} in male mice, while markedly increasing the enzyme activities in male rats. It appears more likely, therefore, that species- and sex-specific differences in the total hepatic cytochrome P-450 isoenzyme populations produce the reversed sex-dependent pattern of hexobarbital hydroxylase and aminopyrine *N*-demethylase.

Adult mice exhibit clear and consistent sexual differences in the activity of certain drug-metabolizing hepatic microsomal monooxygenases. In the male, gonadal androgens suppress the hydroxylation of hexobarbital and the *N*-demethylation of aminopyrine to levels approximately 50–70% of females or gonadectomized males [1]. Testicular depression of monooxygenase activity, however, is dependent upon an intact hypothalamic–pituitary axis. Hypophysectomy eliminates sexually dimorphic patterns of hepatic drug metabolism in mice and, unlike in gonadectomized subjects, exogenous androgen therapy fails to produce any suppressive effect [2]. Studies in rats, which also exhibit marked sex differences in the rate of liver hexobarbital and aminopyrine metabolism, have conclusively identified growth hormone and its ultradian pattern of release as the primary pituitary variable necessary for the expression of sex-specific constituent cytochrome P-450 isoenzymes [3, 4]. Male cytochrome P-450 isoenzymes are preferentially expressed in hypophysectomized rats of either sex when exogenous growth hormone is administered in twice daily subcutaneous injections. The same total dose of growth hormone administered tonically via subcutaneously positioned osmotic pumps results in the preferential expression of female cytochrome P-450 isoenzymes. Yet, when male and female patterns of hepatic xenobiotic metabolism are compared in adult rats and mice, the orientation is opposite (i.e. male > female in rats; female > male in mice) and the magnitude of difference is much lower in mice.

* Correspondence: Dr. James N. MacLeod, Laboratories of Biochemistry, Department of Animal Biology, School of Veterinary Medicine, University of Pennsylvania, 3800 Spruce St., Philadelphia, PA 19104-6048.

The current study was designed to determine if the reversed orientation of sex-dependent hepatic hexobarbital and aminopyrine metabolism can be explained by respective differences in growth hormone regulation or function. That is, in hypophysectomized mice, will an intermittent pattern of growth hormone replacement masculinize, and a constant stable infusion of growth hormone feminize, liver hexobarbital hydroxylase and aminopyrine *N*-demethylase activities?

MATERIALS AND METHODS

Animals. Hypophysectomized and sham hypophysectomized control CRL:CD-1(ICR)BR mice were purchased from Charles River Laboratories (Wilmington, MA). The surgeries were performed at 55 days of age and the mice were shipped to our facility 1 week later. After arrival, the mice were maintained in plastic cages on hardwood bedding with water and commercial mouse diet (Prolab 3000, Agway, Inc., Syracuse, NY) provided *ad lib.* Ambient temperature was maintained at 22–23° with a photoperiod of 12 hr light, 12 hr dark. All mice were provided a 2-week acclimation period during which body weights were measured once daily. Completely hypophysectomized mice have a stable or gradually decreasing body weight, while sham and incompletely hypophysectomized CD-1 mice at this age increase in body weight 2–4 g during the same 2-week postoperative period. It is essential that incompletely hypophysectomized individuals, which can approach 25% of the group, be identified and eliminated from the study. Bilateral gonadectomies were performed under pentobarbital anesthesia, 50 mg/kg body weight, administered by intraperitoneal (i.p.) injection.

Table 1. Masculinization of hexobarbital hydroxylase and aminopyrine *N*-demethylase in murine hepatic microsomes by twice daily growth hormone treatment

Surgery	Sex	Treatment	Hexobarbital hydroxylase		Aminopyrine *N*-demethylase			
			V_{max}	K_m	V_{max}^1	K_m^1	V_{max}^2	K_m^2
Sham	M	Diluent	2.0 ± 0.3	2.38 ± 0.52	9.8 ± 1.7	6.54 ± 0.80	5.4 ± 1.1	2.37 ± 0.38
Sham	M	G. H.	1.9 ± 0.3	2.58 ± 0.63	8.4 ± 1.2	6.30 ± 0.89	5.0 ± 1.1	2.15 ± 0.73
Castrate	M	Diluent	2.9 ± 0.4*	3.22 ± 0.73	12.9 ± 2.2	7.31 ± 1.28	6.0 ± 1.6	2.62 ± 0.66
Castrate	M	G. H.	2.4 ± 0.4	2.75 ± 0.51	9.8 ± 2.2	7.20 ± 1.79	4.6 ± 1.0	2.07 ± 0.42
Hypox	M	Diluent	3.3 ± 0.5†	3.40 ± 0.68	16.1 ± 3.5*	7.82 ± 2.36	9.0 ± 2.5*	2.79 ± 0.77
Hypox	M	G. H.	2.1 ± 0.3‡	3.22 ± 0.50	10.2 ± 2.0§	7.21 ± 2.83	6.8 ± 1.4§	3.05 ± 1.36
Castrate + Hypox	M	Diluent	3.4 ± 0.3†	3.00 ± 0.42	19.0 ± 2.9†	8.79 ± 1.55	10.4 ± 2.8*	2.77 ± 0.67
Castrate + Hypox	M	G. H.	2.2 ± 0.2‡	3.44 ± 0.41	9.7 ± 2.3§	6.97 ± 1.68	5.4 ± 1.1§	2.48 ± 0.63
Sham	F	Diluent	3.1 ± 0.4†	2.89 ± 0.61	13.8 ± 2.6*	7.04 ± 1.21	8.5 ± 1.7*	2.53 ± 0.59

Male (M) and female (F) Crl:CD-1 mice were either sham hypophysectomized, gonadectomized (castrate), hypophysectomized (hypox), or both gonadectomized and hypophysectomized (castrate + hypox) at 55 days of age. Following a 3-week recovery period, the mice received either 0.03 M $NaHCO_3$ in 0.15 M NaCl (pH 9.5) diluent or growth hormone (G. H.) (0.08 I.U./100 g body wt/day) divided into two subcutaneous injections administered 12 hr apart for 7 days prior to being killed. The results are the means ± SD of at least eight mice per group. V_{max}: apparent maximal velocity (nmol/min/mg protein). K_m: apparent Michaelis constant (10^{-4} M).

*† Significantly different compared to sham hypophysectomized diluent-treated males: *$P < 0.05$, and †$P < 0.01$.

‡§ Significantly different compared to diluent animals of the same surgery group: ‡$P < 0.01$, and §$P < 0.05$.

Rat growth hormone (rat GH-B-10, AFP-8570 C, obtained from the NIADDK) was administered for 7 consecutive days at a dose of 0.08 I.U./100 g body weight/day. The total daily dose was divided into either two or four subcutaneous (s.c.) injections, given once every 12 or 6 hr, respectively, or infused continuously through dorsal subcutaneously positioned Alzet osmotic pumps, model 2001 (Alza Corp., Palo Alto, CA). Growth hormone was solubilized in a solution of 0.03 M $NaHCO_3$ and 0.15 M NaCl (pH 10.8) at a concentration of 0.556 mg/ml and quickly adjusted to pH 9.5. Growth hormone solutions were passed through a 0.2 μm Nalgene disposable filter (Nalge Co., Rochester, NY) prior to placement in sterile vials or the osmotic pumps filled under sterile conditions. All vials of growth hormone were stored during the entire 7-day injection period at 37° to approximate conditions of the surgically implanted osmotic pumps. Separate vials of growth hormone were used for each day of injection and no evidence of bacterial contamination was ever observed. Diluent groups received only buffer prepared, stored, and administered under comparable, but separate conditions. The pattern of diluent administration had no effect on hepatic enzyme activities.

Osmotic pumps were positioned in recipient mice under light ether anesthesia. A dorsal subcutaneous pouch on one side of the spinal column was prepared through a 1.0 cm incision in the contralateral paralumbar skin. Distancing the incision from the subcutaneous pouch prevented problems with closure and healing of the wound due to pump-induced skin tension and displacement. Post-recovery, the mice appeared unaffected by the physical presence of the pump.

Hepatic drug-metabolizing enzymes. All mice were decapitated at 10:00 a.m. (±15 min). The final injections of growth hormone were given at 1:00 a.m. or 7:00 a.m. for the two and four injections/day groups respectively. Liver microsomes were prepared by differential centrifugation as previously described [5] and stored at −70° until assayed the next morning for enzyme activities. Hexobarbital hydroxylase, which catalyzes the conversion of hexobarbital (5-cyclohexenyl-3,5-dimethyl[2-^{14}C]barbituric acid; DuPont NEN Research Products, Boston, MA) to 3-hydroxyhexobarbital, was assayed by our modification [5] of the radioenzyme procedure of Kupfer and Rosenfeld [6]. Aminopyrine *N*-demethylase activity was determined by our adaptation [7] of the sensitive radiometric assay of Poland and Nebert [8] in which the polar formaldehyde product complexed with semicarbazide is separated from nonmetabolized substrate, aminopyrine (4-dimethyl[^{14}C]-aminoantipyrine; DuPont NEN Research Products), by chloroform extraction. Aminopyrine *N*-demethylase exhibited the previously described biexponential kinetics [7, 8] resulting in two V_{max} and K_m values. In both assays, product formation was linear with respect to time and microsomal protein concentration. Sufficient counts were accumulated to achieve a within-sample standard deviation of less than 2.0%. Microsomal protein was quantified by the method of Lowry *et al.* [9], using bovine serum albumin as a standard.

Growth hormone assay. Serum growth hormone concentrations were determined by double antibody radioimmunoassay procedures [10, 11] from trunk blood samples collected at the time of sacrifice. Growth hormone was radioiodinated using chloramine-T, and purified by gel filtration chromatography. Polyethylene glycol 6000 was used as a precipitating agent. The rat growth hormone RIA kit and highly purified mouse growth hormone for the standard curve were provided by the National Hormone and Pituitary Program and Dr. A. F. Parlow.

Table 2. Regulation of sex-dependent hexobarbital hydroxylase and aminopyrine *N*-demethylase in hypophysectomized male mice by the pattern of growth hormone administration

Treatment	Hexobarbital hydroxylase		Aminopyrine *N*-demethylase			
	V_{max}	K_m	V_{max}^1	K_m^1	V_{max}^2	K_m^2
Diluent	3.2 ± 0.5	3.39 ± 1.01	16.1 ± 3.3	7.16 ± 2.57	8.9 ± 2.6	2.79 ± 0.77
Growth hormone, 2×/day	2.2 ± 0.2*	3.35 ± 0.42	9.7 ± 2.0*	7.29 ± 2.03	6.4 ± 1.6	2.08 ± 0.68
Growth hormone, 4×/day	3.0 ± 0.3	3.16 ± 0.64	13.2 ± 1.7	8.97 ± 2.74	8.7 ± 1.6	3.66 ± 1.04
Growth hormone, continuous	3.2 ± 0.4	3.57 ± 0.91	12.9 ± 1.7	7.61 ± 2.21	8.3 ± 2.0	3.51 ± 0.56

Male Crl:CD-1 mice were hypophysectomized at 55 days of age. Following a 3-week recovery period, they were administered growth hormone (0.08 I.U./100 g body wt/day) for 7 consecutive days. The total daily dose was divided into either two or four subcutaneous injections, given once every 12 or 6 hr, respectively, or infused continuously through dorsal subcutaneously positioned osmotic pumps. Diluent mice received non-growth hormone containing buffer twice daily. The results are the means ± SD of at least eight mice per group. V_{max}: apparent maximal velocity (nmol/min/mg protein). K_m: apparent Michaelis constant (10^{-4} M).

* Significantly different compared to diluent animals: $P < 0.01$.

Enzyme kinetics and statistics. Apparent maximal velocities (V_{max}) and Michaelis constants (K_m) were determined from linear regression models of the data graphically represented by the method of Hofstee [12]. All Hofstee plots had positive correlation coefficients exceeding 0.95 and were statistically significant ($P < 0.05$). Experimental groups were compared for significant differences by analysis of variance and the Scheffé method for multiple comparisons using SAS/PC version 6.02 (SAS Institute, Inc., Cary, NC).

RESULTS

Hypophysectomy increased hepatic microsomal hexobarbital hydroxylase and aminopyrine *N*-demethylase activities in male mice, eliminating the normal sexually dimorphic patterns of these enzymes (Table 1). A similar, but less dramatic, feminization resulted from testectomy. However, combined surgical removal of the gonads and pituitary in adult male mice did not produce any apparent additive effects, as enzyme activities increased to levels comparable to those produced by hypophysectomy alone.

Growth hormone treatment to sham-hypophysectomized animals did not affect the activities of hepatic hexobarbital hydroxylase or aminopyrine *N*-demethylase. In contrast, for testectomized and/or hypophysectomized male mice, the administration of 0.08 I.U. growth hormone/100 g body weight divided in two equal subcutaneous doses given 12 hr apart masculinized the activities, i.e. lowered the V_{max}, of these enzymes (Table 1). Yet, the same total dose administered in four equal aliquots every 6 hr or infused continuously by subcutaneously positioned osmotic pumps failed to produce a significant masculinization effect (Table 2). As expected, serum growth hormone was not detectable in diluent-treated hypophysectomized animals. Mice given injections twice daily received their last growth hormone treatment approximately 9 hr prior to sacrifice, by which time serum growth hormone concentrations had dropped to an average of less than 2.0 ng/ml. Hypophysectomized mice receiving growth hormone either four times daily (last treatment 3 hr prior to being killed) or through continuous infusion had serum concentrations around 10 ng/ml at the time of sacrifice (Table 3).

Table 3. Serum growth hormone concentration

Treatment	Growth hormone (ng/ml)
Diluent	Not detectable
Growth hormone, 2×/day	1.34 ± 1.62
Growth hormone, 4×/day	16.13 ± 5.14
Growth hormone, continuous	8.88 ± 3.61

Hypophysectomized Crl:CD-1 mice were treated with growth hormone (0.08 I.U./100 g body wt/day) for 7 consecutive days. The final injections of growth hormone were given approximately 9 and 3 hr prior to blood collection for the two and four injections/day groups respectively. "Continuous" mice received a constant growth hormone infusion through subcutaneously positioned osmotic pumps. Diluent mice received non-growth hormone containing buffer twice daily. The results are the means ± SD of at least eight mice per group.

DISCUSSION

The orientation of sex differences in the hepatic microsomal metabolism of hexobarbital and aminopyrine is opposite in rats and mice. The apparent maximal velocities of hexobarbital hydroxylase and aminopyrine *N*-demethylase are higher in female than in male mice [1]. In rats, however, the same monooxygenases have three to five times higher activities in males than females [13, 14]. These sex differences are hormonally regulated in both species. In male rats, the presence of testicular androgens in neonatal and adult life [15] results in a masculine ultradian pattern of growth hormone release from the anterior pituitary. As a result, systemic growth hormone concentrations in postpubertal male rats are characterized by regular fluctuations on a 3- to 4-hr cycle with peak serum values usually exceeding 200 ng/ml interposed by low trough values of less than 5 ng/ml [16]. Female rats, however, maintain

more constant serum growth hormone concentrations with an irregular and more frequent release pattern [17]. These androgen-mediated changes in plasma growth hormone concentrations observed in adult male rats result in sex-dependent differences in the amount and isozymic forms of hepatic monooxygenases [3, 18, 19], which produce sexually dimorphic characteristics of liver drug metabolism.

The current study was designed to examine the hormonal regulation of drug-metabolizing hepatic monooxygenases in the mouse and to investigate the basis of the opposite orientation of sex differences when compared to the rat. The data demonstrated that the basic hormonal axis in the two species is conserved. Testosterone, as reported earlier [1, 5, 20, 21], masculinizes patterns of hepatic drug metabolism. Yet rather than a direct effect at the liver, the gonadal steroid acts through the hypothalamus and pituitary. Hypophysectomy eliminates the sexually dimorphic pattern of hepatic drug metabolism in mice and rats and blocks the ability of exogenous testosterone treatment to masculinize enzyme activities [2, 22]. Considering this relationship, and the fact that hypophysectomy produces marked testicular atrophy due to a lack of gonadotropin stimulation, it is not surprising that simultaneous gonadectomy and hypophysectomy did not result in hepatic monooxygenase changes different from hypophysectomy alone.

As in the rat, growth hormone appears to be the major pituitary product regulating sex-dependent hepatic monooxygenases in mice [23, 24]. Although to our knowledge circadian or ultradian changes in plasma growth hormone concentrations have not been documented in the mouse, exogenous growth hormone replacement by various administration schedules suggests marked similarities in the two species. A total daily dose of 0.08 I.U. growth hormone per 100 g body weight divided into two subcutaneous injections 12 hr apart masculinized the levels of hepatic microsomal hexobarbital hydroxylase and aminopyrine *N*-demethylase in hypophysectomized and/or gonadectomized mice. In contrast, the maintenance of more stable systemic growth hormone levels either through continuous infusion using osmotic pumps or a more frequent injection schedule resulted in higher apparent V_{max} values, comparable to those observed in female mice. Although the plasma half-life of growth hormone is reported to be approximately 5–7 min [25], a four times daily injection schedule of exogenous growth hormone resulted in sufficiently stable serum concentrations to feminize hepatic monooxygenase profiles presumably due to the gradual uptake of growth hormone from the subcutaneous deposition site [26]. Twice daily prolactin injections to hypophysectomized mice, in agreement with previous studies in rats [27, 28], failed to have any effect on the hepatic monooxygenases studied (data not shown). Thus, the hormonal components of the gonadal-hypothalamic-pituitary-hepatic axis involved with the regulation of constituent hepatic cytochrome P-450 monooxygenases appear to be similar in rats and mice. A cyclic fluctuation of systemic growth hormone levels, influenced by testosterone through hypothalamic factors [29], masculinized the kinetic parameters of hexobarbital hydroxylase and aminopyrine *N*-demethylase. Where the two species differ is that a masculinization of the kinetic parameters of these enzymes in mice resulted in a decreased apparent V_{max}, whereas in rats a masculinization indicated a marked elevation of the apparent V_{max} as compared to normal female values.

Individual sex-dependent cytochrome P-450 isoenzymes which catalyze specific steroid hydroxylations have been characterized from the livers of both mice and rats [30–32], and in certain cases have been shown to be under growth hormone regulation [18, 19, 24]. Since the rate of microsomal metabolism of a given xenobiotic substrate reflects the combined activities of multiple cytochrome P-450 isoenzymes [33], the demonstration that the components of the hormonal pathways regulating hepatic cytochrome P-450-dependent drug metabolism are similar in rats and mice provides strong support for the theory that the opposite orientation of sex differences in enzyme activities (i.e. higher activities of hepatic hexobarbital hydroxylase and aminopyrine *N*-demethylase in male rats and female mice) is a result of species- and sex-specific differences in the total cytochrome P-450 isoenzyme population of the liver. That is, the reversed sexually dimorphic pattern between the two species in the total rate of hexobarbital and aminopyrine metabolism by hepatic microsomes as measured *in vitro* reflects relative, quantitative or qualitative, differences in their liver cytochrome P-450 isoenzyme populations, rather than a variation in the gonadal-hypothalamic-pituitary-liver hormonal axis.

Acknowledgements—The authors graciously acknowledge the rat growth hormone radioimmunoassay kit and mouse growth hormone received from the National Hormone and Pituitary Program and Dr. A. F. Parlow. We are similarly grateful for the technical assistance of Ms. Sandra Hirst. This study was supported by grants from the United States Public Health Service (HD-16358 and GM-26222). J. N. M. received fellowship support as a VMSTP trainee (National Institutes of Health 5-T32-GM-07170) and from the Pharmaceutical Manufacturers Association Foundation, Inc.

REFERENCES

1. MacLeod JN, Sorensen MP and Shapiro BH, Strain independent elevation of hepatic monooxygenase enzymes in female mice. *Xenobiotica* **17**: 1095–1102, 1987.
2. Shapiro BH, Pituitary-dependent masculinization of hepatic hexobarbital hydroxylase in Crl:CD-1(ICR)BR mice. *Life Sci* **36**: 1169–1174, 1985.
3. Mode A, Gustafsson J-A, Jansson J-O, Eden S and Isaksson O, Association between plasma level of growth hormone and sex differentiation of hepatic steroid metabolism in the rat. *Endocrinology* **111**: 1692–1697, 1982.
4. Mode A, Norstedt G, Eneroth P and Gustafsson J-A, Purification of liver feminizing factor from rat pituitaries and demonstration of its identity with growth hormone. *Endocrinology* **113**: 1250–1260, 1983.
5. Shapiro BH and Szczotka SM, Androgenic repression of hexobarbitone metabolism and action in Crl:CD-1(ICR)BR mice. *Br. J Pharmacol* **81**: 49–54, 1984.
6. Kupfer D and Rosenfeld J, A sensitive radioactive assay for hexobarbital hydroxylase in hepatic microsomes. *Drug Metab Dispos* **1**: 760–765, 1973.

7. Shapiro BH, Bardales RM and Lech GM, Perinatal induction of hepatic aminopyrine *N*-demethylase by maternal exposure to phenytoin. *Pediatr Pharmacol* **5**: 201–207, 1985.
8. Poland AP and Nebert DW, A sensitive radiometric assay of aminopyrine *N*-demethylation. *J Pharmacol Exp Ther* **184**: 269–277, 1973.
9. Lowry OH, Rosebrough NJ, Farr AL and Randall RJ, Protein measurement with the Folin phenol reagent. *J Biol Chem* **193**: 265–275, 1951.
10. Sinha YN, Selby FW, Lewis UJ and Vanderlaan WP, Studies of GH secretion in mice by a homologous radioimmunoassay for mouse GH. *Endocrinology* **91**: 784–792, 1972.
11. Eigenmann JE and Eigenmann RY, Radioimmunoassay of canine growth hormone. *Acta Endocrinol (Copenh)* **98**: 514–520, 1981.
12. Hofstee BHJ, Non-inverted versus inverted plots in enzyme kinetics. *Nature* **184**: 1296–1298, 1959.
13. Kato R and Gillette JR, Sex differences in the effects of abnormal physiological states on the metabolism of drugs by rat liver microsomes. *J Pharmacol Exp Ther* **150**: 285–291, 1965.
14. Shapiro BH, Lech GM and Bardales RM, Persistent defects in the hepatic monooxygenase system of adult rats exposed, perinatally, to maternally administered phenytoin. *J Pharmacol Exp Ther* **238**: 68–75, 1986.
15. Jansson J-O, Ekberg S, Isaksson OGP and Eden S, Influence of gonadal steroids on age- and sex-related secretory patterns of growth hormone in the rat. *Endocrinology* **114**: 1287–1294, 1984.
16. Tannenbaum GS and Martin JB, Evidence for an endogenous ultradian rhythm governing growth hormone secretion in the rat. *Endocrinology* **98**: 562–569, 1976.
17. Eden S, Age- and sex-related differences in episodic growth hormone secretion in the rat. *Endocrinology* **105**: 555–560, 1979.
18. MacGeoch C, Morgan ET, Halpert J and Gustafsson J-A, Purification, characterization and pituitary regulation of the sex-specific cytochrome P-450 15β-hydroxylase from liver microsomes of untreated rats. *J Biol Chem* **259**: 15433–15439, 1984.
19. Morgan ET, MacGeoch C and Gustafsson J-A, Hormonal and developmental regulation of expression of the hepatic microsomal 16α-hydroxylase P-450 apoprotein in the rat. *J Biol Chem* **260**: 11895–11898, 1985.
20. Noordhoek J, The effect of castration and testosterone on some components of the microsomal drug metabolising enzyme system in mice. *FEBS Lett* **24**: 255–259, 1972.
21. Kato R, Sex-related differences in drug metabolism. *Drug Metab Rev* **3**: 1–32, 1974.
22. Kramer RE, Greiner JW, Rumbaugh RC, Sweeney TD and Colby HD, Requirement of the pituitary gland for gonadal hormone effects on hepatic drug metabolism in rats. *J Pharmacol Exp Ther* **208**: 19–26, 1979.
23. MacLeod JN and Shapiro BH, Elevated hepatic hexobarbital hydroxylase activity in growth hormone deficient C57BL/6J "Little" mice. *Fed Proc* **45**: 934, 1986.
24. Noshiro M and Negishi M, Pretranslational regulation of sex-dependent testosterone hydroxylases by growth hormone in mouse liver. *J Biol Chem* **261**: 15923–15927, 1986.
25. Frohman LA and Bernardis LL, Growth hormone secretion in the rat: Metabolic clearance and secretion rates. *Endocrinology* **86**: 305–312, 1970.
26. Jansson J-O, Albertsson-Wikland K, Eden S, Thorngren K-G and Isaksson O, Effect of frequency of growth hormone administration on longitudinal bone growth and body weight in hypophysectomized rats. *Acta Physiol Scand* **114**: 261–265, 1982.
27. Gustafsson J-A and Stenberg A, Influence of prolactin on the metabolism of steroid hormones in rat liver and adrenals. *Acta Endocrinol (Copenh)* **78**: 545–553, 1975.
28. Kramer RE, Greiner JW and Colby HD, Divergence of GH actions on hepatic drug metabolism in the presence and absence of the pituitary gland. *Life Sci* **17**: 779–786, 1975.
29. Jansson J-O, Eden S and Isaksson O, Sexually dimorphism in the control of growth hormone secretion. *Endocr Rev* **6**: 128–150, 1985.
30. Harada N and Negishi M, Mouse liver testosterone 15α-hydroxylase (cytochrome P-450$_{15\alpha}$). Purification, regioselectivity, stereospecificity, and sex-dependent expression. *J Biol Chem* **259**: 1265–1271, 1984.
31. Harada N and Negishi M, Mouse liver testosterone 16α-hydroxylase (cytochrome P-450$_{16\alpha}$). Purification, regioselectivity, stereospecificity, and immunochemical characterization. *J Biol Chem* **259**: 12285–12290, 1984.
32. Waxman DJ, Rat hepatic cytochrome P-450 isoenzyme 2c. *J Biol Chem* **259**: 15481–15490, 1984.
33. Conney AH, Induction of microsomal cytochrome P-450 enzymes. *Life Sci* **39**: 2493–2518, 1986.