Proc. Natl. Acad. Sci. USA
Vol. 88, pp. 5227–5231, June 1991
Biochemistry

Evidence from dwarf rats that growth hormone may not regulate the sexual differentiation of liver cytochrome P450 enzymes and steroid 5α-reductase

PETER BULLOCK*, BRIAN GEMZIK*, DONALD JOHNSON†, PAUL THOMAS‡, AND ANDREW PARKINSON*§

*Department of Pharmacology, Toxicology and Therapeutics, Center for Environmental and Occupational Health, and †Department of Gynecology and Obstetrics, University of Kansas Medical Center, Kansas City, KS 66103; and ‡Department of Chemical Biology and Pharmacognosy, Rutgers University College of Pharmacy, Piscataway, NJ 08855

Communicated by Ronald W. Estabrook, March 18, 1991 (received for review December 29, 1990)

ABSTRACT **Differences in the pattern of growth hormone (GH) secretion in mature rats (i.e., "continuous" secretion in females versus "pulsatile" secretion in males) are thought to be the underlying cause of sex-dependent differences in a subpopulation of liver microsomal P450 enzymes and steroid 5α-reductase. A new strain of dwarf rats (NIMR/AS) has recently been shown to have low or undetectable levels of circulating GH due to a selective defect in pituitary GH synthesis. We have measured the levels and/or activity of IIA1 (P450a), IIA2 (P450m), IIC11 (P450h), IIC12 (P450i), IIIA2 (a P450p isozyme), and steroid 5α-reductase in liver microsomes from male and female dwarf rats, to test the hypothesis that the expression of these sexually dimorphic enzymes is regulated by GH. In mature rats, the levels of liver microsomal IIA2, IIC11, and IIIA2 were higher in male than in female dwarf rats, whereas the levels of activity of IIA1, IIC12, and steroid 5α-reductase were greater in female than in male dwarf rats. These sex differences resulted from age-related changes in either male dwarf rats (i.e., an increase in IIC11 and IIA2 and a decrease in IIA1) or female dwarf rats (i.e., an increase in IIC12 and 5α-reductase and a decrease in IIIA2). The magnitudes of these sex-dependent, age-related changes were essentially indistinguishable from those observed in normal rats. These unexpected results suggest that GH is not the pituitary factor responsible for regulating the levels of sexually dimorphic, steroid-metabolizing enzymes in rat liver. Alternatively, it is possible that these enzymes are regulated by extremely low levels of GH. In either case, the current model of how steroid-metabolizing enzymes are regulated in rats must be revised to account for the normal sexual differentiation of these enzymes in dwarf rats.**

Liver microsomes from male and female rats contain different amounts of steroid-metabolizing enzymes, such as Δ^4-3-ketosteroid 5α-reductase (steroid 5α-reductase) and several forms of cytochrome P450, including IIA1, IIA2, IIC11, IIC12, and IIIA2 (1–5).¶ These sex differences are evident in mature but not immature rats and are largely the result of a postpubertal expression or suppression of the genes encoding these enzymes. For example, the rate of steroid 5α-reduction and the 15β-hydroxylation of 5α-androstane-3α,17β-diol 3,17-disulfate increases after puberty in female but not male rats (6–9). The latter activity is catalyzed by IIC12, which is considered to be a female-specific P450 enzyme. In contrast, the levels of IIA2 and IIC11 increase postpubertally in male but not female rats, and both of these enzymes are considered male-specific enzymes (7–14). The postpubertal expression of IIC11 is associated with a male-specific, age-dependent increase in the oxidation of testosterone to 2α- and 16α-hydroxytestosterone and androstenedione. (3, 4). Sex differences in the adult levels of IIA1 (female > male) and IIIA2 (male > > female) result from a developmental suppression of these enzymes. After weaning and throughout puberty, the levels of IIA1 decline in male rats to a greater extent than in female rats, and this decline is associated with a decline in testosterone 7α-hydroxylase activity (7, 12–17). Conversely, the levels of IIIA2 decline markedly in female but not male rats, and this decline is associated with a dramatic postpubertal decrease in the rate of testosterone 2β-, 6β-, and 15β-hydroxylation (7, 16–18).

Differences in the pattern of somatotropin (growth hormone, GH) secretion in mature rats (i.e., "continuous" secretion in females versus "pulsatile" secretion in males) are thought to be the underlying cause of sex differences in liver microsomal cytochrome P450 and steroid 5α-reductase (1–3). Sex differences in liver steroid metabolism are abolished by hypophysectomy. Constant infusion of human GH to hypophysectomized rats, which mimics the relatively continuous secretion of GH in female rats (19), increases the levels of IIC12 and 5α-reductase, which causes a feminization of liver steroid metabolism (6, 20, 21). In contrast, periodic infusion of GH to hypophysectomized rats, which mimics the pulsatile pattern of GH secretion in male rats (19), increases the levels of IIC11 and causes a masculinization of liver steroid metabolism (10, 21, 22). Synthesis of IIC12 can also be induced in cultured primary hepatocytes from male rats by exposure to human or bovine GH (23).

Charlton *et al.* (24) have established a colony of GH-deficient (dwarf) rats. These dwarf rats (derived from an NIMR/AS strain) are homozygous for an autosomal recessive mutation that reduces pituitary GH levels to ≈5% of normal, which reduces circulating GH to barely detectable levels (<5 ng/ml) (24–27). Homozygous dwarf rats have normal or slightly elevated levels of other pituitary hormones, such as prolactin, thyrotropin, follitropin, lutropin, and corticotropin, indicating that dwarfism results from a stable, heritable, and selective defect in GH synthesis and storage (24, 27). We have examined the levels and/or activity of a subpopulation of P450 enzymes and steroid 5α-reductase in liver microsomes from homozygous dwarf rats with virtually undetectable circulating GH. Unexpectedly, our results demonstrate that steroid 5α-reductase and IIA1, IIA2, IIC11, IIC12, and IIIA2 are expressed in a sex- and age-dependent manner in dwarf rats and that the magnitude of the sex differences observed in mature dwarf rats is comparable to that observed in normal rats.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviation: GH, growth hormone.

§To whom reprint requests should be addressed.

¶In previous publications, we have used the nomenclature system of Ryan and Levin (4), in which P450a, -b, -e, -h, -i, -m, and -p correspond to IIA1, IIB1, IIB2, IIC11, IIC12, IIA2, and IIIA1. P450 IIIA2 is a constitutively expressed, developmentally regulated isozyme of IIIA1 (4, 5).

MATERIALS AND METHODS

Maintenance and Breeding of Dwarf Rats. The dwarf rats used in this study have been described in detail (24). Although the GH deficiency was first identified in Lewis rats, it was successfully introduced into NIMR/AS rats at Mill Hill and Oxford, England. Four females and two males from this latter strain of dwarf rats were obtained through Harlan–Sprague–Dawley and provided the base for our colony. The rats were allowed free access to Purina Rodent Chow (no. 5008) and water and were maintained at 23 ± 1°C with a 14-hr light/10-hr dark cycle. Female rats displayed normal 4-day estrous cycles. Pups were born on day 23 of pregnancy, and the average litter size was 7.5 ± 0.6 (n = 12). Rats were weaned when 25 days old.

Protein Purification and Antibody Production. P450 IIA1, IIA2, IIB1, IIB2, IIC11, and IIIA1 and NADPH–cytochrome P450 reductase were purified as described (4, 12, 28). Polyclonal antibodies against these enzymes were raised in rabbits and were subjected to immunoabsorption chromatography to remove antibodies that crossreacted with P450 enzymes in other gene families (29). The immunoabsorbed antibodies were not monospecific but recognized two or more distinct enzymes belonging to the same P450 gene subfamily (29). A mouse monoclonal antibody (H70) specific for IIC11 was prepared as described (30). Immunostaining of Western blots was quantified by scanning densitometry (Shimadzu CS-9000).

Steroid Oxidation and 5α-Reduction. The pathways of testosterone oxidation catalyzed by liver microsomes were determined by HPLC (12, 16). Conditions to measure the 5α-reduction of testosterone were similar to those for measuring testosterone oxidation except that liver microsomes were incubated at 23°C for 20 min with antibody against NADPH–cytochrome P450 reductase (5 mg of IgG per mg of microsomal protein) to inhibit cytochrome P450.

The 15β-hydroxylation of 5α-androstane-3α,17β-diol 3,17-disulfate was determined as described (9), except that unreacted substrate and product were resolved by HPLC. Substrate was kindly provided by Wayne Levin (Hoffmann–La Roche).

RESULTS

The mature dwarf rats used in this study weighed approximately half as much as normal rats. Charlton *et al.* (24) have shown that the irregular but frequent pulses of GH characteristic of normal female rats are abolished in female dwarf rats, and likewise the prominent 3-hourly pulses of GH typical of normal male rats are abolished in male dwarf rats. We confirmed that serum levels of GH were consistently low (2.9 ± 0.5 ng/ml) in dwarf rats, as were the pituitary levels of GH. Pituitary levels of GH in female (1.1–5.8 μg/mg) and male (5.6–15 μg/mg) dwarf rats were the same as those reported by Charlton *et al.* (24).

Liver microsomes were prepared from immature (3-week-old) and mature (3- to 4-month-old) female and male dwarf rats (n = 4 or 5 per group). Cytochrome P450 levels were similar to those previously reported for normal rats, whereas cytochrome b_5 levels were slightly higher than normal (Fig. 1; refs. 12, 16, 31, and 32). In contrast, NADPH–cytochrome c reductase activity was 10–50% less than that reported for normal rats (32). Liver microsomes from mature male dwarf rats catalyzed the fastest rate of cytochrome c reduction, and this reflected higher levels of NADPH–cytochrome P450 reductase (Fig. 2). Low levels of NADPH–cytochrome P450 reductase in liver microsomes from normal pre-weanling rats have been reported (17).

Thyroid Hormone Status of Dwarf Rats. The thyroid hormone status of dwarf rats was assessed because liver mi-

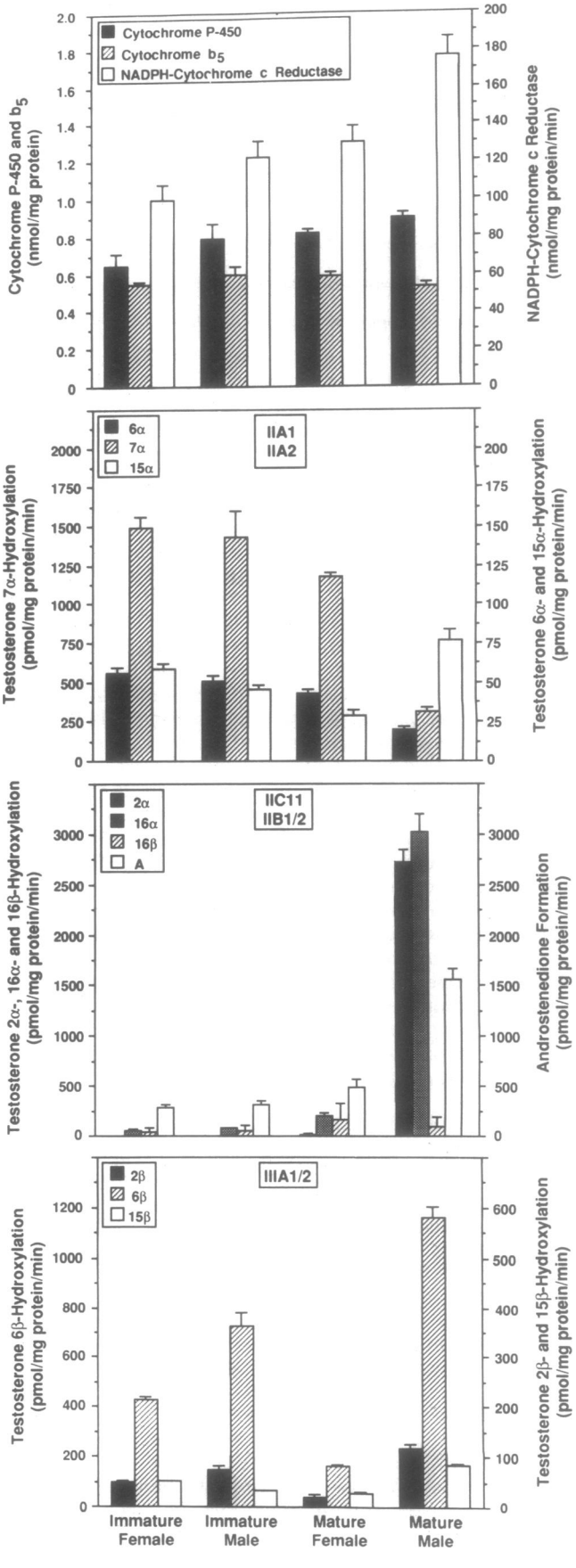
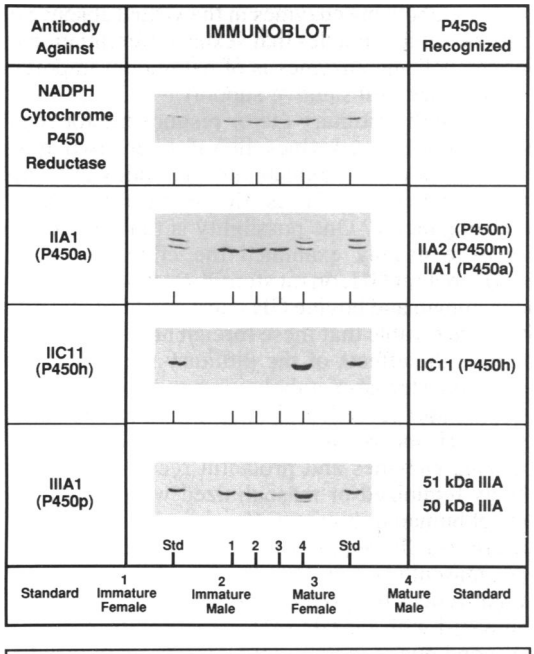


FIG. 1. Cytochrome P450 and b_5 levels, NADPH–cytochrome c reductase activity, and testosterone oxidation by liver microsomes from NIMR/AS dwarf rats. A, androstenedione formation. Values are mean ± SE.

crosomes from dwarf rats have slightly elevated levels of cytochrome b_5 and diminished levels of NADPH–cytochrome P450 reductase, which is a characteristic of hypothyroid rats (31). Serum thyroxine and triiodothyronine in dwarf rats were within normal ranges (Table 1). Previous studies have shown that dwarf rats have normal levels of serum or pituitary thyrotropin, follitropin, lutropin, corticotropin, prolactin, and testosterone (24, 27).

Testosterone Oxidation. In Fig. 1, the metabolites of testosterone generated by liver microsomes are arranged according to the catalytic activity of IIA1/2 (6α-, 7α-, and 15α-hydroxylation), IIC11/IIB1 (2α-, 16α-, and 16β-hydroxylation, and 17-oxidation to androstenedione), and IIIA1/2 (2β-, 6β-, and 15β-hydroxylation). However, the only reliable indicators of the catalytic activity of IIA1, IIC11, and IIB1 are testosterone 7α-, 2α-, and 16β-hydroxylation, respectively. All pathways of testosterone oxidation were stimulated 20–140% when liver microsomes (0.5 mg/ml) were incubated with purified NADPH–cytochrome P450 reductase (0.5 nmol/ml) (results not shown). Stimulation was observed regardless of the source of liver microsomes; hence, the qualitative aspects of the results shown in Fig. 1 were not influenced by the slightly lower-than-normal levels of NADPH–cytochrome P450 reductase in liver microsomes from dwarf rats.

Regulation of P450 IIC11 (P450h). In normal rats, IIC11 is present only in mature male rats, and its expression is thought to be dependent on a pulsatile pattern of GH secretion (1–3, 10, 20–22). We predicted that IIC11 would not be expressed



P-450 Levels (pmol/mg protein)	Immature		Mature	
	Female	Male	Female	Male
IIA1	70	60	60	20
IIA2	-	-	-	25
IIC11	-	-	-	360
IIIA1/2	100	130	10	160
Total P-450	650	790	820	900

FIG. 2. Immunoblots of liver microsomes (10 μg of protein) from NIMR/AS dwarf rats probed with antibodies against NADPH–cytochrome P450 reductase or various P450 enzymes. Lanes marked "Std" contained purified NADPH–cytochrome P450 reductase (0.1 pmol), IIA1 (0.25 pmol) and IIA2 (0.25 pmol), IIC11 (2 pmol), or IIIA1 (1 pmol).

Table 1. Serum levels of thyroid hormones in NIMR/AS dwarf rats and normal Holtzman rats

Rat strain and age status	Thyroxine, μg/dl		Triiodothyronine, ng/dl	
	Female	Male	Female	Male
Dwarf immature	3.3 ± 0.5	4.6 ± 0.7	92 ± 6	104 ± 12
Dwarf mature	3.4 ± 0.1	3.2 ± 0.6	97 ± 12	68 ± 10
Normal mature*	3.3 ± 0.7	5.3 ± 0.5	109 ± 15	100 ± 18

Values are mean ± SD (n = 3–7).
*For mature male Sprague–Dawley rats, normal levels of serum thyroxine and triiodothyronine are 3–7 μg/dl and 25–100 ng/dl, respectively (33).

in the GH-deficient dwarf rats. However, IIC11 was specifically expressed in adult male dwarf rats, just as it is in normal rats (Figs. 1 and 2). Testosterone 2α-hydroxylation, which is catalyzed specifically by IIC11, and IIC11 as shown by reactivity with monoclonal antibody H70 were essentially undetectable in liver microsomes from immature dwarf rats and from mature female dwarf rats. The levels and activity of IIC11 in liver microsomes from mature male dwarf rats were comparable to or slightly greater than values reported for normal mature male rats (7, 16, 17).

Regulation of P450 IIA1 (P450a). GH appears to exert opposing effects on IIA1 levels depending on its pattern of secretion. A pulsatile pattern is thought to suppress IIA1 levels, whereas a continuous pattern of GH secretion is thought to elevate IIA1 levels (34). Consequently, in normal rats, the levels of IIA1 are high in immature rats; they remain high in female rats but decline after weaning and throughout puberty in male rats (7, 12, 17, 34). We predicted that IIA1 would remain at pre-weaning levels in both male and female dwarf rats. However, IIA1 was suppressed in mature male dwarf rats, but not female dwarf rats, just as in normal rats (Fig. 2). Changes in the levels of IIA1 were paralleled by changes in the rate of formation of 6α- and 7α-hydroxytestosterone (Fig. 1). The levels and catalytic activity of IIA1 were comparable to values for liver microsomes from normal rats (13, 17).

Regulation of P450 IIA2 (P450m). In normal rats, both IIA2 and IIC11 are male-specific proteins, and their postpubertal expression is neonatally imprinted by testosterone (11). Despite these similarities, IIA2 and IIC11 are regulated quite differently. Expression of IIC11 is thought to be dependent on regular surges of GH secretion in male rats (1–3). In contrast, the factor that stimulates IIA2 synthesis after puberty has not been identified (11). However, GH is thought to antagonize this factor by suppressing the synthesis of IIA2, because the levels of IIA2 increase in both male and female rats following hypophysectomy (11). In female rats, the continuous pattern of GH secretion completely suppresses IIA2 synthesis. In male rats, the suppressive effect of GH is presumably relaxed during the periodic troughs in circulating GH levels. Based on this model of IIA2 regulation, we anticipated exceptionally high levels of IIA2 in mature dwarf rats of both sexes, because GH-deficient rats would be unable to suppress the postpubertal synthesis of IIA2. However, IIA2 was expressed only in mature male dwarf rats, just as it is in normal rats (Fig. 2). IIA2 was detected on immunoblots probed with antibody against IIA1 because IIA1 and IIA2 are 88% identical in amino acid sequence (14). The levels of immunoreactive IIA2 in liver microsomes from mature male dwarf rats were comparable to their normal counterparts (12). Testosterone 15α-hydroxylase activity was a poor index of IIA2 in liver microsomes from dwarf rats (Fig. 1), just as it is in normal rats (13).

Regulation of P450 IIIA2. In normal rats, the levels of IIIA2 are high in immature rats; they remain high in mature male rats but decline abruptly and markedly after puberty in

female rats (7, 17, 18, 35). GH is thought to suppress the levels of IIIA2, and a continuous pattern of GH secretion exerts a greater suppressive effect than a pulsatile pattern of secretion (possibly because the low trough levels provide periodic relief from GH suppression) (11, 18, 20, 36, 37). Based on this model of IIIA2 regulation, we anticipated high levels of IIIA2 in immature and mature dwarf rats of both sexes, because these GH-deficient rats would be unable to suppress the expression of IIIA2. However, a 51-kDa protein recognized by anti-IIIA1 was suppressed in mature female dwarf rats, just as it is in normal rats (Fig. 2). Based on reports by Gonzalez *et al.* (35) and Waxman *et al.* (37), this 51-kDa protein is most likely IIIA2. However, the antibody used recognizes at least two 51-kDa proteins (i.e., IIIA1 and IIIA2); hence, the identity of the 51-kDa IIIA protein in dwarf rats is not certain. The putative IIIA2 protein was not suppressed in mature male rats, which resulted in a marked sex difference (male > > female) in testosterone 2β-, 6β-, and 15β-hydroxylase activity (Fig. 1). Although the regulation of IIIA2 was qualitatively similar between normal and dwarf rats, the absolute levels and catalytic activity of IIIA2 in liver microsomes from dwarf rats were $\approx$50% of the corresponding values reported for normal rats (7, 17). The reason for this difference is unknown.

Antibody against IIIA1 recognizes a 50-kDa IIIA1 protein (28), which may be identical to $P450_{6\beta\text{-}2}$ purified by Nagata *et al.* (38). This 50-kDa protein was not expressed in preweanling dwarf rats and was expressed only in mature male dwarf rats (Fig. 2), which suggests that the 50-kDa IIIA protein is regulated in dwarf rats as it is in normal rats.

Regulation of P450 IIC12 (P450i) and Steroid 5α-Reductase. P450 IIC12, which catalyzes the 15β-hydroxylation of 5α-androstane-3α,17β-diol 3,17-disulfate (9), and 5α-reductase are considered female-specific enzymes (1–3). The high levels of these enzymes in liver microsomes from mature female rats are thought to be dependent on a continuous pattern of GH secretion (1–3, 6, 20, 23). Accordingly, we predicted that high levels of IIC12 and 5α-reductase would not be expressed in the GH-deficient dwarf rats. However, Fig. 3 indicates that high IIC12 activity and high 5α-reductase activity are expressed in adult female dwarf rats, just as they are in normal rats. In mature dwarf rats, the activities of IIC12 and 5α-reductase were at least 10 times greater in liver microsomes from female rats compared with those from male rats. The activities of IIC12 and 5α-reductase in liver microsomes from mature female dwarf rats were comparable to their normal counterparts.

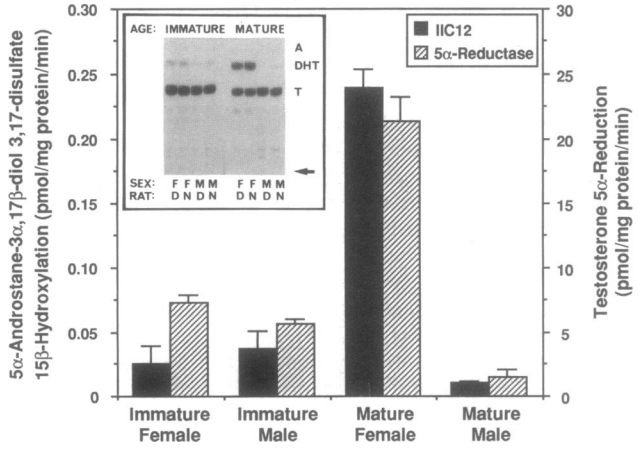


FIG. 3. Testosterone 5α-reduction and 5α-androstane-3,17-diol 3,17-disulfate 15β-hydroxylation by liver microsomes from NIMR/AS dwarf rats. Values are mean ± SE. (*Inset*) An autoradiogram of a TLC plate used to separate [^{14}C]testosterone (T) from 5α-dihydrotestosterone (DHT) and androstenedione (A). D, dwarf rats; N, normal Sprague–Dawley rats; F, female; M, male.

Regulation of IIB1 (P450b). The major phenobarbital-inducible P450 enzyme, IIB1, is not normally present in liver microsomes from untreated rats (4, 15). In mature rats, the synthesis of IIB1 is thought to be suppressed by GH, because the levels of IIB1 increase following hypophysectomy (39). Accordingly, we anticipated high constitutive levels of IIB1 in liver microsomes from GH-deficient dwarf rats. Liver microsomes from dwarf rats had barely detectable IIB1 (results not shown) and were no more active than their normal counterparts at catalyzing the 16β-hydroxylation of testosterone, which is a reliable indicator of IIB1 (Fig. 1).

DISCUSSION

The results show that GH-deficient dwarf rats display the same sex-specific expression of liver steroid-metabolizing enzymes as normal rats. These results were unexpected because sex-specific differences in the pattern of GH secretion are thought to be responsible for the sexual differentiation of liver steroid-metabolizing enzymes (1–3). There are three possible explanations for the normal sexual differentiation of steroid-metabolizing enzymes in dwarf rats: (*i*) contrary to current dogma, GH is not the feminizing/masculinizing hormone secreted by the pituitary; (*ii*) GH is the feminizing/masculinizing hormone secreted by the pituitary, but extremely low levels of circulating GH are sufficient to regulate the expression of liver steroid-metabolizing enzymes; (*iii*) our current theory of liver enzyme regulation by GH is correct, but a "backup" system ensures the normal sexual differentiation of steroid-metabolizing enzymes in the virtual absence of GH. This third possibility implies that sexual differentiation of liver steroid-metabolizing enzymes is of paramount importance, a concept that lacks substantive support.

If GH is not the pituitary factor responsible for regulating steroid-metabolizing enzymes in rat liver, two questions arise. First, how can we explain the large body of compelling evidence implicating GH as the bona fide feminizing/masculinizing factor? One possibility is that previous investigators have usually examined the effects of human or bovine GH, not rat GH, on rat steroid-metabolizing enzymes. Although human and bovine GH exert somatogenic effects in rats, it is conceivable that these foreign hormones also mimic or modulate the effects of the pituitary factor that actually regulates liver steroid-metabolizing enzymes. This possibility is not without precedent, because human GH mimics the effects of GH and prolactin in rats. Furthermore, steroid-metabolizing enzymes and prolactin receptor levels can be completely feminized or masculinized with relatively small amounts of human or bovine GH, whereas larger amounts of rat GH are usually required to achieve even partial feminization or masculinization (10, 20, 40). However, this difference might reflect the greater stability of human and bovine GH compared with rat GH (41).

The second question is: If not GH, what pituitary factor is ultimately responsible for regulating steroid-metabolizing enzymes in rat liver? Mode *et al.* (41) isolated the feminizing/masculinizing factor from rat pituitary and demonstrated its apparent identity with GH. However, the pituitary factor was not purified to homogeneity, and Mode *et al.* (41) recognized the possibility that a contaminant in the GH preparation might actually be the feminizing/masculinizing factor. An explanation that would account for many of these observations is that the feminizing/masculinizing factor is similar but not identical to GH. Rat pituitary appears to contain several electrophoretically distinct forms of GH with different growth-promoting and immunochemical properties (42).

Jeffrey *et al.* (43) reported that liver carbonic anhydrase III is sexually differentiated in dwarf rats (male > > female), just as it is in normal rats. The levels of carbonic anhydrase III

were masculinized by intermittent treatment of normal female rats with somatostatin, but not somatoliberin, both of which cause a pulsatile pattern of GH release. Interestingly, intermittent treatment of female dwarf rats with somatostatin, but not somatoliberin, also masculinized liver carbonic anhydrase III levels. Norstedt *et al.* (44) observed a partial feminization of liver steroid-metabolizing enzymes and prolactin receptors after decreasing somatostatin in male rats. Conversely, increasing somatostatin in female rats caused a partial masculinization of prolactin receptor levels (44). These results suggest that trough levels of GH, rather than peak levels, are important for masculinizing liver enzymes. However, another interpretation is that carbonic anhydrase III, steroid-metabolizing enzymes, and/or prolactin receptors are regulated by direct effects of somatostatin on the liver.

If GH is the pituitary hormone responsible for regulating steroid-metabolizing enzymes in rat liver, the unexpected results obtained with dwarf rats necessitate a revision of our current model. The dwarf rats used in this study are not absolutely deficient in GH, and although stunted they grow larger than hypophysectomized rats (24). It is possible, therefore, that steroid-metabolizing enzymes can be regulated by exceedingly low levels of GH, perhaps because these effects are mediated by high-affinity GH receptors in rat liver. The possibility that low levels of GH are sufficient to regulate liver steroid-metabolizing enzymes has been proposed previously. Waxman *et al.* (45) demonstrated that liver microsomal IIC12 and steroid 5α-reductase were expressed normally in mature female rats treated neonatally with monosodium glutamate at 4 g/kg, which abolished circulating GH levels (<2 ng/ml). Similar treatment of male rats abolished circulating GH and the postpubertal expression of liver microsomal IIC11, IIA2, and IIIA2, which supports a role for GH in the expression of these male-specific enzymes. However, Shapiro *et al.* (46) demonstrated that treatment of neonatal male rats with monosodium glutamate at only 2 g/kg did not abolish the expression of IIC11, IIA2, and IIIA2 in adult male rats, and this treatment diminished but did not abolish the pulsatile pattern of GH secretion. Neonatal treatment of rats with monosodium glutamate destroys 80–90% of the neurons in the arcuate nucleus of the hypothalamus, which disrupts several endocrine systems. In contrast, the NIMR/AS dwarf rats appear to have a selective defect in GH synthesis (24–27). Therefore, dwarf rats have the potential to provide a simple model to address the question of whether extremely low levels of GH can regulate liver steroid-metabolizing enzymes. The dwarf rats will also be invaluable in identifying the feminizing/masculinizing factor, in the event that GH is not the pituitary hormone responsible for regulating steroid-metabolizing enzymes in rat liver.

This work was supported by Grants ES03765 and GM37044 from the National Institutes of Health (NIH). B.G. was supported by NIH Training Grant ES07079, and A.P. is the recipient of NIH Research Career Development Award ES00166.

1. Zaphiropoulos, P. G., Mode, A., Norstedt, G. & Gustafsson, J.-A. (1990) *Trends Pharmacol. Sci.* **10,** 149–153.
2. Skett, P. (1988) *Pharmacol. Ther.* **38,** 269–304.
3. Waxman, D. J. (1988) *Biochem. Pharmacol.* **37,** 71–84.
4. Ryan, D. E. & Levin, W. (1989) *Pharmacol. Ther.* **45,** 153–239.
5. Gonzalez, F. J. (1989) *Pharmacol. Rev.* **40,** 243–288.
6. Zaphiropoulos, P. G., Mode, A., Strom, A., Moller, C., Fernandez, C. & Gustafsson, J.-A. (1988) *Proc. Natl. Acad. Sci. USA* **85,** 4214–4217.
7. Waxman, D. J., Dannan, G. A. & Guengerich, F. P. (1985) *Biochemistry* **24,** 4409–4417.
8. Kamataki, T., Maeda, K., Yamazoe, Y., Nagai, T. & Kato, R. (1983) *Arch. Biochem. Biophys.* **225,** 758–770.
9. Ryan, D. E., Dixon, R., Evans, R. H., Ramanathan, L., Thomas, P. E., Wood, A. W. & Levin, W. (1984) *Arch. Biochem. Biophys.* **233,** 636–642.
10. Morgan, E. T., MacGeoch, C. & Gustafsson, J.-A. (1985) *J. Biol. Chem.* **260,** 11895–11898.
11. Waxman, D. J., LeBlanc, G. A., Morrissey, J. L., Staunton, J. & Lapenson, D. P. (1988) *J. Biol. Chem.* **263,** 11396–11406.
12. Arlotto, M. P., Greenway, D. J. & Parkinson, A. (1989) *Arch. Biochem. Biophys.* **270,** 441–457.
13. Arlotto, M. P. & Parkinson, A. (1989) *Arch. Biochem. Biophys.* **270,** 458–471.
14. Matsunaga, T., Nagata, K., Holsztynska, E. J., Lapenson, D. P., Smith, A., Kato, R., Gelboin, H. V., Waxman, D. J. & Gonzalez, F. J. (1988) *J. Biol. Chem.* **263,** 17995–18002.
15. Thomas, P. T., Reik, L. M., Ryan, D. E. & Levin, W. (1981) *J. Biol. Chem.* **256,** 1044–1052.
16. Sonderfan, A. J., Arlotto, M. P., Dutton, D. R., McMillen, S. K. & Parkinson, A. (1987) *Arch. Biochem. Biophys.* **255,** 27–41.
17. Eberhart, D. C. & Parkinson, A. (1990) *Toxicologist* **10,** 123 (abstr.).
18. Yamazoe, Y., Murayama, N., Shimada, M., Yamauchi, K., Nagata, K., Imaoka, S., Funae, Y. & Kato, R. (1988) *J. Biochem. (Tokyo)* **104,** 785–790.
19. Jansson, J.-O., Eden, S. & Isaksson, O. (1985) *Endocr. Rev.* **6,** 128–150.
20. Mode, A., Norstedt, G., Simic, B., Eneroth, P. & Gustafsson, J.-A. (1981) *Endocrinology* **108,** 2103–2108.
21. Mode, A., Wiersma-Larsson, E., Strom, A., Zaphiropoulos, P. G. & Gustafsson, J.-A. (1989) *J. Endocrinol.* **120,** 311–317.
22. Yamazoe, Y., Shimada, M., Kamataki, T. & Kato, R. (1986) *Jpn. J. Pharmacol.* **42,** 371–382.
23. Guzelian, P. S., Li, D., Schuetz, E. G., Thomas, P., Levin, W., Mode, A. & Gustafsson, J.-A. (1988) *Proc. Natl. Acad. Sci. USA* **85,** 9783–9787.
24. Charlton, H. M., Clark, R. G., Robinson, I. C. A. F., Porter Goff, A. E., Cox, B. S., Bugnon, C. & Bloch, B. A. (1988) *J. Endocrinol.* **119,** 51–58.
25. Skottner, A., Clark, R. G., Fryklund, L. & Robinson, I. C. A. F. (1989) *Endocrinology* **124,** 2519–2526.
26. Carmignac, D. F. & Robinson, I. C. A. F. (1990) *J. Endocrinol.* **127,** 69–75.
27. Bartlett, J. M., Charlton, H. M., Robinson, I. C. A. F. & Nieschlag, E. (1990) *J. Endocrinol.* **126,** 193–201.
28. Halvorson, M., Greenway, D., Eberhart, D., Fitzgerald, K. & Parkinson, A. (1990) *Arch. Biochem. Biophys.* **277,** 166–180.
29. Gemzik, B. & Parkinson, A. (1991) *Methods Enzymol.*, in press.
30. Reik, L. M., Maines, S. L., Ryan, D. E., Levin, W., Bandiera, S. & Thomas, P. E. (1987) *J. Immunol. Methods* **100,** 123–130.
31. Rozman, K., Hazelton, G., Klaassen, C. D., Arlotto, M. & Parkinson, A. (1985) *Toxicology* **37,** 51–63.
32. Gorski, J. R., Arlotto, M. P., Klaassen, C. D. & Parkinson, A. (1985) *Carcinogenesis* **6,** 617–624.
33. Depaolo, L. V. & Masoro, E. J. (1989) in *The Clinical Chemistry of Laboratory Animals*, eds. Loeb, W. F. & Quimby, F. W. (Pergamon, New York), pp. 279–308.
34. Waxman, D. J., Morrissey, J. J. & LeBlanc, G. A. (1989) *Endocrinology* **124,** 2954–2966.
35. Gonzalez, F. J., Song, B. J. & Hardwick, J. P. (1986) *Mol. Cell. Biol.* **6,** 2969–2976.
36. Waxman, D. J., Morrissey, J. J. & LeBlanc, G. A. (1989) *Mol. Pharmacol.* **35,** 519–525.
37. Waxman, D. J., Ram, P. A., Notani, G., LeBlanc, G. A., Alberta, J. A., Morrissey, J. J. & Sundseth, S. S. (1990) *Mol. Endocrinol.* **4,** 447–454.
38. Nagata, K., Gonzalez, F. J., Yamazoe, Y. & Kato, R. (1990) *J. Biochem. (Tokyo)* **107,** 718–725.
39. Yamazoe, Y., Shimada, M., Murayama, N. & Kato, R. (1987) *J. Biol. Chem.* **262,** 7423–7428.
40. Norstedt, G., Mode, A., Eneroth, P. & Gustafsson, J.-A. (1981) *Endocrinology* **108,** 1855–1861.
41. Mode, A., Norstedt, G., Eneroth, P. & Gustafsson, J.-A. (1983) *Endocrinology* **113,** 1250–1260.
42. Russell, S. M., Vodian, M. A., Hughes, J. P. & Nicoll, C. S. (1978) *Life Sci.* **23,** 2373–2382.
43. Jeffrey, S., Carter, N. D., Clark, R. G. & Robinson, I. C. A. F. (1990) *Biochem. J.* **266,** 69–74.
44. Norstedt, G., Mode, A., Hokfelt, T., Eneroth, P., Elde, R., Ferland, L., Labrie, F. & Gustafsson, J.-A. (1983) *Endocrinology* **112,** 1076–1090.
45. Waxman, D. J., Morrissey, J. J., MacLeod, J. N. & Shapiro, B. H. (1990) *Endocrinology* **126,** 712–720.
46. Shapiro, B. H., MacLeod, J. N., Pampori, N. A., Morrissey, J. J., Lapenson, D. P. & Waxman, D. J. (1989) *Endocrinology* **125,** 2935–2944.