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Feminization of Hepatic Cytochrome P450s by Nominal Levels of Growth Hormone in the Feminine Plasma Profile

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SUMMARY

The feminine profile of continuous growth hormone secretion was restored at various concentrations to hypophysectomized, thyroxine-supplemented female rats to determine the minimum signaling concentrations of the hormone required to maintain female-like expression levels of gender-dependent hepatic cytochrome P450s (P450s). Rat growth hormone was infused by intraperitoneally implanted osmotic minipumps, and the resulting circulating concentrations and profiles were determined by radioimmunoassay of serially collected plasma samples. Restoration of feminine growth hormone profiles at 3% of physiological concentration completely suppressed male-specific CYP2C11, CYP2C13, CYP2A2, and CYP3A2. Although significant levels of female-dependent isoforms were expressed at this growth hormone concentration, their full expression required somewhat higher plasma concentrations of the hormone; CYP2A1 and 5α-reductase were increased to normal female levels with only 6–12% of physiological concentrations of the hormone, normal expression levels of CYP2C12 required ~12-25% physiological hormone levels, and CYP2C7 required ~25-50% of the normal growth hormone profile to attain female-like expression levels. When determined, protein and specific catalytic activities were in agreement with mRNA levels, supporting the conclusion that growth hormone regulates gender-dependent expression of P450 isoforms by transcription initiation. There was little effect of gender, hypophysectomy, or growth hormone replacement on CYP2C6, growth hormone receptor, and growth hormone-binding protein mRNAs. In contrast, insulin-like growth factor-1 mRNA was sexually dimorphic (male > female), virtually disappeared after hypophysectomy, and was restored to female-like levels with plasma growth hormone concentrations equaling 12–25% of normal. These findings demonstrate the effectiveness of nominal growth hormone concentrations (undetectable by available radioimmunoassay) in an otherwise feminine plasma profile to maintain female-like expression levels of gender-dependent P450s.

Gender differences in hepatic drug metabolism occur in numerous species, including fishes, birds, and mammals. From the few species in which studies have been extended to the molecular level, it seems that sexual dimorphisms in drug metabolism are due to the existence of multiple forms of hepatic P450s whose gender-dependent expression is regulated by growth hormone (1). Rat liver, which has received the preponderance of investigational attention, is known to contain at least a dozen sex-dependent isoforms of P450 that are regulated by the gender-dependent profiles of circulating growth hormone (2–4). Male rats secrete growth hormone in episodic bursts (~200-300 ng/ml of plasma) every 3.5–4 hr. Between the peaks, growth hormone levels are undetectable. In female rats, the hormone pulses are more frequent and irregular and are of lower magnitude than those in males, whereas the interpulse concentrations of growth hormone are always measurable (1–3).

In the rat, P450 responses to growth hormone regulation are approximately as variable as the number of growth hormone-dependent isoforms. That is, expression of the major female-specific CYP2C12 (as well as the non-P450 5α-reductase) is dependent on the feminine profile of continuous growth hormone secretion. Exposure to the masculine profile of episodic hormone release, as well as the absence of the hormone from the circulation (e.g., hypophysectomy), results in the complete suppression of CYP2C12 (5–8). In a somewhat similar vein, female-predominant CYP2C7 expression is also dependent on the feminine growth hormone profile and is completely suppressed in the hypophysectomized rat. However, exposure to the masculine profile allows expression of CYP2C7 at 25–40% normal female levels (2, 7, 9, 10). Expression of the major male-specific CYP2C11 requires the episodic "on/off" masculine profile of growth hormone secretion. Although the feminine pattern of continuous hormone secretion blocks CYP2C11 expression, total growth hormone depletion from the circulation allows CYP2C11 expression at 15–25% of intact male levels (2, 8, 11, 12). After hypophysec-

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ABBREVIATIONS: P450, cytochrome P450; GHR, growth hormone receptor; GHBP, growth hormone-binding protein; IGF-1, insulin-like growth factor-1; rGH, rat growth hormone; MSG, monosodium glutamate.

tomy, female-predominant CYP2A1 (male/female, ~1:3) concentrations decline but remain above male levels and are restored to intact female-like levels with continuously administered growth hormone (13, 14). Although the expression levels of CYP2C7, CYP2C11, CYP2C12, and CYP2A1 are greatest when exposed to their gender-dependent growth hormone profiles, other isoforms are optimally expressed in the absence of growth hormone. Male-specific CYP2A2 and CYP3A2 are maximally expressed in the hypophysectomized rat, disappear when growth hormone is secreted constantly, but are only partially suppressed, relative to the high levels observed in hypophysectomized rats, under the influence of episodic growth hormone (13, 15, 16). Male-specific CYP2C13 is optimally expressed when exposed to the masculine hormone profile or under conditions of no growth hormone, whereas the feminine growth hormone profile completely suppresses CYP2C13 (2, 8). Although there are additional examples, it becomes clear that the expression or suppression of each isoform of P450 is likely to be regulated by a different "signal" in the sexually dimorphic growth hormone profile. These signals may be recognized by the hepatocyte in the frequencies and/or durations of the pulse and interpulse periods. Alternatively, perhaps the hepatocyte can monitor the mean plasma concentration of the hormone. In the latter case, we have reported that a 70–85% reduction in the feminine growth hormone profile has little, if any, effect on the levels of gender-dependent P450 isoforms normally expressed in the female liver (17, 18). That is, isoforms dependent on the "continuous" feminine profile (e.g., CYP2C12, CYP2A1, CYP2E1) were expressed at normal or above-normal female-like levels, whereas male-specific isoforms (e.g., CYP2C11, CYP2A2, CYP3A2) usually suppressed by the continuous growth hormone profile remained suppressed by the subnormal concentrations of growth hormone.

In the current study, we restored, at various concentrations, the feminine pattern of continuous growth hormone secretion to hypophysectomized, thyroxine-supplemented female rats to identify the minimal level or levels of circulating growth hormone needed to feminize expression of various male- and female-dependent isoforms of P450 as well as GHR, GHBP, and IGF-1 mRNAs.

Materials and Methods

Animals. Animals were housed in the University of Pennsylvania Laboratory Animal Resources facility under the supervision of certified laboratory animal medicine veterinarians and were treated according to a research protocol approved by the university's Institutional Animal Care and Use Committee. Female rats [Crl:CD (SD)BR] were hypophysectomized by the vendor (Charles River Laboratories, Wilmington, MA) at 8 weeks of age and were observed in our facilities for 5 weeks. The effectiveness of the surgery was verified by the lack of weight gain over this period and the absence of pituitaries or fragments at necropsy at the end of the study (i.e., 102–107 days old).[1] Hormone replacement experiments with rGH (1.8 IU/mg) via intraperitoneally implanted osmotic pumps (Alza, Palo Alto, CA), were started when the rats were 13–14 weeks old and continued for 6 days (19). Concurrently, all hypophysectomized animals received thyroxine continuously via separate subcutaneously implanted osmotic pumps at a dosage (0.8 μg/hr/kg of body weight) that produced the euthyroidism (20) required for maintaining normal concentrations of NADPH-cytochrome P450 reductase, a microsomal enzyme requisite for the expression of P450 catalytic activity (21). At the time of necropsy, the pumps were removed and found to contain the expected residual amounts of growth hormone and thyroxine.

Repetitive blood samples (10 μl) were obtained at 15-min intervals from unrestrained, unstressed, and completely conscious rats outfitted with our mobile catheterization apparatus (19, 22). Six-hour plasma growth hormone profiles were determined by using a radioimmunoassay with a sensitivity of 2–3 ng/ml. Procedural details and statistical validation of the assay have been reported previously (23).

RNA analysis. Total hepatic RNA was isolated by using a single-step guanidinium thiocyanate method (24). Ten micrograms of RNA was electrophoresed under formaldehyde-denaturing conditions on 1% agarose and transferred to GeneScreen nylon membranes (DuPont-New England Nuclear, Boston, MA). The Northern blots were probed and reprobed with either ^{32}P-labeled oligonucleotide probes or CYP2C11/cDNA (25) probes, using hybridization and high stringency washing conditions as described previously (26). The nucleotide sequence of oligonucleotide probes for CYP2A1, CYP2A2, CYP2C6, CYP2C7, CYP2C12, CYP2C13 (26), CYP3A2 (27), and steroid 5α-reductase (7) have been reported. We used antisense oligonucleotide sequence 5′-CTC-AGC-ATC-TGG-AGC-GGT-ATC-TGC-3′ to identify GHR mRNA. This probe is complementary to the cDNA nucleotides 1934–1957 bp of GHR (GenBank Accession No. J04811) (28) and does not recognize GHBP mRNA. To identify GHBP, we used antisense sequence 5′-GTT-GTC-AAT-CTC-TTG-ATG-TGG-GTG-CTG-3′ complementary to the splice variant cDNA nucleotides 995-1021 bp (GenBank Accession No. S49003) encoding the GHBP hydrophilic tail (28), which does not recognize GHR mRNA (29). IGF-1 mRNA was detected using rat antisense oligonucleotide probe 5′-ATA-GCC-TGT-GGG-CTT-GTT-GAA-GTA-AAA-GCC-3′ complementary to the 22 to 31 amino acid residues from B and C domains, respectively (30). The consistency of RNA loadings between samples was confirmed by ethidium bromide staining of 18*S* and 28*S* ribosomal RNAs and was verified using an 18*S* oligonucleotide probe (31). The hybridized mRNA signals were quantified by scanning the autoradiographs and normalized to the 18*S* rRNA signals in each lane.

Western blots. Hepatic microsomes were prepared from individual rat livers (32) and then assayed for individual P450s by Western blotting and/or by measurement of their selective catalytic activities (26, 33). Briefly, 10 μg of microsomal protein was electrophoresed on 0.75-mm-thick sodium dodecyl sulfate-polyacrylamide (7.5%) gels and electroblotted onto nitrocellulose filters. The blots were probed with monoclonal anti-rat CYP2C11 (Oxford Biomedical Research, Oxford, MI) and anti-rat CYP2C12/13 (kindly provided by Dr. Marika Rönnholm, Huddinge University Hospital, Huddinge, Sweden) mouse IgG, polyclonal anti-rat CYP2C7 (kindly provided by Dr. Stelvio M. Bandiera, The University of British Columbia, Canada), and anti-rat CYP3A1/2 (Human Biologics, Phoenix, AZ) rabbit IgG and detected with an enhanced chemiluminescence kit (Amersham, Arlington Heights, IL) (34).

Testosterone metabolism. Testosterone 2α- and 6β-hydroxylases, reflective of the activity levels of CYP2C11 and CYP3A2 proteins, respectively, and female-specific testosterone 5α-reductase were assayed according to our methods as described previously (35).

Results

Growth hormone replacement. Growth hormone administration at the rate of 20 μg of rGH/hr/kg of body weight produced mean circulating concentrations (36.6 ± 6.8 ng/ml, mean ± standard deviation) in hypophysectomized female

[1] Despite the absence of detectable pituitary tissue examined by dissecting scope at necropsy, 30–40% of the "hypophysectomized" females exhibited inappropriate body weight gain at 2–3 weeks after surgery, necessitating their exclusion from the study. In contrast, we found that <15% of male rats show any body weight gain after hypophysectomy (personal observations).

rats that were statistically no different from those observed in intact female rats (39.8 ± 15.2 ng/ml) (Fig. 1). There was a proportional relationship between the dose of growth hormone replacement by intraperitoneally implanted osmotic minipumps and the resulting mean plasma concentration of rGH. That is, when the dose of rGH administration was reduced from 20 μg to 10 μg of rGH/hr/kg of body weight, the mean plasma concentration declined to ~50% of normal (17.0 ± 3.3 ng/ml). Another reduction in growth hormone replacement to 5 μg of rGH/hr/kg of body weight produced circulating hormone levels that were ~25% of normal (8.9 ± 1.8 ng/ml). A further 50% reduction in the rate of growth hormone administration to 2.5 μg of rGH/hr/kg of body weight resulted in a decline of plasma rGH to ~12% of normal (4.2 ± 0.5 ng/ml). Growth hormone replacement at rates of 1.25 and 0.625 μg of rGH/hr/kg of body weight produced circulating growth hormone concentrations that were below the statistical sensitivity of the assay. However, because the higher rates of growth hormone replacement resulted in proportional and predictable plasma hormone levels, we thought it reasonable to extrapolate (by linear regression) circulating rGH concentrations for the hypophysectomized females with osmotic pumps secreting 1.25 and 0.625 μg of rGH/hr/kg of body weight to ~6% (2.0 ± 0.4 ng/ml) and ~3% (1.0 ± 0.2 ng/ml) of normal circulating concentrations, respectively (Fig. 1).

Hepatic CYP2C12. The female specificity of CYP2C12 was illustrated by its expression in intact female liver and its absence in male liver (Fig. 2). Furthermore, with the disappearance of the feminine pattern of continuous growth hormone secretion in the hypophysectomized female rat, expression of the isoform was no longer detectable. Restoration of only 3% of the levels characteristic of the feminine profile of growth hormone secretion was capable of restoring CYP2C12 expression (i.e., mRNA and protein) to ~40% of normal. When plasma growth hormone concentrations were increased to ~6% of normal, expression levels of CYP2C12 were elevated by an additional 30%. Restoration of the continuous growth hormone secretory profiles to 12–25% of female-like levels was sufficient to fully restore expression levels of CYP2C12 mRNA and protein.

Hepatic 5α-reductase. Although not a P450 enzyme, hepatic gender- and growth hormone-dependent expressions of 5α-reductase are comparable to those of CYP2C12 (17, 26). This similarity was illustrated by an almost lack of expression of 5α-reductase mRNA and dependent testosterone 5α-reductase activity in liver from intact male and hypophysectomized female rats (Fig. 3). Exhibiting an even greater hormone response than CYP2C12, 5α-reductase mRNA and its catalytic activity were restored to 75% and 60% of normal, respectively, by only 3% of the mean concentration of female-like circulating growth hormone profiles. An increase in

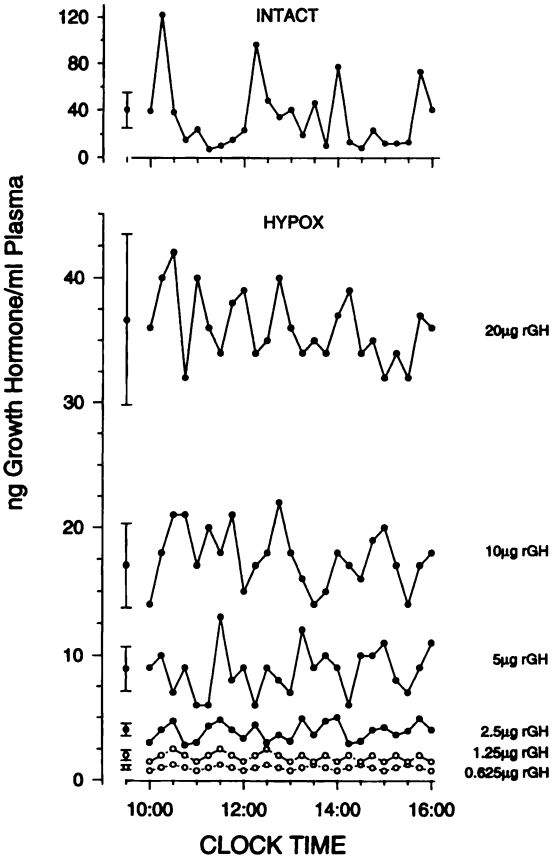

Fig. 1. Plasma levels of circulating rGH obtained from individual undisturbed catheterized intact and hypophysectomized (*HYPOX*) rGH-replaced female rats at 15-min intervals for 6 consecutive hours. Hypophysectomized rats were implanted intraperitoneally with osmotic minipumps set to continuously deliver rGH at the rates (μg of rGH/hr/kg of body weight) indicated (*right*). Depicted next to each infusion rate are the resulting circulating profiles and calculated plasma rGH mean concentrations (*single point* with ± standard deviation *error bar*) normalized by subtracting plasma values obtained from hypophysectomized rats. ●, Values determined by radioimmunoassay; ○, below the sensitivity of the radioimmunoassay; estimates extrapolated from linear regression analysis of the measurable values. Similar findings were obtained from three to four additional animals in each treatment group.

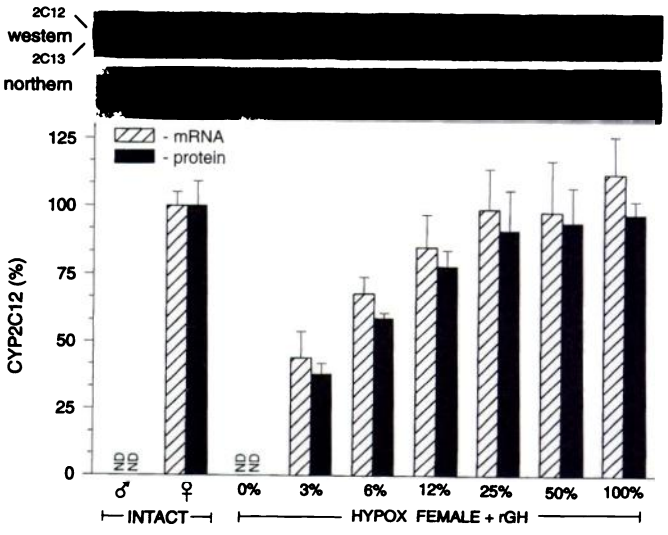

Fig. 2. Relative hepatic CYP2C12 mRNA and protein levels in intact male (♂) and female (♀) rats and hypophysectomized (*HYPOX*) rGH-replaced female rats. The levels of rGH replacement by continuous infusion are presented as a percentage of the normal feminine plasma growth hormone profile illustrated in Fig. 1 and determined in Results. *Top*, Western blot analysis of two livers per treatment group with antibody to CYP2C12 protein and cross-reactive with CYP2C13. *Middle*, Northern blot analysis of two livers per treatment group with a ^{32}P-labeled oligonucleotide probe specific for CYP2C12 mRNA. *Bottom*, relative CYP2C12 mRNA and protein levels determined by laser densitometry of actual Northern radiographs and Western enhanced chemiluminescence radiographs of at least five different livers for each treatment group (mean ± standard deviation). *ND*, not detected.

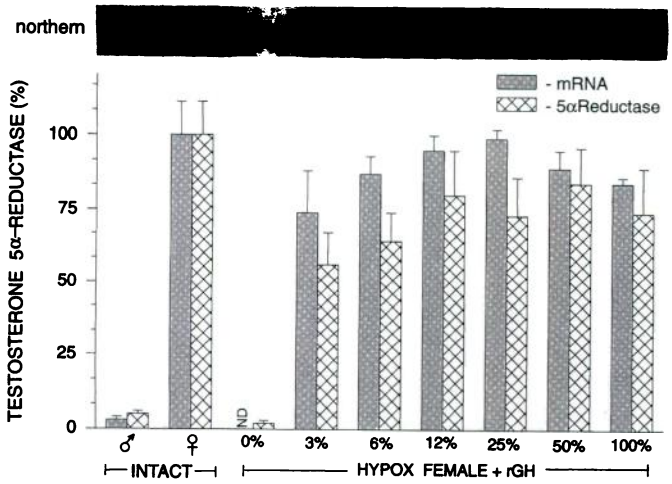

Fig. 3. Relative hepatic testosterone 5α-reductase mRNA and catalytic activity levels in intact male (♂) and female (♀) rats and hypophysectomized (*HYPOX*) rGH-replaced female rats. Levels of rGH replacement by continuous infusion are presented as a percentage of the normal feminine plasma growth hormone profile illustrated in Fig. 1 and determined in Results. *Top*, Northern blot analysis of two livers per treatment group with a ^{32}P-labeled oligonucleotide probe specific for 5α-reductase mRNA. *Bottom*, relative 5α-reductase mRNA levels determined by laser densitometry of the actual Northern radiographs and the microsomal testosterone 5α-reductase activity of at least five different livers for each treatment group (mean ± standard deviation). *ND*, not detected.

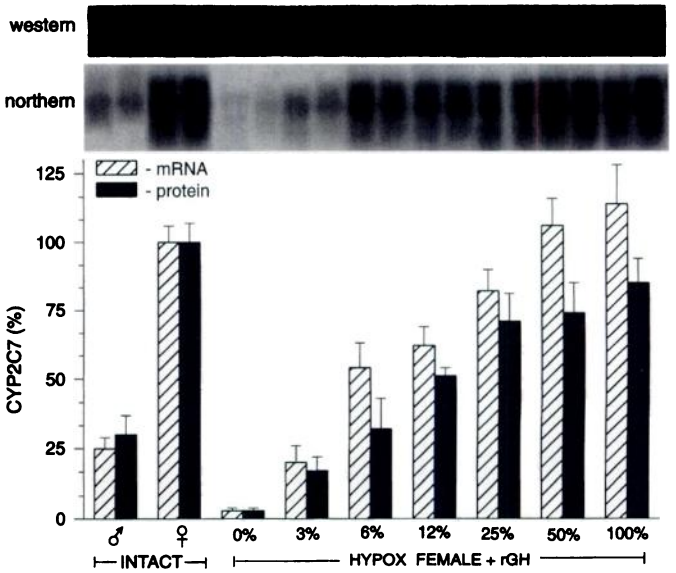

Fig. 4. Relative hepatic CYP2C7 mRNA and protein levels in intact male (♂) and female (♀) rats and hypophysectomized (*HYPOX*) rGH-replaced female rats. The levels of rGH replacement by continuous infusion are presented as a percentage of the normal feminine plasma growth hormone profile illustrated in Fig. 1 and determined in Results. *Top*, Western blot analysis of two livers per treatment group with antibody to CYP2C7 protein. *Middle*, Northern blot analysis of two livers per treatment group with a ^{32}P-labeled oligonucleotide probe specific for CYP2C7 mRNA. *Bottom*, relative CYP2C7 mRNA and protein levels determined by laser densitometry of actual Northern radiographs and Western enhanced chemiluminescence radiographs of at least five different livers for each treatment group (mean ± standard deviation).

plasma growth hormone levels to ~6% of normal further elevated 5α-reductase expression, and 12% of normal growth hormone concentrations seemed to fully restore hepatic expression of the reductase.

Hepatic CYP2C7. In contrast to female-specific CYP2C12, CYP2C7 is more appropriately designated as female predominant because the isoform is also expressed in male liver, albeit at significantly lower concentrations than in female liver (2, 7, 9, 10). In this regard, we found that males expressed CYP2C7 at ~25% of female levels (Fig. 4). Hypophysectomy reduced CYP2C7 expression in female livers to barely detectable levels. In comparison to female-specific CYP2C12 and 5α-reductase, CYP2C7 seemed to be less sensitive to growth hormone regulation. Restoration of 3% of the growth hormone levels characteristic of the feminine plasma profile increased hepatic CYP2C7 mRNA and protein to 20% of normal, which was below the expression levels found in intact males. Although increases in the circulating concentrations of rGH produced commensurate increases in CYP2C7 mRNA and protein, it was not until hormone levels approached 50% of normal that CYP2C7 mRNA was expressed at 100% of intact levels. Protein concentrations, however, seemed to lag behind transcript levels. With the possible exception of females treated with physiological (i.e., 100%) concentrations of growth hormone, CYP2C7 protein levels never seemed to quite reach prehypophysectomy levels during the 7-day treatment period.

Hepatic CYP2C11. Like its female counterpart, CYP2C12, CYP2C11 is considered a male-specific isoform because its expression is limited to males (2, 8, 11, 12). We measured hepatic CYP2C11 expression at the mRNA, protein, and specific catalytic levels (i.e., CYP2C11-dependent testosterone 2α-hydroxylase) and found virtually no expression in intact female rats (Fig. 5). Expression levels in hypophysectomized rats were ~25% of the intact male level. Administration of the feminine profile of continuous growth hormone secretion at 3% of the normal concentrations completely blocked CYP2C11 expression. All dosages of osmotic pump-delivered growth hormone were as equally effective as the lowest dosage, 0.625 μg of rGH/hr/kg of body weight.

Hepatic CYP3A2. CYP3A2 is a male-specific isoform whose expression (mRNA, protein, and specific testosterone 6β-hydroxylase activity) were nearly undetectable in intact female liver (Fig. 6). Although the complete elimination of circulating growth hormone by hypophysectomy resulted in an overexpression of CYP3A2, restorations of the feminine secretory growth hormone profile at 3%, 6%, 12%, 25%, 50%, and 100% of normal were equally and completely effective in suppressing CYP3A2 expression.[2]

Other gender-dependent hepatic P450 isoforms. CYP2A2 and CYP2C13 are male-specific isoforms (8, 15, 16), with transcripts that were undetectable in intact female liver but expressed at normal or above-normal male-like levels in livers of hypophysectomized female rats (Fig. 7). Restoration of the feminine plasma growth hormone profile at all administered concentrations, including 3% of normal, completely

[2] The presence of minimal, although apparently measurable, concentrations of CYP3A2 protein in the absence of CYP3A2 mRNA can be accounted for by the fact that neither sodium dodecyl sulfate-polyacrylamide gel electrophoresis nor our antibody can resolve CYP3A1 from CYP3A2 proteins (36). Because CYP3A1 is basically an inducible, growth hormone-independent isoform (4, 36), it is possible that in the absence of CYP3A2, we observed the constitutive expression of trace amounts of CYP3A1 on our Western blots (Fig. 6). [The occasional occurrence of a small band below CYP3A2/1 has been reported to be a cross-reactive, unidentified 50-kDa protein (36).]

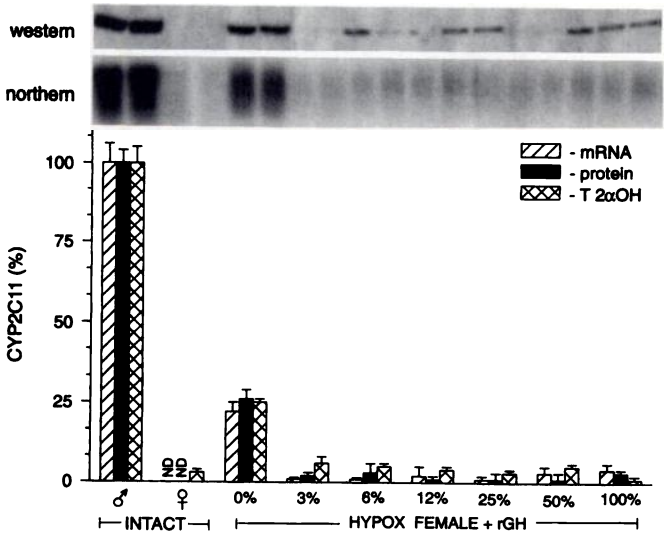

Fig. 5. Relative hepatic CYP2C11 mRNA, protein, and catalytic activity levels in intact male (♂) and female (♀) rats and hypophysectomized (*HYPOX*) rGH-replaced female rats. The levels of rGH replacement by continuous infusion are presented as a percentage of the normal feminine plasma growth hormone profile illustrated in Fig. 1 and determined in Results. *Top*, Western blot analysis of two livers per treatment group with antibody to CYP2C11 protein. *Middle*, Northern blot analysis of two livers per treatment group with a ^{32}P-labeled CYP2C11-cDNA probe specific for CYP2C11 mRNA. *Bottom*, relative CYP2C11 mRNA and protein levels determined by laser densitometry of actual Northern radiographs and Western enhanced chemiluminescence radiographs and microsomal CYP2C11-dependent testosterone 2α-hydroxylase (*T 2αOH*) levels of at least five different livers for each treatment group (mean ± standard deviation). *ND*, not detected.

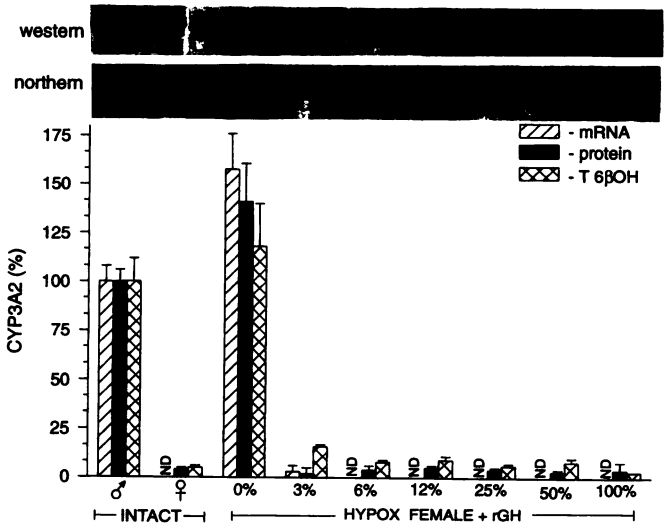

Fig. 6. Relative hepatic CYP3A2 mRNA, protein, and catalytic activity levels in intact male (♂) and female (♀) rats and hypophysectomized (*HYPOX*) rGH-replaced female rats. The levels of rGH replacement by continuous infusion are presented as a percentage of the normal feminine plasma growth hormone profile illustrated in Fig. 1 and determined in Results. *Top*, Western blot analysis of two livers per treatment group with antibody to CYP3A2 protein and cross-reacting with CYP3A1 protein. *Middle*, Northern blot analysis of two livers per treatment group with a ^{32}P-labeled oligonucleotide probe specific for CYP3A2 mRNA. *Bottom*, relative CYP3A2 mRNA and protein levels determined by laser densitometry of actual Northern radiographs and Western enhanced chemiluminescence radiographs and microsomal CYP3A2-dependent testosterone 6β-hydroxylase (*T 6βOH*) levels of at least five different livers for each treatment group (mean ± standard deviation). *ND*, not detected.

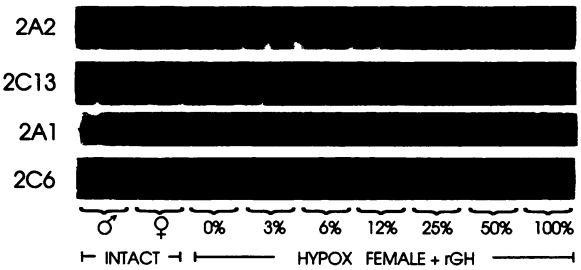

Fig. 7. Relative hepatic CYP2A2, CYP2C13, CYP2A1, and CYP2C6 mRNA levels in intact male (♂) and female (♀) rats and hypophysectomized (*HYPOX*) rGH-replaced female rats. The levels of rGH replacement by continuous infusion are presented as a percentage of the normal feminine plasma growth hormone profile illustrated in Fig. 1 and determined in Results. Northern blot analyses of two livers per treatment group with a ^{32}P-labeled oligonucleotide probe specific for each mRNA. At least five different livers were analyzed for each treatment group.

suppressed expression of CYP2A2 and CYP2C13 mRNA. CYP2C13 protein levels (Fig. 2) and CYP2A2-dependent microsomal testosterone 15α-hydroxylase activity (not presented) were in agreement with the mRNA findings.

CYP2A1 is a female-predominant isoform whose mRNA levels were several-fold higher in liver from intact females than in liver from intact males (Fig. 7). Hypophysectomy reduced CYP2A1 mRNA in female rat liver to concentrations that were intermediate between those of intact males and intact females. Restoration from 3–6% of the normal feminine plasma growth hormone concentrations seemed to fully restore female-like expression levels of CYP2A1 mRNA. CYP2A1-dependent testosterone 7α-hydroxylase activities were in agreement with the mRNA levels (data not reported).

Hepatic CYP2C6 mRNA concentrations were somewhat greater in intact females than in intact males (Fig. 7). Although hypophysectomy seemed to have little effect on CYP2C6 expression in female rats, restoration of circulating growth hormone levels at 3%, 6%, and 12% of normal seemed to cause a slight overinduction of the transcript. For reasons that are unclear, restoration of feminine growth hormone concentrations at 50% and 100% of normal were not as effective as lower levels in stimulating expression of CYP2A1 and CYP2C6.

Hepatic non-P450 mRNAs. We observed very little sexually dimorphic expression of hepatic GHR and GHBP mRNAs (female ≥ male) (Fig. 8). In this regard, hypophysectomy, with or without growth hormone replacement, had minimal effects on the expression levels of the transcripts. In contrast, hepatic concentrations of IGF-1 mRNA were clearly greater in intact males than in intact females (Fig. 8). Although IGF-1 mRNA was nearly undetectable after hypophysectomy, restoration of the feminine circulating growth hormone profile at 3% and 6% of normal was only slightly effective in restoring IGF-1 expression. In fact, plasma growth hormone had to be restored to 25% of normal to induce female-like concentrations (which were still considerably below that observed in males) of IGF-1 mRNA. [The similar concentrations of 18*S* rRNA observed for all the treatment groups (Fig. 8) verifies the high consistency and integrity of RNA loading on the Northern blots.]

Body and kidney weights. Hypophysectomy caused a dramatic loss in relative kidney weights and arrested body

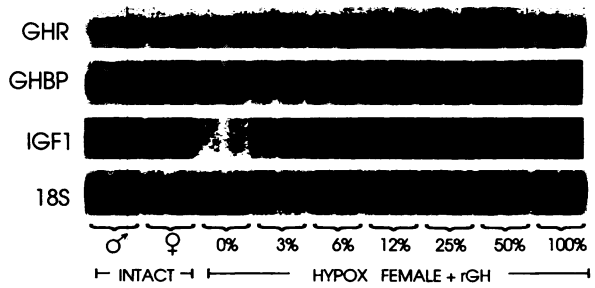

Fig. 8. Relative hepatic GHR, GHBP, and IGF-1 mRNA levels in intact male (♂) and female (♀) rats and hypophysectomized (*HYPOX*) rGH-replaced female rats. The levels of rGH replacement by continuous infusion are presented as a percentage of the normal feminine plasma growth hormone profile illustrated in Fig. 1 and determined in Results. Northern blot analyses of two livers per treatment group with ^{32}P-labeled oligonucleotide probes specific for each mRNA. Bottom, the same Northern blot reanalyzed with a ^{32}P-labeled oligonucleotide probe specific for 18S rRNA used as a control to indicate equal loading of the RNA in all lanes. At least five different livers were analyzed for each treatment group.

weight gain (Table 1). Although as little as 0.625 μg of rGH/hr/kg of body weight (3% of the normal) induced a maximal effect on kidney weight gain, this dosage, as well as 1.25 μg of rGH/hr/kg body of weight (6% of the normal), was completely ineffective in stimulating body weight gain. In fact, it required a dosage of 5.0–10.0 μg of rGH/hr/kg of body weight (25% and 50% of the normal) to restore normal body weight gain in the hypophysectomized female rats.

Discussion

Previously, in an attempt to identify the fundamental signaling elements in the plasma growth hormone profile (which could be considered the initial signal, albeit extrahepatic, in the signal transduction pathway regulating P450 expression), we used the MSG-treated rat. Neonatal administration of MSG, depending on the dose, can produce variable degrees of specific growth hormone deficiency in adult rodents that had otherwise generally normal endocrine levels (35). Results from these studies have demonstrated that a ≤90% reduction in the pulse heights of the masculine growth hormone plasma profile have no inhibitory effect on the male-like expression levels of CYP2C11, CYP2A2, and CYP3A2 (23, 25, 35). Furthermore, a similar MSG-induced reduction in the feminine growth hormone profile was found to have no inhibitory effect on female-like expression levels of CYP2C12, CYP2A1, CYP2E1, and 5α-reductase (17, 18). Recent experiments using the severely growth hormone-deficient dwarf rat have reported findings similar to those observed in the earlier MSG studies (2). Although investigations using the MSG-treated and dwarf rats have not identified the requisite signals in the growth hormone profiles regulating the major sex-dependent P450 isoforms, they have at least eliminated what seemed to be likely candidates: physiological pulse amplitudes and mean concentrations.[3]

Although the commonly used hypophysectomized rat is a model of multiple hormone deficiencies, it has certain advantages over the MSG-treated and dwarf rats. Both the MSG-treated and dwarf rats are models of lifelong growth hormone deficiency, which includes the critical developmental period when the neuroendocrine-hepatic axis differentiates and hepatic P450s are irreversibly imprinted (38). In contrast, the hypophysectomized animal experiences a very limited period of growth hormone depletion, which may make it more responsive to the corrective effects of growth hormone replacement (39). Perhaps more importantly, the hypophysectomized rat is completely growth hormone ablated, exhibiting maximal suppression of growth hormone-dependent P450 isoforms (2, 3, 22) and thus presenting an optimal base-line in which to examine the effects of growth hormone administration. In contrast, the MSG-treated and dwarf rats are not totally growth hormone depleted and can express normal levels of growth hormone-dependent P450s.

Although studies with hypophysectomized rats have enabled us to identify the growth hormone-devoid interpulse as the primary signal in the masculine episodic growth hormone profile regulating CYP2C11 expression (40), such signaling studies require that certain procedures, which are often overlooked, be followed.

Because we have found that plasma growth hormone concentrations too low to be assayable are capable of maintaining the feminine hepatic profile of P450 isoforms without influencing body weight gain, it is essential that the completeness of the hypophysectomy be confirmed.[1] Residual pituitary fragments insufficiently large to affect growth could still alter P450 expression. Before using hypophysectomized rats, we first determine that no body weight gain occurs for ≥4-5 postsurgical weeks. Although pituitary fragments at 2 weeks after surgery may be too small to influence body weight, a 4–5-week wait usually allows residual somatotrops sufficient time to regenerate and secrete enough growth hormone to measurably increase body weight. Perhaps the best marker for growth hormone ablation in female rats is the complete suppression of hepatic CYP2C12 and testosterone 5α-reductase mRNAs.

Because specific patterns of growth hormone secretion reg-

TABLE 1

Body weight gains and kidney weights of hypophysectomized female rats treated with continuously administered rGH

Female rats, hypophysectomized at 55 days of age, were implanted intraperitoneally with osmotic minipumps delivering a continuous infusion of rGH at rates of 0.625–20.0 μg of rGH/hr/kg of body weight. Control hypophysectomized females were implanted with pumps delivering an equivalent volume of rGH-solubilizing solution. All hypophysectomized animals received a second osmotic pump that continuously released 0.8 μg of thyroxine/hr/kg of body weight. Rats were killed on the seventh day of infusion. Values are mean ± standard deviation for at least five rats.

	rGH	Body weight gain	Kidney weight
	μg/hr/kg of body weight	*g/week*	*g/kg of body weight*
Intact		9.5 ± 2.1[a]	7.45 ± 0.50[a]
Hypophysectomized	0	−1.1 ± 0.9	5.06 ± 0.35
Hypophysectomized	0.625	−0.2 ± 2.3	6.71 ± 0.21[a]
Hypophysectomized	1.25	−2.3 ± 4.3	6.37 ± 0.28[a]
Hypophysectomized	2.5	3.0 ± 2.2[a]	6.60 ± 0.30[a]
Hypophysectomized	5.0	7.3 ± 3.1[a]	7.02 ± 0.36[a]
Hypophysectomized	10.0	15.0 ± 3.2[a]	7.00 ± 0.59[a]
Hypophysectomized	20.0	14.8 ± 3.0[a]	6.97 ± 0.29[a]

[a] $p < 0.01$ compared with control hypophysectomized animals not receiving rGH replacement.

[3] Although the pulse amplitude in the masculine growth hormone profile does not seem to be the essential signal directing CYP2C11 expression (25, 35), it is the growth hormone signal that suppresses phenobarbital induction of CYP2B1 and CYP2B2 (37). Clearly, the same "element" in the circulating sexually dimorphic growth hormone profile that signals expression of one P450 gene may go unrecognized by another P450 gene.

ulate expression of individual P450s, it seems worthwhile to know the resulting plasma profiles produced by administered growth hormone. For example, the often-reported observation that two daily subcutaneous injections of growth hormone can masculinize hepatic P450s (1–3) may have led to the erroneous conclusion that it is the elevated, short-lived peaks in the plasma hormone profile of the male rat that signals masculinization of the isoforms. To the contrary, monitored subcutaneous injections of growth hormone were found to produce low-amplitude, very long-lived plasma plateaus that had no resemblance to the endogenous pulse (22). Unfortunately, the vast majority of reports do not monitor renaturalized plasma growth hormone profiles, making it difficult to know the kind of plasma profile to which the hepatocyte was exposed.

Last, when trying to identify what might be very subtle signals in the sexually dimorphic growth hormone patterns regulating expression of individual P450 isoforms, it seems prudent to use species-specific growth hormone (in this case, rGH) instead of the much more widely administered human and bovine growth hormones, which are not necessarily equally effective (5, 13, 41).

In agreement with earlier reports (2, 42, 43), we observed no gender- or growth hormone-dependent effects on GHR and GHBP mRNAs, whose expression may be growth hormone regulated by post-transcriptional events (2, 44). As expected (42, 45), hepatic IGF-1 mRNA levels were sexually dimorphic (male > female), declined to very low concentrations after hypophysectomy, and were restored to normal female-like expression levels with the continuous administration of growth hormone. Although we found that 5 μg of rGH/hr/kg of body weight (25% of normal) could restore female-like levels of IGF-1 mRNA, the continuous administration of 10 times this amount of an equivalently active bovine growth hormone preparation was similarly effective in restoring hepatic IGF-1 mRNA in female hypophysectomized rats (45).

The gender-dependent expression levels and responses to hypophysectomy of male-specific CYP2C11, CYP2C13, CYP2A2, and CYP3A2 in this study are in agreement with earlier findings (see introductory paragraphs). Our observation that as little as 3% of the circulating feminine growth hormone profile could completely suppress CYP2C11, CYP2C13, CYP2A2, and CYP3A2 expression illustrates the profound sensitivity of these male-specific P450 genes to the inhibitory effects of continuous growth hormone.[4] Previous studies in which human growth hormone was infused (via subcutaneously placed osmotic pumps) at a rate of ~35-fold our rate of 0.625 μg of rGH/hr/kg of body weight demonstrated equally effective suppression of CYP2C11, CYP2C13, CYP2A2, and CYP3A2 expression in hypophysectomized female rats (2, 8, 11, 13). With the assumption of similar pharmacokinetics for human growth hormone and rGH, the earlier studies using human growth hormone would have restored the physiological feminine profile of plasma growth hormone, indicating the suppressive nature of normal feminine levels of the hormone.

Although subnormal concentrations of circulating growth hormone restored normal levels of female-dependent CYP2C12, CYP2C7, CYP2A1, and 5α-reductase, it is clear that the male-specific isoforms were more sensitive to the suppressive effects of the hormone. In all cases, restoration of the feminine growth hormone profile at 3% of the physiological concentration induced significant, albeit below normal, increases in the female-dependent P450s. CYP2A1 and 5α-reductase were restored to normal female levels with only 6–12% of physiological concentrations of the hormone; normal expression levels of CYP2C12 required ~12-25% physiological hormone levels; and CYP2C7 mRNA required ~25-50% of normal growth hormone levels to reach female-like concentrations (while CYP2C7 protein remained somewhat below normal at all growth hormone concentrations). Not surprisingly, these findings suggest that the suppression of P450s is more sensitive to growth hormone regulation than is P450 expression. That is, in suppression, one need interrupt only one step in the expression mechanism, whereas induction of expression requires the harmonious activation of all steps in the sequence. Furthermore, it seems that each of the female-dependent P450s requires a different signaling concentration of plasma growth hormone for normal expression: CYP2A1 requires the lowest concentration, and CYP2C7 requires the highest plasma concentration of the hormone. Perhaps the expression of each isoform is regulated by a somewhat different growth hormone-dependent cellular transduction mechanism.

Previous studies in which hypophysectomized female rats were infused with human growth hormone at concentrations reflecting physiological or higher levels increased CYP2C12 protein to 40–60% of normal (5, 6) and mRNA to ~100% of normal (8), 5α-reductase mRNA and its catalytic activity to ~70% of normal (6, 7), CYP2C7 mRNA to 60–100% of normal (7, 9), and CYP2A1 mRNA to near normal (6, 13, 14). Although in many of these earlier studies, hepatic enzyme concentrations were not restored to prehypophysectomy levels,[5] they did establish the importance of the continuous feminine growth hormone profile in directing CYP2C12, CYP2C7, CYP2A1, and 5α-reductase expression. Our observations extend these studies and indicate that the signaling concentrations in the feminine plasma growth hormone profile regulating expression of the female-dependent P450 isoforms and 5α-reductase are remarkably below physiological concentrations.

The effectiveness of low circulating growth hormone concentrations in feminizing P450 expression may be explained by the high affinity of the growth hormone receptor for the hormone [$K_d = 10^{-10}$ M (46)], which corresponds to half-maximal saturation of the membrane receptor at a plasma growth hormone concentration of 2 ng/ml or only 6% of the normal feminine level. Restoration of circulating growth hormone levels to hypophysectomized female rats at 6% of physiological concentration very effectively initiates expression of female-dependent CYP2C12, CYP2A1, and 5α-reductase to levels approaching, if not reaching, normal. However, the suppressive effects of growth hormone on male-specific CYP2C11, CYP2C13, CYP2A2, and CYP3A2 occur at what

[4] The fact that continuous exposure to growth hormone produced similar effects on P450 proteins and their specific catalytic activities as on mRNA levels supports the concept that growth hormone regulates expression of gender-dependent isoforms at the transcriptional level (2, 3).

[5] This was possibly a result of the use of human growth hormone instead of rGH (5, 13, 41) and the likely inconsistent absorption kinetics of growth hormone when administered via subcutaneously rather than intraperitoneally implanted osmotic pumps (19).

might be considered nominal plasma concentrations. As little as 3% of the normal feminine concentration of the hormone completely suppresses expression of these male-dependent isoforms. If we consider earlier studies in which we reported higher mean growth hormone concentrations in intact females (47, 48), then the infusion of 0.625 μg of rGH/hr/kg of body weight might actually be closer to 2% of the normal concentration. Furthermore, because 2–3% of the physiological growth hormone concentration was so completely effective in blocking CYP2C11, CYP2C13, CYP2A2, and CYP3A2, it is not unreasonable to speculate that even half of this concentration could be effective. Thus, plasma concentrations of growth hormone continuously binding ≤10% of the growth hormone receptor may be sufficient to signal the suppression of the male-specific isoforms.

The fact that extremely low levels of circulating growth hormone can effectively feminize hepatic P450 isoforms might suggest an inherent inefficiency in the production and secretion of growth hormone in female rats. However, unlike many other hormones with specific target tissues, growth hormone has a global effect on the body and seems to regulate functions in most cells. Thus, although remarkably subnormal levels of plasma growth hormone are capable of maintaining normal female-like levels of CYP2C11, CYP2C12, CYP2C13, CYP2A1, CYP2A2, and CYP3A2, we have also observed that these hormone concentrations are insufficient to maintain body weight gain or induce near-normal levels of CYP2C7. Perhaps the secretory profiles and their concentrations of growth hormone normally found in rats represent a compromise that ensures that an adequate response is made by all growth hormone-regulated tissues.

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References

1. Shapiro, B. H., A. K. Agrawal, and N. A. Pampori. Gender differences in drug metabolism regulated by growth hormone. *Int. J. Biochem. Cell. Biol.* **27:**9–20 (1995).
2. Legraverend, C., A. Mode, T. Wells, I. Robinson, and J.-Å. Gustafsson. Hepatic steroid hydroxylating enzymes are controlled by the sexually dimorphic pattern of growth hormone secretion in normal and dwarf rats. *FASEB J.* **6:**711–718 (1992).
3. Waxman, D. J. Regulation of liver specific steroid metabolizing cytochromes P450: cholesterol 7α-hydroxylase, bile acid 6β-hydroxylase, and growth hormone-responsive steroid hormone hydroxylases. *J. Steroid Biochem. Mol. Biol.* **43:**1055–1072 (1992).
4. Schenkman, J. B. Steroid metabolism by constitutive cytochromes P450. *J. Steroid Biochem. Mol. Biol.* **43:**1023–1030 (1992).
5. MacGeoch, C., E. T. Morgan, and J.-Å. Gustafsson. Hypothalamo-pituitary regulation of cytochrome $P450_{15\beta}$ apoprotein levels in rat liver. *Endocrinology* **117:**2085–2092 (1985).
6. Waxman, D. J., J. J. Morrissey, and G. A. LeBlanc. Female-predominant rat hepatic P-450 forms j (IIE1) and 3 (IIA1) are under hormonal regulatory controls distinct from those of the sex-specific P-450 forms. *Endocrinology* **124:**2954–2966 (1989).
7. Ram, P. A., and D. J. Waxman. Pretranslational control by thyroid hormone of rat liver steroid 5α-reductase and comparison to the thyroid dependence of two growth hormone-regulated CYP2C mRNAs. *J. Biol. Chem.* **265:**19223–19229 (1990).
8. Legraverend, C., A. Mode, S. Westin, A. Ström, H. Eguchi, P. G. Zaphiropoulos, and J.-Å. Gustafsson. Transcriptional regulation of rat P-450 2C gene subfamily members by the sexually dimorphic pattern of growth hormone secretion. *Mol. Endocrinol.* **6:**259–266 (1992).
9. Westin, S., A. Ström, J.-Å. Gustafsson, and P. G. Zaphiropoulos. Growth hormone regulation of the cytochrome P-450 IIC subfamily in the rat: inductive, repressive and transcriptional effects on P-450f (IIC7) and $P\text{-}450_{PB1}$ (IIC6) gene expression. *Mol. Pharmacol.* **38:**192–197 (1990).
10. Bandiera, S., and C. Dworschak. Effects of testosterone and estrogen on hepatic levels of cytochrome P450 2C7 and P450 2C11 in the rat. *Arch. Biochem. Biophys.* **296:**286–295 (1992).
11. Morgan, E. T., C. MacGeoch, and J.-Å. Gustafsson. Hormonal and developmental regulation of expression of the hepatic microsomal steroid 16α-hydroxylase cytochrome P-450 apoprotein in the rat. *J. Biol. Chem.* **260:** 11895–11898 (1985).
12. Janeczko, R., D. J. Waxman, G. A. LeBlanc, A. Morville, and M. Adesnik. Hormonal regulation of levels of the messenger RNA encoding hepatic P450 2c (IIC11), a constitutive male-specific form of cytochrome P450. *Mol. Endocrinol.* **4:**295–303 (1990).
13. Waxman, D. J., P. A. Ram, G. Notani, G. A. LeBlanc, J. A. Alberta, J. J. Morrissey, and S. S. Sundseth. Pituitary regulation of male-specific steroid 6β-hydroxylase P-450 2a (gene product IIIA2) in adult rat liver: suppressive influence of growth hormone and thyroxine acting at a pretranslational level. *Mol. Endocrinol.* **4:**447–454 (1990).
14. Yamazoe, Y., X. Ling, N. Murayama, D. Gong, K. Nagata, and R. Kato. Modulation of hepatic level of microsomal testosterone 7α-hydroxylase, P-450a (P450IIA1), by thyroid hormone and growth hormone in rat liver. *J. Biochem. (Tokyo)* **108:**599–603 (1990).
15. Waxman, D. J., G. A. LeBlanc, J. J. Morrissey, J. Staunton, and D. P. Lapenson. Adult male-specific and neonatally programmed rat hepatic P-450 forms RLM2 and 2a are not dependent on pulsatile plasma growth hormone for expression. *J. Biol. Chem.* **263:**11396–11406 (1988).
16. Waxman, D. J., P. A. Ram., N. A. Pampori, and B. H. Shapiro. Growth hormone regulation of male-specific rat liver P450s 2A2 and 3A2: induction by intermittent growth hormone pulses in male but not female rats rendered growth hormone deficient by neonatal monosodium glutamate. *Mol. Pharmacol.* **43:**790–797 (1995).
17. Waxman, D. J., J. J. Morrissey, J. N. MacLeod, and B. H. Shapiro. Depletion of serum growth hormone in adult female rats by neonatal monosodium glutamate treatment without loss of female-specific hepatic enzymes P450 2d (IIC12) and steroid 5α-reductase. *Endocrinology* **126:**712–720 (1990).
18. Pampori, N. A., and B. H. Shapiro. Subnormal concentrations in the feminine profile of circulating growth hormone enhance expression of female-specific CYP2C12. *Biochem. Pharmacol.* **47:**1999–2004 (1994).
19. Pampori, N. A., A. K. Agrawal, and B. H. Shapiro. Renaturalizing the sexually dimorphic profile of circulating growth hormone in hypophysectomized rats. *Acta Endocrinol.* **124:**283–289 (1991).
20. Emerson, C. H., R. Lew, L. E. Braverman, and W. J. DeVito. Serum thyrotropin concentrations are more highly correlated with serum triiodothyronine concentrations than with serum thyroxine concentrations in thyroid hormone-infused thyroidectomized rats. *Endocrinology* **124:**2415–2418 (1989).
21. Ram, P. A., and D. J. Waxman. Thyroid hormone stimulation of NADPH P450 reductase expression in liver and extrahepatic tissues. *J. Biol. Chem.* **267:**3294–3301 (1992).
22. MacLeod, J. N., and B. H. Shapiro. Repetitive blood sampling in unrestrained and unstressed mice with a chronic indwelling right atrial catheterization apparatus. *Lab. Anim. Sci.* **38:**603–608 (1988).
23. Shapiro, B. H., J. N. MacLeod, N. A. Pampori, N. A. Morrissey, J. J. Lapenson, and D. J. Waxman. Signalling elements in the ultradian rhythm of growth hormone regulating expression of sex-dependent forms of hepatic cytochrome P450. *Endocrinology* **125:**2935–2944 (1989).
24. Chomczynski, P., and N. Sacchi. Single step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. *Anal. Biochem.* **162:**156–159 (1987).
25. Pampori, N. A., and B. H. Shapiro. Over-expression of CYP2C11, the major male-specific form of hepatic cytochrome P450, in the presence of nominal pulses of circulating growth hormone in adult male rats neonatally exposed to low levels of monosodium glutamate. *J. Pharmacol. Exp. Ther.* **271:**1067–1073 (1994).
26. Waxman, D. J. Rat hepatic P450IIA and P450IIC subfamily expression using catalytic, immunochemical and molecular probes. *Methods Enzymol.* **206:**249–267 (1991).
27. Ram, P. A., and D. J. Waxman. Hepatic P450 expression in hypothyroid rats: differential responsiveness of male-specific P450 forms 2a (IIIA2), 2c (IIC11), and RLM2 (IIA2) to thyroid hormone. *Mol. Endocrinol.* **5:**13–20 (1991).
28. Baumbach, W. R., D. L. Horner, and J. S. Logan. The growth hormone-binding protein in rat serum is an alternatively spliced form of the rat growth hormone receptor. *Genes Dev.* **3:**1199–1205 (1989).
29. Gabrielsson, B. G., D. F. Carmignac, D. M. Flavell, and I. C. A. F. Robinson. Steroid regulation of growth hormone (GH) receptor and GH-binding protein messenger ribonucleic acids in the rat. *Endocrinology* **136:**209–217 (1995).
30. Murphy, L. J., G. I. Bell, M. L. Duckworth, and H. G. Friesen. Identifica-

tion, characterization and regulation of a rat complementary deoxyribonucleic acid which encodes insulin-like growth factor I. *Endocrinology* **121:**684–691 (1987).
31. Ramsden, R., K. M. Sommer, and C. J. Omiecinski. Phenobarbital induction and tissue-specific expression of the rat *CYP2B2* gene in transgenic mice. *J. Biol. Chem.* **268:**21722–21726 (1993).
32. Shapiro, B. H., and S. M. Szczotka. Androgenic repression of hexobarbitone metabolism and action in Crl:CD-1(ICR)BR mice. *Br. J. Pharmacol.* **81:**49–54 (1984).
33. Agrawal, A. K., N. A. Pampori, and B. H. Shapiro. Thin-layer chromatographic separation of regioselective and stereospecific androgen metabolites. *Anal. Biochem.* **224:**455–457 (1995).
34. Pampori, N. A., M. K. Pampori, and B. H. Shapiro. Dilution of the chemiluminescence reagents reduces the background noise on Western blots. *Biotechniques* **18:**588–590 (1995).
35. Pampori, N. A., A. K. Agrawal, D. J. Waxman, and B. H. Shapiro. 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).
36. Gemzik, B., D. Greenway, C. Nevins, and A. Parkinson. Regulation of two electrophoretically distinct proteins recognized by antibody against rat liver cytochrome P450 3A1. *J. Biochem. Toxicol.* **7:**43–52 (1992).
37. Shapiro, B. H., N. A. Pampori, D. P. Lapenson, and D. J. Waxman. Growth hormone-dependent and -independent sexually dimorphic regulation of phenobarbital-induced hepatic cytochromes P450 2B1 and 2B2. *Arch. Biochem. Biophys.* **312:**234–239 (1994).
38. Gustafsson, J.-Å., P. Eneroth, Å. Pousette, P. Skett, C. Sonnenschein, Å. Stenberg, and A. Åhlén. Programming and differentiation of rat liver enzymes. *J. Steroid Biochem.* **8:**429–443 (1977).
39. Shapiro, B. H., N. A. Pampori, P. A. Ram, and D. J. Waxman. 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).
40. Waxman, D. J., N. A. Pampori, P. A. Ram, A. K. Agrawal, and B. H. Shapiro. Interpulse interval in circulating growth hormone patterns regulates sexually dimorphic expression of hepatic P450. *Proc. Natl. Acad. Sci. USA* **88:**6868–6872 (1991).
41. Ström, A., A. Mode, P. Zaphiropoulos, A.-G. Nilsson, E. Morgan, and J.-Å. Gustafsson. Cloning and pretranslational hormonal regulation of testosterone 16α-hydroxylase (P-450$_{16\alpha}$) in male rat liver. *Acta Endocrinol.* **118:**314–320 (1988).
42. Mathews, L. S., B. Enberg, and G. Norstedt. Regulation of rat growth hormone receptor gene expression. *J. Biol. Chem.* **264:**9905–9910 (1989).
43. Carmignac, D. F., B. G. Gabrielsson, and I. C. A. F. Robinson. Growth hormone binding protein in the rat: effects of gonadal steroids. *Endocrinology* **133:**2445–2452 (1993).
44. Bick, T., Z. Hochberg, T. Amit, O. G. P. Isaksson, and J.-O. Jansson. Roles of pulsatility and continuity of growth hormone (GH) administration in the regulation of hepatic GH-receptors, and circulating GH-binding protein and insulin-like growth factor-I. *Endocrinology* **131:**423–429 (1992).
45. Isgaard, J., L. Carlsson, O. G. P. Isaksson, and J.-O. Jansson. Pulsatile intravenous growth hormone (GH) infusion to hypophysectomized rats increases insulin-like growth factor-I messenger ribonucleic acid in skeletal tissue more effectively than continuous GH infusion. *Endocrinology* **123:**2605–2610 (1988).
46. Leung, D. W., S. A. Spencer, G. Cachianes, R. G. Hammonds, C. Collins, W. J. Henzel, R. Barnard, M. J. Waters, and W. I. Wood. Growth hormone receptor and serum binding protein: purification, cloning and expression. *Nature (Lond.)* **330:**537–543 (1987).
47. Agrawal, A. K., N. A. Pampori, and B. H. Shapiro. Neonatal phenobarbital-induced defects in age- and sex-specific growth hormone profiles regulating monooxygenases. *Am. J. Physiol.* **268:**E439–E445 (1995).
48. Agrawal, A. K., and B. H. Shapiro. Phenobarbital induction of hepatic *CYP2B1* and *CYP2B2*: pretranscriptional and post-transcriptional effects of gender, adult age and phenobarbital dose. *Mol. Pharmacol.* **49:**523–531 (1996).

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