INFUSION OF GENDER-DEPENDENT PLASMA GROWTH HORMONE PROFILES INTO INTACT RATS: EFFECTS OF SUBCUTANEOUS, INTRAPERITONEAL, AND INTRAVENOUS ROUTES OF RAT AND HUMAN GROWTH HORMONE ON ENDOGENOUS CIRCULATING GROWTH HORMONE PROFILES AND EXPRESSION OF SEXUALLY DIMORPHIC HEPATIC CYP ISOFORMS

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ABSTRACT:

The dramatic sexual dimorphism in rat hepatic CYPs is determined by gender differences in the circulating GH profiles. Accordingly, each responsive isoform of CYP is induced or suppressed by different components, i.e., signaling elements, in the GH profiles. It was the purpose of this study to determine whether the signaling elements in the sexually dimorphic plasma GH profiles identified in GH-depleted rats are recognized by the hepatic CYPs in intact rats exposed to a multiplicity of signals contained in the normal gender-dependent GH profiles. To accomplish this goal, we imposed (via osmotic minipumps) the continuous feminine GH profile upon normal male rats and superimposed (via intraatrial catheters) the episodic masculine profile upon normal females. Monitored circulating GH profiles indicated that the administered GH had little or no effect on the normally secreted gender-dependent endogenous profiles. Basically, we observed that the degree of constancy of GH in the circulation (continuous in females and episodic in males) is the primary determinant establishing sexually dimorphic expression of eight hepatic CYPs in intact rats. However, the characteristic expression levels of each isoform observed in male and female rat liver are determined by an interaction of more subtle signals in the GH profiles reflected in the concentration and persistence of the feminine continuous profile as well as the frequency, duration, and amplitude of pulse and interpulse periods in the masculine episodic profile. In the course of the study, unexpected findings led us to compare the effectiveness of s.c.- and i.p.-infused GH and rGH with hGH. Briefly, male- and female-dependent hepatic CYPs were undoubtedly most responsive to rGH infused by i.p.-implanted osmotic pumps.

Gender differences in hepatic drug metabolism occur in numerous species, including fish, birds, and mammals. From the few species in which studies have been extended to the molecular level, it appears that sexual dimorphisms in drug metabolism are caused by the existence of multiple forms of hepatic CYPs, the gender-dependent expression of which is regulated by the hormone GH. In the rat, CYP responses to GH regulation are almost as variable as the number of GH-dependent isoforms. In this regard, we have found that the expression as well as suppression of each isoform of CYP is likely to be regulated by a different “signal” in the sexually dimorphic GH profile. These signals may be recognized by the hepatocyte in the frequencies and/or durations of the pulse and interpulse periods. Alternatively, the hepatocyte can monitor the mean plasma concentration of the hormone (Pampori and Shapiro, 1994a,b; Agrawal and Shapiro, 2000a). For example, expression of the major male-specific CYP2C11 is dependent upon the masculine episodic GH profile but is completely suppressed by the feminine profile of continuous hormone secretion. The requisite inductive signal in the masculine plasma profile is a minimum 2.5 h of GH absence during the interpulse, whereas the pulse heights can vary from 5 to 500% of normal without affecting CYP2C11 levels (Waxman et al., 1991; Agrawal and Shapiro, 2000a). Expression of the major female-
specific CYP2C12 is dependent upon the feminine GH profile, but only 10% of physiologic plasma concentration of the hormone is required for normal CYP2C12 expression. In contrast, female expression levels of CYP2C7 are dependent upon the presence of near physiologic plasma GH concentrations (Pampori and Shapiro, 1994a, 1996). Not to belabor the point (for additional examples see Pampori and Shapiro, 1996), it could be stated that each isoform of rat CYP responds to a different signaling element in the sexually dimorphic GH profiles.

As an additional complexity, responsiveness to these “occult signals” in the GH profiles are gender-imprinted. That is, renaturalizing the masculine GH profile in hypophysectomized rats is much more effective in restoring such male-specific isoforms as CYP2C11 expression in male than in female rats (Shapiro et al., 1993). Moreover, the feminine GH profile is considerably more effective at inducing female-dependent isoforms like CYP2C12 expression in hypophysectomized females than in hypophysectomized males (Pampori and Shapiro, 1999). Finally, the signal that induces a particular isoform is very different from the signal that suppresses its expression (Pampori and Shapiro, 1996; Agrawal and Shapiro, 2000a).

In our studies to identify the intrinsic signal elements in the GH plasma profiles regulating expression of gender-dependent CYP isoforms, we have relied upon both the multi-hormone-deficient (including GH) hypophysectomized rat and the selective GH-deficient (i.e., neonatally monosodium glutamate-treated) rat. Advantageously, these animal models allowed us to examine the inductive and suppressive effects of autonomously restored components in the circulating GH profiles. However, the GH signals do not exist in isolation. That is, while a continuous GH plasma profile of ~5 ng/ml is a sufficient signal to restore physiologic expression levels (mRNA, protein, and catalytic activity) of CYP2C12, the isoform is normally expressed in the presence of continuous GH whose plasma concentrations vary from ~10 to 100 ng/ml (Pampori and Shapiro, 1996). To determine the physiologic efficacy of the GH signals identified from GH-deficient rats, we imposed these signals on the endogenous circulating GH profiles of normal rats. We administered the continuous feminine GH plasma profile to normal males and the masculine episodic pattern to normal females, and then monitored the resulting plasma GH profiles and effects on the expression of gender-dependent hepatic CYP isoforms. In the course of this investigation, unexpected results warranted an extension of our study to include an examination of the routes of GH administration and a comparison of the effects of rGH (rat GH) and hGH (human GH) on rat CYP expression.

Materials and Methods

Animals. Animals were housed in the University of Pennsylvania Laboratory Animal Resources facility under the supervision of certified veterinarians and were treated according to a research protocol approved by the University’s Institutional Animal Care and Use Committee. Rats [Crl:CD(SD)BR] were received from the vendor at 8 weeks of age and observed in our facility for an additional 5 weeks. At 13 weeks of age, the female-like plasma profile of continuous GH secretion was replicated in normal male rats by implanting osmotic minipumps (Alza Corp., Palo Alto, CA) either subcutaneously (s.c.) in the intrascapular region or anchored by two stitches in the peritoneal wall (i.p.). The s.c.-implanted pumps were calibrated to deliver either 0.65 or 2.6 μg/kg b.wt./h of rGH, whereas the i.p.-implanted pumps were set to deliver either 1.25 or 10 μg/kg b.wt./h of rGH or hGH. Control rats received an equivalent volume of vehicle (5 μl/kg b.wt./h) for a continuous 7 days. The masculine-like episodic plasma GH profile was achieved in normal female rats by administering 6 pulses per day, one every 4 h, of rGH (40 μg/kg b.wt./pulse) through an external pump attached to an indwelling atrial catheter (Pampori et al., 1991a). Control rats received an equivalent volume of vehicle (450 μl/kg b.wt./pulse) for 7 days.

On the 4th day of GH or vehicle treatment, repetitive blood samples (10 μl) were obtained at 15-min intervals from unrestrained, unstrained, and completely conscious rats outfitted with our mobile catheterization apparatus (MacLeod and Shapiro, 1988; Pampori et al., 1991a). Eight-hour plasma rGH and hGH patterns were determined using radioimmunooassays that were highly specific for either rGH or hGH and having sensitivities of 1 to 3 ng/ml. Procedural details and statistical validation of the assays have been reported previously (Shapiro et al., 1989).

On the morning of the 7th day of hormone or vehicle treatment, rats were euthanized; the livers were quickly removed and perfused with ice-cold saline. Each liver was quickly minced; a portion reserved for mRNA determination was plunged into liquid nitrogen and subsequently stored at −70°C. The remaining minced liver was used for microsomal preparation.

RNA Analysis. Total hepatic RNA was isolated with a single-step guanidinium thiocyanate method (Chomczynski and Sacchi, 1987). 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 32P-labeled oligonucleotides, with hybridization and high stringency washing conditions as described previously (Waxman, 1991). The nucleotide sequence of oligonucleotide probes for CYP2A1, CYP2A2, CYP2C6, CYP2C7, CYP2C11, CYP2C12, CYP2C13 (Waxman, 1991), and CYP3A2 (Ram and Waxman, 1991) have been reported. The consistency of RNA loadings between samples was confirmed by ethidium bromide staining of 18S and 28S ribosomal RNAs and was verified with an 18S oligonucleotide probe (Ramsden et al., 1993). The hybridized mRNA signals were quantified by scanning the autoradiographs and normalized to the 18S rRNA signals in each lane.

Western Blots. Hepatic microsomes were prepared from individual rat livers (Shapiro et al., 1989) and then assayed for individual CYPs by Western blotting and/or by measurement of their selective catalytic activities (Waxman, 1991; Agrawal et al., 1995). 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, MD) 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 Biologic, Phoenix, AZ) rabbit IgG and detected with an enhanced chemiluminescence kit (Amersham, Arlington Heights, IL) (Pampori et al., 1995).

The specificity of the antibodies has been discussed elsewhere (Pampori and Shapiro, 1996). Briefly, antibodies against CYP2C7 and CYP2C11 have been found to be highly specific with no detectable cross-reactivities with known rat CYPs. Antibodies to CYP2C12 strongly react with CYP2C13 protein. However, not only is CYP2C13 a male-specific isoform (in contrast to the female specificity of CYP2C12), but its location on the blot is easily distinguished from CYP2C12. Although CYP3A1 and CYP3A2 proteins are recognized by the anti-rat CYP3A1/2, the former is basically an inducible, GH-independent isoform only marginally expressed constitutively, whereas the latter is a major male-specific isoform.

Catalytic Activity. Testosterone metabolites, including 2α- and 16α-, 7α-, 15α-, and 6β-hydroxylases, reflective of the activity levels of CYP2C11, CYP2A1, CYP2A2, and CYP3A2 proteins, respectively (Schenkman, 1992), and female-specific testosterone 5α-reductase (coincident with CYP2C12) were assayed according to our methods as described previously (Pampori et al., 1991b; Agrawal et al., 1995). Male-predominant, multi-CYP-dependent microsomal hexobarbital hydroxylase was measured as reported in Shapiro and Szczotka (1984).

Statistics. All data were subjected to ANOVA, and differences were determined with t statistics and the Bonferroni procedure for multiple comparison.

5 At the time of necropsy, the osmotic pumps were removed and found to contain the expected residual amounts of rGH, hGH, or vehicle. Similarly, hormone levels in the residual volumes of the spent syringes (changed daily) delivering the masculine pulsatile GH profile were measured by radioimmunooassay and found to contain 98 ± 7% (mean ± S.D.) of the expected values.
Results

CYP2A2, CYP2C11, CYP2C13, and CYP3A2 mRNAs, proteins, and/or specific catalytic activities were basically unexpressed in female liver (Fig. 1), explaining the characterization of the isoforms as male-specific. Continuous infusion by s.c.-implanted osmotic minipumps of 0.65 μg of rGH/kg b.wt./h [restoring about 3% of the typical feminine plasma GH profile when administered to hypophysectomized rats (Pampori and Shapiro, 1999)] had no repressive effect on hepatic CYP2A2, CYP2C11, and CYP3A2 in male rats. When the s.c.-infused dose of rGH was increased 4-fold to 2.6 μg/kg b.wt./h, CYP2A2, CYP2C11, and CYP3A2 mRNAs, proteins, and/or specific testosterone hydroxylations were reduced 35 to 45%. In contrast, neither dose of rGH had a repressive effect on CYP2C13 (Fig. 1) or male-predominant (M:F, 7:1) multi-CYP-dependent hexobarbital hydroxylase (data not presented).

Even less effective than its repressive effects on the male-specific CYP isoforms, s.c. infusion of 0.65 μg of rGH/kg b.wt./h had no inductive effects on female-predominant CYP2A1, CYP2C6, and CYP2C7 and female-specific CYP2C12 mRNAs, proteins, and/or catalytic activities when administered to normal male rats (Fig. 2).

These findings are in dramatic contrast to our previous reports (Pampori and Shapiro, 1996, 1999) demonstrating a much greater responsiveness of both male- and female-dependent CYP isoforms to the repressive and inductive effects, respectively, of just nominal levels of continuously infused rGH. Our earlier studies, however, used hypophysectomized rats in which the feminine profile of continuous GH secretion was restored by osmotic minipumps implanted i.p. To determine whether the route of rGH administration was responsible for these different effects on CYP expression, we repeated the experiment infusing rGH via i.p.-implanted pumps. Moreover, since the vast majority of reports studying GH regulation of rat CYP expression administered hGH, we compared the effectiveness of i.p.-infused hGH with rGH.

As in the first study, the expected gender differences in hepatic CYP levels were evident in the control rats. That is, CYP2C11 and 3A2 as well as their specific catalytic activities (testosterone 2α-hydroxylase and testosterone 6β-hydroxylase, respectively) were expressed nearly exclusively in males. On the other hand, CYP2C12 and its associated testosterone 5α-reductase were basically present only in female liver. Female-predominant CYP2C7 was expressed in the usual male to female ratio of ~1:3 (Table 1).
Continuous i.p. infusion of rGH at a concentration as low as 1.25 \(\mu\)g/kg b.wt./h [restoring about 6% of the typical feminine plasma GH profile (Pampori and Shapiro, 1996, 1999)] reduced expression of male-specific CYP2C11 and CYP3A2 by 50 to 70% (Table 1). In contrast, infusion of the same dose of hGH was only half as effective as rGH in suppressing CYP2C11 and CYP3A2. An 8-fold increase in rGH to 10 \(\mu\)g/kg b.wt./h suppressed CYP2C11 and CYP3A2 to just 10% of normal male-like concentrations. The same i.p.-infused dose of hGH was nearly as suppressive as rGH, reducing protein levels of the male-specific isoforms to 20 to 30% of normal.

In agreement with the greater repression of male-specific CYPs produced by i.p.-infused rGH, the homologous GH proved to be a considerably more effective inducer of female-dependent CYP isoforms than the human hormone (Table 1). At an infusion dose of 1.25 \(\mu\)g/kg b.wt./h, rGH elevated CYP2C12 and CYP2C7 levels to \(~50\%\) of normal. Increasing the rGH dose by 8-fold increased the concentrations of the two isoforms to 70 to 80% of typical female-like levels. In contrast, i.p. infusion of the same doses of hGH induced CYP2C12 and CYP2C7 only one-half or two-thirds as effectively as did rGH.\(^6\)

It is the pattern of GH secretion that determines the sexually dimorphic expression of constituent CYPs in the rat. Since often subtle changes in the GH profile can substantially alter the expression of individual isoforms (Pampori and Shapiro, 1996; Agrawal and Shapiro, 2000a), we investigated whether i.p. infusion of rGH and hGH produced different effects on the endogenous plasma GH profile in exposed male rats. Normal male and female rats implanted i.p. with vehicle-releasing osmotic minipumps (i.e., controls) exhibited typical sexually dimorphic plasma GH profiles (Fig. 3). In the females, profiles were characterized by frequent and irregularly occurring pulses (\(~40–100\) ng of rGH/ml of plasma) and brief interpulse periods containing \(~10\) to 20 ng of rGH/ml. In contrast, control male rats secreted GH in episodic bursts (\(~200\) ng/ml of plasma) every 3 to 4 h separated by prolonged interpulses containing no detectable hormone levels. With the possible exception of the interpulse GH concentrations falling within the range of assay sensitivity (\(~1–3\) ng/ml), constant i.p. infusion of 1.25 \(\mu\)g of rGH/kg b.wt./h had no detectable effect on the normal endogenous masculine GH profile. In the case of the same dose of hGH infusion, use of a specific radioimmunoassay suggested a persistent concentration of \(~1\) ng/ml of plasma, which was within or below the assay sensitivity. Otherwise, the typical masculine GH profile was undisturbed. Continuous infusion of 10 \(\mu\)g of rGH/kg b.wt./h may have slightly reduced endogenous pulse amplitudes but had little if any effect on the periodicity of the GH episodic bursts. However, infusion of this

\(^6\) Although mRNA levels for all the CYP isoforms measured in this experiment comparing i.p. infusion of rGH and hGH were in agreement with protein and catalytic activities, the data are not presented because of an insufficient number of analyzed sample.
higher dose of rGH did elevate interpulse hormone concentrations to near female-like levels of ~10 ng/ml. Finally, with the exception of somewhat elevated pulse amplitudes, the pattern of endogenous rGH in males infused with 10 μg of hGH/kg b.wt./h was indistinguishable from controls. Determination of specific hGH levels, however, revealed a continuous presence at ~10 ng/ml of hGH in the plasma.

The ability of continuously infused rGH to near-totally feminize hepatic CYPs (i.e., repress male-dependent isoforms and induce female forms) despite continued secretion of the masculine episodic GH profile raised the question of whether the opposite effect of administering the episodic GH profile to otherwise normal females would then masculinize hepatic CYPs. Accordingly, we replicated the typical masculine episodic GH profile into normal female rats by use of an external rGH-dispensing pump attached to an indwelling atrial catheter (Pampori and Shapiro, 1991a). The resulting circulating GH profiles were characterized by the typical hour-long burst of GH (~200 ng/ml) every 4 h, superimposed on the endogenous female profile of continuous GH secretion ranging from ~80 ng/ml (pulses) to 10 to 20 ng/ml (interpulses) (Fig. 4). In spite of the imposition of male-like periodicity on the femline plasma GH profile, CYP2C6 and CYP2C12 were unchanged from their usual high female levels while female-predominant CYP2A1 and CYP2C7 were suppressed ~20 to 30%, but they remained at levels 2 to 3 times greater than found in males (Table 2). Male-specific CYP2A2, CYP2C11, CYP2C13, and CYP3A2 as well as male-predominant hexobarbital hydroxylase were even less responsive to the administered masculine GH profile. In fact, the feminine level of these enzymes (which was basically undetectable) remained undetectable in the treated female rats.

Discussion

Continuous infusion of 0.65 μg of rGH/kg b.wt./h by s.c.-implanted osmotic minipumps had no suppressive effect on male-specific CYP2A2, CYP2C11, CYP2C13, and CYP3A2 in intact male rats. Moreover, although a 4-fold increase in the infused GH dose resulted in some (albeit incomplete) suppression, the results were in drastic contrast to the effectiveness of the same doses when administered to hypophysectomized male rats. In this regard, a continuous infusion of 2.5 μg of rGH/kg b.wt./h completely suppressed CYP2A2, CYP2C11, CYP2C13, and CYP3A2 expression in hypophysectomized male rats, with lower hormone doses being nearly as repressive (Pampori and Shapiro, 1996, 1999). Furthermore, the complete ineffectiveness of continuously s.c.-infused rGH to induce female-dependent isoforms (CYP2A1, CYP2C6, CYP2C7, and CYP2C12) at a dose of 2.6 μg/kg b.wt./h in intact males was similarly inconsistent with results using hypophysectomized rats (Pampori and Shapiro, 1996, 1999). One procedural difference between the present study and our earlier reports is that rGH was administered to the hypophysectomized rats via i.p.-implanted minipumps. This procedural difference might not seem significant, considering that in other laboratories continuous GH is invariably and effectively administered by s.c.-implanted minipumps. However, in these other studies GH was administered at infusion doses ~10 times greater than our highest dose (Mode et al., 1981; Waxman et al., 1989). Accordingly, we repeated the experiment by infusing rGH by i.p.-implanted osmotic minipumps at a low dose (intermediate to the 0.65- and 2.6-μg s.c. doses) and a higher dose (10 μg of GH/kg b.wt./h) sufficient to monitor in the circulation. Clearly, i.p. infusion of 1.25 μg of rGH/kg b.wt./h was more effective in suppressing the measured male-specific CYPs (CYP2C11 and CYP3A2) in control males than twice the hormone dose administered via s.c.-implanted minipumps. Moreover, the 1.25-μg i.p.-infused dose of rGH induced significant expression levels of female-dependent CYP2C7 and CYP2C12 in contrast to a lack of induction following infusion of twice the rGH dose by the s.c. route. In agreement with the differential effects induced by s.c.- and i.p.-infused rGH, administration of insulin at equal supraphysiologic doses by s.c.- and i.p.-implanted osmotic minipumps produced significantly different glucose, fatty acid, and triglyceride plasma levels. Moreover, serum levels of insulin varied by ~50% between the two routes of administration (Kazumi et al., 1986).

rGH administered by i.p.-implanted minipumps produces dose-dependent continuous and constant plasma rGH levels in hypophysectomized (Pampori and Shapiro, 1996, 1999) and intact rats (Fig. 3), and suppression of male-dependent CYPs and expression of female-dependent isoforms require continuous exposure to the hormone [albeit at different concentrations for each isoform (Pampori and Shapiro, 1996)]. Therefore, we conclude that infusion of subphysiologic doses of rGH via s.c.-implanted minipumps, like s.c.-injected rGH.
FIG. 3. Plasma concentrations of circulating rGH and hGH (○●○●○●) obtained from individual, undisturbed, catheterized rGH-, hGH-, or vehicle-infused normal male and female rats at 15-min intervals for 8 consecutive h.

Rats were implanted peritoneally with osmotic minipumps set to continuously deliver either 5 μl of vehicle/kg b.wt./h (top panels), 1.25 μg of rGH or hGH/kg b.wt./h (middle panels), or 10 μg of rGH or hGH/kg b.wt./h (bottom panels) for 7 days. Similar findings were obtained from two to three additional animals in each treatment group.
Masculine-like plasma GH profiles were administered to normal female rats by infusing 40 µg of rGH/kg b.wt./pulse six times per day, once every 4 h, for 7 days, through an external pump attached to an indwelling atrial catheter (MacLeod and Shapiro, 1988; Pampori et al., 1991a). Control male and female rats were infused with the vehicle, 450 µl/kg b.wt./pulse. Similar findings were obtained from two to three additional animals in each treatment group.

Our observation that i.p.-implanted, rather than the consistently used s.c.-implanted, osmotic minipumps more effectively regulate rat CYP expression raised a related question regarding the effectiveness of hGH preparations used in the vast majority of rat CYP studies. Although readily available and certainly effective, unlike rGH, hGH binds to both the GH and prolactin receptors of rat liver (Postel-Vinay, 1976). Earlier studies comparing the effects of continuously infused rGH with rGH reported that the human form was a substantially more effective inducer of CYP2C12 in hypophysectomized rats (Mode et al., 1981; MacGeoch et al., 1985). However, note that the rGH used in these studies represented initial isolations by the National Institute of Arthritis, Diabetes, Digestive, and Kidney Diseases contaminated with unstabilizing proteases (Mode et al., 1983), a serious problem subsequently corrected. In the present study, when rGH and hGH were i.p.-infused at the same continuous dose, they produced similar subtle changes in the endogenous masculine GH profile. In agreement with short-term studies continuously infusing i.v. hGH into intact male rats (Clark et al., 1988), we found that infused rGH as well as hGH imposed a measurable interpulse baseline on an otherwise typical episodic profile. That is, in spite of the infused hormone, the endogenous masculine episodic profile was characterized by a secretory burst every 3 to 4 h with undetectable concentrations of GH in the interpulse intervals. The elevated baseline in the hormone-treated rats was due solely to the exogenously administered GH. Interestingly, when infused at the same dose, rGH and hGH elevated baseline GH concentrations to the same degree. Administration of 1.25 µg/kg b.wt./h of either rGH or hGH increased baseline concentrations of the hormone to barely detectable levels within the minimum sensitivity of the assays (i.e., 1–3 ng/ml of plasma). Infusion of rGH and hGH at 10 µg/kg b.wt./h increased, as previously estimated (Wells et al., 1994), the baseline to ~10 ng/ml.7 Significantly, these findings demonstrate that any differences in the response of rat hepatic CYPs to rGH and hGH are unlikely to be a result of differences in the circulating growth hormone profiles, the single most important regulator of gender-dependent CYP expression. Accordingly, and in agreement with interspecies effects of NADPH-cytochrome P450 reductase (Sharma et al., 1995), our results indicate that both male- and female-dependent rat CYP isoforms are more responsive to the regulatory effects of their homologous GH than hGH. Whereas continuous i.p. infusion of hGH repressed male-specific hepatic CYPs and induced the female-dependent isoforms at similar dose-dependent levels reported earlier (Mode et al., 1981; Waxman et al., 1989; Wells et al., 1994), rGH infusion was often twice as effective.

The initial purpose of this study was to determine whether the “hidden” signaling elements in the sexually dimorphic GH profiles identified in hypophysectomized rats could still be recognized by hepatic CYPs in intact rats exposed to normal circulating gender-dependent GH profiles. Observations using hypophysectomized rats have demonstrated that the continuous presence of GH in the circulation characteristic of the female plasma profile maximizes expression of female-dependent CYPs and suppresses male-dependent isoforms. In the case of the masculine episodic GH profile, the periodic absence of the hormone from the circulation allows for expression of high levels of male-dependent CYPs and suppression or only partial expression of female-dependent CYP isoforms. This being the basic premise, each CYP appears to “define” episodic and continuous differently. Some isoforms recognize a plasma profile as episodic when GH secretion is interrupted for a little over an hour, whereas other CYPs will only respond to an episodic profile when GH is

7 As a result of possible differences in metabolism and/or distribution, i.p. GH infusion of 1.25 µg and 10 µg/kg b.wt./h increased plasma concentrations of the hormone in intact rats to only half as much as observed in hypophysectomized rats (Pampori and Shapiro, 1998).
absent from the circulation for almost 3 h (Agrawal and Shapiro, 2000b). Episodic regulation of some CYP isoforms requires full physiologic pulse heights, while others respond to nominal pulse amplitudes of only ~5% of normal. As a final example, some isoforms will respond fully only when the continuous profile is secreted at physiologic concentrations, whereas other CYPs are completely imprinted to be less responsive to the masculine episodic plasma GH profile than in male liver (Shapiro et al., 1993, 1994).

Since it is impossible to impose an episodic profile on an already continuous feminine profile, we examined the effects of the characteristic masculine periodic (once every 4 h) high amplitude GH pulses superimposed on the typical feminine GH profile. In general, imposition of the male-like periodic high amplitude GH pulses on the feminine profile had little effect on altering the sexually dimorphic pattern of CYP isoforms expressed in female rat liver. Male-specific isoforms that were undetectable in female liver remained so in the episodic GH-treated females. Still, notwithstanding the presence of continuous plasma GH concentrations in the females, the imposition of periodic male-like pulses produced an incomplete (20–40%) but significant masculinization (i.e., suppression) of female-predominant CYP2A1 and CYP2C7. The effectiveness of the superimposed male-like GH pulses to even partially masculinize female-predominant CYPs is all the more relevant considering that both constitutive and inducible CYP isoforms in female liver are imprinted to be less responsive to the masculine episodic plasma GH profile than in male liver (Shapiro et al., 1993, 1994).

In contrast to the modest alterations in CYP expression induced by imposing the masculine episodic plasma GH profile on the intact female, addition of a continuous concentration of plasma GH to the masculine episodic profile dramatically feminized expression of the gender-dependent isoforms. In fact, what would represent ~6% of the normal feminine plasma GH concentration produced by 1.25 μg of rGH/kg b.wt/h (Pampori and Shapiro, 1996) suppressed male-specific CYPs by 50 to 70% and induced female-dependent isoforms 30 to 50%. Imposition of a continuous concentration of GH representing ~50% of the normal female profile nearly completely feminized hepatic CYP expression in intact male rats.

In summary, the present results demonstrate that gender-dependent signals in the sexually dimorphic plasma GH profiles identified in hypophysectomized rats can effectively regulate hepatic CYP expression in intact animals. In fact, some exogenously administered GH signals are sufficiently potent to counter or reverse the effects of
signals in the endogenous plasma GH profiles. In this regard, we have observed that the “discriminators” for each CYP isoform programmed to recognize and respond to (i.e., by induction or suppression) selective GH plasma signals are particularly sensitive to the constancy of the circulating profile. The persistence of the hormone in the circulatory characteristic of the feminine GH profile and the episodic or intermittent presence of hormone characteristic of the masculine GH profile appear to be the primary determinants that establish a basic sexual dimorphism in hepatic CYP expression. The actual expression levels of individual isoforms characteristic of male and female rat liver are determined by a combination of more subtle signals in the GH profile reflected in the concentration and persistence of the continuous profile and the frequency, duration, and amplitudes of the pulse and interpulse periods of the episodic profile. It is the presence and subsequent recognition of all these competing GH signals that ultimately determines the expression of individual isoforms and establishes the sexually dimorphic nature of rat CYPs.

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