Attenuated Expression of Episodic Growth Hormone-Induced CYP2C11 in Female Rats Associated with Suboptimal Activation of the Jak2/Stat5B and Other Modulating Signaling Pathways

Ravindra N. Dhir, Chellappagounder Thangavel, and Bernard H. Shapiro

Laboratories of Biochemistry, University of Pennsylvania, School of Veterinary Medicine, Philadelphia, Pennsylvania

Received June 28, 2007; accepted August 1, 2007

ABSTRACT:
Inherent sex differences in various parameters of growth, musculoskeletal function, metabolism, and cytochrome P450 (P450)-dependent drug metabolism have been reported in rats and humans administered typical intermittent/episodic growth hormone (GH) replacement therapy. Having infused and monitored the identical physiologic masculine (episodic) growth hormone profile to both hypophysectomized male and female rats, we observed that induction levels of hepatic CYP2C11 were 35 to 40% lower in females. Associated with the reduced expression of the P450 isoform in the episodic GH-treated females was 50% less activation of Stat5B to the CYP2C11 promoter. Because the Jak2/Stat5B signaling pathway mediates the effects of the masculine GH profile on its target cells, we conclude that the lower induction level of CYP2C11 in females exposed to the masculine GH profile is probably due, at least in part, to the suboptimum activation of the Jak2/Stat5B pathway. In addition to the reduced activation of the Jak2/Stat5B pathway, we observed lower activational levels of mitogen-activated protein kinase (p44/p42) and, indirectly, nuclear factor-κB in the episodic GH-treated females that may be involved in attenuating the activity of the Jak2/Stat5B pathway diminishing CYP2C11 expression levels.

Whereas males and females secrete the same daily amounts of growth hormone (GH), the secretory patterns in various species examined, including rats, mice, and humans (Shapiro et al., 1995), are sexually dimorphic—characterized as “continuous” for females and “episodic” for males. In the case of rats, males secrete GH in episodic bursts (~200–300 ng/ml plasma) every 3.5 to 4 h. Between the peaks, GH levels are undetectable. In females the hormone pulses are more frequent and irregular and are of lower magnitude than in males, whereas the interpulse concentrations of GH are always measurable (Legraverend et al., 1992; Shapiro et al., 1995). These sex differences in the circulating GH profiles and not sexual differences in GH concentrations, per se, are responsible for observed sexual dimorphisms ranging from body growth to the expression of hepatic enzymes (Legraverend et al., 1992; Shapiro et al., 1995). In this regard, rat, as well as murine and human liver each contain sex-dependent isoforms of P450 that are regulated by the sex-dependent profiles of circulating GH (Legraverend et al., 1992; Shapiro et al., 1995; Dhir et al., 2006).

Sex-dependent, hepatic P450s in the rat are generally divided into three groups: male-specific isoforms only found in male liver, female-specific isoforms only expressed in female liver, and sex (generally female)-predominant P450s found in both sexes, but at higher levels in one sex. Essentially, there are four major male-specific isoforms in rat liver: CYP2C11, CYP2C13, CYP2A2, and CYP3A2. Expression of the major male-specific CYP2C11 comprising >50% of the total hepatic pool of P450 in male rats (Morgan et al., 1985) requires the episodic “on/off” masculine profile of GH secretion. Although the feminine pattern of continuous hormone secretion blocks CYP2C11 expression, total GH depletion from the circulation allows CYP2C11 expression at ~25% of intact male levels (Morgan et al., 1985; Pampori and Shapiro, 1996; Agrawal and Shapiro, 2000). Although expression of CYP2C11 is greatest when exposed to its sex-dependent GH profile, other male-specific isoforms are optimally expressed in the absence of GH. CYP2A2 and CYP3A2 are maximally expressed in the hypophysectomized (HYPOX) rat and disappear when GH is secreted constantly but are only minimally suppressed under the influence of episodic GH (Waxman, 1992; Pampori and Shapiro, 1996; Agrawal and Shapiro, 2000). Expression of the major female-specific isoforms, e.g., CYP2C12, are solely dependent on the feminine profile of continuous GH secretion (Waxman, 1992; Legraverend et al., 1992). The sex-predominant isoforms, probably the most abundant, if not sole, group in most species including mice and humans (Shapiro et al., 1995; Dhir et al., 2006), can be represented by CYP2A1 in the rat. After HYPOX, female-predominant CYP2A1

ABBREVIATIONS: GH, growth hormone; P450, cytochrome P450; HYPOX, hypophysectomized; Jak, Janus kinase; Stat, signal transducer and activator of transcription; MAPK, mitogen-activated protein kinase; NF-κB, nuclear factor-κB; IκB, inhibitory κB; rGH, rat growth hormone; PCR, polymerase chain reaction; ChIP, chromatin immunoprecipitation assay; Erk, extracellular signal-regulated kinase.
(male/female, ~1:3) concentrations decline by 50 to 60% in both sexes and are restored to sex-dependent intact levels by renatursing the sex-dependent GH profiles (Pampori and Shapiro, 1996; Agrawal and Shapiro, 2000).

Although HYPOX female rats will respond to the masculine episodic GH profile with an induction of male-dependent P450 isoforms and suppression of female-dependent isoforms, and HYPOX male rats will respond to the feminine continuous GH profile with an induction of female-dependent P450 isoforms and concomitant suppression of male-dependent isoforms, the responses are inherently limited by the sex of the rat. That is, regardless of the restored GH profile, female hepatocytes, either in vitro or in vivo (Legraverend et al., 1992; Shapiro et al., 1993; Waxman et al., 1995; Thangavel et al., 2006) cannot express male-like levels of male-specific P450s, nor can male hepatocytes, either in vitro (Thangavel et al., 2004) or in vivo (Pampori and Shapiro, 1999) be induced to express female-like levels of female-dependent P450s. Accordingly, regulation of male-specific CYP2C11 by episodic GH appears to involve the activation of the Janus kinase (Jak2)/signal transducers and activators of transcription (Stat5B) pathway (Waxman and O’Connor, 2006), in addition to other possible signaling pathways, such as mitogen-activated protein kinase (MAPK) (Verma et al., 2005) and nuclear factor-κB (NF-κB) (Iber et al., 2000), which are examined here as the basis for the inherent sex-dependent response of CYP2C11 to episodic GH.

**Materials and Methods**

**Antibodies and Chemicals.** Antibodies were purchased against Stat5A, Stat5B, inhibitory κ-β (1κB-α), and β-actin; also purchased were horseradish peroxidase-conjugated anti-goat (Santa Cruz Biotechnology, Inc., Santa Cruz, CA), anti-phospho-Jak2 (Chemicon International, Temecula, CA), anti-activated MAPK (Cell Signaling Technology Inc., Beverly, MA), antibodies against CYP2C11 (Oxford Biomedical Research, Oxford, MA), and CYP3A2 (BD Gentest, Woburn, MA), and, finally, horseradish peroxidase-conjugated anti-mouse and anti-rabbit (GE Healthcare, Chalfont St. Giles, Buckinghamshire, UK). Recombinant rat GH (rGH) and materials used to assay plasma rGH were obtained from the National Hormone and Paint Program and Dr. A. F. Parlow (Harbor-UCLA Medical Center, Torrance, CA). Other chemicals of molecular biology grade were purchased either from Sigma-Aldrich (St. Louis, MO) or from Roche Diagnostics (Indianapolis, IN).

**Animals.** Animals were housed in the University of Pennsylvania Laboratory Animal Resources Facility under the supervision of a certified laboratory animal medicine veterinarian. These animals were treated according to a protocol approved by the University’s Institutional Animal Care and Use Committee. HYPOX male and female Crl:CD (SC) BR Sprague-Dawley rats were obtained from Charles River Laboratories (Wilmington, MA). Rats were HYPOX by the supplier at 8 weeks of age and maintained for 4 to 5 weeks on HYPOX by the supplier at 8 weeks of age and maintained for 4 to 5 weeks on commercial rat pellets and 5% sucrose drinking water. These animals were housed under conditions of denatured conditions on a SDS-polyacrylamide gel electrophoresis system. We used 1.5-mm, 10% SDS-polyacrylamide gels for CYP2C11, CYP3A2, 1κB-α, Stat5A, Stat5B, and activated MAPK and 4 to 10% gradient gels (SDS-polyacrylamide gel electrophoresis) for phospho-Jak2. Western blot analyses were performed as reported previously (Verma et al., 2005). The blots were analyzed with an FluorChem 8800 gel documentation system (Alpha Innovations, San Leandro, CA) using a visible light source. Densitometric units were obtained as integrated density values as calculated by the software supplied with the gel documentation system. Equal loading of protein was confirmed by using Ponceau S staining and Western blot analysis for the expression of β-actin. Furthermore, protein values were normalized to two control samples repeatedly run on all blots.

**Reverse Transcription-PCR.** CYP2C11, CYP3A2, and CYP2A1 gene expressions were determined by reverse transcription-PCR. Total cellular RNA was extracted from liver tissues using TRIzol reagent (Invitrogen, Carlsbad, CA). The concentration and purity of the isolated RNA were quantitated using spectrophotometry by measuring absorbance at 260 and the 260/280 nm ratio, respectively. One microgram of RNA from each sample was reverse-transcribed in a 20-μl reaction volume. One microliter of cDNA was PCR-amplified according to our previous description (Verma et al., 2005). PCR primers for CYP2C11, CYP3A2 (Morris and Davila, 1996), and CYP2A1 (Geng and Strobel, 1993) were synthesized by Sigma-Genosys (The Woodlands, TX). The final PCR product was quantified with a FluorChem 8800 gel documentation system using a UV lamp. Densitometric units were obtained as integrated density values as calculated by the software supplied with the gel documentation system. Equiloading of protein was confirmed by using Ponceau S staining and Western blot analysis for the expression of β-actin. Furthermore, protein values were normalized to two control samples repeatedly run on all blots.

**Surgical Implantation of Catheter, GH Treatment, and Assay.** Indwelling right atrial catheters were implanted by methods described previously (Pampori et al., 1991). After 3 days, the unrestrained and unstressed, cathe-

**Catalytic Activity.** Testosterone 7α-hydroxylase, reflective of the activity level of CYP2A1, was assayed according to our methods as described previously (Agrawal et al., 1995).

**ChiP.** A ChiP assay was performed on a portion of the freshly isolated minced liver that had been incubated in Dulbecco’s minimal essential medium containing 1% formaldehyde for 10 min at room temperature. This cross-linking reaction was stopped by addition of glycine to a final concentration of 0.125 M. The tissue was washed three times with ice-cold Dulbecco’s phosphate-buffered saline containing 5 mM EDTA. The nuclei were subsequently isolated and lysed, and the ChiP procedure was performed as described previously (Chia et al., 2006). Briefly, the lysate was sonicated to generate DNA fragments with an average length of 100 to 1000 base pairs. After removal of cell debris by centrifugation, the chromatin concentration was
Renaturalization of the Circulating Episodic rGH Profile. The pattern of circulating rGH observed from serial blood samples collected from control rats infused with rGH vehicle (Fig. 1) exhibited typical sexually dimorphic GH profiles (Shapiro et al., 1995). Intact males secreted GH in episodic bursts (~250 ng/ml plasma) about every 3.5 to 4 h. Between the peaks, GH levels were undetectable. In intact females the hormone pulses occurred more frequently and irregularly and were of lower magnitude than those in males, whereas the interpulse concentrations of GH were always measurable. Intraperitoneal infusion of male and female HYPOX rats with 40 μg of rGH/kg b.wt. every 3.5 to 4 h produced an episodic circulating GH profile that was nearly identical to the hormone profile observed in intact male rats (Fig. 1).

Sexually Dimorphic Expression of P450 Isoforms by the Renaturalized Episodic GH Profile. Because restoration of the masculine GH profile to HYPOX rats is not, in itself, evidence of the treatment’s effectiveness, we examined expression levels of three GH-dependent P450 isoforms in the treated animals. We chose P450s that were each regulated differently by the episodic GH profile. As
reported (Pampori and Shapiro, 1996; Agrawal and Shapiro, 2000), we found that expression of the major male-specific CYP2C11 requires exposure to the episodic “on/off” masculine profile of GH secretion (Fig. 2). Although the feminine pattern of continuous hormone secretion nearly completely blocked CYP2C11 expression, total GH depletion from the circulation (e.g., HYPOX) allowed for CYP2C11 expression at 20 to 30% of intact male levels. Renaturalization of the masculine GH profile for almost 2½ days not only induced CYP2C11 expression in HYPOX males and females but also demonstrated the inherent sexually dimorphic responsiveness of CYP2C11 in which the isoform is more responsive to the inductive effects of the episodic profile in males than in females (Fig. 2). We observed, as previously reported (Waxman, 1992; Pampori and Shapiro, 1996; Agrawal and Shapiro, 2000) that male-specific CYP3A2 is maximally expressed in the HYPOX rat, almost disappears when GH is secreted continuously (i.e., the female pattern), but is only minimally expressed in the HYPOX rat, almost disappears when GH is secreted continuously (i.e., the female pattern), but is only minimally suppressed under the influence of episodic GH. Administration of the masculine GH profile to HYPOX rats for ~2½ days was sufficient to suppress CYP3A2 expression to male-like intact levels (Fig. 2). Also in agreement with previous reports (Pampori and Shapiro, 1996; Agrawal and Shapiro, 2000), after hypophysectomy, female-predominant CYP2A1 (male/female, ~1:3) concentrations declined ~65% in both sexes. Although not as effective as the feminine GH profile, ~2½ days of exposure to the masculine episodic GH profile increased CYP2A1 concentrations in both sexes ~50% above HYPOX levels (Fig. 2).

Although administration of the masculine episodic GH profile to HYPOX rats for almost 2½ days was not sufficient to completely restore all the P450 isoforms to levels observed in intact males [this might require up to 6 days of treatment (Agrawal and Shapiro, 2000)], it does indicate that the rGH regimen was capable of activating signaling pathways responsible for expression of the P450 isoforms.

Sexually Dimorphic Recruitment of Cytoplasmic Stat5A and Stat5B by the Episodic rGH Profile. After phosphorylation of Jak2, the next step in episodic GH induced signal transduction is recruitment of cytoplasmic Stat5 molecules for activation and nuclear translocation. In agreement with earlier reports (Waxman and O’Connor, 2006), after the last rGH pulse we observed that cytoplasmic concentrations of total Stat5B and the much less abundant Stat5A declined precipitously (~80%) in males to a nadir level within 5 min of the pulse (Fig. 4). Thereafter, total Stat5B and total Stat5A returned to prepulse levels after 60 and 100 min, respectively. Although there was no statistically significant sex differences in cytoplasmic Stat5B concentrations immediately preceding the rGH pulse, total Stat5A was somewhat (~25%, P < 0.01) higher in females at this time. The decrease in cytoplasmic Stat5A and Stat5B after the rGH pulse in females occurred as rapidly as in males but with a much smaller magnitude of decline (~30%, P < 0.01) than that observed in males. The concentrations of total Stat5A and total Stat5B in the hepatic

**Fig. 2.** Sex-dependent regulation of hepatic isoforms of cytochrome P450 by the masculine episodic rGH profile infused into HYPOX male and female rats. The masculine circulating profile was renaturalized by i.v. administration of 14 pulses of rGH, 1 pulse every 4 h, by use of our pulse simulator apparatus described elsewhere (Pampori et al., 1991). HYPOX rats were infused with either hormone or rGH diluent. Intact rats received no treatment. Rats were euthanized within 60 min of the final pulse, and expression levels were determined for hepatic CYP2C11 mRNA and protein (top), CYP3A2 mRNA and protein (middle), and CYP2A1 mRNA and CYP2A1-dependent testosterone 7α-hydroxylase (T 7αOH) (bottom). Values presented are the means ± S.D. of at least five animals for each treatment. *, p < 0.01 compared with diluent-treated HYPOX rats of the same sex. †, p < 0.01 comparing males with females receiving the same treatment.

<table>
<thead>
<tr>
<th>Isoform</th>
<th>MALE</th>
<th>FEMALE</th>
<th>MALE</th>
<th>FEMALE</th>
<th>MALE</th>
<th>FEMALE</th>
</tr>
</thead>
<tbody>
<tr>
<td>CYP2C11 (%)</td>
<td>120</td>
<td>120</td>
<td>120</td>
<td>150</td>
<td>120</td>
<td>150</td>
</tr>
<tr>
<td>CYP3A2 (%)</td>
<td>120</td>
<td>120</td>
<td>120</td>
<td>150</td>
<td>120</td>
<td>150</td>
</tr>
<tr>
<td>CYP2A1 (%)</td>
<td>120</td>
<td>120</td>
<td>120</td>
<td>150</td>
<td>120</td>
<td>150</td>
</tr>
</tbody>
</table>

**Table 2.** Sex-dependent regulation of hepatic isoforms of cytochrome P450 by the masculine episodic rGH profile infused into HYPOX male and female rats. The masculine circulating profile was renaturalized by i.v. administration of 14 pulses of rGH, 1 pulse every 4 h, by use of our pulse simulator apparatus described elsewhere (Pampori et al., 1991). HYPOX rats were infused with either hormone or rGH diluent. Intact rats received no treatment. Rats were euthanized within 60 min of the final pulse, and expression levels were determined for hepatic CYP2C11 mRNA and protein (top), CYP3A2 mRNA and protein (middle), and CYP2A1 mRNA and CYP2A1-dependent testosterone 7α-hydroxylase (T 7αOH) (bottom). Values presented are the means ± S.D. of at least five animals for each treatment. *, p < 0.01 compared with diluent-treated HYPOX rats of the same sex. †, p < 0.01 comparing males with females receiving the same treatment.
cytoplasm remained consistently higher in females for 45 to 60 min after the rGH pulse (Fig. 4).

**Sex Differences in Episodic GH-Directed Stat5B Binding to the CYP2C11 Promoter.** Because the masculine episodic rGH profile is the sole regulator of CYP2C11 expression (Legraverend et al., 1992; Waxman, 1992; Shapiro et al., 1995) and is significantly more effective when administered to males than to females (Shapiro et al., 1993; Thangavel et al., 2006), we examined the binding kinetics of phospho-Stat5B to the CYP2C11 promoter by ChIP assay (Fig. 5). In agreement with the expected sequence of signaling events, the maximum binding of activated Stat5B to the hepatic promoter occurred about 45 min after the rGH pulse in both sexes. However, about twice the amount of the transcription factor was bound to the CYP2C11 promoter in males than in females. PCR amplification of a negative control using primers flanking the CYP2C11 promoter at a genomic sequence not including the Stat5B binding site demonstrated no measurable nonspecific binding (Fig. 5). In confirmation of the ChIP results, using Southern blotting, we observed that twice as much of the Stat5B-binding motif of the CYP2C11 promoter bound to the activated transcription factor in hepatocytes from male rats (Fig. 5).

**Sexually Dimorphic Activation and Translocation of MAPK by the Episodic rGH Profile.** Having previously observed an association between episodic GH induction of CYP2C11 and the activation of extracellular signal-regulated kinases (Erk1 and Erk2) (Verma et al., 2005), we investigated whether the relationship might be sexually dimorphic. Just preceding the last rGH pulse (zero time), livers from male and females rats expressed low, but equal concentrations of cytoplasmic phospho-Erk1 and phospho-Erk2 (Fig. 6). Within 5 min after the rGH pulse, cytoplasmic levels of the activated kinases increased 3-fold in males, declining to prestimulation baseline concentrations within 30 min. In contrast, cytoplasmic phospho-Erk1 and phospho-Erk2 levels appeared unresponsive to the rGH pulse in females exhibiting approximately the same concentration during the 100-min observational period.

Unlike the cytoplasmic findings, nuclear levels of phospho-Erk1 and phospho-Erk2 immediately preceding the rGH pulse were twice as high in the males as in the females, suggesting, in males, persistence of residual levels from the previous rGH pulse.1 Basically, nuclear levels of phospho-Erk1 and phospho-Erk2 in males reflected the episodic rGH-induced changes in cytoplasmic levels: a 2-fold elevation in the kinases 5 min after the rGH pulse with levels returning to baseline within 25 min of the MAPK peaks (Fig. 6). For females, unlike their cytoplasmic concentrations that were unaffected by episodic rGH, nuclear levels of the activated kinases were significantly ($P < 0.01$) increased 5 min after the rGH pulse. Thereafter, nuclear phospho-Erk1 and phospho-Erk2 returned to prestimulatory baseline levels within 25 min. Nevertheless, the amount of activated MAPK observed in the nucleus, at every time point, was 2- to 4-fold higher in the male hepatocytes.

**Sex Differences in the Level of Cytoplasmic IκB-α by the Episodic rGH Profile.** Because activation of the NF-κB pathway has been associated with the regulation of CYP2C11 (Iber et al., 2000) and CYP2C11 expression is dependent upon the episodic GH profile (Shapiro et al., 1995), we connected the “dots” and examined the sexually dimorphic response of IκB-α to the masculine GH profile. There was no sex difference in cytoplasmic IκB-α concentration at zero time just preceding the final pulse of the ~2.5 days of episodic rGH treatment (Fig. 7). However, in the case of males, there was a dramatic decline (75%) in cytoplasmic IκB-α levels within 5 min of the rGH pulse. Thereafter, protein levels were slowly restored to the prepulse concentration after 100 min. The response in the females was quite different. There was neither a dramatic nor immediate rGH-induced decline in IκB-α levels in females (Fig. 7). Rather, we observed a gradual decline in the concentration of the protein inhibitor (20%) requiring 60 min to reach its nadir and an additional 40 min to return to preactivation levels.

1 Because this is an in vivo study, and MAPK is known to mediate the activities of numerous factors in addition to GH, it is possible that the elevated nuclear phospho-Erk1 and phospho-Erk2 baselines in male liver were due to some normally occurring, but confounding, male-specific “signal” other than GH.
Fig. 5. Sex-dependent levels of Stat5B binding to the CYP2C11 promoter in HYPOX rats administered the masculine episodic rGH profile. The masculine circulating GH profile was renaturalized in HYPOX male and female rats by infusing, via an indwelling i.a. catheter, 14 pulses of rGH, 1 pulse every 4 h. Rats were euthanized at different time points between 0 and 100 min after the last pulse. Graphic quantitation of Stat5B binding to the CYP2C11 putative promoter (ChIP assay, left) and the occupied Stat5B-binding motif in the CYP2C11 promoter (Southern blot, right) as well as representative ChIP assay and Southern blots (bottom) were determined by procedures described under Materials and Methods. Values presented are the means ± S.D. of at least five animals at each time point. *, p < 0.01, comparing males with females at the same time point. Absolute values should not be compared between panels.

Fig. 6. Sex-dependent phospho-Erk1 and phospho-Erk2 levels in HYPOX rats after i.v. administration of the masculine episodic rGH profile. The masculine circulating GH profile was renaturalized in HYPOX male and female rats by infusing, via an indwelling i.a. catheter, 14 pulses of rGH, 1 pulse every 4 h. Rats were euthanized at different time points between 0 and 100 min after the last GH pulse. Phospho-Erk1 and phospho-Erk2 were measured in the cytoplasmic and nuclear fractions of livers using Western blot analysis as described under Materials and Methods. Values presented are the means ± S.D. of at least five animals at each time point. *, p < 0.01, comparing males with females at the same time point. Absolute values should not be compared between panels.
Because the differential response of hepatic P450 isoforms in male and female rats to the episodic GH profile is physiologic (Shapiro et al., 1993; Waxman et al., 1995), it seemed germane to investigate the possible mechanism(s) responsible for this sex dimorphism under in vivo physiologic GH conditions. Moreover, the use of HYPOX rats allowed us to examine the selective role of GH in the absence of any possible confounding effects resulting from sex differences in other hormones, such as glucocorticoids and sex steroids. Accordingly, we successfully restored the typical as well as an indistinguishable masculine episodic circulating GH profile in both HYPOX male and female rats for ~2.5 days that effectively induced male-like levels of hepatic female predominant CYP2A1 as well as male-specific CYP2C11 and CYP3A2 in both sexes. Because, as expected from previous reports (Shapiro et al., 1993; Thangavel et al., 2006), the inductive response of CYP2C11 to the masculine GH profile was significantly greater in male than in female rats and the actions of the GH profile are mediated, at least in part, by the Jak2/Stat5B signal transduction pathway (Verma et al., 2005; Waxman and O’Connor, 2006), we examined episodic rGH-induced activation of the Jak2/Stat5B pathway in both sexes.

GH signaling in liver by the episodic profile (in contrast to the continuous profile) is initiated by hormone binding to and the resulting activation of GH receptors on the surface of target cells. This allows for the recruitment and/or activation of two molecules of Jak2, which then cross-phosphorylate each other as well as phosphorylating the receptor on key tyrosine residues. Stat5B, a latent transcription factor, binds to these phosphorylated receptor docking sites, undergoes Jak2-catalyzed tyrosine phosphorylation, homodimerizes, and translocates to the nucleus where it binds to promoter sites initiating transcription of GH-regulated genes (Waxman and O’Connor, 2006). For this signal transduction pathway to be optimally effective, threshold levels of Stat5B (and possibly Jak2) have to be activated, translocated into the nucleus, and then bind to the promoter of GH-regulated genes (Choi and Waxman, 2000; Verma et al., 2005). Because all of these signal transduction events were blunted by at least 50% in the females, it seems reasonable to conclude that the sexually dimorphic response of hepatic CYP2C11 to episodic GH is due to a failure by the hormone to stimulate an optimum cascade of signal transducers in the target cells of females.

The reason(s) for this reduced responsiveness of the Jak2/Stat5B signaling pathway to episodic GH in females is far from clear. Because activation of the Jak2/Stat5B pathway, alone, is not sufficient to explain the actions of the episodic GH profile, other signaling factors have been considered. Previous studies using transfected cell lines (Waxman and O’Connor, 2006) and primary hepatocytes (Thangavel and Shapiro, 2007) have implicated hepatocyte nuclear factors and suppressors of cytokine signaling, respectively, in regulating the sexually dimorphic responsiveness of the Jak2/Stat5B pathway to the masculine GH profile. In an earlier in vivo study (Verma et al., 2005), HYPOX male rats were infused with single, but variable, doses of rGH whose resulting plasma pulse amplitudes varied from the physiologic (~250 ng/ml plasma) to the barely detectable (~3 ng/ml of plasma). Whereas all the doses of rGH stimulated Stat5B phosphorylation and its nuclear translocation (albeit, at considerably lower levels in males exposed to the smallest rGH pulses), only those rGH doses stimulating an activation and nuclear accumulation of MAPK also exhibited an induction of CYP2C11 transcription. The present findings demonstrate a similar correlation between episodic GH-induced expression of CYP2C11 and the activation and accompanying nuclear translocation of Erk1 and Erk2. That is, females who responded to the episodic GH profile with lower expression levels of CYP2C11 than males also exhibited lower levels of activated nuclear translocated Erk1 and Erk2. Although the present finding supports earlier reports of GH stimulation of MAPK phosphorylation (Pircher et al., 1999; Piwien-Pilipuk et al., 2002), the involvement of Erk1 and Erk2 in episodic GH regulation of CYP2C11 expression remains circumstantial. Clearly, the masculine GH profile regulates the sex-dependent expression of numerous genes other than CYP2C11 (Ahluawalia et al., 2004) whose induction may be MAPK-dependent. Nevertheless, Erk activation/deactivation cycles are differentially regulated by the circulating profiles of hormones such as gonadotropin-releasing hormone (Kanasaki et al., 2005), and p42/p44 MAPK is a known down-regulator of the Jak/Stat signaling pathway (Valgeirs-döttrir et al., 1999; Piwien-Pilipuk et al., 2002), suggesting the possibility that MAPK may be involved in the deactivation and subsequent activation cycling of Stat5B by the masculine GH profile requisite for inducing expression of CYP2C11 (Waxman and O’Connor, 2006). Whether the suboptimal activation and translocation of Erk1 and Erk2 by episodic GH in females is responsible for the reduced expression of CYP2C11 is not clear. But, were MAPK involved in CYP2C11 expression, it is possible that although the lower levels of nuclear Erk1 and Erk2 in the females permitted some cycling of Stat5B, it was insufficient for optimum CYP2C11 expression. Irrespective of any possible role for Erk1 and Erk2 in CYP2C11, the results do demonstrate an inherent sex difference in the response of the MAPK signaling pathway to the masculine GH profile.

Another signal transduction pathway implicated in CYP2C11 expression is inflammatory-induced NF-κB (Ibler et al., 2000). There are, however, no reports examining a relationship between sex, GH, and NF-κB in regulating CYP2C11 expression. Accordingly, we conducted an initial experiment to probe a possible association. Members of the NF-κB family share a highly conserved homology domain that is responsible for DNA binding, dimerization, and interaction with IκBs. The activity of NF-κB is tightly regulated by its interaction with inhibitory IκB proteins. NF-κB is sequestered in the cytoplasm.
in an inactive form associated with inhibitory IκB proteins. This interaction blocks the ability of NF-κB to enter the nucleus and bind to DNA. After exposure to stimuli, usually proinflammatory agents, the NF-κB signaling cascade is activated as a result of the phosphorylation and subsequent polyubiquitination-dependent degradation of IκB. This allows the translocation of unmasked NF-κB from the cytoplasm to the nucleus where it binds to NF-κB response elements in target genes and regulates their transcription. Functioning through a feedback loop, one of the target genes activated by NF-κB is that encoding IκB-α. Newly synthesized IκB-α can enter the nucleus, remove NF-κB from DNA, and export the complex back to the cytoplasm to restore its original latent state and prepare for another activation cycle (Liu and Malik, 2006).

Our findings indicate that the masculine GH profile induces a rapid decline in cytoplasmic IκB-α only in male rats. Cytoplasmic concentrations of the inhibitory protein in females were unaffected by the episodic GH profile. The decline in cytoplasmic IκB-α in males was presumably a result of its rapid degradation, leading to the activation and nuclear translocation of NF-κB. Although results are contradictory (Yi et al., 2006), there is evidence that GH can activate NF-κB (Jeay et al., 2000). Persistent activation of the NF-κB pathway during inflammation can negatively regulate CYP2C11 expression by occupying a NF-κB-responsive element in the regulatory sequence of the CYP2C11 gene (Iber et al., 2000). However, activated Stat5B appears to reduce NF-κB activity (Han et al., 2007) by directly (Iber et al., 2000), or by indirectly competing for a nuclear factor(s) necessary for NF-κB-mediated activation of its target promoters (Luo and Yu-Lee, 2000), e.g., CYP2C11. CYP2C11 expression is absolutely dependent upon episodic or cycling events. Only the masculine episodic GH profile can induce CYP2C11 expression. The episodic GH profile is responsible for the optimum, albeit episodic (on/off), Stat5B activation and binding to the CYP2C11 promoter. Although speculative, the possibility that the GH pulse in the episodic profile activates NF-κB by stimulating IκB-α degradation exists. NF-κB competes with Stat5B at the CYP2C11 promoter, where levels are already quickly declining from that induced by the previous pulse. Transcription of the CYP2C11 gene is temporarily interrupted. Subsequently, concentrations of activated Stat5B once again increase in the nucleus, competing and replacing NF-κB on the CYP2C11 promoter and again inducing CYP2C11 transcription. Thus, the requirement of episodic (on/off) stimulation for CYP2C11 expression is fulfilled. Why the episodic GH profile is presumably unable to activate the NF-κB pathway in females is not clear.

Our results suggest that reduced induction levels of CYP2C11 in female rats exposed to the masculine GH profile is probably due, at least in part, to suboptimum activation of the Jak2/Stat5B signaling pathway. Whether the reduced activation of the MAPK and/or NF-κB pathways, also observed in females exposed to the episodic GH profile, are directly involved in the reduced expression levels of CYP2C11 is suggestive. However, the findings do demonstrate inherent sex-dependent differences in the responsiveness of the Jak2/Stat5B, MAPK, and NF-κB signal transduction pathways to the circulating masculine GH profile. The effects of this sexual dimorphic response to GH is not limited to the expression of male- and female-dependent P450 isoforms in rats (Shapiro et al., 1993; Waxman et al., 1995; Pampori and Shapiro, 1999; Thangavel et al., 2004). In the case of humans, GH deficiency causes numerous abnormalities in growth rates; lean body mass; cardiovascular, bone, adipose tissue, and muscle function; protein, carbohydrate, lipid, and electrolyte metabolism (Kuromaru et al., 1998; Span et al., 2001; Hubina et al., 2004; Jørgensen and Christiansen, 2005); and expression levels of hepatic insulin-like growth factor-1, insulin-like growth factor binding protein, growth hormone binding protein (Johansson et al., 1999; Hubina et al., 2004; Soares et al., 2004; Jørgensen and Christiansen, 2005), and cytochrome P450-dependent drug-metabolizing enzymes (Gil Berglund et al., 2002; Dhir et al., 2006). However, hormone replacement therapy has clearly demonstrated an intrinsic, irreversible sexually dimorphic response. Daily injections, evoking the masculine-like episodic GH profile, the most feasible, yet efficacious therapeutic approach, are significantly more effective in correcting these GH deficiency abnormalities in men (or boys) and women (or girls) (Kuromaru et al., 1998; Johansson et al., 1999; Span et al., 2001; Gil Berglund et al., 2002; Hubina et al., 2004; Soares et al., 2004; Dhir et al., 2006), suggesting that intrinsic sex differences in GH-activated signal transduction pathways in humans are similar to those in rats.

References
Pampori NA and Shapiro BH (1999) Gender differences in the responsiveness of the sex-

Address correspondence to: Dr. Bernard H. Shapiro, Laboratories of Biochemistry, School of Veterinary Medicine, University of Pennsylvania, 3800 Spruce St., Philadelphia, PA 19104-6048. E-mail: shapirob@vet.upenn.edu