Developmental changes in the human GH receptor and its signal transduction pathways

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Abstract

We previously reported the presence of functional human GH receptors (hGHRs) in the human fetal hepatocyte (FH) as early as the first trimester. Interestingly, fetal serum levels of hGH are in the acromegalic range, yet certain hGH-dependent factors are expressed at very low levels (IGF-I, IGF-binding protein-3), suggesting that fetal liver has limited responsiveness to hGH. To determine whether this is due to the fetal tissue levels of hGHR or factors in the hGH/hGHR axis that might influence hGHR function, we compared hGHR isoforms and downstream signaling proteins in FH versus human adult liver (HAL). Immunoprecipitation/immunoblotting (IB) analyses found similar precursor and mature hGHR forms while RT-PCR assays of truncated (T) hGHR1–279, dominant negative for the full-length (FL) receptor, showed similar T/FL mRNA ratios in FH and HAL. IB demonstrated that Janus kinase (JAK) 2, signal transducers and activators of transcription (STAT(1, 3, 5A/B)), and suppressors of cytokine signaling (SOCS(1, 2, 3, cytokine-inducible SH2-containing protein (CIS))) proteins were detectable in all FH and HAL tested (12 weeks of fetal age to 60 years); the levels were similar (STAT5B) or lower (JAK2/STAT1/STAT3/STAT5A: 38–53%, SOCS/CIS: 58–76%) in FH compared with HAL. Our studies to date demonstrate that, during hepatocyte development, hGHR levels are lower in the fetal cells but the hGHR isoforms, including the relative amount of truncated versus FL, remain unchanged. The JAK2/STAT/SOCS signaling molecules are present in the FH as early as the first trimester. However, they are generally at <50% level in postnatal liver. These data suggest that low expression of both hGHR and major hGHR signaling components may explain the limited responsiveness of the fetal cells to the high circulating levels of hGH.


Introduction

The human growth hormone receptor (hGHR), a member of class I cytokine receptor superfamily, is a 620 amino acid peptide containing an extracellular hormone-binding region, a single transmembrane segment, and a cytoplasmic signaling domain. It is encoded by a single gene spanning more than 300 kb on chromosome 5p13.1–12 (Leung et al. 1987, Barton et al. 1989, Wei et al. 2006). Three hGHR isoforms have been reported to date: full-length (FL), exon 3 deleted (d3), and truncated (T). Transcripts for all three isoforms have been detected in human tissues from early in fetal life (Esposito et al. 1994, Zogopoulos et al. 1996b, Goodyer et al. 2001a).


Two truncated hGHRs, 1–277 and 1–279, are produced at low levels in all human tissues examined, including fetal liver and placenta, with variable truncated to FL receptor (T/FL) ratios among the tissues (Dastot et al. 1996, Ballesteros et al. 2000, Fisker et al. 2001, 2004, Goodyer et al. 2001a). These truncated receptors are capable of binding hGH with high affinity, but they do not activate intracellular signal transduction pathways and, in fact, act as dominant-negative receptors (Ross et al. 1997, Ayling et al. 1999, Iida et al. 1999).

GH binds with high affinity to two molecules of hGHR, forming a stable heterotrimer complex (De Vos et al. 1992, Frank 2002, Gent et al. 2003, Waters et al. 2006, Brooks et al. 2008). Because the GHR is characterized by the absence of intrinsic catalytic activity within its intracellular domain, signal transduction through this receptor is mediated primarily by Janus kinase (JAK) 2, a cytoplasmic tyrosine kinase that...
Developmental changes in hGH signaling

However, their functions during human gestation remain unclear. Only a subset of newborns with hGH deficiency or hGHR dysfunction have decreased birth length, suggesting that, while fetal hGH can affect fetal growth, the presence of other fetal growth factors (e.g., insulin, placental lactogen, prolactin) ensures relatively normal intrauterine growth (Gluckman et al. 1992, Wit & van Unen 1992, Woods et al. 1997, Jensen et al. 2005, Mehta et al. 2005, Osafo et al. 2005).

Interestingly, fetal plasma levels of immunoreactive hGH are significantly elevated relative to the normal adult, up to 150 ng/ml at mid-gestation (Kaplan et al. 1976). This is likely due to the greater sensitivity of the human fetal pituitary somatotrope to its hypothalamic stimulatory factor, GH-releasing hormone (GHRH), than its inhibitory factor, somatostatin, throughout in utero development (Delitala et al. 1978, Goodyer et al. 1993a,b). Similar hGH levels postnatally cause highly elevated serum levels of insulin-like growth factor-I (IGF-I) and IGF-binding protein-3 (IGFBP-3), resulting in gigantism in the prepubertal child and acromegaly in the adult (Kato et al. 2002). However, circulating levels of IGF-I and IGFBP-3 in the fetus are low, reaching significant levels only late in the third trimester (Butler & Le Roith 2001, Gohlke et al. 2004), suggesting a blunted responsiveness of fetal tissues to hGH during earlier stages in development, at either the hGH receptor or the signaling level.

hGHR mRNA and protein have been identified in a wide variety of human tissues from the first trimester of fetal life (Hill et al. 1992, Simard et al. 1996, Zogopoulos et al. 1996a,b). There is a decrease in lung, kidney, and intestinal levels of hGHR postnatally and a significant increase in liver when early to mid-gestation fetal versus adult tissues are compared (Goodyer et al. 2001a). We and others have previously demonstrated that the hGHR present in fetal hepatocytes (FH) specifically binds hGH, and that these cells have biological responses (proliferation, IGF production, glucose uptake) following exposure to hGH, although the effects are lower compared with adult hepatocytes (Strain et al. 1987, Hill et al. 1988, 1989, Goodyer et al. 2001a).

In the present study, we have tested the hypothesis that, in addition to lower tissue levels of hGHR, other factors, including the hGHR protein forms (FL versus truncated) and the JAK/STAT and SOCS/CIS families of downstream signal transduction molecules, may be responsible for the limited responsiveness of FHs to the high circulating levels of hGH.

Materials and Methods

Materials

A rabbit antibody raised against the intracellular domain of the hGHR (AL47) was a generous gift from Dr Stuart Frank (University of Alabama, Birmingham, AL, USA) (Zhang et al. 2001). Antibodies directed against hGH signaling proteins were obtained from the following
commercial sources: JAK2 from Imgenex (IMG-3007; San
Diego, CA, USA); SOCS1 (ab3691-100) and SOCS2
(ab3692-100) from Abcam (Cambridge, MA, USA);
SOCS3 (sc-9023) and CIS (sc-1529) from Santa Cruz
Biotechnology, Inc. (Santa Cruz, CA, USA); and STAT1
(UBI 06-501), STAT3 (UBI 06-596), STAT5A (UBI
06-553), and STAT5B (UBI 06-554) from Upstate
Biotechnology, Inc. (Lake Placid, NY, USA). Rabbit
and mouse IgGs were obtained from Santa Cruz. A mouse
monoclonal antibody for calnexin (C45520), the immuno-
blot loading control, was purchased from Transduction
Laboratories (Lexington, KY, USA). The HRP-labeled
secondary antibodies used were anti-rabbit (NEF812) and
anti-mouse (NEF822) from Perkin–Elmer Life Sciences Inc.
and two human postnatal hepatoma cell lines, HepG2
human embryonic kidney epithelial cells (HEK293 epithelial cells, ATCC),
and two human postnatal hepatoma cell lines, HepG2
(redistributed (Mini Trans-blot Cell; Bio-Rad Laboratories, Inc.) to
proteins were resolved by SDS-PAGE (8–15%) and trans-
ferred (15–60 years, donors were all males). Ethics approval for the
study was obtained from local institutional research ethics
boards, in accordance with the Canadian Council on Health
Sciences Research Involving Human Subjects guidelines.
Written informed consent was obtained in each case. Four
primate cell lines were analyzed: CV1 (African green
monkey kidney fibroblasts, ATCC), human embryonic
kidney epithelial cells (HEK293 epithelial cells, ATCC),
and two human postnatal hepatoma cell lines, HepG2
(HEK293, and HepG2 cells were cultured in DMEM (Invitrogen) supplemented with 10%
fetal bovine serum (FBS), 100 IU/ml penicillin G, and
1.6 mg/ml gentamicin sulfate. HepG2 cells were cultured in
Earle’s MEM (Invitrogen) supplemented with 10% FBS,
7.5 mM HEPES, 100 IU/ml penicillin G, and 1.6 mg/ml
gentamicin sulfate. All cells were maintained at 37 °C with
5% CO₂ in a humid environment.

FH preparations
Fetal hepatic tissues were minced and dispersed by
collagenase, followed by gravity separation, yielding >95%
pure hepatocytes, as described previously (Zogopoulos et al.
1996a). The isolated hepatocytes were plated onto collagen-
coated Petri dishes in William’s E medium (Invitrogen) with
10% FBS, 100 IU/ml penicillin G, 1.6 mg/ml gentamicin
sulfate, and 39.2 μg/ml demethylasone. Two hours after
plating, the hepatocytes were rinsed thoroughly (three to five
times) with 1× PBS to remove all remaining traces of
hematopoietic cells, replaced with fresh, complete William’s
E medium with 39.2 μg/ml dexamethasone, and incubated
overnight. After 24 h, hepatocytes were washed with 1× PBS and collected by scraping. The hematopoietic cells
were pelleted, flash frozen, and stored at −80 °C until extracted for RNA.

Protein and RNA extractions
Homogenized tissue fragments or cell pellets were solubilized in ice-cold lysis buffer (50 mM Tris (pH 7.5), 0.1% Triton
X-100, 150 mM NaCl, and 2 mM EDTA) containing protease and phosphatase inhibitors (complete cocktail tablets
(Roche Diagnostics)) for 15 min on ice. To remove insoluble
material, lysates were centrifuged at 13 000 g for 10 min at
4 °C. The supernatants were collected and protein concentra-
tions measured with the Bradford kit (Bio-Rad Labora-
tories, Inc.), using BSA as a standard. Alternatively, tissue
fragments and the cell pellets were extracted for RNA using the
TRIzol method (Invitrogen).

RT-PCR assay for FL versus truncated hGHR
For each RT reaction, hepatic total RNA (5 μg) was reverse transcribed for 1 h at 42 °C in the presence of 200 U Super-
script II (Invitrogen) and a specific exon 9 reverse primer (9AS)
(5'-TAATCTTGTGAATGACTG-3') designed to recognize both the FL and truncated (1–279) hGHR mRNAs
(Booher et al. 2001a). RT products (3 μl) were amplified for 35 cycles
with 1.25 U Taq DNA polymerase (Invitrogen), hGHR
 exon 7 sense (7S: 5'-TAAGAAGATATGAGGGTG-3'), and hGHR exon 9 antisense primers. The cycles
consisted of one cycle at 94 °C for 2 min, followed by 35 cycles of
94 °C for 30 s, 52 °C for 30 s, and 72 °C for 1 min, ending
with a final elongation at 72 °C for 10 min. In each assay, an
aliquot of H₂O was amplified in parallel as a negative PCR
control. PCR products were separated on 10% polyacrylamide
gels and stained with SybrGold (Molecular Probes, Eugene,
OR, USA). Densitometric analyses were carried out using the
Bio–Rad GelDoc system.

Immunoprecipitation and immunoblotting
Liver lysate protein (0.5–1 mg) were incubated by rotation in
lysis buffer described above with anti-hGHR (Al47, 1:250)
overnight at 4 °C, followed by the addition of 50 μl protein
G-agarose beads (Amersham Biosciences, Baie, D’urfe, QC,
Canada) for 1 h at 4 °C. Antibody complexes were washed
three times with lysis buffer followed by a single wash with
50 mM Tris (pH 8.0). hGHR immunoprecipitates or
50–100 μg whole cell extracts were boiled for 5 min in
Laemmli loading buffer (50 mM Tris (pH 6-8), 2% SDS, 0-1% bromophenol blue, 10% glycerol, and 100 mM DTT) and
proteins were resolved by SDS-PAGE (8–15%) and trans-
ferred (Mini Trans-blot Cell; Bio-Rad Laboratories, Inc.) to
polyvinylidene fluoride Immobilon-P transfer membranes
(Millipore Corporation, Mississauga, ON, Canada).
Developmental changes in hGH signaling

The membranes were blocked overnight at 4°C with 5% non-fat dry milk in PBST buffer, and then incubated with the appropriate primary antibody (hGHR AL47 (1:2000), JAK2 (1:700), STAT1 (1:1000), STAT3 (1:1000), STAT5A (1:1000), STAT5B (1:1000), SOCS1 (1:1000), SOCS2 (1:1000), SOCS3 (1:200), CIS (1:100), or calnexin (1:1000)) for 3 h at room temperature. After washing three times with PBST, the membranes were incubated with an anti-mouse (1:2000), anti-rabbit (1:1000), or anti-goat (1:1000) IgG conjugated to horseradish peroxidase for 1 h at room temperature. Protein bands were visualized by an enhanced chemiluminescence detection system (Perkin–Elmer Life Sciences Inc).

To probe the same membrane for another protein, the membranes were incubated for 30 min at 50°C with stripping buffer (100 mM β-mercaptoethanol, 2% SDS, 62-5 mM Tris (pH 6-8)). Calnexin was used to normalize for protein loading; pilot western blots showed a linear increase in calnexin protein levels over 20–80 μg of both fetal and postnatal hepatic protein (Supplementary Figure 1, see Supplementary data in the online version of the Journal of Endocrinology at http://joe.endocrinology-journals.org/content/vol198/issue1/). Densitometric analyses were carried out using the Bio-Rad GelDoc system.

Statistical analyses

Data are presented as Mean±S.E.M. Differences between two groups were analyzed using Student’s t-test (two tailed). Differences among several groups were analyzed using ANOVA followed by Bonferroni’s multiple group comparison test.

Results

Analysis of hGHR molecular forms in human FHs versus postnatal liver

We previously analyzed hGHR mRNAs from fetal and postnatal hepatocytes and found transcripts for FL, truncated (1–279), and exon 3 forms at both developmental stages (Zogopoulos et al. 1996b, Goodyer et al. 2001a). In the present study, FH (n = 2, 13-8, and 15 weeks FA) and postnatal liver (n = 2, 15, and 47 years) samples were analyzed for hGHR protein by immunoprecipitation (IP) followed by immunoblotting (IB), using the same antibody (AL47) (Fig. 2). Two major forms of the hGHR, precursor (∼70 kDa) and mature (∼120 kDa), were detected in all cases. Since the antibody used for the IP/IB analyses was directed against the intracellular domain of the hGHR, we were only able to identify the FL, as opposed to the truncated, forms (Zhang et al. 2001). These data demonstrate that the FL hGHR forms are similar at the two developmental stages.
Truncated (1–279) hGHR mRNA in fetal tissues

To place these T/FL ratios in a biological context, we also assessed the relative abundance of T and FL hGHR mRNAs in other fetal tissues. Like the fetal liver, all nine other tissues predominantly expressed the FL hGHR transcript (Fig. 3E and F). The dominant-negative T hGHR mRNA was also detected in all tissues, but T/FL hGHR mRNA ratios varied considerably, from a mean of 0.002 in skin to means of 0.53 and 0.65 in kidney cortex and medulla respectively (Fig. 3E and F).

Characterization of T/FL hGHR mRNA isoforms in human and primate cell lines

Four different human and primate cell lines (CV1, HEK293, HepG2, and Huh7) that are routinely used as cell models to examine hGHR binding and signaling pathways were also analyzed for the different hGHR isoforms. The Huh7 hepatoma and CV1 cell lines demonstrated relatively high T/FL ratios (Huh7: 1.48 ± 0.26, CV1: 1.27 ± 0.19), while the HEK293 cell ratios were quite low (0.19 ± 0.05) (Fig. 3G and H).

Figure 3 Analysis of full-length (FL) and truncated (T) (1–279) hGHR mRNA. (A) A specific primer in exon 9 (9AS) was designed to recognize both the FL and T forms of hGHR mRNA for RT-PCR assays. (B) Pilot RT-PCR assays showed that there were no significant differences in T versus FL hGHR amplicon amplification over a wide range of PCR cycle number, in both fetal and postnatal hepatic samples (n = 3 each). (C and D) Comparison of FL and T hGHR mRNAs in intact fetal liver (n = 12, 12.7–19.5 weeks), postnatal liver (n = 3, 37–60 years), fetal hepatocytes (n = 7, 11.5–17.5 weeks), and fetal liver hematopoietic cells (n = 5, 12.7–19.5 weeks) by RT-PCR. (C) Representative polyacrylamide gel of PCR products detects FL hGHR mRNA at 252 bp and T hGHR mRNA at 226 bp. (D) Comparative data for intact postnatal liver, intact fetal liver, fetal hepatocytes, and fetal hematopoietic cells, expressed as a ratio of T/FL transcripts (Mean ± S.E.M.). Statistical differences are calculated relative to fetal hepatocytes: ***P < 0.001. (E and F) Comparison of FL and T hGHR mRNAs in various fetal tissues. (E) Representative polyacrylamide gel of PCR products. pBS-SK<sup>+</sup>/Hpa II marker is indicated at 242 bp. CNS, central nervous system. (F) T hGHR mRNA is detected in a wide variety of fetal tissues. Each tissue type was tested using three different samples and the final data are expressed as a ratio of T/FL transcripts (Mean ± S.E.M.). Statistical differences are calculated relative to intact fetal liver: *P < 0.05, **P < 0.01. (G and H) Comparison of FL and T hGHR mRNAs in various primate cell lines. (G) Representative polyacrylamide gel of PCR products, pBS-SK<sup>+</sup>/Hpa II marker is indicated at 242 bp. (H) Graphic analysis of T/FL hGHR mRNA in three cell lines and human adult liver (HAL) (HepG2 band densities were too low to be accurately analyzed). Each sample was tested using three to five different RNA pools (Mean ± S.E.M.). Statistical differences are calculated relative to HAL: *P < 0.01, ***P < 0.001.
Unfortunately, the HepG2 band densities were too low to be analyzed accurately.

Expression of JAK/STAT proteins in human fetal hepatocytes and intact liver versus postnatal liver

JAK2 and STAT1, STAT3, STAT5A, and STAT5B are major members of the hGH/hGHR intracellular signaling cascade (Lanning & Carter-Su 2006). To determine whether they are expressed in lower amounts in FHs, thereby decreasing responsiveness to hGH, we analyzed their levels in human intact fetal livers \((n=3, 10.75–18\) weeks), FHs \((n=3, 12–18\) weeks), and postnatal livers \((n=3, 15–60\) years). These five proteins were found in all of the samples tested demonstrating that they are expressed as early as the third month of fetal life (Fig. 4A and B). All but STAT5B were lower in FHs compared with postnatal liver; this difference was significant for STAT1 \((42\%, P<0.05)\) (Fig. 4A). Although a similar trend was seen for JAK2 \((38\%)\), STAT3 \((38\%)\), and STAT5A \((53\%)\), they did not reach statistical significance, likely due to the small sample size. Unfortunately, hepatocyte preparations require substantial amounts of fetal liver tissue and these are in limited supply.

Interestingly, we observed quite different levels of the JAK/STATs when we analyzed intact human fetal versus postnatal liver samples (Fig. 4B). While STAT1 and STAT3 were still lower than human adult liver (HAL) \((129\%)\), JAK2, STAT5A, and STAT5B were markedly increased \((100\%, P<0.06; 680\%, P<0.01; \text{and } 130\%, P<0.03\) respectively). These results, along with the T/FL hGHR data, indicate that the presence of hematopoietic cells in the intact fetal liver can markedly influence tissue biological expression patterns. In addition, the data suggest that the JAK2/STAT5A and STAT5B pathways may be very important for fetal hematopoietic cell function. Because of the limited number of hepatocyte preparations, we were, however, unable to carry out parallel studies in isolated hematopoietic cells.

Expression of SOCS proteins in human fetal hepatocytes and intact liver versus postnatal liver

SOCS proteins inhibit hGHR signaling in hGH target cells, including the hepatocytes (Flores-Morales et al. 2006). To test whether increased levels might be responsible for a lower response to hGH in fetal tissues, we compared the levels of SOCS/CIS proteins in human fetal livers \((n=3, 10.75–18\) weeks), FHs \((n=3, 12–19\) weeks), and postnatal livers \((n=3, 15–60\) years). The four SOCS proteins were detected in all of these samples (Fig. 5A and B). There were markedly lower levels of the inhibitory proteins in the FHs relative to the postnatal

![Figure 4](https://example.com/figure4.png)

**Figure 4** Analysis of JAK/STAT proteins. Representative immunoblots of JAK/STAT and calnexin expression in (A) fetal hepatocytes and (B) fetal liver. Relative abundance of JAK2 \((120\text{ kDa})\), STAT1 \((91\text{ kDa})\), STAT3 \((92\text{ kDa})\), STAT5A \((95\text{ kDa})\), and STAT5B \((92\text{ kDa})\) proteins in (A) fetal hepatocytes \((n=3, 12–18\) weeks) or (B) fetal liver \((n=3, 10.75–18\) weeks) \((Mean \pm S.E.M)\). Data are normalized to calnexin \((90\text{ kDa})\) and compared with postnatal liver (HAL: \(n=3, 15–60\) years). Statistical differences are expressed relative to HAL: *\(P<0.05\), **\(P<0.03\), ***\(P<0.01\).
livers: SOCS1 (61%), SOCS2 (76%), SOCS3 (61%), and CIS (58%), although these changes did not achieve statistical significance, again likely due to limited sample numbers (Fig. 5A). Thus, human FHs do contain the four SOCS family members from as early as 12 weeks of FA, but they are present at less than half the levels observed in postnatal livers.

When we analyzed the SOCS levels in intact human fetal livers, we observed that CIS was significantly decreased compared with the postnatal livers (CIS: 67%, P<0.04), while there were consistent decreases in the other family members: SOCS1 (31%), SOCS2 (41%), and SOCS3 (35%) (Fig. 5B). In contrast to the JAK/STAT results, these data are similar to the findings obtained for the FHs, suggesting that the SOCS/CIS family members are present at similar levels in both the fetal hematopoietic and hepatic cells.

Discussion

Although pivotal roles for GH, its receptor and their downstream signal transduction pathways have been well established in postnatal tissues, their functions during gestation remain unclear (Hill 1992, Symonds et al. 2001, Waters & Kaye 2002). Surprisingly, fetal serum levels of GH are tenfold higher than those in the postnatal period in both primates and subprimates (Gluckman et al. 1981). We have previously demonstrated that this may be because human fetal somatotropes have a much higher sensitivity to GHRH than somatostatin (Goodyer et al. 1993a,b). In corroboration, clinical studies have shown that newborns have an exaggerated response to GHRH and a delayed and blunted response to somatostatin (Delitala et al. 1978, Shimano et al. 1985). Postnatally, these elevated hGH levels would cause high serum levels of IGF-I and IGFBP-3, resulting in gigantism in a prepubertal child and acromegaly in an adult (Kato et al. 2002). However, in the fetus, serum IGF-I and IGFBP-3 levels are very low or undetectable until late in the third trimester (Butler & Le Roith 2001, Gohlke et al. 2004). These data suggest that fetal tissues have a relatively low responsiveness to hGH.

In subprimates, the onset of significant GHR mRNA expression occurs late in gestation (Adams et al. 1990, Walker et al. 1992, Ymer & Herington 1992). In the human fetus, ubiquitous transcription of the hGHR gene has been documented from as early as the first trimester; postnatal changes in hGHR mRNA expression are tissue specific, with decreases in the lung, kidney, and intestine but a sixfold increase in liver after birth (Zogopoulos et al. 1996a,b, Goodyer et al. 2001b). Binding (Goodyer et al. 2001a), immunohistochemical (Hill 1992, Hill et al. 1992, Simard et al. 1996), and IB experiments (present study) have identified functional hGHR protein in multiple human tissues from as early as 9–11 weeks of fetal life. In the present study, we asked whether there are factors other than the levels of hGHR, which may be responsible for the decreased responsiveness to hGH in fetal tissues. We focused on...
several hGHR isoforms and their downstream signaling pathway molecules, comparing their levels in FHs versus postnatal liver.

Based on its amino acid sequence, the nascent hGHR is predicted to be ~70 kDa in size while, when resolved on SDS-PAGE gels under reducing conditions, the mature hGHR migrates at 100–140 kDa (Hocquette et al. 1989, 1990, Alele et al. 1998, Zhang et al. 2001, Cowan et al. 2005). These differences are due primarily to the presence of five asparagine-linked glycosylation sites within the extracellular domain, a variable number of which are glycosylated in the mature hGHR (Harding et al. 1994). In addition, hGHRs can be modified by ubiquitination, a step that can target the receptor for internalization and degradation (Govers et al. 1997, Strous et al. 1997, van Kerkhof et al. 2002). cDNA sequencing (Zogopoulos et al. 1996a) and now IP and IB experiments indicate no major size differences between the fetal and postnatal hepatic hGHR: the mRNA sequences are identical and both the immature and mature forms of the fetal hGHR in hepatocytes have very similar SDS–PAGE migration patterns as the postnatal hGHR.

Ross et al. (1997) previously demonstrated that cells expressing a T/FL GHR cDNA ratio of 1:10 exhibited a 10–30% decrease in FL GHR activity, while a 1:1 T/FL ratio resulted in a 40–80% inhibition and a 10:1 ratio completely blocked activity of the FL GHR. When we examined the T/FL hGHR mRNA ratios in FHs and postnatal liver, we found that they were similar and quite low (~1:12), making it unlikely that the truncated form plays a role in the functional hGHR differences observed at these two developmental stages. However, we observed a relatively high T/FL hGHR mRNA ratio in hepatic hematopoietic cells (1:4), fetal kidney tissues (~1:2), and two primate cell lines (~1:4:1), suggesting that the dominant-negative isoforms of hGHR may play a functionally significant role in these cells and tissues, decreasing the ability of hGH to activate its intracellular signaling and, thus, its biological effectiveness. This is especially interesting in the case of the kidney, given the links between hGH and kidney function (Rabkin & Schaefer 2004, Reddy et al. 2007). It would be interesting, for example, to compare T/FL hGHR mRNA ratios in kidney tissue biopsies under normal versus diabetic conditions, to determine whether the T/FL ratio shifts from a high to low level with progression of the diabetic state, leading to increased responsiveness to hGH and diabetic nephropathy.

The only previous study of JAKs and STATs in human liver was undertaken by Hellgren et al. (1999): they determined by western blots, immunohistochemistry, and IP that JAK1, JAK2, JAK3, and TYK2 are all present in adult hepatocytes, and that the hGHR specifically associates with JAK1, JAK2, and TYK2. Our analysis of the hGHR downstream signaling factors demonstrates that JAK2 and STAT1, STAT3, STAT5A, and STAT5B are present in FHs from the 12th week of fetal life. With the exception of STAT5B, FHs contain ~40–50% lower levels of the JAK/STAT proteins compared with postnatal liver. Whether this actually creates a functional difference in hGH signaling is impossible to know with our present data. To determine this, future experiments should examine the relative amounts of hGHR/JAK/STAT molecules that are phosphorylated or the relative responses of target genes following hGH treatment of fetal versus postnatal hepatocytes.

There have been two fetal versus postnatal liver analyses of JAK/STATs in the rat (Shoba et al. 1999, Phornphutkul et al. 2000). Contrary to our data (Goodyer et al. 2001a), Phornphutkul et al. (2000) found no difference in GH binding levels between late gestation (d17, d19) fetal and postnatal rat livers. In vitro or in vivo GH treatments caused a similar phosphorylation of GHR, JAK2, STAT1, and/or STAT5 in the fetal and postnatal hepatocytes. Finally, GH was able to enhance CAT expression of two GHRE reporter vectors transferred into FHs, leading Phornphutkul et al. (2000) to conclude that hepatic GH signaling mechanisms are functional in the late gestation rat. Similar to our data (Goodyer et al. 2001a), Shoba et al. (1999) found a marked increase in GHR mRNA and protein in fetal (d17) compared with postnatal (4 months) rat livers. Over the same time frame, surprisingly, JAK2, STAT1, and STAT3 protein levels dropped while STAT5 levels remained unchanged. These last findings are quite different from our present hepatocyte and intact human liver data and may represent major species differences.

One interesting note is that we observed significantly elevated levels of STAT5A and STAT5B expression in the human intact fetal liver when compared with the FHs, suggesting that this is due to the hematopoietic cell population. Several published reports support this conclusion. STAT5A–/–STAT5B–deficient mice are severely anemic due to significant decreases in their hepatic erythroid precursor cell population (Socolovsky et al. 1999), demonstrating the importance of these two STATs for murine fetal liver hematopoiesis. The recent findings, by Rosenfeld et al., of immune deficiencies in two patients with STAT5B mutations indicate an important role for STAT5B in the human immune system as well (Rosenfeld et al. 2005, Hwa et al. 2005).

The SOCS proteins are well recognized to act as inhibitors of GHR signaling (Flores-Morales et al. 2006). Although we had hypothesized that there might be increased levels in the FHs, our IB studies of FH lysates showed ~60–75% lower levels of all of the SOCS family members compared with postnatal liver. In the only other human study, Dey et al. (1998) examined a CLONTECH panel of fetal and adult human tissues; they also showed a lower level of SOCS2 mRNA levels in the fetal when compared with the postnatal liver. In the only animal study to date, Gentili et al. (2006) have reported that there is a significant increase in SOCS3 mRNA expression in the ovine fetal liver during late gestation (d125–d145), and that this expression is regulated by circulating levels of PRL but STAT5 does not appear to be involved.

One obvious explanation for the lower expression of the SOCS proteins in the human fetal liver is the lower amounts of hGHR, JAK2, and most of the STATs in the fetal cells. It
is well known that transcription of the SOCS genes is upregulated by hGH and other cytokines through the STATs: SOCS1 has STAT1, STAT3, and STAT6 binding sites while SOCS2, SOCS3, and CIS promoters have multiple STAT5 consensus response elements (Matsumoto et al. 1997, Adams et al. 1998, Davey et al. 1999, He et al. 2003, Hebenstreit et al. 2003, Gatto et al. 2004). However, an interesting caveat is that SOCS2 has dose-dependent effects on GHR signaling: at low concentrations it inhibits GH-induced STAT5-dependent gene transcription while restoration of GH signaling is observed at higher concentrations, demonstrating that SOCS2 functions by regulating cell sensitivity to GH (Favre et al. 1999, Greenhalgh et al. 2002). This interesting finding suggests that the relatively low levels of SOCS2 in the human FHs may, in fact, contribute to the lower response to hGH.


Several other aspects have yet to be investigated in any species. These include whether in the fetal liver: 1) the GH/GHR complex is working through alternative intracellular signaling pathways to regulate fetal-specific biological outcomes (e.g., the MAPK and PI3-kinase cascades, Ca²⁺ channels), 2) there are desensitization processes (e.g., more rapid dephosphorylation of the activated receptor, endocytosis, proteolysis) that influence GH responsiveness in general, and 3) gender has an effect on the levels of the signaling proteins, given that the fetal testes is producing significant amounts of testosterone at 10–20 weeks of fetal life (Tapanainen et al. 1981, Frank 2001, Herrington & Carter-Su 2001, Zhu et al. 2001, Choi et al. 2006).

In conclusion, our studies to date demonstrate that there are lower levels of hGH in fetal versus postnatal hepatocytes but that the hGH isoforms are unchanged, including the relative amounts of the truncated dominant-negative (hGH₁₋₂₇₉) form. The JAK2/STAT/SOCS signaling molecules are present in the human hepatocyte as early as the first trimester. However, they are generally at <50% the level in postnatal perfused liver, suggesting that decreased hGH signaling ability may help to explain the relatively low cell responsiveness to the very high fetal serum hGH levels.

Declaration of Interest

The authors have no conflict of interest that would prejudice the research described in this article. C G G is a member of the Research Institute of the McGill University Health Centre, which is supported in part by the Fonds de Recherches en Santé du Québec.

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