Development of Hormone Receptors Within the Fetus

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Many factors affect the rate of fetal growth and development in addition to overall fetal well-being. In eutherian mammals, the placenta serves as the primary mediator and modulator of those factors that ultimately determine development rate. The placenta accomplishes this task in the following ways: (a) by serving as the site of nutrient and waste transfer between the mother and fetus; (b) by serving as a barrier against the maternal immune system and pathogens; and (c) by functioning as an endocrine organ. Regarding this latter function, the placenta is capable of synthesizing and secreting a plethora of protein and steroid hormones, growth factors, cytokines, and other bioactive molecules (1-3), many of which are produced at extraplacental sites as well. However, some are synthesized only by the placenta and are "true" placental hormones, such as the placental lactogens (1-3).

Assigning specific biologic roles to any placental hormone is difficult, because classic ablation-replacement experiments are not feasible. Emphasis has therefore been placed on identifying the receptor for each placental hormone—its location and mechanism of action. Considerable effort has been expended on determining whether or not the placental lactogens act through structurally distinct receptors, or if they act through the growth hormone or prolactin receptor. As yet, firm conclusions cannot be drawn about the existence of distinct placental lactogen receptors, which has hampered our understanding of the function of this placental hormone. In some species (human and sheep), it has been suggested (4) that placental lactogen serves to modulate maternal and fetal metabolism, possibly by stimulating the expression of the insulin-like growth factors (IGFs). If this hypothesis is correct, it provides a direct link between placental hormone production and fetal growth regulation, because evidence now in hand clearly shows the importance and developmental pattern of the IGF system within the fetus (2). This chapter focuses on our current knowledge of the development of fetal growth hormone/placental lactogen receptors and their mechanism of action, with emphasis on humans and sheep.

STRUCTURE AND FUNCTION OF PLACENTAL LACTOGEN

Placental lactogens are members of the growth hormone/prolactin gene family, but they differ considerably from species to species in their primary structure. Primate
placental lactogens are structurally more similar to growth hormone than they are to prolactin, with human placental lactogen (hPL) showing 87% amino acid sequence identity with pituitary-derived human growth hormone and only 23% amino acid sequence identity with human prolactin (1,2). In rodents, the placental members of this family are structurally more similar to prolactin than they are to growth hormone (5), and in the species examined thus far, there are two major types of placental lactogen (PL-1 and PL-2), which differ in structure and secretion pattern (1,5). As for rodents, the placental lactogens synthesized by domestic ruminants are structurally more similar to prolactin than they are to growth hormone, but as in primates, only a single structural type of placental lactogen has been identified (6).

The exact biologic role of the placental lactogens is not well defined for any species, and the diversity shown between species in primary structure may impart diverse biologic functions. As shown in Fig. 1, there may be multiple sites of action for placental lactogens within the maternal system, and the importance of the various sites is likely to be different between species. For example, there appears to be direct luteotropic actions by one or more of the mouse placental lactogens (7) and by bovine placental lactogen (8), whereas evidence for direct luteal support by human or sheep placental lactogens is lacking. This example of functional diversity may result from the gestational requirement for corpus luteum-derived progesterone in the former species, or the lack of a requirement for it in the latter. Species divergence in the structure-function relationship for placental lactogens may be a reflection of evolutionary adaptation. Bovine and ovine placental lactogens are structurally more similar to each other (approximately 66%) (6) than they are to other members of this gene family, yet the divergence in primary structure between the ruminant placental lactogens is greater than the divergence in primary structure between bovine and ovine growth hormone or between bovine and ovine prolactin (approximately 99% amino acid sequence identity). Comparison of the sequence similarities between

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**FIG. 1.** Schematic diagram depicting potential sites of action for placental lactogen within the maternal and fetal systems. These include luteotropic and mammotrophic actions within the mother and metabolic actions within the mother and fetus.
these ruminant placental lactogens revealed a non-synonymous substitution rate greater than the synonymous substitution rate (9), suggesting that the more rapid rate of evolution between bovine and ovine placental lactogens may have resulted from adaptive rather than neutral mutations. On the other hand, there appear to be interspecific commonalities in function, as there is direct and indirect evidence of mammotropic actions for placental lactogens in primates, rodents, and ruminants (1,10,11).

As implied in Fig. 1, placental lactogens may modulate maternal and fetal metabolism by exerting action on maternal and fetal liver, as well as other metabolic tissues. Grumbach et al. (12) initially proposed that placental lactogen serves as an insulin antagonist, thereby inducing peripheral tissue insulin resistance and increased lipolysis and proteolysis within the mother, with the net result of providing additional glucose and amino acids for transport to the fetus. All these responses are normal adaptations in metabolism that occur in pregnant women, and there is experimental evidence (13) to support Grumbach's hypothesis (12). However, for obvious reasons, \textit{in vivo} data from humans to support this hypothesis are lacking, and many of the data acquired \textit{in vitro} were obtained using heterologous systems. Even in the pregnant sheep model, in which \textit{in vivo} approaches are feasible, there is a dearth of experimental evidence supporting the role of placental lactogen as a major modulator of maternal and fetal metabolism (6).

As alluded to in the introductory section, our lack of understanding of the specific biologic role of placental lactogen is at least in part a consequence of the inability to use classic ablation-replacement approaches in defining the function of placental hormones. Further exacerbating the difficulty in defining specific functions is the fact that in both humans and sheep, the endogenous concentration of placental lactogen (2,13) is quite high relative to the $K_d$ of the placental lactogen binding sites described in maternal and fetal tissues. Therefore, administration of additional placental lactogen into what may already be a saturated or near-saturated system is unlikely to yield easily interpretable results. There have been cases in which deletions within the human growth hormone-placental lactogen gene cluster resulted in low to undetectable concentrations of human placental lactogen and human placental growth hormone (hGH-V), yet by all indications pregnancy outcome was normal (13). These data imply that production of placental lactogen is not an absolute requirement for normal pregnancy outcome. It is entirely possible that placental lactogen, hGH-V, and fetal pituitary-derived growth hormone serve within a redundant system aimed at providing the homeorrhetic environment required during pregnancy. Such redundancy is becoming more commonplace as the "true" biology of various systems is described, and definition of the location, mechanism of action, and structural identity of the receptor through which each hormone acts may help determine the role of each hormone within this potentially redundant system.

GROWTH HORMONE / PLACENTAL LACTOGEN RECEPTORS

Many of the placental lactogens were originally purified using heterologous growth hormone or prolactin receptor assays, and both human placental lactogen
(hPL) and ovine placental lactogen (oPL) will bind the growth hormone receptor of their species (14,15). This has led to the suggestion that, at least in these species, placental lactogen exerts its actions through the growth hormone receptor. As yet, no specific placental lactogen receptor has been structurally characterized, and it is likely that in humans and sheep the placental lactogen receptor is either closely related to the growth hormone receptor, or indeed is the growth hormone receptor. Therefore, a brief description of this receptor and its mechanism of action is warranted before the available information related to the existence of fetal growth hormone/placental lactogen receptors is examined.

The growth hormone receptor is a member of the cytokine receptor superfamily, and is comprised of three domains: a 246-amino acid extracellular domain that binds to and is dimerized by a single molecule of growth hormone; a short transmembrane segment; and a 350-amino acid intracellular domain required for signal transduction events (16,17). A single molecule of growth hormone is bound by two molecules of the growth hormone receptor (1:2 stoichiometry) (17), and dimerization of the receptor is essential for signal transduction to take place. Binding of growth hormone to its receptor initiates a signal transduction pathway that involves tyrosine phosphorylation of multiple cellular peptides (Fig. 2). The identification of Janus kinase 2 (JAK2) as a growth hormone receptor-associated, growth hormone-activated tyrosine kinase, established tyrosine phosphorylation as the initial step in the signal transduction of growth hormone (18).

Growth hormone has been observed to stimulate phosphorylation of tyrosine residues within insulin receptor substrate-1 (IRS-1) (19), which is critical not only in insulin signaling but also in providing a binding site for the regulatory subunit of phosphatidylinositol 3'-kinase. Additionally, growth hormone stimulates tyrosine

![FIG. 2. Schematic diagram of the signal transduction cascade induced by growth hormone binding to its receptor. The signal transduction cascade is also depicted as to how it may be activated by placental lactogen. JAK2, Janus kinase 2; Stat, signal transducer/activator of transcription protein.](image)
phosphorylation of latent cytoplasmic transcription factors designated as signal transducer/activator of transcription (Stat) proteins-1 (Stat-1) and -3 (Stat-3), and their activation (Fig. 2) leads to transactivation of target genes (20–22). These Stat proteins mediate the transcriptional response stimulated by multiple growth factors and cytokines. Co-precipitation and growth hormone receptor mutagenesis experiments indicate that growth hormone activation of Stat-1, Stat-3, and IRS-1 require their interaction with JAK2 rather than growth hormone receptor, supporting the hypothesis that JAK2 is the initial signaling molecule for growth hormone. It has recently been shown that intermittent pulses of growth hormone stimulate the tyrosine phosphorylation, translocation to the nucleus, and activation of DNA binding of Stat-5, suggesting that Stat-5 is an additional intracellular mediator of the stimulatory effects of growth hormone (23). As yet, the signal transduction pathways induced by placental lactogen binding to its target tissues have not been examined (Fig. 2).

**Fetal Growth Hormone Receptors**

The lack of effect of growth hormone deficiency on human fetal growth rate resulting from anencephaly or congenital absence of the pituitary gland led to the conclusion that fetal development occurs independently of fetal pituitary-derived growth hormone (24). This has been thought to result from a lack of growth hormone receptor within the fetus, but the growth hormone receptor or its messenger RNA has now been identified in fetal tissues from a variety of species. These more recent data, coupled with clinical data inferring impaired *in utero* growth of infants with idiopathic growth hormone deficiency (25) or growth hormone receptor dysfunction (26), suggest that in fact growth hormone has actions within some fetal tissues.

Hill *et al.* (27) showed specific binding of growth hormone to human fetal liver microsomes obtained at midgestation, but could not demonstrate specific binding to the microsomal fraction of fetal skeletal muscle. However, specific binding sites for hPL were identified in both fetal liver and skeletal muscle (27). The growth hormone binding sites in fetal liver showed a sixfold greater affinity for growth hormone than for placental lactogen, and the placental lactogen binding sites showed a ninefold greater affinity for placental lactogen than for growth hormone, suggesting that the respective binding sites (receptors) are different. These results were supported by the immunohistochemical localization of the growth hormone receptor in fetal liver, pancreas, kidney, skin, and cerebral cortex, but not in fetal skeletal or cardiac muscle, adrenal gland, intestine, lung, or epiphyseal growth plate (28).

The messenger RNA encoding the human growth hormone receptor was recently identified (29) in the same tissues in which the growth hormone receptor was immunolocalized (28), as well as in tissues in which the growth hormone receptor could not be immunolocalized (muscle, adrenal gland, intestine, and lung). Both exon 3-retaining and exon 3-deleted transcripts were detected, and the relative expression pattern of the two growth hormone receptor isoforms appeared to be individual-specific rather than tissue-specific and may be developmentally regulated (29), with
exon 3-retaining transcripts predominating in later gestation. Deletion of exon 3 from the growth hormone receptor messenger RNA results in a 22-amino acid deletion at the amino terminus of the growth hormone receptor, but this deletion does not alter the binding affinity of the growth hormone receptor for growth hormone (30), such that its deletion probably does not account for the absence of detectable growth hormone receptor (28) or growth hormone binding (27) in some tissues (e.g., skeletal muscle). The growth hormone receptor messenger RNA identified in various fetal tissues (29) was not examined for the potential variation in exon 1 sequences that exists in human growth hormone receptor messenger RNA transcripts (16,31). Eight different sequences have been described (31) for the exon 1 region of the human growth hormone receptor messenger RNA, and although exon 1 contains only 5' untranslated sequence (5'-UTR), it is possible that use of one or more of these variant 5'-UTRs could provide for posttranscriptional regulation (inhibition of translation) of the growth hormone receptor in tissues like fetal skeletal muscle.

A different scenario appears to exist in fetal mice and rats. Messenger RNA encoding the growth hormone receptor or the growth hormone binding protein (GHBP) have been identified in fetal mouse (32) and rat (33) tissues as early as embryonic days 12 and 14, and the amount of growth hormone receptor/GHBP immunolocalized in fetal rat tissues increased from day 12 to 18 of gestation (33). However, the identity of the ligand for fetal mouse and rat growth hormone receptor remains in question, as fetal pituitary-derived growth hormone does not appear until very late in gestation (about day 19) (34). It appears unlikely that the ligand is mouse or rat placental lactogen, at least not PL-2, as mouse growth hormone does not compete for mouse PL-2 binding sites on hepatic membranes (35). Recently, Southard et al. (36) showed that mouse growth hormone receptor messenger RNA contains two different 5'-UTRs (exon 1 sequences): one (L1) was preferentially used in pregnant maternal liver transcripts, whereas the other (L2) was predominant in nonpregnant and fetal tissues. The L2 5'-UTR contains an AUG translation initiation codon with features of a preferred start site (37), followed by a stop codon generating a short open reading frame preceding the "normal" growth hormone receptor open reading frame. The GC-rich content of the L2 5'-UTR, coupled with the short open reading frame encoded within L2, may potentially inhibit translation (38) of the growth hormone receptor, which begins within the exon 2-derived sequence. Therefore, the growth hormone receptor messenger RNA found in mouse fetal liver (day 16), as early as embryonic day 14 (32), may not be efficiently translated into the growth hormone receptor, because only L2-containing growth hormone receptor messenger RNA was detected (36). Posttranscriptional regulation of the growth hormone receptor messenger RNA, as a function of the particular 5'-UTR within the messenger RNA, has yet to be demonstrated.

Fetal sheep have long been considered to be growth hormone receptor-deficient until the immediate periparturient period (1,2,6). However, recent cross-linking and immunoprecipitation data indicate that the ovine growth hormone receptor is present in fetal liver between 125 to 135 days of gestation (39). The amount of specific ovine growth hormone binding to fetal hepatic microsomes was still quite low in
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these samples (1.2 ± 0.4%), and others (40) have concluded from saturation analyses of day 105 to 120 fetal liver microsomes that specific binding of growth hormone is nonexistent. Growth hormone receptor messenger RNA has been detected in fetal liver and skeletal muscle tissues as early as day 60 of gestation (41,42), but the major growth hormone receptor messenger RNA transcript present during midgestation is about 300 base pairs larger (Fig. 3) than the growth hormone receptor messenger RNA expressed in maternal liver (42). This difference in growth hormone receptor messenger RNA size appears to result from use of a variant 5'-UTR up through 120 days of gestation (42), in that the 5'-UTR encoded by the adult liver, specifically exon 1A (43), was not detectable until after day 120 (42). Recently, exon 1B was isolated and characterized from postnatal skeletal muscle, another tissue that does not appear to express exon 1A (44). Two of the authors (L. C. Richter and R. V. Anthony, unpublished data) have determined, using reverse transcriptase polymerase chain reactions and Southern hybridizations, that the 5'-UTR sequence present in fetal liver and skeletal muscle, as well as the placenta, is derived from exon 1B. Exon 1B sequence is detectable in fetal liver messenger RNA on days 60, 90, 105, 120, and 135 of gestation, which coincides with our earlier detection of exons 2 to 10 from day 60 on (42). In contrast to our earlier results (42), some individual samples obtained at 120 days of gestation have been found to express exon 1A, suggesting that a developmental switch in ovine growth hormone receptor gene transcription or primary transcript splicing occurs around 120 days of gestation.

Combined, the available information on growth hormone receptor gene transcription in human, rodent, and ruminant fetal tissues indicates that developmental

FIG. 3. Northern hybridization analysis of poly(A)$^+$ RNA obtained from day 105 fetal liver (lane 1), day 135 fetal liver (lane 2), and day 100 maternal liver (lane 3). The electrophoretic gel was blotted onto a nylon membrane and hybridized to a bovine growth hormone receptor complementary DNA. From Pratt and Anthony (42), with permission.
switches occur in growth hormone receptor gene transcription or in subsequent splicing events. As discussed earlier, in the human fetus there appears to be an individual-dependent switch between exon 3-deleted and exon 3-retaining growth hormone receptor messenger RNA as gestation progresses (29). Whether or not there is a developmental switch in the use of the various 5'-UTRs transcribed from the human growth hormone receptor gene (16,31) has yet to be determined. There appears to be a commonality between mice (36) and sheep (42) in the use of 5'-UTR sequences in the growth hormone receptor messenger RNA expressed in fetal liver. As depicted in Fig. 4 for fetal sheep liver, exon 1B (analogous to L2 in mice) (36) is expressed throughout most of gestation and into adult life (44), but exon 1A is not expressed until around 120 days of gestation. Both exon 1B in sheep (44) and L2 in mice (36) contain a translation initiation codon that meets the requirements of a preferred site (37). This initiation codon is followed by a translation stop codon, creating an autonomous open reading frame that precedes the "normal" growth hormone receptor open reading frame. Within the sheep exon 1A sequence (Fig. 4) lie two potential initiation codons, but neither conforms to the requirements (a purine at position -3 and a G at position -4) of a preferred initiation site (37) and are probably not used. Additionally, both exons 1B and L2 have a high GC content.

**FIG. 4.** Diagrammatic representation of the developmentally regulated transcription of the ovine growth hormone receptor gene. Exon 1B is transcribed throughout gestation, but it is not until approximately 120 days of gestation that exon 1A is transcribed. Exon 1B contains an autonomous open reading frame that may inhibit translation initiation of the growth hormone receptor within exon 2. The translation initiation sites within exon 1A do not conform to the conditions of a preferred site, and do not appear to be used. The bent arrows indicate the transcriptional start sites for exons 1B and 1A, respectively.
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which could create sufficient secondary structure to interfere with the assembly of
the preinitiation complex for translation (38). Both an autonomous open reading
frame and excessive secondary structure within the 5'-UTR probably hinder the
efficient translation of growth hormone receptor messenger RNA, a common occur-
rence in proto-oncogenes, growth factors, and growth factor receptor genes (38).
Cell lines need to be established that express growth hormone receptor messenger
RNA containing this type of 5'-UTR (e.g., exon 1B or L2) to examine the effect
on translation efficiency. If these messenger RNAs are not efficiently translated into
a functional growth hormone receptor, it could explain why it is difficult to show
specific growth hormone binding to fetal liver microsomes in species such as the
sheep. Additionally, the transcriptional regulation involved in these apparent devel-
opmental switches needs to be examined.

Fetal Placental Lactogen Receptors

Specific binding sites for hPL have been identified in fetal liver and skeletal
muscle (27) that appear to be distinct from the hGH binding sites identified in fetal
liver. Although hPL and hGH share 87% primary amino acid sequence identity, hPL
binds the hGH receptor with 2300-fold lower affinity (14), indicating that the fetal
liver and skeletal muscle hPL binding sites are not the growth hormone receptor.
As yet, no attempts at purification or structural characterization of the fetal hPL
binding sites have been reported.

Numerous studies have examined the binding of oPL to maternal and fetal hepatic
microsomes (2,6). Specific high-affinity binding (K_d = 0.12 to 0.5 nM) of oPL to fetal
liver microsomes has been demonstrated (40,45), whereas ovine growth hormone or
prolactin shows negligible specific binding to these membranes (40,46). During
quantification of oPL binding sites by saturation analyses (40), the concentration of
the fetal liver binding site did not change with increasing gestational age when data
were expressed per milligram of microsomal protein. However, when the concentra-
tion was expressed per milligram of DNA (i.e., per cell), the concentration did
increase with increasing gestational age, indicating that as the fetus ages and develops
there are increased numbers of oPL binding sites per cell. Additionally, saturation
analyses using radiolabeled oPL, prolactin, or growth hormone as the ligand (40)
and fetal hepatic microsomes (days 105 to 120 gestational age) as the source of
receptors show saturable binding kinetics for oPL, but no specific binding of oPRL
or growth hormone. Although a partial purification of this binding site has been
reported (45), no amino acid sequence data are available on this receptor.

Recent cross-linking and immunoprecipitation studies indicate that oPL and ovine
growth hormone are able to bind an identical or quite similar receptor present in
fetal liver at 125 to 135 days of gestation (39). However, the amount of specific
binding of ovine growth hormone to these microsomes (1.2 ± 0.4%) was consid-
ernably less than the amount of specific binding of oPL (7.6% ± 2.4%). These investiga-
tors (39) suggested that the difference in the amount of specific binding between
oPL and ovine growth hormone might be explained by oPL interacting with the growth hormone receptor in a 1:1 stoichiometry rather than the 1:2 stoichiometry shown by growth hormone binding the growth hormone receptor. Precedence for placental lactogen binding in a 1:1 stoichiometry with the growth hormone receptor was provided by the data of Staten et al. (47) using bovine placental lactogen (bPL) and the bovine growth hormone receptor. It was observed that bPL bound the bovine growth hormone receptor in a 1:1 stoichiometry rather than the 1:2 stoichiometry shown with bovine growth hormone (47). Additionally, a monoclonal antibody raised against the extracellular domain of the bovine growth hormone receptor competes with bovine growth hormone binding to the growth hormone receptor, but does not compete with bPL binding to the growth hormone receptor, suggesting that the binding sites of the two ligands are not exactly the same. However, when the ovine growth hormone receptor was expressed in CHO cells, growth hormone showed a greater affinity ($K_d = 0.30 \text{ nM}$) than did oPL ($K_d = 0.76 \text{ nM}$) for this receptor, yet both ligands bound approximately the same number of ovine growth hormone receptor molecules per cell (15). These latter data are not consistent with the suggestion (39) that oPL is binding the growth hormone receptor within fetal liver in a 1:1 stoichiometry. In other words, if the common receptor for ovine growth hormone and oPL present in late-gestation fetal liver (39) is the ovine growth hormone receptor, then one must ask why ovine growth hormone is not bound with affinity equal to or greater than that of oPL.

As yet, firm conclusions cannot be drawn about the structural identity of the receptor in fetal tissues through which placental lactogen acts. There are several possibilities related to the identity of the fetal placental lactogen receptor, as shown in Fig. 5. One possibility is that placental lactogen acts by binding to a single monomer of the growth hormone receptor (Fig. 5A) in a 1:1 stoichiometry, as has been demonstrated for bPL (47). If this is the case, it is not clear why it has been difficult to show significant specific binding of growth hormone to fetal liver microsomes until late gestation, as this hypothesis assumes that functional growth hormone receptors are present. Furthermore, activation of the signal transduction pathways of the human growth hormone receptor (see above) requires growth hormone-induced dimerization (17) of the growth hormone receptor, and preliminary evidence (48) suggests that when bPL binds the growth hormone receptor in a 1:1 stoichiometry (Fig. 2), JAK2 does not undergo tyrosyl phosphorylation. The requirement for dimerization could be met by placental lactogen inducing a heterodimer between a growth hormone receptor monomer and an as yet to be described placental lactogen-specific monomer (Fig. 5B). However, this hypothesis again assumes the availability of growth hormone receptor monomers in fetal tissues, which would also allow specific binding of growth hormone to occur. In short, the hypotheses as to the identity of the fetal placental lactogen receptor depicted in Fig. 5 A,B do not coincide with the lack of specific growth hormone binding in sheep fetal liver and human fetal skeletal muscle.

Another possibility is that the placental lactogen receptor is a modified form of
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FIG. 5. Diagrammatic representation of the potential identity of the placental lactogen (PL) receptor found in fetal tissues. A: Placental lactogen may act by binding to one monomer of the growth hormone receptor (GHR). B: Placental lactogen may act by binding to one monomer of the growth hormone receptor and to a placental lactogen-specific monomer, generating a heterodimer. C: Placental lactogen may act by binding to two monomers of a growth hormone receptor variant (GHR-V) resulting from an amino terminal extension encoded by a variant 5' UTR in the growth hormone receptor messenger RNA. D: Placental lactogen may act through binding a structurally distinct receptor (PLR).

the growth hormone receptor (GHR-V) (Fig. 5C). As discussed earlier, variant 5'-UTRs of the growth hormone receptor messenger RNA are expressed in fetal tissues. Both the mouse L2 5'-UTR and the sheep 5'-UTR encoded by exon 1B contain preferred translation initiation sites that precede the initiation site used in translating the growth hormone receptor (36,44). However, these "upstream" initiation sites are followed by translation stop codons, forming an autonomous open reading frame. It is possible that as yet undescribed 5'-UTRs exist in fetal tissues possessing similar upstream translation initiation sites that are in-frame with the initiation site in exon 2, and in the absence of an intervening stop codon, a growth hormone receptor could be translated that possesses an amino terminal extension. Such an amino terminal extension (Fig. 5C) could alter the conformation of the growth hormone receptor such that it no longer recognizes growth hormone with high affinity, but rather preferentially binds placental lactogen. As yet, no such growth hormone receptor 5'-UTR has been described in any species.

The final possibility (Fig. 5D) is that the placental lactogen receptor is a structurally distinct receptor and is not directly derived from either the growth hormone receptor or prolactin receptor genes but rather from a closely related gene. This is an inherently attractive hypothesis that coincides with most of the receptor binding data obtained with human and sheep fetal tissues. The structural characterization of such a receptor awaits its purification or complementary DNA isolation by expression cloning methods. Insight into the identity of the fetal placental lactogen binding site may be gained by examining the ability of placental lactogen to activate the
JAK2/Stat signal transduction pathway (Fig. 2). The ability of placental lactogen to activate this system in cells expressing the "normal" growth hormone receptor, or growth hormone receptor encoded by messenger RNA containing the various 5'-UTRs, could be compared with the ability of placental lactogen to activate this system in primary fetal cell cultures. If placental lactogen activates the signal transduction cascade coupled to the growth hormone receptor—and the same pathway in fetal cells—it would be important to ascertain if the activation of fetal cells could be blocked with antibodies raised against the growth hormone receptor. In short, if the ability of placental lactogen to activate cells expressing the growth hormone receptor could be blocked by antibodies raised against the growth hormone receptor, but its ability to activate fetal cells could not be blocked with these same antibodies, strong evidence would be provided for a structurally distinct receptor.

SUMMARY

Placental-fetal hormonal interactions play an important role in determining fetal growth rate and overall well-being. Placental lactogen is a member of the growth hormone/prolactin gene family that is thought to serve as an important mediator of the in utero environment, but it may be only one component of a redundant system that includes fetal pituitary-derived growth hormone. As yet, a specific placental lactogen receptor has not been structurally characterized, but the available evidence in humans and sheep indicates that such a receptor exists in fetal tissues. Additional effort needs to be directed toward the isolation and structural characterization of the fetal placental lactogen receptor to clarify the role of placental lactogen in fetal development. Furthermore, more evidence is becoming available to suggest that fetal development is not entirely independent of fetal pituitary-derived growth hormone, and that transcription of the growth hormone receptor gene may be a developmentally regulated event. What appears to be developmentally regulated use of various growth hormone receptor 5'-UTRs, on further analysis should provide insight into the developmental switches that are important for the transition from fetal to postnatal life. A thorough understanding of the transcriptional regulation of these developmental switches will provide new insight into fetal growth regulation, and could provide the basis for future interventions to treat intrauterine growth retardation or care for premature infants.

REFERENCES


28. Hill DJ, Riley SC, Bassett NS, Waters MJ. Localization of the growth hormone receptor, identified.
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42. Pratt SL, Anthony RV. The growth hormone receptor messenger ribonucleic acid present in ovine fetal liver is a variant form. Endocrinology 1995;136:2150–2155.


DISCUSSION

Dr. Soothill: One aspect of concern relating to growth hormone in the fetus is the Laron type of growth hormone receptor deficiency syndrome, in which the babies have apparently
almost normal weight. Do you have any idea about the switching of the growth hormone receptor variants in such cases?

Dr. Anthony: The 5'-UTR variants have not been looked at to any extent in the fetus. The exon 3 deletion has been looked at, and you find both exon 3-containing and exon 3-deleted forms within fetal tissues, and there is some indication that there may be some developmental switching going on there; however, it appears to be almost an individual-specific phenomenon rather than a tissue-specific phenomenon. The exon 3-deleted form has the same affinity for growth hormone or growth hormone variant as does the exon 3-containing form, so there doesn't seem to be a functional problem with that form of the receptor. But with these 5'-UTR variants, we don't yet know if any one of those are expressed proportionally more in fetal life in humans.

Dr. Soothill: I mentioned families with Laron syndrome specifically because if you found that they did produce one of the variants in pregnancy, that might be the explanation why those children have normal birth weight; until we have an explanation for that, we have to assume that growth hormone is not very important in the fetus.

Dr. Anthony: I agree with you. But I am not sure we have necessarily found all the variants. I can't rule out the possibility that there are other variants.

Dr. Battaglia: I have been pushing my obstetrical colleagues in Colorado to look at epiphyseal growth. You know that obstetricians measure femur length, which is really looking at the solid bone, but all the trophic peptides seem to act on the epiphyses, and with new imaging, you can look at epiphyseal growth in much more detail. So my question is, in terms of receptor development in fetal tissues, were you talking only about liver, or is this a broader phenomenon of growth hormone action? What is the developmental sequence if you take several target tissues? Is the developmental sequence always the same?

Dr. Anthony: From the standpoint of the growth hormone receptor, it seems that we see the same thing in fetal skeletal muscle, although we don't see the onset of exon 1A in skeletal muscle. That seems to be a liver-specific exon that is produced late in gestation and in adult life. We also see this in the placenta, but we haven't had a chance to go back and look at some of the other tissues that we have in the freezer, and I don't know about bone. Almost all David Hill's work was done in fetal liver and skeletal muscle; there hasn't been a broad-spectrum examination of other tissues. Mike Freemark at Duke is doing that with the prolactin receptor in the fetal rat, and Caroline McMillen's laboratory in Adelaide is doing it with the prolactin receptor (1), and I think they have planned to do it with the growth hormone receptor. I think we will know more about the distribution across tissues within the next year or two.

Dr. Chard: Can we assume from what you and others have said that there is general agreement that a growth hormone or growth hormone-like compound plays a key role in the intrauterine growth of the sheep fetus? We have heard all the reservations and doubts about that in the human. Is it clearer in the sheep? What about the hypophysectomized sheep?

Dr. Anthony: Unfortunately, it is probably not clear in the sheep! The hypophysectomized fetus does grow, but not totally normally. It is interesting that one of the things you see with those is a higher fat deposition. There was some work done several years ago in primary cultures; they challenged fetal sheep adipocytes with growth hormone and in fact they were responsive. In the fetal sheep you tend to see subcutaneous fat deposition until almost the analogous time when we start to pick up the adult exon 1A form of the growth hormone receptor, so possibly the fat is a target tissue that most of us have overlooked. There is plenty of fetal pituitary-derived growth hormone in the fetal sheep, and I think that many of us who may have discounted its role are rethinking that to some extent, especially late in gestation.
I think there is evidence of early placentally derived growth hormone, but that is in the first 50 days of gestation, so it is not as clear.

Dr. Girard: The receptor is there, but you need to have the protein in the phosphorylation cascade to be present and to be capable of doing the job.

Dr. Anthony: I can state that from the standpoint of placental lactogen, steps 1 and 3 are activated as early as 100 days of gestation. We have not collected hepatocytes earlier than that, and we tried to sort that out with growth hormone as well. One problem is that we know that placental lactogen will bind to growth hormone receptor and vice versa, so when you do those experiments, sorting that out is not easy. So our approach is to take the adult form of the growth receptor message and the fetal form and make cell lines of those, and then we're doing challenges with growth hormone and placental lactogen. At the same time, we are doing the same experiments with cultured fetal hepatocytes. If we can block the action with an antibody against the growth hormone receptor (which might block the action of growth hormone but not of placental lactogen in the fetal hepatocyte), then hopefully we will be able to sort some of these things out. But I don't believe that the limiting step will be some of the signal transduction cascade components, because many of these are used in other systems that are probably active much earlier in gestation.

Dr. Soares: You mentioned some experiments done with bovine placental lactogen showing that it failed to activate the cascade. What sort of system were they working on? In that system, does bovine placental lactogen actually have an effect, or is it just able to bind to receptors?

Dr. Anthony: In 1993, Staten showed that in the case of bovine placental lactogen when it bound to bovine growth hormone receptor you didn't get receptor dimerization. They followed this up by making a stable cell line expressing growth hormone receptor in BHK21 cells and then challenged them with both growth hormone and placental lactogen, looking for phosphorylation by Western blotting.

Dr. Godfrey: I think there are human data indicating that growth hormone may play a role in fetal growth; they come from a large Scandinavian series of children with growth hormone deficiency who, although they were of normal birth weight, were short at birth. So I think it is very important that we address the effects on specific fetal compartments.

Dr. Anthony: I think we are dealing with a redundant system that shows up in a lot of biologic systems. If you lack of one or other hormone, you may see some subtle changes or some important things may take place, but if you lack a single hormone you are not going to have severe intrauterine growth retardation.

Dr. Owens: There are two studies that may cast light on the importance of growth hormone or placental lactogen-like protein for growth in the fetal sheep. One is by Alan Bell at Ithaca, who a number of years ago had a graduate student who infused purified ovine placental lactogen into fetal sheep for several weeks and wrote it up as a thesis. I have seen the data in abstract form only, but apparently there was no effect on fetal growth, which was a bit disappointing. That may just be telling us that increasing placental lactogen above normal levels cannot promote growth, but placental lactogen may still be required. The other is a more recent study by Michael Bauer in Auckland (2); he infused bovine growth hormone into fetal sheep for 10 days and found a marked increase in placental weight and fetal weight at the end of the treatment. What would be interesting to know there is to what extent bovine growth hormone is growth hormone-like or prolactin-like in the sheep.

Dr. Anthony: The data from Alan Bell were in abstract form. The one thing that they did see and reported in that abstract was that there was about a 40% increase in fetal circulating IGF-1, but there was no effect on IGF binding protein. Alan told me they also saw a 40%
increase in IGF-2. There were some effects on the placenta, but it was very hard to sort out exactly what they were. That infusion was for 14 days and started at 122 days of gestation, and I think that by that point you may be too late to show really dramatic effects. In contrast, if you look at the 10-day infusion from the Auckland group’s experiments, I would like to think it was given at a window of opportunity at which growth hormone may begin to be quite important. I think timing is very important. What stage of gestation are you at, and is it the proper stage to address what is going on?

Dr. Ogata: You haven’t mentioned the insulin receptors. IGF acts in part through insulin receptors in the fetus, at least in the rat. Is there a role here for the insulin receptor? In the old days, we would say that insulin is the growth hormone of the fetus.

Dr. Anthony: I think undoubtedly there is a role for the insulin receptor. I am not sure if there is any developmental regulation of the insulin receptor in this situation. I don’t think so.

Dr. Marini: It is very important that we should think of the very preterm fetus as suffering from a syndrome of placental deprivation from an endocrinologic point of view. We neonatologists tend to think only in terms of the placenta carrying nutrients to the fetus and cleaning the fetus, but the placenta has an important endocrine role in the formation of receptors in the fetus. For instance, if you think about the high incidence of osteopenia in the very small preterm baby, and if you realize the role that estrogen plays in bone mineralization, then the fact that these babies are deprived of estrogen for about 3 months when they should have been supplied with it by the placenta in fetal life is an important practical point.

REFERENCES