The human growth hormone family of peptide hormones is encoded by five genes, pituitary growth hormone (hGH-N), and four placentaally expressed genes, growth hormone variant (hGH-V), chorionic somatomammotropin A and B (hCS-A, hCS-B), and prolactin (hPrl). As part of an effort to define the local effects of the placentaally expressed members of the GH/Prl family of hormones on the placenta, we have identified an isoform (hGHRd3) of the growth hormone receptor expressed in the placentall villi. hGHRd3 mRNA differs from the liver GHR mRNA by the deletion of a 66-base pair segment encoding exon 3. In this study we show that hGHRd3 mRNA encodes a stable and functional receptor. hGHRd3 mRNA is efficiently translated and processed in a rabbit reticulocyte lysate translation system as well as in an in vivo Xenopus laevis oocyte expression system. In Xenopus oocytes hGHRd3 is stably integrated into the cell membrane and binds and internalizes ligand as efficiently as hGHR. hGHRd3 binds all three of the placentaally expressed members of the GH/Prl gene family (hGH-V, hCS, and hPrl) as well as both the 22 and 20 kDa isoforms of the pituitary hGH-N. The results of the present study strongly support the expression of a functional hGHRd3 isoreceptor in the placenta which may serve in autocrine, paracrine, and/or endocrine activation.

The human growth hormone/prolactin family of peptide hormones is encoded by five genes: growth hormone (hGH-N), hGH-variant (hGH-V), chorionic somatomammotropin A and B (hCS-A, hCS-B), and prolactin (hPrl). The GH and CS genes are clustered on chromosome 17 (George et al., 1981) while the single hPrl gene is located on chromosome 6 (Owerbach et al., 1981). The expression of each of these genes is tissue-specific: hGH-N is expressed exclusively by somatotrope cells of the anterior pituitary (Whilhelmi, 1961) while hCS-A, hCS-B, and hGH-V are expressed exclusively by the syncytiotrophoblasts of the placental villi (Cooke et al., 1988a; Liebhaber, 1989; Cooke et al., 1991). In contrast to this highly restricted pattern of expression, hPrl is expressed in a number of tissues, most prominently lactotrope cells of the anterior pituitary, endometrium, placental decidua, and lymphocytes (Cooke, 1993; Clements et al., 1983; and DiMattia et al., 1990). While the functions of hGH-N and hPrl are well established, the roles of the placentaly expressed hCS, hGH-V, and hPrl are less well understood.

The functions of GH and Prl are mediated by specific binding to a set of cell surface receptors belonging to the cytokine receptor superfamily (Cosman et al., 1990). Receptors in this superfamily, which encompass receptors for a broad spectrum of growth factors and peptide hormones, are characterized by a large amino-terminal extracellular domain which contains four highly conserved cysteine residues, a single transmembrane domain, and a cytoplasmic domain of variable size. Although no kinase or other enzyme activities have been assigned to these receptors, several, including the erythropoietin and GH receptors, are phosphorylated in response to ligand binding (Foster et al., 1988; Taga and Kishimoto, 1992; and Linnekin et al., 1992). As a general rule these receptors dimerize upon ligand binding, then internalize as a ligand-receptor complex. The mode of signal transduction for this superfamily of receptors remains undefined.

The expression of certain GH/Prl genes by the placenta raises the question of whether they might be acting locally on this rapidly growing organ as well as on more distant tissues. hGH-V, the most recently characterized of the hormones in this group, binds to both somatogen and lactogen receptors with a binding profile that defines it as the most purely somatogen human hormone (Ray et al., 1990). Considering the placental expression of this potent somatogen as well as the two locally expressed lactogens, hCS and hPrl, it is necessary to consider the potential for autocrine and/or paracrine activities in addition to their effects on distant tissues. Such local activities would necessitate the presence of a corresponding set of receptors on the surface of placental cells.

As part of an effort to define the local effects of the GH/Prl family of hormones on the placenta, we recently screened a placental cDNA library to detect expression of the hGH receptor (hGHR) mRNA. We found that the placental villi are enriched for hGHR mRNA. Structural analysis of the placental villous hGHR mRNA revealed that it differed from the previously defined hepatic hGHR mRNA by the selective deletion of a 66 bp segment encoded by exon 3 (Urbanek et al., 1992). This placental hGHR mRNA isoform, hGHRd3 mRNA, predicts the expression of a corresponding hGHRd3 isoform that differs from the hepatic hGHR by a deletion of 22 amino acids within the extracellular domain of the recep-
tor. While others have shown that there is no substantial effect of this region on hGH-N binding by bacterially expressed extracellular domains of the hGHR (Bass et al., 1991), the significance of this finding with respect to the biological activity of the full-length, processed, transmembrane receptor has not been addressed. Determining whether the hGHRd3 mRNA can encode a stable membrane-bound receptor and establishing its ligand binding profile is of central importance to understanding the potential roles of the placenta expressed hormones. The studies reported in the present paper demonstrate that the full-length, transmembrane hGHRd3 isoform encodes a stable and functional GH receptor. These results establish the potential for a GH-GHR axis within the placenta.

**EXPERIMENTAL PROCEDURES**

**Materials**—Restriction and modification enzymes were purchased from New England Biolabs (Beverly, MA), Boehringer Mannheim, USA; Biochemicals (Cleveland, OH), and Amersham, USA. The Avian myeloblastosis virus reverse transcriptase was purchased from Life Sciences (St. Petersburg, FL), and Taq DNA polymerase from Perkin-Elmer-Cetus. Each enzyme was used according to the manufacturer's specifications, [γ-32P]ATP (5000 Ci/mmol), and [α-32P]dATP (400 and 3000 Ci/mmol) were purchased from Amersham. Nucleotides were supplied by the DNA synthesis facility of the Howard Hughes Medical Institute at the University of Pennsylvania. [35S]-hGH-N was purchased from New England Nuclear (Wilmington, DE). Recombinant 22-kDa placenta. Osteocytes were nonviable by visual inspection were discarded prior to analysis. RNAs were analyzed by electrophoresis on a 6% acrylamide, 8 M urea gel and analyzed by autoradiography to document its quantity and integrity.

**In vitro Transcription and Translation**—In vitro transcriptions were carried out using 1 μg of linearized plasmid DNA or 0.5 μg of cDNA fragment as template. The 20-μl transcription reaction was incubated in the presence of 1.5 μl of canine pancreatic microsomal membranes (CPMM) (Promega Corporation) and translated for 2.5 h. N-Linked carbohydrate residues were removed by incubating the translation reaction with 2 units of endoglycosidase H (endo H) at 37 °C for 6 h in 0.1% SDS and 0.1 M sodium citrate, pH 5.5. Aliquots of each reaction (1 or 5 μl) were analyzed directly or following immunoprecipitation on 8% SDS-PAGE.

**X. laevis Oocyte Injections**—Experiments utilizing X. laevis were reviewed and approved by the Institutional Animal Care and Use Committee of the University of Pennsylvania. Care of X. laevis and procedures for oocyte harvesting followed standard protocol (Coleman, 1984; Gurdon and Wakefield, 1986; Huet and Marbaix, 1986).

**in vitro** translations were carried out in rabbit reticulocyte lysate in a 15-μl reaction volume at 30 °C for 2 h as detailed previously (Lieberhaver et al., 1984). Where noted, translations were carried out in the presence of 1.5 μl of canine pancreatic microsomal membranes (CPMM) (Promega Corporation) and translated for 2.5 h. N-Linked carbohydrate residues were removed by incubating the translation reaction with 2 units of endoglycosidase H (endo H) at 37 °C for 6 h in 0.1% SDS and 0.1 M sodium citrate, pH 5.5. Aliquots of each reaction (1 or 5 μl) were analyzed directly or following immunoprecipitation on 8% SDS-PAGE.

**Analysis of Injected mRNA**—To assess the stability of the injected RNAs, transcripts were labeled to a high specific activity, injected, and incubated as detailed above. At the noted times oocytes were snap-frozen on dry ice and stored at −70 °C until processed. Oocytes were sequenced and analyzed at room temperature for 45 min in 75 μl of RNA extraction buffer/oocyte (2% SDS, 20 mM NaCl, 5 mM MgCl2, 0.2 M Tris, pH 7.5, and 1 mg/ml proteinase K).

**Receptor Binding Assays**—Oocytes injected with hGHR mRNA, hGHRd3 mRNA, or un.injected controls were incubated overnight at room temperature and washed with fresh MBSh. An experimental sample consisted of five identically injected oocytes incubated at an additional 4 h at 4 °C in 50 μl of MBSh containing 2 mg/ml BSA, approximately 25,000 counts/min [35S]-hGH-N, and the indicated concentrations of unlabeled hormones. Unbound [35S]-hGH was subsequently removed as described with a few modifications (Peacock et
RESULTS

Synthesis of hGHR and hGHrd3 mRNA—We have previously demonstrated that hGHR gene transcripts are alternatively spliced in the placental villi to exclude exon 3. This hGHrd3 mRNA predicts the expression of an hGH receptor isoform with a 22-amino-acid deletion in its extracellular domain. In the present study we attempt to express this putative hGHrd3 receptor in a membrane-associated form and determine whether it can bind GH or GH-related hormones. These studies were carried out by microinjecting hGH and hGHrd3 mRNAs into X. laevis oocytes. Initial attempts to clone full-length hGH and hGHrd3 cDNAs for use as templates for in vitro transcription were unsuccessful. Numerous full-length GH cDNAs were isolated either by screening a placental cDNA library (Urbanek et al., 1992) or by RT-PCR cloning from placent al RNA. In each case full-length inserts contained one or more rearrangements, missense mutations, or nonsense mutations (data not shown). After screening a large number of such clones, we concluded that the bacterial strains that we were using (Escherichia coli HB101 and DH5a) could not support the expression of an intact hGH cDNA. To circumvent this problem full-length expression templates were generated by direct transcription of amplified full-length cDNAs using a SOE/PCR strategy (see “Experimental Procedures”). In these templates the 5'-NTR of the hGH cDNA was replaced by the full 5'-NTR of the human ß-globin cDNA to maximize translational efficiency in the X. laevis oocyte. For convenience these mRNAs are referred to as hGH and hGHrd3 mRNAs with the understanding that the 5'-NTRs are ß-globin in origin. The receptors encoded by each chimeric mRNA contain only hGH sequences. The full-length hGH and hGHrd3 transcripts generated from the SOE/PCR cDNA templates contain only trace amounts of prematurely terminated transcripts (Fig. 1A). The expected 66 base difference in their sizes can be clearly discerned on agarose gel analysis (data not shown).

In Vitro Translation of hGH and hGHrd3 Transcripts—To establish that the SOE/PCR-generated transcripts can be translated, the synthetic hGH and hGHrd3 mRNAs were incubated in rabbit reticulocyte lysate in the presence of [35S]methionine. Incubation duration was prolonged to 2 h to obtain maximal levels of synthesis (Fig. 1B). An 84-kDa product was specifically generated by translation of the hGH, hGHrd3 and hGH mRNAs. Although the predicted size for the unprocessed GHR is 77 kDa, previous studies indicate that deglycosylated GHR from IM9 cells migrates at 95 kDa on SDS-PAGE gels, significantly slower than predicted (Kelly et al., 1991). To demonstrate appropriate processing of the encoded proteins by the endoplasmic reticulum, a step critical to membrane receptor biogenesis, the in vitro translations were carried out in the presence of CPM (Fig. 1C). hGH and hGHrd3 have identical NH2 termini encompassing their signal peptide cleavage sites and they both contain N-linked glycosylation consensus sites (four and five sites, respectively). Translation of the hGHrd3 mRNA in the presence of CPM results in an apparent net increase of 10 kDa (84–94 kDa). A parallel increase is seen in the hGH mRNA translated under identical conditions (Fig. 1C). Treatment with endo H, which cleaves the unmodified N-linked sugar residues, decreases the size of the translation products to the unprocessed preproteins. These results are consistent with in vivo expression and processing of the predicted hGH and hGHrd3 by their respective synthetic RNAs.

Stability of the hGHrd3 and hGH mRNAs in X. laevis Oocytes—To study the receptors on an intact cell surface, the two synthetic mRNAs were separately injected into the cytoplasm of X. laevis oocytes. To assess the utility of this
FIG. 1. In vitro transcription and translation of hGHR and hGHRd3 cDNA templates. A, in vitro transcribed hGHR and hGHRd3 mRNA. The hGHR and hGHRd3 templates prepared by the SOE/PCR reactions see "Experimental Procedures" were transcribed by SP6 polymerase in the presence of [cY-~*P]CTP, resolved on a denaturing acrylamide gel, and autoradiographed. hGHR and hGHRd3 transcripts migrate at their predicted sizes and are indicated by the arrow (DNA molecular weight markers not shown). B, in vitro translation of hGHR and hGHRd3 transcripts. Aliquots of hGHRd3 or hGHR transcription reactions (1 or 5 μl) were translated in a rabbit reticulocyte lysate in vitro translation system in the presence of [35S]methionine. Aliquots of the translation reaction were assayed by SDS-PAGE after a 2-h incubation. The positions of protein molecular size standards are indicated on the left of the autoradiograph, and the location of the hGHR/hGHRd3 specific bands is shown on the right by the double arrows. The identity and quantity of transcript used in each reaction is indicated at the top. C, effects of microsomes and endo H on the sizes of the hGHRd3 (left panel) and hGHR (right panel) in vitro translation products. hGHR and hGHRd3 transcripts were translated in the rabbit reticulocyte lysate system in the presence (+) or absence (−) of CPMM. An aliquot of each sample translated in the presence of CPMM was subsequently incubated with endo H. The locations of molecular size standards are indicated between the two autoradiographs. Treatment with CPMM or endo H is indicated above the respective lanes. The locations of each preprotein (prehGHRd3 or prehGHR), the glycosylated isoforms (glycosylated hGHRd3 or glycosylated hGHR), and the deglycosylated, mature proteins (hGHRd3 or hGHR) are indicated.

FIG. 2. Specific binding of hGH-N to Xenopus oocytes expressing hGHR and hGHRd3. A, binding of recombinant 22-kDa hGH-N to hGHR and hGHRd3. The percent [125I]-hGH-N bound as a function of quantity of unlabeled competitor present in the incubation is illustrated. 100% binding is defined as the amount of [125I]-hGH-N bound in the absence of cold competitor. Binding to hGHR is indicated by the open boxes and binding to hGHRd3 by the closed boxes. The amount of binding observed with uninjected oocytes is shown by the closed circles. The average values and standard deviations from three experiments are shown. B, binding of recombinant 20-kDa hGH-N to hGHR and hGHRd3. The axes and symbols are the same as in panel A. The average values and standard deviations from three experiments are shown.

system for expression and comparison of the hGHRd3 relative to the hGHR mRNA and to optimize the incubation time, the relative stabilities of these two mRNAs were assessed post-injection. Internally 32P-labeled transcripts were injected into Xenopus oocytes, and mRNA was extracted 0, 10, 20, and 30 h post-injection and directly analyzed by gel electrophoresis (data not shown). Each transcript was coinjected with the highly stable human β-globin mRNA as an internal control for the efficiency of RNA harvest. The absolute and relative levels of hGHR and hGHRd3 mRNAs did not change substantially over 30 h of incubation. The stability of the hGHR and hGHRd3 mRNAs suggested that an overnight incubation of the injected oocytes would be a reasonable time for their expression and the assembly of their encoded receptors on the oocyte membrane.

X. laevis Oocyte Binding Assays—A competition binding assay was established to measure the interaction of ligands with the hGH receptors assembled on the surface of intact microinjected oocytes. The sensitivity and accuracy of the assay was established by determining the binding of recombinant GH to oocytes injected with the mRNA for the hGHR; the binding affinity for this interaction is well established in a number of systems (Lesniak et al., 1977; Hocquette et al., 1989; Fuh et al., 1990; Spencer et al., 1990; and Dusquesnoy et al., 1991). Oocytes injected with hGHR mRNA and incubated overnight to allow expression of the hGHR were subsequently incubated with 125I-hGH-N along with known amounts of competing unlabeled hGH. The relationship between 125I-hGH binding to the amount of cold competitor establishes the competition binding curve (Fig. 2). 100% binding, defined as the amount of binding which occurs in the absence of cold competitor, is between 5-10% of the total 125I-hGH-N added to the incubation, and the amount of binding to uninjected oocytes is generally between 5-10% of the maximal specific binding seen in oocytes injected with receptor mRNA. 50% maximal binding or EC50 of hGH for the hGHR expressed on the surface of the injected oocytes is 14
pressed in Xenopus oocytes. hCS binds equally to hGHR and hGHRd3, but its affinity is approximately 1000-fold lower than the placental decidua. To further assess the ability of lactoexpressed by the placental villi, hCS, to bind to hGHRd3. Fig. 3B is a competition binding curve in which hCS competes for hGHR with the same affinity (Fig. 3A). The EC_{50} occurs at 39 ± 15 × 10^{-10} M for hGHRd3 and 64 ± 9 × 10^{-10} M for hGHR. Although the hGHRd3 has a slightly higher affinity for 20-kDa hGH-N than does hGHR in each of three independent binding experiments, the degree of variability in our system makes it difficult to state with certainty whether this difference is significant. The binding studies in Fig. 2 clearly demonstrate that the hGHRd3 mRNA encodes a stable receptor protein in Xenopus oocytes that binds the two isoforms of pituitary hGH-N. The hGHRd3 therefore has the potential to act as a functional hGH receptor.

Placental Hormone Binding Assays—The alternatively spliced hGHRd3 mRNA is selectively expressed in placental syncyial cells. To assess its ability to bind and potentially mediate the action of placationally expressed hormones of the GH gene cluster, we carried out a series of competition binding studies with each of the placently expressed members of the family (hCS, hGH-V, and Prl). In these studies the binding was measured by the ability of the placental hormones to compete for binding with 125I-hGH-N. In each case the hGH and the hGHRd3 were compared. These studies are summarized in Fig. 3. Conditioned media from a mouse fibroblast cell line stably transfected with the hGH-V gene was used as the source of hGH-V. The results of the binding studies using the hGH-V media demonstrate that hGH-V binds hGHRd3 and hGHR with the same affinity (Fig. 3A). The EC_{50} occurs at 10 ± 1 × 10^{-10} M for hGHRd3 and 12 ± 2 × 10^{-10} M for hGHR. Thus the binding affinities of hGH-V for the two receptors are quite similar to the affinities of hGH-N.

We next assessed the ability of the most abundant protein expressed by the placental villi, hCS, to bind to hGHRd3. Fig. 3B is a competition binding curve in which hCS competes with labeled hGH-N for binding to hGHR or hGHRd3 expressed in Xenopus oocytes. hCS binds equally to hGHR and hGHRd3, but its affinity is approximately 1000-fold lower than that of the 22-kDa hGH-N. EC_{50} occurs at 11 ± 1.7 × 10^{-7} M for hGHRd3 and 17 ± 4.2 × 10^{-7} M for hGHR.

The prolactin gene is expressed in both the pituitary and the placental decidua. To further assess the ability of lactogens to bind to the hGHRs, oPrl was used as a prolactin source due to its availability in pure form. As seen in Fig. 3C, oPrl competes effectively with 22-kDa hGH-N for binding to hGHR or hGHRd3. There is no difference in the ability of oPrl to bind to the two forms of the hGHR. EC_{50} occurs at 214 ± 162 × 10^{-10} M for hGHRd3 and 237 ± 4 × 10^{-10} M for hGHR. The affinity of oPrl for the hGHRs is, therefore, 10-20-fold weaker than that of hGH-N.

Internalization of Hormone Receptor Complexes—Ligand binding to the GHR is followed by its internalization (Roupa and Herrington, 1989). To determine whether this post-binding internalization also occurs after ligand binding to hGHRd3, we established an internalization assay using the Xenopus oocytes based on protocols of previous investigators (Rodriguez et al., 1992; Peacock et al., 1988). Oocytes were injected with hGHRd3 or hGHR mRNA and incubated overnight to permit receptor synthesis and assembly on the cell surface. The receptors were then loaded with 125I-hGH-N at 4 °C, and internalization was measured over time by determining the proportion of radioactive tracer that remained on the surface of the oocyte after shifting the cells to RT. Oocytes injected with hGHRd3 or hGHR mRNAs were studied in parallel. The rates of internalization of labeled ligand-bound to the hGHR and hGHRd3 are shown in Fig. 4. The ligand-bound hGHRd3 internalized with the same kinetics as the hGHR. The internalization of the receptor-ligand complex as assayed by the increasing resistance of hormone to release with acid started almost immediately after shifting the oocytes to RT and was complete after approximately 50 min. Essentially the same results were generated in three additional experiments. These data suggest that the initial post-ligand binding steps mediating receptor internalization are identical for hGHRd3 and hGHR.

DISCUSSION

The importance of the exon 3-encoded region of the hGHR is unknown. All sequenced GHRs in mammalian species conserve this region while it is specifically absent in Prl receptor mRNAs (Boutin et al., 1988; Davis and Linzer, 1989; Smith et al., 1989; Adams et al., 1990; Cioff et al., 1990; Edery et al., 1989; Hauser et al., 1990; and Urbanek et al., 1992). This pattern of evolutionary conservation suggests that the loss or retention of exon 3 by alternative splicing could have a major impact on receptor expression and/or function. In addition to affecting hGH-N ligand binding, deletion of exon 3-encoded sequences might affect receptor processing, transport, stability, binding to other related ligands, and/or signal transduction.

In this study we show that the exon 3-deleted isoform of hGH encodes a stable and functional receptor. We demonstrate that hGHRd3 mRNA can be efficiently translated in an in vitro rabbit reticulocyte lysate translation system as well as in an in vivo X. laevis oocyte expression system. Furthermore, the latter system is demonstrated to be appropriate for ligand binding studies. In Xenopus oocytes we demonstrate that hGHRd3 is stably integrated into the cell membrane and efficiently binds and internalizes ligand. Our data indicate that hGHRd3 is as efficient as hGHR at the level of ligand binding or internalization. hGHRd3 binds all three of the placentially expressed members of the GH/Prl gene family, hGH-V, hCS, and Prl, as well as the 22- and 20-kDa isoforms of the pituitary hGH-N.

Of special significance is the finding that hGHRd3 is expressed in placental tissue as are two potential ligands, hGH-V and hCS. The high affinity of hGH-V for the receptor...
Function of the Placental hGH Receptor (hGHRd3)

Fig. 3. Binding of placentally expressed members of the GH/Prl gene cluster to hGHR and hGHRd3. A, binding of recombinant hGH-V to injected oocytes. The axes and symbols are the same as in Fig. 2. Each point represents the average and standard deviation of five oocytes each. For each hormone one representative binding study is shown, although at least two independent experiments for each yielded identical results. B, binding of purified hCS in parallel experiments. C, binding of purified oPrl in parallel experiments.

Fig. 4. Internalization of hGH-N and receptor in a complex. Oocytes injected with hGHR or hGHRd3 mRNA were incubated at 4°C with 125I-hGH. The oocytes were then brought to room temperature and the receptor-hormone complex allowed to internalize for increasing amounts of time at room temperature. The ratio of acid releasable (surface-bound) 125I-hGH-N to the total 125I-hGH-N bound expressed as a percent is shown as a function of time. Each point represents the average and standard deviation of three data points utilizing five oocytes each. Internalization of the hGHRd3 complex is indicated by closed boxes and that of hGHR by open boxes.

makes its potential for local interaction quite evident. Although hCS has only 1/1000 the affinity for hGHRd3 compared to hGH-V, a case can also be made for its potential significance as a ligand since hCS is found in the sera of pregnant women during the second and third trimester at levels that are approximately 1000-fold that of normal serum hGH-N and 300-fold that of hGH-V at term (Frankenne et al., 1988; Phillips and Vencak-Jones, 1989; Walker et al., 1991). The fact that hGHRd3 may serve as a receptor for both hGH-V and hCS supports their potential to act in an autocrine fashion in the syncytial cells of the placental villi. What effect this autocrine stimulation might mediate is open to speculation, but the rapid growth of the placenta presents an obvious possibility.

The validity of the Xenopus oocyte expression system for the study of GHR and ligand interactions is supported by several lines of evidence. The EC50 values that we obtained for hGHR using this approach correspond quite closely with those obtained in other systems (Lesniak et al., 1977; Hocquette et al., 1988; Fuh et al., 1990; Spencer et al., 1990; Dusquesnoy et al., 1991). In our system, only oPrl has a significantly different binding affinity for the GHR than has been reported in other studies. Monkey kidney COS cells transfected with the rabbit GHR have an affinity for oPrl that is approximately 50-fold weaker than that for hGH-N (Spencer et al., 1990). In IM9 cells, a human lymphoblast cell line that has hGH binding activity, oPrl competes approximately 1000-fold less efficiently for receptor binding than does hGH-N (Lesniak et al., 1977). The reasons for discrepancy among these results are not clear. Our results are reproducible with several different batches of hormone. Each oPrl sample that we used contained less than 0.1% contamination with other anterior pituitary hormones as determined by radioimmunoassay (see “Experimental Procedures”). The strong competition observed with oPrl cannot, therefore, be due to contamination with oGH. The differences in results may reflect details of each experimental model. For example, IM9 lymphocytes may express a multitude of yet uncharacterized receptors that could compete for oPrl binding and shift the binding curve to the right. Lymphocytes have recently been characterized as possessing Prl receptors, and the structure of the rat Nb2 lymphoma cell Prl receptor is unique, containing a short cytoplasmic tail (Pellegrini et al., 1992; Ali et al., 1992). In the COS cell experimental model, competition studies were carried out by binding to the rabbit GHR which may have a different affinity for hGH than does the hGHR.
Alternative splicing has been shown to impart different signal transduction activities to receptors (Pin et al., 1992; Nakamura et al., 1992; Doherty et al., 1992; Sokka et al., 1992; Danoff et al., 1991; and Sommer et al., 1990); however, in all these cases the alternatively spliced domain has been localized to the intracellular region. More recently, studies by Chiba et al. (1993) using chimeric cytokine receptors showed that the specificity of the signal transduction pathway which was activated in response to ligand binding was determined by the extracellular domain of the receptor. It is believed that the activation of the signaling pathway may be mediated via an accessory protein which recognizes specific extracellular domains. It is therefore possible that the presence or absence of exon 3 may allow the interaction of different accessory proteins which may activate different signal transduction pathways.

Another possible difference between the biological activity of hGHR3 and hGHR in vivo may be at the level of expression via translational efficiency. In this study we used the β-globin 5'-NTR instead of the endogenous GH receptor 5'- NTR and therefore did not address the significance of different endogenous 5'-NTRs of the hGHR mRNA (Leung et al., 1987; Godowski et al., 1989). Such differences, possibly reflecting utilization of alternative promoters, might affect the subsequent splicing as well as translational efficiency of the processed mRNA (Moldave, 1985; Ratner et al., 1987; Kozak, 1987; London et al., 1987; Kozak, 1988).

Finally, immunochemical studies directly support the expression of GHRs in the fetus and placenta. These studies have shown that GHRs are expressed at significant levels in fetal liver, pancreas, cerebral cortex, and epidermis as well as second trimester placenta (Hill et al., 1992). Immunoreactive GHR was also detected in the syncytiotrophoblast of the placental villi as early as 8 weeks of gestation, and strong staining for the GHR was detected at term (Hill et al., 1992). Decidual cells also stain for the GHR while cytotrophoblast, chorionic trophoblast, and amnion do not. This would indicate that hGHR3 protein, which our earlier studies have shown is the only GHR mRNA isoform expressed in the placental villi (Urbanke et al., 1992), is translated at significant levels in the placenta. Taken together, the results of the present studies in the context of these previous reports strongly support the expression of a functional hGHR3 isoform in the placenta. The function(s) of this receptor may serve in autocrine, paracrine, and/or endocrine activation can now be addressed.
Function of the Placental hGH Receptor (hGHRd3)

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