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Expression of Somatostatin Receptor SST4 in Human Placenta and Absence of Octreotide Effect on Human Placental Growth Hormone Concentration during Pregnancy*

PHILIPPE CARON, LOUIS BUSCAIL, ALBERT BECKERS, JEAN-PIERRE ESTÈVE, AHMED IGOUT, GEORGES HENNEN, AND CHRISTIANE SUSINI

Service d'Endocrinologie et Maladies Métaboliques (P.C.) and INSERM U-151, Institut Louis Bugnard (L.B., J.-P.E., C.S.), IFR31, Centre Hospitalier Universitaire Rangueil, Toulouse, France; and Service d'Endocrinologie (A.B.) et de Biochimie (A.I., G.H.), Centre Hospitalier Universitaire de Liège, Liège, Belgium

ABSTRACT

In pregnancy, the human placenta GH acts as a growth-promoting hormone and appears to be the main stimulator of insulin-like growth factor I (IGF-I) secretion. In a woman with a TSH-secreting macroadenoma, successful treatment with the somatostatin analog octreotide was conducted during the first month and the second half of pregnancy without side-effects on placental and fetal development. As observed in normal pregnancy, both serum placental GH and IGF-I levels increased throughout pregnancy and dropped sharply after delivery. In placental membranes from both treated and healthy untreated patients, we demonstrated the presence of high affinity binding sites for somatostatin-14 (K_d, 4.6 and 5.3 nmol/L; binding capacity, 1.53 and 1.35 pmol/mg protein, respectively). These receptors displayed low affinity for octreotide (IC_{50}, 1.2–2 μmol/L), suggesting the presence of SST1 and/or SST4 receptors. We found that messenger ribonucleic acids of these two subtypes were expressed in both human placental tissue and purified human cytotrophoblast cells. Finally, the SST1-selective analog, des-AA^{1,2,5}[D-Trp^8,IAmp^9]S-14 had low affinity for placental somatostatin receptors. These results argue in favor of the presence of the SST4 subtype in human placenta. At the doses administered, octreotide did not bind to placental somatostatin receptors. Our results may explain the absence of changes in both human placental GH and IGF-I concentrations that we observed during octreotide treatment. (*J Clin Endocrinol Metab* **82:** 3771–3776, 1997)

HUMAN PLACENTA has been shown to express the GH-V gene and to produce placental GH (hPGH), which is distinguishable from pituitary GH using specific monoclonal antibodies (1). It appears likely that hPGH is biologically active as a growth-promoting hormone; in the second half of pregnancy, serum insulin-like growth factor I (IGF-I) increases when hPGH concentrations are high, whereas pituitary GH secretion is suppressed (2, 3). In nonpregnant women, pituitary GH secretion is pulsatile as a result of the actions of neurotransmitters and neuropeptides, with both stimulatory (GHRH) and inhibitory (somatostatin) effects. It has been shown that hPGH has a sustained, rather than episodic, secretion (4, 5). However, little is known about factors that regulate hPGH secretion (6). Octreotide, the first somatostatin analog introduced for clinical practice, is routinely used for the treatment of GH- and TSH-secreting pituitary adenomas. The various actions of octreotide are mediated through specific membrane receptors, and to date, five human somatostatin receptor subtypes have been cloned and characterized (7, 8). Recently, we reported the first woman with a TSH-secreting macroadenoma treated with octreotide during the second half of pregnancy (9). The present work was undertaken to study hPGH and IGF-I secretions during somatostatin analog therapy. To explain the absence of an octreotide effect on hPGH and IGF-I serum concentrations, the expression of somatostatin receptors was studied in human placenta.

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Address all correspondence and requests for reprints to: Dr. Philippe Caron, Service d'Endocrinologie et Maladies Métaboliques, CHU Rangueil, 1, avenue J. Poulhés, 31054 Toulouse Cedex 4, France.

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Subjects and Methods

Patient

Detailed clinical, endocrine, and neuroradiological features of the patient were reported recently (9). Briefly, a 31-yr-old infertile woman with hyperthyroidism caused by a TSH-secreting macroadenoma was treated with a continuous sc infusion of 300 μg octreotide/day. She became euthyroid, and the macroadenoma decreased in size. Subsequently, the woman was found to be pregnant, and octreotide was stopped at 1 month of gestation. At 6 months, she was hyperthyroid, serum TSH was elevated, a visual field examination was abnormal, and magnetic resonance imaging showed an enlargement of the macroadenoma. After obtaining written informed consent, octreotide therapy was administered again at the same dose (continuous sc infusion of octreotide at 300 μg/day). Thereafter, serum TSH and thyroid hormone concentrations returned to normal, the visual fields improved rapidly, and the size of the macroadenoma decreased. Maternal serum octreotide concentrations during the seventh month of pregnancy were 890 ± 150

pg/mL (mean ± SD); octreotide therapy was continued until an elective cesarean section was performed at 8 months gestation. Examination of the placenta was unremarkable. At birth, the newborn (weight, 3300 g; length, 51 cm) was normal. The cord serum TSH, thyroid hormone, PRL, and GH concentrations were in the normal range.

In vivo studies

Blood samples for measurements of hPGH and IGF-I concentrations were obtained before pregnancy, subsequently every 15 days during gestation, and after elective cesarean section. hPGH was assayed using an immunoradiometric assay with specific monoclonal antibodies raised against recombinant hPGH (10) and purified recombinant hPGH as standard (11). No cross-reactivity with human placental lactogen or pituitary GH was found. The detection limit of the assay was 0.5 μg/L. The intra- and interassay coefficients of variation were 3.3% and 8.5%, respectively. Plasma IGF-I concentrations were measured, after ethanol-acid extraction, using an IGF-I RIA kit from Nichols Institute Diagnostics (San Juan Capistrano, CA). The intra- and interassay coefficients of variation were less than 5.2% and 11.2%, respectively. The pituitary GH concentration was measured using a double monoclonal antibody method (Elisa hGH, Cis-Bio-International, Gif-sur-Yvette, France). The intra- and interassay coefficients of variation were less than 2.8% and 4.4%, respectively. Octreotide concentrations were determined using a specific RIA (12).

In vitro studies

Materials. Octreotide, somatostatin-14 (S-14), and Tyr^{11}-S-14 were obtained from Sandoz (Basel, Switzerland). The stable somatostatin analog RC-160 was a gift from A. V. Schally, Tulane University (New Orleans, LA). Des-AA^{1,2,5}[D-Trp^{8},IAmp^{9}]S-14 was synthesized, as previously described (13), by Drs. C. Hoeger and J. Rivier at The Salk Institute for Biological Studies (La Jolla, CA). Soybean trypsin inhibitor, bacitracin, and BSA were purchased from Sigma (Saint Quentin-Fallavier, France). guanosine 5′-[γ-thio]triphosphate (GTP[γ-S]) was obtained from Boehringer Mannheim (Meylan, France). Tyr^{11}-S-14 was radioiodinated and purified by HPLC as previously described (14). Ribonuclease-free deoxyribonuclease I was obtained from Eurogentec (Seraing, Belgium). Moloney murine leukemia virus reverse transcriptase was purchased from Life Technologies (Cergy Pontoise, France). RNAble and oligonucleotides were obtained from Bioprobe (Montreuil s/Bois, France). RNAsin was purchased from Promega (Charbonnieres, France). *Taq* polymerase was obtained from Beckman (Gagny, France).

Tissue samples. Placental samples were obtained from the patient treated with the somatostatin analog octreotide for a TSH-secreting pituitary macroadenoma and from four healthy adult women after normal pregnancy and delivery of term infants. Tissue samples were immediately frozen in liquid nitrogen for 15 min, and stored at −80 C until use. The study protocol was approved by the hospital ethical committee.

Binding studies. Placental samples were washed, roughly cut, and homogenized using a Teflon potter in 20 mmol/L HEPES buffer (pH 7.4) containing 0.3 mg/mL soybean trypsin inhibitor. The homogenate was centrifuged at 26,000 × *g* for 30 min at 4 C. After centrifugation, the pellet was resuspended in the same buffer and homogenized again with a Dounce homogenizer (Kontes Co., Vineland, NJ). The obtained crude membranes were immediately analyzed for protein concentration by the Bradford method (15) and kept at −80 C until use. Before the binding study, acid washing was performed to eliminate endogenous and exogenous ligand: 300 μL acetic acid (0.2 mol/L)-sodium chloride (0.5 mol/L) solution were added to 500 μL crude membranes for 2 min at 4 C. The membranes were then washed three times with 500 μL 20 mmol/L HEPES buffer, 1 mg/mL BSA, 0.3 mg/mL soybean trypsin inhibitor, and 0.5 mg/mL bacitracin (pH 7.4). The binding study was performed in the same buffer as previously described (14). For saturation curves, 10–15 μg membrane proteins were incubated at 25 C for 90 min with 20 pmol/L to 1 nmol/L labeled [^{125}I]Tyr^{11}-S-14 (SA, 900 Ci/mmol; 1 Ci = 37 gigabecquerels); nonspecific binding was determined in the presence of 1 μmol/L S-14. In ligand competition experiments, membranes were incubated under the same conditions with 30 pmol/L [^{125}I]Tyr^{11}-S-14 and varying concentrations of unlabeled peptide analogue.

Ribonucleic acid (RNA) isolation and reverse transcription-PCR (RT-PCR). Total RNA was extracted by the modified procedure of Chomczynski and Sacchi (16). Human placental tissue was homogenized with a Polytron tissue homogenizer (Brinkmann Instruments, Westbury, NY) in RNAble, and RNA was extracted as previously described (17). Before RT, 1 μg total RNA was submitted to a ribonuclease-free deoxyribonuclease I treatment as described previously (18) to eliminate genomic DNA regarding the intronless structure of SST genes. RT and PCR were conducted on a DNA Thermal Cycler (Trio-Thermobloc-Biometra, Gottingen, Germany) as previously described (18). The locations of the oligonucleotide primer pairs used to amplify each complementary DNA are shown with respect to the translational start site in the DNA sequence of SST(s) and external control β-actin as follows: hSST1 sense (nucleotides 622–636) and antisense (nucleotides 1022–1036) primers, hSST2 sense (nucleotides 1–23) and antisense (nucleotides 1081–1104) primers, hSST3 sense (nucleotides 721–741) and antisense (nucleotides 1148–1167) primers, hSST4 sense (nucleotides 843–862) and antisense (nucleotides 1099–1120) primers, hSST5 sense (nucleotides 539–557) and antisense (nucleotides 994-1011) primers, and β-actin sense (nucleotides 506–529) and antisense (nucleotides 998-1021) primers (19–22). Amplified fragments were separated by 7% PAGE and stained with ethidium bromide. Only samples positive for actin were considered for SST messenger RNA (mRNA) expression. To confirm that PCR products resulted from complementary DNA templates rather than genomic DNA, parallel RT-PCR reactions were carried out for each sample in the absence of reverse transcriptase during the RT procedure. These procedures and PCR reactions on water were used as a negative control of the reaction. Positive controls were also used using amplification of hSST plasmids as described previously (18). Two separate runs of PCR reaction were performed for each tissue specimen and for the five subtypes. RT-PCR analysis were also performed on total RNA extracted from human purified villous cytotrophoblast cells isolated from term placentas of healthy women as previously described (23).

Results

In vivo studies

Figure 1 shows serum hPGH and IGF-I concentrations before pregnancy, throughout gestation, and after elective cesarean section in the woman treated with octreotide for a

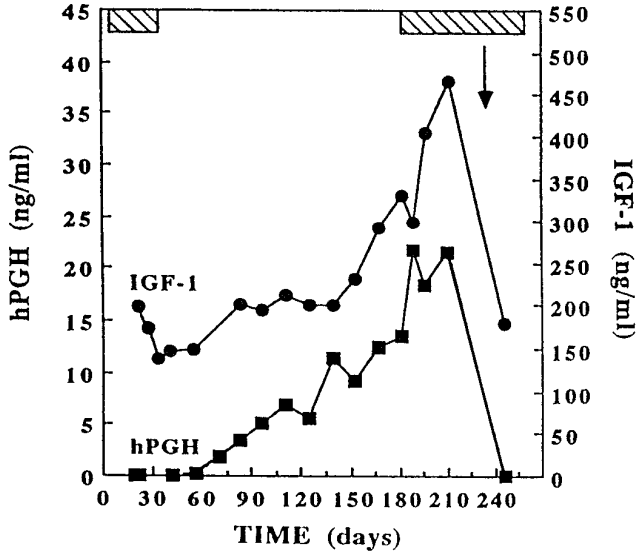

FIG. 1. Serum hPGH and IGF-I concentrations during pregnancy in a woman with a TSH-secreting macroadenoma treated with octreotide. ▨, Octreotide was given at a dose of 300 μg/day by continuous sc infusion. The *arrow* indicates the time of elective cesarean section.

TSH-secreting macroadenoma. hPGH was undetectable in serum before gestation and during the first month of pregnancy. Then, the hPGH concentration increased and was maximal during the third trimester of pregnancy. After delivery, serum hPGH levels decreased rapidly in maternal serum. Likewise, IGF-I concentrations increased throughout pregnancy and dropped sharply after delivery. During the third trimester of pregnancy (*i.e.* during treatment with octreotide), pituitary GH levels were suppressed (pituitary GH, 0.7 ± 0.1 μg/L).

In vitro studies

[^{125}I]Tyr^{11}-S-14 specifically bound to placental crude membranes obtained from both treated and healthy untreated patients. The Scatchard plot of the saturation curve revealed the presence of one class of high affinity binding sites with K_d of 4.6 ± 1 and 5.3 ± 1.6 nmol/L and maximal binding capacities (B_{max}) of 1.53 ± 0.15 and 1.35 ± 0.15 pmol/mg protein for placentas of treated (Fig. 2A) and healthy untreated (Fig. 2B) patients, respectively (mean ± SE of three experiments performed in triplicate). Competitive inhibition in the presence of unlabeled analogs revealed that the concentrations producing half-maximal inhibition of binding (IC_{50}) were 7.1 ± 1.5 nmol/L for S-14 and 1.2 ± 0.3 μmol/L for SMS 201–995 in the treated patient (Fig. 3A). Similar results were obtained in healthy untreated patients; the IC_{50} were 9.3 ± 0.7 nmol/L, 0.46 ± 0.3 μmol/L, and 2 ± 1 μmol/L for S-14, RC-160, and SMS 201–995, respectively (Fig. 3B). The somatostatin receptors present on human placental membranes bound with low affinity to the synthetic stable analogs

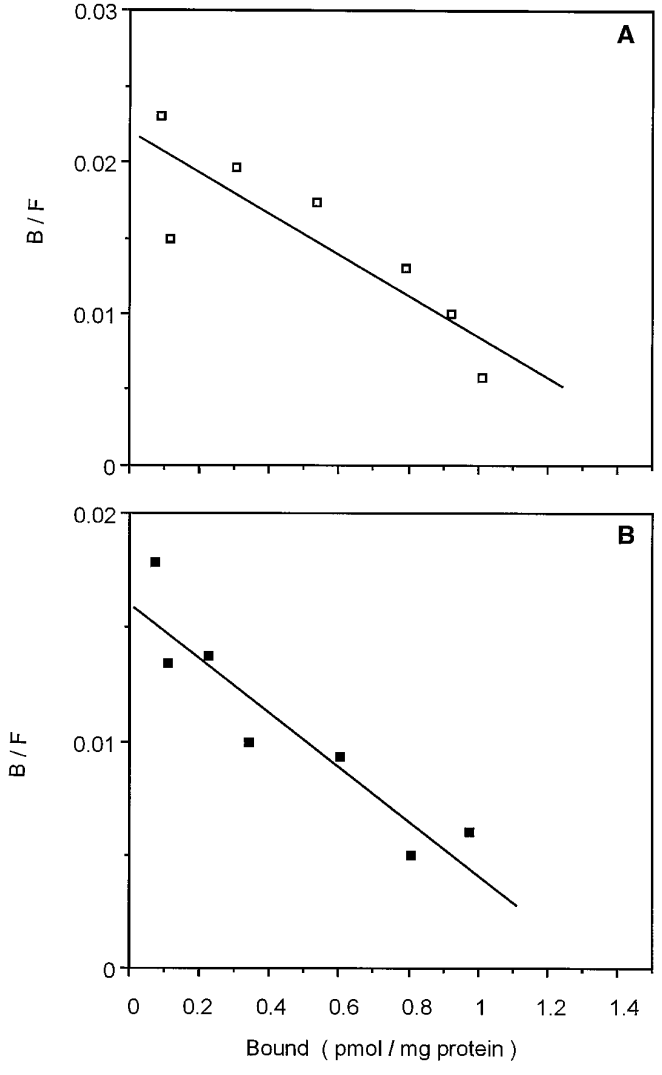

FIG. 2. Linear Scatchard plot of the [^{125}I]Tyr^{11}-S-14 binding saturation curve in membrane preparations from human placentas (A, placenta from a patient treated with octreotide; B, placenta from a healthy untreated patient). Both plots indicate one class of high affinity binding site. B/F, Bound/free ratio. Each *curve* is representative of three experiments performed in triplicate.

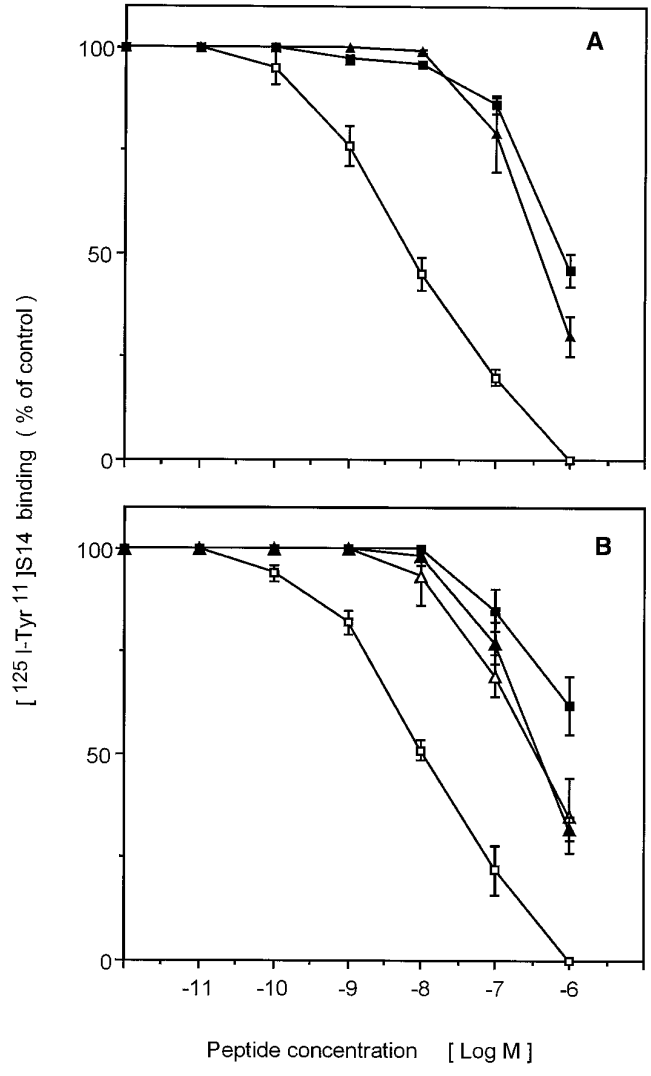

FIG. 3. Concentration dependence of the inhibition of [^{125}I]Tyr^{11}-S-14 binding by somatostatin analogs. Membranes from human placentas (A, octreotide-treated patient; B, healthy untreated patients) were incubated with [^{125}I]Tyr^{11}-S-14 and the indicated concentration of S-14 (□), SMS 201–995 (■), RC-160 (△), and des-AA^{1,2,5}[D-Trp^{8},IAmp^{9}]S-14 (▲). Results are expressed as a percentage of the maximal specific binding observed in the absence of competitor and are the mean of three separate experiments performed in triplicate. For healthy untreated patients (B) results are the mean of three separate experiments performed in triplicate on placental membrane preparations from three patients.

SMS 201–995 and RC-160. This pharmacological profile was related to that previously observed for the human cloned SST1 and SST4 receptors (7, 8). To pharmacologically differentiate SST1 and SST4 receptors (that could be expressed in human placenta), we investigated the ability of the selective agonist for SST1 receptor, des-AA^{1,2,5}[D-Trp^{8},IAmp^{9}]S-14 (13), to inhibit [^{125}I]Tyr^{11}-S-14 specific binding to human placental membranes. As shown in Fig. 3, A and B, des-AA^{1,2,5}[D-Trp^{8},IAmp^{9}]S-14 displaced [^{125}I]Tyr^{11}-S-14 binding with low affinity, as the observed IC_{50} were 0.64 ± 0.2 and 0.4 ± 0.1 μmol/L in treated and healthy untreated patients, respectively. These results rule out the possibility of pharmacologically active SST1 receptors and suggest the presence of SST4 receptors on human placenta. In addition, incubation of placental membranes from the treated patient with increasing concentrations of the nonhydrolyzable GTP analog, GTP[γ-S], reduced [^{125}I]Tyr^{11}-S-14 binding to placental somatostatin receptors (Fig. 4). Similar results were obtained in membranes from placentas of healthy untreated patients (data not shown). Magnesium chloride at concentrations of 5 and 10 mmol/L in binding buffer inhibited specific [^{125}I]Tyr^{11}-S-14 binding by 39 ± 6% and 59 ± 4%, respectively, compared to the control values assayed without magnesium chloride in the binding buffer (mean ± SE; n = 3).

We finally examined simultaneously the mRNA expression of the five somatostatin receptor subtypes in human placental tissue and purified human cytotrophoblast cells from healthy untreated patients. As shown in Fig. 5A, SST1, SST4, and SST5 mRNAs were expressed in whole placenta. The three subtypes were also expressed in cytotrophoblast cells; SST4 was more clearly detected than SST1 and SST5 (Fig. 5B). A second run of the experiment confirmed the expression of these three mRNA subtypes; SST2 and SST3 mRNAs were not detected.

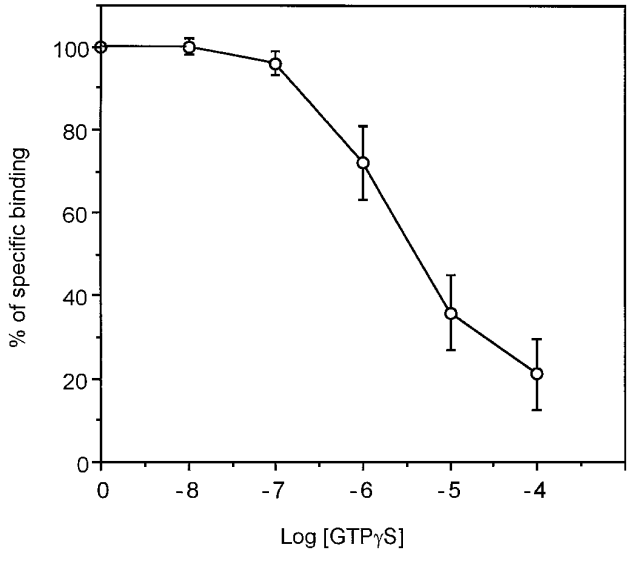

FIG. 4. Effect of the stable GTP analog GTP[γ-S] on the binding of [^{125}I]Tyr^{11}-S-14 to human placental membranes from a patient treated with octreotide. Membranes were incubated with [^{125}I]Tyr^{11}-S-14 and the indicated concentrations of GTP[γ-S]; nonspecific binding was determined in the presence of 1 μmol/L S-14. Results are expressed as the percentage of specific binding obtained in membranes incubated in the absence of GTP[γ-S] and are the mean ± SE of two separate experiments performed in triplicate.

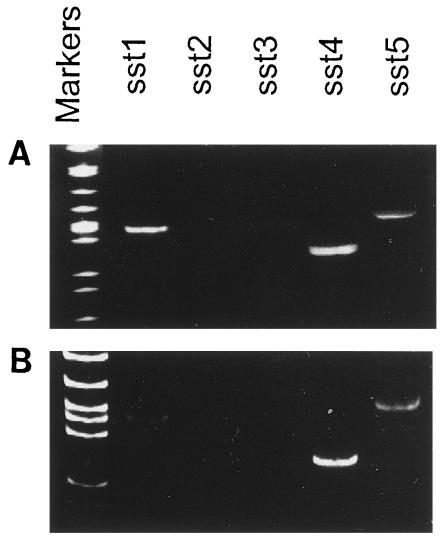

FIG. 5. Expression of somatostatin receptor genes SST1–5 in human placenta. A polyacrylamide ethidium bromide-stained gel shows SST1–5 mRNA expression after RT-PCR analysis on total RNAs extracted from untreated patients. A, Placental tissue; B, purified cytotrophoblast cells. The expected lengths for PCR products were 415 bp (hSST1), 1105 bp (hSST2), 447 bp (hSST3), 278 bp (hSST4), and 473 bp (hSST5). DNA size markers (pGEM markers, Promega), from *top* to *bottom*, are 2645, 1605, 1198, 676, 517, 460, 396, 350, 222, 179, and 126 bp (A) and 676, 517, 460, 396, 350, and 222 bp (B). Results are representative of two runs of RT-PCR experiments.

Discussion

High estrogen levels, normally present during pregnancy, are known to promote growth of the normal pituitary and of PRL-secreting macroadenomas (24). In patients with pituitary tumors, transphenoidal surgery is rarely indicated in the case of symptomatic tumor growth and is associated with an increased risk of fetal loss during the first and second trimesters of gestation (25). In pregnant women with macroprolactinomas, bromocriptine is used successfully to reduce tumor size (24), and in the majority of cases, no neonatal abnormalities have been reported with dopamine agonist treatment during pregnancy. In most patients with GH-secreting pituitary tumors, GH hypersecretion is stable, and no evolution of the adenoma has been reported during pregnancy (5). To date, two acromegalic women have been treated with octreotide during the first month of gestation, and the pregnancies were maintained to term, with normal neonates (26, 27). In our patient with a TSH-secreting macroadenoma, symptomatic tumor growth was observed after 6 months of gestation, and octreotide therapy was administered again. Exposure of the fetus to octreotide during the first month as well as the last trimester of gestation did not induce any malformation and did not affect placental or fetal development.

Increased IGF-I levels seem to be important for the regulation of pregnancy-associated tissue and placental growth (28, 29). During normal pregnancy, the increase in serum IGF-I levels is independent of the maternal somatotrophs,

and hPGH appears to be the main stimulator of IGF-I secretion during the second half of pregnancy when pituitary GH secretion is suppressed (3). hPGH is secreted by placenta, and levels increase during normal pregnancy (1–3). The regulation of hPGH in humans has been sparsely investigated and does not appear to be under GHRH control (6). To our knowledge, no information exists regarding the effects of somatostatin or its analogs on hPGH and IGF-I secretion in humans. In the patient with a TSH-secreting macroadenoma given octreotide during the third trimester of gestation, serum hPGH and IGF-I levels were higher in late pregnancy than in first and second trimesters of gestation and decreased rapidly after elective cesarean section. Therefore, changes in hPGH and IGF-I levels were comparable to those reported during normal pregnancy (2, 3). The normal neonate at birth suggests physiological IGF-I secretion during pregnancy and implies that IGF-I levels were biologically sufficient for normal fetal development. Finally, reinstitution of octreotide therapy was associated with a normalization of thyroid parameters and a new reduction of the macroadenoma, whereas hPGH and IGF-I secretions were similar to those observed during normal pregnancy. Therefore, in our patient treated during late pregnancy, hPGH and IGF-I secretions were not influenced by sc infusion of octreotide at a dose previously reported to control TSH hypersecretion in patients with GH- and TSH-secreting pituitary adenomas (30, 31).

Five somatostatin receptor subtypes and one splice variant have been cloned from human and rodent (7, 8, 19–21). After expression of human SST1 to five gene clones in mammalian cell lines, we and others demonstrated that all five human receptors bind with high affinity to S-14 (IC_{50}, 0.1–2.3 nmol/L). However, they display a distinct profile for binding with clinically used somatostatin analogs, SMS 201–995 (octreotide), BIM 23014 (lanreotide), and RC-160 (vapreotide). These analogs bind with high affinity to SST2, SST3, and SST5 (IC_{50}, 0.1–22 nmol/L) and with low affinity to SST1 and SST4 (IC_{50}, 200 to 1000 nmol/L) (7, 8, 19–21). The similar pharmacological properties observed for SST1 and SST4 receptors might be related to the high degree of amino acid sequence homology between these two subtypes (19, 21). In placental cell membranes from patients treated, or not, with octreotide, we demonstrated the presence of somatostatin receptors that bound S-14 with high affinity. These results differ from those previously reported, describing high capacity, but low affinity, binding sites in plasma cell membranes of human placenta (32).

In the present work, we describe receptors that display a low affinity for the somatostatin analogs SMS 201–995 and RC-160, accounting for the possible presence of SST1 and SST4 subtypes (7, 8, 19, 21, 33–36). To pharmacologically distinguish the two receptors in human placenta, we used the recently characterized SST1-selective analog des-$AA^{1,2,5}$[D-Trp^{8},$IAmp^{9}$]S-14, which has been found to inhibit binding of [^{125}I]Tyr^{11}-S-14 to human SST1 expressed in COS-7 and CHO (Chinese hamster ovary) cells with IC_{50} of 1.8 and 4.9 nmol/L, respectively (13). Conversely, in these cells, the analog inhibits [^{125}I]Tyr^{11}-S-14 binding to the other recombinant receptors with an IC_{50} from 0.63–1 μmol/L; the IC_{50} for human SST4 is 0.87 μmol/L (13). This latter affinity was related to that we observed in the present study for somatostatin receptors present in human placenta. Unfortunately, among various ligands previously synthesized, there are no highly selective compounds for human SST4 receptor (34, 36). Nevertheless, our results argue in favor of the presence of this subtype in human placenta. Moreover, we found that the vapreotide displays a higher affinity than octreotide for placental somatostatin receptors. This result is in accordance with that obtained with the recombinant human SST4 expressed in mammalian cells (21, 33, 35).

As expected for a G protein-coupled receptor, the binding was sensitive to GTP analogs, but this sensitivity was less marked than we and others observed for SST2 receptor (7, 8, 36, 37). This characteristic has been previously described for the SST1/SST4 receptor subclass that appeared poorly or not sensitive to GTP analogs or pertussis toxin treatment (34, 37, 38). Magnesium is theoretically required to promote the formation of high affinity agonist-receptor complex. We found in the present study that the binding of [^{125}I]Tyr^{11}-S-14 to somatostatin receptor of human placenta was reduced by the addition of magnesium chloride. The sensitivity of binding to magnesium has been previously investigated for SST1 and SST2 receptors stably expressed in CHO cells. Although somatostatin binding to SST2 was enhanced by magnesium chloride, that of SST1 was decreased (37). This atypical binding property was also observed for placental somatostatin receptors and could represent one of the characteristics of the SST1/SST4 receptor subclass.

Finally, we detected SST4 mRNA in human placental tissue and villous cytotrophoblast cells. We also found expression of SST4 mRNA in syncytiotrophoblast cells (data not shown). These findings represent an argument in favor of the expression of this subtype in placental villi. In addition, we found expression of SST1 and SST5 mRNA. However, the pharmacology of somatostatin receptors described in the present work differs from that of SST1 and SST5. Moreover, the measured mRNA levels do not necessarily reflect the amount of functional receptor protein on the cell surface. Based on pharmacological properties and gene expression, we conclude that SST4 is predominantly expressed in human placenta.

In the patient treated with octreotide during late pregnancy, serum analog concentrations were periodically determined and were in the nanomolar range. At the measured concentrations, binding of the analog to somatostatin receptors should require high affinity receptors. At these concentrations, the analog does not interact with the somatostatin SST4 receptors present in human placenta. The exact function of these receptors remains to be elucidated, especially for their possible involvement in placenta physiology. However, the presence of somatostatin receptors with low affinity for octreotide in human placenta may provide an explanation for the absence of changes in both hPGH and IGF-I concentrations observed during octreotide treatment.

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