0026-895X/94/030417-11$03.00/0

MOLECULAR PHARMACOLOGY, 45:417-427

Molecular Cloning, Functional Characterization, and Chromosomal Localization of a Human Somatostatin Receptor (Somatostatin Receptor Type 5) with Preferential Affinity for Somatostatin-28

ROSEMARIE PANETTA, MICHAEL T. GREENWOOD, ANIA WARSZYNSKA, LIDIA L. DEMCHYSHYN, ROBERT DAY, HYMAN B. NIZNIK, COIMBATORE B. SRIKANT, and YOGESH C. PATEL

Fraser Laboratories, Departments of Medicine and Neurology and Neurosurgery, McGill University, Royal Victoria Hospital, and the Montreal Neurological Institute, Montreal, Quebec, Canada H3A 1A1 (R.P., M.T.G., A.W., C.B.S., Y.C.P.), Laboratory of Molecular Neurobiology, The Clarke Institute of Psychiatry, Toronto, Ontario, Canada M5T 1R8 (L.L.D., H.B.N.), and Clinical Research Institute of Montreal, Montreal, Quebec, Canada H2W 1R7 (R.D.)

Received June 3, 1993; Accepted December 14, 1993

SUMMARY

Using a combination of polymerase chain reaction and genomic library screening we have cloned a human gene for a subtype of the somatostatin (SST) receptor (SSTR) termed human SSTR5 (hSSTR5), which is located on chromosome 16. The predicted amino acid sequence of hSSTR5 displays 75% sequence identity with a recently identified rat SSTR [*Mol. Pharmacol.* **42**:939–946 (1992)], suggesting that it is the human homologue of this receptor. hSSTR5 consists of a 363-residue polypeptide exhibiting a putative seven-transmembrane domain topology typical of G protein-coupled receptors. The receptor displays considerable sequence identity to hSSTR1 (42%), hSSTR2 (48%), hSSTR3 (47%), and hSSTR4 (46%). Membranes prepared from COS-7 cells transiently expressing the hSSTR5 gene bound ^{125}I-Leu^{8},D-Trp^{22},Tyr^{25}-SST-28 (^{125}I-LTT-SST-28) with high affinity and in a saturable manner. SST-14, SST-28, and various synthetic SST peptide agonists produced dose-dependent inhibition of radioligand binding with the following rank order of potency: LTT-SST-28 > SST-28 > D-Trp^{8}-SST-14 > SST-14 ≈ RC-160 ≈ BIM 23014 > MK-678 > SMS 201-995. hSSTR5 bound SST-28 with a 12.6-fold greater affinity (K_i = 0.19 nM), compared with SST-14 (K_i = 2.24 nM), indicating that the receptor is SST-28 selective. Addition of GTP, guanosine-5′-*O*-(3-thio)triphosphate, Na^+ ions, or pertussis toxin greatly reduced ^{125}I-LTT-SST-28 binding, thereby indicating that hSSTR5 is coupled to pertussis toxin-sensitive G proteins. Both SST-14 and SST-28 displayed dose-dependent inhibition of forskolin-stimulated cAMP accumulation, consistent with functional coupling of the receptor to adenylyl cyclase inhibition. Northern blot analysis of SSTR5 mRNA revealed a 2.4-kilobase transcript in normal rat pituitary and GH_3 rat pituitary tumor cells and a 4.0-kilobase transcript in normal human pituitary. Reverse transcriptase polymerase chain reaction revealed expression of the hSSTR gene in fetal human pituitary and hypothalamus but not in human cerebral cortex. *In situ* hybridization of the rat pituitary showed that SSTR5 mRNA is selectively localized in the anterior lobe. SSTR5 mRNA was not expressed in four human pituitary tumors (somatotroph adenoma, prolactinoma, and chromophobe adenomas) or in a human insulinoma. Although hSSTR5 displays ~75% sequence identity with rat SSTR5, the two receptors display significantly different pharmacological profiles, especially with respect to their binding affinities for the SST analogue SMS 201-995.

Mammalian SST consists of two naturally occurring bioactive peptides, SST-14 and SST-28, which are produced in different proportions in neurons and secretory cells through differential processing of a common pro-SST precursor (1–3). These two peptides have various physiological roles, such as controlling endocrine and exocrine secretion, functioning as a neurotransmitter or neuromodulator, and regulating cell proliferation as anti-peptide growth factors (1, 2, 4). The biological actions of SST-14 and SST-28 overlap virtually completely, although each peptide displays some selectivity. For instance, SST-28 is more potent than SST-14 in inhibiting growth hormone, thyroid-stimulating hormone, and insulin secretion, whereas SST-14 is selective with respect to inhibiting glucagon

This work was supported by Medical Research Council of Canada Grants MT-10411 (Y.C.P.), PG-11121 (H.B.N.), and MT-11268 (R.D.). M.T.G. was supported by a postdoctoral fellowship from the Fonds de la Recherche en Santé du Quebec.

Sequences reported in this paper have been deposited in GenBank under accession numbers L14865 and X74828.

ABBREVIATIONS: SST, somatostatin; SSTR, somatostatin receptor; rSSTR, rat somatostatin receptor; hSSTR, human somatostatin receptor; PCR, polymerase chain reaction; SDS, sodium dodecyl sulfate; kb, kilobase(s); bp, base pair(s); GTPγS, guanosine-5′-*O*-(3-thio)triphosphate; LTT-SST-28, Leu^{8},D-Trp^{22},Tyr^{25}-somatostatin-28.

secretion, gastric exocrine secretion, splanchnic blood flow, and intestinal motility (5). The actions of SST are mediated by high affinity plasma membrane receptors that are coupled via G proteins to adenylyl cyclase, K^+ and Ca^{2+} ion channels, and protein tyrosine phosphatase (4–11). Pharmacological studies have suggested that SSTRs are heterogeneous and feature subtypes selective for SST-14 and SST-28. Receptors that show preference for SST-14 binding occur in cerebrocortical synaptosomal membranes, adrenal cortex, exocrine pancreas, islet α cells, and GH_3/GH_4C_1 cells, whereas SST-28-selective sites are found in the normal pituitary, AtT-20 pituitary tumor cells, and islet β cells (5, 6, 8). The recent cloning of the SSTR has confirmed the existence of molecular subtypes but additionally revealed a far greater genetic diversity in this receptor family than was previously suspected (12–23). Five distinct SSTR subtype genes have already been identified, four of which (with the exception of SSTR2) appear to be intronless, at least in the coding segment (12, 20, 21). SSTR2 features two separate isoforms, i.e., a long (SSTR2A) and a short (SSTR2B) variant formed through alternate mRNA splicing (24, 25). The protein products of the five cloned receptors exhibit differing selectivities for SST-14 and SST-28. Three of the receptors (SSTR1, SSTR2, and SSTR4) are somewhat selective for SST-14 binding (12, 17, 21). SSTR3, on the other hand, shows species-specific binding selectivity for the two SST peptides. rSSTR3 binds SST-28 with 2.6-fold higher potency than SST-28 (16), whereas the mouse variant of SSTR3 interacts equally with the two peptides (15). The binding affinity of hSSTR3 is controversial, in that this receptor has been reported to be 8-fold more selective for SST-28 than SST-14 binding in one study (18) but 2-fold more potent for SST-14 than SST-28 in another (20). In contrast, a fifth receptor recently cloned from rat pituitary by O'Carroll *et al.* (19) (here referred to as rSSTR5)[1] exhibits 30-fold greater affinity for SST-28 than SST-14 and appears to be the first SSTR subtype that is truly SST-28 selective.

In the present study, we have used a combination of PCR and genomic library screening to clone a SST-28-specific hSSTR subtype gene, whose transcript is expressed predominantly in the anterior pituitary. This gene maps to human chromosome 16. Based on amino acid sequence identity, the receptor protein encoded by the gene is the human isoform of rSSTR5 and is functionally coupled to adenylyl cyclase. Although hSSTR5, like the rat homologue, displays marked selectivity for binding to SST-28, compared with SST-14, it diverges significantly from the rodent receptor with respect to its overall pharmacological profile and affinity for other SST agonists.

Materials and Methods

Peptides. Synthetic SST-14 was obtained from Ayerst Laboratory Montreal. SST-28, D-Trp^8-SST-14, and LTT-SST-28 were from Bachem (Marina Del Ray, CA). Other SST analogues were received as gifts from the following sources: SMS 201-995 (Sandoz Pharmaceuticals, Basel, Switzerland), MK-678 (Merck Frosst, Canada), RC-160 (A. V. Schally, Tulane University, New Orleans, LA), and BIM 23014 (IPSEN Biotech, Paris, France).

Isolation and sequencing of the hSSTR5 gene. Rat anterior pituitary mRNA was used as a template for first-strand cDNA synthesis. cDNA fragments were generated by PCR amplification using oligonucleotide primers corresponding to highly conserved sequences found in the first cytoplasmic loop (5′-TATGCCAAGATGAAGAC-3′) and the seventh transmembrane domain (antisense, 5′-ATAT/GAGG/AATG/AGGGTTGGCA/GCAGCTGTT-3′) of the recently cloned hSSTR1, hSSTR2, and hSSTR4 (12, 21). Reverse transcription was performed as described, with 1 μg of total RNA, 1 mM concentrations of each deoxynucleoside triphosphate, 0.1 units of RNasin, 100 pmol of random hexamers, and 200 units of Moloney murine leukemia virus reverse transcriptase, followed by 25 cycles of PCR with 20 pmol of oligonucleotide primers and 2.5 units of *Thermus aquaticus* polymerase (Cetus) (26, 26a). Products of 0.7 kb were generated, cloned into PCR vector II (Invitrogen), and sequenced. One PCR product, RP30, represented a novel sequence homologous to the SSTR family. It was radiolabeled and used as a probe to screen a λFIX II human genomic library (Stratagene). Five hundred thousand independent plaques were lifted onto Zeta Probe membranes (Bio-Rad) and hybridized at 65° for 24 hr in 1 mM EDTA, 0.5 M NaH_2P0_4, pH 7.2, 7% SDS. The membranes were then washed in 1 mM EDTA, 40 mM NaH_2P0_4, pH 7.2, 5% SDS, at 65° twice for 60 min, followed by two additional washes in the same solution containing 1% SDS. The filters were then exposed to Kodak XAR-5 film at −80° for 3 days, using intensifying screens. Two independent clones (λRP1 and λRP2) were amplified and DNA was isolated by repeated phenol/chloroform extraction (27). The nucleotide sequence of both DNA strands was determined using the dideoxy chain termination method of Sanger *et al.* (28), with a T7 Pharmacia sequencing kit. A variety of restriction sites in or near the SSTR5 coding region (Fig. 1) were used to subclone various portions of the gene. These subclones were used as sequencing templates. Nested deletions generated using a Erase-a-base kit (Pharmacia) were also used as sequencing templates. In addition, oligonucleotides derived from established sequence data were used to complete the sequence. Sequence analysis and comparisons were performed using DNA Strider and PC Gene software. DNA probes, for screening as well as for Northern blot analysis, were radiolabeled with [α-^{32}P]dCTP by random priming using a Quick Prime kit (Pharmacia).

Expression and radioligand binding analysis. The mammalian expression vector pRc/CMV-hSSTR5 (see Results) was constructed by subcloning a 5.5-kb *Hind*III fragment, containing the complete open reading frame of the novel SSTR gene, into the pRc/CMV vector (Invitrogen). DNA from the resulting construct was used to transfect COS-7 cells by the calcium phosphate method (29). Cells were cultured in D-75 flasks in Dulbecco's modified Eagle medium with 5% fetal bovine serum and were transfected at ≈70% confluency with 100 μg of plasmid DNA. Plasma membrane fractions were prepared 48 hr later and used in radioligand binding studies with ^{125}I-LTT-SST-28 or ^{125}I-Tyr^{11}-SST-14 ligands, as described previously (5, 8). Binding increased linearly with time up to 20 min and remained constant up to 60 min. Saturation experiments were performed with increasing concentrations of ^{125}I-LTT-SST-28 (2–2000 pM) under equilibrium binding conditions. Specific binding was defined as the difference between the counts bound in the absence and in the presence of 100 nM SST-28. Competition analysis was carried out by incubating membranes with ^{125}I-LTT-SST-28 (≈60 pM) and increasing concentrations of SST peptides. Data were analyzed by nonlinear least square regression analysis using INPLOT 4.03 (GraphPAD, San Diego, CA). Binding data were obtained from 10 separate transfections of COS-7 cells in pools of 20 flasks/transfection.

Receptor coupling to G proteins and adenylyl cyclase. To determine G protein coupling of the expressed receptor, binding experiments were carried out in the presence of GTP or GTPγS (10^{-7} to 10^{-4} M), in the absence or presence of 100 nM Na^+ or after pretreatment of transfected cells with pertussis toxin (100 nM). Coupling of hSSTR5

[1] The rSSTR described as SSTR4 by O'Carroll *et al.* (19) is structurally and pharmacologically (selectivity for SST-28) distinct from the first rSSTR4 described by Bruno *et al.* (17) and therefore should more appropriately be designated as another SSTR. To avoid confusion and to be consistent with several recent publications (21–23, 50), we have retained the term SSTR4 for the receptor reported by Bruno and co-workers and have referred to the receptor described by O'Carroll and co-workers as rSSTR5 in this article.

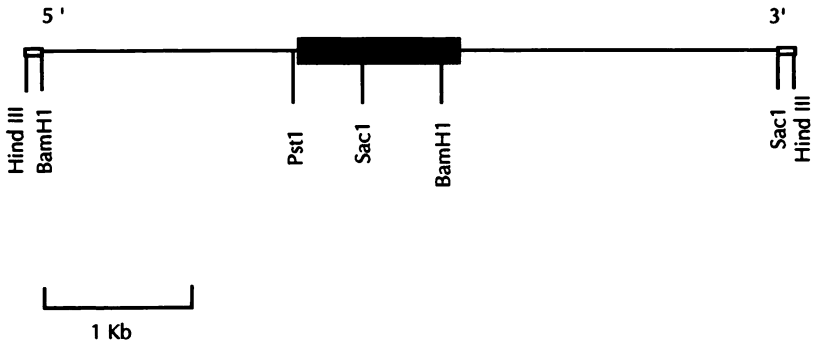

Fig. 1. Restriction endonuclease map of the hSSTR5 genomic clone. A 5.5-kb *Bam*HI-*Sac*I human genomic fragment is shown. *Shaded box*, location of the hSSTR5 coding sequence; *open boxes*, pBluescript vector sequence. Relevant restriction endonuclease sites used for subcloning and constructing the expression vector are also depicted.

to adenylyl cyclase in COS-7 cells was investigated by determining the effects of SST-14 and SST-28 in inhibiting basal and forskolin-stimulated cAMP accumulation. Transfected cells were preincubated in medium containing 500 μM 3-isobutyl-1-methylxanthine, washed, and subsequently exposed for 30 min at 37° to 1 μM forskolin, with or without SST-14 or SST-28. Cells were extracted in 0.1 N HCl, lyophilized, and assayed for cAMP by radioimmunoassay using a rabbit antibody, succinyl-cAMP ^{125}I-Tyr-methyl ester, and cAMP standards, as described previously (30).

Analysis of SSTR5 mRNA by RNA blots and reverse transcriptase PCR. Total RNA was isolated by the acid guanidinium/thiocyanate phenol/chloroform extraction method from normal rat tissues, tissues from 16- and 18-week aborted male human fetuses, postmortem human pituitaries, surgically removed samples of human cerebral cortex, human pituitary tumors (somatotroph adenoma, prolactinoma, and chromophobe adenoma), and human insulinoma, and GH_3 pituitary tumor cells (31). Poly(A)$^+$ RNA was isolated from postmortem human pituitaries using the Poly-A Tract mRNA isolation system (Promega). RNA was denatured in formamide/formaldehyde, fractionated on a 1.5% agarose-formaldehyde gel, and transfered to Nytran nylon membranes using the Vacugene blotting system (LKB Pharmacia). The blots were probed with the ^{32}P-labeled 0.7-kb PCR product RP30 or a 1.1-kb *Pst*I-*Bam*HI genomic fragment from λRP1, containing most of the hSSTR5 coding region (Fig. 1). After hybridization in 50% formamide at 42°, blots were washed at 65° and exposed to Kodak XAR-5 film at −80° for 1–4 weeks, with intensifying screens. Reverse transcriptase PCR was performed on 1-μg total RNA samples using the protocol described above for isolating rat pituitary SSTR5 cDNA. Twenty picomoles of oligonucleotide primers corresponding to sequences in the amino-terminal segment (5′-ATGGAGCCCCTGTT CCCA-3′) and carboxyl-terminal segment (antisense, 5′-GGTCTGCA TAAGCCCGTTGG-3′) of hSSTR5 were used for the initial 25-cycle amplification. Ten microliters of the product obtained were subjected to an additional 25 cycles of PCR using 40 pmol of a different set of internal primers corresponding to sequences in the first cytoplasmic loop (5′-CGCCAAGATGAAGACCG-3′) and the sixth transmembrane domain (antisense, 5′-GCAGCCAACATCCCGC-3′) of hSSTR5. Ten-microliter aliquots of the PCR mixtures were fractionated by electrophoresis on 1.5% agarose gels, stained with ethidium bromide, and visualized under UV illumination. The specificity of the PCR product was confirmed by hybridization with a ^{32}P-labeled hSSTR5 genomic DNA.

***In situ* hybridization.** Pituitaries from male CD rats were rapidly removed and frozen at −30° to −50° on dry ice/isopentane. Sections (15 μm) were cut on a cryostat (Hacker Instruments), thaw mounted on polylysine-coated slides, and later fixed for 60 min in 4% formaldehyde in 0.1 M phosphate buffer, pH 7.4. *In situ* hybridization was performed according to a previously published protocol (32). Cryosections were pretreated with proteinase K (1 μg/ml) for 10 min and hybridized with ^{35}S-labeled probes (3.4 × 10^5 dpm/μl) in 30–50 μl of hybridization buffer (75% formamide, 10% dextran sulfate, 3× standard saline citrate, 50 mM $NaPO_4$, pH 7.4, 1× Denhardt's solution, 0.1 mg/ml yeast RNA) at 55° for 16 hr. Labeled sense and antisense probes were generated from pRP30 using T7 and SP6 RNA polymerase. After hybridization, sections were washed, treated with RNase A, dehydrated in alcohol, dried, dipped in Kodak NTB_2 nuclear emulsion, and exposed in the dark at 4° for 4 weeks. The slides were then developed, counterstained with cresyl violet, dehydrated in graded alcohols, dipped in xylene, and coverslipped. Control sections were pretreated with RNase A or hybridized with labeled sense-strand probe of the same specific activity.

Chromosomal location of the SSTR5 gene. Amplification of a 193-bp region flanking both the 3′ translated and untranslated nucleotides was performed to determine the chromosomal location of the hSSTR5 gene. Oligonucleotide primer (5′-ACGGGCTTATGCAGAC-CAGCAAGCTGT-3′), antisense primer (5′-TGTGTGGCAGACGGT-TACAGGGAT-3′) (300 nM) encompassing nucleotides +1200 and +1413, and target DNA (250 ng) from panels of hybrid human-hamster somatic cell lines (Bios, New Haven, CT) were subjected to 25 cycles of PCR with 2.5 units of *T. aquaticus* polymerase and 200 μM deoxynucleoside triphosphates. Each PCR cycle consisted of denaturation for 1 min at 94°, annealing for 1.5 min at 60°, and extension for 1 min at 72°. Aliquots (10 μl) were then subjected to an additional 25 cycles of PCR under the same conditions. Aliquots of amplified DNA were electrophoresed in a 2.5% agarose gel containing 0.1% ethidium bromide, to determine the presence of a 190-bp fragment. Only human genomic DNA was amplified under the conditions used. An oligonucleotide probe, located internally to the amplified fragments, was radiolabeled with [γ-^{32}P]ATP and Southern blot analysis was performed on the amplified products.

Results

Cloning and amino acid sequence of hSSTR5. To clone novel SST-28-selective receptors we used PCR to amplify mRNA from rat anterior pituitary, using oligonucleotide primers corresponding to conserved sequences present in SSTR1, SSTR2, and SSTR4. Three different PCR amplification products were identified. Sequence analysis revealed that two of these corresponded to the previously characterized rSSTR1 and rSSTR2 sequences, whereas the third represented a novel clone showing strong sequence identity to the known SSTRs. It subsequently proved to be a partial cDNA of the newly cloned rSSTR5 (19). The rSSTR5 PCR product (RP30) was used as a probe to screen a λFIX human genomic library (27). Of 500,000 independent plaques screened, two positive plaques were identified.[2] Restriction mapping revealed identical 15-kb inserts in both clones. A 2.7-kb *Bam*HI fragment and an overlapping 2.8-kb *Sac*I fragment that hybridized to the rSSTR5 PCR product were isolated, cloned into pBluescript, and partially sequenced. This analysis showed that both fragments contain incomplete but overlapping portions of the coding sequence of the hSSTR5 gene (Fig. 1). The complete nucleotide sequence of the coding region contains a single open

reading frame of 1089 bp, which predicts a protein of 363 amino acids (M_r = 39,128) (Fig. 2). A hydropathy plot of this protein sequence showed seven hydrophobic membrane-spanning domains separated by stretches of hydrophilic amino acids typical of G protein-coupled receptors. hSSTR5 showed 75% amino acid sequence identity to the rSSTR5 sequence. Comparison of the sequence with those of the other members of the hSSTR family revealed 42%, 48%, 46%, 47%, and 46% sequence identity to SSTR1, SSTR2A, SSTR2B, SSTR3, and SSTR4, respectively (Fig. 3). The sequences of the five proteins showed the greatest similarity in the putative membrane-spanning domains and diverged most at their amino and carboxyl termini. There are three putative *N*-linked glycosylation sites (at Asn-13 and Asn-26 in the amino-terminal segment and at Asn-184 in the second extracellular loop), three consensus sequences for phosphorylation by cAMP-dependent protein kinase A (at Ser-239 and Thr-244 in the third cytoplasmic loop and at Ser-322 in the carboxyl-terminal segment), and three sites for protein kinase C phosphorylation [in the second cytoplasmic loop (Ser-145), the third cytoplasmic loop (Ser-239), and the cytoplasmic carboxyl-terminal domain (Thr-357)]. A consensus sequence identifying a potential site for coupling to G proteins (33) is located in the 22-residue region of Lys-224 to Arg-245, within the third cytoplasmic loop, and is made up of two basic residues at the amino-terminal side (KVR) and the RKVTR motif at the carboxyl-terminal end of the loop.

Pharmacological characterization of hSSTR5. To characterize the protein product encoded by the cloned SSTR gene, the expression vector pRc/CMV-hSSTR5 was constructed. The *Bam*HI and *Sac*I genomic fragments were fused *in vitro* at a common *Bam*HI site (Fig. 1). Because of the absence of an appropriate restriction endonuclease site at the 3′ end of the reconstituted gene in pBluescript, a *Hind*III site was created in the pBluescript polylinker by *in vitro* mutagenesis using the method of Kunkel *et al* (34), with the synthetic oligonucleotide 5′-GACGGCCAGTAAGCTTGCGTAATACG-3′. The resulting 5.5-kb *Hind*III fragment was subcloned into the *Hind*III site of pRc/CMV vector. Membranes prepared from COS-7 cells transiently expressing hSSTR5 bound ^{125}I-LTT-SST-28 with high affinity. Analysis of saturation isotherms revealed a B_{max} value of 162 ± 30 fmol/mg of protein and a K_d value of 0.055 nM (Fig. 4A). No specific binding was observed in nontransfected or mock-transfected COS-7 cells. Competition experiments revealed that SST-28 bound to hSSTR5 with a 12.6-fold greater affinity than did SST-14 (Fig. 4B; Table 1). In addition to the two naturally occuring SST peptides, several synthetic SST analogues were tested for their affinity for hSSTR5. These included several compounds, such as SMS 201–995, BIM 23014, and MK-678, that act as selective or superactive agonists at endogenous SST binding sites and are currently used clinically for diagnostic and therapeutic purposes (35, 36). These agonists inhibited binding of ^{125}I-LTT-SST-28 in a dose-dependent manner, but the low Hill coefficients of the inhibition curves for conformationally restricted SST analogues suggested their interaction with dual affinity states of the expressed hSSTRs. The K_i values for the high affinity binding sites showed the following rank order of potency: LTT-SST-28 > SST-28 > D-Trp^8-SST-14 > SST-14 ≈ RC-160 ≈ BIM 23014 > MK-678 > SMS 201–995. RC-160 and BIM 23014 appeared to be as potent as SST-28 at low concentrations but, surprisingly, failed to completely saturate the receptor at higher concentrations.

Coupling of hSSTR5 to G proteins was investigated by the ability of GTP and GTPγS to inhibit binding of ^{125}I-LTT-SST-28 to hSSTR5 expressed in COS-7 cells. Both GTP and GTPγS inhibited ^{125}I-LTT-SST-28 binding in a dose-dependant manner (10^{-7} to 10^{-4} M), with maximal inhibition of 44% and 68%, respectively (Fig. 5). Sodium ions also caused a 40% inhibition of specific binding. Inclusion of Na^+ with GTP led to an additional decrease in binding (70%). Additionally, pretreatment of these cells with pertussis toxin resulted in 66% reduction in radioligand binding. Both SST-14 and SST-28 displayed dose-dependent inhibition of forskolin-stimulated cAMP accumulation in COS-7 cells transiently expressing hSSTR5, indicating that the receptor is negatively coupled to adenylyl cyclase (Fig. 6). Consistent with its higher affinity for receptor binding, SST-28 was also more potent in its effect on adenylyl cyclase at each dose tested. SST-28 at 10^{-7} M inhibited forskolin-stimulated cAMP by 65 ± 4%, compared with 48 ± 2.5% inhibition by the same concentration of SST-14. Neither SST-14 nor SST-28 influenced basal cAMP levels in hSSTR5-tranfected COS-7 cells. Maximal inhibition of forskolin-stimulated cAMP was observed at 10^{-7} M SST-28, compared with 10^{-6} M for SST-14. The ED_{50} values for inhibition of forskolin-stimulated cAMP were 0.35 ± 0.06 nM and 4.8 ± 0.7 nM for SST-28 and SST-14, respectively.

Tissue and cellular expression of SSTR5. The pattern of expression of SSTR5 mRNA was examined in human and rat tissues by Northern blot analysis, reverse transcriptase PCR, and *in situ* hybridization. A 2.4-kb transcript was expressed at high levels in the normal rat pituitary, GH_3 cells (Fig. 7A), and the mouse pituitary (data not shown). A weakly labeled SSTR5 mRNA band of identical size was also seen in rat cerebral cortex but not in hypothalamus, pancreas, adrenal gland, stomach, kidney, or muscle (data not shown). A RNA blot of postmortem human pituitary revealed expression of a 4-kb SSTR5 transcript (Fig. 7B). Of four human pituitary adenomas analyzed, neither the secretory tumors (one prolactinoma and one somatotroph adenoma) nor the chromophobe adenomas expressed SSTR5 mRNA. Likewise, a single human insulinoma examined was devoid of SSTR5 gene expression. Because of the relatively weak tissue expression of hSSTR5 mRNA detected by Northern analysis, reverse transcriptase PCR was performed on additional human samples. Specific hSSTR5 PCR products conforming to the predicted 1.1-kb (primary PCR) and 0.6-kb (secondary PCR) sizes were detected in postmortem human pituitary, fetal human pituitary, and fetal human hypothalamus, but not in human cerebral cortex (Fig. 7C). Control samples subjected to PCR without reverse transcriptase showed no detectable SSTR5 product. *In situ* hybridization revealed abundant expression of SSTR5 mRNA in the rat anterior pituitary, with only weak signals in the intermediate and posterior lobes (Fig. 8). No specific labeling was detected in control sections probed with sense-strand RNA.

Chromosomal location of hSSTR5. PCR amplification of hybrid human-hamster somatic lines for the hSSTR5 gene identified chromosome 16 as being the only chromosome positive for this gene (Fig. 9).

Discussion

Using degenerate oligonucleotides corresponding to conserved sequences found in SSTRs, we have synthesized from

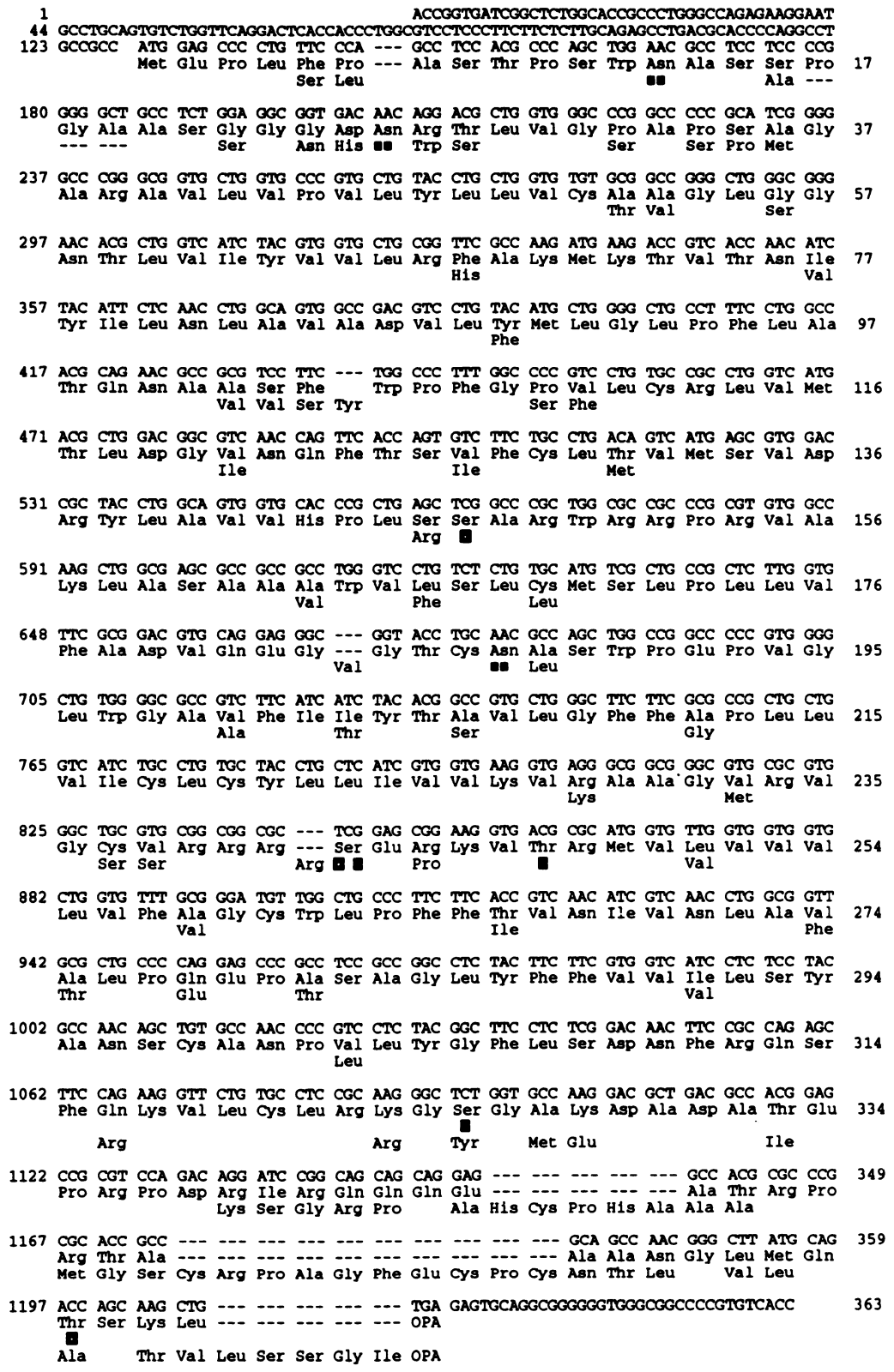

Fig. 2. Nucleotide and deduced amino acid sequences of hSSTR5. The nucleotide sequence is numbered on the *left*; the amino acid sequence is shown on the *right*. rSSTR5 amino acid residues that differ from hSSTR5 are shown. – – –, Gaps that exist between the two sequences. Potential *N*-glycosylation (■ ■), cAMP-dependent protein kinase (■), and protein kinase C (■) sites are indicated on the sequence.

Fig. 3. Alignment of the five hSSTR subtypes. The amino acid sequences of the five hSSTRs are aligned, using the single-letter amino acid code. Amino acid residues conserved within the five sequences are *boxed*. The putative transmembrane domains are indicated in the sequences. The amino acid residues are numbered on the *right*. – – –, Gaps in the sequences that were inserted to achieve maximum alignment.

rat pituitary mRNA a PCR product corresponding to a SSTR. The full length cDNA corresponding to this receptor subtype (which we call rSSTR5) has recently been cloned in rats (19). The rSSTR5 PCR product was used as a probe to isolate a novel SSTR gene from a human genomic library. The predicted amino acid sequence of the cloned human gene shows ~75% sequence identity to rSSTR5 (19). Although this represents a relatively high degree of homology, it is significantly lower than the 86–99% sequence identity that exists between the human and rodent forms of the other SSTR subtypes (SSTR1–4) (12–22, 37). A great deal of the sequence difference that exists between rSSTR5 and our cloned SSTR is located in the carboxyl-terminal domain. In addition, the intracellular carboxyl-terminal segment of rSSTR5 is significantly longer (70 versus 55 amino acids). Accordingly, we re-examined the nucleotide sequence encoding the carboxyl-terminal domain of rSSTR5 and found that the published rSSTR5 sequence contains an error due to the omission of a cytidine residue at position 1034 (Fig. 10A). This results in a frame-shift that changes amino acid residues 351–383 of the carboxyl-terminal segment. The revised sequence of rSSTR5 results in a significantly smaller protein (363 residues, compared with 383 residues) whose carboxyl-terminal sequence is identical in size to the corresponding segment of hSSTR5 and displays a high degree of similarity within this domain (Fig. 10B). Furthermore, the overall sequence identity between the rat and human receptors is increased to 80%. Chromosomal localization studies have mapped the hSSTR5 gene to chromosome 16. Because the other four

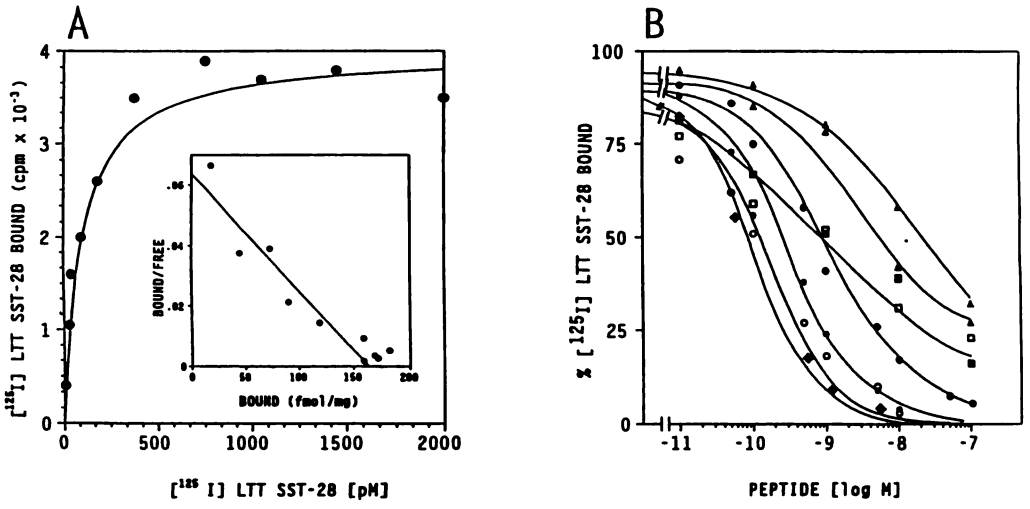

Fig. 4. A, Saturation isotherm of ^{125}I-LTT-SST-28 binding to membranes prepared from COS-7 cells transiently expressing hSSTR5. Membranes were incubated with increasing concentrations of ^{125}I-LTT-SST-28 and assayed for receptor binding activity in the absence or presence of 100 nM SST-28 to define specific binding. Scatchard analysis of the binding data (*inset*) indicated the presence of a single class of high affinity sites, with a maximum binding capacity (B_{max}) of 162 ± 30 fmol/mg of protein and estimated affinity constant (K_d) of 55 ± 10 pM. Data represent the mean of three separate experiments performed in duplicate. B, Competitive dose-dependent inhibition by SST agonists of ^{125}I-LTT-SST-28 binding to hSSTR5. Membranes were incubated with 60 pM ^{125}I-LTT-SST-28 and the indicated concentrations of LTT-SST-28 (♦), SST-28 (●), SST-14 (○), D-Trp^8-SST-14 (°), SMS 201-995 (▲), MK-678 (△), RC-160 (■), or BIM 23014 (□) under equilibrium conditions, as described in the text. These peptides exhibited the following rank order of potency: LTT-SST-28 > SST-28 > D-Trp^8-SST-14 > SST-14 ≈ RC-160 ≈ BIM 23014 > MK-678 > SMS 201-995. The results are representative of three experiments performed in duplicate.

TABLE 1

Comparison of potencies of SST agonists for binding to hSSTR5

K_i values represent the inhibitory concentrations of the agonists required for half-maximal inhibition of ^{125}I-LTT-SST-28 binding (mean ± standard error), three experiments. Relative affinity for the high affinity binding site (site 1) is expressed as a ratio of the K_i value to that of SST-28, taken as 1.

Agonist	K_i		Relative affinity for site 1
	Site 1	Site 2	
	nM		
SST-28	0.19 ± 0.03		1
LTT-SST-28	0.11 ± 0.03		1.72
SST-14	2.24 ± 0.36		0.085
D-Trp^8-SST-14	0.28 ± 0.02		0.68
SMS 201-995	14.16 ± 3.1	112 ± 16	0.013
MK 678	5.02 ± 0.80	82 ± 7	0.038
RC 160	1.7 ± 0.26	37 ± 4	0.112
BIM 23014	2.76 ± 0.37	101 ± 9	0.069

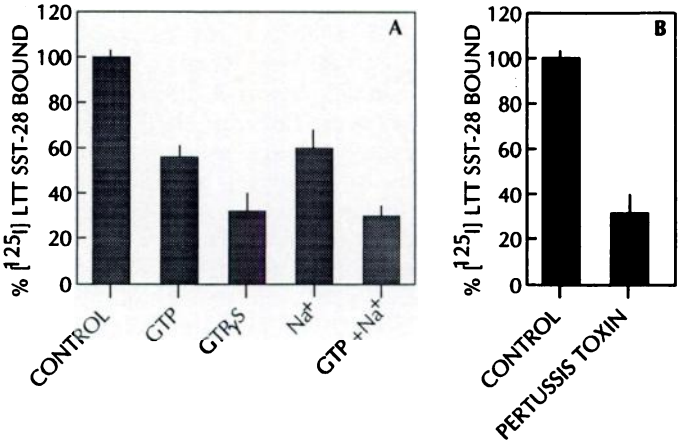

Fig. 5. hSSTR5 is coupled to a pertussis toxin-sensitive G protein, as shown by the inhibition of ^{125}I-LTT-SST-28 binding by guanine nucleotides as well as by Na^+ and pertussis toxin. GTP and GTPγS inhibited radioligand binding over the concentration range of 10^{-7} to 10^{-4} M. Only the maximal inhibition of specific binding by the nucleotides is shown. The inhibitory effect of 100 mM NaCl was tested in the absence or presence of 100 μM GTP. Pretreatment of cells with 100 nM pertussis toxin also decreased specific binding of ^{125}I-LTT-SST-28. Data shown are the mean ± standard error of three experiments.

hSSTR genes, SSTR1–4, are located on chromosomes 14, 17, 22, and 20, respectively, this means that each of the hSSTR genes segregates to a different chromosome (20, 21, 38).

A comparison of the amino acid sequence of hSSTR5 with that of the four other hSSTR subtypes shows that these receptors are closely related in size and structure (12, 18, 20–22). The sequences of the five proteins diverge most at their amino and carboxyl termini and show the greatest similarity within the putative membrane-spanning domains, where hSSTR5 exhibits 55%, 61%, 69%, and 59% identity with hSSTR1, hSSTR2, hSSTR3, and hSSTR4, respectively. Of the individual transmembrane segments, the regions of highest and lowest identity are within the seventh and fourth transmembrane domains, respectively. hSSTR5, like hSSTR1, hSSTR2, and hSSTR4, contains a conserved cysteine residue 12 amino acids downstream from the seventh transmembrane domain in the cytoplasmic carboxyl-terminal segment, which may serve as a potential site for a palmitoyl membrane anchor similar to that described for the human β_2-adrenergic receptor (39). hSSTR5 is comparable to the other SSTRs with respect to potential sites in the second and third cytoplasmic loops and the carboxyl-terminal segment for receptor regulation through phosphorylation by protein kinase A and protein kinase C.

The agonist profile of the receptor protein encoded by hSSTR5 revealed selectivity for SST-28, with a SST-28 to

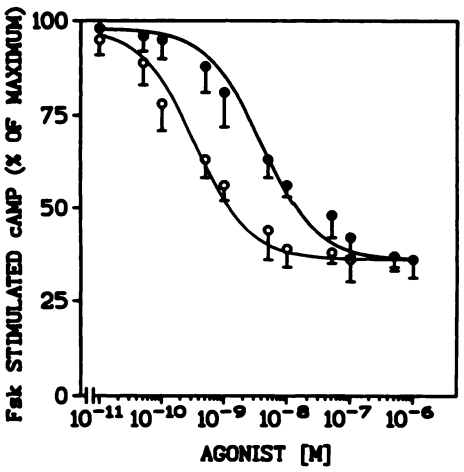

Fig. 6. hSSTR5 is negatively coupled to adenylyl cyclase. SST-14 (●) and SST-28 (○) inhibited forskolin (*Fsk*)-stimulated cAMP accumulation in COS-7 cells expressing hSSTR5. COS-7 cells were plated in six-well plates at a density of 2×10^5 and were transfected with hSSTR5 cDNA when the cells reached ~70% confluency. Inhibition of forskolin (1 μM)-stimulated cAMP accumulation by SST-14 and SST-28 was measured 48 hr later, as described in Materials and Methods. Data shown are the mean ± standard error of four experiments. Basal cAMP content was 7 ± 0.6 pmol/well, whereas the maximal forskolin-stimulated level was 43 ± 3 pmol/well.

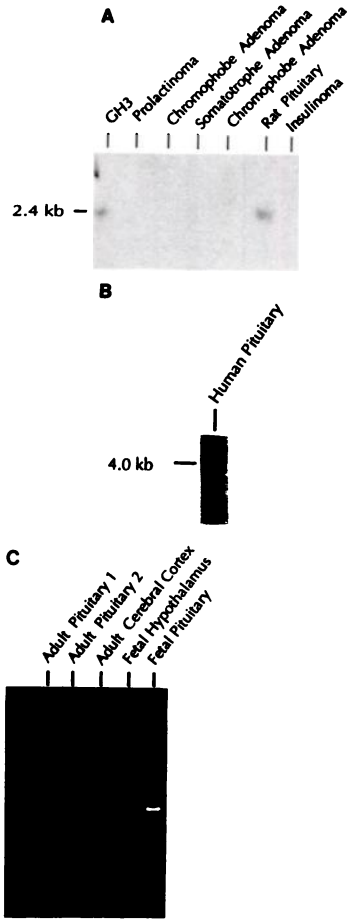

Fig. 7. Northern blot analysis of SSTR5 mRNA in rodent and human tissues and tumor cells. A, 50 μg of total RNA from normal rat pituitary, GH_3 rat pituitary tumor cells, human pituitary tumors (somatotroph adenoma, prolactinoma, or chromophobe adenoma), or human islet tumor (insulinoma) were hybridized to a ^{32}P-labeled rSSTR5 cDNA (RP30) synthesized by PCR. A 2.4-kb transcript is present in normal rat pituitary and GH_3 cells. No transcript is detectable in the human tumors. B, 3 μg of poly(A)$^+$ RNA from human postmortem pituitary were probed with a ^{32}P-labeled hSSTR5 DNA fragment. A weakly labeled 4-kb transcript is seen. C, 1 μg of total RNA from adult pituitary, adult cerebral cortex, fetal hypothalamus, or fetal pituitary was subjected to RT-PCR using hSSTR5-specific primers, as described in Materials and Methods. The PCR products were separated on an agarose gel, stained with ethidium bromide, and photographed under UV illumination. A specific band of 578 bp was detected in all tissues examined except adult cerebral cortex. *Left lane*, DNA markers, i.e., *Hae*III-digested φX174 phage DNA (1.35, 1.08, 0.87, 0.6, and 0.3 kb).

SST-14 potency ratio of 12:1. This contrasts with a SST-28 to SST-14 potency ratio of 30:1 reported for the rat homologue (19). This difference cannot be explained by the use of different radioligands (^{125}I-LTT-SST-28 versus ^{125}I-Tyr^{11}-SST-14) in the two studies, because the relative affinities of the various SST analogues for binding to hSSTR5 were found to be the same with either radioligand (data not shown). A direct comparison of the inhibitory potencies of SST-28 and SST-14 in these studies is not feasible, because single IC_{50} values rather than K_i values were reported for the rat homologue of hSSTR5 despite very low Hill coefficients for the competition curves, indicating multiple affinity states of that receptor (19). Nevertheless, the selectivity of hSSTR5 and rSSTR5 for SST-28 distinguishes this subtype from SSTR1, SSTR2, and SSTR4, all of which bind SST-14 more than SST-28. On the other hand, SSTR3 displays species-dependent selectivity for either SST-28 or SST-14, suggesting that this receptor may be an intermediate form between the SST-14- and SST-28-selective receptor subfamilies. In this respect it is interesting that hSSTR5 shares 69% amino acid identity with hSSTR3 in the seven transmembrane domains (18, 20). A second pharmacological difference between hSSTR5 and rSSTR5 resides in their ligand selectivity for the conformationally restricted octapeptide and hexapeptide SST analogues. Analysis of saturation isotherms indicates the presence of a single population of high affinity hSSTR5 binding sites. Scatchard analysis of competition experiments with these analogues, however, suggests their interaction with a second low affinity form of hSSTR5, with 10-fold lower K_i values. Such dual-affinity behavior is also implied by the Hill coefficients of $\ll 1$ in the case of rSSTR5 (19). Overall, the four conformationally restricted analogues show a lower affinity for hSSTR5 than does SST-28, the putative endogenous ligand for this receptor. It should be noted, however, that the inhibition curves obtained with RC-160 and BIM 23014 differ from those of SMS 201-995 and MK-678. This is the first report describing a divergent pattern of binding to a cloned SSTR between SMS 201-995 and MK-678 on one hand and RC-160 and BIM 23014 on the other.

The structural differences underlying the differing pharmacological profiles of hSSTR5 and rSSTR5, or indeed those that confer SST-14 or SST-28 selectivity in a SSTR, remain to be clarified. Interspecies variation in ligand selectivity for a receptor homologue is not uncommon. For instance, the recently described hSSTR4 features an agonist profile different from that of rSSTR4, although the two receptors share ~86% sequence identity (21-23). Likewise, a single amino acid difference between the rodent and human forms of the 5-hydroxytryptamine type 1B receptor has been reported to lead to a dramatic alteration in their ligand-binding properties (40). Within the seventh transmembrane domain, the sequence YANSCANPILY is completely conserved in each of the rat,

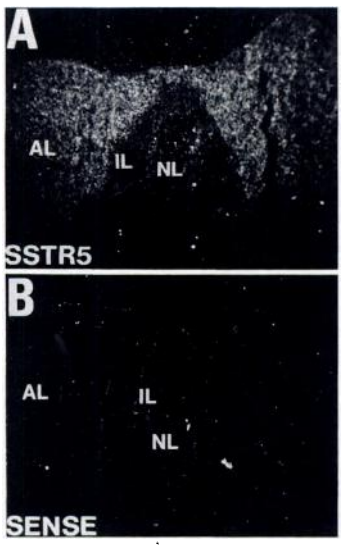

Fig. 8. Localization of SSTR5 mRNA in rat pituitary by *in situ* hybridization with ^{35}S-labeled sense and antisense RNA probes. A, Dark-field photomicrograph showing dense message levels diffusively distributed throughout the anterior lobes (*AL*). In contrast, the intermediate lobes (*IL*) and posterior lobes (*NL*) show very low grain densities. B, There is no specific labeling in a control section probed with sense-strand RNA.

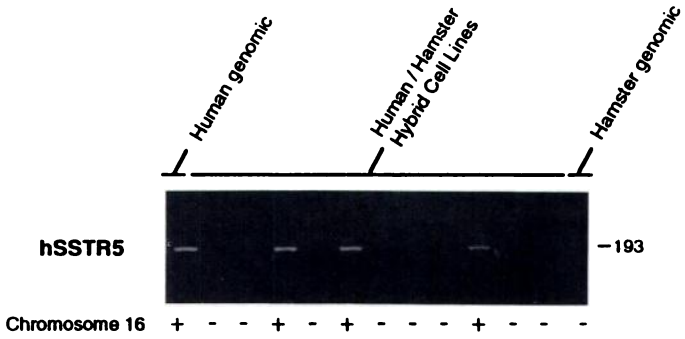

Fig. 9. Chromosomal location of the hSSTR5 gene. An ethidium bromide-stained gel of hybrid hamster-human somatic cell lines (BIOS Corp.) was analyzed for the presence of hSSTR5 DNA. Oligonucleotide primers flanking 3′ translated and untranslated sequences of hSSTR5 were used to generate a 190-bp PCR product.

mouse, and human forms of SSTR1, SSTR2, SSTR3, and SSTR4 (Fig. 3). This sequence is not present in other seven-transmembrane domain receptors, although some of the residues are highly conserved, suggesting that the YANSCANPILY motif is unique to the SSTR family (41). In the case of SSTR5, the rat and human receptor sequences differ by a single amino acid in this region. Whether this substitution in itself is sufficient to explain SST-14 versus SST-28 selectivity remains to be determined.

hSSTR5, like rSSTR5, was found to be coupled to a pertussis toxin-sensitive G protein. This receptor subtype is thus comparable to three other members of the SSTR family, SSTR2, SSTR3, and SSTR4, that have been reported also to be coupled to pertussis toxin-sensitive G proteins (18, 21, 37). A putative consensus sequence for G protein coupling exists in the third cytoplasmic loop in each of these receptors (33) but is not present in SSTR1, the only member of this receptor group whose G protein interaction thus far remains uncertain (12, 14). Our data demonstrate that hSSTR5, like the rat homologue, is negatively coupled to adenylyl cyclase. Furthermore, the potencies of SST-28 and SST-14 for inhibition of forskolin-stimulated cAMP accumulation paralleled their binding affinities, suggesting functional coupling of the receptor to adenylyl cyclase. In the case of the other SSTRs, initial reports suggested that SSTR1 and SSTR2 (expressed in COS-1 and CHO-DG44 cells) do not signal via adenylyl cyclase (42), but subsequent studies have shown that both of these receptors (expressed in CHO-K1 or COS-7 cells), as well as SSTR3 and SSTR4, are linked to inactivation of adenylyl cyclase (18, 43–45). This means that all five SSTRs signal through inhibition of adenylyl cyclase and that there is no selectivity for coupling to adenylyl cyclase between the SST-14- and SST-28-type receptors. Whether any of these receptors activates other signaling pathways, e.g., ion channels or phosphoprotein tyrosine phosphatase, remains to be determined.

The endogenous tissue expression of the SSTR5 gene appears to be relatively selective. RNA blots of rat tissues revealed a single 2.4-kb transcript in the pituitary, as reported previously (19), as well as in cerebral cortex. Outside the pituitary and cerebral cortex, we found no detectable expression of SSTR5 mRNA in rat pancreas, stomach, adrenal gland, kidney, hypothalamus, or muscle. Northern analysis showed a single 4-kb SSTR5 transcript in human pituitary. Reverse transcriptase PCR confirmed that, in addition to adult pituitary, this gene is expressed in fetal pituitary and hypothalamus but not in adult human cerebral cortex. This is unlike the other cloned SSTRs, which are more widely expressed in brain and peripheral tissues (SSTR1, brain, stomach, and intestine; SSTR2, brain, pituitary, adrenal, pancreas, intestine, and kidney; SSTR3, brain, pituitary, stomach, and intestine; and SSTR4, brain, pituitary, pancreas, adrenal, stomach, and kidney) (13–22, 37). By *in situ* hybridization, the rSSTR5 transcript was diffusely localized to the anterior lobe of the pituitary. Based on previous pharmacological and *in vitro* autoradiographic studies, at least three of the normal pituitary cell types, i.e., somatotrophs, lactotrophs, and thyrotrophs, express functional SSTRs (46). Additional experiments using dual-labeling *in situ* hybridization will be required to determine which of these subsets of pituitary cells express SSTR5. The finding of mRNA for SSTR5, a SST-28-selective receptor subtype, in the pituitary correlates with the known preference of pituitary membrane SSTRs for SST-28 binding. On the other hand, whereas SSTR5 appears to be relatively selective for the pituitary, the pituitary is not selective for this receptor, because all of the other four SSTRs are also expressed in the pituitary, albeit in different amounts (SSTR2 $\gg$ SSTR4 > SSTR1 = SSTR3) (13–21, 37). Analysis of human and rodent pituitary tumors for SSTR5 gene expression disclosed a number of unusual findings. SSTR5 mRNA was highly expressed in GH_3 cells, although these cells have been reported to display a preference for SST-14 binding sites (47). Because GH_3 cells also express large amounts of the SST-14-selective subtype SSTR2 (25), conceivably the overall SST-14 selectivity must be determined by a relatively higher proportion of SST-14 binding sites, compared with SST-28 binding sites. Paradoxically, SSTR5 mRNA was undetectable in a human insulinoma, although normal islet β cells and hamster insulinoma cells are known to exhibit SST-28-selective binding sites (48, 49). This is similar to AtT-20 mouse pituitary cells, which also display a preference for SST-28 binding but show poor expression of SSTR5 mRNA, raising the possibility that other SST-28-selective SSTR genes may exist (50). In contrast

A

```
GAC AAC TTC CGC CAG AGC TTC CGG AAG GTT CTG TGC CTA CGT AGA   969
Asp Asn Phe Arg Gln Ser Phe Arg Lys Val Leu Cys Leu Arg Arg   323

GGA TAC GGT ATG GAG GAT GCG GAC GCC ATA GAG CCA CGG CCA GAC  1014
Gly Tyr Gly Met Glu Asp Ala Asp Ala Ile Glu Pro Arg Pro Asp   338

                         *
AAG AGT GGG CGG CCT CAG GCC ACA CTG CCC ACA CGC AGC TGC GAG  1059
Lys Ser Gly Arg Pro Gln Ala Thr Leu Pro Thr Arg Ser Cys Glu   353

GCC AAT GGG CTC ATG CAG ACC AGC AGG ATT TGA                  1092
Ala Asn Gly Leu Met Gln Thr Ser Arg Ile ---                   363
```

B

```
rSSTR5 (published)   DNFRQSFRKVLCLRRGYGMEDADAIEPRPDKSGRPQAHCPHAAARPMGSCRPAGFECPCNTLGVLQASTVLSSGI
                     :::::::::::::::::::::::::::::::::::::::  :       :
rSSTR5 (revised)     DNFRQSFRKVLCLRRGYGMEDADAIEPRPDKSGRPQATLPTRSCEANGLMQTSRI
                     ::::::::.:::::::.: : .::::.::::::.  ..:.. ..:. .:::::::::..
hSSTR5               DNFRQSFQKVLCLRKGSGAKDADATEPRPDRIRQQQEATRPRTAAANGLMQTSKL
```

Fig. 10. Revised nucleotide and amino acid sequences of the carboxyl-terminal tail of rSSTR5. A, Corrected nucleotide and amino acid sequences of the carboxyl-terminal tail of rSSTR5. The nucleotides and amino acid residues are numbered in accordance with the published sequence (19). *, Position of the cytidine residue missing from the published sequence (nucleotide 1034). Total RNA, isolated from rat pituitaries, was reverse transcribed into single-stranded cDNA. PCR was used to amplify the 3′ end of rSSTR5 using oligonucleotides based on the published sequence (nucleotides 547–567, 5′-GGCACCTGCAACCTGAGCTG-3′; nucleotides 1085–1066, 3′-CGAGTACGTCTGGTCGTCC-5′) (19). The PCR products were subcloned into PCRII (Invitrogen) for sequence analysis. B, Amino acid sequences of the carboxyl-terminal domain of the published (19) and revised rSSTR5 sequences were aligned manually. *, Position of the frame shift in the published rSSTR5 sequence. The revised rSSTR5 sequence was aligned with the corresponding hSSTR5 sequence using FASTA.

to the finding of normal pituitary expression of SSTR5, four human pituitary tumors, of both secretory and nonsecretory types, were devoid of hSSTR5 mRNA. These same tumor samples have been previously reported to express mRNA for the SST-14-selective SSTR genes, in particular SSTR2 (25). Although this is a small sample size, it raises the possibility of differential expression of SST-14- rather than SST-28-type receptors in human pituitary tumors. This in turns implies that the action of analogues such as SMS 201-995, which are clinically used for treatment of pituitary tumors, is targeted to SST-14-type receptors such as SSTR2, which not only are abundantly expressed but also interact with high affinity with this agonist (13, 35).

Acknowledgments

We thank Dr. Denis Banville (National Research Council of Canada Biotechnology Research Institute, Montreal) for providing the human genomic library and Dr. J. Bruno (State University of New York, Stonybrook, NY) for pointing out a potential error in the published rSSTR5 sequence. Dr. Cynthia Goodyer (Montreal Children's Hospital) provided fetal human tissues. We are grateful to Valerie Burns and Olga Dembinska for technical assistance and to Maria Correia for secretarial help.

References

1. Reichlin, S. Somatostatin. *N. Engl. J. Med.* **309**:1495–1501, 1556–1563 (1983).
2. Patel, Y. C. General aspects of the biology and function of somatostatin, in *The Role of Somatostatin: Basic and Clinical Aspects* (E. E. Muller, M. O. Thorner, and C. Weil, eds.), Vol. 4. Springer-Verlag, Berlin, 1–16 (1992).
3. Patel, Y. C., and W. O'Neil. Peptides derived from cleavage of prosomatostatin at carboxyl and amino-terminal segments. *J. Biol. Chem.* **263**:745–751 (1988).
4. Pan, M. G., T. Florio, and P. J. C. Stork. G protein activation of a hormone-stimulated phosphatase in human tumor cells. *Science (Washington D. C.)* **256**:1215–1217 (1992).
5. Srikant, C. B., and Y. C. Patel. Somatostatin receptor: evidence for structural and functional heterogeneity, in *Somatostatin: Basic and Clinical Status* (S. Reichlin, ed.). Plenum Press, New York, 89–102 (1987).
6. Patel, Y. C., K. K. Murthy, E. Escher, D. Banville, J. Spiess, and C. B. Srikant. Mechanism of action of somatostatin: an overview of receptor function and studies of the molecular characterization and purification of somatostatin receptor proteins. *Metabolism* **39** (Suppl. 2):63–69 (1990).
7. Srikant, C. B., and Y. C Patel. Somatostatin receptors: identification and characterization in rat brain membranes. *Proc. Natl. Acad. Sci. USA* **78**:3930–3934 (1981).
8. Srikant, C. B., and Y. C. Patel. Receptor binding of somatostatin-28 is tissue specific. *Nature (Lond.)* **297**:259–260 (1981).
9. Koch, B. D., J. B. Blalock, and A. Schonbrunn. Characterization of the cyclic AMP-independent actions of somatostatin in GH cells: an increase in potassium conductance is responsible for both the hyperpolarization and the decrease in intracellular free calcium produced by somatostatin. *J. Biol. Chem.* **263**:216–225 (1988).
10. Koch, B. D., and A. Schonbrunn. Characterization of the cyclic AMP-independent actions of somatostatin in GH cells: an increase in potassium conductance initiates somatostatin-induced inhibition of prolactin secretion. *J. Biol. Chem.* **263**:226–234 (1988).
11. Lewis, D. L., F. F. Weight, and A. Luini. A guanine nucleotide-binding protein mediates the inhibition of voltage-dependent calcium current by somatostatin in a pituitary cell line. *Proc. Natl. Acad. Sci. USA* **83**:9035–9039 (1986).
12. Yamada, Y., S. R. Post, K. Wang, H. S. Tager, G. I. Bell, and S. Seino. Cloning and functional characterization of a family of human and mouse somatostatin receptors expressed in brain, gastrointestinal tract and kidney. *Proc. Natl. Acad. Sci. USA* **89**:251–255 (1992).
13. Kluxen, F. W., C. Bruns, and H. Lubbert. Expression cloning of a rat brain somatostatin receptor cDNA. *Proc. Natl. Acad. Sci. USA* **89**:4618–4622 (1992).
14. Li, X. J., M. Forte, R. A North, C. A. Ross, and S. H. Snyder. Cloning and expression of a rat somatostatin receptor enriched in brain. *J. Biol. Chem.* **267**:21307–21312 (1992).
15. Yasuda, K., S. Rens-Domiano, C. D. Breder, S. F. Law, C. B. Saper, T. Reisine, and G. I. Bell. Cloning of a novel somatostatin receptor, SSTR3, coupled to adenylyl cyclase. *J. Biol. Chem.* **267**:20422–20428 (1992).
16. Meyerhof, W., I. Wulfsen, C. Schonrock, S. Fehr, and D. Richter. Molecular cloning of a somatostatin-28 receptor and comparison of its expression pattern with that of a somatostatin-14 receptor in brain. *Proc. Natl. Acad. Sci. USA* **89**:10267–10271 (1992).
17. Bruno, J. F., Y. Xu, J. Song, and M. Berelowitz. Molecular cloning and fuctional expression of a brain-specific somatostatin receptor. *Proc. Natl. Acad. Sci. USA* **89**:11151–11155 (1992).
18. Yamada, Y., T. Reisine, S. F. Law, Y. Ihara, A. Kubota, S. Kagimoto, M. Seino, Y. Seino, G. I. Bell, and S. Seino. Somatostatin receptors, an expanding gene family: cloning and functional characterization of human SSTR3, a protein coupled to adenylate cyclase. *Mol. Endocrinol.* **6**:2136–2142 (1992).
19. O'Carroll, A. M., S. J. Lolait, M. Konig, and L. C. Mahan. Molecular cloning and expression of a pituitary somatostatin receptor with preferential affinity for somatostatin-28. *Mol. Pharmacol.* **42**:939–946 (1992).
20. Corness, J. D., L. L. Demchyshyn, P. Seeman, H. H. M. Van Tol, C. B. Srikant, G. Kent, Y. C. Patel, and H. B. Niznik. A human somatostatin receptor (SSTR3), located on chromosome 22, displays preferential affinity for somatostatin-14 like peptides. *FEBS Lett.* **321**:279–284 (1993).
21. Demchyshyn, L. L., C. B. Srikant, R. K. Sunahara, G. Kent, P. Seeman, H. H. M. Van Tol, R. Panetta, Y. C. Patel, and H. B. Niznik. Cloning and expression of a human somatostatin-14 selective receptor variant (SSTR4) located on chromosome 20. *Mol. Pharmacol.* **43**:894–901 (1993).

22. Rohrer, L., F. Raulf, C. Bruns, R. Buettner, F. Hofstaedter, and R. Schule. Cloning and characterization of a fourth human somatostatin receptor. *Proc. Natl. Acad. Sci. USA* **90**:4196–4200 (1993).
23. Xu, Y., J. Song, J. F. Bruno, and M. Berelowitz. Molecular cloning and sequencing of a human somatostatin receptor, hSSTR4. *Biochem. Biophys. Res. Commun.* **193**:648–652 (1993).
24. Vanetti, M., M. Kouba, X. Wang, G. Vogt, and V. Hollt. Cloning and expression of a novel mouse somatostatin receptor (SSTR2B). *FEBS Lett.* **311**:290–294 (1992).
25. Patel, Y. C., M. Greenwood, G. Kent, R. Panetta, and C. B. Srikant. Multiple gene transcripts of the somatostatin receptor SSTR2: tissue selective distribution and cAMP regulation. *Biochem. Biophys. Res. Commun.* **192**:288–294 (1993).
26. Innis, M. A., and D. H. Gelfand. Optimization of PCRs, in *PCR Protocols: A Guide to Methods and Application* (M. A. Innis, D. H. Gelfand, J. J. Sninsky, and T. J. White, eds.). Academic Press, New York, 3–12 (1990).
26a. Kawasaki, E. S. Amplification of RNA, in *PCR Protocols: A Guide to Methods and Application* (M. A. Innis, D. H. Gelfand, J. J. Sninsky, and T. J. White, eds.). Academic Press, New York, 21–27 (1990).
27. Sambrook, J., E. F. Fritsch, and T. Maniatis. *Molecular Cloning: A Laboratory Manual.* Cold Spring Harbor Laboratory, Cold Spring Harbor, NY (1989).
28. Sanger, F., S. Nicklen, and A. R. Coulson. DNA sequencing with chain-terminating inhibitors. *Proc. Natl. Acad. Sci. USA* **74**:5463–5467 (1977).
29. Gorman, C. M., L. F. Moffet, and B. H. Howard. Recombinant genomes which express chloramphenicol acetyltransferase in mammalian cells. *Mol. Cell. Biol.* **2**:1044–1051 (1982).
30. Patel, Y. C., D. N. Papachristou, H. H. Zingg, and E. M. Farkas. Regulation of islet somatostatin and gene expression: selective effects of adenosine 3′,5′-monophosphate and phorbol esters in normal islets of Langerhans and in a somatostatin-producing rat islet clonal cell line, 1027B2. *Endocrinology* **128**:1754–1762 (1991).
31. Chomczynski, P., and N. Sacchi. Single-step method of RNA isolation by acid guanidium thiocynate phenol-chloroform extraction. *Anal. Biochem.* **162**:156–158 (1987).
32. Day, R., M. K.-H. Shafer, S. J. Watson, M. Chretien, and N. G. Seidah. Distribution and regulation of the prohormone convertases PC1 and PC2 in the rat pituitary. *Mol. Endocrinol.* **6**:485–497 (1992).
33. Takashi, O., and I. Nishimoto. Detection of G protein activator regions in M_4 subunit muscarinic, cholinergic, and α_2-adrenergic receptors based upon characteristics in primary structure. *J. Biol. Chem.* **267**:8342–8346 (1992).
34. Kunkel, T. A., J. D. Roberts, and R. A. Zakour. Rapid and efficient site-specific mutagenesis without phenotypic selection. *Methods Enzymol.* **154**:367–382 (1987).
35. Nutt, R. F., D. Colton, R. Saperstein, and D. F. Veber. Side chain configurations of somatostatin analogues when bound to receptors, in *Somatostatin: Basic and Clinical Status* (S. Reichlin, ed.). Plenum Press, New York, 83–88 (1987).
36. Lamberts, S. W. J., E. P. Krenning, and J.-C. Reubi. The role of somatostatin and its analogs in the diagnosis and treatment of tumors. *Endocr. Rev.* **12**:450–482 (1991).
37. Bell, G., and T. Reisine. Molecular biology of somatostatim receptors. *Trends Neurosci.* **16**:34–38 (1993).
38. Yamada, Y., I. Stoffel, R. Espinosa, K.-S. Xiang, M. Seino, S. Seino, M. M. LeBeau, and G. I. Bell. Human somatostatin receptor genes: localization to human chromosome 14, 17, and 22 and identification of simple tandem repeat polymorphisms. *Genomics* **15**:449–452 (1993).
39. Caron, M. G., R. J. Lefkowitz, and M. Bouvier. Palmitoylation of the human β_2-adrenergic receptor. *J. Biol. Chem.* **264**:7564–7569 (1989).
40. Oksenberg, D., S. A. Marsters, B. F. O'Dowd, H. Jin, S. Havlik, S. J. Peroutka, and A. Ashkenazi. A single amino acid difference confers major pharmacological variation between human and rodent 5-HT_{1B} receptors. *Nature (Lond.)* **360**:161–163 (1992).
41. Probst, W. C., L. A. Snyder, D. I. Schuster, J. Brosius, and S. C. Sealfon. Sequence alignment of the G-protein coupled receptor superfamily. *DNA Cell Biol.* **11**:1–20 (1992).
42. Rens-Domiano, S., S. F. Law, Y. Yamada, S. Seino, G. I. Bell, and T. Reisine. Pharmacological properties of two cloned somatostatin receptors. *Mol. Pharmacol.* **42**:28–34 (1992).
43. Patel, Y. C., M. T. Greenwood, A. Warszynska, R. Panetta, and C. B. Srikant. All five cloned human somatostatin reactors (USSTR- 1-5) are functionally coupled to adenylyl cyclase. *Biochem. Biophys. Res. Commun.* **198**: 605–612 (1994).
44. Strnad, J., M. Eppler, M. Corbett, and J. R. Hadcock. The rat SSTR2 somatostatin receptor subtype is coupled to inhibition of cyclic AMP accumulation. *Biochem. Biophys. Res. Commun.* **191**:968–976 (1993).
45. Hershberger, R. E., B. L. Newman, T. Florio, P. J. Stork, and D. Cook. The somatostatin receptors SSR1 and SSR2 mediate the inhibition of adenylyl cyclase in CHO cells, in *Program of the United States Endocrine Society 75th Annual Meeting.* Endocrine Society Press, Bethesda, MD., 476 (1993).
46. Morel, G., P. Leroux, and G. Pelletier. Ultrastructural autoradiographic localization of somatostatin-28 in the rat pituitary gland. *Endocrinology* **116**:1015–1020 (1985).
47. Murthy, K. K., C. B. Srikant, and Y. C. Patel. Evidence for multiple protein constituents of the somatostatin receptor in pituitary tumor cells: affinity cross-linking and molecular characterization. *Endocrinology* **125**:948–956 (1989).
48. Amherdt, M., Y. C. Patel, and L. Orci. Selective binding of somatostatin-14 and somatostatin-28 to islet cells revealed by quantitative electron microscopic autoradiography. *J. Clin. Invest.* **80**:1455–1458 (1987).
49. Maletti, M., M. Andersson, J.-C. Marie, G. Rosselin, and V. Mutt. Solubilization and partial purification of SST-28 preferring receptors from hamster pancreatic β cells. *J. Biol. Chem.* **267**:15620–15625 (1992).
50. Patel, Y. C., R. Panetta, E. Escher, M. T. Greenwood, and C. B. Srikant. Expression of multiple somatostatin receptor genes in AtT-20 cells: evidence for a novel somatostatin-28 selective receptor subtype. *J. Biol. Chem.*, in press.

Send reprint requests to: Yogesh C. Patel, Fraser Laboratories, Department of Medicine and Neurology and Neurosurgery, Royal Victoria Hospital, 687 Pine Avenue West, Room M3.15, Montreal, Quebec H3A 1A1, Canada.