0026-895X/93/060894-08$03.00/0

MOLECULAR PHARMACOLOGY, 43:894-901

Cloning and Expression of a Human Somatostatin-14-Selective Receptor Variant (Somatostatin Receptor 4) Located on Chromosome 20

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Received January 28, 1993; Accepted April 1, 1993

SUMMARY

Based on pharmacological, biochemical, and molecular criteria, multiple somatostatin receptor (SSTR) subtypes selective for somatostatin (SST)-14 and -28 have been postulated to exist in both the brain and periphery. We report here on the cloning and characterization of a human gene encoding a new member of the guanine nucleotide-binding protein-linked SSTR family, termed human (h)SSTR4. The 388-amino acid protein, with a predicted molecular mass of~ 42 kDa, displays sequence similarity, particularly within putative transmembrane domains, with the recently cloned hSSTR1 (69%), hSSTR2 (56%), and hSSTR3 (58%). Membranes prepared from COS-7 cells transiently expressing the hSSTR4 gene bound ^{125}I-[Leu^{8},D-Trp^{22},Tyr^{25}]SST-28 in a saturable manner with high affinity (~60 pM) and with a pharmacological profile and rank order of potency ([D-Trp^{8}]SST-14 > SST-14 > SMS 201-995 > SST-28 > MK-678) indicative of a SST-14-selective receptor. K_i values for the inhibition of ^{125}I-[Leu^{8},D-Trp^{22},Tyr^{25}]SST-28 binding to the expressed receptor by these somatostatinergic peptides were 0.3, 1.1, 1.4, 2.2, and 6.5 nM, respectively. High affinity agonist binding to hSSTR4 was significantly reduced by GTP and pertussis toxin, indicating association of the expressed receptor with pertussis toxin-sensitive guanine nucleotide-binding proteins. Northern blot analysis revealed the presence of an SSTR4 mRNA species of ~4 kilobases in select regions of the monkey brain, including the hippocampus, hypothalamus, cortex, and striatum, with little or no receptor mRNA detected in either the olfactory tubercle, medulla, cerebellum, or amygdala. The SSTR4 gene maps to human chromosome 20. These findings document the existence of a novel human SSTR gene. Although the hSSTR4 displays an overall deduced amino acid homology of 86% with the recently reported rat homolog [*Proc. Natl. Acad. Sci. USA* **89**:11151-11155 (1992)], the two gene products possess distinctive pharmacological profiles and affinities for the SST agonists SMS 201-995 and MK-678.

SST, a neuropeptide originally isolated from the hypothalamus as a growth hormone-inhibitory substance, is now known to be a multifunctional peptide located in most brain regions as well as in peripheral organs such as the endocrine pancreas, gastrointestinal tract, thyroid, adrenal glands, kidneys, reproductive organs, and lymphoid tissue (1-3). There are two naturally occurring bioactive SST products, SST-14 and SST-28. These two peptides are produced in different proportions by various SST-producing cells and regulate such diverse physiological processes as endocrine and exocrine secretion, neurotransmission, smooth muscle contractility, nutrient absorption, and cell division (2-4). Disorders of SST function may contribute to the pathophysiology of several diseases including diabetes mellitus and Alzheimer's, Huntington's, and Parkinson's disease dementia (5, 6).

The actions of SST are mediated by high affinity plasma membrane receptors that are coupled via G proteins to several membrane effector systems, notably adenylate cyclase, K^+ channels, and voltage-dependent Ca^{2+} channels (7-16). SSTRs occur in varying densities throughout the central nervous system and in the pituitary, endocrine and exocrine pancreas, gastrointestinal tract, adrenal glands, thyroid, and kidneys (3,

This work was supported in part by grants from the Medical Research Council of Canada (PG-11121 and MT-10411) and the National Institute on Drug Abuse (DA-07223-01). R.K.S. holds a Medical Research Council Studentship. H.H.M.V.T. and H.B.N. are Career Scientists of the Ontario Ministry of Health.

ABBREVIATIONS: SST, somatostatin; SSTR, somatostatin receptor; LTT SST-28, [Leu^{8},D-Trp^{22},Tyr^{25}]SST-28; rSSTR, rat somatostatin receptor; hSSTR, human somatostatin receptor; mSSTR, mouse somatostatin receptor; SDS, sodium dodecyl sulfate; PCR, polymerase chain reaction; G protein, guanine nucleotide-binding protein; kb, kilobase(s); SSC, standard saline citrate; bp, base pair(s); HEPES, 4-(2-hydroxyethyl)-1-piperazine-ethanesulfonic acid.

7–12). Pharmacological studies have suggested that the SSTRs are heterogeneous and may exhibit subtypes selective for SST-14 or SST-28 (8, 12, 17–23). This heterogeneity can be explained by the presence of multiple molecular forms of the SSTR and their differential interaction with transmembrane signaling systems (12, 17–27). Photoaffinity labeling and purification studies have provided evidence for the existence of at least three different molecular species of SSTR proteins, which are expressed in a tissue-specific manner and which exhibit selective agonism for SST-14 or SST-28 (25, 26, 28–30). These include a 58-kDa form present in most tissues and additional 32-kDa and 80-kDa polypeptide species unique to the brain and pituitary, respectively.

Recent molecular cloning studies have confirmed the existence of multiple SSTR forms and have revealed a large gene family composed at present of five distinct genes (30–39). These include SSTR1 (31, 33), SSTR2 (31, 32), and SSTR3 (34, 35, 37, 39), which have been structurally characterized in human, rat, and mouse, together with a rSSTR4 (36). A fifth member of this group, isolated from the rat by O'Carroll *et al.* (38), has also been designated rSSTR4. This receptor, however, is structurally and pharmacologically (selectivity for SST-28) distinct from the first described rSSTR4 (36) and therefore should more appropriately be termed rSSTR5 (see Results and Discussion).

Using a strategy based on the PCR and low stringency homology screening (40), we report here on the isolation and characterization of a hSSTR gene, termed hSSTR4. The gene encodes a protein that shares considerable amino acid homology (~45%) with cloned hSSTR1, hSSTR2, and hSSTR3 and is localized on chromosomes 20. The hSSTR4 expressed in transfected COS-7 cells exhibits a pharmacological profile consistent with previously observed SST binding sites selective for the SST-14 peptide. Although hSSTR4 displays an overall amino acid homology of 86% with the recently reported rat homolog (36), the two gene products appear to differ with respect to their pharmacological profiles and affinities for SST agonists, specifically SMS 201–995 and MK-678.

Materials and Methods

Peptides. Synthetic SST-14 was obtained from Ayerst Laboratories (Montreal, Canada). SST-28, [D-Trp^8]SST-14, and LTT SST-28 were from Bachem (Marina Del Ray, CA). The octapeptide SST analog SMS 201–995 was a gift of Sandoz Pharmaceuticals (Basel, Switzerland).

Cloning of the SSTR4 gene. Five microliters of human genomic λEMBL3-SP6-T7 library (Clontech) were subjected to PCR amplification using degenerate primers, under conditions described previously (40). DNA from contiguous gel slices, ranging from 300 to 700 bp, were subcloned into the *Sma*I site of the plasmid pSP73 (Promega), transformed, and selected on ampicillin plates. Bacterial colonies were lifted onto nitrocellulose filters (Millipore) and hybridized under low stringency conditions (40, 41) at 42° for 24 hr with an [α-^{32}P]dCTP (NEN)-labeled fragment encoding the human dopamine D2 or D5 receptor (42, 43). Filters were washed at 50° for 1 hr in a 2× SSC/1% SDS. Hybridizing colonies were selected and sequenced by the dideoxynucleotide chain termination method using Sequenase version 2.0 (United States Biochemical) and 7-deaza-GTP. Two PCR products were identified, HG2–3 and HGA1–22, that contained 400-bp inserts displaying considerable sequence homology to each other.

The two fragments were radiolabeled with [α-^{32}P]dCTP by nick translation (Amersham) and were used to probe the human genomic λEMBL3 library to obtain full length clones. The filters were hybridized with radiolabeled HGA1–22 and then HG2–3 at 42° for 24 hr and were washed at 50° for 1 hr in 0.1× SSC/1% SDS.

From these two screenings, 12 positive colonies were isolated and analyzed by restriction endonuclease and Southern blot analysis. In addition to the recently cloned hSSTR1, hSSTR2, and hSSTR3 genes, a unique 1.4-kb *Sac*I fragment (LDIII) was isolated, subcloned into pSP73, and characterised by sequence analysis. The deduced amino acid sequence indicated that the *Sac*I fragment did not contain the full coding sequence of the receptor. A partial *Sac*I digestion was performed from phage DNA, and a 1.7-kb *Sac*I genomic fragment was subcloned into pSP73 and subsequently shown to contain the entire coding sequence of LDIII.

Expression and radioligand binding analysis. The mammalian expression vector containing hSSTR4 was constructed by inserting the 1.7-kb *Sac*I fragment of the human genomic clone LDIII into Bluescript (Stratagene), from which a 1.7-kb *Hind*III/*Not*I fragment was excised and subcloned into pRC/CMV (Invitrogen). The LDIII-pRC/CMV vector was transfected in COS-7 cells as described (44).

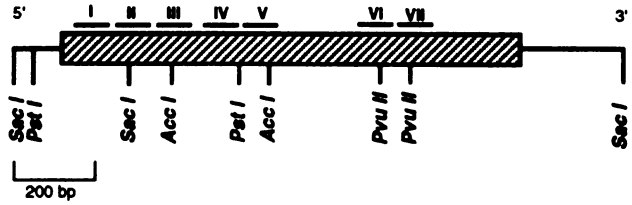
Radioligand binding studies were carried out on membranes prepared from transfected COS-7 cells, as follows. Cells were harvested, washed with Ca^{2+}/Mg^{2+}-free phosphate-buffered saline, and homogenized in ice-cold 10 mM Tris·HCl, pH 7.4, containing 2.5 mM dithiothreitol. After removal of nuclear debris, plasma membrane fractions were obtained by centrifugation of the homogenate at 20,000 × *g* for 20 min. The pellets were then washed twice with 10 mM Tris·HCl, resuspended, and stored at −80°. ^{125}I-LTT SST-28 was radioiodinated to a specific activity of approximately 1050 Ci/mmol by modification of the chloramine-T method and was purified by reverse phase HPLC on a μBondapak C-18 column (8). Binding studies were carried out for 30 min at 30° with 20–40 μg of membrane protein and ^{125}I-LTT SST-28 in 50 mM HEPES-KOH buffer, pH 7.5, containing 5 mM Mg^{2+}, 0.02% bovine serum albumin, 200 kallikrein inhibitor units/ml aprotinin, 0.02 μg/ml phenylmethylsulfonyl fluoride, and 0.02 μg/ml bacitracin. Incubations were terminated by addition of 1 ml of ice-cold HEPES-KOH containing 0.2% bovine serum albumin, rapid centrifugation, and washing. Radioactivity associated with membrane pellets was quantitated in a LKB γ counter. Specific binding was defined as the difference between total binding and binding in the presence of 100 nM SST-14.

Saturation experiments were performed with increasing concentrations of ^{125}I-LTT SST-28 (2–1200 pM) under equilibrium binding conditions. Competition analyses were carried out by incubating membranes with ^{125}I-LTT SST-28 (~60 pM) and increasing concentration of SST peptides. Estimated B_{max} and K_i values were obtained using the LIGAND computer program (45).

Northern blot analysis. Poly(A)$^+$ RNA was isolated from several monkey brain regions by the guanidium-isothiocyanate method, as described (41). Samples were denatured in glyoxal and dimethylsulfoxide, electrophoresed in a 1% agarose gel, and transferred to nylon membranes (Hybond; Amersham). The blots were probed with a [α-^{32}P]dCTP-labeled 1.7-kb *Sac*I fragment encoding hSSTR4 and were hybridized under high stringency conditions as described for genomic library screening. The blots were then washed twice for 10 min with 2× SSC/1% SDS at 20° and twice for 15 min with 0.2× SSC/1% SDS at 50°. The blots were exposed at −75° to XAR-5 film (Kodak).

Chromosomal location of the SSTR4 gene. Nested amplification of a 500-bp region flanking both the 3′ translated and untranslated nucleotides was performed to determine the chromosomal location of the hSSTR4 gene. Oligonucleotide primers 5′-CGCTGCTGCCCTCC TGGAAGGT-3′ and 5′-GAGGTTCAGGAATGATTAAA-3′ (300 nM), encompassing nucleotides 985 to 1435, and target DNA (250 ng) from panels of hybrid human-hamster somatic cell lines (BIOS, New Haven, CT) were subjected to 20 cycles of PCR containing 2.5 units of *Thermus aquaticus* polymerase (Cetus), under conditions described previously (39). Aliquots (2 μl) were then subjected to an additional 20 cycles of PCR with 300 ng of the primers 5′-CGAAGACTGCATCTCCTGAAT -3′ and 5′-GAGGTTCAGGAATGATTAATA-3′, encompassing

A



B

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                                                                    +1
5' - GAGCTCTCTGGCGCACGGCTAGCTCCGCCGCGCTCAGCTGCCCTGCGCCGGCACCCCTGGTC ATG AGC GCC
                                                                    Met Ser Ala
10                                                                          69
CCC TCG ACG CTG CCC CCC GGG GGC GAG GAA GGG CTG GGG ACG GCC TGG CCC TCT GCA GCC
Pro Ser Thr Leu Pro Pro Gly Gly Glu Glu Gly Leu Gly Thr Ala Trp Pro Ser Ala Ala
70                                                                          129
AAT GCC AGT AGC GCT CCG GCG GAG GCG GAG GAG GCG GTG GCG GGG CCC GGG GAC GCG CGG
Asn Ala Ser Ser Ala Pro Ala Glu Ala Glu Glu Ala Val Ala Gly Pro Gly Asp Ala Arg
▲130                                                                        189
GCG GCG GGC ATG GTC GCT ATC CAG TGC ATC TAC GCG CTG GTG TGC CTG GTG GGG CTG GTG
Ala Ala Gly Met Val Ala Ile Gln Cys Ile Tyr Ala Leu Val Cys Leu Val Gly Leu Val
190                                                                         249
GGC AAC GCC CTG GTC ATC TTC GTG ATC CTT CGC TAC GCC AAG ATG AAG ACG GCT ACC AAC
Gly Asn Ala Leu Val Ile Phe Val Ile Leu Pro Tyr Ala Lys Met Lys Thr Ala Thr Asn
250                                                                         309
ATC TAC CTG CTC AAC CTG GCC GTA GCC GAC GAG CTC TTC ATG CTG AGC GTG CCC TTC GTG
Ile Tyr Leu Leu Asn Leu Ala Val Ala Asp Glu Leu Phe Met Leu Ser Val Pro Phe Val
310                                                                         369
GCC TCG TCG GCC GCC CTG CGC CAC TGG CCC TTC GGC TCC GTG CTG TGC CGC GCG GTG CTC
Ala Ser Ser Ala Ala Leu Arg His Trp Pro Phe Gly Ser Val Leu Cys Arg Ala Val Leu
370                                                                         429
AGC GTC GAC GGC CTC AAC ATG TTC ACC AGC GTC TTC TGT CTC ACC GTG CTC AGC GTG GAC
Ser Val Asp Gly Leu Asn Met Phe Thr Ser Val Phe Cys Leu Thr Val Leu Ser Val Asp
430                                                                         489
CGC TAC GTG GCC GTG GTG CAC CCT CTG CGC GCG GCG ACC TAC CGG CGG CCC AGC GTG GCC
Arg Tyr Val Ala Val Val His Pro Leu Arg Ala Ala Thr Tyr Arg Arg Pro Ser Val Ala
490                                                                 □       549
AAG CTC ATC AAC CTG GGC GTG TGG CTG GCA TCC CTG TTG GTC ACT CTC CCC ATC GCC ATC
Lys Leu Ile Asn Leu Gly Val Trp Leu Ala Ser Leu Leu Val Thr Leu Pro Ile Ala Ile
550                                                                         609
TTC GCA GAC ACC AGA CCG GCT CGC GGC GGC CAG GCC GTG GCC TGC AAC CTG CAG TGG CCA
Phe Ala Asp Thr Arg Pro Ala Arg Gly Gly Gln Ala Val Ala Cys Asn Leu Gln Trp Pro
610                                                                         669
CAC CCG GCC TGG TCG GCA GTC TTC GTG GTC TAC ACT TTC CTG CTG GGC TTC CTG CTG CCC
His Pro Ala Trp Ser Ala Val Phe Val Val Tyr Thr Phe Leu Leu Gly Phe Leu Leu Pro
670                                                                         729
GTG CTG GCC ATT GGC CTG TGC TAC CTG CTC ATC GTG GGC AAG ATG CGC GCC GTG GCC CTG
Val Leu Ala Ile Gly Leu Cys Tyr Leu Leu Ile Val Gly Lys Met Arg Ala Val Ala Leu
730                                                                         789
CGC GCT GGC TGG CAG CAG CGC AGG CGC TCG GAG AAG AAA ATC ACC AGG CTG GTG CTG ATG
Arg Ala Gly Trp Gln Gln Arg Arg Arg Ser Glu Lys Lys Ile Thr Arg Leu Val Leu Met
790                                 ■               □                       849
GTC GTG GTC GTC TTT GTG CTC TGC TGG ATG CCT TTC TAC GTG GTG CAG CTG CTG AAC CTC
Val Val Val Val Phe Val Leu Cys Trp Met Pro Phe Tyr Val Val Gln Leu Leu Asn Leu
850                                                                         909
TTC GTG ACC AGC CTT GAT GCC ACC GTC AAC CAC GTG TCC CTT ATC CTC AGC TAT GCC AAC
Phe Val Thr Ser Leu Asp Ala Thr Val Asn His Val Ser Leu Ile Leu Ser Tyr Ala Asn
910                                                                         969
AGC TGC GCC AAC CCC ATT CTC TAT GGC TTC CTC TCC GAC AAC TTC CGC CGA TCC TTC CAG
Ser Cys Ala Asn Pro Ile Leu Tyr Gly Phe Leu Ser Asp Asn Phe Arg Arg Ser Phe Gln
970                                                                        1029
CGG GTT CTC TGC CTG CGC TGC TGC CTC CTG GAA GGT GCT GGA GGT GCT GAG GAG GAG CCC
Arg Val Leu Cys Leu Arg Cys Cys Leu Leu Glu Gly Ala Gly Gly Ala Glu Glu Glu Pro
1030                                                                       1089
CTG GAC TAC TAT GCC ACT GCT CTC AAG AGC AAA GGT GGG GCA GGG TGC ATG TGC CCC CCA
Leu Asp Tyr Tyr Ala Thr Ala Leu Lys Ser Lys Gly Gly Ala Gly Cys Met Cys Pro Pro
1090                                                                       1149
CTC CCC TGC CAG CAG GAA GCC CTG CAA CCA GAA CCC GGC CGC AAG CGC ATC CCC CTC ACC
Leu Pro Cys Gln Gln Glu Ala Leu Gln Pro Glu Pro Gly Arg Lys Arg Ile Pro Leu Thr
1150
AGG ACC ACC ACC TTC TGA GGAGCCCTTCCCCTACCCACCCTGCCGTTGGCACCTCCCAAGGGGTGGGCACCAT
Arg Thr Thr Thr Phe OPA

TCCTACAGCCCCGAAGACTGCATCTCCTGAATGCTCACCTAAGCTCCTCCTCCTGTTCCTTCCAGCAGCCCATGTACCT
GCCGGAGAGTGTAGAACTCTTCTCGTCGCTCCTCCT -3
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Fig. 1. A, Restriction map of the hSSTR4 genomic clone. Relevant endonuclease restriction sites of the 1.7-kb *Sac*I genomic clone are indicated. *Hatched areas*, coding region of the clone, with the positions of putative transmembrane domains *marked with lines and numbered with Roman numerals*. B, Nucleotide and deduced amino acid sequences of the hSSTR4 gene. Nucleotides of the LDIII genomic clone are numbered beginning with the codon for the putative initiation methionine, with the deduced amino acid sequence presented below the nucleotide sequence. ▲, Putative *N*-linked glycosylation sites. Potential phosphorylation sites for protein kinase A (cAMP dependent) (□) and protein kinase C (■) are shown.

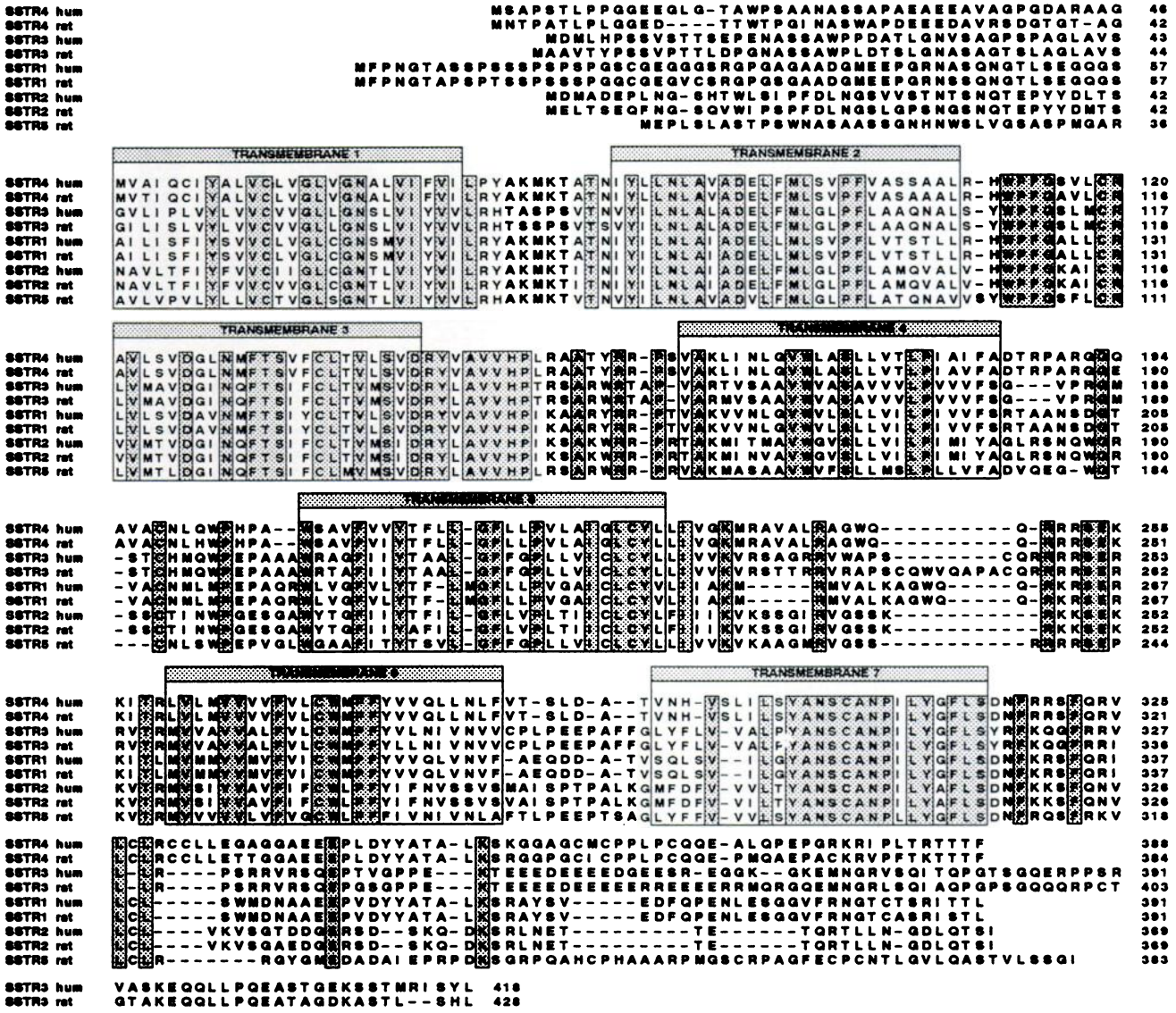

Fig. 2. Alignment of deduced amino acid sequence of hSSTR4 and other members of the SSTR family. *Boxed and shaded areas*, conserved amino acid residues between hSSTR4 and hSSTR1, rSSTR1, hSSTR2, rSSTR2, hSSTR3, rSSTR3, rSSTR4 (36), and rSSTR5, previously termed SSTR4 (38), proteins. Single-letter code is used to denote amino acids.

nucleotides 1233 to 1435, or 5′-CGCTGCTGCCTCCTGGAAGGT-3′ and 5′-ATTCAGGAGATGCAGTCTTCG-3′, encompassing nucleotides 985 to 1254, under the same conditions. Samples of amplified DNA were electrophoresed in a 1.2% agarose gel, transferred to nylon membranes (Zeta-probe; Bio-Rad), and hybridized with [γ-^{32}P] ATP-labeled oligonucleotide probes located internally to the flanking regions of hSSTR4, under the same conditions as described for genomic library screening. The blots were washed twice with 2× SSC/1% SDS at 20° for 10 min and once with 1× SSC/1% SDS at 50° for 10 min and were subjected to autoradiography for 1 hr with XAR-5 film.

Results and Discussion

PCR amplification of human genomic DNA with degenerate oligonucleotide primers corresponding to conserved segments of transmembrane domains 3 and 6 of the dopamine receptor gene family generated many clones, as described previously (40). Two 400-bp genomic fragments, HG2–3 and HGA1–22, were isolated and displayed a deduced amino acid sequence with strong homology to the G protein-linked peptide receptor subfamily. During genomic library screening to obtain full length clones, a third 1.4-kb *Sac*I fragment (LDIII) was isolated that showed, upon sequencing, considerable homology to the first two PCR products, HG2–3 and HGA1–22. These two products were found to have complete sequence identity with the first two reported members of the SSTR family, termed SSTR1 and SSTR2 (31, 32). It was therefore concluded that LDIII was also a potential member of the SSTR family.

After partial *Sac*I digestion a 1.7-kb fragment was isolated; upon nucleotide sequencing it revealed consensus sequences for a putative initiating methionine followed by a long open reading frame of 1164 nucleotides, encoding a 388-amino acid protein with an estimated relative molecular mass of 41,856. Fig. 1 depicts the restriction map and nucleotide and deduced amino acid sequences of the genomic clone.[1] The size of this receptor

[1] Sequences reported in this paper have been deposited in GenBank (accession number L07061).

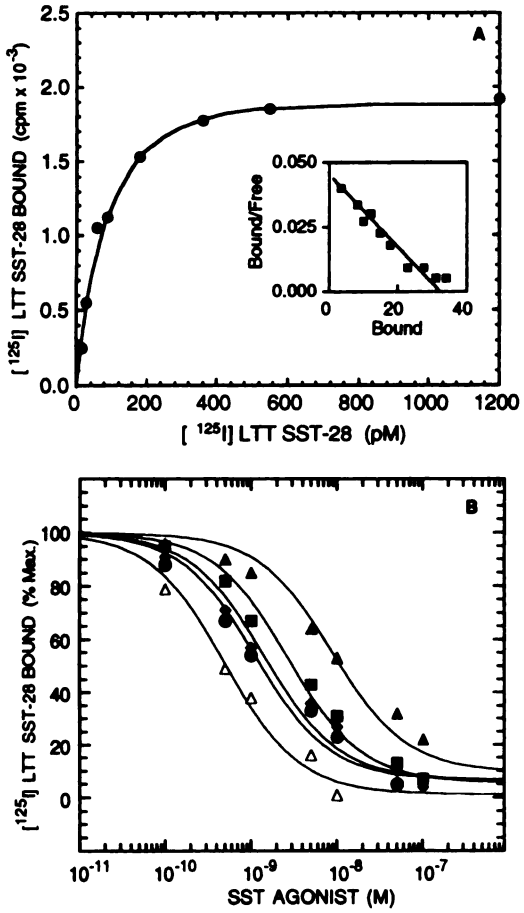

Fig. 3. A, Saturation isotherms of ^{125}I-LTT SST-28 binding to membranes prepared from COS-7 cells transfected with hSSTR4 DNA. Membranes prepared from COS-7 cells transfected with the hSSTR4 gene were incubated with increasing concentrations of radioligand and assayed for receptor binding activity in the absence or presence of 100 nM LTT SST-28 to define nonspecific binding, as described in Materials and Methods. Scatchard analyses of the binding data (*inset*) revealed a binding capacity (B_{max}) of 33 ± 5 fmol/mg of protein and estimated affinity (K_d) of 57 ± 10 pM for SSTR4. Data represent means ± standard errors of three determinations, each conducted in duplicate. B, Concentration-dependent competitive inhibition by SST analogs of ^{125}I-LTT SST-28 binding to the expressed hSSTR4. Membranes were incubated with ^{125}I-LTT SST-28 and the indicated concentrations of SST-14 (●), SST-28 (■), [D-Trp^8] SST-14 (△), SMS 201-995 (♦), or MK-678 (▲). Data are representative of three separate experiments performed in duplicate.

TABLE 1

Relative affinities of SST analogs for binding to the expressed hSSTR4

Inhibitory constants (K_i) of SST agonists for ^{125}I-LTT SST-28 binding to membranes prepared from COS-7 cells transfected with the hSSTR4 gene were determined. Values represent the means ± standard errors of three independent experiments, each conducted in duplicate.

Peptide	K_i	Hill coefficient
	nM	
[D-Trp^8]SST-14	0.32 ± 0.04	0.76
SST-14	1.09 ± 0.19	0.75
SMS 201-995	1.36 ± 0.17	0.74
SST-28	2.20 ± 0.24	0.77
MK-678	6.50 ± 1.60	0.64

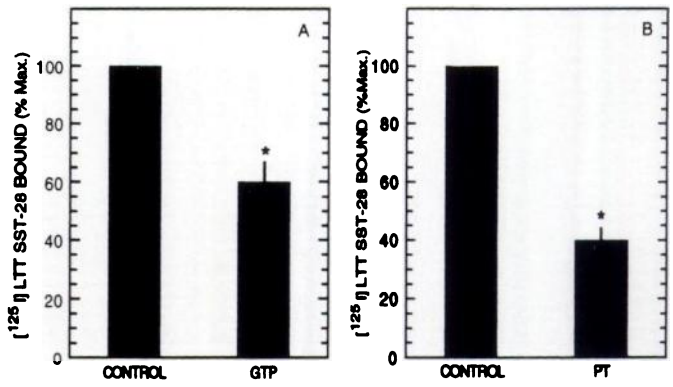

Fig. 4. Evidence for association with G proteins for hSSTR4 expressed in COS-7 cells. A, Membranes of COS-7 cells expressing hSSTR4 were incubated with ^{125}I-LTT SST-28 in the absence or presence of 100 μM GTP. Values are expressed as percentage of maximum specific binding of the radioligand in the absence of GTP (three experiments). Total binding was 2350 ± 210 cpm and nonspecific binding (in the presence of 100 nM SST-14) was 825 ± 97 cpm. B, Effect of pretreatment of membranes with pertussis toxin (*PT*). ^{125}I-LTT SST-28 binding to membranes was assessed after incubation for 2 hr at 30° with or without 100 ng/ml pertussis toxin in 20 mM Tris·HCl, pH 7.5 (three experiments). *, Binding was significantly different from controls ($p < 0.05$), using Student's *t* test.

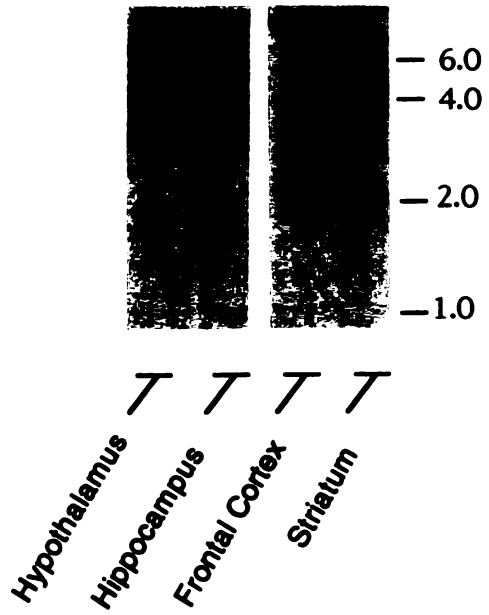

Fig. 5. Northern blot analysis of SSTR4 mRNA in monkey brain. Poly(A)$^+$ (3–5 μg/lane) RNA samples from various monkey brain regions were denatured, electrophoresed, transferred to nylon membranes, and hybridized with a 1.7-kb *Sac*I fragment encoding the entire hSSTR4 gene. Nylon membranes were exposed for 6 days at −80° with one intensifying screen.

protein (termed hSSTR4) is comparable to that of SSTR1, SSTR2, and SSTR3 and is in good agreement with the size of the principal form of the native SSTR identified by cross-linking and solubilization studies (25–28, 30). The hSSTR4 does not appear to contain any introns within its coding region, as reported for both hSSTR1 and hSSTR3 (31, 37, 39). This is in contrast to SSTR2, which has been shown to contain a single intron at the 3′ end of both the murine (46) and human genes.[2]

[2] 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).

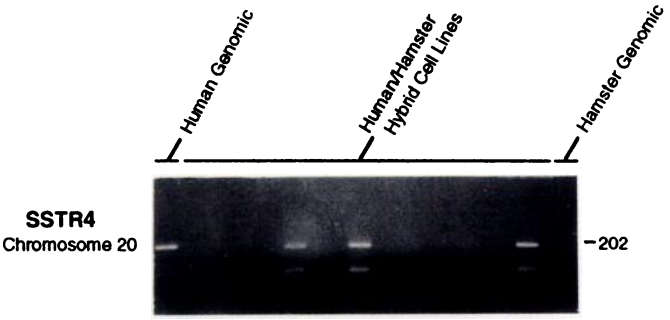

Fig. 6. Chromosomal location of the hSSTR4. Ethidium bromide-stained agarose gel of hybrid human-hamster somatic cell lines (BIOS) analyzed for the presence of hSSTR4 DNA using the PCR, as described in Materials and Methods, is shown. Oligonucleotide primers flanking the 3′ translated and untranslated sequences of the hSSTR4 gene were used to generate an amplified product of approximately 200 bp.

Hydrophobicity analysis of the deduced amino acid sequence of this gene reveals the presence of seven putative transmembrane domains, characteristic of members of the superfamily of G protein-coupled receptors. Moreover, as with other members of the SSTR family, hSSTR4 contains consesus sequences for one potential *N*-linked glycosylation site within the extracellular amino acid region (Asn^{24}). As illustrated in Fig. 1B, three putative consensus sequences for potential phosphorylation by protein kinase C and protein kinase A were found in the second and third cytoplasmic loops of hSSTR4.

As summarized in Fig. 2. the greatest degree of amino acid sequence homology between hSSTR4 and other SSTRs occurs within the seven putative transmembrane domains, where hSSTR4 shares 69%, 56%, 58%, and 57% homology with hSSTR1, hSSTR2, hSSTR3, and rSSTR5, respectively. Overall, the amino acid sequence homology is 57% between hSSTR4 and hSSTR1, 44% with hSSTR2, 37% with hSSTR3, and 40% with rSSTR5. The hSSTR4 and rSSTR4 display ~86% amino acid sequence identity, with a particularly striking 97% homology within membrane-spanning domains. Most of the sequence divergence between these two receptors occurs at the extracellular amino terminus, as well as in the cytoplasmic carboxyl-terminal tail.

To ascertain the pharmacological nature of the hSSTR4 gene product, membranes prepared from COS-7 cells transiently expressing the LDIII-pRC/CMV clone were assessed for their ability to bind SST. As depicted in Fig. 3A, ^{125}I-LTT SST-28 exhibited specific and saturable binding to the expressed hSSTR4. Scatchard transformation of the binding data revealed a single class of high affinity binding sites, with an estimated K_d of 57 ± 10 pM and B_{max} of 33 ± 5 fmol/mg of protein (Fig. 3A, *inset*). No specific binding of ^{125}I-LTT SST-28 was observed in nontransfected COS-7 cells or cells transfected with the vector alone. This is in contrast to the findings of O'Carroll *et al.* (38), who have observed displaceable ^{125}I-[Tyr^{11}]SST-14 binding sites in mock-transfected COS-7 cells. ^{125}I-LTT SST-28 binding was inhibited in a concentration-dependent manner by SST-14, SST-28, and synthetic SST-14 analogs with the following rank order of potency: [D-Trp^8]SST-14 > SST-14 > SMS 201-995 > SST-28 > MK-678 (Fig. 3B; Table 1). The hSSTR4 bound SST-14 with a 2-fold higher affinity than SST-28 (K_i of 1.09 ± 0.19 nM and 2.2 ± 0.24 nM, respectively; see Table 1). Likewise, rSSTR1, mSSTR1, and hSSTR1 (31, 33), as well as mSSTR2 and hSSTR2 (31) (but not rSSTR2) (32), have all been reported to be SST-14 selective, with K_i or IC_{50} values for SST-14 and SST-28 ranging from 0.8 to 2.4 nM and from 4 to 5 nM, respectively. On the other hand, SST-14 and SST-28 appear to display species-specific selectivity for SSTR3, with SST-28 > SST-14 for hSSTR3 and rSSTR3 (35, 37) but SST-28 ≈ SST-14 for mSSTR3 (34). This is in contrast to the recently cloned rSSTR5, which exhibits a striking 30-fold greater affinity for SST-28 than for SST-14 (38). The variable binding selectivities that have been reported for SSTR2 and SSTR3 may have arisen from the use of different radioligands and expression systems, as suggested by our own data showing that hSSTR3 is selective for SST-14 when assessed with radiolabeled ^{125}I-LTT SST-28 (39), in contrast to SST-28-selective binding of the same receptor tested using ^{125}I-CGP23996 as radioligand (37).

The agonist profiles of hSSTR4 and rSSTR4 reveal a striking difference between the two species. First, whereas hSSTR4 binds the conformationally restricted analogs SMS 201-995 and MK-678 with relatively high affinities (1.36 and 6.5 nM, respectively), the rat gene product fails to recognize these congeners even at micromolar concentrations (36). Although native rat neuronal SSTRs with poor affinity for these analogs have been described (23), it is unknown whether human brain membranes express SSTRs with similar pharmacological properties. Second, [D-Trp^8]SST-14 shows higher affinity than SST-14 for hSSTR4, whereas it binds to rSSTR4 with a 2-fold lower potency (36). It remains to be determined whether sequence divergence between these receptors accounts for such disparity in their pharmacological profiles.

GTP, at a concentration of 100 μM, inhibited ^{125}I-LTT SST-28 binding to hSSTR4 (Fig. 4A). Likewise, treatment of COS-7 cells expressing hSSTR4 with pertussis toxin significantly reduced high affinity agonist binding (Fig. 4B). These data indicate that hSSTR4 associates with pertussis toxin-sensitive G proteins in COS-7 cells, and they support the existence of multiple agonist affinity states for various SST analogs, as suggested by Hill coefficients considerably less than unity (see Table 1). The hSSTR4 joins three other members of the SSTR family, mSSTR2, mSSTR3, and rSSTR5, that have similarly been shown to interact with pertussis toxin-sensitive G proteins (38, 47). Preliminary data indicate that in transfected COS-7 cells hSSTR4 inhibits forskolin-stimulated cAMP levels (48), suggesting that hSSTR4 (like SSTR3, SSTR5, and probably SSTR2) is functionally coupled to adenylyl cyclase (37, 38).

Northern blot analysis of poly(A)$^+$ RNA from various brain regions probed with the 1.7-kb *Sac*I fragment of SSTR4 demonstrated tissue-specfic distributions. As depicted in Fig. 5, hSSTR4 mRNA transcripts of approximately 4.0 kb in length were observed in monkey hippocampus, hypothalamus, cortex, and striatum. Little or no receptor mRNA was detected in either the olfactory tubercle, medulla, cerebellum, or amygdala (data not shown). The neuronal distribution of hSSTR4 mRNA is very similar to that reported for the rat gene (36). SSTR4 mRNA distribution in the brain is distinct from that of other SSTRs (49). As shown in Fig. 6, PCR amplification of hybrid human-hamster somatic cell lines for the hSSTR4 gene revealed chromosome 20 to be the only chromosome to which this gene segregates. The hSSTR3 was found to map to chromosome 22 (39), compared with hSSTR1 and hSSTR2, which

were found to segregate with chromosomes 14 and 17, respectively (data not shown).

The findings described above document the existence of a novel human homolog of the SSTR gene family. The newly cloned hSSTR4 is closely related to hSSTR1 and hSSTR2 in size, structure, and selectivity for SST-14 binding. It shows less amino acid similarity to hSSTR3 and rSSTR5, a SST-28-preferring receptor. Furthermore, the human form of SSTR4 is quite distinct from its rat homolog in its pharmacological profile. Whether this receptor subtype is differentially regulated and/or preferentially coupled to membrane signaling pathways, compared with other members of the SSTR family, remain to be determined.

Acknowledgments

We thank Anne Tirpak, Michael Chung, and Ania Warzsynska for their excellent technical assistance.

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