Proc. Natl. Acad. Sci. USA
Vol. 90, pp. 4196–4200, May 1993
Biochemistry

Cloning and characterization of a fourth human somatostatin receptor

(GTP-binding-protein-coupled receptor/somatostatin receptor gene)

LUCIA ROHRER*†, FRIEDRICH RAULF*‡, CHRISTIAN BRUNS*, REINHARD BUETTNER§, FERDINAND HOFSTAEDTER§, AND ROLAND SCHÜLE*¶

*Preclinical Research, Sandoz Pharma, Ltd., 4002 Basel, Switzerland; and §Department of Pathology, University of Regensburg Medical School, 8400 Regensburg, Federal Republic of Germany

Communicated by Roger Guillemin, January 22, 1993

ABSTRACT **We have isolated a gene coding for a fourth human somatostatin (somatotropin release-inhibiting factor) receptor. This additional somatostatin receptor (hSSTR4) is specifically expressed in human fetal and adult brain and lung tissue. The deduced amino acid sequence of the receptor displays both sequence and structural homology to three cloned somatostatin receptors as well as to other members of the family of GTP-binding-protein-coupled seven-helix transmembrane-spanning receptors. Pharmacological characterization of the expressed receptor reveals specific, high-affinity binding of somatostatin 14 and somatostatin 28. Surprisingly, several well-characterized synthetic somatostatin analogs fail to exhibit high-affinity binding to hSSTR4, indicating the existence of pharmacologically different receptor subtypes. Our data suggest that the diverse biological effects exerted by somatostatin are mediated by a family of receptors with discrete patterns of expression and different pharmacological properties.**

Somatostatin (somatotropin release-inhibiting factor) is a cyclic tetradecapeptide initially isolated from hypothalamic extracts (1). Subsequent studies revealed that it is widely distributed in the body: it occurs in the central nervous system and several peripheral tissues like stomach, intestine, and pancreas. Somatostatin displays diverse biological functions as a modulator of various endocrine and exocrine secretion processes, such as inhibition of growth hormone, insulin, gastrin, glucagon, and secretion release (2). In the central nervous system, somatostatin functions as a neurotransmitter and may be an important regulator of motor activity and cognitive processes (3, 4). In addition, somatostatin shows antiproliferative effects *in vitro* and *in vivo* and might be an important modulator of cellular proliferation (5).

Somatostatin exerts its biological effects by binding to specific membrane receptors (6–8). High-affinity somatostatin receptors (SSTRs) have been demonstrated in various tissues such as different brain regions, pancreas, adrenal cortex, and several tumors [e.g., endocrine breast, lung, or brain tumors (9–12)]. Biochemical analysis demonstrated that SSTRs are coupled to GTP-binding proteins (G proteins) (13, 14). Subsequent pharmacological studies suggested the existence of at least two different receptor subtypes (15–19). Recently, molecular cloning confirmed the existence of three different SSTRs, SSTR1, SSTR2, and SSTR3, all members of the seven-helix membrane-spanning receptor superfamily (20–23).

Here we report cloning and characterization of a gene encoding an additional human SSTR (hSSTR), hSSTR4,‖ with distinct tissue distribution and pharmacological properties different from the other known SSTRs. Our data suggest that the diverse regulatory effects mediated by somatostatin are exerted by a family of receptors with discrete patterns of expression and pharmacological properties.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

EXPERIMENTAL PROCEDURES

General Methods. Standard procedures were carried out as described by Sambrook *et al.* (24). Sequence analyses were performed by using the Genetics Computer Group software programs (26).

Cloning of the SSTR4 Gene. A human genomic library (Stratagene) was screened with the entire coding region of the ^{32}P-labeled hSSTR1 gene (kindly provided by Graeme I. Bell, University of Chicago). The filters were hybridized under standard conditions with 1×10^6 cpm of ^{32}P-labeled probe per ml at 42°C for 16 h. The final washing was performed in 0.2× standard saline citrate (SSC) at 65°C for 1 h. Several overlapping phages with restriction patterns clearly different from phages containing the parental hSSTR1 gene were isolated. One of these clones (P5) was chosen for further analysis. The 19-kb insert of P5 was analyzed by restriction mapping and Southern blot hybridizations. A 2.1-kb *Xba* I/*Nae* I fragment of P5, containing the entire hSSTR4 coding sequence, was subcloned into pBluescript II KS(+) and subsequently sequenced. The sequence of both strands was confirmed according to Tabor and Richardson (27).

Reverse Transcription (RT) and PCR. For the RT–PCRs, 0.5 μg of human poly(A)$^+$ RNA (Clontech) was reverse transcribed using random hexamer oligonucleotide primers. PCR amplifications of the first-strand cDNA product were carried out according to the manufacturer's protocol (Perkin–Elmer/Cetus) with the exception that the Mg^{2+} concentration varied from 1 to 4 mM depending on the primer set. The following temperature profile was used for amplification: an initial denaturing step at 95°C for 2.5 min and then 40 cycles consisting of 95°C for 20 sec, 60°C for 30 sec, and 72°C for 90 sec. The following primers were used: 1A (GGAACTCTATGGTCATCTA) and 1B (GAGGGCCACCATGCGCATCTT) for hSSTR1, 2A (TCATCAAGGTGAAGTCCTCTGG) and 2B (AGATACTGGTTTGGAGGTCTCCA) for hSSTR2, 3A (AGAACGCCCTGTCCTACTGGCC) and 3B (TGAAGCGGTAGGAGAGGAAGCC) for hSSTR3, 4A (ATGGTCGCTATCCAGTGC), 4B (GTGAGACAGAA-

Abbreviations: G protein, GTP-binding protein; RT, reverse transcription; GPR, G-protein-coupled receptor; SSTR, somatostatin receptor; hSSTR, human SSTR.

†Present address: Laboratorium für Biochemie I, ETH, 8092 Zürich, Switzerland.
‡To whom reprint requests should be addressed at: Sandoz Pharma Ltd., PCR 386/609, P.O. Box, CH-4002 Basel, Switzerland.
¶Present address: Tumor Biology Center, University of Freiburg, 7800 Freiburg, Federal Republic of Germany.

‖The sequence reported in this paper has been deposited in the GenBank data base (accession no. L07833).

GACGCTGGTGAACAT), and 4C (GGGCTCCTCA-GATGGT) for hSSTR4, and actin-1 (ATGCCTCTGGTCG-TACCACGGGCATTG) and actin-2 (CTTGCTGATCCA-CATCTGCTGGAAGGTG) for β-actin.

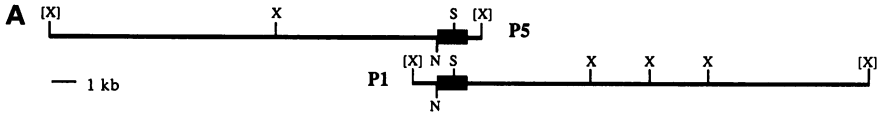
Northern Blot Analysis. Northern blots (Clontech) were hybridized with receptor subtype-specific probes, generated by PCR using cloned hSSTR DNAs as templates. Amplifications were performed according to the manufacturer's protocols. The following primers were used: 1A and 1B for hSSTR1, 2A and 2B for hSSTR2, 3A and 3B for hSSTR3, and 4A and 4C for hSSTR4. The amplified PCR products were randomly labeled with [α-^{32}P]dCTP. Hybridizations were performed under standard conditions in 50% formamide at 42°C for 16 h. Final washes were done in 0.1× SSC at 55°C for 45 min.

Heterologous Expression of hSSTR4. A 2.1-kb *Xba* I/*Nae* I fragment of phage P5 containing the entire coding sequence of the hSSTR4 gene was inserted into the eukaryotic expression vector pcDNA I (Invitrogen). The resulting construct pcDNA-H4 was transfected into COS-1 cells by using the standard DEAE-dextran technique. COS-1 cells were grown in Dulbecco's modified Eagle's medium supplemented with 10% (vol/vol) fetal calf serum and 2 mM glutamine.

Pharmacological Characterization. Binding studies were performed with cell membranes from transiently transfected COS-1 cells as described (21). Ten micrograms of membrane protein was incubated in 10 mM Hepes (pH 7.5), 5 mM $MgCl_2$, bacitracin (20 μg/ml), 0.5% bovine serum albumin, and ^{125}I-labeled [Tyr^{11}]somatostatin 14 {hereafter referred to as [Tyr^{11}(^{125}I)]somatostatin 14} (30,000 cpm) with or without various concentrations of unlabeled somatostatin 14, somatostatin 28, SMS 201–995 (Octreotide; ref. 28), MK-678 (29), or RC-160 (30) for 1 h at room temperature. Specific binding was measured as total [Tyr^{11}(^{125}I)]somatostatin 14 binding minus the amount of radioligand bound in the presence of 100 nM somatostatin 14 (31). Inhibition curves were analyzed, and IC_{50} values were calculated using the curve-fitting program of De Lean (32).

RESULTS

Cloning and Amino Acid Sequence of a Fourth hSSTR. In an attempt to isolate and characterize additional members of the SSTR family, we used the coding region of the hSSTR1 gene as a probe to screen a human genomic library. Under stringent hybridization conditions, 12 positive clones were identified. Restriction mapping analysis indicated that most of them were independently derived recombinant phages covering the entire genomic locus of the hSSTR1 gene (data not shown). However, two phages (P1 and P5) hybridized significantly more weakly and exhibited a restriction pattern incompatible with the hSSTR1 locus (Fig. 1*A*). A 2.1-kb *Xba* I/*Nae* I fragment from phage P5 was subcloned and sequenced. As shown in Fig. 1*B*, the fragment contained an



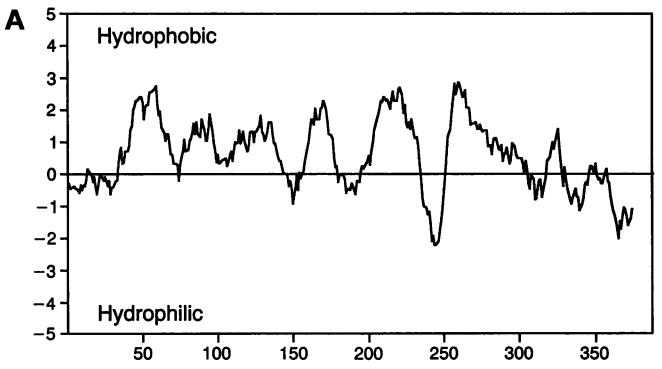

```
B  5'CTGCGCGCCAGCCCCCGCCCTGGGCCCGCCGCCCGAGCTCTCTGGCGCAGCGCTAGCTCCGCCGCGCTGAGCTGCCCTGCG   81
   CCGGCACCCCTGGTCATGAGCGCCCCCTCGACGCTGCCCCCCGGGGGCGAGGAAGGGCTGGGGACGGCCTGGCCCTCTGCA  162
 1                 MetSerAlaProSerThrLeuProProGlyGlyGluGluGlyLeuGlyThrAlaTrpProSerAla
   GCCAATGCCAGTAGCGCTCCGGCGGAGGCGGAGGAGGCGGTGGCGGGGCCCGGGGACGCGCGGGCGGCGGGCATGGTCGCT  243
 23 AlaAsnAlaSerSerAlaProAlaGluAlaGluGluAlaValAlaGlyProGlyAspAlaArgAlaAlaGlyMetValAla
   ATCCAGTGCATCTACGCGCTGGTGTGCCTGGTGGGGCTGGTGGGCAACGCCCTGGTCATCTTCGTGATCCTTCGCTACGCC  324
 50 IleGlnCysIleTyrAlaLeuValCysLeuValGlyLeuValGlyAsnAlaLeuValIlePheValIleLeuArgTyrAla
   AAGATGAAGACGGCTACCACCATCTACCTGCTCAACCTGGCCGTAGCCGACGAGCTCTTCATGCTGAGCGTGCCCTTCGTG  405
 77 LysMetLysThrAlaThrThrIleTyrLeuLeuAsnLeuAlaValAlaAspGluLeuPheMetLeuSerValProPheVal
   GCCTCGTCGGCCGCCCTGCGCCACTGGCCCTTCGGCTCCGTGCTGTGCCGCGCGGTGCTCAGCGTCGACGGCCTCAACATG  486
104 AlaSerSerAlaAlaLeuArgHisTrpProPheGlySerValLeuCysArgAlaValLeuSerValAspGlyLeuAsnMet
   TTCACCAGCGTCTTCTGTCTCACCGTGCTCAGCGTGGACCGCTACGTGGCCGTGGTGCACCCTCTGCGCGCGGCGACCTAC  567
131 PheThrSerValPheCysLeuThrValLeuSerValAspArgTyrValAlaValValHisProLeuArgAlaAlaThrTyr
   CGGCGGCCCAGCGTGGCCAAGCTCATCAACCTGGGCGTGTGGCTGGCATCCCTGTTGGTCACTCTCCCCATCGCCATCTTC  648
158 ArgArgProSerValAlaLysLeuIleAsnLeuGlyValTrpLeuAlaSerLeuLeuValThrLeuProIleAlaIlePhe
   GCAGACACCAGACCGGCTCGCGGCGGCCAGGCCGTGGCCTGCAACCTGCAGTGGCCACACCCGGCCTGGTCGGCAGTCTTC  729
185 AlaAspThrArgProAlaArgGlyGlyGlnAlaValAlaCysAsnLeuGlnTrpProHisProAlaTrpSerAlaValPhe
   GTGGTCTACACTTTCCTGCTGGGCTTCCTGCTGCCCGTGCTGGCCATTGGCCTGTGCTACCTGCTCATCGTGGGCAAGATG  810
212 ValValTyrThrPheLeuLeuGlyPheLeuLeuProValLeuAlaIleGlyLeuCysTyrLeuLeuIleValGlyLysMet
   CGCGCCGTGGCCCTGCGCGCTGGCTGGCAGCAGCGCAGGCGCTCGGAGAAGAAAATCACCAGGCTGGTGCTGATGGTCGTG  891
239 ArgAlaValAlaLeuArgAlaGlyTrpGlnGlnArgArgArgSerGluLysLysIleThrArgLeuValLeuMetValVal
   GTCGTCTTTGTGCTCTGCTGGATGCCTTTCTACGTGGTGCAGCTGCTGAACCTCGTCGTGACCAGCCTTGATGCCACCGTC  972
266 ValValPheValLeuCysTrpMetProPheTyrValValGlnLeuLeuAsnLeuValValThrSerLeuAspAlaThrVal
   AACCACGTGTCCCTTATCCTCAGCTATGCCAACAGCTGCGCCAACCCTATTCTCTATGGCTTCCTCTCCGACAACTTCCGC 1053
293 AsnHisValSerLeuIleLeuSerTyrAlaAsnSerCysAlaAsnProIleLeuTyrGlyPheLeuSerAspAsnPheArg
   CGATCCTTCCAGCGGGTTCTCTGCCTGCGCTGCTGCCTCCTGGAAGGTGCTGGAGGTGCTGAGGAGGAGCCCCTGGACTAC 1134
320 ArgSerPheGlnArgValLeuCysLeuArgCysCysLeuLeuGluGlyAlaGlyGlyAlaGluGluGluProLeuAspTyr
   TATGCCACTGCTCTCAAGAGCAAAGGTGGGGCAGGGTGCATGTGCCCCCCACTAAAATGCCAGCAGGAAGCCCTGCAACCA 1215
347 TyrAlaThrAlaLeuLysSerLysGlyGlyAlaGlyCysMetCysProProLeuLysCysGlnGlnGluAlaLeuGlnPro
   GAACCCGGCCGCAAGCGCATCCCCCTCACCAGGACCACCACCTTCTGAGGAGCCCTTCCCCTACCCACCCTGCGTGGCCAC 1296
374 GluProGlyArgLysArgIleProLeuThrArgThrThrThrPhe*  388
   CTCCCAAGGGGTGGGACCATTCCTACAGCCCCGAAGACTGCATC 3' 1340
```

FIG. 1. (*A*) Restriction map alignment of the two overlapping genomic λ clones P1 and P5. The open reading frame encoding hSSTR4 is indicated by a filled box. X, *Xba* I; S, *Sal* I; N, *Nae* I. Restriction sites generated by the cloning procedure are shown in brackets. (*B*) Nucleotide and deduced amino acid sequences of hSSTR4. The putative transmembrane domains (I–VII) are underlined. The putative N-linked glycosylation site at position Asn-24 is marked with a star. The potential phosphorylation sites at Ser-161 and Ser-253 in the second and third cytoplasmic loops are indicated by filled circles. Characteristic residues conserved in all G-protein-coupled receptors (GPRs) are emphasized in boldface type. The cysteine residues in the first and second extracellular loops known to form disulfide bridges in other GPRs are indicated by double underlining. The putative palmitoylation site at Cys-327 is marked by boldface type and double underlining.

1167-bp open reading frame, encoding a protein of 388 amino acids with a predicted molecular size of 42 kDa. Hydropathicity analysis (33) of the protein sequence suggested the presence of seven hydrophobic, putatively membrane-spanning regions (Fig. 2*A*). Thus this receptor shares structural features of the receptor superfamily with seven transmembrane domains. Many members of this superfamily are also G-protein-coupled receptors (GPRs; for review, see ref. 34), and indeed amino acid residues that are conserved in the GPR family are also conserved in the sequence of this receptor. Three important motifs should be noted (Fig. 1*B*). The sequences G^{64}NXXV in the first transmembrane domain and N^{307}PIXXY in the seventh transmembrane domain of this receptor are typically conserved residues in GPRs (34). In addition the "DRY" sequence element in L^{140}XXDRY is considered to be functionally important in coupling to G proteins (35). Two consensus sites for serine phosphorylation (Ser^{162} and Ser^{253}) could be identified in the second and third intracellular loop of the new receptor (Fig. 1*B*). Both sequences perfectly match the recognition motif of the multifunctional calmodulin-dependent protein kinase II (36). The serine at position 253 also matches the consensus site for cAMP-dependent protein kinases (36). In analogy to other GPRs, the carboxyl-terminal Cys^{327} in the receptor sequence is a potential palmitoylation site (34). Therefore Cys^{327} may attach the receptor's carboxyl terminus to the cell membrane via a palmitoyl anchor. Two other amino-terminal cysteines located at position Cys^{119} and Cys^{199} might form a disulfide bridge between the first and second extracellular loop (37). In addition the amino terminus contains a consensus sequence (N^{24}XS/T) for N-glycosylation (38), which might be implicated in high-affinity ligand binding (39).

Sequence comparison of this gene with other GPRs indicated that it is most similar to the hSSTR genes. As demonstrated in Fig. 2*B*, the amino acid sequence showed 58%, 43%, and 41% identity (78%, 66%, and 67% similarity) with the sequences of hSSTR1, hSSTR2, and hSSTR3, respectively. The sequence of the four proteins showed greatest similarity in the putative membrane-spanning regions, whereas their amino and carboxyl termini completely diverge. Together these data suggest that we have isolated a fourth hSSTR, hSSTR4.

Pharmacological Properties of hSSTR4. To determine the pharmacological properties of hSSTR4, we performed binding experiments with membranes prepared from COS-1 cells transiently expressing hSSTR4. [$Tyr^{11}(^{125}I)$]somatostatin 14 exhibited high-affinity and saturable binding to the expressed receptor. The affinity constant (K_d) was calculated to be 1.1 ± 0.1 nM. Membranes prepared from COS-1 cells transfected with the parental expression vector pcDNA I showed no



B

```
                                                                    <---- TM I --
hSSTR4  M******SAPSTLP***PGGEEGLGTAWPSAANASSAPAEAEEAVAGPGDARA**AGMVAIQCIYALVCL   59
hSSTR1  MFPNGTASSPSSSPSPSPGSCGEGGGSRGPGAGAADGMEEPGRNASQNGTLSEGQGSAILISFIYSVVCL   70
hSSTR2  M****DM********A**DEPLNGSHTWLSIPFDLNGSVVSTNTSNQTEPYYDLTSNAVLT*FIYFVVCI   55
hSSTR3  M****DMLHPSSVSTT**SEPENASSAW***PPDATLGNVSAGPSPA*****GLAVSGVLIPLVYLVVCV   56
        *                                                           *  **

        ---------->          <----- TM II --------->                    <---
hSSTR4  VGLVGNALVIFVILRYAKMKTATTIYLLNLAVADELFMLSVPFVASSAALRHWPFGSVLCRAVLS  124
hSSTR1  VGLCGNSMVIYVILRYAKMKTATNIYILNLAIADELLMLSVPFLVTSTLLRHWPFGALLCRLVLS  135
hSSTR2  IGLCGNTLVIYVILRYAKMKTITNIYILNLAIADELFMLGLPFLAMQVALVHWPFGKAICRVVMT  120
hSSTR3  VGLLGNSLVIYVVLRHTASPSVTNVYILNLALADELFMLGLPFLAAQNALSYWPFGSLMCRLVMA  121
          ** **  ** * **       *  * **** **** **  **       *  ****   ** *

        --- TM III -------->                  <-------- TM IV -------->
hSSTR4  VDGLNMFTSVFCLTVLSVDRYVAVVHPLRAATYRRPSVAKLINLGVWLASLLVTLPIAIFADTRP  189
hSSTR1  VDAVNMFTSIYCLTVLSVDRYVAVVHPIKAARYRRPTVAKVVNLGVWVLSLLVILPIVVFSRTAA  200
hSSTR2  VDGINQFTSIFCLTVMSIDRYLAVVHPIKSAKWRRPRTAKMITMAVWGVSLLVILPIMIYAGLRS  185
hSSTR3  VDGINQFTSIFCLTVMSVDRYLAVVHPTRSARWRTAPVARTVSAAVWVASAVVVLPVVVFSGVP*  185
        **  * ***  **** * *** *****  *  *  *       **  *  * **

                          <-------- TM V ---------->
hSSTR4  ARGGQAVACNLQWPHPA**WSAVFVVYTFLLGFLLPVLAIGLCYLLIVGKMRAVALRAGW****QQR  250
hSSTR1  NSDG*TVACNMLMPEPAQRWLVGFVLYTFLMGFLLPVGAICLCYVLIIAKMRMVALKAGW****QQR  262
hSSTR2  NQWGRS*SCTINWPGESGAWYTGFIIYTFILGFLVPLTIICLCYLFIIIKVKSSGIRV****GSSKR  247
hSSTR3  **RGMS*TCHMQWPEPAAAWRAGFIIYTAALGFFGPLLVICLCYLLIVVKVRSAGRRV*WAPSCQRR  248
          *  *  *  *  *  **  **  *  * ***  *  *                  *

                  <-------- TM VI ----->                <------- TM VII -------
hSSTR4  RRSEKKITRLVLMVVVVFVLCWMPFYVVQLLNLVVTSLDAT****VNHVSLILSYANSCANPILYG  312
hSSTR1  KRSERKITLMVMMVVMVFVICWMPFYVVQLVNVFAEQDDAT****VSQLSVILGYANSCANPILYG  324
hSSTR2  KKSEKKVTRMVSIVVAVFIFCWLPFYIFNVSSVSMAISPTPALKGMFDFVVVLTYANSCANPILYA  313
hSSTR3  RRSERRVTRMVVAVVALFVLCWMPFYVLNIVNVVCPLPEEPAFFGLYFLVVALPYANSCANPILYG  314
          **  *  *  **  *  ** ***                          * ***********

        ->
hSSTR4  FLSDNFRRSFQRVLCLRCCLLEGAGGAEEEPLDYYATALKSKGGAGCMCPPLKCQQEALQPEPG*RKRIP  381
hSSTR1  FLSDNFKRSFQRILCLS**WMDNAA***EEPVDYYATALKSRA*****YSVEDFQPENLESGGVFRNGTC  384
hSSTR2  FLSDNFKKSFQNVLCLV*****KVSGTDDGERS***DSKQDKSRLNETTETQRTLLNGDLQTSI       369
hSSTR3  FLSYRFKQGFRRVL*****************LR***PSRRVRSQEPTVGPPEKTEEEDEEEEDGEESREG  364
        *** *  *  *

hSSTR4  LTRTTTF                                                          388
hSSTR1  TSRITTL                                                          391
hSSTR2                                                                   369
hSSTR3  GKGKEMNGRVSQITQPGTSGQERPPSRVASKEQQLLPQEASTGEKSSTMRISYL           418
```

FIG. 2. Amino acid analysis of hSSTR4. (*A*) Hydrophobicity analysis according to Kyte and Doolittle (33) revealed seven hydrophobic regions, suggesting the presence of seven membrane-spanning domains. (*B*) Comparison of the amino acid sequences of the known SSTRs (PILEUP program of the Genetics Computer Group program package; ref. 26). The seven predicted transmembrane domains (TM I–VII) are noted. Identical and conservative substituted amino acids are shown in boldface type. Stars denote identical amino acids.

specific binding of the radioligand (data not shown). As shown in Fig. 3, binding of [Tyr^{11}(^{125}I)]somatostatin 14 was displaced by somatostatins 14 and 28 in a monophasic manner, which is characteristic for binding to a single class of receptors. The inhibitory concentrations for half-maximal inhibition (IC_{50}) were calculated to be 1.0 nM and 1.1 nM for somatostatins 14 and 28, respectively. Surprisingly, the synthetic somatostatin analogs SMS 201–995 and RC-160 exhibited extremely low-affinity (>100 nM) binding to hSSTR4. In addition MK-678, another somatostatin analog, failed to displace [Tyr^{11}(^{125}I)]somatostatin 14 binding at all. These results clearly distinguish hSSTR4 from rat and human SSTR2, which bind these somatostatin analogs with subnanomolar affinities (refs. 20 and 21; C.B., unpublished data).

Tissue Distribution of hSSTR4. To compare the distinct expression pattern of all four hSSTRs, we used receptor subtype-specific probes to perform Northern blot and RT–PCR analysis. As shown in Fig. 4, a 4.6-kb hSSTR4 transcript was detected in brain and to a lesser extent in lung tissue. No expression could be detected in heart, placenta, liver, skeletal muscle, kidney, or pancreas. A single 4.3-kb hSSTR1 transcript was also detected in brain and lung. hSSTR2 mRNAs of 8.9 kb and 2.4 kb were expressed in brain, whereas only a single 4.9-kb hSSTR3 transcript could be detected in brain and pancreas. To examine further the expression pattern of hSSTR4, we performed a series of RT–PCR experiments. As shown in Fig. 4*B*, hSSTR4 is only expressed in fetal and adult brain and lung tissue. In accordance with previous data, we observed additional hSSTR1 gene expression in fetal kidney, fetal liver, and adult pancreas (20).

DISCUSSION

In this study, we describe the cloning and characterization of a fourth hSSTR (hSSTR4). Sequence analysis revealed that hSSTR4 is an additional member of the seven-helix membrane-spanning receptor superfamily, most closely related to hSSTR1. hSSTR4 binds somatostatin 14 and somatostatin 28 with nanomolar affinities, but interestingly fails to bind synthetic somatostatin analogs with high affinities.

The cloning and characterization of four unique but structurally closely related SSTR subtypes suggest that SSTRs form a distinct subgroup of GPRs. Like many other GPR family members, all four SSTRs lack introns in their coding regions. In addition to the great similarities in the putative membrane-spanning regions, SSTR4 shares several conserved sequence elements typical for GPRs. For example the DRY motif, which is considered to be functionally important in coupling to G proteins (35), is located in a similar position in GPRs and in hSSTR4.

With four hSSTRs and several mouse and rat homologs now cloned, (20–23) it is interesting to search for structural features specific for SSTRs. For example, the motif NXFTS located in the third transmembrane domain is found exclusively in all known SSTRs but not in other GPRs. In addition, the putative phosphorylation site RXXSE in the third intra-

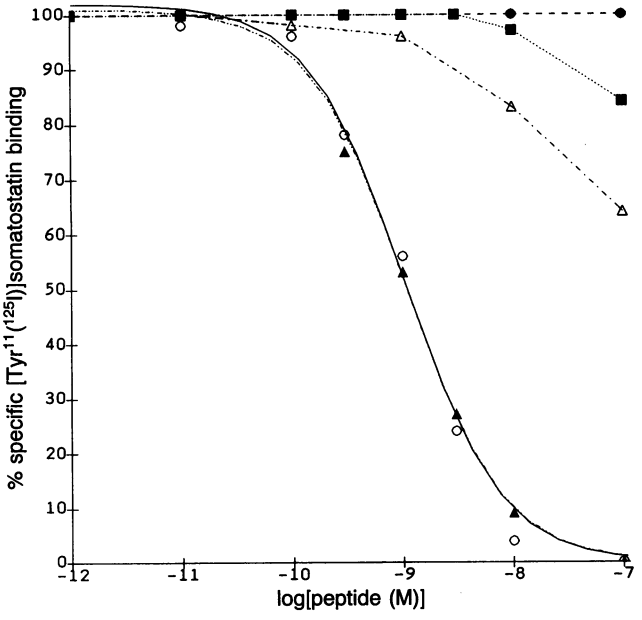


FIG. 3. Concentration dependence for the inhibition of [Tyr^{11}(^{125}I)]somatostatin 14 binding by various somatostatin analogs. Membranes prepared from COS-1 cells transiently transfected with the receptor cDNA were incubated with [Tyr^{11}(^{125}I)]somatostatin 14 and the indicated concentrations of somatostatin 14 (○), somatostatin 28 (▲), SMS 201–995 (Octreotide; ■), MK-678 (●), and RC-160 (△). Results are expressed as a percentage of the maximal specific binding observed in the absence of competitor. Data are mean values of three determinations.

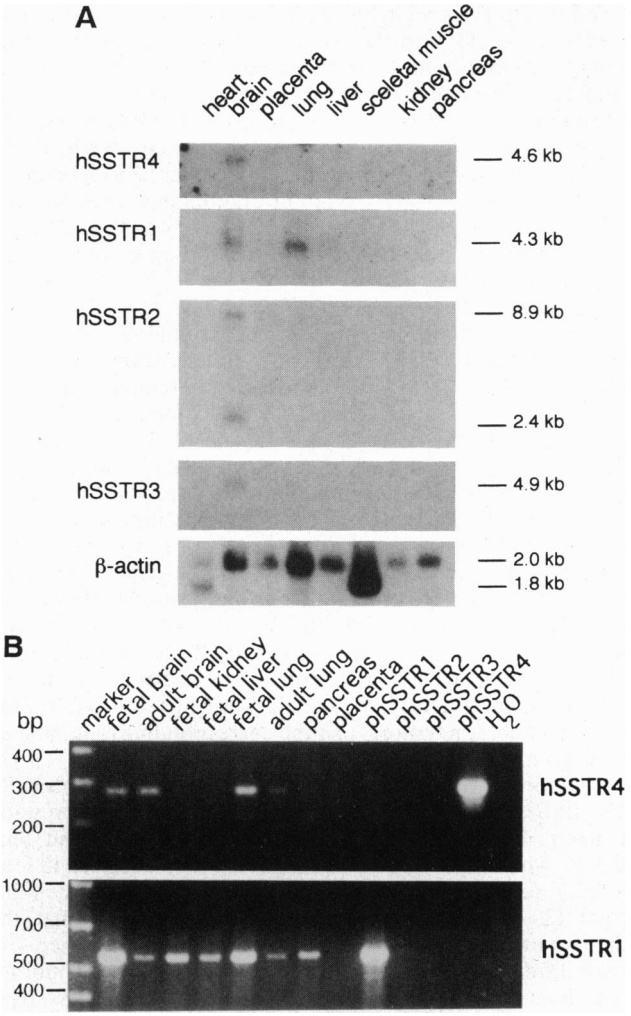


FIG. 4. Tissue distribution of hSSTRs. (*A*) A multiple-tissue Northern blot with 2 μg of poly(A)$^+$ RNA of human adult heart, brain, placenta, lung, liver, skeletal muscle, kidney, and pancreas was subsequently hybridized with SSTR subtype-specific ^{32}P-labeled probes. β-Actin was used as a control. Exposure times were 8 days for hSSTR4, 3 days for hSSTR1, 6 days for hSSTR2 and hSSTR3, and 30 min for β-actin at −80°C. (*B*) RT–PCR analyses were performed with primers specific for hSSTR4 (primers 4A and 4B) and hSSTR1 (primers 1A and 1B). PCR products were separated on 2% agarose gels. The expected length of the PCR products was 274 bp (hSSTR4) and 519 bp (hSSTR1).

cellular loop and the motif YANSCANPILY in the seventh transmembrane domain are specifically conserved in SSTRs only. The discovery of these SSTR-specific motifs should help to design mutagenesis experiments to elucidate the role of certain amino acid residues in ligand interaction and signal transduction.

hSSTRs share an interesting arrangement of putative protein phosphorylation sites. As mentioned above, the recognition motif for calmodulin-dependent protein kinase II at Ser^{253} is present in all four hSSTRs, whereas the consensus motif around Ser^{162} is only found in hSSTR1 and hSSTR4. In contrast, the consensus motif for cAMP-dependent protein kinases are detected only in hSSTR3 and hSSTR4. Although the biological relevance remains to be elucidated, these potential phosphorylation sites are predicted to be located within intracellular loops and may therefore be accessible to protein kinases. The different arrangement of protein kinase recognition sites might also indicate coupling of individual receptors to multiple effector systems.

The carboxyl-terminal loop of hSSTR4 contains a putative site for palmitoylation ($LC^{327}L$). This motif is absent in hSSTR3 but is found in hSSTR1, hSSTR2, and in many other GPRs. In analogy to the β_2-adrenergic receptor, this motif might be important in anchoring the receptor to the cell membrane via a palmitoyl anchor (40). Sequence analysis of the amino-terminal extracellular loop of hSSTR4 revealed the presence of only one consensus sequence (NXS/T) for N-glycosylation. In contrast, the three other hSSTRs contain multiple sites for N-glycosylation. Interestingly, recent studies suggested that the carbohydrate component of somatostatin receptors may be involved in promoting high-affinity ligand binding (39).

Pharmacological studies performed with hSSTR4 demonstrated high-affinity binding of both somatostatin 14 and somatostatin 28, whereas several synthetic somatostatin analogs like SMS 201–995 exhibited a greatly reduced affinity or failed to bind at all. A similar pharmacological profile was obtained with only hSSTR1 (refs. 20 and 22; data not shown). These findings are in marked contrast to the pharmacological profile of SSTR2, which binds synthetic somatostatin analogs like SMS 201–995 with subnanomolar affinities (22). Our results might have important therapeutical and diagnostic implications, since somatostatin analogs have been used recently for the treatment of various tumors (41) and as radioligands for tumor imaging *in vivo* (42). However, our data imply that tumor cells expressing SSTR4 or SSTR1 will not bind currently available somatostatin analogs with high affinity. Consequently such analogs might fail to inhibit the growth of certain tumors, and the corresponding radiolabeled analogs might not detect them *in vivo*.

In addition to a similar pharmacological profile, hSSTR1 and hSSTR4 showed a distinct but overlapping expression pattern. Both receptors are expressed in brain and lung tissue, whereas hSSTR1 transcripts are also detected in fetal kidney, fetal liver, pancreas, and the human gastrointestinal tract (20–22). In summary, our results suggest that the distinct expression patterns of all four hSSTRs, together with their different pharmacological properties, might account for the diverse biological effects mediated by SSTRs. It is tempting to speculate that receptor-subtype-selective ligands might result in tissue-specific actions of somatostatin receptors. Furthermore, the cloning of four hSSTRs provides the molecular basis to elucidate the structural requirements for these functional differences.

Note Added in Proof. After this manuscript was submitted, two different rat somatostatin receptors were published, both termed rSSTR4 (25, 43). While one receptor (43) represents clearly the rat homolog to our human receptor, the second one represents a new SSTR subtype.

We thank Dr. Graeme I. Bell for plasmids and exchange of information prior to publication. We are grateful to W. Bauer for synthesizing all somatostatin analogs. We also thank Désirée Eckert, Christine Zeschnigk, and Silvia Seegers for expert technical assistance and Trevor J. Petcher for critical review of the manuscript.

1. Brazeau, P., Vale, W., Burgus, R., Ling, N., Butcher, M., Rivier, J. & Guillemin, R. (1973) *Science* **179,** 77–79.
2. Reichlin, S. (1983) *N. Engl. J. Med.* **309,** 1495–1506, 1556–1563.
3. Epelbaum, J. (1986) *Prog. Neurobiol. (New York)* **27,** 63–100.
4. Martin-Iversen, M., Radke, J. & Vincent, S. (1986) *Pharmacol. Biochem. Behav.* **24,** 1707–1714.
5. Schally, A. V. (1988) *Cancer Res.* **48,** 6977–6985.
6. Reubi, J. C., Perrin, M. H., Rivier, J. E. & Vale, W. (1981) *Life Sci.* **28,** 2191–2198.
7. Zeggari, M., Viguerie, N., Susini, C., Esteve, J. P., Vaysse, N., Rivier, J., Wuensch, E. & Ribet, A. (1986) *Peptides (New York)* **7,** 953–959.
8. Reubi, J. C., Perrin, M. H., Rivier, J. E. & Vale, W. (1982) *Biochem. Biophys. Res. Commun.* **105,** 1538–1545.
9. Srikant, C. B. & Patel, Y. C. (1982) *Endocrinology* **110,** 2138–2144.
10. Maurer, R. & Reubi, J. C. (1986) *Mol. Cell. Endocrinol.* **45,** 81–90.
11. Srikant, C. B. & Patel, Y. C. (1981) *Proc. Natl. Acad. Sci. USA* **78,** 3930–3934.
12. Sakamoto, C., Goldfine, I. D. & Williams, J. A. (1984) *J. Biol. Chem.* **259,** 9623–9627.
13. He, H.-T., Rens-Domiano, S., Martin, J.-M., Law, S. F., Borislow, S., Woolkalis, M., Manning, D. & Reisine, T. (1990) *Mol. Pharmacol.* **37,** 614–621.
14. Law, S. F., Manning, D. & Reisine, T. (1991) *J. Biol. Chem.* **266,** 17885–17897.
15. Reubi, J. C. (1984) *Neurosci. Lett.* **49,** 259–263.
16. Reubi, J. C. (1985) *Life Sci.* **36,** 1829–1836.
17. Reisine, T. & Guild, S. (1985) *J. Pharmacol. Exp. Ther.* **235,** 551–557.
18. Tran, V. T., Beal, M. F. & Martin, J. B. (1985) *Science* **228,** 492–495.
19. Raynor, K. & Reisine, T. (1989) *J. Pharmacol. Exp. Ther.* **251,** 510–517.
20. Yamada, Y., Post, S. R., Wang, K., Tanger, S. H., Bell, G. I. & Seino, S. (1992) *Proc. Natl. Acad. Sci. USA* **89,** 251–255.
21. Kluxen, F., Bruns, C. & Luebbert, H. (1992) *Proc. Natl. Acad. Sci. USA* **89,** 4618–4622.
22. Yasuda, K., Rens-Domiano, S., Breder, C. D., Law, S. F., Saper, C. B., Reisine, T. & Bell, G. I. (1992) *J. Biol. Chem.* **267,** 20422–20428.
23. Yamada, Y., Reisine, T., Law, S. F., Ihara, Y., Kubota, A., Kagimoto, S., Seino, M., Seino, Y., Bell, G. I. & Seino, S. (1993) *Mol. Endocrinol.*, in press.
24. Sambrook, J., Fritsch, E. F. & Maniatis, T. (1989) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Lab., Plainview, NY), 2nd Ed.
25. O'Carroll, A.-M., Lolait, S. J., König, M. & Mahan, L. C. (1992) *Mol. Pharmacol.* **42,** 939–946
26. Devereux, J., Haeberli, P. & Smithies, O. (1984) *Nucleic Acids Res.* **12,** 387–395.
27. Tabor, S. & Richardson, C. C. (1987) *Proc. Natl. Acad. Sci. USA* **84,** 4767–4771.
28. Bauer, W., Briner, U., Doepfner, W., Haller, R., Huguenin, R., Marbach, P., Petcher, T. J. & Pless, J. (1982) *Life Sci.* **31,** 1133–1140.
29. Veber, D. F., Holly, F. W., Paleveda, W. J., Nutt, R. F., Bergstrand, S. J., Torchiana, M., Glitzer, M. S., Saperstein, R. & Hirschmann, R. (1978) *Proc. Natl. Acad. Sci. USA* **75,** 2636–2640.
30. Cai, R. Z., Szoke, B., Lu, R., Fu, B., Redding, T. W. & Schally, A. V. (1986) *Proc. Natl. Acad. Sci. USA* **83,** 1896–1900.
31. Bruns, C., Dietel, M. M., Palacios, J. M. & Pless, J. (1990) *Biochem. J.* **265,** 39–44.
32. De Lean, A. (1979) Ph.D. thesis (Howard Hughes Med. Inst., Duke Univ., Durham, NC).
33. Kyte, T. J. & Doolittle, R. F. (1982) *J. Mol. Biol.* **157,** 105–132.
34. Probst, W. C., Snyder, L. A., Schuster, D. I., Brosius, J. & Seaflon, S. C. (1992) *DNA Cell Biol.* **11,** 1–20.
35. Fraser, C. M., Chung, F. Z., Wang, C. D. & Venter, J. C. (1988) *Proc. Natl. Acad. Sci. USA* **85,** 5478–5482.
36. Kemp, B. E. & Pearson, R. B. (1990) *Trends Biochem. Sci.* **15,** 342–346.
37. Strosberg, A. D. (1991) *Eur. J. Biochem.* **196,** 1–10.
38. Kornfeld, R. & Kornfeld, B. E. (1985) *Annu. Rev. Biochem.* **54,** 631–664.
39. Rens-Domiano, S. & Reisine, T. (1991) *J. Biol. Chem.* **266,** 20094–20102.
40. O'Dowd, B. F., Hanatowich, M., Caron, M. G., Lefkowitz, R. J. & Bouvier, M. (1989) *J. Biol. Chem.* **264,** 7564–7569.
41. Weckbecker, G., Liu, R., Tolcsvai, L. & Bruns, C. (1992) *Cancer Res.* **52,** 4973–4978.
42. Lamberts, S. W. J., Bakker, W. H., Reubi, J.-C. & Krenning, E. P. (1990) *Metabolism* **39,** Suppl. 2, 152–155.
43. Bruno, J. F., Xu, Y., Song, J. & Berelowitz, M. (1992) *Proc. Natl. Acad. Sci USA* **89,** 11151–11155.