A human somatostatin receptor (SSTR3), located on chromosome 22, displays preferential affinity for somatostatin-14 like peptides

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We report here on the cloning of a human intronless gene encoding a member of the G-protein linked somatostatin (SST) receptor subfamily, termed SSTR3. Based on the deduced amino acid sequence, this gene encodes a 418 amino acid protein displaying sequence similarity, particularly within putative transmembrane domains, with the recently cloned human SSTR1 (62%), SSTR2 (64%) and SSTR4 (58%) receptors. Membranes prepared from COS-7 cells transiently expressing the human SSTR3 gene bound [^{125}I]Leu^8,D-Trp^{22},Tyr^{25} SST-28 in a saturable manner with high affinity (~ 200 pM) and with rank order of potency (D-Trp^8 SST-14 > SST-14 > SMS-201-995 > SST-28) indicative of a somatostatin-14 selective receptor. The pharmacological profile of the expressed human SSTR3 receptor is similar but not identical to that reported for the rat homolog [(1992) J. Biol. Chem. 267, 20422] where the peptide selectivity is SST-28 ≧ SST-14 ≫ SMS-201-995. Northern blot analysis reveals the presence of an SSTR3 mRNA species of ~ 5 kb in various regions of the monkey brain, including the frontal cortex, cerebellum, medulla, amygdala, with little or no SSTR3 mRNA detectable in brain regions such as the striatum, hippocampus, and olfactory tubercle. The SSTR3 receptor gene maps to human chromosome 22. The existence of at least four distinct human genes encoding somatostatin-14 selective receptors with diverse pharmacological specificities may help to account for some of the multiple biological actions of somatostatin under normal and pathological conditions.

Polymerase chain reaction; Neuropeptide; SST-14; SST-28

1. INTRODUCTION

Somatostatin (somatotropin release-inhibiting factor or SRIF), a tetradecapeptide derived from presomatostatin, is a multifunctional peptide located in both the central nervous system and peripheral tissue [1–3]. Originally isolated from the hypothalamus [1], it has been identified in most brain regions as well as such organs as the pituitary, pancreas, adrenals, gastrointestinal system and thyroid. Functionally, somatostatin regulates the secretion of various hormones and secretory substances, including growth hormone, gastrin, glucagon and insulin. However, it can also act as a neuromodulator in the CNS, modulating neuronal activity by facilitating the release of several neurotransmitters including dopamine and serotonin [4]. Two forms of somatostatin exist, SST-14 and SST-28, and are believed to mediate

somatostatin's functions in a tissue-specific manner. It has been postulated that a malfunction in somatostatin metabolism contributes to the maintenance of several disorders including Alzheimer's and Parkinson's dementia [5,6].

Somatostatin is believed to mediate its effects via specific cell surface receptors which are coupled to GTP-binding proteins (see [3] and [4] for reviews). Pharmacological studies have demonstrated the presence of at least two distinct receptor subtypes which possess high affinity, saturable binding sites selective for the two native and biologically active forms of somatostatin. These findings suggest that the two peptides, SST-14 and SST-28, may therefore have different functions, mediated by different receptors and that these receptors are expressed in a tissue-specific manner. Somatostatin receptors have been observed in many tissues including brain, pituitary, pancreas, adrenals and various 'cell lines such as GH3, GH4C1 and AtT-20 pituitary tumour cells.

Recent photoaffinity labeling and purification studies have also provided evidence for the existence of multiple somatostin receptors in the CNS and periphery. These reports have postulated a diverse population of receptors ranging in polypeptide size from 27 to 200 kDa [7,8]. It is unclear however, whether this observed diver-

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Abbreviations: SST, somatostatin; SSTR, somatostatin receptor; G, guanine nucleotide.

sity is due to receptor heterogeneity or experimental variability. Ambiguity therefore still remains in the number and structure of the somatostatin receptor family which has only begun to be elucidated by the cloning of its receptor subtypes [9].

Using a strategy based on low stringency screening for genes encoding members of the superfamily of receptors which couple to G-protein binding [10], we report here the isolation of a third human intronless somatostatin receptor gene (SSTR3) located on chromosome 22. The gene encodes for a protein which shares considerable amino acid homology to the recently cloned human somatostatin receptors SSTR1, SSTR2 [11] and SSTR4 [12]. The expressed receptor when transfected in COS-7 cells displays a pharmacological profile consistent with previously observed somatostatin binding sites selective for the SS-14 peptide in native membranes, but not with the recently cloned rat homolog [13,14] of human SSTR3.

2. MATERIALS AND METHODS

2.1. Peptides
Synthetic SST-14 was obtained from Ayerst Laboratories, Montreal. SST-28, [D-Trp^8]SST-14 and [Leu^8,D-Trp^{22},Tyr^{25}]SST-28 (LTT SS-28) were from Bachem (Marina Del Ray, CA). The octapeptide somatostatin analog SMS-201-995 was a gift of Sandoz Pharmaceuticals (Basel, Switzerland).

2.2. Cloning of the SSTR3 receptor gene
A fragment encoding transmembrane domains one to five of the human dopamine D5 receptor [15] was radiolabelled with [α-^{32}P]dCTP by nick translation (Amersham) and used to probe a human genomic λEMBL3 library. Nylon filters (Colony/Plaque Screen, DuPont) were hybridized under low stringency conditions [15] at 42°C for 24 h with [α-^{32}P]dCTP (NEN) labeled fragment. Filters were washed twice at 50°C for 1 h in a 2 × standard saline citrate (SSC/1% SDS solution). Hybridizing colonies were selected and sequenced with the dideoxynucleotide chain termination method using Sequenase version 2.0 (US Biochemical) and 7-deaza-GTP.

From this screening, positive colonies were systematically isolated and analyzed by restriction endonuclease and Southern blot analysis. A 5.5 kb BamHI fragment (JCI) was isolated, subcloned into pSP73 and characterised by sequence analysis.

2.3. Expression and radioligand binding analysis
The mammalian expression vector containing the SSTR3 receptor was constructed by inserting a 2.1 kb NcoI/HindIII fragment of the human genomic clone JCI into the expression vector pCD-ps.,

Radioligand binding studies were carried out on membranes prepared from JCI-pCD-ps transfected COS-7 cells using methods previously described [12,15]. Cells were harvested, washed with Ca^{2+}/Mg^{2+}-free PBS 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°C. [^{125}I]LTT STT-28 was radioiodinated by modification of the chloramine-T method and purified by reverse phase HPLC on a μBondapak C-18 column to a specific activity of approximately 1,050 Ci/mmol (1 Ci = 37 GBq) [16].

Binding studies were carried out with [^{125}I]LTT SST-28 in 50 mM HEPES-KOH buffer (pH 7.5) containing 5 mM Mg^{2+}, 0.02% BSA, 200 kalliketin inhibitor units of Aprotinin/ml and 0.02 μg/ml each of

phenylmethyl-sulfonylfluoride and bacitracin, incubated with 20–40 μg membrane protein for 30 min at 30°C. Incubations were terminated by adding 1 ml of ice-cold HEPES-KOH containing 0.2% BSA, rapid centrifugation and washing. Radioactivity associated with membrane pellets was quantitated in an LKB gamma spectrometer. Specific binding was defined as the difference between total binding and binding in the presence of 100 nM SST-14.

Saturation experiments were performed under equilibrium conditions with increasing concentrations of [^{125}I]LTT SST-28 (2.5–1,000 pM). Competition analyses were carried out by incubating membranes with [^{125}I]LTT SST-28 (~ 60 pM) and increasing concentrations of SST peptides. Estimated B_{max} and K_i values were obtained using the computer program LIGAND as previously described [15].

2.4. Northern blot analysis
Poly(A)$^+$ RNA was isolated from several monkey brain regions by the guanidium-isothiocyanate method as previously described [10]. Samples were denatured in glyoxal and dimethyl sulfoxide, electrophoresed in a 1% agarose gel and transferred to nylon membrane (Hybond, Amersham). The blots were probed with an [α-^{32}P]dCTP labelled 2.1 kb NcoI/HindIII fragment encoding the SSTR3 receptor and hybridized under high stringency conditions. The blots were then washed twice for 10 min in 2 × SSC/1% SDS at 20°C and twice for 15 min at 0.5 × SSC/1% SDS at 50°C. The blots were exposed at −75°C to XAR-5 film (Kodak).

2.5. Chromosomal location of the SSTR3 receptor gene
Amplification of a 410 bp region flanking both the 5′ translated and untranslated nucleotides of the human SSTR3 gene was performed to determine its chromosomal location. Oligonucleotide primers 5′-CAGCACAGAGAAGCCATTCTCTGCTGT-3′ and 5′-TAGGAC-AGGGCGTTCTGG-3′ (encompassing nucleotides −72 and 340 within the 5′ translated and untranslated regions) and target DNA (250 ng) from panels of hybrid human–hamster somatic cell lines (Bios, New Haven, CT) were submitted to 20 cycles of PCR containing 2.5 units of Taq polymerase (Cetus) under the following conditions: denature, 1 min at 94°C; annealing, 60°C 1.5 min; primer extension, 72°C 1.5 min. Aliquots (2 μl) were then resubmitted to an additional 20 cycles of PCR under the same conditions. Samples of amplified DNA were electrophoresed in a 1.2% agarose gel, transferred to nylon (Zeta-probe, Bio-Rad) and hybridized with a [γ-^{32}P]ATP labelled oligonucleotide probe (5′-GTTCTGATCCCCCTGGTCTAC-3′) located internally (nucleotide 133) to the amplified regions of SSTR3 under high stringency conditions. The blots were washed twice with 2 × SSC/1% SDS at 20°C for 10 min, once in 1 × SSC/1% SDS at 50°C for 10 min and exposed to autoradiography for 1 h to XAR-5 film.

3. RESULTS AND DISCUSSION

Low stringency screening of human genomic DNA with conserved segments of transmembrane one to five of the dopamine receptor gene family, generated many clones as previously described [10]. A 5.5 kb BamHI fragment (JCI) was isolated, which upon nucleotide sequencing, showed considerable deduced amino acid homology to the G-protein coupled peptide receptor family, and more specifically, to recently cloned members of the somatostatin receptor family [11,12].

Nucleotide sequence analysis of JCI revealed consensus sequences for a putative initiating methionine [17] followed by an open reading frame of 1,254 nucleotides encoding a 418 amino acid protein with an estimated molecular mass of 45,817. Fig. 1a and 1b depict the restriction map, nucleotide and deduced amino acid sequence of the genomic clone, termed SSTR3, respec-
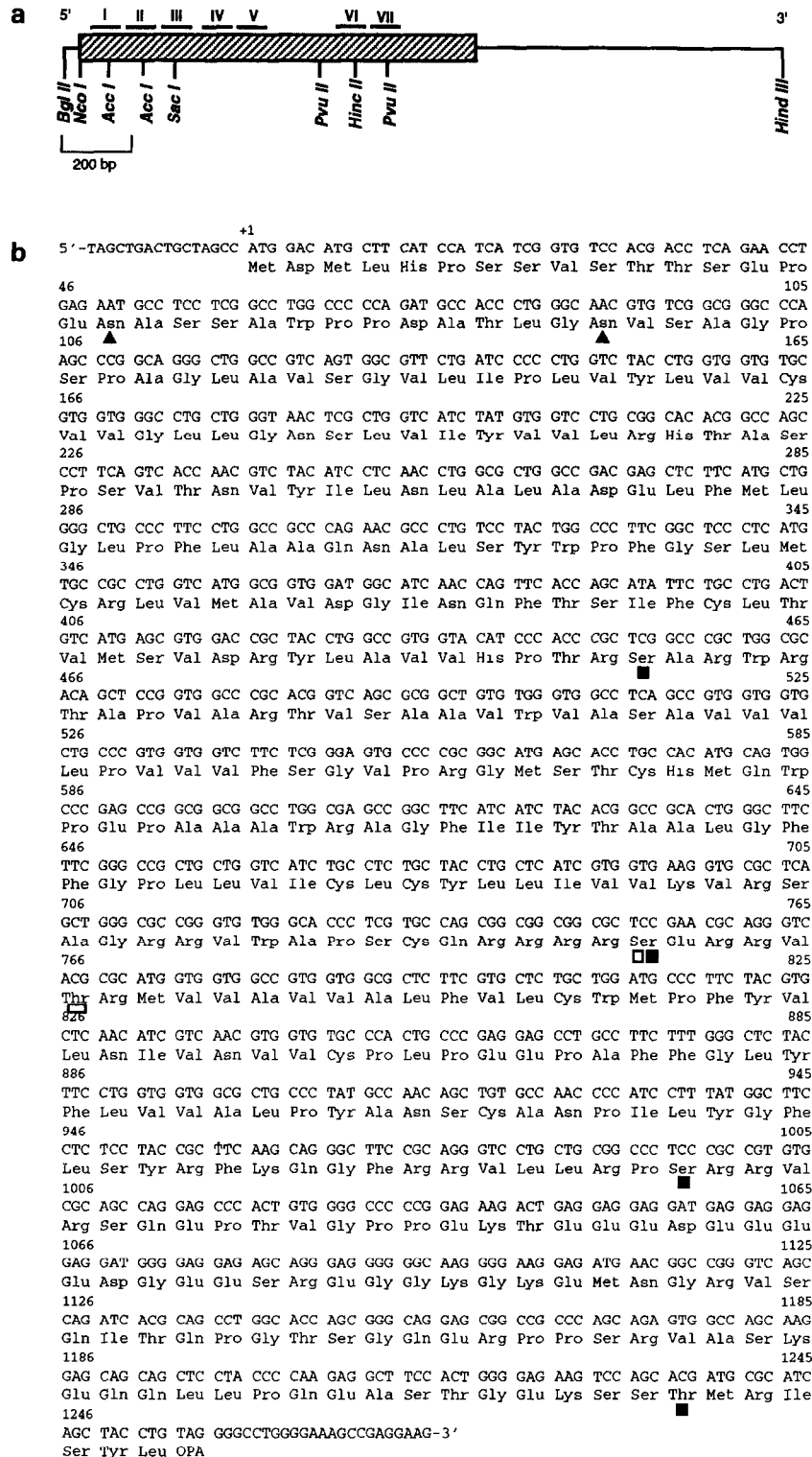
a

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5'   I   II  III  IV  V        VI VII                                    3'

    Bgl II                                              Hind III
    Nco I   Acc I  Acc I  Sac I      Pvu II Hinc II Pvu II

    |_____|
      200 bp
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b

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                                  +1
5'-TAGCTGACTGCTAGCC ATG GAC ATG CTT CAT CCA TCA TCG GTG TCC ACG ACC TCA GAA CCT
                    Met Asp Met Leu His Pro Ser Ser Val Ser Thr Thr Ser Glu Pro
46                                                                           105
GAG AAT GCC TCC TCG GCC TGG CCC CCA GAT GCC ACC CTG GGC AAC GTG TCG GCG GGC CCA
Glu Asn Ala Ser Ser Ala Trp Pro Pro Asp Ala Thr Leu Gly Asn Val Ser Ala Gly Pro
106  ▲                                                   ▲
AGC CCG GCA GGG CTG GCC GTC AGT GGC GTT CTG ATC CCC CTG GTC TAC CTG GTG GTG TGC
Ser Pro Ala Gly Leu Ala Val Ser Gly Val Leu Ile Pro Leu Val Tyr Leu Val Val Cys
166                                                                          225
GTG GTG GGC CTG CTG GGT AAC TCG CTG GTC ATC TAT GTG GTC CTG CGG CAC ACG GCC AGC
Val Val Gly Leu Leu Gly Asn Ser Leu Val Ile Tyr Val Val Leu Arg His Thr Ala Ser
226                                                                          285
CCT TCA GTC ACC AAC GTC TAC ATC CTC AAC CTG GCG CTG GCC GAC GAG CTC TTC ATG CTG
Pro Ser Val Thr Asn Val Tyr Ile Leu Asn Leu Ala Leu Ala Asp Glu Leu Phe Met Leu
286                                                                          345
GGG CTG CCC TTC CTG GCC GCC CAG AAC GCC CTG TCC TAC TGG CCC TTC GGC TCC CTC ATG
Gly Leu Pro Phe Leu Ala Ala Gln Asn Ala Leu Ser Tyr Trp Pro Phe Gly Ser Leu Met
346                                                                          405
TGC CGC CTG GTC ATG GCG GTG GAT GGC ATC AAC CAG TTC ACC AGC ATA TTC TGC CTG ACT
Cys Arg Leu Val Met Ala Val Asp Gly Ile Asn Gln Phe Thr Ser Ile Phe Cys Leu Thr
406                                                                          465
GTC ATG AGC GTG GAC CGC TAC CTG GCC GTG GTA CAT CCC ACC CGC TCG GCC CGC TGG CGC
Val Met Ser Val Asp Arg Tyr Leu Ala Val Val His Pro Thr Arg Ser Ala Arg Trp Arg
466                                                      ■                    525
ACA GCT CCG GTG GCC CGC ACG GTC AGC GCG GCT GTG TGG GTG GCC TCA GCC GTG GTG GTG
Thr Ala Pro Val Ala Arg Thr Val Ser Ala Ala Val Trp Val Ala Ser Ala Val Val Val
526                                                                          585
CTG CCC GTG GTG GTC TTC TCG GGA GTG CCC CGC GGC ATG AGC ACC TGC CAC ATG CAG TGG
Leu Pro Val Val Val Phe Ser Gly Val Pro Arg Gly Met Ser Thr Cys His Met Gln Trp
586                                                                          645
CCC GAG CCG GCG GCG GCC TGG CGA GCC GGC TTC ATC ATC TAC ACG GCC GCA CTG GGC TTC
Pro Glu Pro Ala Ala Ala Trp Arg Ala Gly Phe Ile Ile Tyr Thr Ala Ala Leu Gly Phe
646                                                                          705
TTC GGG CCG CTG CTG GTC ATC TGC CTC TGC TAC CTG CTC ATC GTG GTG AAG GTG CGC TCA
Phe Gly Pro Leu Leu Val Ile Cys Leu Cys Tyr Leu Leu Ile Val Val Lys Val Arg Ser
706                                                                          765
GCT GGG CGC CGG GTG TGG GCA CCC TCG TGC CAG CGG CGG CGG CGC TCC GAA CGC AGG GTC
Ala Gly Arg Arg Val Trp Ala Pro Ser Cys Gln Arg Arg Arg Arg Ser Glu Arg Arg Val
766                                                   □■                      825
ACG CGC ATG GTG GTG GCC GTG GTG GCG CTC TTC GTG CTC TGC TGG ATG CCC TTC TAC GTG
Thr Arg Met Val Val Ala Val Val Ala Leu Phe Val Leu Cys Trp Met Pro Phe Tyr Val
826                                                                          885
CTC AAC ATC GTC AAC GTG GTG TGC CCA CTG CCC GAG GAG CCT GCC TTC TTT GGG CTC TAC
Leu Asn Ile Val Asn Val Val Cys Pro Leu Pro Glu Glu Pro Ala Phe Phe Gly Leu Tyr
886                                                                          945
TTC CTG GTG GTG GCG CTG CCC TAT GCC AAC AGC TGT GCC AAC CCC ATC CTT TAT GGC TTC
Phe Leu Val Val Ala Leu Pro Tyr Ala Asn Ser Cys Ala Asn Pro Ile Leu Tyr Gly Phe
946                                                                         1005
CTC TCC TAC CGC TTC AAG CAG GGC TTC CGC AGG GTC CTG CTG CGG CCC TCC CGC CGT GTG
Leu Ser Tyr Arg Phe Lys Gln Gly Phe Arg Arg Val Leu Leu Arg Pro Ser Arg Arg Val
1006                                                             ■           1065
CGC AGC CAG GAG CCC ACT GTG GGG CCC CCG GAG AAG ACT GAG GAG GAG GAT GAG GAG GAG
Arg Ser Gln Glu Pro Thr Val Gly Pro Pro Glu Lys Thr Glu Glu Glu Asp Glu Glu Glu
1066                                                                        1125
GAG GAT GGG GAG GAG AGC AGG GAG GGG GGC AAG GGG AAG GAG ATG AAC GGC CGG GTC AGC
Glu Asp Gly Glu Glu Ser Arg Glu Gly Gly Lys Gly Lys Glu Met Asn Gly Arg Val Ser
1126                                                                        1185
CAG ATC ACG CAG CCT GGC ACC AGC GGG CAG GAG CGG CCG CCC AGC AGA GTG GCC AGC AAG
Gln Ile Thr Gln Pro Gly Thr Ser Gly Gln Glu Arg Pro Pro Ser Arg Val Ala Ser Lys
1186                                                                        1245
GAG CAG CAG CTC CTA CCC CAA GAG GCT TCC ACT GGG GAG AAG TCC AGC ACG ATG CGC ATC
Glu Gln Gln Leu Leu Pro Gln Glu Ala Ser Thr Gly Glu Lys Ser Ser Thr Met Arg Ile
1246                                                                   ■
AGC TAC CTG TAG GGGCCTGGGGAAAGCCGAGGAAG-3'
Ser Tyr Leu OPA
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Fig. 1. (a) Restriction map of the human somatostatin SSTR3 genomic clone. Relevant endonuclease restriction sites of the 2.1 kb NcoI/HindIII genomic clone are indicated. Hatched areas denote the coding region of clone with the position of putative transmembrane domains underlined and numbered by Roman numerals. (b) Nucleotide and deduced amino acid sequence of the human SSTR3 receptor gene. Nucleotides of JCI 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 are indicated by closed triangles (▲). Potential phosphorylation sites for protein kinase A (cAMP dependent) and protein kinase C are indicated by open (□) and closed (■) squares, respectively.
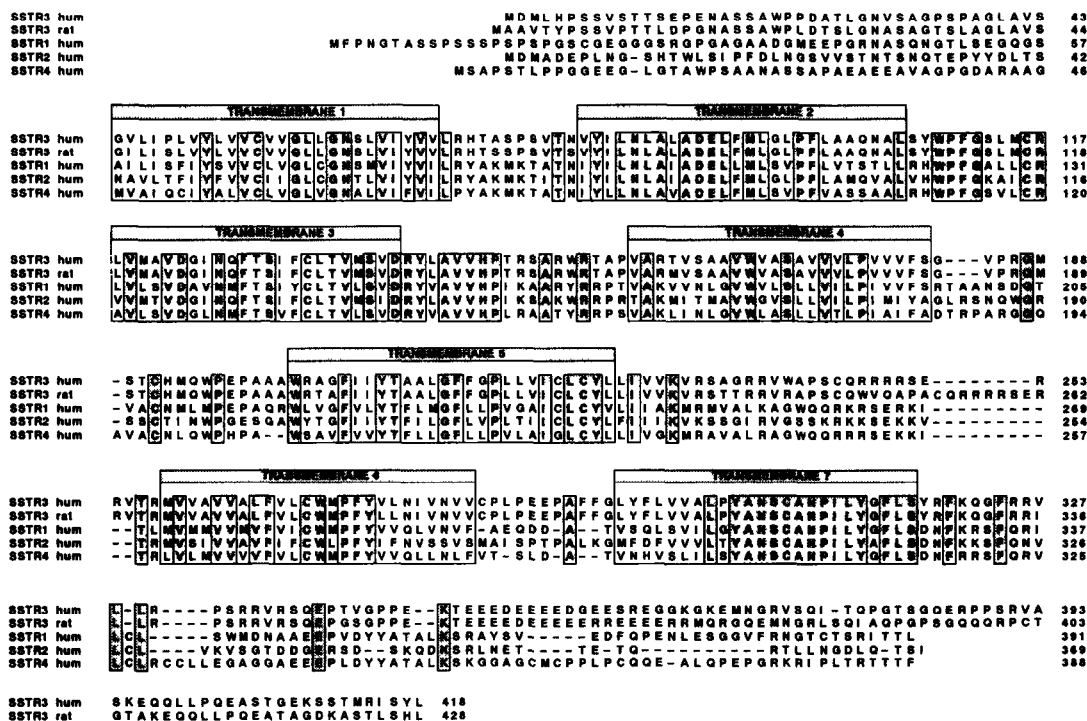
Fig. 2. Alignment of deduced amino acid sequence of the human SSTR3 receptor, and other members of the somatostatin receptor family. Boxed and shaded areas denote the conserved amino acid residues between the human SSTR3 receptor and the human and rat SSTR1, SSTR2 and SSTR4 proteins. Single letter code used to denote amino acids.

tively. As with other members of the human somatostatin receptor gene family, the SSTR3 appears to be intronless within its coding region.

Hydrophobicity analysis of SSTR3 reveals the presence of seven putative transmembrane domains, characteristic of members of the superfamily of receptors which couple to guanine nucleotide binding proteins [18]. As outlined in Fig. 1b, SSTR3 contains consensus sequence for two potential N-linked glycosylation sites in the amino-terminus at Asn^{17} and Asn^{30}. Moreover, five putative phosphorylation sites for cAMP-dependent protein kinase A and protein kinase C were found in the second, third cytoplasmic loops and in the 3' carboxyl terminus. Regions within the second and third cytoplasmic loop have been implicated in G-protein coupling [19,20], while serine and threonine residues within the carboxy tail may act as potential sites for receptor specific kinases [21].

Based on the deduced amino acid sequence, the greatest degree of amino acid homology between SSTR3 and other cloned somatostatin receptors (SSTR1, SSTR2, SSTR4) occur within these putative transmembrane domains (Fig. 2). Within these transmembrane domains, SSTR3 shares 62%, 64% and 58% homology with SSTR1, SSTR2 and SSTR4, respectively. Overall sequence homology between SSTR3 and the human SSTR1 is 41%, 39% with the SSTR2, and 37% with the SSTR4 receptor. Moreover, as shown in Fig. 2, the human SSTR3 is 97% homologous, within putative

transmembrane domains, with the recently cloned rat SSTR3 [13,14] and displays an overall amino acid homology of $\sim$ 80%. It is of interest to note, that a conserved Cys residue which is found within the carboxy tail of most members of the G-linked receptor family, including SSTR1, SSTR2 and SSTR4, and which is a site for palmitoylation [22,23] is absent from both the human and rat SSTR3s. Mutation of this Cys residue, at least for the β adrenergic receptor [23] has been shown to modify receptor G-protein interactions. Moreover, the rat and human SSTR3s also differ within the size of the third cytoplasmic loop; the rat containing 8 additional amino acids. Both rat and human SSTR3s contain stretches of acidic amino acid residues within the COOH tail (AA 349–360), the functional significance of which, if any, is unknown at present. Acidic peptides are, however, substrates for phosphorylation by receptor specific kinases (see [21]).

In order to ascertain the pharmacological nature of this gene product, membranes from COS-7 cells transiently expressing JCI-pCDps clones were measured for the presence of high affinity [^{125}I]LTT SST-28 binding. As depicted in Fig. 3, the expressed receptor bound radioligand in a saturable manner and with high affinity. Scatchard transformation of the binding data of SSTR3 (Fig. 3 inset) revealed a single class of high affinity binding sites, with an estimated K_d of 210 ± 11 pM and a receptor density (B_{max}) of 189 ± 11 fmol/mg protein (n = 3). No specific binding of radioligand was
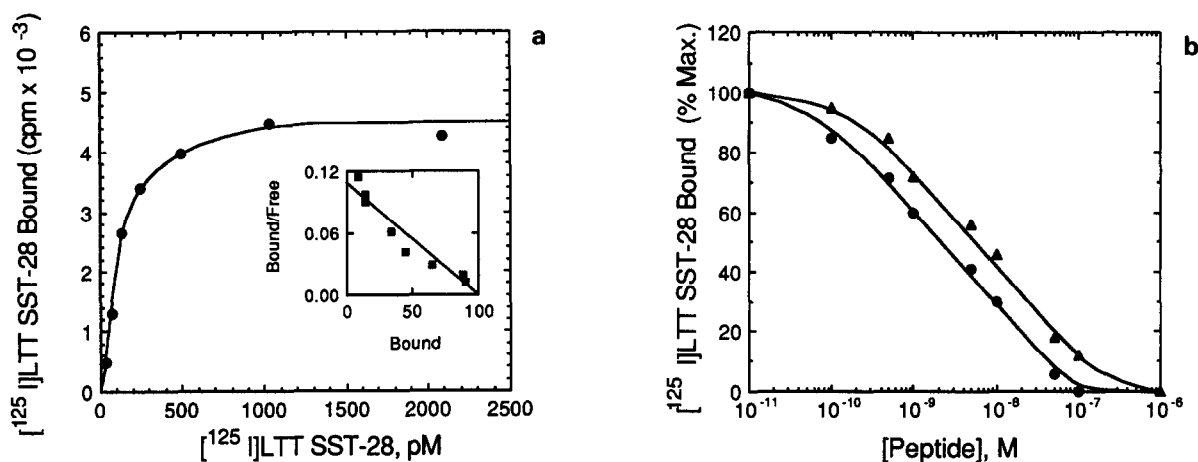
Fig. 3. (a) Saturation isotherms of [^{125}I]LTT SST-28 binding to membranes prepared from COS-7 cells transfected with SSTR3 DNA. Membranes prepared from COS-7 cells transfected with the human SSTR3 gene were incubated with increasing concentrations of radioligand and assayed for receptor binding activity in the absence or presence of 100 nM LTT SST-28 to define non-specific binding as described in section 2. Scatchard analysis of the binding data (shown in inset) revealed a binding capacity (B_{max}) of 189 ± 11 fmol/mg protein and an estimated affinity (K_d) of 210 ± 11 pM for SSTR3. Data represent mean ± S.E.M. of 3 determinations each conducted in duplicate. (b) Pharmacological specificity of the cloned SSTR3. The concentration-dependent competitive inhibition by somatostatin analogs of [^{125}I]LTT SST-28 binding to the expressed SSTR3 revealed binding affinity for SST-14 > SST-28 in each experiment. Membranes were incubated with ~ 60–80 pM [^{125}I]LTT SST-28 and with the indicated concentrations of SST-14 (●) or SST-28 (▲) under equilibrium binding conditions as described in section 2 (n = 3). K_i values are listed in Table I.

observed in nontransfected cells (data not shown). Various peptides (SST-14, SMS 201-995 and [D-Trp^8]SST-14) inhibited [^{125}I]LTT SST-28 binding in a concentration-dependent and uniphasic manner over a concentration range of 10^{-10}–10^{-7} M (Fig. 3b). The relative estimated dissociation [K_i] values for these ligands are listed in Table I and reveal a rank order of potency for the SSTR3 of [D-Trp^8]SST-14 > SST-14 > SMS 201-995 > SST-28.

Pharmacologically, some important differences between the human and rat [13,14] SSTR3 receptors are evident. While the human SSTR3 receptor displays affinities for the SST-14 peptide ~ fourfold higher than SST-28, the rat homolog appears to display 2- to 3-fold higher affinity for SST-28 than SST-14. Thus, the rank order of potency for the human SSTR3 is indicative of

Table I
Relative binding affinities of SS peptides for the cloned human SSTR3

Peptide	K_i (nM)	SSTR3/SSTR4
[D-Trp^8]SST-14	0.56 ± 0.10	1.75
SST-14	2.09 ± 0.45	1.92
SMS 201-995	5.79 ± 1.10	4.26
SST-28	7.94 ± 1.26	3.61

Inhibitory constants (K_i) of various compounds for [^{125}I]LTT SST-28 binding are listed in order of potency for the human SSTR3 receptor. Transfection of COS-7 cells with the SSTR3 receptor gene was performed as described in section 2. Dissociation constants were obtained using the computer program LIGAND. Ratio for estimated K_i values for the cloned human SSTR3 and SSTR4s expressed in COS-7 cells. Values represent the means of three independent experiments each conducted in duplicate.
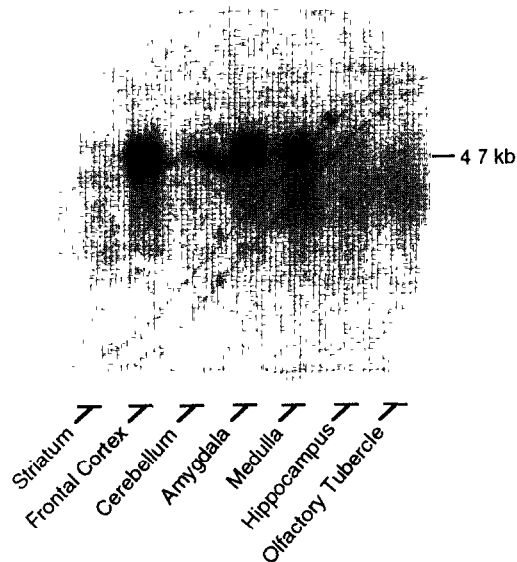
a SST-14 preferring receptor (see above), while the rat SSTR3 displays, with respect to SST-28 and SST-14, an inverted order of potency (SST-28 > SST-14 ⋙ SMS-201-995). At present it is difficult to ascertain whether sequence divergence among the rat and human SSTR3s may be implicated in the maintenance of these pharmacological profiles. The relative affinities for various somatostatinergic agents for SSTR3 are lower, however, than that for the human SSTR4. In any event, the size and sequence of the third cytoplasmic loop of SSTR3 is similar to those of the SSTR1, SSTR2 and SSTR4 receptors, suggesting that it might be coupled to guanine nucleotide binding proteins in a similar manner. Preliminary data appear to indicate that in COS-7 cells the human SSTR3 receptor, as does the rat SSTR3, inhibits forskolin stimulated adenylate cyclase activity [24].

Northern blot analysis of poly(A)$^+$ RNA from various primate brain regions probed with the 2.1 kb NcoI/HindIII of SSTR4 revealed widely divergent distributions. As depicted in Fig. 4, SSTR3 mRNA transcripts of approximately 5.0 kb in length were observed in monkey frontal cortex. Lower levels of mRNA expression were observed in the cerebellum, amygdala and medulla. As depicted in Fig. 4 two SSTR3 mRNA transcripts were evident in these regions and may possibly be associated with multiple transcription or polyadenylation sites for the SSTR3 gene [25]. Little or no SSTR3 mRNA was detected in the monkey striatum, hippocampus, and olfactory tubercle. The brain distribution of the SSTR3 mRNA appears distinct from that of SSTR4, and SSTR2s [13].

PCR amplification of hybrid human–hamster so-

Fig. 4. Northern blot analysis of SSTR3 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 2.1 kb NcoI/HindIII fragment encoding the entire SSTR3 gene. Nylon membranes were exposed for 4 days at −80°C with one intensifying screen.

matic cell lines for SSTR3 showed chromosome 22 to be the only chromosome to which this gene segregates (Fig. 5), as compared to SSTR1, SSTR2 and SSTR4, which were found on chromosomes 14, 17 and 20, respectively (data not shown).

The above findings document the existence of another member of the somatostatin receptor gene family. The cloned human SSTR3 receptor, despite less overall amino acid similarity, is closely related in size, structure and selectivity for SST-14 peptides with other members of the human SSTR family. Whether or not these receptors display distinctive tissue specific patterns of expression, differential regulation and/or coupling to subtype specific membrane signalling pathways remains to be
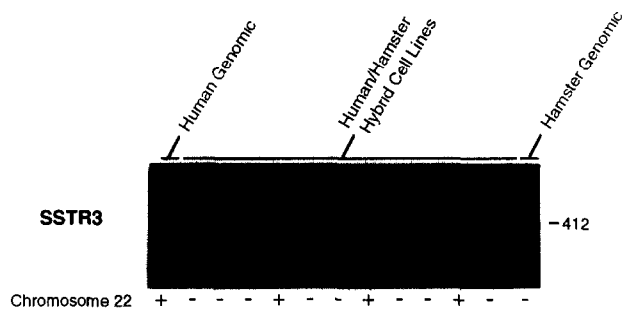
Fig. 5. Chromosomal location of the human SSTR3 receptor gene. Ethidium bromide stained agarose gel of hybrid hamster–human somatic cell lines (BIOS Corp.) analyzed for the presence of SSTR3 receptor DNA using the PCR as described in section 2. Oligonucleotide primers flanking 5′ translated and untranslated sequence of the SSTR3 gene were used to generate an amplified product of approximately 410 bp.

determined. The present study points to a remarkable degree of genetic diversity within the SS-14 receptor gene subclass alone, and predicts an extensive receptor gene family which will need to be fully characterised at the molecular level for understanding the biological functions of the somatostatin receptor systems in health and disease.

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