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Characterization of Cloned Human Somatostatin Receptor SSTR5

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SUMMARY

The recent molecular cloning of the genes encoding six distinct somatostatin (SRIF) receptor subtypes from various species has allowed for the individual expression and characterization of these receptors in mammalian cells. In the present study, we have cloned the human homologue of the SRIF receptor subtype SSTR5 (formerly termed SSTR4) and characterized its pharmacological and functional properties, as well as its distribution. Although there is 80.5% sequence homology between the cloned rat and human SSTR5 receptors, their pharmacological profiles differ. We have labeled both rat and human SSTR5, expressed in Chinese hamster ovary (CHO-K1) cells, with ^{125}I-Tyr^{11}-SRIF and performed inhibition studies using SRIF analogues of differing structures, including cyclic penta-, hexa-, and octapeptide SRIF analogues. Whereas rat SSTR5 bound compounds in all structural classes with high to moderate affinities, human SSTR5 bound most SRIF analogues with much lower affinity, with the exceptions of SRIF, SRIF-28, and L-362,855. Like rat SSTR5, human SSTR5 mediated the inhibition by SRIF of forskolin-stimulated cAMP accumulation. However, the clinically used SRIF analogue SMS 201-995, which potently inhibited cAMP formation via interaction with rat SSTR5, did not inhibit cAMP accumulation in cells expressing human SSTR5. The distribution of expression of human SSTR5 mRNA, as analyzed by reverse transcription-polymerase chain reaction, shows selective expression in small intestine, heart, adrenal, cerebellum, pituitary, placenta, and skeletal muscle but not in kidney, liver, pancreas, uterus, thymus, testis, spleen, lung, thyroid, ovary, or mammary gland. The structural differences between cloned rat and human SSTR5 receptors suggest useful strategies for identifying regions of this receptor subtype that may be involved in ligand binding specificities. Identification of subtype-selective SRIF analogues may lead to more specific pharmacological therapeutic interventions.

SRIF is a 14-amino acid-containing hormone/neurotransmitter that modulates a wide variety of biological processes. SRIF was originally isolated from mammalian hypothalamus and characterized as a potent physiological regulator of GH secretion from the anterior pituitary (1). SRIF was subsequently shown to be broadly, yet discretely, localized throughout the central nervous system, where it acts as a neurotransmitter (2). SRIF is also found in various other tissues including the pancreas and gut, where it regulates multiple physiological processes and the release of endocrine and exocrine secretions (3, 4). At the cellular level, SRIF is an inhibitory regulator of adenylyl cyclase (5-7) and both stimulates and inhibits ionic conductances, through K^+ and Ca^{2+} channels, respectively (8-10). These actions of SRIF are mediated via pertussis toxin-sensitive G proteins. SRIF also regulates the activity of tyrosine phosphatases, the Na^+/H^+ antiport, and cellular proliferation through mechanisms that are insensitive to pertussis toxin (11-12).

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The actions of SRIF are mediated via membrane-bound receptors, of which six distinct SRIF receptor subtypes have been definitively demonstrated by molecular cloning. Yamada *et al.* (13) first reported the cloning of the human and mouse genes encoding two SRIF receptor subtypes (SSTR1 and SSTR2). These receptors are differentially expressed in various tissues and are pharmacologically distinct (14, 15). More recently, a splice variant of SSTR2 has been identified (16) that has ligand specificities similar to those of the unspliced form but, unlike the unspliced form, efficiently couples to adenylyl cyclase in an inhibitory manner (17). Yasuda *et al.* (18) reported the cloning of a third SRIF receptor from mouse, SSTR3. Recently, two additional SRIF receptor subtypes, SSTR4 and SSTR5, were cloned from rat (19, 20). These receptors differ from SSTR1, SSTR2, and SSTR3 in predicted amino acid sequence, tissue distribution, and pharmacological properties (15, 21). In particular, SSTR5 is the only receptor that has higher affinity for SRIF-28 than for SRIF. The cloning of these receptors from other species was subsequently reported (22-28).

ABBREVIATIONS: SRIF, somatostatin; GH, growth hormone; SA, c[Aha-Phe-D-Trp-Lys-Thr(Bzl)]; SSC, standard saline citrate; RT, reverse transcription; PCR, polymerase chain reaction; EGTA, ethylene glycol bis(β-aminoethyl ether)-*N,N,N′,N′*-tetraacetic acid.

Initial characterizations confirmed a unique pharmacological profile for the cloned SRIF receptor subtype rat SSTR5 (19, 21). We have now tested the pharmacological specificities of human SSTR5 with a variety of SRIF analogues that have been widely used both experimentally and clinically. Whereas human SSTR5 exhibits higher affinity for SRIF-28 than for SRIF, as does rat SSTR5, this study demonstrates significant pharmacological differences between rat and human SSTR5. In previous studies we have shown that rat SSTR5 mediates the inhibition of forskolin-stimulated cAMP accumulation (19, 21), and this functional coupling is preserved for human SSTR5. We have also identified the distribution of expression of mRNA encoding this receptor.

Experimental Procedures

Materials. SRIF, SRIF-28, SRIF-28(1–14), [des-Ala^1,des-Gly^2, His^{4,5},D-Trp^8]-SRIF, [D-Trp^8]-SRIF, and SA were obtained from Bachem (Torrance, CA). MK-678, L-362,823, L-362,855, and L-362,862 were the gifts of Dr. D. Veber (Merck, West Point, PA). SMS 201–995 was obtained from Sandoz (Basel, Switzerland). CGP 23996 was the gift of Dr. B. Petrack (Ciba-Geigy, Rahway, NJ). All other peptides were the gifts of Dr. D. Coy (Tulane University, New Orleans, LA) and Biomeasure, Inc. (Hopkinton, MA). ^{125}I-Tyr^{11}-SRIF was obtained from Amersham (Arlington Heights, IL).

Cloning. A 583-base pair *Sma*I-*Pst*I fragment of the rat SSTR5 cDNA, random prime labeled with [α-^{32}P]dCTP (3000 Ci/mmol; NEN), was used to screen a human cosmid library constructed in pWE15 (Stratagene, La Jolla, CA; courtesy of Dr. Tom Bonner, Laboratory of Cell Biology, National Institute of Mental Health, Bethesda, Maryland). Pools of recombinants were screened by Southern blot analysis [hybridization in 3× SSC (20× SSC is 3 M NaCl, 0.3 M sodium citrate, pH 7.0) at 60° and washing in 1× SSC at 60°] (29). One clone (HC1211) was isolated and analyzed by restriction mapping. An ~4-kilobase *Eco*RI insert of this clone, containing the entire human SSTR5 coding sequence, was subcloned into pCDNA1/AMP (Invitrogen). Overlapping fragments were subcloned into M13 and sequenced by the Sanger dideoxynucleotide chain termination method using Sequenase (United States Biochemicals). Plasmid preparations were also prepared and both strands of the double-stranded plasmid templates were sequenced to verify the sequence. Sequence analysis was performed with Genetics Computer Group software (University of Wisconsin). Plasmid DNA (15 μg) and pcDneo (1.5 μg) were co-transfected into CHO-K1 cells (CCL61; American Type Culture Collection), and stable cell lines expressing human SSTR5 were selected as described previously (30).

Radioligand binding. Receptor binding assays with human and rat SSTR5 were performed using membranes from CHO-K1 cells stably expressing these cloned SRIF receptors, as described previously (6). For radioligand binding assays, cells were harvested in 50 mM Tris·HCl, pH 7.8, containing 1 mM EGTA, 5 mM $MgCl_2$, 10 μg/ml leupeptin, 10 μg/ml pepstatin, 200 μg/ml bacitracin, and 0.5 μg/ml aprotinin (buffer 1) and were centrifuged at 24,000 × *g* for 7 min at 4°. The pellet was homogenized in buffer 1 using a Polytron homogenizer (Brinkmann) (setting 2.5, 30 sec). The homogenate was then centrifuged at 48,000 × *g* for 20 min at 4°. The pellet was homogenized in buffer 1 and this membrane preparation was used for the radioligand binding studies. Cell membranes (5–15 μg of protein) were incubated with ^{125}I-Tyr^{11}-SRIF (0.1 nM; specific activity, 2000 Ci/mmol), in a final volume of 200 μl, for 30 min at 25°, in the presence or absence of competing peptides. Nonspecific binding was defined as the radioactivity remaining bound in the presence of 0.1 μM SRIF. For the saturation studies, increasing concentrations of ^{125}I-Tyr^{11}-SRIF (0.01–0.15 nM) were incubated in the presence or absence of 0.1 μM SRIF. The binding reaction was terminated by the addition of ice-cold 50 mM Tris·HCl buffer, pH 7.8, and rapid filtration over Whatman GF/C glass fiber filters. The filters were then washed with 12 ml of ice-cold Tris·HCl buffer and the bound radioactivity was counted in a γ counter (80% efficiency). Data from radioligand binding studies were used to generate inhibition curves. IC_{50} values were obtained from curve-fitting performed by the mathematical modeling program FITCOMP (31), which is available on the National Institutes of Health-sponsored PROPHET system.

Studies examining the abilities of these peptides to inhibit forskolin-stimulated adenylyl cyclase activity were performed as described previously (21). Briefly, cells used for cAMP accumulation studies were subcultured in 12-well culture plates. Culture medium was removed from the wells and replaced with 500 μl of fresh medium containing 0.5 mM isobutylmethylxanthine. Cells were incubated for 20 min at 37°. Medium was then removed and replaced with fresh medium containing 0.5 mM isobutylmethylxanthine, with or without 10 μM forskolin and various concentrations of peptides. Cells were incubated for 30 min at 37°. Medium was then removed, and cells were sonicated in the wells in 500 μl of 1 N HCl and frozen for subsequent determination of cAMP content by radioimmunoassay. Samples were thawed and diluted in cAMP radioimmunoassay buffer before analysis of cAMP content using the commercially available assay kit from NEN/DuPont (Wilmington, DE).

RT-PCR. Human poly(A)$^+$ RNA was obtained from Clontech. Human pituitary total RNA was purified by the RNAstat60 (Tel Test B) method (courtesy of Dr. M. Brownstein, Laboratory of Cell Biology, National Institute of Mental Health, Bethesda, Maryland). Before RT, residual genomic DNA was removed by digestion with RQ DNase (Promega). RQ DNase (5 units) and RNAsin (10 units; Promega) were added to the RNA samples (~1 μg in 40 mM Tris, pH 7.9, 10 mM NaCl, 6 mM $MgCl_2$) and digestion was carried out at 37° for 15 min. Reactions were terminated by phenol extraction and the RNA was precipitated with ethanol and resuspended in 10 μl of distilled water. The DNase-treated RNA was reverse transcribed in a 20-μl reaction volume using random hexamer oligonucleotide primers (100 pmol) and Superscript II reverse transcriptase (100 units; BRL). Corresponding control samples containing no reverse transcriptase were included for each tissue. After an initial incubation for 10 min at room temperature, the reactions were allowed to continue at 42° for 45 min and were then heated to 95° for 5 min. cDNA was stored at −20° and 1-μl aliquots were used for PCR.

PCR was carried out using primers (24-mers and 21-mers, respectively) for human SSTR5 (RT4–1, 5′-GCCACGCAGAACGCCGCGTCCTTC-3′; RT4–2, 5′-CCGCACGCAGCCCACGCGCACGCC-3′) and β-actin (sense, 5′-ATCATGAAGTGTGACGTGGAC-3′; antisense, 5′-AACCGACTGCTGTCACCTTCA-3′) (32). Amplifications for human SSTR5 were carried out in a reaction volume of 50 μl with 50 pmol of each primer, 0.2 mM each of dATP, dCTP, dGTP, and dTTP, and 2.5 units of *Thermus aquaticus* polymerase (Perkin-Elmer, Norwalk, CT). Thirty cycles of 95° for 45 sec and 72° for 1.5 min (one-step annealing and extension) were carried out, followed by a final extension at 72° for 8 min. Amplifications for β-actin were carried out in a reaction volume of 100 μl with 25 pmol of each primer. Twenty-five cycles of 95° for 45 sec, 60° for 1 min, and 72° for 30 sec were performed. Products were analyzed on 1.6% agarose gels containing 1 μg/ml ethidium bromide. For human SSTR5, the gels were then Southern blotted and hybridized with a 48-base pair oligodeoxynucleotide probe directed at an internal sequence between the human SSTR5 PCR primers. This probe (h4A, 5′-CCTGGCAGTGGTGCACCCGCTGAGCTCGGCCCGCTGGCGCCGCCCGCG-3′) was tailed on the 3′ end with terminal deoxynucleotidyl transferase (Boehringer Mannheim) and [α-^{32}P]dATP (3000 Ci/mmol; NEN). The blots were hybridized for 16–18 hr in 1× SSC, 2× Denhardt's solution (0.1% bovine serum albumin, 0.1% Ficoll 400, 0.1% polyvinylpyrrolidone), at 60° with 2 × 10^6 cpm/ml h4A probe and were washed for 20 min at 60° in 1× SSC/0.1% sodium dodecyl sulfate, followed by 3 × 20 min at 60° in 0.5× SSC/0.1% sodium dodecyl sulfate. Blots were exposed to Kodak XAR film with intensifying screens at −80° for the times indicated in

the figure legends. For β-actin, the product was viewed on a 300-nm UV transilluminator.

Results

Using a *Sma*I-*Pst*I fragment of the rat SSTR5 as a probe, the human homologue of this receptor was cloned from a human cosmid library. The nucleotide and deduced amino acid sequences of clone HC1211, the human SSTR5 subtype, are identical to those of the SRIF receptor subtype recently published by Yamada *et al.* (33). There is 52, 53, 45, and 49% sequence homology between human SSTR5 and the other cloned SRIF receptor subtypes, i.e., human SSTR2, human SSTR3, human SSTR1, and human SSTR4, respectively. Within the putative membrane-spanning domains of human SSTR5 there are only three nonconservative (Glu^{87} to valine, Leu^{102} to alanine, and Val^{163} to alanine) and three conservative (Val^{118} to leucine, Val^{170} to methionine, and Ile^{302} to valine) substitutions in amino acids that are found in all other four human SSTR subtypes. There is 80.5% sequence homology between human and rat SSTR5. The area of highest sequence homology between the two receptors resides in the putative transmembrane domains and the areas of least homology are found at the extracellular amino terminus and the intracellular carboxyl terminus. A comparison of the deduced amino acid sequences for these two receptors is shown in Fig. 1.

To compare the pharmacological profiles of the recently cloned rat and human SRIF receptor SSTR5, we tested a variety of analogues of SRIF for binding to these receptors. The genes encoding these receptor subtypes were stably expressed in CHO-K1 cells as described previously (19, 30). SRIF receptor subtypes were labeled with ^{125}I-Tyr^{11}-SRIF. The binding of this radioligand to each receptor was of high affinity and saturable (Fig. 2). Scatchard analysis of saturation experiments showed that the K_d for ^{125}I-Tyr^{11}-SRIF binding to human SSTR5 expressed in CHO-K1 cells was 0.11 nM, with a B_{max} value of 196 fmol/mg of protein. Similar analysis of binding studies with rat SSTR5 revealed a K_d of 0.09 nM, with a B_{max} value of 593 fmol/mg of protein. All data were best fit by a single-site analysis. No specific binding was detectable in untransfected CHO-K1 cells.

We next performed inhibition studies to compare the pharmacology of rat and human SSTR5. The binding of ^{125}I-Tyr^{11}-SRIF to rat and human SSTR5 was inhibited with various concentrations of SRIF analogues of vastly differing structures (15, 21). As shown in Table 1, SRIF potently inhibited radioligand binding to both rat and human SSTR5, with IC_{50} values of 0.29 and 0.16 nM, respectively. SRIF-28 was more potent than SRIF at both of these receptors. Likewise, BIM 23003, a 12-amino acid-containing analogue of SRIF, bound to both receptors with high affinity. The hexapeptide analogues MK-678 and BIM 23027 bound to rat SSTR5 with high affinities but bound to human SSTR5 with 10–100-fold lower affinities.

Octapeptide analogues, such as the clinically used SMS 201–995, were also tested for binding affinity for rat and human SSTR5. As shown in Table 1, octapeptide analogues inhibited ^{125}I-Tyr^{11}-SRIF binding to rat SSTR5 with IC_{50} values in the subnanomolar to low nanomolar range. These same analogues bound with lower affinities to the human homologue of this receptor subtype, with most displaying at least 100-fold lower affinity for human SSTR5 than for rat SSTR5. Smaller CGP

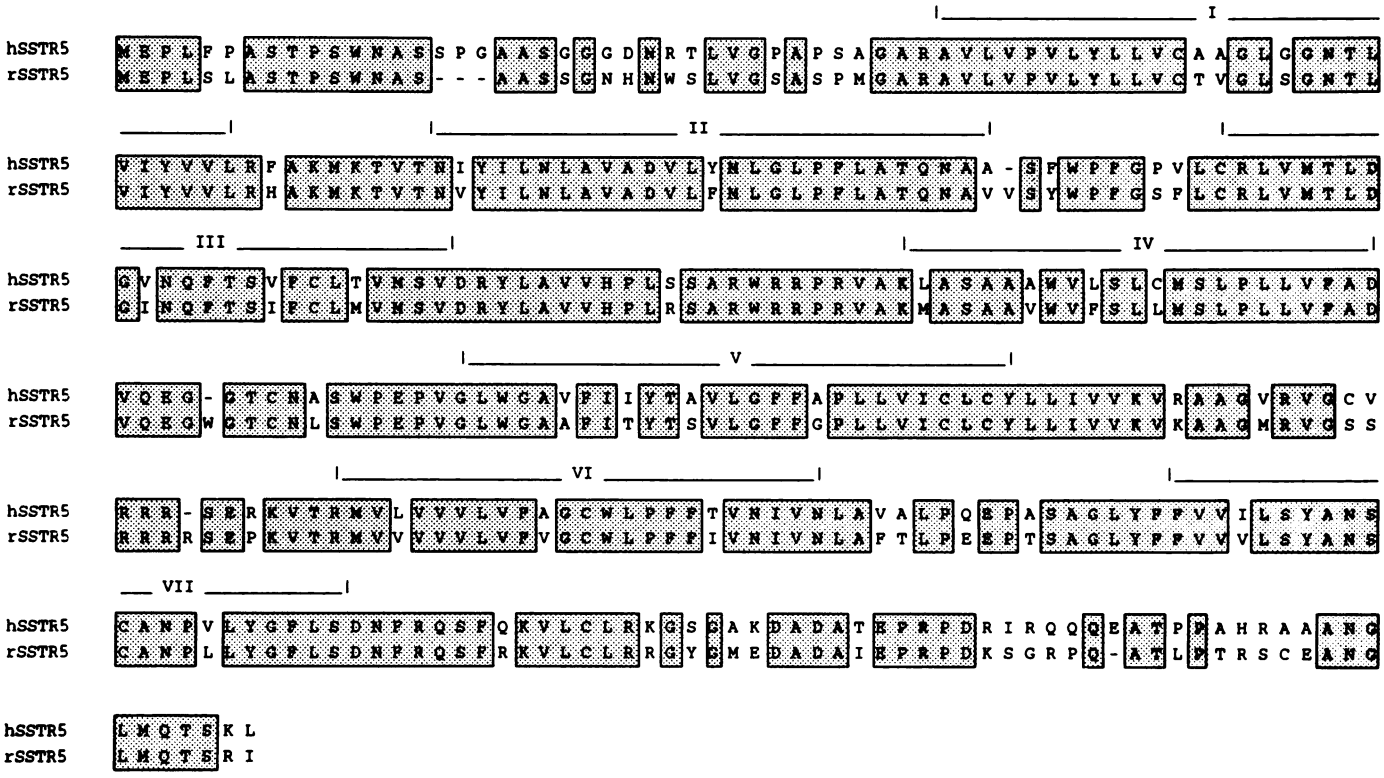

Fig. 1. Comparison of the deduced amino acid sequence of human SSTR5 with that of the previously characterized rat SSTR5. *Dashes*, gaps introduced to maximally align sequences. Primary sequence identities are *boxed* and *shaded*. The proposed seven membrane-spanning hydrophobic sequences (*I-VII*) are indicated by *solid lines*.

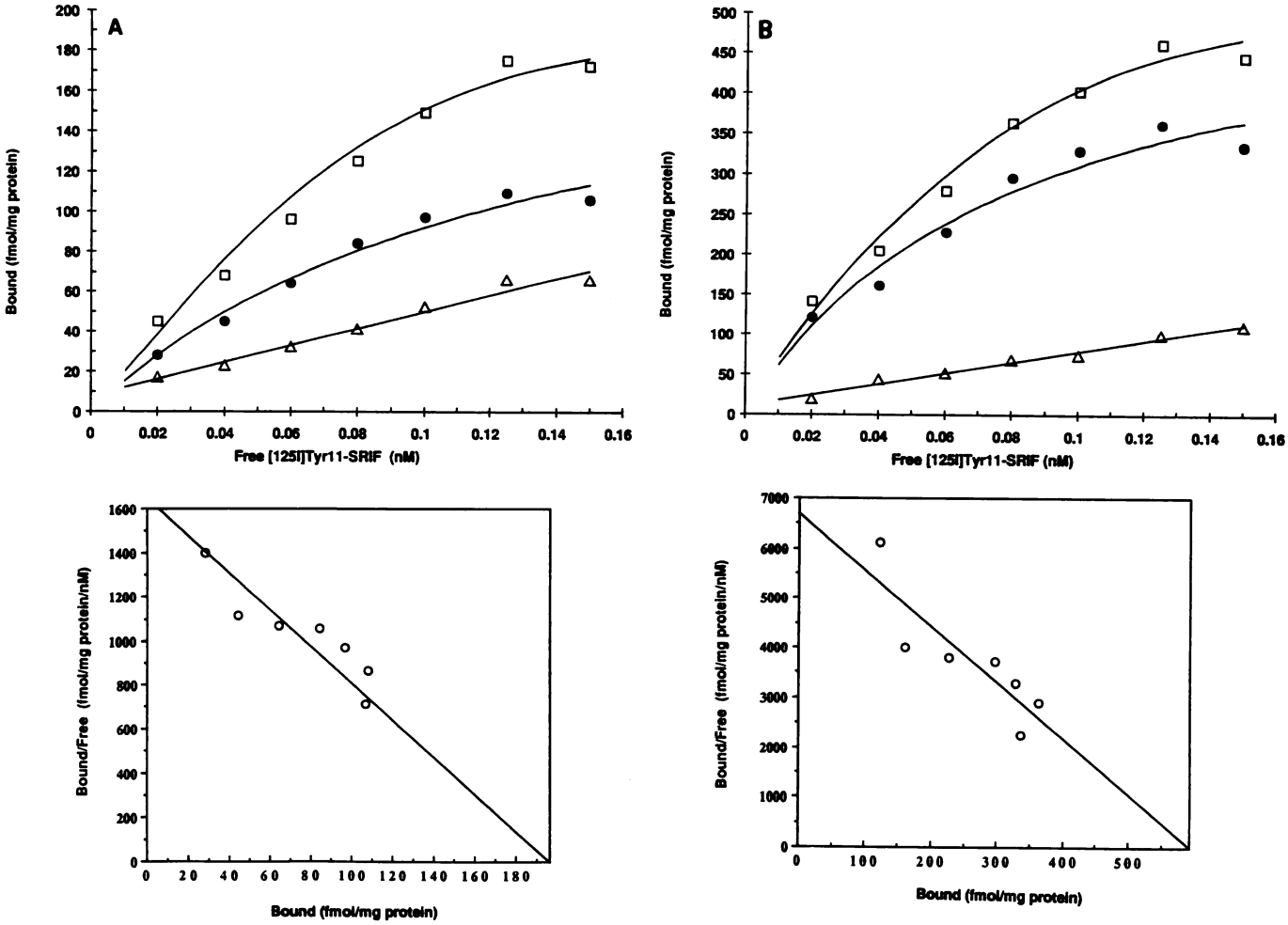

Fig. 2. Saturable binding of ^{125}I-Tyr^{11}-SRIF to the cloned human SSTR5 and rat SSTR5 receptors. Membranes from CHO-K1 cells stably expressing the cloned human SSTR5 receptor (A) or the cloned rat SSTR5 receptor (B) were incubated for 30 min at 25° with increasing concentrations of ^{125}I-Tyr^{11}-SRIF, in the presence (△) or absence (□) of 0.1 μM SRIF, to determine specific binding (●). *Upper*, saturation isotherms of representative experiments; *lower*, linearization of the saturation isotherm data. Analysis of the saturable binding to human SSTR5 revealed that ^{125}I-Tyr^{11}-SRIF bound to a single site, with a K_d of 0.11 nM and a B_{max} of 196 fmol/mg of protein. Analysis of the saturable binding to rat SSTR5 receptor revealed that ^{125}I-Tyr^{11}-SRIF bound to a single site, with a K_d of 0.09 nM and a B_{max} of 593 fmol/mg of protein. Experiments were conducted in triplicate and the results of two or three independent experiments were similar.

23996-like analogues, such as SA and the structurally related pentapeptide c[Aha-Phe-D-Trp-Lys-Ser(Bzl)], also interacted with rat SSTR5 with higher affinities than for human SSTR5, albeit with moderate to low affinities at both receptors. The hexapeptide analogue L-362,862, cyclized via a carbon bridging, interacted with high affinities with both rat and human SSTR5, being somewhat more potent at the former. In contrast, L-362,855, which differs from L-362,862 by only one amino acid, demonstrated higher affinity for human SSTR5 than for rat SSTR5, being the only compound other than SRIF to bind with higher affinity to human SSTR5 than to rat SSTR5. The linear compound BIM 23052 displayed approximately 1000-fold lower affinity for human SSTR5 than for rat SSTR5.

To determine whether human SSTR5 mediates the inhibition of adenylyl cyclase activity, as we have previously shown for rat SSTR5, we tested the effects of SRIF and SMS 201–995 on forskolin-stimulated (10 μM) cAMP accumulation in cells expressing human and rat SSTR5. SRIF and SMS 201–995 maximally inhibited forskolin-stimulated cAMP accumulation in CHO-K1 cells expressing rat SSTR5 by 89 ± 7%, with EC_{50} values of 12 and 84 nM, respectively (Fig. 3A). In CHO-K1 cells expressing human SSTR5, SRIF maximally inhibited forskolin-stimulated cAMP accumulation by 82 ± 1%, with a potency of 2.1 nM (Fig. 3B). In contrast, SMS 201–995 (Fig. 3B) did not significantly inhibit cAMP accumulation in cells expressing human SSTR5, in accordance with its lower affinity as determined in radioligand binding studies.

Previous studies by Yamada *et al.* (33) were unable to detect human SSTR5 mRNA in human tissues by RNA blotting because of the low abundance of this mRNA. Therefore, RT-PCR, using subtype-specific primers, was used to characterize the distribution of mRNA encoding human SSTR5 in human tissues, because this is a more sensitive approach to detect mRNA. A 2-hr exposure of PCR blots probed with the human SSTR5-specific 48-mer oligodeoxynucleotide showed that mRNA for human SSTR5 was present in the heart, small intestine, adrenal, pituitary, and cerebellum (Fig. 4A), whereas 1-week exposure additionally detected mRNA expression in skeletal muscle and placenta (data not shown). No expression was detected in fetal or adult kidney, liver, pancreas, uterus,

TABLE 1

Potencies (mean ± standard error) of SRIF analogues to inhibit ^{125}I-Tyr^{11}-SRIF binding to cloned rat and human SRIF receptor subtype SSTR5

Peptide	IC_{50}	
	Rat SSTR5	Human SSTR5
	nM	
SRIF	0.29 ± 0.04	0.16 ± 0.03
SRIF-28	0.05 ± 0.009	0.05 ± 0.01
BIM 23003	1.8 ± 0.13	2.9 ± 0.31
Hexapeptides		
BIM 23027	2.2 ± 0.30	176 ± 31
MK-678	1.3 ± 0.25	23 ± 7
Octapeptides		
SMS 201-995	0.20 ± 0.01	32 ± 3.9
BIM 23014	0.51 ± 0.11	14 ± 1.5
BIM 23023	1.3 ± 0.26	28 ± 3.4
BIM 23034	0.48 ± 0.07	437 ± 78
BIM 23059	3.2 ± 0.38	38 ± 5.8
BIM 23060	1.3 ± 0.10	41 ± 7.4
L-362,823	3.0 ± 0.55	273 ± 33
CGP 23996-like peptides		
SA	42 ± 8	757 ± 181
c[Aha-Phe-D-Trp-Lys-Ser(Bzl)]	103 ± 13	301 ± 17
L-362,862	3.8 ± 0.5	17 ± 2.3
L-362,855	0.27 ± 0.05	0.016 ± 0.007
Linear peptide		
BIM 23052	0.0037 ± 0.0005	3.6 ± 0.8

testis, spleen, lung, thyroid, or ovary with up to a 3-week exposure. Corresponding control samples subjected to PCR without prior RT yielded no detectable levels of human SSTR5 (data not shown). β-Actin mRNA was also amplified from the same cDNA samples and was present in all samples tested (Fig. 4B).

Discussion

In the present study, we describe the cloning and characterization of a human homologue of rat SSTR5. Although the human homologue is only 80.5% identical, it has higher affinity for SRIF-28 than SRIF, which is a unique characteristic of SSTR5 among all other receptors. Furthermore, human SSTR5 mRNA, like rat SSTR5 mRNA, is expressed in the pituitary. The results of this study show that comparison of the pharmacological specificities of the cloned SRIF receptor subtype SSTR5 from rat and human reveals surprising and important differences and these differences are reflected functionally. The original postulation of the existence of subtypes of SRIF receptors was based on pharmacological studies identifying SRIF receptors that are differentially sensitive to the native peptides SRIF and SRIF-28 (34, 35). SSTR1, SSTR2, and SSTR3 each possessed relatively similar affinities for SRIF and SRIF-28, indicating that none is the "SRIF-28-preferring receptor." In contrast, rat SSTR5 possesses higher affinity for SRIF-28 than for SRIF, and this pharmacological difference is preserved in its human homologue. However, important pharmacological differences are demonstrated between these clones. Specifically, clinically used octapeptide analogues such as SMS 201–995 (Sandostatin) and BIM 23014, which display high affinity for rat SSTR5, show much lower affinities for its human homologue. In addition, conformationally constrained hexapeptide analogues such as MK-678 also bind to human SSTR5 with lower affinities. We had previously shown that the linear compound BIM 23052 and the CGP 23996-like compounds SA and its analogues bind selectively to rat SSTR5 versus human SSTR1, mouse SSTR2, mouse SSTR3, and human SSTR4. These analogues bind with lower affinities to human SSTR5 than to rat SSTR5, suggesting that they may not be selective agents for targeting SSTR5-mediated processes clinically. We previously found L-362,855 to be >10,000-fold selective for rat SSTR5, and the higher potency of this compound for human SSTR5 further supports the selectivity of this compound for SSTR5 in both humans and rodents. Because of this high specificity, we propose that L-362,855 be considered the prototypical SSTR5-specific ligand.

Comparison of the affinities of rat and human SSTR5 for the rigid cyclic SRIF analogues L-362,855 and L-362,862 revealed that L-362,862 bound to both receptors with similar potencies, whereas L-362,855 was more potent at human SSTR5. In fact, L362,855 was >1000-fold more potent than L-362,862 at human SSTR5 but only 14-fold more potent at rat SSTR5. These peptides differ by only one amino acid residue, with the tryptophan of L-362,855 being replaced by a *p*-chlorophenylalanine in L-362,862. This major potency difference between such structurally related peptides suggests that the tryptophan is essential for maintaining high potency of SRIF ligands for SSTR5. Further investigations of the nature of the structural requirements for SSTR5 selectivity may be useful in the development of more specific SSTR5 agonists and antagonists.

As further characterization of the human SSTR5, the tissue distribution of its corresponding mRNA was investigated. Analysis by RT-PCR demonstrated a broad distribution of expression of human SSTR5 mRNA, which was observed in heart, small intestine, adrenal, cerebellum, skeletal muscle, and placenta. However, human SSTR5 mRNA was expressed in pituitary, as is true of rat SSTR5 mRNA. Although studies on the distribution of the other human SRIF receptor subtypes did not use the range of tissues or the method used in this study, the pattern of human SSTR5 mRNA expression differs from that of human SSTR2 (present in the kidney), human SSTR3 (present in the pancreas), and human SSTR4 (present in the lung) (13, 26, 28). Yamada *et al.* (33) recently reported the cloning of the human homologue of rat SSTR5 but were unable to detect mRNA for this receptor in human tissues, including liver, kidney, gastrointestinal tract, and placenta, by Northern analysis. This lack of detection is likely due to low abundance of SSTR5 mRNA in human tissues.

Although species-specific differences in SRIF receptor mRNA distribution exist, it is interesting to note the presence of human SSTR5 mRNA in the human heart, because it has been suggested that a SRIF-28-preferring receptor may mediate negative ionotrophy in the guinea pig atrium (36). Furthermore, previous studies have shown that SRIF-28 is significantly more potent than SRIF in blocking insulin release, which is characteristic of an SSTR5-mediated mechanism (3). Rossowski and Coy (37) have shown that SSTR5-selective agonists inhibit insulin release *in vivo* in rats as potently as does SRIF. In contrast, SSTR2 and SSTR3 agonists were either much less potent or inactive at blocking insulin release. The lack of detectable SSTR5 mRNA in pancreas may be due to the selective expression of SSTR5 mRNA in a small portion of the pancreas, the islets, which could be undetectable when the entire pancreas is measured. Our pharmacological studies suggest that the high selectivity of L-362,855 for the human SSTR5

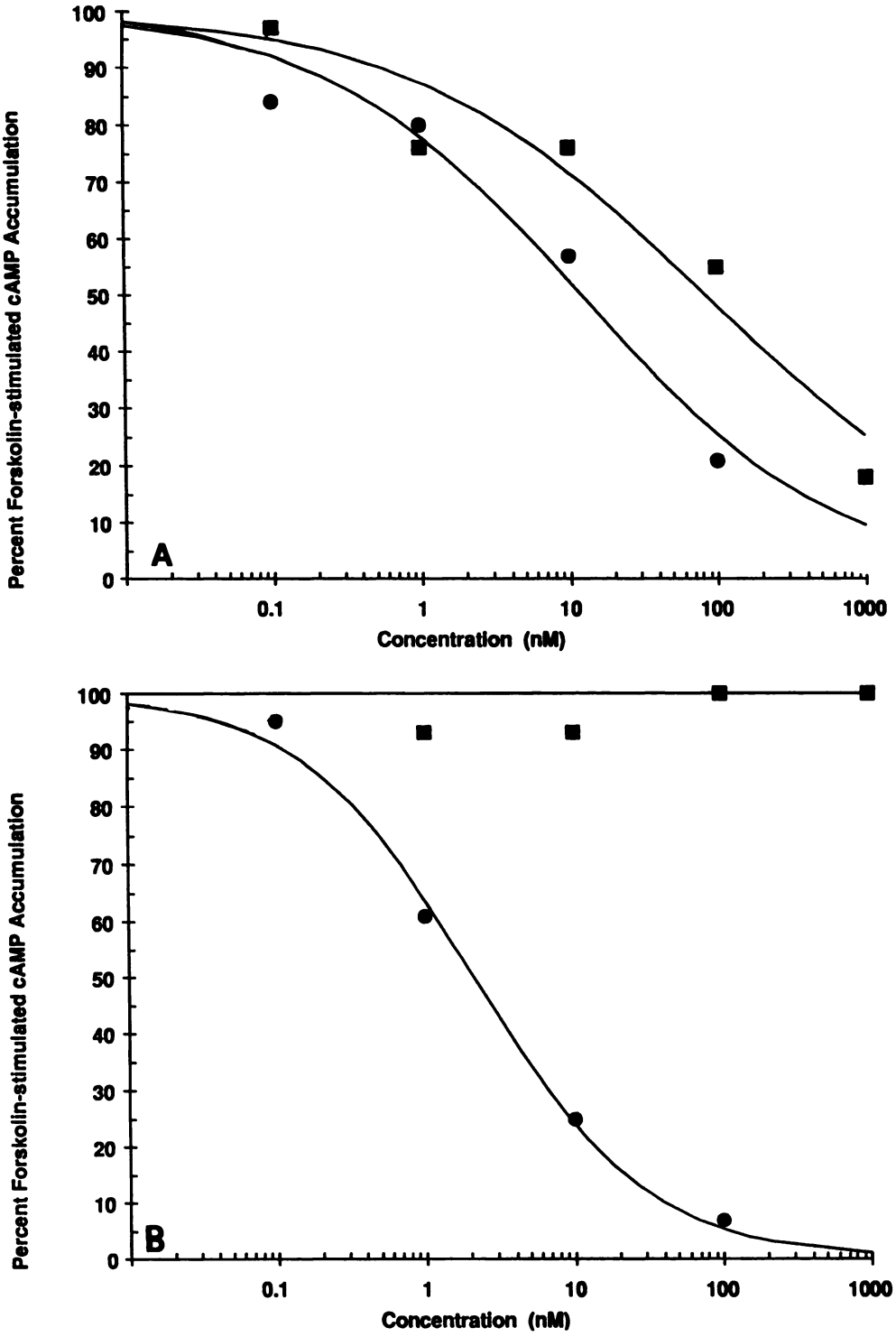

Fig. 3. Effects of various concentrations of SRIF and SMS 201–995 on forskolin-stimulated cAMP accumulation in cells expressing rat or human SSTR5. Forskolin-stimulated (10 μM) cAMP accumulation in CHO-K1 cells stably expressing rat SSTR5 (A) or human SSTR5 (B) was tested in the presence of various concentrations of SRIF (●) or SMS 201–995 (■), as described in Experimental Procedures. SRIF inhibited cAMP accumulation via rat and human SSTR5 receptors with EC_{50} values of 12 and 2.1 nM, respectively. SMS 201–995 dose-dependently inhibited cAMP accumulation in cells expressing rat SSTR5, with an EC_{50} value of 84 nM, but was without effect on cells expressing human SSTR5. Data are the means of three independent experiments.

receptor might render it a useful drug for the production of negative ionotropic effects or in the treatment of hyperinsulinemia.

The presence of human SSTR5 mRNA in the pituitary, in addition to its preferential affinity for SRIF-28 over SRIF-14, suggests that it may be the "SRIF-28 receptor" described in pituitary membrane preparations (for review, see Ref. 38). Human SSTR5 is unlikely, however, to mediate the inhibition of GH release from somatotrophs. Correlational analysis has shown previously that the affinities of various SRIF analogues

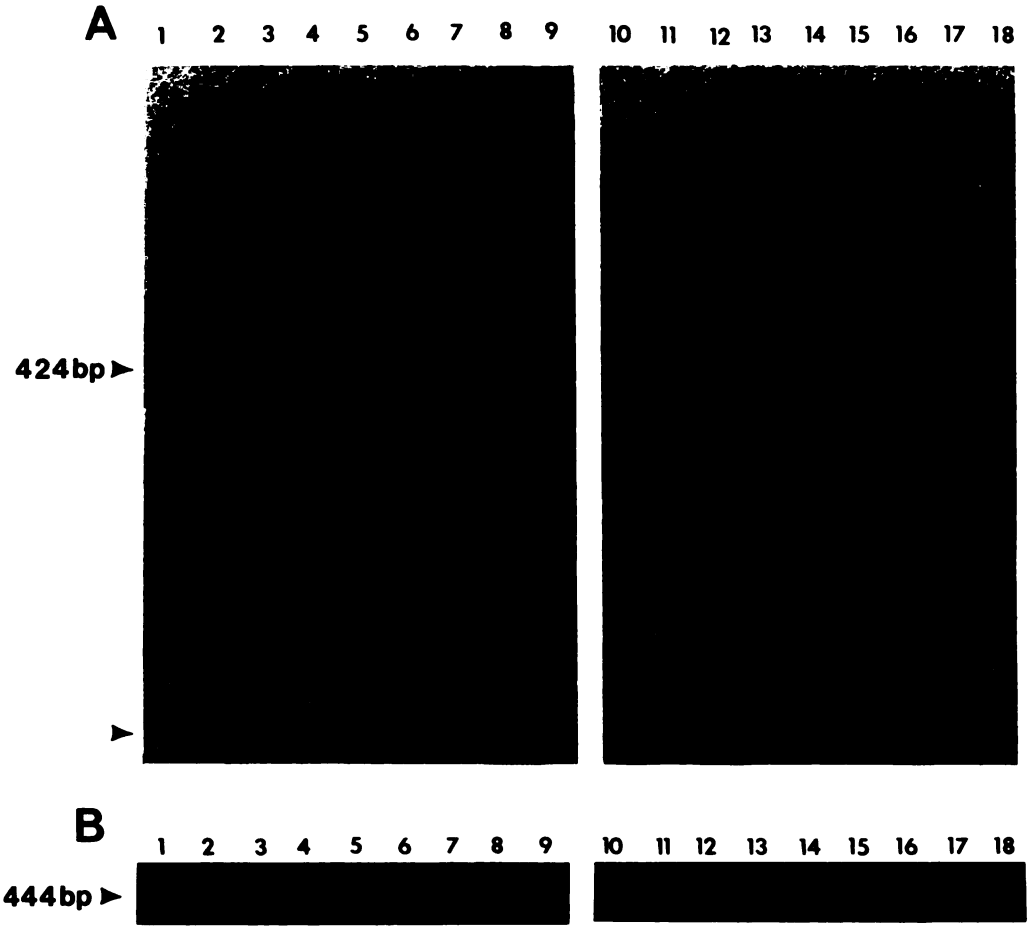

Fig. 4. Tissue distribution of human SSTR5. RT-PCR analysis was performed with primers specific for the human SSTR5 receptor (primers RT4–1 and RT4–2; see Experimental Procedures) (A) and β-actin (sense and antisense primers; see Experimental Procedures) (B). PCR products were separated on 1.6% agarose gels. A, For human SSTR5 the gels were Southern blotted and hybridized with a [α-^{32}P]dATP-labeled oligodeoxynucleotide probe directed at an internal sequence between the human SSTR5 PCR primers. The expected size of the PCR product was 424 base pairs (*arrowhead*). The human RNA samples [all poly(A)$^+$ RNA except for pituitary, which was total RNA] used were from heart (*lane 1*), kidney (*lane 2*), fetal kidney (*lane 3*), liver (*lane 4*), fetal liver (*lane 5*), skeletal muscle (*lane 6*), pancreas (*lane 7*), small intestine (*lane 8*), uterus (*lane 9*), adrenal (*lane 10*), testis (*lane 11*), pituitary (*lane 12*), spleen (*lane 13*), lung (*lane 14*), placenta (*lane 15*), cerebellum (*lane 16*), thyroid (*lane 17*), and ovary (*lane 18*). Exposure against Kodak XAR film for 2 hr (*top*) or 16 hr (*bottom*) at −80° is shown. B, For β-actin the ethidium bromide-stained gels are shown. The expected size of the PCR product was 444 base pairs (*arrowhead*). *Lanes 1-18* are as for human SSTR5.

to inhibit binding to SSTR2 but not to SSTR1, SSTR3, SSTR4, or SSTR5 are highly correlated with the affinities of these compounds to inhibit GH release *in vitro* (15, 21). The pharmacological differences between rat SSTR5 and human SSTR5 do not alter this hypothesis, because compounds that are potent inhibitors of GH release, such as MK-678 and the octapeptide analogues, do not bind potently to human SSTR5 and, conversely, compounds that bind potently to human SSTR5, such as L-362,855 and BIM 23052, are relatively less potent in GH inhibition.

The results of the present study indicate that important pharmacological differences exist between species with regard to the SRIF receptor subtype SSTR5. This is the first clear demonstration of species variations in the pharmacological properties of a cloned SRIF receptor. Extracellular regions of these receptors that may contain the ligand binding domains diverge in predicted amino acid sequence, in particular in the extracellular amino terminus, which is likely to be the underlying structural basis for the pharmacological specificities observed with rat and human SSTR5. Studies are underway to test this hypothesis and to further delineate regions of the receptor involved in ligand binding. These results, coupled with more extensive pharmacological analyses of the other cloned human SRIF receptor subtypes, will provide structural information that should be useful in the rational design of compounds with even greater experimental and clinical specificities. Identification of such compounds, coupled with the knowledge of the regional distribution of the expression of these receptor subtypes in human tissues, may lead to more specific compounds for experimental and therapeutic purposes.

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