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Characterization of Cloned Somatostatin Receptors SSTR4 and SSTR5

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SUMMARY

The recent molecular cloning of the genes and cDNAs encoding multiple somatostatin (SRIF) receptor subtypes has allowed for the individual expression of these receptors in mammalian cells and characterization of their respective pharmacological profiles. Previously, we fully described and compared the pharmacological properties of the first three SRIF receptor subtypes, SRIF receptor type (SSTR)1, SSTR2, and SSTR3. In the present study, we have investigated the properties of the newly cloned SRIF receptor subtypes SSTR4 and SSTR5 with regard to pharmacological profiles, the regulation of high affinity agonist binding to these receptors by stable GTP analogues, Na^+, or prior exposure to agonists, and the inhibition of forskolin-stimulated cAMP accumulation mediated by these receptors. We labeled SSTR4 and SSTR5 expressed in Chinese hamster ovary (CHO-K1) and COS-1 cells, respectively, with the metabolically stable SRIF analogue ^{125}I-CGP 23996. Radioligand binding competition studies were performed using SRIF analogues of differing structures, including hexapeptide analogues similar to MK-678, octapeptide analogues similar to SMS 201-995, pentapeptide analogues similar to c[Ahep-Phe-D-Trp-Lys-Thr(Bzl)], and linear SRIF analogues. SSTR4 bound compounds in all structural classes with high to moderate affinities, and several compounds were identified that are >100-fold selective for SSTR4, compared with the other cloned SRIF receptors, including the linear SRIF analogue BIM-23052 and the CGP 23996-like SRIF analogue L-362,855. In contrast, SSTR5 bound very few SRIF analogues with high affinity. Both receptors could be regulated by prior exposure to agonist. In addition, agonist binding to SSTR4 was reduced by stable GTP analogues, Na^+, and pertussis toxin, but agonist binding to SSTR5 was not affected by these treatments. SSTR4 is efficiently coupled to the inhibition of adenylyl cyclase activity, whereas SSTR5 appears not to couple to this cellular effector system. Such differences between the cloned SRIF receptors provide useful strategies for identifying regions of these receptor subtypes that may be involved in ligand-binding specificities and G protein and cellular effector system coupling. The identification of subtype-selective SRIF analogues may lead to more specific therapeutic interventions.

SRIF is a cyclic tetradecapeptide hormone/neurotransmitter that mediates multiple 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 has been subsequently localized throughout the central nervous system, where it acts as a neurotransmitter (2). In the central nervous system, SRIF has been shown to both positively and negatively regulate neuronal firing (3, 4), to affect the release of other neurotransmitters (5, 6), and to modulate motor activity and cognitive processes (7-9). SRIF is also found in various other tissues including the pancreas and gut, where it regulates multiple physiological processes and endocrine and exocrine secretions (10, 11).

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SRIF affects multiple cellular processes. Previous studies have shown that SRIF is an inhibitory regulator of adenylyl cyclase in different tissues (12-14). SRIF also regulates the conductance of ionic channels, including both K^+ and Ca^{2+} channels (15-17). 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 pertussis toxin-insensitive mechanisms (18-20).

The actions of SRIF are mediated via membrane-bound receptors. The existence of several different SRIF receptors has been shown in pharmacological, biochemical, and functional studies using a variety of SRIF analogues. The fact that multiple SRIF receptor subtypes exist has now been definitively demonstrated by molecular cloning. Yamada *et al.* (21) first reported the cloning of the human and mouse genes encoding

ABBREVIATIONS: SRIF, somatostatin; CHO, Chinese hamster ovary; GH, growth hormone; NMDG, *N*-methyl-D-glucamine; SA, c[Ahep-Phe-D-Trp-Lys-Thr(Bzl)]; EGTA, ethylene glycol bis(β-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid; SSTR1-5, somatostatin receptor types 1-5.

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two SRIF receptor subtypes, SSTR1 and SSTR2, which are differentially expressed in various tissues. Characterization of the pharmacological properties of these receptors indicates that they are pharmacologically distinct (22, 23) and correspond to two SRIF receptors, $SRIF_2$ and $SRIF_1$, respectively, that we previously characterized in rat brain (8, 14, 24, 25). Yasuda *et al.* (26) reported the cloning of a third SRIF receptor, SSTR3. Subsequent reports of the molecular cloning of the genes encoding these receptors in other species have now appeared (27–33). We previously characterized the pharmacological specificities, regulation, G protein coupling, and effector system coupling of SSTR1, SSTR2, and SSTR3 (22, 23, 26).

Recently reported has been the molecular cloning of two more SRIF receptor subtypes, SSTR4 and SSTR5, which differ from SSTR1, SSTR2, and SSTR3 in predicted amino acid sequence, tissue distribution, and pharmacological properties. O'Carroll *et al.* (34) have cloned rat SSTR4, and Bruno *et al.* (35) have cloned rat SSTR5. Rohrer *et al.* (36) have recently reported the cloning of human SSTR5. Initial characterizations confirmed a unique pharmacological profile for these most recently cloned SRIF receptors. In contrast to the pharmacological profiles of SSTR1, SSTR2, and SSTR3, SSTR4 was found to have higher affinity for SRIF-28 than for SRIF (34). In addition, the SRIF analogue SMS-201–995 demonstrated higher affinity for SSTR4 than did SRIF, and the SRIF analogues MK-678 and CGP 23996 demonstrated a slightly lower affinity than did SRIF. The pharmacological properties of both rat SSTR5 and human SSTR5 differ from those of the cloned SRIF receptor subtypes SSTR2, SSTR3, and SSTR4 in that they show little or no affinity for the SRIF analogue SMS-201–995 (35, 36). Rohrer *et al.* (36) also demonstrated that human SSTR5 does not bind MK-678 and binds the SRIF analogue RC-160 with very low affinity. We have extended these initial studies by testing the pharmacological specificities of these receptors with a battery of SRIF analogues that have been widely used both experimentally and clinically. These include cyclic hexapeptides, cyclic disulfide-containing octapeptides, cyclic CGP 23996-like compounds, and linear SRIF analogues. The present study, in comparison with previous studies on SSTR1, SSTR2, and SSTR3, reveals several peptides that are highly specific for individual SRIF receptor subtypes. These results provide structural information that should be useful in the design of compounds with even greater experimental and clinical specificities. Furthermore, we have shown that SSTR4 and SSTR5 differ from one another with regard to sensitivity to Na^+, nonhydrolyzable GTP analogues, and pertussis toxin, as well as in the mediation of the inhibition of adenylyl cyclase activity.

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-363,301, L-363,376, L-363,572, 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). CGP 23996 was iodinated as described previously (37).

Isolation of the human SSTR5 gene. The human SSTR5 clone lHX5–1 was isolated from a human genomic library (catalogue no. 946203; Stratagene, La Jolla, CA) by hybridization with a polymerase chain reaction-generated fragment of the rat SSTR5 gene (35). The sequence of the SRIF receptor-encoding region of this genomic clone was identical to that reported by Rohrer *et al.* (36) and confirmed that this clone encoded the human homologue of rat SSTR5. A 1.4-kilobase *NheI-NdeI* fragment containing the protein-coding region of the intronless human SSTR5 gene was subcloned into the pCMV-based expression vector pCMV6c (21) to generate pCMV-HX. The plasmid pCMV-HX was used for transient expression studies in COS-1 cells as well as to generate a stable cell line expressing human SSTR5 by cotransfection of CHO cells (strain DG44) together with pSV2neo DNA, as described previously (21).

Radioligand binding assays. Receptor binding assays on cloned SRIF receptors were performed using membranes from CHO-K1 cells stably expressing rat SSTR4 or from COS-1 cells transiently expressing human SSTR5, as described previously (22, 23, 26). In some studies, we also used CHO (DG44) cells stably expressing SSTR5. For radioligand binding assays, cells were harvested in 50 mM Tris·HCl, pH 7.8, containing 1 mM EGTA, 5 mM $MgCl_2$, 10 mg/ml leupeptin, 10 mg/ml pepstatin, 200 mg/ml bacitracin, and 0.5 mg/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 Brinkmann Polytron (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 mg of protein) from COS-1 cells transiently expressing SSTR5 were incubated with ^{125}I-CGP 23996 (0.2 nM; specific activity, 505 Ci/mmol) for 90 min at 25° in the presence or absence of competing peptides, stable GTP analogues, Na^+, or NMDG, in a final volume of 200 ml. Cell membranes (5–15 mg of protein) from CHO-K1 cells expressing SSTR4 were incubated for 60 min at 37°. Nonspecific binding was defined as the radioactivity remaining bound in the presence of 1 mM SRIF. For the saturation studies, increasing concentrations of ^{125}I-CGP 23996 (0.05–1.5 nM) were incubated in the presence or absence of 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 (38), available on the National Institutes of Health-sponsored PROPHET system.

The effect of pertussis toxin pretreatment of cells expressing either SSTR4 or SSTR5 on subsequent agonist binding to membranes or on cAMP accumulation was also tested. Cells were treated with either control medium or 100 ng/ml pertussis toxin 18 hr before radioligand binding or cAMP accumulation studies.

To determine the effects of previous exposure of the receptors to high concentrations of agonist, cells were incubated for 2 hr in the presence or absence of 1 μM SRIF. The cell culture medium was then removed; cells were washed twice and then harvested and assayed as described above. We previously demonstrated that the loss of agonist labeling is not simply due to a masking of sites by residual peptide (39).

cAMP accumulation studies. Studies examining the potencies of these peptides to inhibit forskolin-stimulated cAMP accumulation were performed as described previously (22, 39). Briefly, cells used for cAMP accumulation studies were subcultured in 12- or 24-well culture plates. CHO cells expressing SSTR4 were subcultured 24 hr before the experiments and COS-1 cells were transfected 72 hr before the experiments. Culture medium was removed from wells and 500 μM of fresh medium containing 0.5 mM isobutylmethylxanthine were added to each well. 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 were frozen for subsequent determination of cAMP content by radio-

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immunoassay. 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).

Results

To identify analogues of SRIF that possess selectivity for the recently cloned SRIF receptors SSTR4 and SSTR5, we examined the pharmacological profiles of each receptor subtype. The genes encoding these receptor subtypes were either stably expressed in CHO-K1 cells, as for SSTR4, or transiently expressed in COS cells, as for SSTR5, as described previously (22, 34, 35). SRIF receptor subtypes were labeled with ^{125}I-CGP 23996, a metabolically stable SRIF analogue that has been extensively characterized (24, 37). The binding of this radioligand to each receptor was saturable and of high affinity. Analysis of saturation experiments by nonlinear regression showed that the K_d for ^{125}I-CGP 23996 binding to SSTR4 expressed in CHO-K1 cells was 0.6 nM, with a B_{max} value of 5.2 pmol/mg of protein. ^{125}I-CGP 23996 bound to SSTR5 expressed transiently in COS cells with a K_d of 0.88 nM and a B_{max} of 10.7 pmol/mg of protein. All data were best fit by a single-site analysis, and these values represent the average of two individual experiments. No specific binding was detectable in untransfected CHO-K1 or CHO (DG44) cells or in vector-transfected COS-1 cells.

We next performed inhibition studies to characterize the pharmacology of each SRIF receptor subtype and to identify subtype-selective agents. The binding of ^{125}I-CGP 23996 to SRIF receptor subtypes was inhibited with various concentrations of SRIF analogues of vastly differing structures, including both cyclic and linear compounds. The structures of these SRIF analogues are presented in Table 1.

As shown in Table 2, SRIF-28 was more potent than SRIF in inhibiting radioligand binding to SSTR4, with IC_{50} values of 0.23 and 0.86 nM, respectively. Both peptides also bound potently to SSTR5. Likewise, other larger analogues of SRIF bound to both receptor subtypes with high affinities. The hexapeptide analogues bound only to SSTR4, with more moderate to low affinities, demonstrating no appreciable effect on binding to SSTR5 at concentrations of peptide as high as 1 μM. These hexapeptides bind potently to SSTR2, some with low picomolar affinities (22, 23). The cyclic heptapeptide BIM-23030 also bound to SSTR4 with relatively high affinity but to SSTR5 with lower affinity.

Octapeptide analogues, such as the clinically used SMS 201–995, were also tested for binding affinity with each of the SRIF receptor subtypes. As shown in Table 2, only three of the analogues significantly inhibited ^{125}I-CGP 23996 binding to SSTR5, and only at concentrations higher than 0.1 μM, indicating that they bound to this receptor subtype with very low affinity. In contrast, most of these compounds bound to SSTR4 with high affinity. Many of these compounds also bound to SSTR2 with extremely high affinities, in the range of 1–10 pM (23).

We also tested smaller CGP 23996-like analogues for their abilities to interact with each of the SRIF receptor subtypes. The peptide SA and structurally related pentapeptides III-V interacted with SSTR4, albeit with moderate to low affinities, but did not bind to SSTR5. Compound II, which lacks the benzyl substituent, did not interact with either of the cloned receptors. Hexapeptide analogues L-362,862 and L-362,855, also cyclized via a carbon bridging, interacted with SSTR4 with very high affinities and with SSTR5 with more moderate affinities. These two peptides bind to SSTR2 and SSTR3 with affinities similar to that for SSTR5 but do not interact with SSTR1 (23).

SRIF is a cyclic peptide, and various studies have shown that reduction of the disulfide bridge and consequent linearization of the peptide result in a loss of binding and functional activity of the peptide (40). We tested a variety of smaller linear compounds for their abilities to interact with the cloned SRIF receptors. Interestingly, most of these compounds were found to interact with SSTR4 with high affinities, whereas those that interacted with SSTR5 did so with moderate to low affinities. Only BIM-23052 interacted with SSTR4 with subnanomolar affinity. This compound was highly potent and selective for SSTR4.

We also investigated whether agonist labeling of SSTR4 and SSTR5 could be regulated by SRIF pretreatment of cells expressing these receptors. We exposed cells to agonists for 2 hr, washed the cells to remove unlabeled agonist, and examined the ability of ^{125}I-CGP 23996 to bind to membranes from these cells. This treatment was previously shown to regulate high affinity agonist binding to SSTR2 and SSTR3 but not to SSTR1 (22, 26). SRIF pretreatment of CHO-K1 cells expressing SSTR4 produced a 45% loss of subsequent agonist labeling of the SRIF receptor by ^{125}I-CGP 23996 (data not shown). SRIF pretreatment of COS-1 cells expressing SSTR5 also decreased the subsequent specific binding of ^{125}I-CGP 23996 to SSTR5 by 74% (data not shown). These findings show that high affinity agonist binding to both SSTR4 and SSTR5 can be regulated by homologous hormone treatment.

We next examined the ability of the nonhydrolyzable GTP analogue GMP-PNP (100 μM) to affect ^{125}I-CGP 23996 binding to SSTR4 and SSTR5. GMP-PNP produced a 77% reduction of specific ^{125}I-CGP 23996 binding to SSTR4, suggesting that this SRIF receptor subtype is coupled to G proteins (data not shown). In contrast, no reduction of specific ^{125}I-CGP 23996 binding to SSTR5 by GMP-PNP was observed. We similarly found no reduction of specific ^{125}I-CGP 23996 binding to CHO (DG44) cells stably expressing SSTR5 or of specific ^{125}I-[Tyr^{11}]-SRIF binding to COS-1 or CHO (DG44) cells expressing SSTR5 (data not shown). Pertussis toxin pretreatment of CHO-K1 cells expressing SSTR4 resulted in a complete loss of specific ^{125}I-CGP 23996 binding to SSTR4, indicating that SSTR4 is coupled to pertussis toxin-sensitive G proteins (data not shown). In contrast, pertussis toxin pretreatment of COS-1 cells expressing SSTR5 did not significantly affect subsequent ^{125}I-CGP 23996 binding to SSTR5 (data not shown). Similarly, no effect of pertussis toxin on ^{125}I-CGP 23996 binding to CHO (DG44) cells stably expressing SSTR5 was observed (data not shown). Taken together, these results indicate that SSTR4 is efficiently coupled to pertussis toxin-sensitive G proteins, whereas SSTR5 appears not to couple to G proteins when transiently expressed in COS-1 or when stably expressed in CHO (DG44) cells.

We also tested whether agonist binding to SSTR4 and SSTR5 was sensitive to Na^+, as has been reported for SRIF receptors (41). As shown in Fig. 1, ^{125}I-CGP 23996 binding to SSTR4 was more sensitive to Na^+ than to NMDG, which has been used to account for nonspecific effects of increasing ionic

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TABLE 1
Structures of SRIF peptide analogues

Analogue	Structure[a]
SRIF	Ala-Gly-c[Cys-Lys-Asn-Phe-Phe-Trp-Lys-Thr-Phe-Thr-Ser-Cys]
BIM-23003	c[Cys-Lys-Asn-*p*-Cl-Phe-Phe-D-Trp-Lys-Thr-Phe-Thr-Ser-Cys]
Hexapeptides	
BIM 23027	c[*N*-Me-Ala-Tyr-D-Trp-Lys-Abu-Phe]
MK-678	c[*N*-Me-Ala-Tyr-D-Trp-Lys-Val-Phe]
L-363,301	c[Pro-Phe-D-Trp-Lys-Thr-Phe]
L-363,572	c[D-Ala-D-Phe-D-Trp-Lys-D-Thr-*N*-Me-D-Phe]
L-363,376	c[Pro-Ala-D-Trp-Lys-Thr-Phe]
Heptapeptide	
BIM-23030	c[MPA-Tyr-D-Trp-Lys-Val-Cys]-Phe-NH_2
Octapeptides	
BIM-23023	D-Phe-c[Cys-Tyr-D-Trp-Lys-Abu-Cys]-Thr-NH_2
BIM-23034	D-Phe-c[Cys-Tyr-D-Trp-Lys-Val-Cys]-Nal-NH_2
BIM-23059	D-Nal-c[Cys-Tyr-D-Trp-Lys-Thr-Cys]-Thr-NH_2
BIM-23060	D-Phe-c[Cys-Tyr-D-Trp-Lys-Thr-Cys]-Nal-NH_2
BIM-23014	D-Nal-c[Cys-Tyr-D-Trp-Lys-Val-Cys]-Thr-NH_2
BIM-23042	D-Nal-c[Cys-Tyr-D-Trp-Lys-Val-Cys]-Nal-NH_2
SMS 201-995	D-Phe-c[Cys-Phe-D-Trp-Lys-Thr-Cys]-Thr-oL
DC23-60	D-Nal-c[Cys-Tyr-D-Trp-Lys-Val-Cys]-Thr-OH
EC5-21	D-Phe-c[Cys-Phe-D-Trp-Lys-Thr-Cys]-Nal-NH_2
NC4-28B	D-Phe-c[Cys-Tyr-D-Trp-Lys-Ser-Cys]-Nal-NH_2
L-362.823	c[Aha-[Cys-Phe-D-Trp-Lys-Thr-Cys]]
CGP 23996-like peptides	
CGP 23996	c[Aha-Lys-Asn-Phe-Phe-Trp-Lys-Thr-Tyr-Thr-Ser]
SA	c[Aha-Phe-D-Trp-Lys-Thr(Bzl)]
II	c[Aha-Phe-D-Trp-Lys-Thr]
III	c[Aha-Phe-D-Trp-Lys-Ser(Bzl)]
IV	c[Ahx-Phe-D-Trp-Lys-Thr(Bzl)]
V	c[Aoc-Phe-D-Trp-Lys-Thr(Bzl)]
L-362,855	c[Aha-Phe-Trp-D-Trp-Lys-Thr-Phe]
L-362.862	c[Aha-Phe-*p*-Cl-Phe-D-Trp-Lys-Thr-Phe]
Linear peptides	
BIM-23049	D-Nal-Ala-Tyr-D-Trp-Lys-Val-Ala-Thr-NH_2
BIM-23050	*N*-Me-D-Ala-Tyr-D-Trp-Lys-Val-Phe-NH_2
BIM-23051	D-Phe-Ala-Phe-D-Trp-Lys-Thr-Ala-Thr-NH_2
BIM-23052	D-Phe-Phe-Phe-D-Trp-Lys-Thr-Phe-Thr-NH_2
BIM-23053	D-Phe-Ala-Tyr-D-Trp-Lys-Val-Ala-Nal-NH_2
BIM-23055	D-Phe-Phe-Tyr-D-Trp-Lys-Val-Phe-D-Phe-NH_2
BIM-23056	D-Phe-Phe-Tyr-D-Trp-Lys-Val-Phe-D-Nal-NH_2
BIM-23057	D-Phe-CPA-Tyr-D-Trp-Lys-Val-Phe-Thr-NH_2
BIM-23058	D-Phe-Phe-Tyr-D-Trp-Lys-Val-Phe-Thr-NH_2
BIM-23063	D-Phe-CPA-Tyr-D-Trp-Lys-Thr-Phe-Nal-NH_2
BIM-23064	D-Phe-CPA-Tyr-D-Trp-Lys-Val-Phe-D-Ala-NH_2
BIM-23065	D-Nal-CPA-Tyr-D-Trp-Lys-Thr-Phe-Thr-NH_2
BIM-23066	D-Phe-*p*-NO_2-Phe-Tyr-D-Trp-Lys-Val-Phe-Thr-NH_2
BIM-23067	D-CPA-Ala-Tyr-D-Trp-Lys-Val-Ala-D-Phe-NH_2
BIM-23068	D-Phe-CPA-Tyr-D-Trp-Lys-Thr-Phe-Thr-NH_2
BIM-23069	D-CPA-Ala-Tyr-D-Trp-Lys-Val-Ala-Nal-NH_2
BIM-23070	D-Phe-Ala-Tyr-D-Trp-Lys-Thr-Ala-Nal-NH_2
Bim-23073	D-Phe-Ala-Tyr-D-Trp-Lys-Val-Ala-D-Nal-NH_2

[a] Abu, aminobutyric acid; Aha, 7-aminoheptanoic acid; Ahx, 6-aminohexanoic acid; Aoc, 8-aminooctanoic acid; CPA, 4-chlorophenylalanine; MPA, 3-mercaptopropionic acid; Nal, β-(2-naphthyl)alanine.

strength (42),[1] indicating that Na^+ can reduce high affinity agonist binding to SSTR4, as was previously reported for SSTR4 transiently expressed in COS-7 cells (34). In contrast, specific ^{125}I-CGP 23996 binding to SSTR5 was equally sensitive to increasing concentrations of Na^+ and NMDG (Fig. 1), indicating no specific Na^+ effect on agonist binding to SSTR5. It has been suggested that the ability of Na^+ to reduce the affinity of receptors for agonist may result from conformational changes that occur upon Na^+ binding, which thereby induce G protein uncoupling. The specific Na^+ effect observed for SSTR4 and not for SSTR5 would be consistent with apparent G protein coupling of SSTR4 but not SSTR5. We have likewise shown a specific Na^+ effect on SSTR2 but not SSTR1.[1]

SSTR4 was previously shown to mediate the inhibition of forskolin-stimulated cAMP accumulation (34). We tested the effects of SRIF and various SRIF analogues on forskolin (10 μM)-stimulated cAMP accumulation in cells expressing SSTR4 and SSTR5. SRIF and SRIF-28 maximally inhibited forskolin-stimulated cAMP accumulation in CHO-K1 cells stably expressing SSTR4 by 63% and 68%, respectively, with potencies of 50 nM and 1 nM (Fig. 2). Likewise, SRIF analogues with high affinities for SSTR4, including BIM-23027, MK-678, SMS 201–995, BIM-23014, BIM-23023, BIM-23034, BIM-23059,

[1] H. Kong, K. Raynor, K. Yasuda, G. Bell, and T. Reisine. Mutation of an aspartate at residue 89 in the somatostatin receptor subtype SSTR2 prevents Na^+ regulation of agonist binding but does not affect apparent receptor/G protein association. Submitted for publication.

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TABLE 2

Potencies of SRIF analogues to inhibit ^{125}I-CGP 23996 binding to cloned SRIF receptor subtypes SSTR4 and SSTR5

These are the average results of three different experiments for each peptide tested, with the standard errors being <10% of the mean.

Peptide	IC_{50}	
	SSTR4	SSTR5
	nM	
SRIF	0.86	1.2
SRIF-28	0.23	0.29
[D-Trp^8]-SRIF	0.002	0.29
[Des-Ala^1,des-Gly^2,His^{4,5},D-Trp^8]-SRIF	0.001	0.10
BIM-23003	0.002	1.8
Hexapeptides		
BIM-23027	6.2	>1000
MK-678	5.5	>1000
L-363,301	3.2	>1000
L-363,572	405	>1000
Heptapeptide		
BIM-23030	3.9	360
Octapeptides		
SMS 201-995	0.57	>1000
NC8-12	6.0	>1000
NC4-28B	1.0	>1000
BIM-23014	0.10	>1000
BIM-23023	0.18	>1000
BIM-23034	0.19	252
BIM-23042	5.2	102
BIM-23059	0.08	>1000
BIM-23060	0.09	>1000
DC23-60	1.5	>1000
EC5-21	1.9	560
L-362,823	1.2	>1000
CGP 23996-like peptides		
SA	51	>1000
II	>1000	>1000
III	188	>1000
IV	34	>1000
V	50	>1000
L-362,862	0.47	44
L-362,855	0.005	63
Linear peptides		
BIM-23049	146	>1000
BIM-23050	26	124
BIM-23051	53	>1000
BIM-23052	0.002	18
BIM-23053	29	>1000
BIM-23055	3.3	254
BIM-23056	43	158
BIM-23057	8.1	219
BIM-23058	1.2	77
BIM-23063	43	>1000
BIM-23064	17	27
BIM-23065	9.5	433
BIM-23066	2.4	245
BIM-23067	15	>1000
BIM-23068	1.1	24
BIM-23069	14	>1000
BIM-23070	10	>1000
BIM-23073	5.9	>1000

DC23–60, L-362,823, and BIM-23052, also inhibited adenylyl cyclase activity to the same maximal extent as did SRIF and SRIF-28 in cells expressing SSTR4, indicating that each of these analogues is a full agonist at this receptor. Furthermore, pretreatment of these cells with pertussis toxin negated the subsequent ability of SRIF or SRIF-28 to inhibit forskolin-stimulated cAMP accumulation. In contrast, SRIF did not affect forskolin-stimulated cAMP accumulation in COS-1 cells expressing SSTR5, consistent with a lack of effect of GMP-

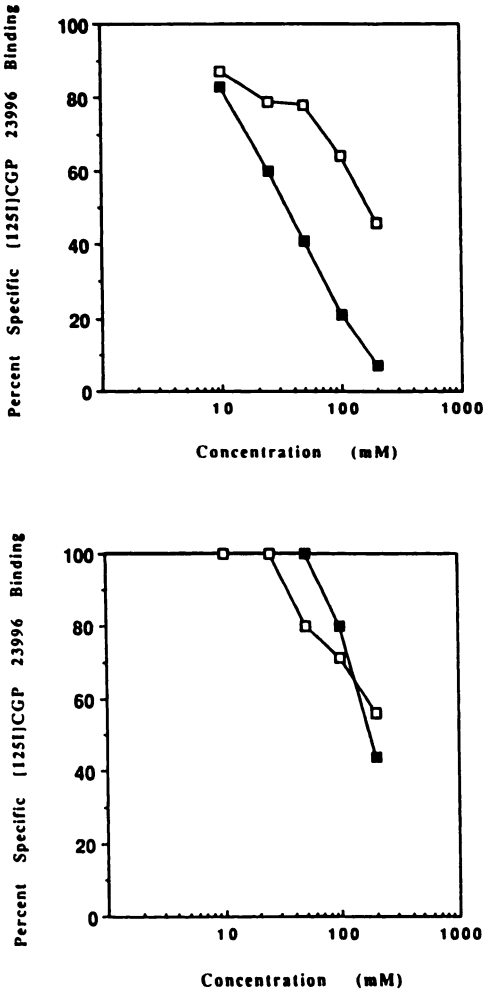

Fig. 1. Regulation by Na^+ of agonist binding to the cloned SRIF receptor subtypes SSTR4 and SSTR5. SRIF receptors in membranes from CHO-K1 cells stably expressing SSTR4 (*top*) or COS-1 cells transiently expressing SSTR5 (*bottom*) were labeled with ^{125}I-CGP 23996 in the absence or presence of various concentrations of Na^+ (■) or NMDG (□). Data are the means ± standard errors of three separate experiments.

PNP, Na^+, and pertussis toxin on agonist binding to this receptor. SRIF did not inhibit forskolin-stimulated cAMP accumulation in CHO (DG44) cells stably expressing SSTR5 or in CHO-K1 cells transiently expressing SSTR5, indicating that the lack of coupling of SSTR5 to adenylyl cyclase is not cell type dependent. These results confirm that SSTR4, but not SSTR5, is coupled via pertussis toxin-sensitive G proteins to the inhibition of adenylyl cyclase activity.

Discussion

The recent molecular cloning of two additional SRIF receptor subtypes has allowed us to express each individually in mammalian cells and to characterize their respective pharmacological profiles, regulation, and G protein and cellular effector coupling. We have identified different structural classes of SRIF analogues that interact with these subtypes. Furthermore, we have identified specific peptide analogues of SRIF that are selective for SRIF receptor subtypes. Coupled with previous results on cloned SSTR1, SSTR2, and SSTR3 (23), we can now identify compounds that are selective for the cloned SRIF receptors SSTR2, SSTR3, and SSTR4. Both SSTR1 and

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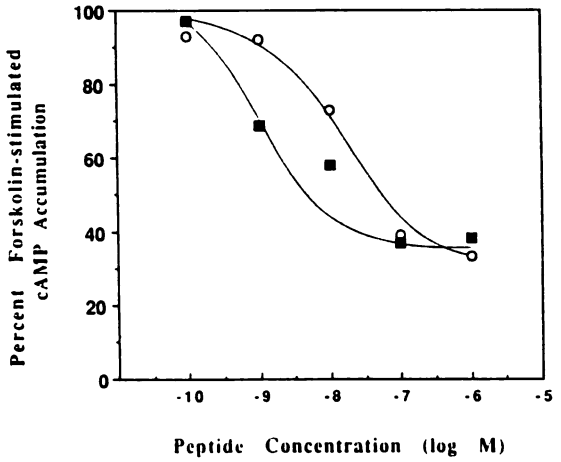

Fig. 2. Effect of various concentrations of SRIF and SRIF-28 on forskolin-stimulated cAMP accumulation in cells expressing SSTR4 or SSTR5. Forskolin (10 μM)-stimulated cAMP accumulation in CHO-K1 cells stably expressing SSTR4 was inhibited by various concentrations of SRIF (○) and SRIF-28 (■), as described in Experimental Procedures. SRIF and SRIF-28 inhibited forskolin-stimulated cAMP accumulation (161.5 pmol/well; basal, 13.4 pmol/well) to a similar maximal extent, by 67% and 62%, respectively. Data are the means ± standard errors of three separate experiments.

TABLE 3

SRIF receptor subtype-selective compounds

Values for SSTR1, SSTR2, and SSTR3 were taken from the report of Raynor *et al.* (23).

Compound	IC_{50}				
	SSTR1	SSTR2	SSTR3	SSTR4	SSTR5
			nM		
BIM-23027	>1000	0.001	2.4	6.2	>1000
NC4-28B	>1000	0.002	112	1	>1000
BIM-23056	>1000	>1000	0.02	43	158
L-362,855	>1000	29	30	0.005	63
BIM-23052	23	32	0.42	0.002	18

SSTR5 bind few SRIF analogues with high affinity, and we have not yet identified compounds that are selective for these SRIF receptor subtypes. We previously identified NC4–28B and BIM-23027 as being selective for SSTR2, and in light of the present results these compounds are still selective for SSTR2, because they exhibit 500- and 2400-fold higher affinity, respectively, for SSTR2 than for the other cloned SRIF receptor subtypes (Table 3). Furthermore, we had identified BIM-23056 as being SSTR3 selective. Although this compound binds to SSTR4, it remains >2000-fold selective for SSTR3 (Table 3). The results of the present study now identify several compounds that are >100-fold selective for SSTR4 over all the other cloned SRIF receptors. These include the linear compounds BIM-23052 and CGP 23996-like L-362,855, which are 210-fold and 5800-fold selective, respectively, for SSTR4 (Table 3). Other CGP 23996-like compounds similar to SA generally demonstrate highest affinity for SSTR4, compared with the other cloned SRIF receptors, consistent with the affinities we have found for these compounds at SRIF receptors expressed in the rat pituitary and in AtT-20 cells.[2]

Before the cloning of these SRIF receptors, subtypes of SRIF receptors had been identified based on pharmacological and functional studies using SRIF analogues. Early studies identified SRIF receptors that were differentially sensitive to the native peptides SRIF and SRIF-28 (10, 43). SSTR1, SSTR2, and SSTR3 each possessed relatively similar affinities for SRIF and SRIF-28, indicating that none is the "SRIF-28-preferring receptor." SSTR4 possesses higher affinity for SRIF-28 than for SRIF, as was previously reported for SSTR4 transiently expressed in COS-7 cells (34). Furthermore, SRIF-28 was 50-fold more potent than SRIF in inhibiting forskolin-stimulated cAMP accumulation mediated via SSTR4, as was shown previously (34). The pharmacological characteristics of SSTR4 are similar to those of SRIF receptors that we previously characterized in both rat pituitary and the mouse adrenocorticotropic cell line AtT-20 (24, 44). In fact, SSTR4 mRNA has been identified in the pituitary by Northern blotting (34).

SSTR4 is expressed in the anterior pituitary but is unlikely to mediate the inhibition of GH release from somatotrophs. Correlational analysis has shown that the affinities of various SRIF analogues to inhibit binding to SSTR2, but not SSTR1 or SSTR3, are highly correlated with the affinities of these compounds to inhibit GH release *in vitro* (23), indicating that SSTR2 may mediate SRIF inhibition of GH secretion. The results of the present study further substantiate this hypothesis, because the correlation of the affinities of SRIF analogues to bind to SSTR4 or SSTR5 and to inhibit GH release is low (r = 0.01 and 0.01, n = 32 and 17, respectively). Furthermore, SSTR5 is unlikely to be relevant to GH release, because mRNA encoding this receptor was not detected in the pituitary by Northern blotting (35). In addition, this receptor has low affinity for MK-678 and SMS 201–995, SRIF analogues that are potent inhibitors of GH release (35, 36).

Many characteristics of human SSTR5 are similar to those previously described for the first SRIF receptor cloned, SSTR1 (Table 4). Pharmacologically, both SSTR1 and SSTR5 are insensitive to small cyclic hexapeptides, such as MK-678, which we originally used to discriminate SRIF receptor subtypes in rat brain (24). Furthermore, with few exceptions, both these receptors are insensitive to cyclic octapeptides, such as SMS 201–995, which Reubi (45) and Tran *et al.* (46) used to discriminate SRIF receptor subtypes in rat brain. Because both SSTR1 and SSTR5 are expressed in the brain, these receptors constitute what have been described as $SRIF_2$ or SS_B receptors. SSTR1 and SSTR5 are similar to one another and are further distinguished from the other cloned SRIF receptors in their apparent lack of efficient coupling to G proteins, as evidenced by the lack of effect of GTP analogues, pertussis toxin, or Na^+ on agonist labeling of these receptors (22)[1] (Table 4). Consistently, neither receptor appears to couple to the inhibition of adenylyl cyclase activity when expressed either transiently in COS-1 cells or stably in CHO (DG44) cells (22). The cellular effector coupling of these receptors is currently unknown.

Interestingly, the amino acid sequences of human SSTR1 and human SSTR5 are 58% identical and 78% similar. This is the highest degree of amino acid sequence similarity between any of the cloned SRIF receptor subtypes. This high degree of sequence homology may provide a structural basis for the similarity of the pharmacological properties of the two receptors. In contrast, SSTR5 has much lower identity (approximately 40–43%) with the other SRIF receptor subtypes, from which its pharmacological properties differ greatly. SSTR4 has higher amino acid sequence homology with SSTR2 and SSTR3

[2] K. Raynor and T. Reisine, unpublished observations.

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TABLE 4
Properties of the five cloned SRIF receptors

	SSTR1	SSTR2	SSTR3	SSTR4	SSTR5
GTP sensitivity	No	Yes	Yes	Yes	No
Pertussis toxin sensitivity	No	Yes	Yes	Yes	No
Na^+ sensitivity	No	Yes	Yes	Yes	No
Adenylyl cyclase coupling	No	No	Yes	Yes	No
Agonist regulation	No	Yes	Yes	Yes	Yes
Tissue distribution of mRNA					
Human	Gastrointestinal tract	Brain, kidney	Brain	:	Brain
Rodent	Brain	Brain, pituitary	Brain, pancreatic islets	Pituitary	Brain

than with SSTR1, consistent with the greater similarities of the pharmacological properties of this receptor and those of SSTR2 and SSTR3. The pharmacological and structural comparisons suggest that there may be subgroups within the SRIF receptor gene family, with SSTR1 and SSTR5 comprising one subfamily and SSTR2, SSTR3, and SSTR4 comprising a second subfamily.

We have now reported that the five cloned SRIF receptor subtypes have distinct properties in terms of ligand specificity, G protein coupling, agonist-induced regulation, Na^+ effect, and inhibition of adenylyl cyclase activity. These results, combined with the unique patterns of distribution of these receptors, indicate that each receptor subtype may mediate distinct but overlapping physiological effects of SRIF. Our studies will also help in the development of more specific SRIF analogues for each receptor subtype, and such analogues may be of greater experimental and therapeutic significance.

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References

1. Brazeau, P., W. Vale, R. Burgus, N. Ling, J. Rivier, and R. Guillemin. Hypothalamic polypeptide that inhibits the secretion of immunoreactive pituitary growth hormone. *Science (Washington D. C.)* **129**:77–79 (1972).
2. Epelbaum, J. Somatostatin in the central nervous system: physiology and pathological modifications. *Prog. Neurobiol.* **27**:63–100 (1986).
3. Renaud, L., J. Martin, and P. Brazeau. Depressant actions of TRH, LHRH and SRIF on activity of central neurons. *Nature (Lond.)* **255**:233–235 (1975).
4. Dodd, J., and J. Kelly. Is somatostatin an excitatory transmitter in the hippocampus? *Nature (Lond.)* **273**:674–677 (1978).
5. Gothert, M. Somatostatin selectively inhibits noradrenaline release from hypothalamic neurons. *Nature (Lond.)* **288**:86–88 (1980).
6. Chesselet, M. F., and T. Reisine. Somatostatin regulates dopamine release in rat striatal slices and cat caudate nuclei. *J. Neurosci.* **3**:232–236 (1983).
7. Havlicek, V., M. Rezek, and H. Freisen. Somatostatin and thyrotropin releasing hormone central effect on sleep and motor systems. *Pharmacol. Biochem. Behav.* **4**:455–459 (1976).
8. Raynor, K., I. Lucki, and T. Reisine. Somatostatin$_1$ receptors in the nucleus accumbens selectively mediate the stimulatory effect of somatostatin on locomotor activity in rats. *J. Pharmacol. Exp. Ther.* **265**:67–73 (1993).
9. Schettini, G. Brain somatostatin: receptor-coupled transducing mechanisms and role in cognitive functions. *Pharmacol. Res.* **23**:203–215 (1991).
10. Mandarino, L., D. Stenner, W. Blanchard, S. Nissen, J. Gerich, N. Ling, P. Brazeau, P. Bohlen, F. Esch, and R. Guillemen. Selective effects of somatostatin-14, -25, and -28 on *in vitro* insulin and glucagon secretion. *Nature (Lond.)* **291**:76–77 (1981).
11. Bloom, S., C. Mortimer, M. Thorner, G. Besser, R. Hall, A. Gomez-Pan, U. Roy, R. Russel, D. Coy, A. Kastin, and A. Schally. Inhibition of gastrin and gastric acid secretion by growth hormone release-inhibiting hormone. *Lancet* **2**:1106–1109 (1974).
12. Chneiweiss, H., P. Bertrand, J. Epelbaum, C. Kordon, J. Glowinski, J. Premont, and A. Enjalbert. Somatostatin receptors on cortical neurons and adenohypophysis: comparison between specific binding and adenylate cyclase inhibition. *Eur. J. Pharmacol.* **138**:249–255 (1987).
13. Schuit, F., and D. Pipeleers. Regulation of adenosine 3′,5′-monophosphate levels in the pancreatic beta cells. *Endocrinology* **117**:834–840 (1985).
14. Raynor, K., and T. Reisine. Differential coupling of somatostatin$_1$ receptors to adenylyl cyclase in the rat striatum vs. the pituitary and other regions of the rat brain. *J. Pharmacol. Exp. Ther.* **260**:841–848 (1992).
15. Wang, H., T. Bogen, T. Reisine, and M. Dichter. Somatostatin-14 and somatostatin-28 induce opposite effects on potassium currents in rat neocortical neurons. *Proc. Natl. Acad. Sci. USA* **86**:9616–9620 (1989).
16. Wang, H., T. Reisine, and M. Dichter. Somatostatin-14 and somatostatin-28 inhibit calcium currents in rat neocortical neurons. *Neuroscience* **38**:335–342 (1990).
17. Raynor, K., H.-L. Wang, M. Dichter, and T. Reisine. Subtypes of brain somatostatin receptors couple to multiple cellular effector systems. *Mol. Pharmacol.* **40**:248–253 (1991).
18. Dokas, L., H. Zweirs, D. Coy, and W. Gispen. Somatostatin and analogs inhibit endogenous synaptic plasma membrane protein phosphorylation *in vitro*. *Eur. J. Pharmacol.* **88**:185–193 (1983).
19. Barber, D., M. McGuire, and M. Ganz. β-Adrenergic and somatostatin receptors regulate Na^+-H^+ exchange independent of cAMP. *J. Biol. Chem.* **264**:21038–21042 (1989).
20. Viguerie, N., N. Tahiri-Jouti, A. Ayral, C. Cambillau, J. Scemama, M. Bastie, S. Knuhtsen, J. Esteve, L. Pradayrol, C. Susini, and N. Vaysse. Direct inhibitory effects of a somatostatin analog, SMS 201–995, on AR4–2J cell proliferation via pertussis toxin-sensitive guanosine triphosphate-binding protein-independent mechanism. *Endocrinology* **124**:1017–1025 (1989).
21. Yamada, Y., S. Post, K. Wang, H. Tager, G. Bell, and S. Seino. Cloning and functional characterization of a family of human and mouse somatostatin receptors expressed in brain, gastrointestinal tract, and kidney. *Proc. Natl. Acad. Sci. USA* **89**:251–255 (1992).
22. Rens-Domiano, S., S. Law, Y. Yamada, S. Seino, G. Bell, and T. Reisine. Pharmacological properties of two cloned somatostatin receptors. *Mol. Pharmacol.* **42**:28–34 (1992).
23. Raynor, K., W. A. Murphy, D. H. Coy, J. E. Taylor, J.-P. Moreau, K. Yasuda, G. I. Bell, and T. Reisine. Cloned somatostatin receptors: identification of subtype selective peptides and demonstration of high affinity binding of linear peptides. *Mol. Pharmacol.* **43**:838–844 (1993).
24. Raynor, K., and T. Reisine. Analogs of somatostatin selectively label distinct subtypes of somatostatin receptors in rat brain. *J. Pharmacol. Exp. Ther.* **251**:510–517 (1989).
25. Raynor, K., D. Coy, and T. Reisine. Analogues of somatostatin bind selectively to brain somatostatin receptor subtypes. *J. Neurochem.* **59**:1241–1250 (1992).
26. Yasuda, K., S. Rens-Domiano, C. Breder, S. Law, C. Saper, T. Reisine, and G. Bell. Cloning of a novel somatostatin receptor, SSTR3, coupled to adenylyl cyclase. *J. Biol. Chem.* **267**:20422–20428 (1992).
27. Bell, G., and T. Reisine. Molecular biology of somatostatin receptors. *Trends Neurosci.* **16**:34–38 (1993).
28. Vanetti, M., M. Kouba, X. Wang, G. Vogt, and V. Hollt. Cloning and expression of a novel mouse somatostatin receptor. *FEBS Lett.* **311**:290–294 (1992).
29. Meyerhof, W., I. Wulfsen, C. Schonrock, S. Fehr, and D. Richter. Molecular cloning of a somatostatin-28 receptor and comparison of its expression pattern with that of a somatostatin-14 receptor in rat brain. *Proc. Natl. Acad. Sci. USA* **89**:10267–10271 (1992).
30. Meyerhof, W., H.-J. Paust, C. Schonrock, and D. Richter. Cloning of a cDNA encoding a novel putative G-protein-coupled receptor expressed in specific rat brain regions. *DNA Cell Biol.* **10**:689–694 (1991).

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31. Kluxen, F.-W., C. Bruns, and H. Lubbert. Expression cloning of a rat brain somatostatin receptor cDNA. *Proc. Natl. Acad. Sci. USA* **89**:4618–4622 (1992).
32. Yamada, Y., T. Reisine, S. Law, I. Yu, A. Kubota, S. Kagimoto, M. Seino, Y. Seino, G. Bell, and S. Seino. Somatostatin receptors, an expanding gene family: cloning and functional characterization of human SSTR3, a protein coupled to adenylyl cyclase. *Mol. Endocrinol.* **6**:2136–2142 (1993).
33. Li, X.-J., M. Forte, R. A. North, C. Ross, and S. Snyder. Cloning and expression of a rat somatostatin receptor enriched in brain. *J. Biol. Chem.* **267**:21307–21312 (1992).
34. O'Carroll, A.-M., S. Lolait, M. Konig, and L. Mahan. Molecular cloning and expression of a pituitary somatostatin receptor with preferential affinity for somatostatin-28. *Mol. Pharmacol.* **42**:939–946 (1992).
35. Bruno, J., Y. Xu, J. Song, and M. Berelowitz. Molecular cloning and functional expression of a novel brain specific somatstatin receptor. *Proc. Natl. Acad. Sci. USA* **89**:11151–11155 (1992).
36. Rohrer, L., F. Raulf, C. Bruns, R. Buttner, and R. Schule. Cloning and characterization of a novel human somatostatin receptor. *Proc. Natl. Acad. Sci. USA*, **90**:4196–4200 (1993).
37. Czernick, A., and B. Petrack. Somatostatin receptor binding in rat cerebral cortex: characterization using a non-reducible somatostatin analog. *J. Biol. Chem.* **258**:5525–5530 (1983).
38. McGonigle, P., and H. Perry. FITCOMP, a program to fit 1-, 2-, and 3-site models to competition binding data, in *PROPHET Public Procedures Notebook*. Bolt, Berabek, and Newman, Inc., Cambridge, MA (1988).
39. Raynor, K., and T. Reisine. Subtypes of somatostatin receptor are expressed in the anterior pituitary cell line GH_3. *J. Pharmacol. Exp. Ther.* **254**:110–116 (1993).
40. Brown, M., J. Rivier, and W. Vale. Somatostatin: analogs with selected biological activities. *Science (Washington D. C.)* **196**:1467–1468 (1977).
41. Mahan, L., and T. Reisine. Molecular mechanisms of somatostatin inhibition of hormone release from AtT-20 cells, in *Somatostatin: Basic and Clinical Status* (S. Reichlin, ed.). Plenum Press, New York, 137–145 (1987).
42. Limbird, L., J. Speck, and S. Smith. Sodium ion modulates agonist and antagonist interactions with the human platelet *alpha*$_2$ adrenergic receptor in membrane and solubilized preparations. *Mol. Pharmacol.* **21**:609–617 (1982).
43. Srikant, C., and Y. Patel. Receptor binding of somatostatin-28 is tissue specific. *Nature (Lond.)* **294**:259–260 (1981).
44. Raynor, K., and T. Reisine. The clonal anterior pituitary cell lines AtT-20 and GH_3 express pharmacologically distinct somatostatin receptor subtypes. *Pharmacologist* **32**:187 (1990).
45. Reubi, J. Evidence for two somatostatin-14 receptor types in rat brain cortex. *Neurosci. Lett.* **49**:259–263 (1984).
46. Tran, V., F. Beal, and J. Martin. Two types of somatostatin receptors differentiated by cyclic somatostatin analogs. *Science (Washington D. C.)* **228**:492–495 (1985).

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