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ACCELERATED COMMUNICATION

Cloned Somatostatin Receptors: Identification of Subtype-Selective Peptides and Demonstration of High Affinity Binding of Linear Peptides

KAREN RAYNOR, WILLIAM A. MURPHY, DAVID H. COY, JOHN E. TAYLOR, JACQUES-PIERRE MOREAU, KAZUKI YASUDA, GRAEME I. BELL, and TERRY REISINE

Department of Pharmacology, University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania 19104 (K.R., T.R.), Peptide Research Laboratories, Department of Medicine, Tulane University Medical Center, New Orleans, Louisiana 70112 (W.A.M., D.H.C.), Biomeasure Inc., Hopkinton, Massachusetts 01748 (J.E.T., J.-P.M.), and Howard Hughes Medical Institute, Departments of Biochemistry and Molecular Biology and Medicine, University of Chicago, Chicago, Illinois 60637 (K.Y., G.I.B.)

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SUMMARY

The recent molecular cloning of the genes encoding three somatostatin (SRIF) receptor subtypes has allowed for the individual expression of these receptors in mammalian cells and characterization of their respective pharmacological profiles. In the present study, we have investigated the affinities of a battery of SRIF analogues to bind to SRIF receptor subtypes SSTR1 (cloned somatostatin complex), SSTR2, and SSTR3, as well as their abilities to inhibit the release of growth hormone from anterior pituitary cells *in vitro*. We labeled SSTR1 and SSTR3 receptors expressed in Chinese hamster ovary and COS-1 cells, respectively, with the metabolically stable SRIF analogue ^{125}I-CGP 23996. SSTR2 receptors expressed in Chinese hamster ovary cells were labeled with the SSTR2-specific radioligand ^{125}I-MK-678. Inhibition 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)] (SA), and linear SRIF analogues. SSTR1 bound SRIF and SRIF-28 with high affinity and the peptide SA and its structural analogues with low affinity. The hexapeptides did not interact with SSTR1 at concentrations as high as 1 μM, and only a few of the octapeptides or linear peptides bound, with very low affinities. In contrast, ^{125}I-MK-678 binding to SSTR2 was potently inhibited by the hexapeptides, octapeptides, and some of the linear compounds, whereas SA and its analogues did not bind to SSTR2. The potencies of the various SRIF agonists to inhibit growth hormone release *in vitro* was highly correlated with their potencies to inhibit radioligand binding to SSTR2, but not to SSTR1 or SSTR3. SSTR3 bound analogues of each class but with moderate to low affinities, with the exception of several linear peptides and one of the octapeptides. We report for the first time the binding affinities of linear analogues of SRIF, some of which display subnanomolar affinities and are highly selective for SRIF receptor subtypes. Most importantly, these studies identify several peptide analogues that are highly potent, specific, and selective for individual subtypes of SRIF receptors. Such information, coupled with the knowledge of the distribution of these receptor subtypes in normal and pathological tissues, will be critical for more specific experimental and therapeutic interventions.

SRIF is a cyclic tetradecapeptide that was originally isolated from mammalian hypothalamus and characterized as a physiological regulator of GH secretion from the anterior pituitary (1). SRIF was 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 the release of endocrine and exocrine secretions (10, 11).

SRIF affects multiple cellular processes. 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 guanine nucleotide-binding proteins. SRIF also regu-

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ABBREVIATIONS: SRIF, somatostatin; GH, growth hormone; CHO, Chinese hamster ovary; EGTA, ethylene glycol bis(β-aminoethyl ether)-*N,N,N',N'*-tetraacetic acid; SA, c[Ahep-Phe-D-Trp-Lys-Thr(Bzl)]; GRF, growth hormone-releasing factor; SSTR, cloned somatostatin receptor.

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lates 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 two SRIF receptor subtypes (SSTR1 and SSTR2), which are differentially expressed in various tissues. Initial characterization of the pharmacological properties of these receptors indicated that the receptors are pharmacologically distinct (22) and correspond to two SRIF receptor subtypes, $SRIF_2$ and $SRIF_1$, respectively, which we have characterized in rat brain (8, 14, 17, 23, 24). Yasuda *et al.* (25) reported the subsequent cloning of a third SRIF receptor subtype, SSTR3, which differs from SSTR1 and SSTR2 in predicted amino acid sequence, tissue distribution, and pharmacological properties. Subsequent reports of the molecular cloning of the genes encoding these receptors in other species have now appeared (26–33).

Initial characterizations confirmed a unique pharmacological profile for each of these receptors (22, 25). We have extended these 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 analogues that are similar to 1) SRIF, in that they are relatively large and cyclic by virtue of a disulfide bridging between two cysteine residues, 2) the clinically used octapeptide analogue SMS 201–995, 3) the cyclic hexapeptide analogue MK-678, which we initially used to distinguish SRIF receptors, 4) SA, which also discriminates distinct SRIF receptors in the brain, and 5) linear SRIF analogues. This study reveals several peptides that are highly specific for individual SRIF receptor subtypes. It is also the first report of high affinity binding of linear SRIF analogues to SRIF receptors. These results provide structural information that should be useful in the design of compounds with even greater experimental and clinical specificities.

Materials and Methods

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 synthesized by D.H.C. and Biomeasure Inc. CGP 23996 and MK-678 were iodinated as described previously (34).

Receptor binding assays on cloned SRIF receptors were performed using membranes from CHO cells (cell line DG44) stably expressing human SSTR1 or mouse SSTR2 or from COS-1 cells transiently expressing mouse SSTR3, as described previously (22, 25). 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 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 (approximately 10 μg of protein) were incubated with ^{125}I-CGP 23996 (0.2 nM; specific activity, 505 Ci/mmol) or ^{125}I-MK-678 (0.05 nM; specific activity, 2200 Ci/mmol) in a final volume of 200 μl, for 90 min at 25°, in the presence or absence of competing peptides. Nonspecific binding was defined as the radioactivity remaining bound in the presence of 1 μM SRIF. For the saturation studies, increasing concentrations of ^{125}I-MK-678 or ^{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 with the mathematical modeling program FITCOMP (35), available on the National Institutes of Health-sponsored PROPHET system.

Studies examining the potencies of these peptides to inhibit GH release *in vitro* were performed as described previously (36). Briefly, anterior pituitaries from adult male rats (200–250 g) were dispersed aseptically by a trypsin/DNase method. The dispersed cells were diluted with sterile Dulbecco's modified Eagle's medium (GIBCO, Grand Island, NY) supplemented with 2.5% fetal calf serum (GIBCO), 3% horse serum (GIBCO), 10% fresh rat serum from the pituitary donors, 1% minimum essential medium nonessential amino acids (GIBCO), 10 ng/ml gentamycin (Sigma Chemical Co., St. Louis, MO), and 10,000 units/ml nystatin (GIBCO). The cells were counted with a hemocytometer and randomly plated at a density of 2×10^5 cells/well (Costar Cluster 24; Rochester Scientific, Rochester, NY). The plated cells were maintained in the Dulbecco's medium described above, in a humidified atmosphere of 95% air/5% CO_2, at 37° for 96 hr. In preparation for hormone challenge, the cells were washed three times with medium 199 (GIBCO). Each dose of secretagogue (diluted in siliconized test tubes) was tested in quadruplicate wells, in a total volume of 1 ml of medium 199 containing 1% bovine serum albumin. Cells were pulsed in the presence of 1 nM GRF(1–29)NH_2, in the presence or absence of various concentrations of SRIF analogues. After 3 hr at 37° in an 95% air/5% carbon dioxide atmosphere, the medium was removed and stored at −20° until the time of the GH radioimmunoassay. GH in plasma and media was measured by a standard double-antibody radioimmunoassay, using components generously supplied by the National Institute of Diabetes and Digestive and Kidney Diseases and the National Hormone and Pituitary Program, University of Maryland School of Medicine.

Results

To identify analogues of SRIF that possess selectivity for the three cloned SRIF receptors, SSTR1, SSTR2, and SSTR3, we examined the pharmacological profiles of each receptor subtype. The genes encoding these receptor subtypes were either stably expressed in CHO cells (DG44), as for SSTR1 and SSTR2, or transiently expressed in COS cells, as for SSTR3 (22, 25). Studies of SSTR3 were carried out in COS cells because of the apparent instability of expression of SSTR3 in CHO cells.

SRIF receptor subtypes were labeled with either ^{125}I-CGP 23996 (SSTR1 and SSTR3) or ^{125}I-MK-678 (SSTR2), both of which are metabolically stable SRIF analogues that have been extensively characterized previously (8, 14, 17, 22–24, 34). The binding of these radioligands to the respective receptors was of high affinity and saturable. Analysis of saturation experiments by nonlinear regression showed that the K_d for ^{125}I-CGP 23996 binding to SSTR1 expressed in CHO cells was 1.2 nM, with a B_{max} value of 276 fmol/mg of protein. ^{125}I-MK-678 bound to SSTR2 expressed in CHO cells with a K_d of 0.23 nM and a B_{max} of 476 fmol/mg of protein. Saturation experiments using ^{125}I-CGP 23996 and SSTR3 expressed transiently in COS cells

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yielded K_d values of 0.36 nM and a B_{max} of 656 fmol/mg of protein. All data were best fit by a single-site analysis, and these values represent the average of two individual experiments.

We next performed inhibition studies to characterize the pharmacology of each SRIF receptor subtype and to identify subtype-selective agents. The binding of these radioligands to SRIF receptor subtypes was inhibited with various concentrations of SRIF analogues of vastly differing structures. The structures of these SRIF analogues are presented in Table 1.

As shown in Table 2, SRIF potently inhibited radioligand binding to SSTR1, SSTR2, and SSTR3, with IC_{50} values of 0.1, 0.28, and 0.08 nM, respectively. SRIF and SRIF-28 were approximately equipotent at each of the receptor subtypes. Likewise, the other larger analogues of SRIF bound to each receptor subtype with relatively high affinity, except for BIM-23003, which appeared somewhat selective for SRIF receptor subtypes, with highest affinity for SSTR2 > SSTR3 > SSTR1. The hexapeptide analogues bound to only SSTR2 and SSTR3 with high to moderate affinity, demonstrating no appreciable effect on binding to SSTR1 at concentrations of peptide as high as 1 μM. BIM-23027 was found to bind to SSTR2 with extremely high affinity and selectivity. The cyclic heptapeptide BIM-23030 also bound to only SSTR2 and SSTR3, being somewhat more selective for the former.

Octapeptide analogues, such as the clinically used SMS 201–995, were also tested for binding affinity at each of the SRIF receptor subtypes. As shown in Table 2, only three of the analogues significantly inhibited ^{125}I-CGP 23996 binding to SSTR1 and only at concentrations higher than 0.1 μM, indicating that they bind with very low affinity to this receptor subtype. In contrast, these compounds bound to SSTR2 with high affinity, with NC4–28B, BIM-23023, BIM-23034, BIM-23059, and BIM-23060 demonstrating very high affinities and high selectivity for SSTR2. The octapeptide compounds also bound to SSTR3 but with more moderate affinities. The exception appears to be L-362,823, which bound to SSTR2 with only moderate affinity but demonstrated the highest affinity for SSTR3 of the octapeptides tested.

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 SSTR1 and SSTR3, albeit with rather low affinities, but not with SSTR2. Of these, compound III showed the greatest selectivity for SSTR1. Compound II, which lacks the benzyl substituent, did not interact with any of the cloned receptors. Hexapeptide analogues L-362,862 and L-362,855, cyclized via a carbon bridging, demonstrated a different pharmacological profile. These compounds interacted with highest affinities with SSTR2 > SSTR3 > SSTR1.

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 (37). We tested a variety of smaller linear compounds for their abilities to interact with the cloned SRIF receptors. Interestingly, many of these compounds were found to interact with SSTR2 and SSTR3 with very high affinities, whereas only three of these interacted with SSTR1, with moderate to low affinities. Only BIM-23068 interacted with SSTR2, with subnanomolar affinity, and this compound displayed high affinity for SSTR3 as well. In contrast, several of these compounds bound to SSTR3 with subnanomolar affinities and high selectivity, including BIM-23058, BIM-23052, and BIM-23056. The latter compound displayed remarkable selectivity for SSTR3, not interacting significantly with either SSTR1 or SSTR2 at concentrations as high as 1 μM.

To identify which receptor subtype might be involved in the regulation of GH release from the anterior pituitary, we tested the potencies of these compounds to inhibit the release of GH from rat anterior pituitary cells in culture. As shown in Table

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
DC 23-60	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.

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TABLE 2

Potencies of SRIF analogues to inhibit radioligand binding to cloned SRIF receptors and to inhibit GH release from anterior pituitary cells *in vitro*, relative to SRIF

Peptide	IC_{50}			GH release[a]
	SSTR1	SSTR3	SSTR2	
		nM		
SRIF	0.10	0.08	0.28	1 (0.05)
SRIF-28	0.07	0.07	0.43	ND[b]
[D-Trp[8]]-SRIF	0.23	0.05	0.005	0.13[c]
[des-Ala[1],des-Gly[2],His[4,5],D-Trp[8]]-SRIF	0.86	0.26	0.006	ND
BIM-23003	14	2.1	0.005	ND
BIM-23027	>1,000	2.4	0.001	ND
MK-678	>1,000	12	0.18	0.02[d]
L-363,301	>1,000	108	8.6	0.59[d]
L-363,572	>1,000	18.9	26	ND
L-363,376	>1,000	244	356	5[d]
BIM-23030	>1,000	133	6.1	23[e]
BIM-23023	>1,000	13.5	0.001	ND
NC4-28B	>1,000	112	0.002	0.003
BIM-23034	>1,000	65	0.002	0.43
BIM-23059	>1,000	7.1	0.008	0.19
BIM-23060	>1,000	17.4	0.012	0.05
DC 23-60	241	166	0.93	1.07
BIM-23014	789	5.6	1.6	0.83
SMS 201-995	>1,000	3	2.4	0.93
BIM-23042	>1,000	207	4.2	11
EC5-21	382	20	4.8	0.22
L-362,823	>1,000	0.08	83	0.81[f]
SA	141	107	>1,000	ND
II	>1,000	>1,000	>1,000	ND
III	80	214	>1,000	ND
IV	183	73	>1,000	ND
V	700	101	>1,000	ND
L-362,862	580	24	8.3	1.4[g]
L-362,855	>1,000	30	29	16.7[g]
BIM-23068	>1,000	0.8	0.15	3.4
BIM-23057	>1,000	4.8	2.4	17
BIM-23066	>1,000	1.1	3.1	159
BIM-23053	>1,000	152	6.1	15
BIM-23070	>1,000	111	7.8	16
BIM-23069	>1,000	143	22	45
BIM-23052	23	0.42	32	77
BIM-23065	>1,000	11	40	17
BIM-23058	>1,000	0.04	46	111
BIM-23073	146	65	60	ND
BIM-23049	>1,000	10	86	435
BIM-23067	>1,000	123	135	417
BIM-23063	>1,000	81	248	100
BIM-23051	>1,000	72	836	2,500
BIM-23064	>1,000	34	948	4,000
BIM-23056	>1,000	0.02	>10,000	12,500
BIM-23050	419	4.7	>10,000	20,000
BIM-23055	>1,000	38	>10,000	11,100

[a] Values for inhibition of GH release are given relative to that for SRIF (=1.0), with the EC_{50} values for SRIF (nM) given in parentheses. Values are the means of at least three different experiments.
[b] ND, not determined.
[c] Data from Ref. 43.
[d] Data from Ref. 44.
[e] Data from Ref. 36.
[f] Data from Ref. 45.
[g] Data from Ref. 46.

2, these peptides inhibited GH release *in vitro* with potencies similar to their binding affinities for SSTR2. Correlational analyses suggest that SSTR2, and not SSTR1 or SSTR3, regulates the release of GH from the anterior pituitary (Fig. 1).

Discussion

The recent molecular cloning of three distinct SRIF receptor subtypes has allowed us to express each individually in mammalian cells and to characterize their respective pharmacological profiles. 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 each of the SRIF receptor subtypes. These results also identify, for the first time, linear compounds of SRIF that bind to SRIF receptors with high affinity.

Before the cloning of these SRIF receptors, subtypes of SRIF receptors had been identified based on pharmacological and

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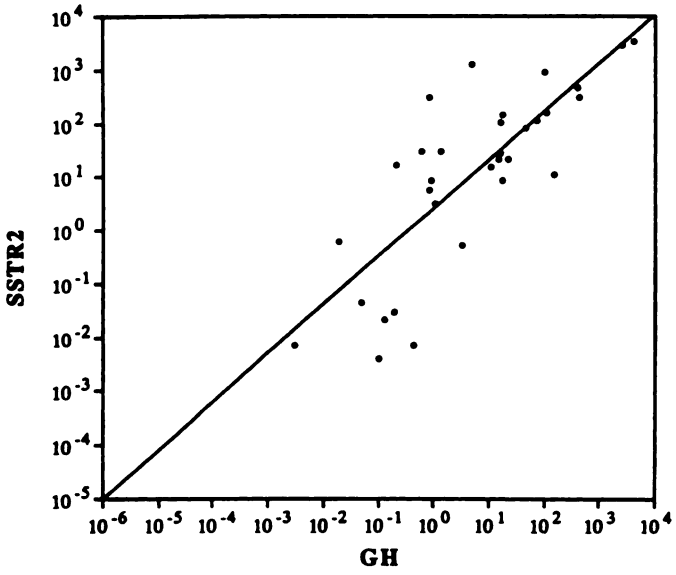

Fig. 1. Correlation plot of relative potencies of SRIF analogues to inhibit ^{125}I-MK-678 binding to SSTR2 and to inhibit GRF-stimulated GH release from anterior pituitary cells. Correlation analyses were performed by plotting the logarithm of the affinities of SRIF analogues for SSTR2 (*SSTR2*) versus the logarithm of the potencies of these compounds to inhibit GRF-stimulated GH release (*GH*). Inhibition of radioligand binding to SSTR2 was highly correlated with the inhibition of GH release (r = 0.93), whereas the correlation of potencies at SSTR3 with inhibition of GH release was much poorer (r = 0.18) (not plotted). Few of the compounds that inhibited GRF-stimulated GH release had any affinity for SSTR1.

functional studies using SRIF analogues. Early studies identified SRIF receptors that were differentially sensitive to the native peptides SRIF and SRIF-28 (38, 39). SSTR1, SSTR2, and SSTR3 each possessed relatively similar affinities for SRIF and SRIF-28, indicating that none is the "SRIF-28-preferring receptor." This latter receptor has now been cloned by O'Carroll *et al.* (33) and has been found to be strongly expressed in the anterior pituitary.

We have extensively characterized two receptor subtypes, $SRIF_1$ and $SRIF_2$, which are expressed in rat brain and are distinguished by their sensitivities to SRIF analogues (8, 14, 17, 23, 24). Thus, $SRIF_1$ receptors are sensitive to hexapeptide analogues, such as MK-678, whereas $SRIF_2$ receptors are insensitive to these analogues. In contrast, SA and its analogues are selective for $SRIF_2$ receptors in the rat brain. Initial characterization of the cloned SRIF receptors demonstrated that these receptors correspond pharmacologically to SSTR2 and SSTR1, respectively (22).

We have now greatly expanded the pharmacological characterization of these receptors to include other compounds of the same general structure, as well as linear peptides. Thus, SSTR1 receptors, like $SRIF_2$ receptors, have very little or no affinity for any of the hexapeptide analogues tested and bind the octapeptides with very low affinity. Also similarly to $SRIF_2$ receptors, SSTR1 binds SA and its analogues but with relatively low affinity. In this regard, compound III was the most potent of these and the only analogue identified with any significant degree of selectivity for SSTR1.

Pharmacological characterization of SSTR2 showed that this receptor was sensitive to a series of hexapeptide analogues with the same rank order of potencies that we have previously shown for $SRIF_1$ receptors (24). By further analogy with $SRIF_1$ receptors, SSTR2 displayed no affinity for SA or any of its analogues. Furthermore, these results show that BIM-23027 binds with even higher affinity and receptor subtype selectivity than does MK-678. SSTR2 also was sensitive to the octapeptide analogues and bound SMS 201–995 and BIM-23014 (otherwise known as SRIF tumor-inhibiting analogue) with affinities similar to those characterized for the $SRIF_1$ receptor. However, these compounds suffer from a lack of specificity, because both SMS 201–995 and BIM-23014 also bind to μ-opiate receptors, with K_i values of 3.4 and 2.0 nM, respectively.[1] The current study identifies octapeptide analogues that have much higher affinities for SSTR2 than do either of these octapeptides and that have low affinities for μ-opiate receptors, namely BIM-23042 and BIM-23060 (K_i values of 429 and 201 nM, respectively). One of the most potent octapeptides for SSTR2 was NC4–28B, and this compound displays no affinity for μ-opiate receptors.

The results from studies on the potencies of these compounds to inhibit the *in vitro* release of GH agree well with the potencies of these analogues to inhibit radioligand binding to SSTR2, suggesting that this receptor is the biologically relevant receptor with regard to regulation of GH release. A novel SRIF receptor has now been cloned by Bruno *et al.* (39). This receptor is unlikely to be relevant to GH release, because mRNA encoding this receptor was not detected in the pituitary by Northern blotting and this receptor has low affinity for MK-678 and SMS 201–995 (39), SRIF analogues that are potent inhibitors of GH release. O'Carroll *et al.* (33) have cloned the gene encoding another SRIF receptor, which is highly expressed in the pituitary. Preliminary results of radioligand binding studies[2] suggest that this receptor is unlikely to mediate effects on GH release, inasmuch as some compounds that were potent in the inhibition of GH release *in vitro* were not potent in the radioligand binding assay and *vice versa*. For example, this receptor binds SMS 201–995 with much higher affinity than MK-678. However, as shown in Table 2, MK-678 is more potent than SMS 201–995 in inhibiting GH release. Furthermore, compound L-362,855 is approximately 200-fold more potent in binding to SSTR4 than is SRIF but is less potent in inhibiting GH release than is SRIF. In addition, the linear compounds, which are >2000-fold less potent than SRIF in inhibiting GH release, bind to SSTR4 with relatively high affinities, with IC_{50} values between 4 and 70 nM.

The pharmacological profile of SSTR3 differed from that of SSTR1 or SSTR2. This receptor bound the hexapeptide, octapeptide, and SA-like analogues but all with moderate to low affinities. An interesting exception was L-362,823, which bound to SSTR3 with high affinity and selectivity. This compound was originally used, in addition to SMS 201–995, by Tran *et al.* (40) to pharmacologically distinguish receptor subtypes in the rat brain. Both of these compounds inhibit ^{125}I-Tyr^{11}-SRIF binding to rat brain with biphasic inhibition curves. These results suggest, however, that these peptides may do so in rat brain via interactions with different populations of SRIF receptors, inasmuch as SSTR1, SSTR2, and SSTR3 are all expressed in rat brain (21, 25, 31).[3]

[1] John Taylor, unpublished observations.
[2] K. Raynor and T. Reisine, unpublished observations.
[3] H. Kong, A. DePaoli, C. Breder, K. Yasuda, G. Bell, and T. Reisine. Differential expression of somatostatin receptor subtypes SSTR1, SSTR2 and SSTR3 in adult rat brain, pituitary and adrenal gland: analysis by RNA blotting and *in situ* hybridization. Submitted for publication.

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Perhaps the most intriguing and surprising result of these studies was the high potency of many of the linear compounds for SSTR2 and SSTR3. Linearization of native SRIF results in a loss of binding and functional activity of the peptide (41). Whereas the cyclic SRIF analogues tested bound to SSTR3 with moderate to low affinities, several of the linear peptides, namely BIM-23052, BIM-23056, and BIM-23058, bound to SSTR3 with high affinity and selectivity, making them ligands of choice for future studies on SSTR3. In fact, BIM-23056 was 30,000-fold selective for SSTR3. Studies are currently underway to iodinate and characterize these peptides as selective radioligands for experimental and potentially clinical purposes.

In the present study, we have identified analogues of SRIF that interact selectively with each of the cloned SRIF receptors. These compounds will be useful for evaluating the functional role of each SRIF receptor subtype. In this regard, SSTR2-specific compounds were highly potent in reducing GRF-stimulated GH secretion from rat anterior pituitary cells in culture. In contrast, several of the SSTR3-specific peptides were much less potent in reducing evoked GH release. Recent RNA blotting and *in situ* hybridization studies[3] have shown that SSTR2 mRNA is selectively expressed in the rat anterior pituitary. In contrast, SSTR1 and SSTR3 mRNAs are expressed at low levels in the pituitary and are not detectable in the anterior lobe. These findings suggest that SSTR2 is more likely to be involved in mediating the effect of SRIF on GH release, consistent with the findings on the effects of SSTR2-specific peptides on GH secretion.

Several SRIF analogues, such as SMS 201–995 and BIM-23014, have been designed for use as antiproliferative peptides for the treatment of acromegaly, adenomas, and pancreatic, breast, and prostate tumors (42). Identification of the SRIF receptor subtypes expressed in these tissues, as well as in normal human tissues, will be important for devising strategies for more specific therapeutic interventions. Our study serves as a foundation for experimental and clinical designs attempting to achieve more specific effects in complex biological systems.

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Send reprint requests to: Dr. Terry Reisine, Department of Pharmacology, University of Pennsylvania, 103 John Morgan Building, 36th and Hamilton Walk, Philadelphia, PA, 19107.

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