Potent Somatostatin Undecapeptide Agonists Selective for Somatostatin Receptor 1 (sst1)

Jean E. Rivier,*,† Carl Hoeger,† Judit Erchegyi,† Jozsef Gulyas,† Ryan DeBoard,† A. Grey Craig,† Steven C. Koerber,† Sandra Wenger,§ Beatrice Waser,§ Jean-Claude Schaer,§ and Jean Claude Reubi§

The Clayton Foundation Laboratories for Peptide Biology, The Salk Institute, La Jolla, California, and Division of Cell Biology and Experimental Cancer Research, Institute of Pathology, University of Berne, Switzerland.

Received January 24, 2001

A family of analogues of des-AA^{1,2,5}-[D-Trp^8/D-2Nal^8]-SRIF that contain a 4-(*N*-isopropyl)-aminomethylphenylalanine (IAmp) at position 9 was identified that has high affinity and selectivity for human somatostatin receptor subtype 1 (sst1). The binding affinities of des-AA^{1,2,5}-[D-Trp^8,IAmp^9]-SRIF (c[H-Cys-Lys-Phe-Phe-D-Trp-IAmp-Thr-Phe-Thr-Ser-Cys-OH], CH-275) (**7**), des-AA^{1,5}-[Tyr^2,D-Trp^8,IAmp^9]-SRIF (CH-288) (**16**), des-AA^{1,2,5}-[Tyr^7,D-Trp^8,IAmp^9]-SRIF (**23**), and des-AA^{1,2,5}-[D-Trp^8,IAmp^9,Tyr^{11}]-SRIF (**25**) are about $^1/_7$, $^1/_4$, $^1/_{125}$, and $^1/_4$ that of SRIF-28 (**1**) to sst1, respectively, about $^1/_{65}$, $^1/_{130}$, $<^1/_{1000}$, and $<^1/_{150}$ that of **1** to sst3, respectively, and about or less than $^1/_{1000}$ that of **1** to the other three human SRIF receptor subtypes. A substitution of D-Trp^8 by D-2Nal^8 in **7** to yield des-AA^{1,2,5}-[D-2Nal^8,IAmp^9]-SRIF (**13**) and in **16** to yield des-AA^{1,5}-[Tyr^2,D-2Nal^8,IAmp^9]-SRIF (**17**) was intended to increase chemical stability, selectivity, and affinity and resulted in two analogues that were less potent or equipotent with similar selectivity, respectively. Carbamoylation of the N-terminus as in des-AA^{1,2,5}-[D-Trp^8,IAmp^9,Tyr^{11}]-Cbm-SRIF (**27**) increased affinity slightly as well as improved selectivity. Monoiodination of **25** to yield **26** and of **27** to yield **28** resulted in an additional 4-fold increase in affinity at sst1. Desamination of the N-terminus of **17** to yield **18**, on the other hand, resulted in significant loss of affinity. Attempts at reducing the size of the ring with maintenance of selectivity failed in that des-AA^{1,4,5,13}-[Tyr^2,D-Trp^8,IAmp^9]-SRIF (**33**) and des-AA^{1,4,5,6,12,13}-[Tyr^2,D-Trp^8,IAmp^9]-SRIF (**34**) progressively lost affinity for all receptors. Both des-AA^{1,2,5}-[D-Trp^8,IAmp^9,Tyr^{11}]-Cbm-SRIF (**27**) and des-AA^{1,2,5}-[D-Cys^3,D-Trp^8,IAmp^9,Tyr^{11}]-Cbm-SRIF (**29**) show agonistic activity in a cAMP assay; therefore, the structural basis for the agonist property of this family of analogues is not contingent upon the chirality of the Cys residue at position 3 as shown to be the case in 18-membered ring SRIF octapeptides. None of the high affinity structures described here showed receptor antagonism. We have prepared the radiolabeled des-AA^{1,2,5}-[D-Trp^8,IAmp^9,^{125}ITyr^{11}]-SRIF (^{125}I-**25**) and des-AA^{1,2,5}-[D-Trp^8,IAmp^9,^{125}ITyr^{11}]-Cbm-SRIF (^{125}I-**27**), used them as in vitro tracers, and found them to be superior to des-AA^{1,5}-[^{125}ITyr^2,D-Trp^8,IAmp^9]-SRIF (^{125}I-**16**) for the detection of sst1 tumors in receptor autoradiography studies.

Introduction

Somatostatin (SRIF) is a major endocrine hormone and physiological inhibitor of growth hormone (GH),[1] glucagon, and insulin secretion.[2] Analogues of somatostatin have been used as drugs for the management of acromegaly and treatment of other pituitary tumors. For digestive diseases, analogues of somatostatin have been used to alleviate the symptoms produced by neuroendocrine tumors, to treat bleeding esophageal varices, refractory diarrheas, acute pancreatitis, pancreatic fistulae, and pancreatic pseudocysts. They are also used to prevent complications from pancreatic surgery and to manage nonvariceal upper digestive bleeding and digestive dumping syndrome. There are reports that SRIF analogues will affect angiogenesis and have a useful analgesic effect. Scintigraphy using SRIF analogues is a unique tool for diagnosis of neuroendocrine tumors, and radionuclide therapy offers promising possibilities to limit tumor growth. For reviews, see references 3−8. The actions of somatostatin are modulated by one or more of the five known membrane-associated receptor subtypes, sst1, sst2, sst3, sst4, and sst5. The actual function, distribution, and specificity of the different SRIF receptors, however, are still far from being fully understood due to the lack of potent and selective agonists and antagonists to each receptor. Analogues presently used in the clinic lack the selectivity necessary for the kind of intervention needed in some cases. Interestingly, most reviews of the field point to the limitations of the agonists presently available, such as poor selectivity and lack of selective antagonists. Despite the recent discovery of sst-selective non-peptide agonists,[9−15] a definite need still exists for potent, long acting receptor-selective ligands (agonists and antagonists) that are amenable to clinical formulation or specific labeling in order to gain a better understanding of the physiological role of SRIF and provide useful clinical tools. Known sst1-selective ligands include the

* Address for correspondence and reprint requests: Jean Rivier, Ph.D., The Salk Institute, The Clayton Foundation Laboratories for Peptide Biology, 10010 N. Torrey Pines Road, La Jolla, CA 92037. Tel: (858) 453-4100. Fax: (858) 552-1546. E-mail: Jrivier@salk.edu.
† The Salk Institute.
§ University of Berne.

10.1021/jm010037+ CCC: $20.00
Published on Web 05/24/2001

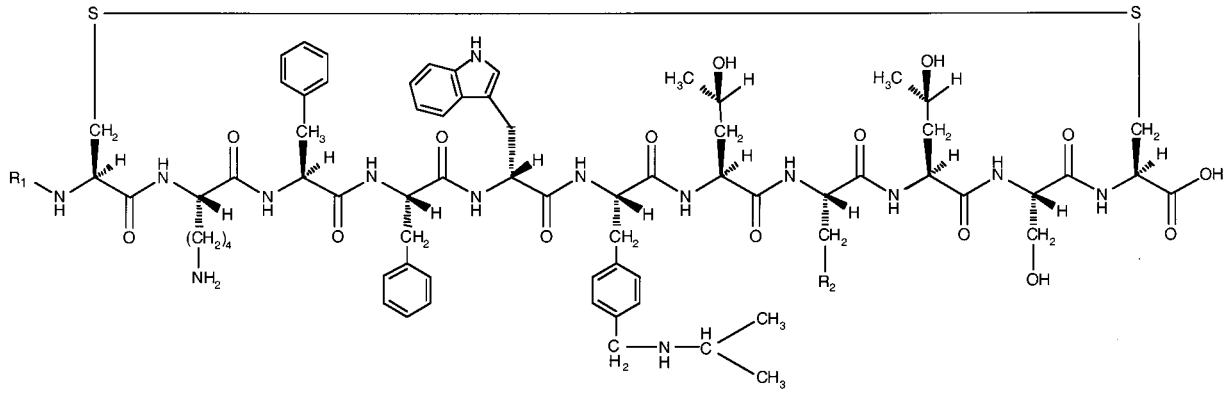

Figure 1. Structure of scaffold of CH-275 (**7**), CH-288 (**16**), **25**, **27**, and **28**. **7** (CH-275): R_1 = H, R_2 = phenyl. **16** (CH-288): R_1 = Tyr-yl, R_2 = phenyl. **25**: R_1 = H, R_2 = 4-hydroxyphenyl. **27**: R_1 = NH_2-CO-, (Cbm), R_2 = 4-hydroxyphenyl. **28**: R_1 = NH_2-CO-, (Cbm), R_2 = 3-iodo-4-hydroxyphenyl.

peptide CH-275 (**7**), its tyrosinated analogue CH-288 (**16**)[16] (see Figure 1), and the non-peptide L-797,591.[9] Several biological studies have been carried out using these analogues to study receptor tissue distributions.[17,18]

We present the rationale that led to the discovery of CH-275 and additional investigations in the structure–activity relationships of sst1-selective analogues that led to more potent, sst1-selective and radioiodinatable analogues. Our hypothesis is that substitutions and scaffolds that would mimic or constrain the multiple bioactive conformations of SRIF will yield high affinity analogues (agonists and antagonists) with selectivity for the different receptors.[19,20]

Results and Discussion

All analogues shown in Table 1 were synthesized either manually or automatically on a chloromethylated resin using the Boc-strategy and diisopropylcarbodiimide (DIC) for amide bond formation and trifluoroacetic acid (TFA) for Boc removal. The peptide resins were treated with hydrogen fluoride (HF) in the presence of scavengers to liberate the fully deblocked crude linear peptides. Cyclization of the cysteines was mediated by iodine in an acidic milieu. Purification was carried out using multiple HPLC steps[21] and characterized by HPLC,[21] capillary zone electrophoresis,[22] and mass spectrometry. The measured masses obtained using LSIMS were within 100 ppm of those calculated for the protonated molecule ions.

The compounds were tested for their ability to bind to 20 μm thick cryostat (Leitz 1720, Rockleigh, NJ) sections of a membrane pellet of cells transfected with the five human sst receptor subtypes. For each of the tested compounds, complete displacement experiments with the universal somatostatin radioligand [Leu^8,D-Trp^{22},^{125}I-Tyr^{25}]-SRIF-28 (30000 cpm/100 μL) using increasing concentrations of the unlabeled peptide ranging from 0.1 to 1000 nM were performed. The unlabeled SRIF-28 was run in parallel using the same increasing concentrations, as control. IC_{50} values were calculated after quantification of the data using a computer-assisted image processing system as described previously.[17,23] Tissue standards (Autoradiographic [^{125}I] microscales, Amersham) that contain known amounts of isotope, cross-calibrated to tissue-equivalent ligand concentrations, were used for quantitation.[24] Advantages of the present method using receptor autoradiography with sectioned cell pellets compared to binding on cell homogenates are, in addition to an economy on cells and a great flexibility, the greater interassay reliability and reproducibility, since sections of the same embedded pellet can be used for successive experiments. As a minor disadvantage, IC_{50} values are somewhat higher than in the homogenate binding assay.[23] The most potent and selective analogues were then evaluated for their agonist/antagonist properties measuring inhibition of cyclic adenosine monophosphate (cAMP) production. The iodinated form of the best sst1 analogues was also used to label sst1-transfected cells and human sst1-expressing tissues.

We have shown that the N-terminus dipeptide of SRIF-14 could be deleted without loss of potency in several functional assays.[25] Similarly, we have shown that the [D-Trp^8] substitution consistently increased the potency of the analogues into which the substitution was introduced.[26] The effect of these deletions and substitution on human ssts selectivity was not considered until recently. It is therefore noteworthy that **2** would show somewhat higher affinity (8-fold) for sst2 and somewhat lower (one-fourth) affinity for sst5 than SRIF-28 while being roughly equipotent on the other three ssts (Table 1).

At the initiation of this project aimed at identifying deletions and substitutions that would yield sst-selective analogues, we first revisited early observations that des-Asn^5-[D-Trp^8]-SRIF and [D-Ser^{13}]-SRIF, to a lesser extent, were more potent at inhibiting insulin than glucagon secretions, with reduced ability to inhibit GH secretion.[27–29] Results presented in Table 1 confirm that **3** has significantly lower affinity for sst2 than for any of the other four receptors which it binds to with very high affinity. This is consistent with the observation that inhibition of GH is mediated in part by sst2.[30] From these data, however, nothing can be said of which receptor mediates inhibition of insulin. In other words, the loss of affinity ($^1/_{17}$) of **3** for sst2 is not sufficient to allow assignment of a function for sst1 because of the residual yet high affinity of **3** for sst3, sst4, and sst5. We hypothesized that this loss of affinity for sst2 was due to a conformational change of the ring resulting from the deletion of Asn^5. It was therefore important,

Table 1. Characterization of SRIF Analogues by HPLC, CE, MS, and Affinities to the Five Human Cloned Somatostatin Receptors

	compound	HPLC[a]	CE[b]	M calcd	M obsd[c] $[M+H]^+$	sst1-IC_{50}[d]	sst2-IC_{50}[d]	sst3-IC_{50}[d]	sst4-IC_{50}[d]	sst5-IC_{50}[d]
1	SRIF-28	>98	>98	3146.48	3147.3	4.2 ± 0.3 (39)	2.4 ± 0.2 (36)	5.3 ± 0.5 (27)	4.0 ± 0.4 (29)	4.4 ± 0.4 (34)
2	Des-AA^{1,2}-[DTrp^8]-SRIF	98	82	1508.66	1509.0	5.4 ± 2.0 (4)	0.33 ± 0.1 (4)	4.3 ± 2.3 (2)	3.5 ± 1.9 (2)	16.7 ± 8.2 (3)
3	Des-Asn^5-[DTrp^8]-SRIF	98	97	1522.67	1523.2	1.0 ± 0.2 (2)	42.5 ± 2.5 (2)	3.4 ± 0.3 (2)	1.7 ± 0.4 (2)	5.0 ± 1.0 (2)
4	Des-AA^{1,2,5}-[DTrp^8,Aph^9(Atz)]-SRIF	95	96	1624.67	1625.7	>1000 (2)	>10K (3)	>10K (2)	>1000 (2)	>10K (2)
5	Des-AA^{1,2,5}−[DTrp^8,HArg^9(Et_2)]-SRIF	>98	>97	1492.76	1493.7	450 ± 70 (2)	>10K (3)	>1000 (2)	>1000 (2)	>10K (2)
6	Des-AA^{1,2,5}-[DTrp^8,Amp^9]-SRIF	97	97	1442.62	1443.3	309 ± 72 (2)	213 ± 33 (3)	273 ± 53 (2)	267 ± 59 (2)	190 ± 60 (2)
7	Des-AA^{1,2,5}-[DTrp^8,IAmp^9]-SRIF CH-275	94	97	1484.66	1485.5	30.9 ± 13 (4)	>10K (2)	345 ± 195 (2)	>1000 (2)	>10K (2)
8	Des-AA^{1,2}-[DTrp^8,IAmp^9]-SRIF	94	98	1598.71	1599.8	443 ± 298 (2)	>1000 (3)	>10K (2)	>10K (2)	>10K (2)
9	Des-AA^{1,2,5}-[DTrp^8,Amp^9(*i*Bu)]-SRIF	97	97	1498.68	1499.3	130 ± 60 (2)	>1000 (3)	381 ± 9.5 (2)	>1000 (2)	>1000 (2)
10	Des-AA^{1,2,5}-[DTrp^8,Amp^9(iBu$_2$)]-SRIF	97	97	1554.74	1555.5	>1000 (2)	>10K (3)	>10K (2)	>10K (2)	>10K (2)
11	Des-AA^{1,2,5}-[D2Nal^8,Amp^9]-SRIF	>97	>98	1453.62	1454.6	347 ± 144 (2)	343 ± 143 (3)	81 ± 25 (2)	>1000 (2)	385 ± 75 (2)
12	Des-AA^{1,2,5}-[D2Nal^8,Amp^9(Me)]-SRIF	85	97	1467.64	1468.8	101 ± 29 (2)	556 ± 78 (3)	280 ± 85 (3)	>10K (3)	>1000 (3)
13	Des-AA^{1,2,5}-[D2Nal^8,IAmp^9]-SRIF	97	>98	1495.67	1496.6	103 ± 34 (3)	>1000 (3)	>1000 (2)	>1000 (2)	>10K (2)
14	Des-AA^{1,2,5}-[DHis^8(^{im}Bzl),IAmp^9]-SRIF	97	>98	1525.69	1526.7	>1000 (3)	>1000 (3)	>1000 (2)	>10K (2)	>10K (3)
15	Des-AA^{1,2,5}-[DTrp^8,IAmp^9,DSer^{13}]-SRIF	97	98	1484.66	1485.6	187 ± 87 (3)	>10K (3)	>1000 (2)	>1000 (2)	>1000 (2)
16	Des-AA^{1,5}-[Tyr^2,DTrp^8,IAmp^9]-SRIF [Tyr^2]-CH-275 = CH-288	97	97	1647.73	1648.8	15.1 ± 2.9 (7)	>1000 (6)	713 ± 43 (4)	>10K (6)	>10K (6)
17	Des-AA^{1,5}-[Tyr^2,D2Nal^8,IAmp^9]-SRIF	98	98	1658.72	1659.6	24.0 ± 7.8 (3)	>1000 (3)	633 ± 88 (3)	>1000 (3)	>1000 (2)
18	Des-AA^{1,5}-[desamino-Tyr^2,D2Nal^8,IAmp^9]-SRIF	97	>98	1643.72	1644.8	563 ± 13 (2)	>1000 (2)	>1000 (2)	>10K (2)	>10K (2)
19	Des-AA^{1,5}-[Tyr^2,DTrp^8,IAmp^9]-Cbm-SRIF	94	98	1690.73	1691.8	15 ± 3.8 (3)	>10K (3)	500 ± 50 (3)	> 1000 (3)	>1000 (3)
20	Des-AA^{1,5}-[ITyr^2,DTrp^8,IAmp^9]-Cbm-SRIF	92	88	1816.64	1817.8	34.7 ± 6.1 (3)	>1000 (3)	290 ± 55 (3)	> 1000 (3)	>1000 (3)
21	Des-AA^{1,5}-[Tyr^2,DCys^3,DTrp^8,IAmp^9]-SRIF [DCys^3]-CH-288	>98	97	1647.79	1648.5	245 ± 105 (2)	>10K (2)	>1000 (2)	>1000 (2)	>10K (2)
22	Des-AA^{1,5}-[Tyr^2,DCys^3,DTrp^8,IAmp^9]-Cbm-SRIF	93	96	1690.73	1691.6	33 ± 6.2 (3)	>10K (3)	>1000 (3)	487 ± 13 (3)	>1000 (3)
23	Des-AA^{1,2,5}-[Tyr^7,DTrp^8,IAmp^9]-SRIF [Tyr^7]-CH-275	96	98	1500.66	1501.5	530 ± 130 (2)	>10K (2)	>10K (2)	>10K (2)	>1000 (2)
24	Des-AA^{1,2,5}-[Phe^7(4NO_2),D2Nal^8,IAmp^9]-SRIF	>98	>98	1540.65	1541.6	35 ± 2.5 (2)	>1000 (2)	975 ± 25 (2)	>1000 (2)	>1000 (2)
25	Des-AA^{1,2,5}-[DTrp^8,IAmp^9,Tyr^{11}]-SRIF [Tyr^{11}]-CH-275	98	96	1500.66	1501.5	17.1 ± 6.0 (5)	>10K (5)	>1000 (5)	>10K (5)	>1000 (5)
26	Des-AA^{1,2,5}-[DTrp^8,IAmp^9,ITyr^{11}]-SRIF	94	96	1626.55	1627.5	3.6 ± 0.7 (3)	>10K (3)	>1000 (3)	>1000 (3)	>1000 (3)
27	Des-AA^{1,2,5}-[DTrp^8,IAmp9,Tyr^{11}]-Cbm-SRIF	>98	>98	1543.66	1544.6	8.1 ± 1.3 (5)	>10K (5)	>1000 (5)	>1000 (5)	>1000 (5)
28	Des-AA^{1,2,5}-[DTrp^8,IAmp^9,ITyr^{11}]-Cbm-SRIF	91	97	1669.57	1670.6	2.5 ± 0.2 (4)	>1000 (4)	618 ± 125 (4)	>1000 (4)	>1000 (4)
29	Des-AA^{1,2,5}-[DCys^3,DTrp^8,IAmp^9,Tyr^{11}]-Cbm-SRIF	>98	98	1543.66	1544.6	17.4 ± 6.9 (3)	>10K (3)	>1000 (3)	>10K (3)	>1000 (3)
30	Des-AA^{1,5}-[desamino-Tyr^2,DCys^3,D2Nal^8, IAmp^9]-SRIF	91	95	1643.72	1644.8	845± 355 (2)	>10K (3)	750 ± 250 (2)	>1000 (2)	>1000 (2)
31	Des-AA^{1,5}-[desamino-Tyr^2,D2Nal^8,IAmp^9, DCys^{14}]-SRIF	>96	>97	1643.72	1644.8	975 ± 25 (2)	>10K (3)	>1000 (2)	>1000 (2)	>10K (2)
32	Des-AA^{1,5}-[desamino-Tyr^2,DCys^3,D2Nal^8, IAmp^9,DCys^{14}]-SRIF	92	97	1643.71	1644.8	>1000 (2)	>1000 (3)	>1000 (2)	>1000 (2)	>1000 (2)
33	Des-AA^{1,4,5,13}-[Tyr^2,DTrp^8,IAmp^9]-SRIF	95	98	1432.60	1433.6	720 ± 170 (2)	>10K (2)	445 ± 155 (2)	>1000 (2)	>10K (2)
34	Des-AA^{1,4,5,6,12,13}-[Tyr^2,DTrp^8,IAmp^9]-SRIF	96	98	1184.48	1185.5	>1000 (2)	>1000 (2)	>1000 (2)	>1000 (2)	>10K (2)
35	Des-AA^{1,2,4,5,12,13}-[DTrp^8]-SRIF	>95	98	1077.44	1078.9	27.3 ± 3.4 (4)	43.8 ± 10 (5)	13.1 ± 3.2 (4)	1.3 ± 0.8 (3)	45.7 ± 27 (3)
36	Des-AA^{1,2,4,5,12,13}-[DTrp^8,IAmp^9]-SRIF	98	98	1168.49	1169.5	>1000 (2)	>10K (3)	>1000 (2)	>1000 (2)	>10K (2)

Abbreviations: Amp, 4-aminomethylphenylalanine; Amp(*i*Bu), 4-(*N*-isobutyl)-aminomethylphenylalanine; Amp(iBu)$_2$, 4-(*N*,*N*-diisobutyl)-aminomethylphenylalanine; Amp(Me), 4-(*N*-methyl)- aminomethylphenylalanine; Aph(Atz), 4-(3-amino-1*H*-2,4-triazol-5-yl)-phenylalanine; His^{im}Bzl, *N*-im-benzyl-histidine; hArg(Et)$_2$, N^G-diethyl-homo-arginine; IAmp, 4-(*N*-isopropyl)-aminomethylphenylalanine; 2Nal, 3-(2-naphthyl)-alanine. [a] Percent purity determined by HPLC as described in Experimental Section. [b] Percent purity determined by capillary zone electrophoresis (CZE) as described in Experimental Section. [c] The calculated mass (M) of the monoisotope compared with the observed $[M+H]^+$ monoisotopic mass. [d] Binding affinities at the five human receptors expressed as IC_{50}. Mean of two or more experiments ± SEM when $N \geq 2$. N = number of experiments and is shown in parentheses.

if we chose this scaffold, to introduce compensatory changes in the conformational or binding properties of the amino acid(s) responsible for SRIF activity.

It has long been recognized that residues 8 and 9 of SRIF were critical for SRIF activity and that substitution of Trp^8 by DTrp^8 and some of its halogenated derivatives was favorable.[26,31] No similar favorable substitution for Lys^9 however had been found. SAR studies investigating the effect of substitutions at position 9 by several other closely related basic amino acids such as Orn and Arg yielded less potent SRIF analogues.[29]

We hypothesized that a favorable substitution (if it could be found) would have the added advantage of conferring some receptor selectivity. We concluded that the amino acid at position 9 would have to be basic (mimicking Lys) and sterically hindered to prevent receptor promiscuity.

We therefore investigated the effect of substitutions by Aph(Atz) (**4**), HArg(Et_2) (**5**), Amp (**6**), IAmp (**7**), Amp(^{i}Bu) (**9**), and Amp(^{i}Bu$_2$) (**10**) in des-AA^{1,2,5}-[DTrp^8]-SRIF. We had shown the advantage of 4-amino-phenylalanine (Aph) over lysine and Aph^8(Atz) over Lys^8(Atz) in the design of potent GnRH antagonists.[32] The low pK_b of Aph(Atz) as compared to that of Lys may explain the low potency of **4** at all receptors. Lys^9 substitution by HArg(Et_2) described by Nestor et al. also in the design of potent GnRH antagonists[32,33] was another lead because of the expected higher pK_b of HArg(Et_2). However, des-AA^{1,2,5}-[DTrp^8,HArg^9(Et_2)]-SRIF (**5**) showed low potency at all receptors as well.

If the pK_b of Lys^9 is to be mimicked, we surmised that 4-aminomethylphenylalanine (Amp), first introduced in a somatostatin analogue by Nutt et al.,[34] would be a better substitution for lysine than residues presented earlier. Molecular modeling and distance calculations of models of Amp and Lys in side chain extended conformations reveal good similarity in Cα-Nω distances of 6.40 and 7.50 Å, respectively. The basicities of the terminal nitrogens in Amp and Lys are, to a good approximation, equivalent, which suggests equivalent electrostatic contributions to binding. Analogue **6**, however, is about 70 times less potent than SRIF-28 on all receptors. Because of our observation that isopropyllysine is a good substitute for arginine at position 8 of GnRH antagonists, we synthesized **7** with a 4-(*N*-isopropyl)-aminomethylphenylalanine (IAmp) at position 9. This analogue has high affinity for sst1 and significantly lower affinity for the other ssts. To gain an appreciation of the contribution of des-Asn^5 and IAmp^9 toward sst1 affinity and selectivity, **8** was synthesized and is 15 times less potent at sst1 although still sst1-selective. Because **3** has high affinity for all receptors except sst2, we conclude that sst1-selectivity of **7** derives from the unique ionic and steric character of IAmp^9 that is not compatible with binding to sst2−5. It is noteworthy that an increase in the bulk of the alkylating group on the 4-aminomethyl functionality as in **9** (isobutyl) and **10** (diisobutyl) correlates with an incremental loss of affinity and selectivity.

NMR data have suggested a unique hydrophobic interaction of the side chains of Trp^8 and Lys^9. Models of SRIF analogues have been presented which are consistent with the observed upfield shift of Lys^8-Hγ by the Trp indole by postulating an interaction or close proximity to these functionalities.[35,36] If this interaction played a critical role in the bioactive conformation of SRIF, any modification of the lysine side chain, as with IAmp, could have a beneficial or deleterious effect depending on the nature of residue 8. Substitution of DTrp^8 by D2Nal^8 to yield **11** suggests that both residues are equivalent since **6** and **11** have affinities that are not statistically different at all five ssts. Methylation of Nω-Amp^9 in **12** had no significant effect on affinity (less than 2-fold) at either of the receptors as compared to the affinity of **11**. Disappointing is the fact that **13**, with D2Nal^8, is 3 times less potent at sst1 than **7**, its DTrp^8 equivalent. Early studies[37] showed that fluorination of the indole group at carbons 5 and 6 had little effect on potency while some loss in potency upon substitution of DTrp^8 by D2Nal^8 was observed by Hirst et al.[38] This early work, which also compared the SAR of diastereomeric pairs of SRIF hexapeptides substituted at position 8, suggested that the indole nitrogen of the Trp side chain had no direct role in receptor binding. This conclusion was based on the observation that analogues with an L amino acid and D amino acids at position 8, although equipotent, are conformationally different and would require that the aromatic side chain at position 8 undergoes rotational inversion along the side chain; thus, a unique presentation of an indole nitrogen would not be required since it is not possible. Additionally, methylation of the indole nitrogen had no effect on potency. Data presented here parallel results from the Merck group;[39] yet they do not offer additional light on the role for either the indole or naphthyl rings in SRIF analogues. Another aromatic moieity such as the DHis^{im}-Bzl, a residue found to be compatible at position 6 in GnRH superagonists,[40] is likely not recognized by ssts for steric reasons since **14** is inactive at all ssts.

Independently of the observation that des-Asn^5-SRIF and des-Asn^5-[DTrp^8]-SRIF were more potent at inhibiting insulin than glucagon secretions, we had also observed that [DSer^{13}]-SRIF had similar activities and the question remained whether those modifications would have an additive effect. Analogue **15**, homologous to **7** with a DSer^{13} substitution, although sst1-selective, is 6 times less potent than **7**.

One of the advantages that peptides offer over nonpeptide ligands is their ability to be labeled in a number of unique ways—be these substitutions with strong UV absorbing or fluorescent moieties, radionuclides, cytotoxic agents, or the like. This property has been particularly useful in the design of SRIF analogues for clinical diagnostic and therapeutic indications. Scintigraphy, using somatostatin analogues to bind to sst-positive tumors, is a unique tool for diagnosis of neuroendocrine tumors and radionuclide therapy.[3,41] Here, we have identified positions where the introduction of an iodinated tyrosine is compatible with high affinity, high selectivity, and low background labeling (**20**, **28**). Ascertaining that the cold monoiodinated derivatives of our somatostatin analogues have the same or higher affinity and selectivity than the parent noniodinated analogues is critical for the interpretation of future studies using the radioactively (^{125}I) monoiodinated tracers. These analogues were synthesized as

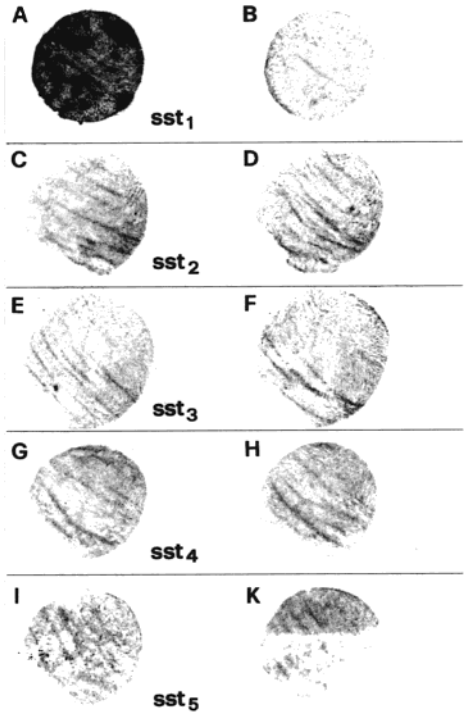

Figure 2. Binding of ^{125}I-**25** to sections of sst1−sst5-expressing cell pellets. All sections were incubated with 8000 cpm/100 μL of ligand. Strong specific labeling is seen only with sst1 cells. A, C, E, G, I: Autoradiograms showing total binding of ^{125}I-**25** to sections of cell pellets expressing sst1 (A), sst2 (C), sst3 (E), sst4 (G), and sst5 (I). B, D, F, H, K: Autoradiograms showing nonspecific binding of ^{125}I-**25** (in the presence of 10^{-6} M **25**).

a result of the observation that a tyrosine residue, introduced at positions 2 (**16**, **17**, **19**, **21**, and **22**), 7 (**23**), and 11 (**25**, **27**, and **29**), was only favorable at positions 2 and 11. For selected peptide structures, see Figure 1. The properties of **16** (high sst1 affinity and selectivity) were reported elsewhere in part on cells bearing SRIF receptors other than human.[16] High background of the corresponding radioiodinated analogue in tissue labeling limited its use in these systems and in our hands.[16] While substituting Phe^7 in **7** by Tyr^7 to yield **23** resulted in significant and complete loss of affinity at sst1 (1/20) and other ssts, respectively, another position 7 substitution such as 4NO$_2$Phe^7 (**24**) in the parent D2Nal^8-containing analogue **13**, retained both selectivity and potency.

Substitution of Tyr^{11} in **25** by ITyr^{11} to yield **26** was also compatible with increased potency. Therefore **25** was developed as an ^{125}I-labeled radioligand. It is an sst1-selective radioligand as illustrated in Figure 2 since only sst1-transfected cells and not sst2−sst5-transfected cells were strongly labeled. This compound was also tested in human tumors known to express sst1, as measured by sst1 mRNA by in situ hybridization. One example of an sst1-expressing mesenchymal tumor is shown in Figure 3. Tumors expressing predominantly sst1, including four gastroenteropancreatic tumors, four prostate carcinomas, and three sarcomas could be specifically labeled with ^{125}I-**25**. While this radioligand still exhibited a measurable nonspecific binding, it was nevertheless found to be a significantly better radioligand to detect sst1 in human tissues than the corresponding ^{125}ITyr^2-containing analogue of **16**.[17]

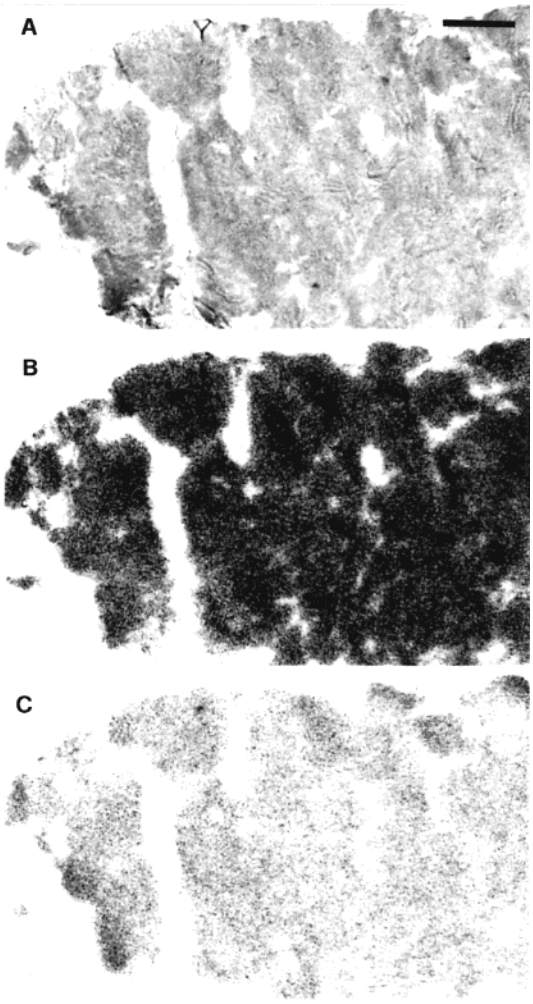

Figure 3. Labeling of sst1 receptors in a human leiomyoma. A: Hematoxylin and eosin stained section. Bar = 1 mm. B: Autoradiogram showing total binding of ^{125}I-**25**. C: Autoradiogram showing nonspecific binding of ^{125}I-**25** (in the presence of 10^{-6} M unlabeled **25**). This tumor was shown previously to have predominantly and abundantly sst1 mRNA with in situ hybridization.

Encouraged by the recent observation that incorporation of a carbamoyl functionality at the N-terminus of SRIF analogues will increase potency of sst3-selective SRIF antagonists without loss of selectivity,[42] we introduced that substitution in both **16** to yield **19** and in **25** to yield **27**, among others. We published earlier that *N*-ureido peptides could be prepared by direct coupling reaction of individually synthesized *N*-ureido amino acids or by the reaction of the resin bound partially deprotected amino peptides with different isocyanates such as tosylisocyanate, benzyl isocyanate, trimethylsilyl isocyanate, and *tert*-butyl isocyanate.[43] The protecting groups of the resulting ureas could be removed during the final anhydrous HF cleavage, except for trimethylsilyl which had already been removed under the last TFA deprotection step of solid phase synthesis. It has been observed that the presence of a base such as DIPEA resulted in a biuret byproduct when tosyl or trimethylsilyl isocyanates were used for the *N*-ureido formation. Very clean products were obtained with benzyl isocyanate, but the removal of the benzyl group from benzylureas required extended HF treatment. The *tert*-butyl protecting group was easily cleavable from

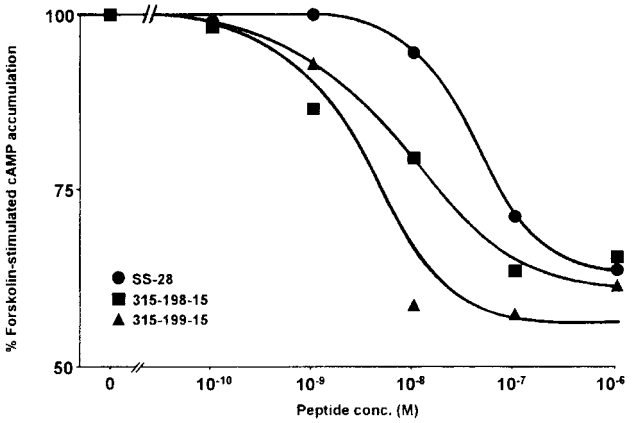

Figure 4. Effect of various concentrations of somatostatin-28 and analogues on forskolin-stimulated cAMP accumulation in cells expressing sst1. Concentration−response curves were obtained with increasing concentrations of somatostatin-28 (round), **27** (square), and **29** (triangle). Data are expressed as a percentage of the 3 μM forskolin response; they are from a typical experiment performed in duplicate and representative of three others. The somatostatin analogues **27** and **29** behave as agonists with equivalent results.

tert-butylureas with HF, not with TFA, and resulted in pure products. Further optimizing our synthetic methodologies, we have found that sodium cyanate (NaOCN) in the presence of glacial acetic acid is also suitable for the introduction of a carbamoyl (Cbm) moiety on a free amino function of an otherwise fully protected peptido resin, resulting in the desired ureido-peptido resin. This is the synthetic strategy that was used here.

The addition of a carbamoyl group at the N-terminus of SRIF analogues derived in part from the observation that desamination of the N-terminus as in **18** and **30**−**32**, although likely to confer resistance to aminopeptidases, resulted in a loss of sst1 affinity (1/10−1/15: compare affinities of **18** with those of **17**). Compounds **19** and **27** retained or had a slightly enhanced affinity and selectivity for sst1 with respect to the other ssts. Compound **27** was ^{125}I-iodinated and gave results similar to those found with ^{125}I-**25** in labeling experiments, both in sst1-transfected cells and in sst1-containing tumors. The cold iodinated **28** had an affinity for sst1 that was higher (IC_{50} = 2.5 nM) than that of the corresponding noniodinated **27** (IC_{50} = 8.1 nM) (Table 1) and comparable to that of the cold iodinated **26**.

With the identification of unique structural modifications that yielded sst1-selective SRIF agonists, we investigated the possibility of converting such agonists into antagonists through the introduction of an L residue at position 1 and DCys residue at position 3, as shown effective by Hocart et al. in cyclo(2−6) octapeptides.[44] We found that **29**, with a DCys residue in position 3, and its homologue **27** (without DCys^3) retained sst1 affinity as well as selectivity in binding assays and were found to be agonists in an adenylate cyclase activity assay (Figure 4). Compounds **27** and **29** inhibited forskolin-stimulated cAMP accumulation by more than 35% at a peptide concentration of 100 nM with EC_{50} (±SEM; n = 3) of 8.34 nM (±1.08) and 6.43 nM (±1.14), respectively, as compared to 29.3 nM (±10.5) for **1**.

Comparing the binding affinities of **22** to those of **19**, one reaches the same conclusion that the DCys^3 modification results in minimal loss of affinity at sst1. While exploring the effect of changes in chirality of the Cys bridge heads in sst1-selective analogues, we also made **31** (with a DCys^{14}) and **32** (with a DCys^{3,14}) that are much less potent at all ssts, suggesting that a well-defined secondary structure is an important requirement for high affinity.

The question remained whether shortening the ring size from 11 (**16**) to 9 (**33**) or 7 (**34**) residues would alter potency or sst selectivity. This approach is in keeping with our original observation that deletion of two residues at a time starting with the residues adjacent to the cysteines would yield cyclic analogues as short as 8 residues long with limited loss of potency as compared to SRIF.[45] This is exemplified with **35** that has high affinity for all five ssts. Interestingly, **33** lost some affinity and selectivity as it is also binding to sst3 while **34** is inactive. Clearly, the uneven deletion of residues 5 (**16**), 4−5 and 13 (**33**), and 4−6 and 12−13 (**34**) was well tolerated in the largest ring (**16**) and conferred sst1 selectivity, whereas it may have altered the overall conformation too drastically in the cases of the significantly smaller **33** and **34**. The hypothesis that IAmp^9 by itself might confer sst1 selectivity to an otherwise potent octapeptide (**35**) was rebutted by the observation that **36** had very low affinity for all ssts.

In conclusion, limited but specific conformational changes in the SRIF molecule will yield analogues with unique receptor selectivity. While selectivity can also be achieved with non-peptide agonists (such as L-797,591),[9] **16**, **19**, **20**, **22**, **25**, **27**, **28**, and **29** have affinities for sst1 similar to that of SRIF-28 and can be radioiodinated. Although there is still nonspecific binding when ^{125}I-**25** or ^{125}I-**27** are used in human tissues, both compounds are significantly better compared to ^{125}I-CH-288 and allow clear identification of sst1-expressing human tumors using in vitro receptor autoradiography.

Experimental Procedures

Instruments. The HF cleavage line was designed in-house and allowed for HF distillation under high vacuum, trapped in liquid nitrogen. Preparative RP-HPLC was accomplished using a Waters Associates (Milford, MA) DeltaPrep 3000 System and Model Shimadzu SPD-6A variable wavelength UV detector and Huston Instruments Omni Scribe chart recorder. The 5 × 30 cm cartridge was packed in the laboratory with reversed-phase 300 Å Vydac C_{18} silica (15−20 μm particle size). Analytical RP-HPLC screening was performed on a Vydac C_{18} column (0.46 × 25 cm, 5 μm particle size, 300 Å pore size) connected to a Rheodyne Model 7125 injector, an Altex 420 HPLC system using two Altex 100A pumps, a Kratos Spectroflow 757 UV detector set to 210 nm, and a Houston Instruments D-5000 strip chart recorder. Quality control HPLC was performed on a Hewlett-Packard Series II 1090 liquid chromatograph system that was connected to a Vydac C_{18} column (0.21 × 15 cm, 5 μm particle size, 300 Å pore size), Controller Model 362, and a Think Jet printer. Capillary zone electrophoresis (CZE) analysis was performed on a Beckman P/ACE System 2050 controlled by an IBM Personal System/2 Model 50Z connected to a ChromJet integrator. LSI-MS measurements were carried out with a JEOL JMS-HX110 double-focusing mass spectrometer (JEOL, Tokyo, Japan) fitted with a Cs^+ gun.

Starting Materials. The Boc-Cys(Mob)-CM resin with a capacity of 0.3−0.5 mequiv/g was obtained according to published procedures.[46] All N^{α}-*tert*-butyloxycarbonyl (BOC) protected amino acids with side chain protection were pur-

chased from Bachem Inc. (Torrance, CA), Chem-Impex Intl. (Wood Dale, IL), or Novabiochem (San Diego, CA). The side chain protecting groups of the different amino acids were as follows: Asn(Xan), Cys(S-*p*-Mob), Lys(ϵ-2ClZ), Ser(Bzl), Thr(Bzl), Tyr(2Br-Z), and ITyr(2Br-Z). Boc-Amp(Fmoc), Boc-IAmp(Z), and desamino-Tyr were synthesized in our laboratory.[47] All N^{ω}-alkylated amino acids were obtained on the resin as described in the literature.[48] Boc-L-HArg(Et_2) acetate was synthesized at the Southwest Foundation for Biomedical Research (under contract NO1-HD-6-2928 with NIH) and made available by the Contraceptive Development Branch, Center for Population Research, NICHD. All reagents and solvents were reagent grade or better and used without further purification.

Peptide Synthesis. Peptides were assembled by the solid phase approach[49] either manually or on a Beckman 990 peptide synthesizer and CS-Bio peptide synthesizer model CS536 (San Carlos, CA). A 3.0 equivalent excess of the protected amino acids based on the original substitution of the resin was used. The couplings on 1−2 g of resin per peptide were mediated by DIC in CH_2Cl_2, or DIC with *N*-hydroxybenzotriazole (HOBt) in dichloromethane and *N*-methyl-pyrrolidinone (NMP), respectively, for 1 h and monitored by the qualitative ninhydrin[50] or bromophenol blue test.[51] Boc removal was achieved with trifluoroacetic acid (60% in CH_2Cl_2, 1−2% ethanedithiol or *m*-cresol) for 20 min. An isopropyl alcohol (1% ethanedithiol or *m*-cresol) wash followed TFA treatment and then successive washes with triethylamine solution (10% in CH_2Cl_2), methanol, triethylamine solution, methanol, and CH_2Cl_2 completed the neutralization sequence. The Fmoc groups were removed with 20% piperidine in NMP in two successive 10 min treatments.

The 4-(*N*-methyl) group to Amp and the 4-(*N*-isobutyl) or 4-(*N*-diisobutyl) groups to Amp were introduced on the resin as described in the literature.[48] Briefly, after the synthesis was completed, the Boc group was not removed from the α-amino group, but the Fmoc group from the side chain nitrogen was removed with 20% piperidine in NMP. The resulting free amino group was alkylated with Dod-Cl (4,4′-dimethoxydityl chloride), followed by reductive methylation or isobutylation of the Dod-alkylated amino group, treating the alkylated resin with formaldehyde or isobutyraldehyde and sodium cyanoborohydride in the presence of acetic acid in NMP. At the end of the reaction, the Dod group and N^{α}-Boc group were removed with TFA (60% in DCM) simultaneously. To obtain the diisobutylamino derivative of Amp, the Dod alkylation was omitted, the resin was treated with isobutyraldehyde and sodium cyanoborohydride immediately after Fmoc removal, and at the end of the synthesis N^{α}-Boc group was removed with TFA. The ureido group at the N-terminus of **19**, **20**, **22**, and **27**−**29** was introduced on the resin as well. The N-terminal Boc group of the fully assembled peptide was deprotected with TFA in the usual manner; after neutralization, the carbamoylation proceeded with NaOCN (100 mg, 0.65 mmol) in NMP (4 mL) and glacial acetic acid (3 mL per gram of initial resin). The mixture was agitated at room temperature for 30 min, and the ninhydrin test indicated a complete reaction. The completed peptide resin was then cleaved from the resin by HF and cyclized by addition of iodine, as described below.

The completed peptides were cleaved from the resin support with simultaneous side chain deprotection by using anhydrous hydrogen fluoride containing the scavengers anisole (10% v/v) and methyl sulfide (5% v/v) for 90 min at 0 °C. The diethyl ether precipitated crude peptides were cyclized in 75% acetic acid (200 mL) by addition of iodine (10% solution in methanol) until the appearance of a stable orange color. Forty minutes later, ascorbic acid was added to quench the excess of iodine.

Purification of Peptides. The lyophilized crude peptides were purified by preparative RP-HPLC[21] using a linear gradient 1% B per 3 min increase from the baseline %B (eluent A = 0.25 N triethylammonium phosphate (TEAP) pH 2.25, eluent B = 60% CH_3CN, 40% A) at a flow rate of 100 mL/min. Purification in TEAP pH 2.25 was followed by a rechromatography in a 0.1% TFA solution and acetonitrile on the same cartridge (gradient of 1% acetonitrile/min). The separations were monitored by analytical RP-HPLC at 215 nm. The fractions containing the pure product were pooled and lyophilized. Each peptide was obtained as a fluffy white powder.

Characterization of SRIF Analogues. The purity of the final peptides was determined by analytical RP-HPLC in TEAP pH 2.5 buffer system. The TEAP pH 2.5 conditions were defined by a 1% B/min gradient slope at 0.2 mL/min from equilibrium A/B where A = 15 mM TEAP (pH 2.5) and B = 60% CH_3CN/40% A. Detection was set at 214 nm.

The peptides were analyzed by CZE as well. CZE analysis employed a field strength of 10−20 kV at 30 °C with a buffer of 15% CH_3CN/85% 100 mM sodium phosphate pH 2.5 on either a Beckman eCAP or a Supelco P15 fused silica capillary (363 μm o.d. × 75 μm i.d. × 50 cm length).

The observed monoisotopic $(M + H)^+$ values of each peptide corresponded with the calculated $(M + H)^+$ values. The mass accuracy was typically better than ±100−20 pp. An accelerating voltage of 10 kV and Cs^+ gun voltage between 25 and 30 kV were employed. The samples were added directly to a glycerol and 3-nitrobenzyl alcohol (1:1) matrix.

Cell Culture. CHO-K1 cells stably expressing human sst1 and sst5 were kindly provided by Drs. T. Reisine and G. Singh (University of Pennsylvania, Philadelphia, PA) and CCL39 cells stably expressing human sst2, sst3, and sst4 by Dr. D. Hoyer (Novartis Pharma, Basel, Switzerland). Cells were grown as described previously.[23] All culture reagents were supplied by Gibco BRL, Life Technologies (Grand Island, NY).

In Situ Hybridization Histochemistry. To control adequacy of the cell material, in situ hybridization for human sst mRNAs was performed on CHO-K1 and CCL39 cells expressing the different sst receptor subtypes. Cell smears were prepared, then used for sst1, sst2, sst3, sst4, and sst5 mRNA detection, as described in detail previously.[23] These control in situ hybridization studies confirmed that the five cell lines used for the study expressed the correct sst mRNA.

Receptor Autoradiography. Cell membrane pellets were prepared, and receptor autoradiography was performed as depicted in detail previously.[23] Each somatostatin analogue was tested for its binding affinity to the five sst1−sst5 subtypes in competitive experiments using ^{125}I-[Leu^8, DTrp^{22}, Tyr^{25}] SRIF-28 as radioligand. IC_{50} values were calculated from the displacement curves.[23]

Moreover, compounds **25** and **27** (Table 1) were ^{125}iodinated at the Tyr^{11} position using the lactoperoxidase method and were HPLC purified (74 TBq/mmol, Anawa, Wangen, Switzerland). Binding studies were performed with these ligands, as reported previously[23] for [Leu^8,DTrp^{22},^{125}ITyr^{25}]-SRIF-28, on cell pellet sections and on tissue sections of sst1-expressing human tumors using 8000 cpm/100 μL of the radioligand.

Adenylate Cyclase Activity. Modulation of forskolin-stimulated adenylate cyclase activity was determined using a radioimmunoassay measuring intracellular cAMP levels by competition binding. Sst1-expressing cells were subcultured in 96-well culture plates at 2×10^4 cells/well and grown for 24 h. Culture medium was removed from the wells, and 100 μL of fresh medium containing 0.5 mM 3-isobutyl-I-methylxanthine (IBMX) was added to each well. Cells were incubated for 30 min at 37 °C. Medium was then removed and replaced with fresh medium containing 0.5 mM IBMX, with or without 3 μM forskolin and various concentrations of peptides. Cells were incubated for 30 min at 37 °C. After removal of medium, cells were lysed and cAMP accumulation was determined using a commercially available cAMP scintillation proximity assay (SPA) system (RPA 538), according to the instructions of the manufacturer (Amersham, Aylesbury, U.K.). In these studies, basal levels of cAMP production were 0.25 ± 0.02 pmol cAMP/well, rising to 3.2 ± 0.2 pmol cAMP/well in the presence of 3 μM forskolin, representing a 12.8 ± 1.2-fold stimulation. cAMP data were expressed as percentage of stimulation over the nonstimulated level. Values of EC_{50} (the agonist concentration causing 50% of its maximal effect) were derived from concentration−response curves.

Acknowledgment. This work was supported in part by NIH grant DK 50124 and the Hearst Foundation. J.R. is the Frederik Paulsen Chair in Neurosciences Pofessor. The authors thank Dr. D. Hoyer (Novartis, Basel, Switzerland) and Drs. T. Reisine and G. Singh (University of Pennsylvania, Philadelphia, PA) for the generous gifts of sst-transfected cells. We are indebted to R. Galyean, L. Cervini, W. Low, D. Kirby, R. Kaiser, and T. Goedken for technical assistance in the synthesis and characterization of the peptides.

References

(1) Brazeau, P.; Vale, W. W.; Burgus, R.; Ling, N.; Butcher, M.; Rivier, J. E.; Guillemin, R. Hypothalamic polypeptide that inhibits the secretion of immunoreactive pituitary growth hormone. *Science* **1973**, *179*, 77−79.

(2) Guillemin, R.; Gerich, J. E. Somatostatin: physiological and clinical significance. *Annu. Rev. Med.* **1976**, *27*, 379−388.

(3) Krenning, E. P.; de Jong, M.; Kooij, P. P. M.; Breeman, W. A. P.; Bakker, W. H.; de Herder, W. W.; van Eijck, C. H. J.; Kwekkeboom, D. J.; Jamar, F.; Pauwels, S.; Valkema, R. Radiolabeled somatostatin analogue(s) for peptide receptor scintigraphy and radionuclide therapy. *Ann. Oncol.* **1999**, *10*, S23−S29.

(4) Eriksson, B.; Öberg, K. Summing up 15 years of somatostatin analogue therapy in neuroendocrine tumors: Future outlook. *Ann. Oncol.* **1999**, *10*, S31−S38.

(5) Sakane, T.; Suzuki, N. The role of somatostatin in the pathophysiology of rheumatoid arthritis. *Clin. Exp. Rheumatol.* **1998**, *16*, 745−749.

(6) Wynick, D.; Bloom, S. R. Clinical review 23: The use of the long-acting somatostatin analogue octreotide in the treatment of gut neuroendocrine tumors. *J. Clin. Endocrinol. Metab.* **1991**, *73*, 1−3.

(7) Ösapay, G.; Ösapay, K. Therapeutic applications of somatostatin analogues. *Expert Opin. Ther. Pat.* **1998**, *8*, 855−870.

(8) Patel, Y. C. Somatostatin and its receptor family. *Front. Neuroendocrinol.* **1999**, *20*, 157−198.

(9) Rohrer, S. P.; Birzin, E. T.; Mosley, R. T.; Berk, S. C.; Hutchins, S. M.; Shen, D.-M.; Xiong, Y.; Hayes, E. C.; Parmar, R. M.; Foor, F.; Mitra, S. W.; Degrado, S. J.; Shu, M.; Klopp, J. M.; Cai, S.-J.; Blake, A.; Chan, W. W. S.; Pasternak, A.; Yang, L.; Patchett, A. A.; Smith, R. G.; Chapman, K. T.; Schaeffer, J. M. Rapid identification of subtype-selective agonists of the somatostatin receptor through combinatorial chemistry. *Science* **1998**, *282*, 737−740.

(10) Berk, S. C.; Rohrer, S. P.; Degrado, S. J.; Birzin, E. T.; Mosley, R. T.; Hutchins, S. M.; Pasternak, A.; Schaeffer, J. M.; Underwood, D. J.; Chapman, K. T. A combinatorial approach toward the discovery of non-peptide subtype-selective somatostatin receptor ligands. *J. Comb. Chem.* **1999**, *1*, 388−396.

(11) Rohrer, S. P.; Schaeffer, J. M. Identification and characterization of subtype selective somatostatin receptor agonists. *J. Physiol. (Paris)* **2000**, *94*, 211−215.

(12) Ankersen, M.; Crider, M.; Liu, S.; Ho, B.; Andersen, H. S.; Stidsen, C. Discovery of a novel non-peptide somatostatin agonist with SST4 selectivity. *J. Am. Chem. Soc.* **1998**, *120*, 1368−1373.

(13) Liu, S.; Crider, A. M.; Tang, C.; Ho, B.; Ankersen, M.; Stidsen, C. E. 2-pyridylthioureas: Novel nonpeptide somatostatin agonists with SST4 selectivity. *Curr. Pharm. Des.* **1999**, *5*, 255−263.

(14) Scicinski, J. J.; Barker, M. D.; Murray, P. J.; Jarvie, E. M. The solid phase synthesis of a series of trisubstituted hydantoin ligands for the somatostatin SST_5 receptor. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 3609−3614.

(15) Zhou, C.; Guo, L.; Morriello, G.; Pasternak, A.; Pan, Y.; Rohrer, S. P.; Birzin, E. T.; Huskey, S.-E. W.; Jacks, T.; Schleim, K. D.; Cheng, K.; Schaeffer, J. M.; Patchett, A. A.; Yang, L. Nipecotic and *iso*-nipecotic amides as potent and selective somatostatin subtype-2 receptor agonists. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 415−417.

(16) Liapakis, G.; Hoeger, C.; Rivier, J.; Reisine, T. Development of a selective agonist at the somatostatin receptor subtype SSTR1. *J. Pharmacol. Exp. Ther.* **1996**, *276*, 1089−1094.

(17) Reubi, J. C.; Schaer, J. C.; Waser, B.; Hoeger, C.; Rivier, J. A selective analogue for the somatostatin sst1-receptor subtype expressed by human tumors. *Eur. J. Pharmacol.* **1998**, *345*, 103−110.

(18) Albers, A. R.; O'Dorisio, M. S.; Balster, D. A.; Caprara, M.; Gosh, P.; Chen, F.; Hoeger, C.; Rivier, J.; Wenger, G. D.; O'Dorisio, T. M.; Qualman, S. J. Somatostatin receptor gene expression in neuroblastoma. *Regul. Pept.* **2000**, *88*, 61−73.

(19) Tran, T.-A.; Mattern, R.-H.; Afargan, M.; Amitay, O.; Ziv, O.; Morgan, B. A.; Taylor, J. E.; Hoyer, D.; Goodman, M. Design, synthesis, and biological activities of potent and selective somatostatin analogues incorporating novel peptoid residues. *J. Med. Chem.* **1998**, *41*, 2679−2685.

(20) Tran, T.-A.; Mattern, R.-H.; Morgan, B. A.; Taylor, J. E.; Goodman, M. Synthesis and binding potencies of cyclic hexapeptide analogs of somatostatin incorporating acidic and basic peptoid residues. *J. Pept.Res.* **1999**, *53*, 134−145.

(21) Miller, C.; Rivier, J. Peptide chemistry: Development of high-performance liquid chromatography and capillary zone electrophoresis. *Biopolymers* **1996**, *40*, 265−317.

(22) Miller, C.; Rivier, J. Analysis of synthetic peptides by capillary zone electrophoresis in organic/aqueous buffers. *J. Pept. Res.* **1998**, *51*, 444−451.

(23) Reubi, J. C.; Schaer, J. C.; Waser, B.; Wenger, S.; Heppeler, A.; Schmitt, J. S.; Mäcke, H. R. Affinity profiles for human somatostatin receptor sst1-sst5 of somatostatin radiotracers selected for scintigraphic and radiotherapeutic use. *Eur. J. Nucl. Med.* **2000**, *27*, 273−282.

(24) Reubi, J. C. In vitro identification of vasoactive intestinal peptide receptors in human tumors: Implications for tumor imaging. *J. Nucl. Med.* **1995**, *36*, 1846−1853.

(25) Rivier, J.; Brazeau, P.; Vale, W.; Guillemin, R. Somatostatin analogs: Relative importance of the disulfide bridge and of the Ala-Gly side chain for biological activity. *J. Med. Chem.* **1975**, *18*, 123−126.

(26) Rivier, J.; Brown, M.; Vale, W. [D-Trp^8]-somatostatin: An analog of somatostatin more potent than the native molecule. *Biochem. Biophys. Res. Commun.* **1975**, *65*, 746−751.

(27) Sarantakis, D.; McKinley, W. A.; Jaunakais, I.; Clark, D.; Grant, N. H. Structure activity studies on somatostatin. *Clin. Endocrinol.* **1976**, *5*, 275s−278s.

(28) Brown, M.; Rivier, J.; Vale, W. W. Somatostatin analogs wih selected biologic activities. *Metabolism* **1976**, *25*, 1501−1503.

(29) Rivier, J.; Brown, M.; Rivier, C.; Ling, N.; Vale, W. Hypothalamic hypophysiotropic hormones: review on the design of synthetic analogs. In *Peptides 1976;* Loffet, A., Ed.; Editions de l'Université: Bruxelles, Belgium, 1976; pp 427−521.

(30) Parmar, R. M.; Chan, W. W.-S.; Dashkevicz, M.; Hayes, E. C.; Rohrer, S. P.; Smith, R. G.; Schaeffer, J. M.; Blake, A. D. Nonpeptidyl somatostatin agonists demonstrate that sst2 and sst5 inhibit stimulated growth hormone secretion from rat anterior pituitary cells. *Biochem. Biophys. Res. Commun.* **1999**, *263*, 276−280.

(31) Meyers, C. A.; Coy, D. H.; Huang, W. Y.; Schally, A. V.; Redding, T. W. Highly active position eight analogues of somatostatin and separation of peptide diastereomers by partition chromatography. *Biochemistry* **1978**, *17*, 2326−2330.

(32) Rivier, J.; Porter, J.; Hoeger, C.; Theobald, P.; Craig, A. G.; Dykert, J.; Corrigan, A.; Perrin, M.; Hook, W. A.; Siraganian, R. P.; Vale, W.; Rivier, C. Gonadotropin releasing hormone antagonists with N^{ω}-triazolyl-ornithine, -lysine or -para-aminophenylalanine residues at positions 5 and 6. *J. Med. Chem.* **1992**, *35*, 4270−4278.

(33) Nestor, J. J., Jr.; Tahilramani, R.; Ho, T. L.; McRae, G. I.; Vickery, B. H. Potent, long-acting luteinizing hormone-releasing hormone antagonists containing new synthetic amino acids: N,N′-dialkyl-D-homoarginines. *J. Med. Chem.* **1988**, *31*, 65−72.

(34) Nutt, R. F.; Curley, P. E.; Pitzenberger, S. M.; Freidinger, R. M.; Saperstein, R.; Veber, D. F. Novel conformationally constrained amino acids as lysine-9 substitutions in somatostatin analogs. In *Peptides: Structure and Function, Proceedings of the Ninth American Peptide Symposium*; Deber, C. M., Hruby, V. J., Kopple, K. D., Eds.; Pierce Chemical Company: Rockford, IL, 1985; pp 441−443.

(35) van den Berg, E. M. M.; Jans, A. W. H.; Van Binst, G. Conformational properties of somatostatin. VI. In a methanol solution. *Biopolymers* **1986**, *25*, 1895−1908.

(36) Wynants, C.; Van Binst, G.; Loosli, H. R. SMS 201−995, a very potent analogue of somatostatin. Assignment of the ^1H 500 MHz NMR spectra and conformational analysis in aqueous solution. *Int. J. Pept. Protein Res.* **1985**, *25*, 608−614.

(37) Nutt, R. F.; Saperstein, R.; Veber, D. F. Structural and conformational studies regarding tryptophan in a cyclic hexapeptide somatostatin analogue. In *Peptides, Structure and Function, Proceedings of the Proceedings of the Eighth American Peptide Symposium*; Hruby, V. J., Rich, D. H., Eds.; Pierce Chemical Company: Rockford, IL, 1983; pp 345−348.

(38) Hirst, B. H.; Shaw, B.; Meyers, C. A.; Coy, D. H. Structure−activity studies with somatostatin: the role of tryptophan in position 8. *Regul. Peptides* **1980**, *1*, 97−113.

(39) Veber, D. F. Design and discovery in the development of peptide analogs. In *Peptides: Chemistry and Biology, Proceedings of the Twelfth American Peptide Symposium*; Smith, J. A., Rivier, J. E., Eds.; Pierce Chemical Company: Rockford, IL, 1991; pp 1−14.

(40) Rivier, J.; Rivier, C.; Perrin, M.; Porter, J.; Vale, W. GnRH Analogs Structure−activity relationships. In *LHRH Peptides as Female and Male Contraceptives*; Zatuchni, G. I., Shelton, J. D., Sciarra, J. J., Eds.; Harper and Row: Philadelphia, PA, 1981; pp 13−23.
(41) Krenning, E. P.; Kwekkeboom, D. J.; Pauwels, S.; Kvols, L. K.; Reubi, J. C. Somatostatin receptor scintigraphy. In *Nuclear Medicine Annual 1995;* Freeman, L. M., Ed.; Raven Press: Ltd.: New York, 1995; pp 1−50.
(42) Reubi, J. C.; Schaer, J.-C.; Wenger, S.; Hoeger, C.; Erchegyi, J.; Waser, B.; Rivier, J. SST3-selective potent peptidic somatostatin receptor antagonists. *Proc. Natl. Acad. Sci. U.S.A.* **2000**, *97*, 13973−13978.
(43) Jiang, G.; Stalewski, J.; Galyean, R.; Dykert, J.; Schteingart, C.; Broqua, P.; Aebi, A.; Aubert, M. L.; Semple, G.; Robson, P.; Akinsanya, K.; Haigh, R.; Riviere, P.; Trojnar, J.; Junien, J. L.; Rivier, J. E. GnRH antagonists: A new generation of long acting analogues incorporating urea functions at positions 5 and 6. *J. Med. Chem.* **2001**, *44*, 453−467.
(44) Hocart, S. J.; Jain, R.; Murphy, W. A.; Taylor, J. E.; Coy, D. H. Highly potent cyclic disulfide antagonists of somatostatin. *J. Med. Chem.* **1999**, *42*, 1863−1871.
(45) Vale, W.; Rivier, C.; Brown, M.; Rivier, J. Pharmacology of TRF, LRF and somatostatin. In *Hypothalamic Peptide Hormones and Pituitary Regulation: Advances in Experimental Medicine and Biology*; Porter, J. C., Ed.; Plenum Press: New York, 1977; pp 123−156.
(46) Horiki, K.; Igano, K.; Inouye, K. Amino acids and peptides. Part 6. Synthesis of the Merrifield resin esters of N-protected amino acids with the aid of hydrogen bonding. *Chem. Lett.* **1978**, *2*, 165−168.
(47) Rivier, J. E.; Jiang, G.; Porter, J.; Hoeger, C.; Craig, A. G.; Corrigan, A. Z.; Vale, W. W.; Rivier, C. L. GnRH antagonists: novel members of the azaline B family. *J. Med. Chem.* **1995**, *38*, 2649−2662.
(48) Kaljuste, K.; Undén, A. New method for the synthesis of *N*-methyl amino acids containing peptides by reductive methylation of amino groups on the solid phase. *Int. J. Pept. Protein Res.* **1993**, *42*, 118−124.
(49) Stewart, J. M.; Young, J. D. Solid-Phase Peptide Synthesis. In *Solid Phase Peptide Synthesis*; Pierce Chemical Co.: Rockford, IL, 1984; p 176.
(50) Kaiser, E.; Colescott, R. L.; Bossinger, C. D.; Cook, P. I. Color test for detection of free terminal amino groups in the solid-phase synthesis of peptides. *Anal. Biochem.* **1970**, *34*, 595−598.
(51) Krchnak, V.; Vagner, J.; Safar, P.; Lebl, M. Noninvasive continuous monitoring of solid phase peptide synthesis by acid−base indicator. *Int. J. Pept. Protein Res.* **1988**, *32*, 415−416.

JM010037+