Receptor-Specific Somatostatin Analogs: Correlations With Biological Activity

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A number of cyclic and linear somatostatin (SRIF) analogs have now been found to have promising levels of selectivity for rodent somatostatin receptors ($rsst_{2,3,5}$), but not sst_1 and sst_4. Comparisons between binding affinities for these and transfected human receptors are just beginning to emerge and we present results from a comparison of affinities of several key families of peptides for sst_2 present on rat AR42J cells and on cells transfected with human (h)sst_2. The typical cyclic octapeptide analogs, octreotide, lanreotide, and RC-160, exhibited similar affinities to SRIF for $rsst_2$, but somewhat lower affinities for the human receptor. Affinities of several analogs for transfected $hsst_5$ were also measured. As with the rat receptor, octreotide-related analogs had low affinity for $hsst_5$. The highly specific $rsst_5$ analog, DC-23-99, was less so for the human receptor; however, a D-Tyr[1] version of DC-23-99 had subnanomolar affinity (K_i, 0.68 nmol/L) and high selectivity. A new extended-ring analog, BIM-23268D, showed superior affinity to DC-23-99 and even to SRIF and SRIF-28 for $hsst_5$ (K_i, 0.38 nmol/L), and had the highest sst_5/sst_2 selectivity ratio of any analog that we have tested thus far.

THE ISOLATION and cloning of five typical seven-transmembrane spanning somatostatin (SRIF) receptors (ssts)[1-4] has dramatically increased research activity in the area of SRIF analog chemistry and pharmacology. Screening[5,6] of existing libraries of analogs on cells transfected with the five rodent (r)ssts has revealed several families of cyclic and linear peptides with considerable selectivity for $rsst_{2,3}$ and $rsst_5$ (new nomenclature) and little affinity for $rsst_1$ and $rsst_4$: SRIF—Ala-Gly-[Cys-Lys-Asn-Phe-Phe-Trp-Lys-Thr-Phe-Thr-Ser-Cys] DC-25-100 D-NaI-[Cys[Tyr-D-Trp-Lys-Val-Cys]-Thr-NH_2 (type 2); NC-8-12—D-Phe-[Cys-Tyr-D-Trp-Lys-Abu-Cys]-NaI-NH_2(type 2); DC-25-12—D-Phe-Phe-Tyr-D-Trp-Lys-Val-Phe-Thr-NH_2 (type 3,5); DC-23-99—D-Phe-Phe-Phe-D-Trp-Lys-Thr-Phe-Thr-NH_2 (type 5); and DC-25-20—D-Phe-Phe-Tyr-D-Trp-Lys-Val-Phe-D -NaI-NH_2 (type 3).

These analogs have now been used extensively to examine which receptors might be specifically involved in normal physiological processes known to be regulated by endogenous SRIF and some of the results are summarized in Table 1 together with literature references.

However, it is clear that additional analog studies are necessary to develop more specific compounds. It has also become apparent that there is a lack of correlation[11] between binding affinities derived from transfected rodent and human receptors, so that existing and new analogs should be examined for binding to cells transfected with human receptors to ensure that the rat data will reasonably extrapolate to human physiological systems.

COMPARISON OF RAT AND HUMAN sst_2-BINDING PROFILES

Table 2 contains a small subset of K_i data obtained from comparison of binding affinities of a large number of SRIF analogs to sst_2 on rat AR42J cells and on cells transfected in the Biomeasure Laboratories with the human (h)sst_2. The three peptides in the octreotide series, octreotide itself, lanreotide, and RC-160, all have similar affinities to SRIF for $rsst_2$; however, the trend appears to be towards slightly lower affinity for the human receptor using these and most similar analogs tested. One of the few analogs to retain full affinity for the human receptor was the cyclic Merck analog, MK-678. A typical linear peptide with high sst_2 affinity, it exhibited a similar binding profile to the octreotide-type analogs.

Thus, one would expect a good correlation between the sst_2-mediated biological activities observed for the octreotide series in rats and humans, and, of course, this has proved to be the case in the numerous clinical trials conducted on several of these analogs. This is serendipitous, since they were developed solely on rat data and, as will be seen, correlations between at least one other species pair of ssts is much poorer.

Table 1. Rodent ssts Probably Associated With Major Biological Activities

Type of Activity	Receptor	Reference
GH inhibition	2	5
Gastric acid inhibition	2	7,8
Glucagon inhibition	2	9
Insulin inhibition	5	9
Amylase inhibition	5	7
Gastric smooth muscle	3	10

Table 2. Comparison of Binding Affinities of Typical High-Potency GH-Release–Inhibiting SRIF Analogs to sst_2 on Rat AR42J Cells and CHO-1 Cells Transfected With the Human Receptor

Analog	K_i $rsst_2$ (nmol/L)	K_i $hsst_2$ (nmol/L)
SRIF	0.17	0.19
SRIF-28	0.23	0.24
Octreotide	0.29	0.57
Lanreotide (BIM-23014)	0.34	0.86
RC-160	0.21	0.69
MK-678	0.16	0.12
D-Phe-Cpa-Tyr-D-Trp-Lys-Thr-Phe-ThrNH_2	0.39	0.75

SRIF ANALOG BINDING TO $hsst_5$

As already mentioned, among the several receptor-specific analogs discovered was DC-23-99, which had selective high affinity for $rsst_5$ and $rsst_6$. However, a subsequent

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0026-0495/96/4508-1020$03.00/0

Table 3. Affinities of Several Standard Type 2 Analogs and Rat Type 5–Specific SRIF Analogs for CHO-1 Cells Transfected With $hsst_5$

Analog	K_i $hsst_5$ (nmol/L)	Ratio $hsst_2/hsst_5$
SRIF	0.88	0.21
SRIF-28	0.38	0.63
Octreotide	7.00	0.08
Lanreotide	5.21	0.17
RC-160	7.53	0.09
DC-23-99 (BIM-23052)	1.23	9.75
L-362,855 (BIM-23208D)	0.76	4.36
D-Tyr^1-DC-23-99 (BIM-23126)	0.65	6.33
Cys-Phe-Phe-D-Trp-Lys-Thr-Phe-CysNH_2 (BIM-23268D)	0.38	40.05

report[12] indicated that its affinity for the cloned human receptor was considerably diminished. Additionally, it was found that DC-23-99 (BIM-23052), the type 5-selective analog, while displaying potent inhibitory effects on insulin release in rats, was almost devoid of effects on perfused human pancreas.[13,14] With these points in mind, we have obtained the affinities of a great many analogs for $hsst_5$ and the results with a few key peptides are shown in Table 3.

As a measure of selectivity, the ratio of sst_2/sst_5 binding is also presented. As with $rsst_5$ and $rsst_6$, the octreotide-related peptides have low affinity for $hsst_5$. Our best $rsst_5$-selective analog, BIM-23052, had substantially lower affinity for the human receptor. The cyclic Merck analog, L-362,856 (BIM-23208D), which had high affinity for the rat receptor,[5] seemed to retain high affinity for the $hsst_5$ in our system as was reported in assays by other groups.[12] Much work has been performed to improve analog affinities for $hsst_5$ and, if possible, retain or improve selectivity for this receptor. A D-Tyr^1 version of DC-23-99 offered some improvement in affinity (Table 3) and retained similar selectivity; however, a new analog (BIM-23268D, Table 3) was much superior to other analogs tested. This had a K_i of 0.38 nmol/L, which was lower than SRIF and equivalent to SRIF-28. Dose-response curves are shown in Fig 1. It also had the highest selectivity of any analogs thus far tested, with 40-fold selectivity for $hsst_6$ over $hsst_2$. The structure of this analog differs from that of the octreotide series in that the disulfide bridge begins and ends at the N and C terminals of the octapeptide, rather than positions 2 and 7. It is thus closely related to the free acid version with the

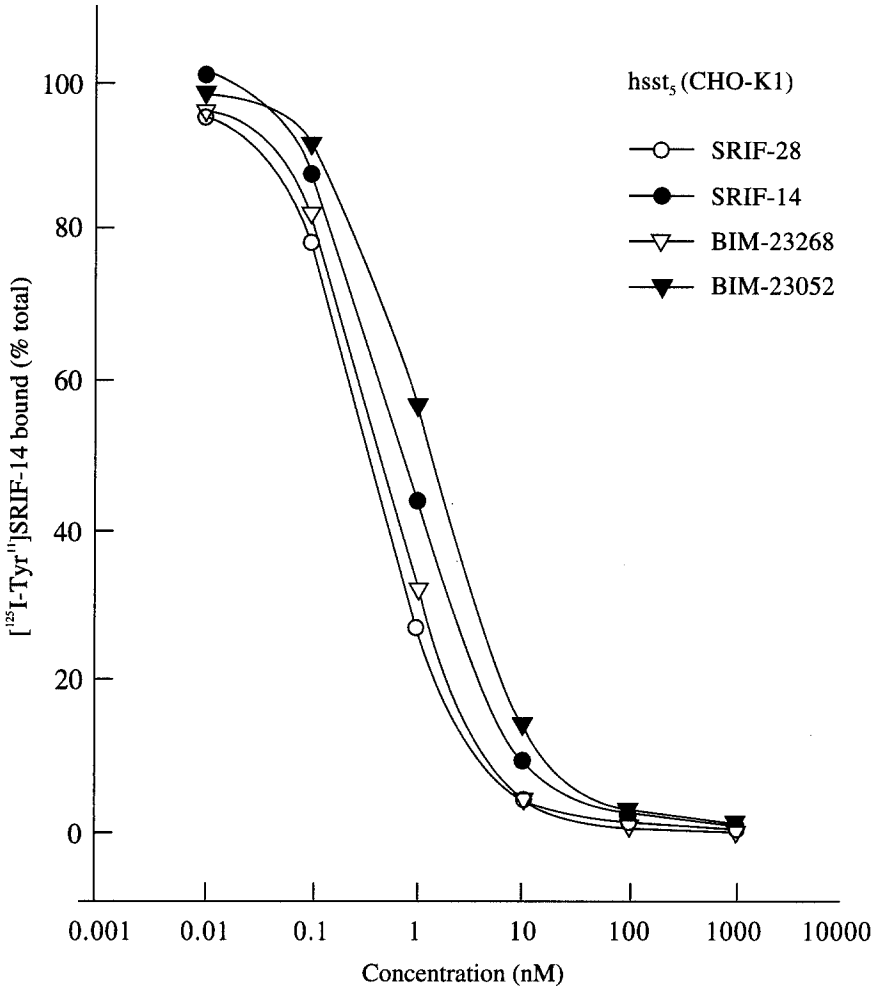

Fig 1. Ability of SRIF-14, SRIF-28, and 2 type 5 receptor-specific analogs to displace radioiodinated SRIF-14 from CHO-K1 cells transfected with the $hsst_5$.

same sequence reported by Vale et al many years ago,[14] which we find has about 10 times less affinity for sst_6. It is expected that the structure of the conformationally restricted peptide amide, differing substantially from the octreotide series, will perhaps enable molecular modeling studies to elucidate some of the unique ligand-binding requirements for the human type 6 receptor.

BIM-23268D has very low potency in the rat inhibition of insulin release assay (Rossowski and Coy, unpublished observations, September 1995), which again points to a major difference in ligand binding requirements between rat and human forms of sst_6. It will be interesting, although admittedly difficult, to determine the biological profile of this analog in humans.

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