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[^{125}I][Tyr^{3}]octreotide labels human somatostatin sst$_2$ and sst$_5$ receptors

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Abstract

Human somatostatin (somatotropin release inhibiting factor = SRIF) receptor subtypes sst$_2$ and sst$_5$ were stably expressed in Chinese hamster lung fibroblast (CCL39) cells. [^{125}I][Tyr^{3}]octreotide labelled with high affinity and in a saturable manner both sst$_2$ ($pK_d = 9.89 \pm 0.02$, $B_{max} = 210 \pm 10$ fmol/mg, $n = 3$) and sst$_5$ sites ($pK_d = 9.64 \pm 0.04$, $B_{max} = 920 \pm 170$ fmol/mg, $n = 3$). The pharmacological profile of sst$_2$ sites established in CCL39 cells using SRIF and various peptide analogues was very similar to that described previously in CHO cells and in human cortex: SRIF$_{14}$ = SRIF$_{28}$ ≥ seglitide > BIM 23014 = RC160 > octreotide > CGP 23996 ≥ L362,855 > BIM 23052 > L361,301 = cortistatin$_{14}$ > BIM 23030 > BIM 23056 > cycloantagonist SA. However, peptides classically perceived as sst$_2$ receptor selective (e.g., seglitide, octreotide, vapreotide) showed also high affinity for human sst$_5$ receptors labelled with [^{125}I][Tyr^{3}]octreotide: SRIF$_{28}$ > seglitide > SRIF$_{14}$ > L361,301 = octreotide > cortistatin$_{14}$ = BIM 23014 = BIM 23052 > L362,855 = RC160 > CGP 23996 > BIM 23056 > cycloantagonist SA > BIM 23030. Further radioligand binding studies were performed with [Leu^{8},D-Trp^{22},^{125}I-Tyr^{25}]SRIF$_{28}$ ([^{125}I]LTT-SRIF$_{28}$) and [^{125}I]CGP 23996. At sst$_2$ receptors, B_{max} values determined with [^{125}I][Tyr^{3}]octreotide, [^{125}I]LTT-SRIF$_{28}$ and [^{125}I]CGP 23996 were in the same range (180–370 fmol/mg). 5′-Guanylyl-imidodiphosphate (GppNHp) displaced all three radioligands to the same extent (85%) and the pharmacological profiles were superimposable. By contrast, at sst$_5$ receptors B_{max} values were very different: [^{125}I][Tyr^{3}]octreotide (920 fmol/mg), [^{125}I]CGP 23996 (3530 fmol/mg) and [^{125}I]LTT-SRIF$_{28}$ (6950 fmol/mg). GppNHp affected [^{125}I][Tyr^{3}]octreotide more than [^{125}I]CGP 23996 binding, whereas [^{125}I]LTT-SRIF$_{28}$ was much less affected. In addition, the affinity values determined in competition experiments at sst$_5$ receptors, varied markedly; whereas SRIF$_{14}$, cortistatin$_{14}$ and SRIF$_{28}$ showed 2-, 4- and 8-fold differences in affinity at sst$_5$ receptors labelled with [^{125}I][Tyr^{3}]octreotide and [^{125}I]LTT-SRIF$_{28}$ compounds such as RC160, L363,301, L362,855, octreotide or CGP 23996 showed between 42- and 123-fold lower affinity when sst$_5$ sites were labelled with [^{125}I]LTT-SRIF$_{28}$. The present data suggest caution to be used when comparing affinity profiles determined in binding studies using different radioligands. In addition, the present results suggest that effects produced by octreotide and related short chain SRIF analogues on hormone release, modulation of tumour growth and central effects may be mediated by either sst$_2$ and/or sst$_5$ receptors.

Keywords: Somatostatin (SRIF); Octreotide; Somatostatin sst$_2$ receptor, recombinant; Somatostatin sst$_5$ receptor, recombinant$_i$; CCL39 Chinese hamster lung fibroblast cells

1. Introduction

Octreotide is a short analogue of the naturally occurring somatostatins (SRIF = somatotropin release inhibiting factor). In mammals, two biologically active forms of somatostatin are derived from the same gene by alternative processing: SRIF$_{14}$ and SRIF$_{28}$ (Reichlin, 1983; Zingg and Patel, 1982; Robbins and Reichlin, 1983; Patel et al., 1985). The complex physiological role of SRIF includes modulation of hormone release, neurotransmission and regulation of other neurotransmitters, as well as inhibition of tumour growth (Reichlin, 1983; Schally, 1988; Buscail et al., 1993, 1994). Recently, a SRIF-related prepropeptide cDNA named preprocortistatin was cloned from rat, mouse and human, and is thought to be cleaved ultimately to cortistatin 14 in rat and to cortistatin 17 in human, respectively, and shares high structural similarity with SRIF$_{14}$ (De Lecea et al., 1996, 1997; Fukusumi et al., 1997). In contrast to SRIF and the preprosomatostatin mRNA, which are widely expressed in brain and periphery (Finley et al., 1981; Reichlin, 1983), expression of preprocortistatin

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mRNA is reported exclusively in brain (De Lecea et al., 1996; Fukusumi et al., 1997).

The various physiological actions of somatostatin are mediated by a class of cell-surface receptors, which belong to the superfamily of G-protein coupled receptors (Bell and Reisine, 1993). Five subtypes have been cloned (Bruno et al., 1992; Kluxen et al., 1992; Li et al., 1992; Meyerhof et al., 1992; O'Carroll et al., 1992, 1994; Rohrer et al., 1993; Vanetti et al., 1992; Yamada et al., 1992a,b, 1993; Yasuda et al., 1992), which are designated as sst_1–sst_5 (Hoyer et al., 1995a). Based on structural and operational features, two classes of SRIF receptors can be distinguished: the $SRIF_1$-family comprising sst_2, sst_3 and sst_5, and the $SRIF_2$-family including sst_1 and sst_4 receptors (Hoyer et al., 1995a). SRIF receptors communicate via G-proteins with distinct signal transduction pathways including inhibition of adenylate cyclase activity (Bell and Reisine, 1993; Patel et al., 1994; Raynor and Reisine, 1992a; Reisine et al., 1995).

SRIF receptors are specifically expressed in brain and many peripheral tissues (Bell and Reisine, 1993; Breder et al., 1992; Kaupmann et al., 1993; Raulf et al., 1994), as well as in a number of tumours (Reubi and Landolt, 1984; Reubi et al., 1987, 1990a,b). The synthetic somatostatin analogue octreotide (SMS 201-995, Sandostatin®, Bauer et al., 1982) has antiproliferative properties in vitro in a number tumour cell lines and in vivo in a variety of hormone-secreting tumours (Cheung and Boyages, 1995; Kubota et al., 1994; Lamberts et al., 1987, 1991; Srikant, 1995). The radionuclide-coupled peptide ([^{125}In]octreoscan, Penteotride®) is used to visualise SRIF receptor-positive tumours (Krenning et al., 1994). Octreotide binds selectively to members of the $SRIF_1$-family of SRIF receptors with preferential affinity to sst_2 and sst_5 receptors (Hoyer et al., 1994; Patel et al., 1994; Raynor et al., 1993a).

The peptide [^{125}I][Tyr^3]octreotide is reported to label exclusively sst_2 receptors in situ (Hoyer et al., 1994; Piwko et al., 1997; Schoeffter et al., 1995). In addition, based on the affinity of octreotide for recombinant receptors (about 5–10 nM), one may not expect the peptide to label sst_5 receptors at the low concentrations used in radioligand binding studies (20–50 pM). In the present study, however, sst_5 receptors stably expressed in CCL39 cells could be labelled with [^{125}I][Tyr^3]octreotide, and the peptide was therefore used as a radioligand to characterise recombinant human sst_5 receptors. Thus, the pharmacological profile of sst_5 sites was established in competition studies and compared to that of sst_2 receptors expressed in the same cell system. Since the sst_5 profile defined using [^{125}I][Tyr^3]octreotide was very similar to that of sst_2 receptors, the studies were extended by using other radioligands in both cell lines, namely [^{125}I]LTT-$SRIF_{28}$ and [^{125}I]CGP 23996. The data suggest that pharmacological profiles determined in binding studies may vary depending on the radioligand and/or the receptor model used.

2. Materials and methods

2.1. Cell culture

CCL39 cells (established line of Chinese hamster lung fibroblasts; American Type Culture Collection) were cultured in a 1:1 mixture of Dulbecco's Modified Eagle's Medium (Seromed, Biochrom, Berlin, Germany; 3.7 g/l $NaHCO_3$; 1.0 g/l sucrose; with stable glutamine) and Ham's F-12 Nutrient Mixture (Seromed; 1.176 g/l $NaHCO_3$; with stable glutamine) supplemented with 10% (v/v) foetal bovine serum (Gibco) and penicillin (100 u/ml final concentration)/streptomycin (100 μg/ml final concentration) (both from Sigma–Aldrich, Deisenhofen, Germany) at 37°C, 5% CO_2 and 95% relative humidity. For passaging, the cells were detached from the cell culture flask by washing with phosphate-buffered saline (PBS) and by brief incubation with trypsin (0.5 mg/ml)/EDTA (0.2 mg/ml) (Gibco). The cells were passaged every 2 days. For storage, the cells were resuspended in medium containing dimethyl sulfoxide (10% final concentration), and frozen in liquid nitrogen.

2.2. Stable transfection

CCL39 cells were used for stable expression of the human SRIF receptor genes. Cells were split 1 day prior to transfection for logarithmic growth. 1.6×10^8 cells resuspended in 800 μl electroporation buffer (272 mM sucrose, 1 mM $MgCl_2$, 7 mM Na_2HPO_4/NaH_2PO_4), pH 7.4, were mixed with 10–80 μg DNA (pcDNAI-$hsst_2$ + pNeo from G.I. Bell, Chicago, USA, or pRc/CMV-$hsst_5$ from S. Seino, Chiba, Japan), and incubated on ice for 10 min. Cells were electroporated at 500 V/25 μF, cooled down to 4°C for 10 min and supplemented with cell culture medium in 260 ml culture flasks. After 2 days, the antibiotic G418 (geneticin sulphate; Gibco) was added to the cell culture medium (0.4 mg/ml 100% active G418 final concentration) for selection of SRIF receptor-expressing cells. Receptor expression of single cell derived colonies was tested by radioligand binding.

2.3. Radioligand binding assay

For crude membrane preparations, cells were harvested by washing with 10 mM HEPES, pH 7.5, scrapping off the culture plates with 4 ml of the same buffer and centrifugation at 4°C for 5 min at $2500 \times g$. The cell pellet was either stored at −80°C or directly used. The cell preparations were resuspended in binding assay buffer (10 mM HEPES, pH 7.5, 0.5% (w/v) bovine serum albumin (BSA) by homogenisation with the Polytron at 50 Hz for 20 s.

In competition experiments, 150 μl of the cell homogenate from CCL39 cells ($hsst_2$: ca. 1.5×10^5 and $hsst_5$: 0.75×10^5 cells) were incubated with 50 μl [^{125}I][Tyr^3]octreotide (2175 Ci/mmol; 25–35 pM; final

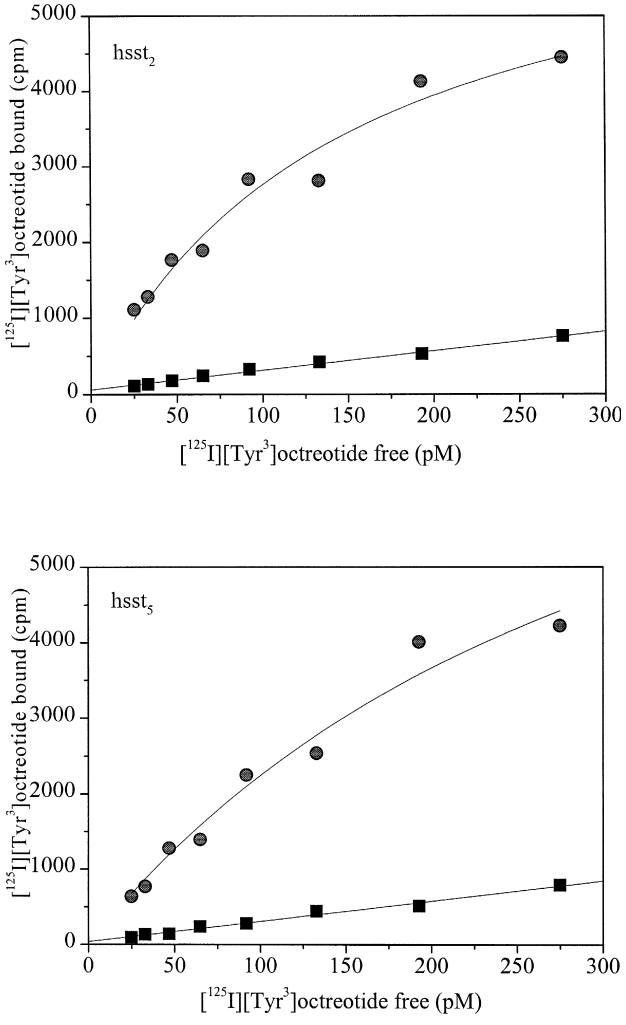

Fig. 1. Saturation curves of [^{125}I][Tyr^3]octreotide binding to membranes prepared from CCL39 cells stably expressing human sst_2 or sst_5 receptors. Crude membrane preparations from sst_2 and sst_5 expressing cells (6 μg or 2 μg per assay, respectively) were incubated with increasing concentrations of [^{125}I][Tyr^3]octreotide and assayed for receptor binding activity. The plots depict specific (●) and non-specific binding (■) expressed as amount of radioligand bound (cpm/assay) vs. free radioligand concentration (pM). The figure shows one representative example of three different experiments.

concentration) in binding assay buffer containing $MgCl_2$ (5 mM) and the protease inhibitor bacitracin (5 μg/ml) and either 50 μl binding assay buffer (total binding) or with 50 μl of various peptide/GppNHp concentrations. Non-specific binding was determined in the presence of $SRIF_{14}$ (1 μM). After 1 h at room temperature, the incubation was terminated by vacuum filtration through glass fibre filters pre-soaked in 0.3% (w/v) polyethyleneimine. The filters were rinsed twice with 5 ml of ice-cold 10 mM Tris–HCl buffer, pH 7.4, and dried. Bound radioactivity was measured in a γ-counter (80% counting efficiency). Data were analysed by nonlinear regression curve fitting with the computer program SCTFIT (De Lean, 1979).

In saturation experiments, 150 μl of cell homogenates were incubated with 50 μl of eight different concentrations (approximately 25–300 pM) of [^{125}I][Tyr^3]octreotide and 50 μl of binding assay buffer (total binding) or 1 μM $SRIF_{14}$ (nonspecific binding). Data were analysed using the computer program SCTFIT (De Lean, 1979). Protein concentration was determined according to Bradford (1976) by means of the BioRad Protein Assay Kit with BSA as a standard. Binding studies with [^{125}I]LTT-$SRIF_{28}$ and [^{125}I]CGP 23996 were performed as described above for [^{125}I][Tyr^3]octreotide.

2.4. Ligands

Names and abbreviations: BIM 23014 (lanreotide, somatuline; D-Nal-c[Cys-Tyr-D-Trp-Lys-Val-Cys]-Thr-NH_2); BIM 23030 (c[Mpr-Tyr-D-Trp-Lys-Val-Cys]-D-Phe-NH_2); BIM 23052 (D-Phe-Phe-Phe-D-Trp-Lys-Thr-Phe-Thr-NH_2); BIM 23056 (D-Phe-Phe-Tyr-D-Trp-Lys-Val-Phe-D-Nal-NH_2); CGP 23996 (c[Asu-Lys-Asn-Phe-Phe-Trp-Lys-Thr-Tyr-Thr-Ser]); cortistatin 14 (Pro-c[Cys-Lys-Asn-Phe-Phe-Trp-Lys-Thr-Phe-Ser-Ser-Cys]-Lys); [Tyr^{10}]cortistatin (Pro-c[Cys-Lys-Asn-Phe-Phe-Trp-Lys-Thr-Lys-Thr-Tyr-Ser-Ser-Cys]-Lys), cycloantagonist (SA, c[Aha-Phe-D-Trp-Lys-Thr(Bzl)]); L363,301 (c[Pro-Phe-D-Trp-Lys-Thr-Phe]); L362,855 (c[Aha-Phe-Trp-D-Trp-Lys-Thr-Phe]); seglitide (MK678; c[N-Me-Ala-Tyr-D-Trp-Lys-Val-Phe]); RC160 (vapreotide; octastatin, D-Phe-c[Cys-Tyr-D-Trp-Lys-Val-Cys]-Trp-NH_2); SMS 201-995 (octreotide; D-Phe-c[Cys-Phe-D-Trp-Lys-Thr-Cys]-Thr-OH); SMS 204-090 ([Tyr^3]octreotide; D-Phe-c[Cys-Tyr-D-Trp-Lys-Thr-Cys]-Thr-OH); $SRIF_{14}$ (Ala-Gly-c[Cys-Lys-Asn-Phe-Phe-Trp-Lys - Thr - Phe-Thr-Ser-Cys]-OH);$SRIF_{28}$(Ser-Ala-Asn-Ser-sn-Pro - Ala-Met-Ala - Pro - Arg - Glu - Arg - Lys - Ala - Gly -

Table 1
Comparison of affinities of SRIF and various SRIF-analogues for human sst_2 and sst_5 receptors labelled with [^{125}I][Tyr^3]octreotide

	CCL39/$hsst_2$	CCL39/$hsst_5$
$SRIF_{28}$	9.99 ± 0.08	10.30 ± 0.25
Seglitide	9.81 ± 0.13	10.18 ± 0.22
$SRIF_{14}$	10.01 ± 0.04	9.87 ± 0.24
[Tyr^{10}]cortistatin	9.00 ± 0.09	9.65 ± 0.22
L361,301	8.39 ± 0.08	9.51 ± 0.14
Octreotide	9.10 ± 0.07	9.48 ± 0.11
$Cortistatin_{14}$	8.35 ± 0.11	9.34 ± 0.23
BIM 23014	9.55 ± 0.03	9.31 ± 0.10
BIM 23052	8.55 ± 0.01	9.28 ± 0.35
L362,855	8.79 ± 0.06	9.17 ± 0.10
RC160	9.50 ± 0.16	9.13 ± 0.35
CGP 23996	8.95 ± 0.07	8.68 ± 0.20
BIM 23056	6.38 ± 0.11	8.32 ± 0.17
Cycloantagonist SA	5.77 ± 0.05	8.25 ± 0.17
BIM 23030	7.94 ± 0.22	7.45 ± 0.18

The data (in this table and Tables 3 and 4) represent the mean of pK_d values ($-\log M$) ± standard error of at least three determinations.

c[Cys-Lys-Asn-Phe-Phe-Trp-Lys-Thr-Phe-Thr-Ser-Cys]-OH); [Leu^8,D-Trp^{22},Tyr^{25}]SRIF$_{28}$ (Ser-Ala-Asn-Ser-Asn-Pro-Ala-Leu-Ala-Pro-Arg-Glu-Arg-Lys-Ala-Gly-c[Cys-Lys-Asn-Phe-Phe-D-Trp-Lys-Thr-Tyr-Thr-Ser-Cys]-OH); [^{125}I]CGP 23996 (c[Lys-Asu-Phe-Phe-Trp-Lys-Thr-(^{125}I-Tyr)-Thr-Ser]); [^{125}I][Tyr^3]octreotide (D-Phe-c[Cys-(^{125}I-Tyr)-D-Trp-Lys-Thr-Cys]-Thr-OH); [Leu^8, D-Trp^{22},^{125}I-Tyr^{25}]SRIF$_{28}$ ([^{125}I]LTT-SRIF$_{28}$ = Ser-Ala-Asn-Ser-Asn-Pro-Ala-Leu-Ala-Pro-Arg-Glu-Arg-Lys-Ala-Gly-c[Cys-Lys-Asn-Phe-Phe-D-Trp-Lys-Thr-(^{125}I-Tyr)-Thr-Ser-Cys]-OH). [Abbreviations: Asu = amino suberic acid; Aha = amino heptanoic acid; Mpr = 3-mercaptopropionic acid; D-Nal = Naphthyl-D-Ala; Bzl = Benzylsubstituent]. 5′-Guanylyl-imidodiphosphate (Gp-pNHp) was from Sigma (St. Louis, Mo). SRIF$_{14}$, SRIF$_{28}$, BIM 23014 and cycloantagonist SA were purchased from Bachem (Bubendorf, Switzerland), RC160 was purchased from Peninsula Laboratories (Heidelberg, Germany), cortistatin$_{14}$ was from Anawa (Wangen, Switzerland). Other ligands were synthesised at Novartis Pharma (Basel, Switzerland). [^{125}I][Tyr^3]octreotide, [^{125}I]LTT-SRIF$_{28}$ and [^{125}I]CGP 23996 were custom labelled by Anawa (Wangen, Switzerland).

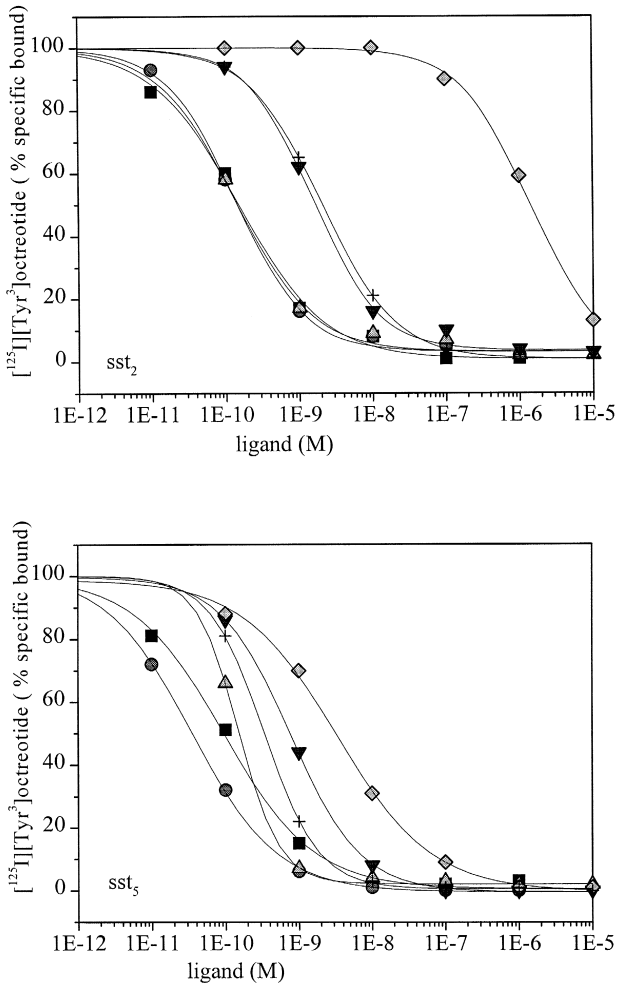

Fig. 2. Competitive radioligand binding on membranes of CCL39 cells expressing human sst$_2$ or sst$_5$ receptors. Crude membrane preparations from sst$_2$ and sst$_5$ transfected cells (6 μg or 2 μg per assay, respectively) were incubated with [^{125}I][Tyr^3]octreotide and the indicated concentrations of SRIF$_{14}$ (■), SRIF$_{28}$ (●), seglitide (△), octreotide (▽) and BIM 23056 (◇) and cortistatin$_{14}$ (+). Data are expressed as percentage of specific binding. The figure shows one representative example of at least three different experiments.

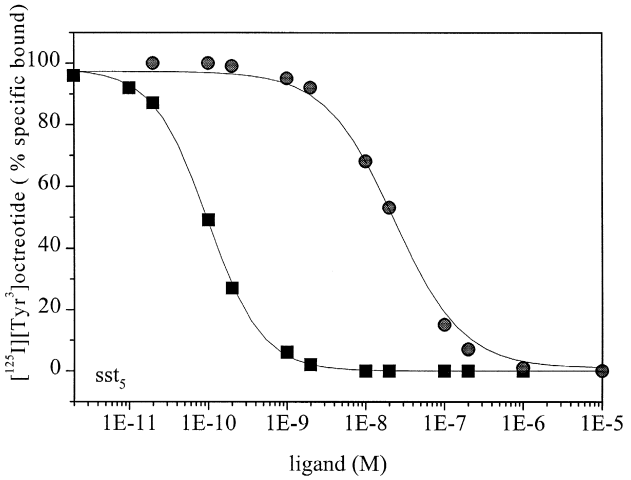

Fig. 3. Competitive displacement by SRIF$_{28}$ and BIM 23056 of radioligand binding at human sst$_5$ receptors: Crude membrane preparations from human sst$_5$ transfected cells (2 μg per assay) were incubated with [^{125}I][Tyr^3]octreotide and 12 concentrations of SRIF$_{28}$ (■) or BIM 23056 (●). Data are expressed as percentage of specific binding. The figure shows one representative example of at least three different experiments.

3. Results

[^{125}I][Tyr^3]octreotide showed high specific binding to the human somatostatin receptor subtypes sst$_2$ and sst$_5$, nonspecific binding was comparatively low. Saturation experiments performed with [^{125}I][Tyr^3]octreotide suggested labelling of a single population of binding sites (Fig. 1) in CCL39 cells expressing human sst$_2$ ($pK_d = 9.89 \pm 0.02$, $B_{max} = 210 \pm 10$ fmol/mg, $n = 3$) and sst$_5$ receptors ($pK_d = 9.64 \pm 0.04$, $B_{max} = 920 \pm 170$ fmol/mg, $n = 3$). No specific binding, i.e., no endogenous SRIF receptors could be detected in non-transfected CCL39

Table 2
Results of saturation experiments performed with different radioligands at human sst$_2$ and sst$_5$ receptors expressed in CCL39 cells

Radioligand	sst$_2$ receptors		sst$_5$ receptors	
	B_{max}	pK_d	B_{max}	pK_d
[^{125}I][Tyr^3]octreotide	210 ± 10	9.89 ± 0.02	920 ± 170	9.64 ± 0.04
[^{125}I]CGP 23996	180 ± 20	9.76 ± 0.06	3530 ± 50	9.52 ± 0.08
[^{125}I]LTT-SRIF$_{28}$	370 ± 60	9.89 ± 0.04	6950 ± 220	10.48 ± 0.04

The data are expressed as B_{max} (fmol/mg) and pK_d values (−log mol/l) ± S.E.M. of at least three experiments.

Table 3
Comparison of affinities of SRIF and various SRIF-analogues for human sst_2 receptors labelled with [^{125}I][Tyr^3]octreotide and other radioligands

Radioligand or tissue	[^{125}I][Tyr^3]-octreotide	[^{125}I]LTT-SRIF$_{28}$	[^{125}I]CGP 23996	Human cortex	CHO cells $hsst_2$
$SRIF_{14}$	10.01 ± 0.04	10.00 ± 0.01	10.10 ± 0.12	10.12	10.50
$SRIF_{28}$	9.99 ± 0.08	9.92 ± 0.03	9.99 ± 0.14	9.66	10.29
Seglitide	9.81 ± 0.13	9.96 ± 0.02	9.82 ± 0.07	10.08	10.64
BIM 23014	9.55 ± 0.03	9.27 ± 0.06	9.43 ± 0.09	8.86	9.67
RC160	9.50 ± 0.16	9.35 ± 0.09	9.56 ± 0.06	8.85	10.23
Octreotide	9.10 ± 0.07	9.19 ± 0.03	9.16 ± 0.23	8.57	9.90
CGP 23996	8.95 ± 0.07	8.58 ± 0.07	9.06 ± 0.05	8.78	9.62
L362,855	8.79 ± 0.06	8.36 ± 0.05	8.69 ± 0.24		
BIM 23052	8.55 ± 0.01	8.30 ± 0.14	8.76 ± 0.46	7.78	8.73
L361,301	8.39 ± 0.08	8.39 ± 0.11	8.28 ± 0.61	8.32	9.27
$Cortistatin_{14}$	8.35 ± 0.11	8.75 ± 0.20	9.04 ± 0.08		
BIM 23030	7.94 ± 0.22	7.77 ± 0.07	7.88 ± 0.03	8.14	9.14
BIM 23056	6.38 ± 0.11	6.33 ± 0.10	6.33 ± 0.14	6.14	6.65
Cycloantagonist SA	5.77 ± 0.05	5.40 ± 0.06	5.80 ± 0.17	5.43	5.90

Human cortex and CHO cell data are from Piwko et al. (1997).

cells (data not shown). RT–PCR revealed only expression of either $hsst_2$ or $hsst_5$ in the stably transfected cells, but not in non-transfected CCL39 cells (data not shown).

[^{125}I][Tyr^3]octreotide was used to determine the affinity of SRIF and a range of SRIF-analogues in competition studies in CCL39 cells expressing $hsst_2$ or $hsst_5$ receptors (Table 1, Figs. 2 and 3); in addition, $hsst_2$ receptor binding data reported previously (Piwko et al., 1997) obtained in CHO cells and human cerebral cortex are also listed (see Table 3). The rank order of potency of [^{125}I][Tyr^3]octreotide labelled $hsst_2$ sites was very similar in human cortex and both CHO and CCL39 cells: $SRIF_{14} = SRIF_{28} >$ seglitide > BIM 23014 = RC160 > octreotide > [Tyr^{10}]cortistatin > CGP 23996 > L362,855 > BIM 23052 > L361,301 = $cortistatin_{14}$ > BIM 23030 > BIM 23056 > cycloantagonist. At $hsst_5$ sites, [^{125}I][Tyr^3]octreotide defined following rank order of affinity: $SRIF_{28}$ > seglitide > $SRIF_{14}$ > [Tyr^{10}] cortistatin = [Leu^8,xd-Trp^{22},Tyr^{25}]$SRIF_{28}$ > L361,301 > octreotide > $cortistatin_{14}$ = BIM 23052 = BIM 23014 > L362,855 = RC160 > CGP 23996 > [Tyr^3]octreotide > BIM 23056 = cycloantagonist > BIM 23030. As expected, the sst_5 receptor showed higher affinity for $SRIF_{28}$ than $SRIF_{14}$. The short cyclic SRIF-analogues seglitide, octreotide, RC160 and BIM 23014 bound all with high affinity (between 0.1 and 1 nM) to both receptor subtypes. There was no evidence from competition experiments that two classes of sites fitted the data better than a single class (Fig. 3). Altogether, the pharmacological profiles of both receptor subtypes as labelled with [^{125}I][Tyr^3]octreotide (see Table 1) are similar with a correlation coefficient of $r^2 = 0.536$ (data not shown).

Since [^{125}I][Tyr^3]octreotide binding was 'atypical', i.e., a number of ligands displayed very similar affinities for both sst_2 and sst_5 receptors, further radioligand binding studies were performed with [^{125}I]LTT-SRIF$_{28}$ and [^{125}I]CGP 23996 at both sst_2 and sst_5 receptors expressing CCL39 cells (see Table 2). [^{125}I]LTT-SRIF$_{28}$ and [^{125}I]CGP 23996 showed high affinity and saturable binding for both sst_2 and sst_5 receptors. Saturation experiments were compatible with the presence of a homogeneous population of recognition sites. At sst_2 receptors, B_{max} values determined with [^{125}I][Tyr^3]octreotide, [^{125}I]LTT-SRIF$_{28}$ and [^{125}I]CGP 23996 were in the same range (180–370 fmol/mg). There were, however, marked differences in B_{max} values at sst_5 receptors (see Table 2): [^{125}I][Tyr^3]octreotide (920 fmol/mg), [^{125}I]CGP 23996 (3530 fmol/mg) and [^{125}I]LTT-SRIF$_{28}$ (6950 fmol/mg). In addition, whereas the affinities of the various ligands for sst_2 sites revealed little differences if any (see Table 3 and top of Fig. 4), there were some notable discrepancies in affinity values at the sst_5 receptors, which appeared to be radioligand dependent (see Table 4 and bottom of Fig. 4). These differences are illustrated in Fig. 4: it can be seen in the top that the competition curves of $SRIF_{14}$ or the 'cycloantagonist' at sst_2 receptors are superimposable whichever radioligand is used. At sst_5 receptors, the competition curves obtained with $SRIF_{14}$ show little variations, whereas those of the cycloantagonist are shifted by a factor 10 from one radioligand to the other. Finally, the effects of GppNHp, a non-hydrolysable GTP analogue, were investigated on the binding of the three radioligands at both sst_2 and sst_5 receptors. GppNHp displaced all three radioligands to the same extent (85%) and similar apparent potency at sst_2 receptors (Fig. 5), whereas again the effects on sst_5 receptors were radioligand dependent. Thus, the binding of [^{125}I][Tyr^3]octreotide was similarly displaced at sst_2 and sst_5 receptors, whereas [^{125}I]CGP 23996 and particularly [^{125}I]LTT-SRIF$_{28}$ binding were less sensitive to the guanine nucleotide analogue.

4. Discussion

Octreotide (SMS 201-995, Sandostatin®) is currently used for treatment of acromegaly, various gastro-intestinal

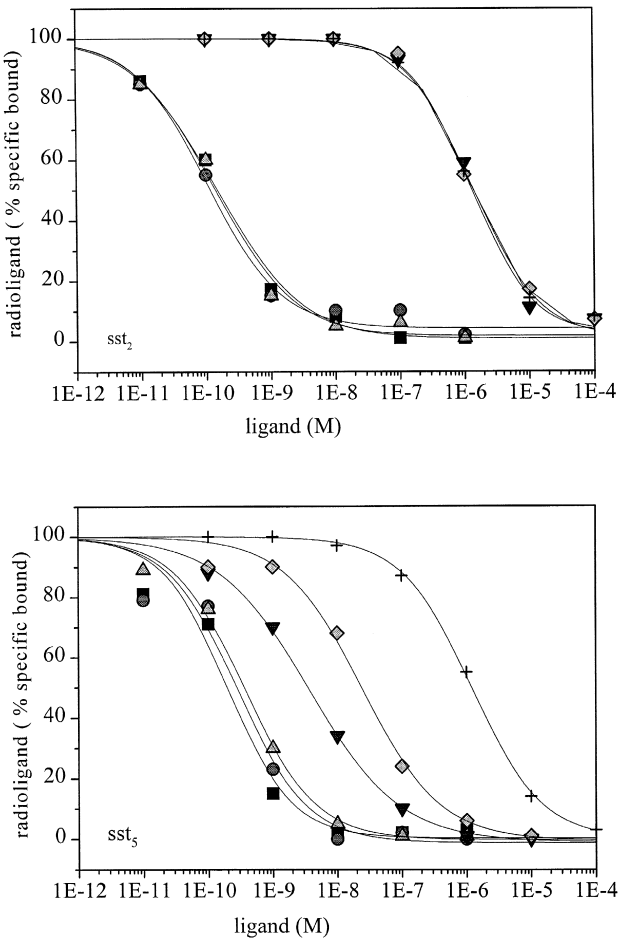

Fig. 4. Binding of [^{125}I][Tyr^3]octreotide, [^{125}I]CGP 23996 and [^{125}I]LTT-SRIF$_{28}$ at sst$_2$ and sst$_5$ receptors is differently affected by SRIF$_{14}$ and the cycloantagonist SA: Crude membrane preparations from sst$_2$ and sst$_5$ transfected cells (6 μg or 2 μg per assay, respectively) were incubated with [^{125}I][Tyr^3]octreotide or [^{125}I]CGP 23996 or [^{125}I]LTT-SRIF$_{28}$ and the indicated concentrations of SRIF$_{14}$ (■, ●, and △ for each radioligand, respectively), and cycloantagonist SA (▼, ◇, and +, respectively). Data are expressed as percentage of specific binding. The figure is representative of at least three different experiments.

disorders and cancer in the gastro-entero-pancreatic system. In addition, since a number of tumours respond to octreotide treatment, a radiolabelled analogue (octreoscan, Penteotride®) is used to visualise SRIF receptor bearing tumours. Octreotide was instrumental in the definition of SRIF receptor subtypes, when Reubi and colleagues (Reubi, 1984, 1985; Reubi and Maurer, 1986) were able to differentiate octreotide-sensitive SRIF binding sites from those which are not sensitive to octreotide, the former were called SS-1 and the latter SS-2. Other ligands were used, such as [^{125}I]MK 678 and [^{125}I]CGP 23996 which according to Raynor et al. (Raynor et al., 1992b) were labelling what was called SRIF-1 and SRIF-2 sites and a number of SRIF$_{14}$ and SRIF$_{28}$ radiolabelled analogues. The situation became more complex in 1992 when five SRIF receptor were cloned, but at least it was then convincingly demonstrated that SRIF receptor subtypes exist (see Bell and Reisine, 1993; Hoyer et al., 1995a). It was then established that SRIF analogues, e.g., [^{125}I][Tyr^{11}]SRIF$_{14}$ or [^{125}I]LTT-SRIF$_{28}$ labelled all subtypes. More surprisingly, it was found that [^{125}I]CGP 23996 also labelled all receptor subtypes (Raynor et al., 1993a,b). By contrast it seemed that [^{125}I]MK 678 or [^{125}I][Tyr^3]octreotide would only label the currently designated sst$_2$ receptor (Kluxen et al., 1992; Raynor et al., 1993a). We have investigated that matter further (Schoeffter et al., 1995) and shown that in rat brain and recombinant cells the populations of sites labelled with these two ligands were (1) superimposable with respect to distribution, (2) had close to identical pharmacological profiles in native tissue (e.g., rat brain or human brain, Piwko et al., 1997), (3) that this profile was identical with that of the recombinant sst$_2$ receptor and (4) that the distribution of sites labelled in the rat or human brain was comparable to that of sst$_2$ receptor mRNA. Therefore, it was felt adequate to conclude that [^{125}I][Tyr^3]octreotide and [^{125}I]MK 678 labelled sites represent sst$_2$ receptors.

On the other hand, we have established that under specific salt conditions (120 mM NaCl) the binding sites labelled with [^{125}I][Tyr^{11}]SRIF$_{14}$ in rat cortex (Hoyer et al., 1995b) had a pharmacological profile that could not be distinguished from that of recombinantly expressed sst$_1$ receptors (although the sst$_4$ profile is very close). Thus, one could assume that the so called SS-2 sites of Reubi (1984, 1985) corresponded to sst$_1$ receptors, although in the lung, sst$_4$ receptor show a similar profile (Schloos et al., 1997). However, a number of findings are disturbing: there is indeed great variation in the data reported by different groups (Raynor et al., 1993a,b; Bruns et al., 1994; Patel and Srikant, 1994) on the pharmacological profile of the different receptors, as illustrated at the Ciba Foundation meeting (see Patel et al., 1995; Bruns et al., 1995 and the discussions therein). For instance, it has been reported that [^{125}I]CGP 23996 (Czernik and Petrack, 1983) was labelling so called SRIF-2 sites with profiles and distribution different from SRIF-1 sites (Raynor and Reisine, 1989; Raynor et al., 1992b, 1993a; Martin et al., 1991), whereas Epelbaum et al. (1985) had noticed that the pharmacology and distribution of the sites labelled with [^{125}I]CGP 23996 was very similar to that of sites labelled with [^{125}I][D-Trp^8]SRIF$_{14}$ and this is fully justified since [^{125}I]CGP 23996 was found later to label all five cloned SRIF receptors.

The present paper shows clearly that [^{125}I][Tyr^3]octreotide labels recombinant human sst$_5$ receptors with high affinity although the radioligand was thought to label exclusively sst$_2$ receptors (Hoyer et al., 1994; Piwko et al., 1997; Schoeffter et al., 1995). The pharmacological profile of [^{125}I][Tyr^3]octreotide labelled human sst$_2$ receptors is very similar in transfected CCL39 and CHO cells and native tissue, i.e., human cerebral cortex. On the other hand, [^{125}I][Tyr^3]octreotide-labelled human sst$_5$ sites bound

Table 4
Comparison of affinities of SRIF and various SRIF-analogues for human sst_5 receptors labelled with [^{125}I][Tyr^3]octreotide and other radioligands

Radioligand	[^{125}I][Tyr^3]octreotide	[^{125}I]LTT-SRIF$_{28}$	[^{125}I]CGP 23996
$SRIF_{28}$	10.30 ± 0.25	9.39 ± 0.22	10.15 ± 0.23
Seglitide	10.18 ± 0.22	8.70 ± 0.26	10.22 ± 0.35
$SRIF_{14}$	9.87 ± 0.24	9.53 ± 0.13	9.82 ± 0.19
[Tyr^{10}]cortistatin	9.65 ± 0.22	8.67 ± 0.24	9.77 ± 0.24
[Leu^8,D-Trp^{22},Tyr^{25}]$SRIF_{28}$	9.60 ± 0.02	8.47 ± 0.02	9.70 ± 0.21
L361,301	9.51 ± 0.14	7.69 ± 0.13	8.77 ± 0.09
Octreotide	9.48 ± 0.11	7.17 ± 0.30	8.96 ± 0.10
$Cortistatin_{14}$	9.34 ± 0.23	8.71 ± 0.02	9.24 ± 0.07
BIM 23014	9.31 ± 0.10	7.76 ± 0.13	9.07 ± 0.04
BIM 23052	9.28 ± 0.35	7.92 ± 0.19	9.59 ± 0.14
L362,855	9.17 ± 0.10	7.17 ± 0.30	8.72 ± 0.04
RC160	9.13 ± 0.35	7.51 ± 0.06	8.72 ± 0.25
CGP 23996	8.68 ± 0.20	6.59 ± 0.41	8.26 ± 0.08
[Tyr^3]octreotide	8.41 ± 0.06	6.49 ± 0.01	8.03 ± 0.05
BIM 23056	8.32 ± 0.17	7.17 ± 0.05	7.77 ± 0.09
Cycloantagonist SA	8.25 ± 0.17	6.38 ± 0.23	7.77 ± 0.06
BIM 23030	7.45 ± 0.18	6.02 ± 0.09	7.09 ± 0.05

$SRIF_{28}$ preferentially compared to $SRIF_{14}$, and somewhat surprisingly, showed very high affinity for the SRIF-analogues octreotide, seglitide, RC160, BIM 23014, BIM 23052 and BIM 23056. Due to these 'atypical' features, the pharmacological profiles of sst_2 and sst_5 receptors expressed in CCL39 cells were further investigated by using [^{125}I]LTT-$SRIF_{28}$ and [^{125}I]CGP 23996. There were differences in B_{max} values (see Table 2): at sst_2 receptors, [^{125}I][Tyr^3]octreotide, [^{125}I]CGP 23996 and [^{125}I]LTT-$SRIF_{28}$ labelled about the same number of sites (180–370 fmol/mg); by contrast, the differences were particularly marked at sst_5 receptors, where [^{125}I][Tyr^3]octreotide labelled 920 fmol/mg, [^{125}I]CGP 23996 recognised 3530 fmol/mg and [^{125}I]LTT-$SRIF_{28}$ 6950 fmol/mg, i.e., seven fold a higher value than [^{125}I][Tyr^3]octreotide. Obviously, such discrepancies have already been reported for peptide receptors, e.g., Neurokinin NK1 or opiate receptors (see Hjorth et al., 1996; Schwartz and Rosenkilde, 1996), but were mainly related to the actual nature of the radioligands used, i.e., agonists vs. antagonists. Such a point can, however, not be made here, since the three radioligands and the non-labelled peptides used behave essentially as agonists (with the limitation that none of the actual radioligands exists as cold iodinated form) when assayed in second messenger tests (inhibition of cAMP production). It is commonly assumed that agonists label a high affinity state of the receptor whereas antagonists label all receptors (high and low affinity states), although it can be debated whether two affinity states exists or whether the receptor–ligand–G protein complex exist under multiple forms. In the present case, we have evaluated the effects of GppNHp on the binding of all three radioligands. GppNHp reduced the binding to sst_2 receptors to the same extent as would be expected, since all three ligands define binding sites with similar profile and similar receptor density. Similarly, the binding of [^{125}I][Tyr^3]-octreotide to sst_5 receptors was highly sensitive to GppNHp, whereas that of [^{125}I]CGP 23996 was less affected, and [^{125}I]LTT-$SRIF_{28}$ binding only weakly inhibited by GppNHp. The data are consistent with [^{125}I][Tyr^3]octreotide labelling only a minor part of the sst_5 receptor population which shows high affinity for many of the agonists tested and is almost entirely inhibited by GppNHp. [^{125}I]CGP 23996 labels significantly more sites, which show intermediate affinity for the synthetic ligands and is only partly affected by GppNHp. By contrast, [^{125}I]LTT-$SRIF_{28}$ labels a very large number of receptors (6950 fmol/mg compared to 920 fmol/mg for [^{125}I][Tyr^3]octreotide); but this binding is only little affected by GppNHp and shows low affinity for most of the synthetic analogues of SRIF. Yet, the sites labelled by all three ligands have high affinity for the endogenous peptides (SRIF and cortistatin, see tables and Fig. 4). This kind of behaviour is very common for 'antagonist' ligands (see Teitler et al., 1990), much less so for agonists binding. However, whereas the binding of [^{125}I] pancreatic peptide (PP) to NPY_4 receptors (Walker et al., 1997) is very sensitive to GppNHp, the binding of [^{125}I]peptide YY is not affected by the guanine nucleotide; PP and PYY are considered as the endogenous agonists. Similarly to the present case, [^{125}I]PYY represented only a very minor fraction of the sites labelled by [^{125}I]PP at NPY4 receptors (Walker et al., 1997). Obviously, one could invoke other reasons to explain apparent differences in B_{max} values in cells expressing homogeneous populations of recombinant G-protein receptors such as receptor dimerisation which could be differently affected by agonists as is suggested for Dopamine D2, β2 adrenoceptors or metabotropic glutamate type 5 receptors (see Hebert et al., 1996; Romano et al., 1996), but there is as yet no positive evidence for such behaviour with SRIF receptors.

As can be taken from Table 3, the use of different radioligands has apparently little influence on the affinity

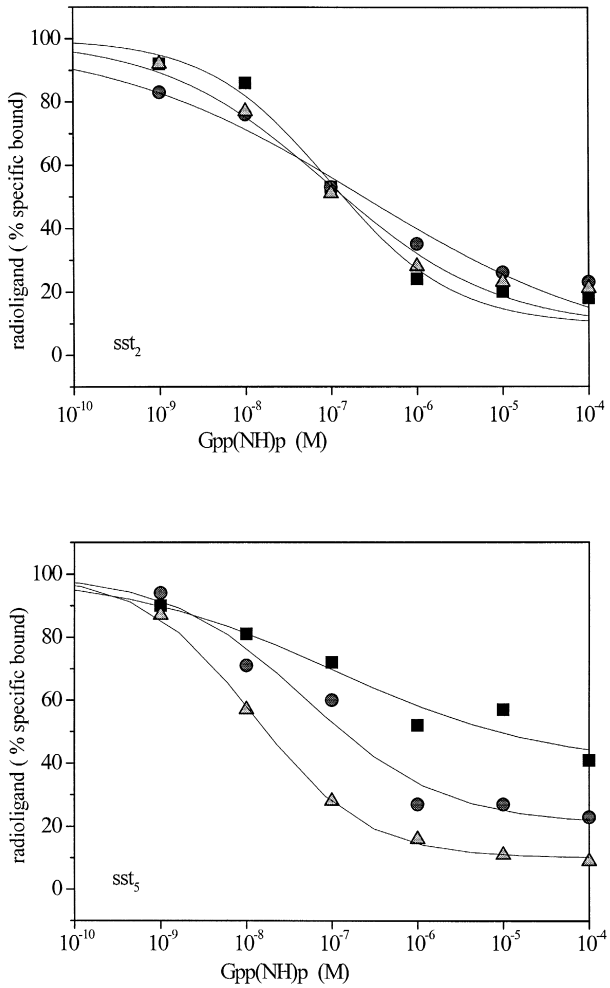

Fig. 5. Binding of [^{125}I][Tyr^3]octreotide, [^{125}I]CGP 23996 and [^{125}I]LTT-SRIF$_{28}$ at sst$_2$ and sst$_5$ receptors is differently affected by the stable guanine nucleotide GppNHp: Crude membrane preparations from sst$_2$ and sst$_5$ transfected cells (6 μg or 2 μg per assay, respectively) were incubated with [^{125}I][Tyr^3]octreotide (△), [^{125}I]CGP 23996 (●) or [^{125}I]LTT-SRIF$_{28}$ (■) and the indicated concentrations of GppNHp at sst$_2$ receptors (top) and sst$_5$ receptors (bottom). Data are expressed as percentage of specific binding. One representative example of at least three independent experiments.

of the tested compounds at hsst$_2$ receptors. Indeed, the correlation coefficients of the profiles defined with the three radioligands are very high: $r^2 = 0.972–0.975$, and the individual values almost identical. In addition, the data are very comparable to those reported previously with the hsst$_2$ receptors expressed in CHO cells and native receptors of human cerebral cortex ($r^2 = 0.939–0.947$, see Piwko et al., 1997). By contrast, the profile determined for human sst$_5$ receptors appeared to be rather radioligand-dependent (Table 4). Thus, whereas the affinity values determined using [^{125}I]CGP 23996 or [^{125}I][Tyr^3]octreotide were similar, affinity values determined using [^{125}I]LTT-SRIF$_{28}$ were clearly lower for some compounds: octreotide, seglitide, L363,301, BIM 23014, BIM 23030, BIM 23052, BIM 23056, CGP 23996, L362,855, RC160, [Tyr^3]octreotide and cycloantagonist (SA) showed up to 100 fold lower affinities. These affinity values were so markedly low that one would not expect [Tyr^3]octreotide or CGP 23996 to label the human sst$_5$ receptors at the low concentrations used here (between 25 and 30 pM, whereas the affinity for the non-labelled compounds are about 1000-fold lower). The correlation coefficient obtained when comparing the three sst$_5$ binding profiles were respectively 0.773, 0.833 and 0.922, lower than observed at sst$_2$ receptors. On the other hand, the putative endogenous peptides SRIF$_{14/28}$ and cortistatin were little affected by the use of different radioligands since the differences in affinity values were about 2-, 4- and 8-fold to the most between the extreme values.

Based on the present results, it would appear that the pharmacological profiles of some G-protein coupled receptors may be radioligand-dependent. The surprising findings are that in two similar situations (i.e., sst$_2$ and sst$_5$ receptors expressed in the same CCL39 cells), the results can be so different. Probably, the various agonists are able to induce different kinds of receptor conformations, although this does not apply to every receptor within one family. Post-transcriptional and post-translational modifications, such as protein phosphorylation or glycosylation, may be cell type-specific and as such may affect receptor conformation and influence the binding properties. Coupling to different G-protein isoforms, which may show different expression pattern depending on the cells, could also be implicated in receptor binding properties (Lefkowitz et al., 1993). In any case, the labelling by [^{125}I][Tyr^3]octreotide with similar affinity of human sst$_2$ and sst$_5$ receptors and the very high affinity of small cyclic peptides for the sst$_5$ receptor suggests that in addition to sst$_2$ receptors, sst$_5$ receptors could be responsible for mediating a number of effects of short SRIF analogues which may have been assigned primarily to sst$_2$ receptors. Similarly, in vivo labelling by octreotide analogues may represent both sst$_2$ and sst$_5$ receptor sites. Finally, from the present data, it is not all too surprising to see differences in affinity reported by different groups at the same recombinant receptor and data obtained by different investigators may not be immediately comparable.

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