Binding Properties of Somatostatin Receptor Subtypes

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In the past few years, five different somatostatin (SRIF) receptor subtypes (sst_{1-5}) have been identified, which form a distinct group in the superfamily of G-protein–coupled receptors. The naturally occurring somatostatins SRIF-28, SRIF-25, and SRIF-14 all reveal high-affinity binding for sst_{1-5}. In contrast, short synthetic analogs that are in clinical use, such as SMS 201-995, RC-160, or BIM 23014, primarily interact with the sst_2 subtype. Some SRIF analogs were previously reported to be selective for one SRIF receptor subtype, eg, the sst_2 (MK 678), the sst_3 (BIM 23056), or the sst_5 (BIM 23052, L362-855) subtype. However, when we studied the binding affinities of these SRIF analogs for human (h) sst_{1-5} expressed in either CHO or COS-1 cells, we were unable to confirm these previously reported selectivities. The absence of sst antagonists is a major drawback for investigating the functional role of each sst subtype. We used site-directed mutagenesis to identify amino acids that determine ligand specificity for sst_2. A single Ser305 to Phe mutation in TM VII increased the affinity of $hsst_1$ for SMS 201-995 nearly 100-fold, and when Gln291 was also exchanged to Asn in TM VII of $hsst_1$, almost full sst_2-like binding of SMS 201-995 was obtained. These data may aid in the design and synthesis of new selective type sst ligands. We have identified the expression of sst subtypes in nonclassical SRIF target tissue such as the lung. The pK_i values for SRIF and various SRIF analogs in rat lung tissue preparations were in close correlation with those obtained for CHO cells expressing the sst_4 subtype. Furthermore, reverse transcriptase polymerase chain reaction (RT-PCR) experiments revealed the predominant expression of mRNA specific for sst_4 in mouse, rat, and human lung tissue, confirmed by autoradiographies of rat lung. No specific binding for [^{125}I]Tyr^3-SMS 201-995 was detected, since SMS 201-995 has low affinity for sst_4. In contrast, specific binding of [^{125}I]SRIF-28 to rat lung sections was demonstrated, which could be displaced by unlabelled SRIF-14 and SRIF-28, indicating specific, high affinity binding of this radioligand to sst_4 receptors.

SOMATOSTATIN (SRIF) is an important regulator of endocrine and exocrine secretion, acts as a neurotransmitter/neuromodulator, affects cognitive and behavioral processes, and possesses antiproliferative properties. All of these effects are mediated by high-affinity SRIF receptors (ssts) on target tissues such as the pituitary, pancreas, gastrointestinal tract, brain, and various types of tumor.

For more than 10 years, ligand-binding studies with radiolabeled SRIF analogs and functional studies have suggested the presence of sst subtypes. For several SRIF target tissues, it has been demonstrated that SRIF-28, SRIF-14, and various short synthetic SRIF analogs, such as SMS 201-995, or MK 678, differ in their binding affinities and their ability to inhibit the release of neurotransmitters and/or hormones. Photoaffinity labeling and purification of receptor proteins provided further evidence for the existence of more than one sst subtype.[1]

The definitive proof of multiple sst receptors has been obtained from molecular cloning of five different genes, so far, coding for sst_{1-5}, that belong to the superfamily of G-protein–coupled seven-transmembrane receptors. The overall percentage of amino acid identity among the sst gene family is at least 46% (65% similarity), with the highest levels of sequence identity found in the transmembrane regions. Human (h) ssts have been expressed in cell lines normally not expressing ssts, to characterize their binding properties. Radioligand-binding studies revealed that various SRIF analogs have distinct affinity profiles for the five ssts.[2,3] These binding studies provide a direct approach in the characterization of ligand-receptor interactions and the search for subtype-selective and/or universal sst ligands.

sst-BINDING STUDIES

The five human sst subtypes were transiently or stably expressed in COS-1 or CHO cells to study their binding properties. The binding of [^{125}I]SRIF-28 and [^{125}I]SRIF-14 to $hsst_{1-5}$ was demonstrated to be of high affinity and saturable. The naturally occurring somatostatins SRIF-28, SRIF-25, and SRIF-14 show little difference in their binding affinities for $hsst_{1-5}$ ($pK_i \geq 9.0$), although the rat sst_5 has been reported to have some preferential affinity for SRIF-28.[4] In contrast, SRIF-22 binds with low affinity to all five hsst subtypes ($pK_i \leq 7.9$). When we investigated the binding properties of short synthetic sst ligands, they showed characteristics either as universal ligands (BIM 23052, BIM 23056, and CGP 23996) or were selective for the sst_2/sst_5 subtype (SMS 201-995, BIM 23014, MK 678, L363-301, L362-855, RC-160, and RC-160II). The binding results obtained with $hsst_{1-5}$, expressed in COS-1 and CHO cells, are summarized in Table 1, where two subgroups of related receptors are clearly distinguishable. The $hsst_1$ and $hsst_4$ subtypes, as already suggested by structural similarities, form a distinct subgroup, since their binding affinity for the short synthetic SRIF analogs is very low or nonexistent. The other subgroup, which consists of $hsst_2$, $hsst_3$, and $hsst_5$, binds short SRIF analogs such as SMS 201-995, BIM 23014, or RC-160 with intermediate to high affinity.

The sst_5 subtype stands out in that it displays: (1) the largest species difference in amino acid sequence, eg, with 19% overall difference between rat and human ssts; and (2) significant difference between rat and human sst_5 in binding affinity for short synthetic SRIF analogs such as SMS 201-995. Affinity values (pK_i) for SMS 201-995 have been reported by different laboratories to be 9.7[4] and 9.2[5] using

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Table 1. Binding Affinities of Natural SRIFs and Synthetic Analogs Towards $hsst_{1-5}$ (pK_i values [−log M])

Peptide	Structure	$hsst_1$	$hsst_2$	$hsst_3$	$hsst_4$	$hsst_5$
SS-14	AGc[CKNFFWKTFTSC]	9.2	9.7	9.6	8.9	9.4
SS-25	SNPAMAPRERKAGc[CKNFFWKTFTSC]	9.1	9.5	9.3	9.0	9.7
SS-28	SANSNPAMAPRERKAGc[CKNFFWKTFTSC]	9.2	9.7	9.5	9.1	9.4
SS-22*	DNTVTSKPLNc[CMNYFWKSRTAC]	6.1	6.9	7.0	<7.0	7.9
CGP 23996	c[*Aha* KNFFWKTYTS]	8.4	9.1	9.4	8.7	8.6
BIM 23052	DFFFDWKTFT-NH_2	8.2	8.1	8.7	7.8	8.8
BIM 23056	DFFYDWKTFD*Nal*-NH_2	7.0	6.7	7.0	7.0	7.9
SA	c[*Aha* FDWKT(*Bzl*)]	6.4	5.6	6.4	5.5	6.5
SMS 201-995	DFc[CFDWKTC]T(*ol*)	6.6	9.5	8.3	<6.0	8.3
BIM 23014	D*Nal* c[CYDWKVC]T-NH_2	6.3	9.3	7.2	<6.0	8.4
MK 678	c[*Me* AYDWKVF]	<6.0	10.1	7.5	<6.0	7.9
RC-160	DFc[CYDWKVC]W-NH_2	6.8	10.1	7.7	6.8	8.3
RC-160II	*Ac* DFc[CYDWKVC]W-NH_2	<7.0	8.8	7.0	<7.0	8.3
L362-855	c[*Aha*FWDWKTF]	<7.0	9.1	8.2	<7.0	10.0
L363-301	c[PFDWKTF]	6.2	9.2	6.9	<7.0	7.4

NOTE. Binding assays were performed with cell membranes from CHO ($hsst_{1-4}$) and COS ($hsst_5$) cells as described.[12]
Abbreviations: *Aha,* 7-aminoheptanoic acid; *Nal,* β-(2-naphthyl) alanine; c, cyclo; *Bzl,* benzyl-; capital letters, amino acids, single letter IUB code.
*From Channel catfish.

rat sst_5, but 8.1,[6] 7.8,[7] and 8.2 (Table 1) using $hsst_5$. Therefore, in humans, short synthetic SRIF analogs, such as SMS 201-995, which are in clinical use, primarily interact with sst_2 as their main target receptor.

Some of the aforementioned SRIF analogs (Table 1) were previously reported to be selective for one sst subtype, eg, the sst_2 (MK 678),[3] the sst_3 (BIM 23056),[3] or the sst_5 subtype (BIM 23052 and L362-855).[3,5] However, when we studied the binding affinities of various SRIF analogs for $hssts_{1-5}$ expressed in either CHO or COS-1 cells, we were unable to confirm the selectivities previously reported. None of these compounds displayed selectivity for a single hsst subtype. In conclusion, there appear to be some differences between the pharmacology of rodent and hsst subtypes. However, species differences do not explain all the differences in receptor subtype selectivities reported previously. Bell et al,[8] for example, reported L365-855 to be highly selective for the $hsst_5$ (IC_{50} = 0.005 nmol/L), which could not be confirmed in our laboratory, also using $hsst_5$ (Table 2).

Despite the fact that many results regarding the binding properties and the distribution of sst subtypes have been reported for brain and peripheral tissue,[11] only a few functional responses can be specifically assigned to a single sst subtype. This is largely because there are few, if any, subtype-selective ligands, especially antagonists, available. Although cyclo[7-aminoheptanoyl-Phe-DTrp-Lys-Thr(Bzl)] (SA) was described to be a somatostatin receptor antagonist,[9] it appears, based on our studies (inhibition of growth hormone [GH] release in vitro from primary cultures of rat pituitary cells), that SA and all other SRIF analogs so far described in the literature are agonists. The absence of sst antagonists is a major drawback for investigating the functional role of each sst subtype.

MOLECULAR NATURE OF sst LIGAND SELECTIVITY

To better understand the molecular nature of selective peptide agonist binding to sst_2, we have used a site-directed mutagenesis approach to identify amino acids in the $hsst_2$ that mediate its specific interaction with short-chain SRIF analogs such as SMS 201-995. The goal of this study was to shift the low affinity of $hsst_1$ for SMS 201-995 (pK_i < 7.0) to a high-affinity binding as observed for $hsst_2$, to identify structural components that determine ligand specificity (gain of function mutation). A single Ser305 to Phe mutation in TM VII increased the affinity of $hsst_1$ for SMS 201-995 nearly 100-fold (Table 3). When this mutation was combined with an exchange of Gln291 to Asn in TM VII of

Table 2. Discrepancies Between Binding Data for sst_{1-5} (IC_{50} values [nmol/L])

Peptide	Bruns* $hsst_1$	Reisine[3] $hsst_1$	Bruns* $hsst_2$	Reisine[3] $msst_2$	Bruns* $hsst_3$	Reisine[3] $msst_3$	Bruns* $hsst_4$	Reisine[3] $hsst_4$	Bruns* $hsst_5$	Reisine[3] $hsst_5$	Reisine[3,5] $rsst_5$
SRIF-14	0.7	0.1	0.2	0.3	0.3	0.1	1.3	1.2	0.5	0.2	0.3/0.86
SRIF-28	0.6	0.1	0.2	0.4	0.3	0.1	7.9	0.3	0.4	0.05	0.05/0.23
SMS 201-995	290	>1,000	0.4	0.4†	5.8	150§	>1,000	>1,000	5.6	32	0.2/0.57
MK 678	>1,000	>1,000	0.1	0.01‡	36	268¶	>1,000	>1,000	13	23	1.3/5.5
L362-855	>100	>1,000	1	29	6.2	30	>100	63	0.1	0.016	0.3/0.005
BIM 23014	500	800	0.5	2	61	6	>1,000	>1,000	4	14	0.5/0.1
BIM 23052	6.3	23	10	32	2.1	0.4	16	18	1.6	4	0.004/0.002
BIM 23056	110	>1,000	250	>1,000	100	0.02	95	160	14.1	nd	43/43

NOTE. Binding assays were performed with cell membranes from CHO ($hsst_{1-4}$) and COS ($hsst_5$) cells as described.[12]
*This paper. Binding assays were performed with cell membranes from CHO ($hsst_{1-4}$) and COS ($hsst_5$) cells as described.[12]
†2.4 nmol/L in refs 8, 13; ‡0.2 nmol/L in refs 8, 13; §3 nmol/L in ref 13; ¶12 nmol/L in ref 13.
Abbreviations: hsst, human sst; msst, mouse sst; rsst, rat sst; nd, not determined.

Table 3. Binding of SRIF-14 and SMS 201-995 to Wild-Type Human sst_1/sst_2, and sst_1 Mutants

Receptor	pIC_{50} SRIF-14	pIC_{50} SMS 201-995
sst_1	8.6 ± 0.1 (3)	6.2 ± 0.1 (3)
sst_2	9.4 ± 0.2 (3)	9.3 ± 0.2 (3)
Mutant Ser305	8.9 ± 0.1 (2)	8.0 ± 0.1 (2)
Mutant Q291/S305	8.9 ± 0.3 (5)	8.5 ± 0.3 (3)

sst_1 (Fig 1), almost full sst_2-like binding of SMS 201-995 was obtained (Table 3). These data allowed a refined molecular modeling of the receptor-ligand interaction.[9] Since the mutated sst_1 receptor also displays high-affinity binding for two other SRIF analogs, RC-160 and BIM 23014, these results demonstrate the important role of individual amino acids in determining the binding specificity of subtype-selective peptide agonists for sst subtypes.

sst EXPRESSION IN LUNG TISSUE

The expression of sst subtypes in classical SRIF target tissues such as the brain, pituitary, pancreas, gastrointestinal tract, and human tumors was shown to be tissue-specific, but overlapping.[2,11] We have also identified the expression of sst subtypes in nonclassical SRIF target tissue, such as the lung.[14] The binding affinities determined for SRIF and various SRIF analogs in membrane preparations from rat lung tissue were in close correlation with those obtained for CHO cells expressing the sst_4 subtype (r = .97) (Fig 2A). In accordance with these binding affinities, reverse-transcriptase polymerase chain reaction (RT-PCR) experiments revealed the predominant expression of mRNA specific for sst_4 in mouse, rat, and human lung tissue (Fig 2B). Receptor autoradiographies with tissue sections from rat lung were in line with these results. Similarly, as concluded from RT-PCR and membrane-binding assays, no specific binding for [^{125}I]Tyr^3-SMS 201-995 was detected,

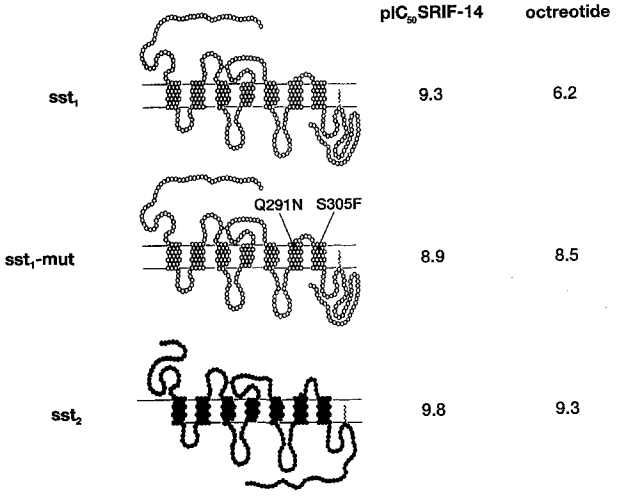

Fig 1. sst_1 mutations conferring high-affinity binding for octreotide (SMS 201-995). The 2 mutations Gln-291 to Asn (Q291N) and Ser-305 to Phe (S303F) were found to be both necessary and sufficient for high-affinity binding of SMS 201-995 to $hsst_1$ mutant. Schematic diagrams of hssts show the locations of the 2 point mutations in the transmembrane domains VI and VII (typical experiment).

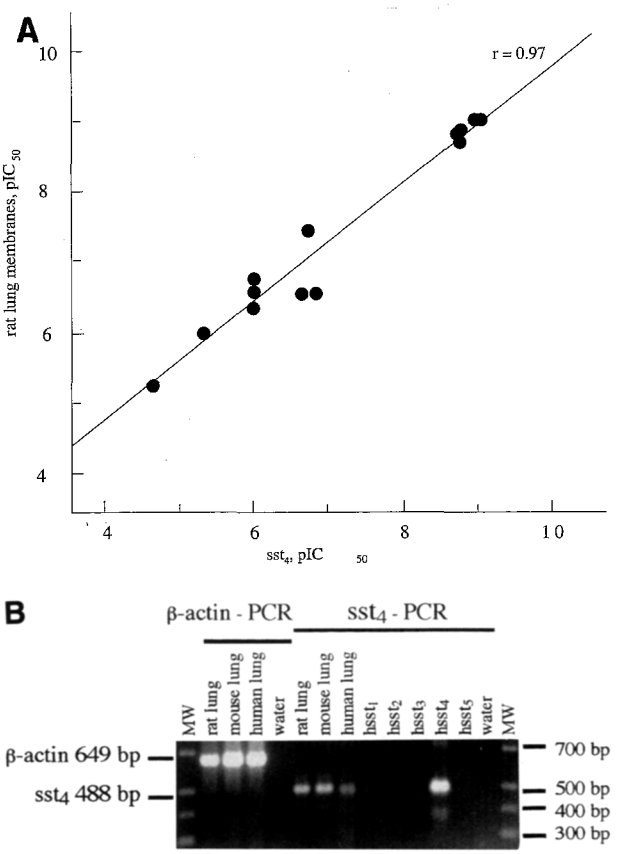

Fig 2. (A) Correlation of the pIC_{50} values of various SRIF analogs determined from competition experiments using rat lung membranes and sst_4 expressed in CHO cell membranes. (B) sst_4 expression in lung: RT-PCR revealed sst_4 mRNA expression in rat, mouse and human lung tissue. Plasmid controls of all 5 hssts demonstrate the sst_4 specificity of the reaction. DNAase treatment of poly(A)$^+$-RNA, reverse transcription, and PCR (with primers HS41/HS44 for sst_4 detection) were performed as described previously.[10]

since SMS 201-995 has low affinity for the sst_4 subtype. In contrast, specific binding of [^{125}I]SRIF-28 to rat lung sections was demonstrated, which could be displaced by unlabeled SRIF-14 and SRIF-28, indicating specific, high-affinity binding of this radioligand (data not shown). The functional role of sst_4 expression in lung tissue remains to be elucidated, although some evidence suggests that ssts might be involved in the modulation of β_2-adrenoreceptor–mediated bronchorelaxation.[15]

CONCLUSION

The availability of the five cloned sst subtypes, $sst_{1\text{-}5}$, will help to further elucidate the physiological role of sst subtypes in the brain and peripheral tissue. Moreover, site-directed mutagenesis studies have allowed the identification of ligand-binding domains and hence the modeling of receptor-ligand interactions. This may aid in the design and synthesis of new sst ligands that bind selectively to distinct sst subtypes. Therefore, the availability of ssts may open up new therapeutic opportunities for the treatment of endocrine and oncological disorders and those of the CNS.

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