Molecular and Functional Properties of Somatostatin Receptor Subtypes

George Liapakis, Melanie Tallent, and Terry Reisine

Identification of the ligand binding domains of the somatostatin (SRIF) receptors may facilitate the rational development of new SRIF ligands. To identify ligand-binding domains of sst_1 and sst_2, we tested a series of chimeras. Using site-directed mutagenesis, we found that to bind with high affinity to sst_2, the sst_2 agonists MK678 and SMS-201-995 require a four amino acid sequence (FDFV) at the border of the third extracellular loop and transmembrane 7. Transference of residue 294 in $msst_2$ to sst_1 conferred onto sst_1 the ability to bind SMS-201-995 and other octapeptides. Cyclic peptides with a phenylalanine adjacent to the D-Trp appear to interact with Phe^{294} of sst_2, whereas hexapeptides with a tyrosine adjacent to the D-Trp, such as MK 678 and BIM 23027, did not interact with the Phe^{294}. We have recently identified a peptide that selectively binds to human (h)sst_1 with 100-fold higher affinity than for the other cloned SRIF receptor subtypes. The second extracellular loop of sst_1 is critical for this peptide to bind. This contrasts with the sites involved in binding of sst_2 agonists and indicates that the two receptors have distinct ligand-binding domains. G proteins couple SRIF receptors to multiple cellular effector systems, including adenylyl cyclase and ionic conductance channels. A critical cellular action of SRIF is the inhibition of Ca^{2+} influx, which may be responsible for its blockade of hormone and neurotransmitter release. Various studies suggest that both sst_2 and sst_5 endogenously expressed in AtT-20 cells can couple to L-type Ca^{2+} channels; the coupling was pertussis toxin–sensitive. The coupling of sst_2 to the Ca^{2+} channels was relatively resistant to desensitisation; 5 hours of pretreatment with MK 678 did not attenuate MK 678 inhibition of the Ca^{2+} current. In contrast, the sst_5 receptors were desensitised by 1 hour of pretreatment with BIM 23052. Thus, the coupling of the two receptors to the Ca^{2+} channel could be differentially regulated. The SRIF receptor subtype coupling to the Ca^{2+} channel could also be distinguished by a unique antagonist, the peptide L362,855, which binds with high affinity to cloned sst_5.

SOMATOSTATIN (SRIF) induces its diverse physiological actions following interaction with a family of receptor subtypes. Five SRIF receptors have been cloned.[1] They have 40% to 45% amino acid sequence identity. They all bind SRIF and SRIF-28 with high affinity. Ligands have been identified that selectively interact with three of the SRIF receptor subtypes, sst_1, sst_2, and sst_5.[2,3] These compounds have been useful in delineating functions of these receptors. Recent studies have suggested that sst_2 may selectively mediate inhibitory effects of SRIF on growth hormone and glucagon release, whereas sst_5 in the rat may selectively mediate inhibitory effects of SRIF on insulin secretion.

Development of SRIF receptor subtype-selective drugs may have important clinical implications in the treatment of metabolic disorders, cancer and CNS diseases, including epilepsy and Alzheimer's disease. Identification of the ligand-binding domains of the SRIF receptors may facilitate the rational development of new SRIF ligands. To identify ligand-binding domains of sst_1 and sst_2, we tested a series of chimeras generated by Drs Dan Fitzpatrick and Richard Vandlen at Genentech (San Francisco, CA) for the binding of selective sst_1 and sst_2 ligands.

LIGAND-BINDING DOMAINS OF SST_1 AND SST_2

Using site-directed mutagenesis, we found that the sst_2 agonists MK678 and SMS-201-995 require a four–amino acid sequence (FDFV) at the border of the third extracellular loop and transmembrane 7 to bind with high affinity to sst_2.[4] The first phenylalanine (residue 294 in $msst_2$) is especially critical for the binding of SMS-201-995 and other octapeptides, since transference of this residue to sst_1 conferred onto sst_1 the ability to bind these peptides. In contrast, this phenylalanine was not essential for the binding of MK 678 and other hexapeptides such as BIM 23027, indicating that these peptides interact with other amino acids in the receptor to bind. Comparison of a series of hexapeptide analogs of MK 678 revealed that those cyclic peptides with a phenylalanine adjacent to the D-Trp appear to interact with Phe^{294} of sst_2. In contrast, those hexapeptides with a tyrosine adjacent to the D-Trp, such as MK 678 and BIM 23027, did not interact with the Phe^{294}. These studies appear to distinguish binding determinants of different classes of hexapeptide analogues of SRIF.

In recent studies in collaboration with Dr Jean Rivier at the Salk Institute (San Diego, CA), we have identified a peptide that selectively binds to sst_1. The peptide exhibits over 100-fold higher affinity for human sst_1 than the other cloned SRIF receptor subtypes. We used this peptide in binding studies on the sst_1/sst_2 chimeras to identify its selective binding domain in sst_1. Our studies revealed that the second extracellular loop of sst_1 is critical for this peptide to bind. This contrasts with the sites involved in binding of sst_2 agonists and indicates that the two receptors have distinct ligand-binding domains. Modeling studies using this information may be useful in the further development of new sst_1 and sst_2 ligands.

G PROTEIN COUPLING OF SRIF RECEPTORS

G proteins couple SRIF receptors to multiple cellular effector systems, including adenylyl cyclase and ionic conductance channels. In an effort to identify which G proteins

From the Department of Pharmacology, University of Pennsylvania School of Medicine, Philadelphia, PA.

Address reprint requests to Professor Terry Reisine, Department of Pharmacology, University of Pennsylvania School of Medicine, 36th Street and Hamilton Walk, Philadelphia, PA 19104-6084.

0026-0495/96/4508-1006$03.00/0

associate with SRIF receptors, Law et al developed an immunoprecipitation technique to solubilize SRIF receptor/G protein complexes, and immunoprecipitate the complexes with antisera directed against the α or β subunits of G proteins.[5] SRIF receptors in rat brain and the cell line AtT-20 that bind the ligand ^{125}I-MK 678 with high affinity were found to predominantly associate with $G_i a1$, $G_i a3$, and $G_o\alpha$, as well as the β1 subunit.[6] The cloned mouse (m)sst_2A expressed in CHO-DG44 cells, which also binds ^{125}I-MK 678 with high affinity, was found to be associated with $G_i a3$ and $G_o\alpha$.[7] The lack of association of this cloned receptor to $G_i\alpha 1$ may explain our inability to show efficient coupling of sst_2A to adenylyl cyclase, since Tallent and Reisine showed that $G_i\alpha 1$ was critical for coupling SRIF receptors to adenylyl cyclase.[8] Furthermore, Law et al[9] showed that $G_i a1$ was critical for coupling the cloned $msst_3$ to adenylyl cyclase. Biochemical studies have shown that SRIF receptors associate with multiple G proteins and functional studies have indicated that different G proteins may couple SRIF receptors to selective cellular effector systems. Thus, specific G proteins may link SRIF receptors to specific cellular effector systems, thereby creating the molecular basis for the diversity of cellular actions of SRIF.

COUPLING OF SRIF RECEPTOR SUBTYPES TO Ca^{2+} CHANNELS

A critical cellular action of SRIF is the inhibition of Ca^{2+} influx, which may be responsible for its blockade of hormone and neurotransmitter release. SRIF reduces Ca^{2+} influx by diminishing Ca^{2+} conductance. In the cell line AtT-20, SRIF has been found to inhibit Ca^{2+} conductance through L-type Ca^{2+} channels. Such cells also express sst_2 and sst_5 mRNA[10] and may therefore express these receptor subtypes. As a result, they can be used as a model system to investigate the coupling of SRIF receptor subtypes to Ca^{2+} channels.

Tallent et al[11] used whole-cell patch clamp techniques to study the effects of SRIF and its analogs on Ca^{2+} currents in AtT-20 cells. They found that MK 678 and other sst_2-selective agonists could reduce the Ca^{2+} currents. Similarly, the sst_5 selective agonist BIM 23052 could also reduce the Ca^{2+} current. These results suggest that both sst_2 and sst_5 endogenously expressed in AtT-20 cells can couple to L-type Ca^{2+} channels.

The coupling of the receptors to the Ca^{2+} channels was pertussis toxin–sensitive. The coupling of sst_2 to the Ca^{2+} channels was relatively resistant to desensitisation, since pretreatment of the cells for up to 5 hours with MK 678 did not attenuate the ability of MK 678 to inhibit the Ca^{2+} current. In contrast, the sst_5 receptors desensitized following 1 hour of pretreatment with BIM 23052. Thus, the coupling of the two receptors to the Ca^{2+} channel could be differentially regulated.

The SRIF receptor subtype coupling to the Ca^{2+} channel could also be distinguished by a novel antagonist. The peptide L362,855 binds with high affinity to cloned sst_5.[3] When applied to AtT-20 cells, it has minimal effects on the Ca^{2+} current. However, it blocked the ability of BIM 23052 to reduce the Ca^{2+} current. Its antagonism of the sst_5 response was selective, since L362,855 did not alter the ability of MK 678 to inhibit the Ca^{2+} current. Preliminary studies have also shown that L362,855 blocks the inhibition of forskolin-stimulated cyclic adenosine monophosphate accumulation by SRIF in CHO cells expressing rat sst_5. L362,855 is the first antagonist at sst_5.

REFERENCES

1. Reisine T, Bell GI: Molecular biology of somatostatin receptors. Endocr Rev 16:427-442, 1995
2. Raynor K, Murphy W, Coy D, et al: Cloned somatostatin receptors: Identification of subtype selective peptides and demonstration of high affinity binding of linear peptides. Mol Pharmacol 43:838-844, 1993
3. Raynor K, O'Carroll A-M, Kong H, et al: Characterisation of cloned somatostatin receptors SSTR-4 and SSTR-5. Mol Pharmacol 44:385-392, 1993
4. Liapakis G, Fitzpatrick D, Codispoti C, et al: Ligand binding domains of the somatostatin receptor SSTR2. Neuroscience 21: 1350, 1995 (abstr)
5. Law S, Woulfe D, Reisine T: Somatostatin receptor activation of cellular effector systems. Cell Signal 7:1-8, 1995
6. Law S, Manning D, Reisine T: Identification of the subunits of GTP binding proteins coupled to somatostatin receptors. J Biol Chem 266:17885-17897, 1991
7. Law S, Yasuda K, Bell GI, et al: Gia3 and Goa selectively associate with the cloned somatostatin receptor subtype SSTR2. J Biol Chem 268:10721-10727, 1993
8. Tallent M, Reisine T: Gia1 selectively couples somatostatin receptors to adenylyl cyclase in the pituitary cell line AtT-20. Mol Pharmacol 41:452-455, 1992
9. Law S, Zaina S, Sweet R, et al: Gia1 selectively couples the somatostatin receptor subtype SSTR-3 to adenylyl cyclase: Identification of the functional domains of this alpha subunit necessary for mediating somatostatin's inhibition of cAMP formation. Mol Pharmacol 45:587-590, 1994
10. O'Carroll A-M, Lolait S, Konig M, et al: Molecular cloning and expression of a pituitary somatostatin receptor with preferential affinity for somatostatin-28. Mol Pharmacol 42:939-946, 1992
11. Tallent M, Liapakis G, O'Carroll A-M, et al: Somatostatin receptor subtypes SSTR-2 and SSTR-5 couple negatively to an L-type Ca^{2+} current in the pituitary cell line AtT-20. Neuroscience 71:1073-1081, 1996