The Cytoplasmic Tail of the Human Somatostatin Receptor Type 5 Is Crucial for Interaction with Adenylyl Cyclase and in Mediating Desensitization and Internalization*

We have investigated the role of the cytoplasmic tail (C-tail) of the human somatostatin receptor type 5 (hSSTR5) in regulating receptor coupling to adenylyl cyclase (AC) and in mediating agonist-dependent desensitization and internalization responses. Mutant receptors with progressive C-tail truncation (∆347, ∆338, ∆328, ∆318), Cys320→Ala substitution (to block palmitoylation), or Tyr304→Ala substitution of a putative NPXXY internalization motif were stably expressed in Chinese hamster ovary K1 cells. Except for the Tyr304→Ala mutant, which showed no binding, all other mutant receptors exhibited binding characteristics (Kd and Bmax) and G protein coupling comparable with wild type (wt) hSSTR5. The C-tail truncation mutants displayed progressive reduction in coupling to AC, with the ∆318 mutant showing complete loss of effector coupling. Agonist pretreatment of wt hSSTR5 led to uncoupling of AC inhibition, whereas the desensitization response of the C-tail deletion mutants was variably impaired. Compared with internalization (66% at 60 min) of wt hSSTR5, truncation of the C-tail to 318, 328, and 338 residues reduced receptor internalization to 46, 46, and 23%, respectively, whereas truncation to 347 residues slightly improved internalization (72%). Mutation of Cys320→Ala induced a reduction in AC coupling, desensitization, and internalization. These studies show that the C-tail of hSSTR5 serves a multifunctional role in mediating effector coupling, desensitization, and internalization. Whereas coupling to AC is dependent on the length of the C-tail, desensitization and internalization require specific structural domains. Furthermore, internalization is regulated through both positive and negative molecular signals in the C-tail and can be dissociated from the signaling and desensitization responses of the receptor.

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The neurohormone somatostatin (SST)1 is synthesized widely in the body and acts as a potent inhibitor of hormone and exocrine secretion as well as a modulator of neurotransmission and cell proliferation (1). These actions are mediated by a family of five G protein-coupled receptors (GPCRs) with seven a helical transmembrane segments termed SSTR1–5 (2). All five SSTRs inhibit adenylyl cyclase. Some of the receptor isotypes also modulate other effectors such as phosphotyrosine phosphatase, K+ and Ca2+ ion channels, a Na+/H+ exchanger, phospholipase C, phospholipase A2, and mitogen-activated protein kinase (2). The five SSTRs display an overlapping pattern of expression throughout the brain and in peripheral organs (2, 3). SSTR2 is the most widely expressed isoform (2, 3). SSTR5 is the predominant subtype in the pituitary and hypothalamus (2–5).

An important property of many GPCRs is their ability to regulate their responsiveness in the presence of continued agonist exposure (6). Such agonist-specific regulation by GPCRs involves a series of discrete cellular steps that include loss of binding affinity and signaling capability due to receptor uncoupling from G proteins (desensitization), receptor internalization, and receptor degradation. Like other GPCRs, SSTRs also appear to be dynamically regulated at the membrane by agonist treatment (2). For instance, during pharmacotherapy with SST analogs in man, the acute effects on pituitary islet and gastric functions subside with continued exposure to the peptide due to the development of tolerance (7). Agonist-dependent internalization of SSTRs occurs in rat pituitary and islet cells and in AtT-20 cells (8–10). In GH3C1 and Rin m5f cells, however, prolonged agonist treatment up-regulates SSTRs (11, 12). These differences may be explained by differential expression of SSTR subtypes since AtT-20 cells express predominantly the SSTR5 subtype, whereas GH3C1 cells are rich in the SSTR1 isotype (13, 14). Furthermore, because pituitary and islet cells or their tumor cell derivatives express multiple SSTR subtypes, it is difficult to determine subtype-selective responses in these systems (4, 5, 19, 20). To circumvent these problems, several recent studies have characterized agonist regulation of individual SSTRs using subtype-selective SST analogs or cell lines stably transfected with SSTR cDNAs (10, 15–18). These studies have shown differential internalization of SSTR2,3,4, and 5 but not of SSTR1 (15–18).

1 The abbreviations used are: SST, somatostatin; LTT SST-28, Leu5-d-Trp28-SST-28; fluo-SST, α-fluoresceinyl-d-Trp28-SST-28; fluro-SST, α-fluoresceinyld-Trp28-SST-14; SSTR, somatostatin receptor; wt hSSTR5, wild type human somatostatin receptor type 5; GPCR, G protein-coupled receptor; TM, transmembrane domain; C-tail, cytoplasmic carboxyl-terminal segment; PCR, polymerase chain reaction; CHO, Chinese hamster ovary; GTPγS, guanosine 5′-O-(thiotriphosphate).
Very little is currently known about the molecular determinants of the desensitization and internalization responses of the SSTR family. For other GPCRs, the presence in the C-tail of Ser and Thr phosphorylation sites as well as Tyr internalization sites is critically important in these processes (6, 19). In the present study we have characterized by mutagenesis the structural domains in the C-tail of hSSTR5 necessary for agonist-dependent desensitization and internalization. We show that the C-tail of hSSTR5 is critical for internalization, receptor coupling to adenylyl cyclase, and acute desensitization responses. Although internalization and signaling both require the C-tail, they are independent functions of the receptor, which appear to be residue-specific in the case of internalization signals is critically important in these processes (6, 19). Very little is currently known about the molecular determinants of the desensitization and internalization responses of the SSTR family. For other GPCRs, the presence in the C-tail of Ser and Thr phosphorylation sites as well as Tyr internalization sites is critically important in these processes (6, 19). In the present study we have characterized by mutagenesis the structural domains in the C-tail of hSSTR5 necessary for agonist-dependent desensitization and internalization. We show that the C-tail of hSSTR5 is critical for internalization, receptor coupling to adenylyl cyclase, and acute desensitization responses. Although internalization and signaling both require the C-tail, they are independent functions of the receptor, which appear to be residue specific in the case of internalization signals is critically important in these processes (6, 19).

EXPERIMENTAL PROCEDURES

Construction of Wild Type Cassette sstr5 cDNA and Mutant sstr5 cDNAs—A hSSTR5 cassette gene consisting of five cDNA fragments corresponding to consecutive segments of hSSTR5 was created as described previously by introducing silent mutations to generate unique restriction sites to facilitate the manipulation of the sequence as discrete restriction fragments (20). Using this construct, a series of point mutations, oligonucleotide primers were used that contain an appropriate restriction fragment in the cassette construct (20). Point mutations were created using the PCR overlap extension technique; for the C-tail-truncated mutants, oligonucleotide primers were used that contain an appropriately placed stop codon (20). Mutated DNA fragments were used to replace the corresponding wild type restriction fragment in the cassette construct in the expression vector pTE8. The structure of the cassette construct and the mutated cDNAs was confirmed by sequence analysis (University Core DNA Service, University of Calgary, Calgary, Alberta, Canada). CHO-K1 cells were transfected with cDNAs for wild type or mutant receptors by the Lipofectin method, and stable G418-resistant nonclonally selected cells were prepared for study.

Binding Assays—CHO-K1 cells expressing wild type and mutant hSSTR5s were cultured to ~70% confluency in D-75 flasks in Ham’s F-12 medium containing 10% fetal calf serum and 700 μg/ml G418. The cells were washed and pelleted by centrifugation, and membranes were prepared by homogenization. Binding studies were carried out for 30 min at 37 °C with 20–40 μg of membrane protein and 2×10−9 M radioligand as described previously (13, 20). To determine G protein coupling of the expressed receptors, the effect of treatment with 10−4 M GTPγS for 30 min on 32P-labeled SST-28 binding to membranes from cells expressing wild type and mutant SSTRs was evaluated. In addition, binding was analyzed after pretreatment of membranes with pertussis toxin (100 ng/ml) for 2 h at 37 °C to determine pertussis toxin sensitivity of the receptor-bound G proteins.

Receptor Coupling to Adenylyl Cyclase—Transfected cells were plated in Falcon 6-well dishes (2×106 cells/well) and used two days later at ~70% confluency. Receptor coupling to adenylyl cyclase was investigated by measuring the dose-dependent inhibitory effects of SST-28 on forskolin-stimulated CAMP accumulation. Cells were exposed to 1 μM forskolin with or without SST-28 (10−6–10−10 M) for 30 min at 37 °C, scraped in 1 ml of ice-cold 0.1 M HCl, and assayed for CAMP by radioimmunoassay. To study agonist-dependent desensitization of adenylyl cyclase response, cells were preincubated for 1 h at 37 °C in binding buffer with or without 100 nM SST-28.
then washed twice with cold binding buffer to remove unbound SST-28. Receptor-bound SST-28 was then stripped by incubation for 10 min at 37 °C in Hanks’ buffered saline acidified to pH 5.0 with 20 mM sodium acetate (acid wash). The cells were washed twice and analyzed along with control cells for receptor coupling to adenylyl cyclase.

Internalization Experiments—Cultured CHO-K1 cells expressing wild type and mutant SSTRs were incubated overnight at 4 °C in binding buffer with 125I-LTT SST-28 (200,000 cpm) with or without 100 nM SST-28 (15). Cells were washed three times with ice-cold HEPES binding buffer containing 5% Ficoll to remove unbound ligand and then warmed to 37 °C for different times (0, 15, 30, and 60 min) to initiate internalization. Surface-bound radioligand was removed by treatment for 10 min with acid wash solution. Internalized radioligand was measured as acid-resistant counts in 0.1 N NaOH extracts of acid-washed cells. Internalization of receptor-bound ligand was also assessed using fluo-SST. This peptide binds with high affinity (IC50 4.9 nM) to SSTRs in rat cortical homogenate and has been reported to undergo agonist-dependent internalization in COS-7 cells transfected with SSTR2A (16). CHO-K1 cells expressing wild type and mutant hSSTR5 receptors were grown to 70% confluency. On the day of the experiment, the culture medium was removed, and the cells were washed and incubated in 1 ml of binding buffer containing 10 nM fluo-SST at 4 °C for 1–2 h. To examine internalization, sister cultures were incubated with fluo-SST under identical conditions for 45 min at 37 °C. At the end of each incubation, media were removed, and the cells were washed, mounted in immunofluor, and viewed under a Zeiss LSM 410 inverted confocal microscope (5, 20). All images were archived on a Bernoulli multitidc and printed on Kodak XLS3000 high resolution printer.

Immunocytochemistry—Confocal immunofluorescence studies were performed to confirm cell surface expression of the Tyr304 → Ala mutant in live unfixed transfected cells using a rabbit polyclonal antibody directed against the amino-terminal 4–11 peptide sequence of hSSTR5 as described previously (5, 20).

RESULTS

Binding Affinity—Table I shows the results of membrane binding analyses of CHO-K1 cells transfected with wt hSSTR5 cassette cDNA and the hSSTR5 C-tail mutant cDNAs. By saturation analysis, wt hSSTR5 displayed high affinity binding with a Kd of 0.31 nM and a Bmax of 162 ± 42 fmol/mg of protein. The Δ328, Δ338, and the Δ347 C-tail truncation mutants as well as the Cys320 → Ala mutants displayed binding affinities of 0.21–0.47 nM, comparable with that of wt hSSTR5. The Δ318 truncation mutant also displayed high affinity ligand binding (Kd 0.89 nM), which, however, was 3-fold lower than that of the wild type receptor. Bmax of the four C-tail deletion mutants ranged between 247 and 352 fmol/mg of protein. 1,2–2-fold higher than that of wt hSSTR5; the Bmax of the Cys320 → Ala mutant (179 ± 51 fmol/mg of protein) was comparable with that of the wild type receptor. No specific binding was observed in membranes prepared from cells transfected with the Tyr304 → Ala mutant. The mutant protein, however, was expressed on the cell membrane as determined by immunocytochemistry using live unfixed cells. Using the hSSTR5 primary antibody, nonpermeabilized CHO-K1 cells expressing wt hSSTR5 showed rhodamine immunofluorescence localized to the cell surface (not shown). Cells transfected with the Tyr304 → Ala mutant also exhibited surface immunofluorescence with this antibody, indicating that the mutant receptor was properly targeted to the plasma membrane. No specific immunofluorescence was detected in nontransfected CHO-K1 cells or in transfected cells probed with preimmune serum or antigen-absorbed primary antibody. To exclude any breach of the plasma membrane and labeling of cytosolic structures beneath the plasma membrane during incubation with primary antibody, parallel immunocytochemistry was performed with antibody to vimentin, an intracellular protein. Under these conditions, vimentin immunoreactivity was detected only in cells permeabilized with 0.2% Triton X-100 but not in intact CHO-K1 cells. These findings suggest that the loss of binding of the Tyr304 → Ala mutant is not due to a failure of the mutant receptor to be localized to the plasma membrane but rather reflects an important structural requirement of the Tyr residue in maintaining a high affinity ligand binding conformation. Loss of agonist binding by this mutant precluded further analysis of the role of the Tyr304 residue as an internalization signal.

G Protein Coupling—To determine whether the hSSTR5 C-tail mutants were coupled to G proteins, the effect of 10−8 M GTPγS on membrane binding was assessed in cells expressing wild type and mutant hSSTR5 receptors (Fig. 2). Pretreatment with GTPγS reduced 125I-LTT SST-28 binding of wt hSSTR5 to 67 ± 2% that of control. The four C-tail truncation mutants as well as the Cys320 → Ala mutant also displayed significant loss of radioligand binding of 50–70% that of control, comparable with that of the wild type receptor. This suggests that the mutant receptors are capable of associating with G proteins. Pretreatment of membranes with pertussis toxin also led to a significant 40–50% reduction of 125I-LTT SST-28 binding to the
wild type and the C-tail mutant SSTRs, suggesting that the mutant receptors associate with pertussis toxin-sensitive G proteins.

**Coupling to Adenylyl Cyclase and Desensitization Responses**—Fig. 3 depicts the results of coupling of the C-tail mutants to adenylyl cyclase. Basal cAMP level in cells expressing the mutant receptors was comparable with that in cells transfected with wt hSSTR5. Compared with the wild type receptor, which showed a maximum of 70 ± 6% inhibition by SST-28 of forskolin-stimulated cAMP accumulation, the C-tail deletion mutants displayed a progressive loss of the ability to inhibit forskolin-stimulated cAMP from 69.8 ± 2% for the Δ347 mutant to 63 ± 3.8% for the Δ338 mutant to 60 ± 3.1% for the Δ328 mutant. The Cys320 → Ala mutant showed only 57 ± 3.4% maximum inhibition of forskolin-stimulated cAMP; the Δ318 showed complete loss of coupling to adenylyl cyclase.

To study agonist-dependent desensitization of adenylyl cyclase responses, parallel studies were carried out in sister cultures preincubated with 100 nM SST-28 for 1 h at 37°C. Surface-bound SST-28 was then removed, and the cells were tested with different concentrations of SST-28 for their ability to inhibit forskolin-stimulated cAMP. Such agonist pretreatment induced a marked loss of the ability of wt hSSTR5 to inhibit forskolin-stimulated cAMP. Such agonist pretreatment induced a marked loss of the ability of wt hSSTR5 to inhibit forskolin-stimulated cAMP from 70 ± 6% in control nontreated cells to 21 ± 5% in treated cells (Fig. 4). The mutant receptors that displayed partial loss of efficiency for adenylyl cyclase coupling also showed variable impairment of their uncoupling responses. The Δ328 and Δ347 mutants both retained some ability to uncouple from adenylyl cyclase, although the range of responses for maximum forskolin-stimulated cAMP inhibition before and after agonist pretreatment (60 ± 3.1% and 40 ± 1.2% for Δ328 mutant; 69.8 ± 2% and 45.4 ± 3.3% for Δ347 mutant) was markedly attenuated compared with the native receptor. The Δ338 mutant displayed virtually complete...
loss of the ability to uncouple from adenylyl cyclase with agonist preexposure (63 ± 4% and 53 ± 2.3% maximum forskolin-stimulated cAMP inhibition). Like the Δ328 and Δ347 mutants, the Cys320→Ala mutant retained some ability to uncouple from adenylyl cyclase inhibition with SST-28 pretreatment but with a blunted response (57 ± 3.4% and 43 ± 2.4% maximum forskolin-stimulated cAMP inhibition) compared with the wild type receptor.

Internalization of Receptor Bound ¹²⁵I-LTT SST-28—Internalization of ¹²⁵I-LTT SST-28 ligand was studied in stably transfected CHO-K1 cells initially treated with ligand for 12 h at 4 °C to allow for equilibrium binding but to limit internalization (Fig. 5). Switching from 4 to 37 °C led to a rapid time-dependent internalization of radioligand that, in the case of wt hSSTR5, reached a maximum of 66 ± 2% at 60 min (Fig. 5). Truncation of the C-tail to 318 and 328 residues produced moderate decreases in receptor internalization to 46% at 60 min. Truncation to 338 residues led to a dramatic loss of radioligand internalization of only 23 ± 3% at 60 min. In contrast, truncation to 347 residues improved the efficiency of internalization even more than that of the wild type receptor (72 ± 3% compared with 66%). This suggests the presence of a positive internalization signal between residues 338 and 347 and a negative signal between 347 and 364 residues and 328 and 338 residues. Mutation of the Cys320 palmitoylation site reduced ligand internalization to 42 ± 3% at 1 h. The extent of the loss of internalization of this mutant was comparable with that of the Δ318 mutant, which also lacked the Cys320 residue and suggests an important function of the palmitoylation anchor in producing the impaired internalization of both these mutant receptors.

The pattern of internalization obtained by radioligand binding was confirmed directly with fluo-SST-14 ligand, whose surface binding and endocytosis in transfected CHO-K1 cells was traced by confocal microscopy. Fig. 6 shows confocal fluorescent images of cells incubated with fluo-SST at either 4 °C (left hand panels) or 37 °C (right hand panels).
Desensitization and Internalization Signals in hSSTR5

Role of C-Tail in Ligand Binding and in Coupling to G Protein and Adenylyl Cyclase—Progressive deletion of the C-tail of hSSTR5 had no effect on high affinity ligand binding, indicating that the C-tail, like that of other GPCRs, does not influence receptor targeting or binding conformation (21). Mutation of the Tyr304 residue, however, produced a receptor protein that was correctly targeted to the plasma membrane but which showed complete loss of binding, suggesting a critical role of Tyr304 in ligand binding through either direct hydrophobic interaction with SST ligand or through an allosteric change in the receptor binding conformation. The C-tail as well as the second and third intracellular loops of several GPCRs have been implicated in G protein interaction (6, 22). Radioligand binding by all of the C-tail mutants of hSSTR5 was inhibited to the same degree as the wild type receptor by GTPγS and pertussis toxin, indicating that the mutant receptors are capable of associating with pertussis toxin-sensitive G proteins and that the C-tail of hSSTR5 is not required for this interaction. Interestingly, despite the ability to associate with G proteins, the four C-tail deletion mutants as well as the Cys320 → Ala mutant displayed reduced efficiency for adenylyl cyclase coupling. This was most pronounced in the case of the ∆318 mutant, which showed a complete loss of the ability to inhibit adenylyl cyclase. Whether this mutant can signal through other effector pathways remains to be seen. There are two other examples of dissociated G protein and effector coupling by C-tail mutants of GPCRs. The first is the C-tail-truncated postaglandin EP3 receptor, which retains the ability to associate with G12 but which shows no forskolin-induced inhibition of cAMP accumulation, identical to the ∆318 hSSTR5 mutant (23). The second is an Ala → Glu substitution in the distal third intracellular loop of the gastrin-releasing peptide receptor, which abrogates phospholipase C coupling while retaining full efficacy for G protein interaction (24). In contrast to the C-tail of hSSTR5, which is required for inhibitory regulation of adenylyl cyclase, the naturally occurring SSTR2B splice variant with a shorter C-tail length than SSTR2A is more efficiently coupled to adenylyl cyclase (25).

Role of C-Tail in Mediating Acute Desensitization—We found that hSSTR5 stably expressed in CHO-K1 cells was desensitized by agonist pretreatment. Phosphorylation of the rat SSTR2A receptor primarily on serine residues and of the rat SSTR3 receptor on both serine and threonine residues in the C-tail has been reported to be crucial for desensitization and internalization of these two subtypes (17, 18). hSSTR5 features three serine (Ser314, Ser325, Ser361) and four threonine (Thr333, Thr347, Thr351, Thr360) residues in the C-tail (Fig. 1). The Ser325 and Thr360 sites fit the consensus sequence for phosphorylation by protein kinase A and protein kinase C, respectively, and the Thr347 position qualifies as a putative G protein-coupled receptor kinase phosphorylation site (26). The third intracellular loop of this receptor displays three additional sites for phosphorylation by second messenger-activated kinases. The ability of the ∆347 mutant to be desensitized by agonist to the same degree as the wild type receptor suggests that the Thr351, Thr360 and Ser361 sites in the distal C-tail play a minimal role in the desensitization response. In contrast, the resistance of the ∆338 mutant to desensitization suggests an important role of Thr347 in the putative G protein receptor kinase phosphorylation site. This role, however, cannot be absolute since the ∆328 mutant, which also lacks the Thr347 residue, underwent significant desensitization. A conserved cysteine residue 11–12 amino acids downstream from the 7th TM is found in the C-tail of most GPCRs and serves as a palmitoylation membrane anchor for a fourth intracellular loop. Palmitoylation induces differential changes in G protein coupling, desensitization, intracellular trafficking, and internalization of different GPCRs (27, 28). In the case of hSSTR5, the Cys320 → Ala mutant displayed poor ability to uncouple from adenylyl cyclase, indicating an important role of C-tail palmitoylation in the desensitization response of this receptor. Overall, these studies suggest that the C-tail plays a prominent role in agonist-induced desensitization of hSSTR5 through both specific motifs, which may serve as sites for phosphorylation, as well as through conformational changes in the C-tail of the agonist-occupied receptor, which may determine its substrate specificity for phosphorylation.

Role of C-Tail in Mediating Receptor Internalization—The C-tail segment of hSSTR5 is not only critical for receptor coupling to adenylyl cyclase and in mediating acute desensitization responses but also plays an important role in regulating agonist-induced receptor internalization. This is in agreement with previous studies that have shown that the C-tail of many other GPCRs, e.g. receptors for angiotensin II1A (29), β2 adrenergic (30), m3 muscarinic (31), luteinizing hormone/human chorionic gonadotrophin (32), parathyroid hormone (33), thyrotrophin releasing hormone (34), neurotensin (35), and cholecystokinin (36), is also involved in internalization. Our results indicate that mutant receptors with variable length C-tails are differentially internalized. Truncation of the C-tail at positions 318 and 328 attenuated receptor internalization only partially from 66 to 46% at 60 min. This suggests that the C-tail distal to position 318, which contains multiple phosphorylation sites including the putative G protein receptor kinase site on Thr347, although important, is not a critical determinant of endocytosis. Furthermore, the comparable rates of internalization of the ∆328 mutant, which contains the putative protein kinase A site at Ser325 and the ∆318 mutant, which does not, excludes a role of the protein kinase A site in agonist-induced hSSTR5 internalization. Truncation of the C-tail to 338 residues led to a dramatic loss of internalization. This mutant has 10 more residues than ∆328 that appear to contain potent negative endocytic signals. The ∆347 deletion mutant internalized slightly more than the wild type receptor, suggesting that the nine-amino acid residue stretch between positions 338 and 347 harbors a positive internalization signal, likely on Thr347 in the putative G protein receptor kinase phosphorylation site. Furthermore, the ability of the ∆347 mutant to internalize more than the wild type receptor argues for a second negative endocytic signal in the extreme C-tail segment distal to residue 347.
Negative endocytic signals have been postulated in the case of the luteinizing hormone/human chorionic gonadotrophin and parathyroid hormone/parathyroid hormone-related protein receptors (32, 33). The EVQ sequence in the membrane-proximal C-tail, which is highly conserved across members of the parathyroid hormone/secretin receptor family has been identified as a negative endocytic signal for this receptor subclass. Point mutations in the 328–338 and 347–363 segment of hSSTR5 C-tail will help to determine whether there are similar structural motifs in this receptor capable of acting as negative endocytic regulators. The palmitoylation-defective hSSTR5 mutant showed reduced internalization comparable with that reported for the thyrotropin-releasing hormone (34) and vasopressin V2 (37) receptors but different from the palmitoylation-deficient luteinizing hormone/human chorionic gonadotrophin receptor, which displays enhanced internalization (38). Tyrosine-based internalization signals on NPXY-type motifs are common to many classes of membrane receptors (39). In the case of GPCRs, a conserved NPXXY sequence at the interface between the VIIth TM and the C-tail serves as an endocytic signal for some receptors (19). In other GPCRs such as the gastrin-releasing peptide and the angiotensin II receptors, signal for some receptors (19). In other GPCRs such as the phinh receptor, which displays enhanced internalization (38).

The luteinizing hormone/human chorionic gonadotrophin and luteinizing hormone/human chorionic gonadotrophin receptors that cannot function as an endocytic signal. 

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**References**

1. Bechtle, S. (1993) *N. Engl. J. Med.* 329, 1495–1501 and 1556–1563
2. Patel, Y. C. (1997) *Trends Endocrinol. Metab.* 8, 398–405
3. Bonifacino, J., Marks, M. S., Ohno, H., and Kirchhausen, T. (1996) *J. Biol. Chem.* 271, 151–156
4. Thomas, W. G., Thekkumkara, T. J., Motel, T. J., and Baker, K. M. (1995) *J. Biol. Chem.* 270, 151–156
5. Hurley, J. H. (1994) *Proc. Natl. Acad. Sci. U. S. A.* 91, 10157–10161
6. Pal et al. (1995) *J. Biol. Chem.* 270, 29004–29011
7. Tolbert, L. M., and Lamm, J. (1998) *J. Neurochem.* 70, 113–119

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**Desensitization and Internalization Signals in hSSTR5**

An important question concerning GPCRs is the relationship between receptor signaling and internalization. Although there has been some controversy in the past, many recent studies have suggested that the two events can be readily dissociated (24, 29, 36, 42, 43). For instance, receptor mutations that inhibit G protein coupling or signaling do not prevent endocytosis (24, 29, 36, 42, 43). The addition of second messengers such as phorbol 12-myristate 13-acetate, Ca²⁺, and cAMP fails to stimulate internalization of mutant thyrotropin-releasing hormone receptors or antagonist-blocked luteinizing hormone/human chorionic gonadotrophin receptors that cannot activate phospholipase C or adenyl cyclase (34, 36). A cholecystokinin antagonist has been reported to induce receptor internalization independent of G protein coupling, signaling events, and receptor phosphorylation (36). The human muscarinic receptor subtype I has been shown to undergo internalization by interaction with antibody against an epitope tagged to the amino terminus, independent of exogenous ligand or second messenger activation (44). The dissociated effects of the hSSTR5 C-tail mutants on adenyl cyclase coupling and internalization lend further support to these arguments. For instance, there was no correlation between the progressive loss of the ability of the C-tail deletion mutants of hSSTR5 to inhibit adenyl cyclase and receptor internalization, which was both inhibited or accelerated. In particular, the Δ318 mutant, which was rendered inert with respect to its ability to inhibit adenyl cyclase, nonetheless exhibited reduced internalization. Although activation of second messenger systems may exert a secondary influence on the internalization process, the collective findings from all of these studies suggest that internalization is an intrinsic property of most receptors, dependent on specific conformational changes rather than receptor signaling capability.

In conclusion, we have shown that the C-tail of hSSTR5 serves a multifunctional purpose in mediating effector coupling, agonist-dependent desensitization, and internalization. Receptor coupling to adenyl cyclase is dependent on the length of the C-tail, whereas desensitization and internalization require specific structural domains. Since SSTR5 is the principal SSTR subtype in tissues such as the pituitary, elucidation of the molecular signals underlying these processes will provide a better understanding of the function of this receptor during prolonged agonist treatment normally and in disease such as pituitary tumors.