Homo- and Heterodimerization of Somatostatin Receptor Subtypes

INACTIVATION OF sst3 RECEPTOR FUNCTION BY HETERODIMERIZATION WITH sst2A

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Several recent studies suggest that G protein-coupled receptors can assemble as heterodimers or hetero-oligomers with enhanced functional activity. However, inactivation of a fully functional receptor by heterodimerization has not been documented. Here we show that the somatostatin receptor (sst) subtypes sst2A and sst3 exist as homodimers at the plasma membrane when expressed in human embryonic kidney 293 cells. Moreover, in coimmunoprecipitation studies using differentially epitope-tagged receptors, we provide direct evidence for heterodimerization of sst2A and sst3. The sst2A-sst3 heterodimer exhibited high affinity binding to somatostatin-14 and the sst2-selective ligand L-779,976 but not to the sst3-selective ligand L-786,778. Like the sst2A homodimer, the sst2A-sst3 heterodimer stimulated guanosine 5′-3-O-(thio)triphosphate (GTPγS) binding, inhibition of adenylyl cyclase, and activation of extracellular signal-regulated kinases after exposure to the sst3-selective ligand L-779,976. However, unlike the sst3 homodimer, the sst2A-sst3 heterodimer did not promote GTPγS binding, adenylyl cyclase inhibition, or extracellular signal-regulated kinase activation in the presence of the sst3-selective ligand L-786,778. Interestingly, during prolonged somatostatin-14 exposure, the sst2A-sst3 heterodimer desensitized at a slower rate than the sst2A and sst3 homodimers. Both sst2A and sst3 homodimers underwent agonist-induced endocytosis in the presence of somatostatin-14. In contrast, the sst2A-sst3 heterodimer separated at the plasma membrane, and only sst2A but not sst3 underwent agonist-induced endocytosis after exposure to somatostatin-14. Together, heterodimerization of sst2A and sst3 results in a new receptor with a pharmacological and functional profile resembling that of the sst2A receptor, however with a greater resistance to agonist-induced desensitization. Thus, inactivation of sst3 receptor function by heterodimerization with sst2A or possibly other G protein-coupled receptors may explain some of the difficulties in detecting sst3-specific binding and signaling in mammalian tissues.

Although G protein-coupled receptors (GPCRs) generally were believed to act as monomeric entities, a growing body of evidence suggests that they may form functionally relevant dimers. The existence of homodimers has been shown for several GPCRs including the β2-adrenergic receptor (1), δ- and κ-opioid receptors (2, 3), the metabotropic glutamate receptor 5 (4), the calcium-sensing receptor (5), the m3 muscarinic receptor (6), and dopamine receptors (7). GPCRs seem to dimerize via different mechanisms. Whereas dimerization of the β2-adrenergic receptor (1) and D2 dopamine receptor (8) occurs via their transmembrane regions, dimerization of the δ-opioid receptor involves the carboxyl terminus (2). In contrast, the metabotropic glutamate receptor 5 (4) and the calcium-sensing receptor (5, 9, 10) appear to be disulfide-linked dimers, and dimerization occurs via their large amino termini. The question of to what extent agonist binding affects dimerization remains controversial. Recent evidence obtained in living cells using bioluminescence resonance energy transfer suggests that the β2-adrenergic receptor exists at the cell surface as a constitutive dimer that is stabilized by agonist binding (11). In contrast, agonist stimulation reduced the level of δ-opioid receptor dimers suggesting that monomerization precedes agonist-induced internalization of this receptor (2). Biochemical and functional studies suggest that GPCRs can also assemble as heterodimers with enhanced functional activity (3, 12–20). Formation of heterodimers between two nonfunctional γ-aminobutyric acid (GABA) receptors, GABARγ1 and GABARγ2, was necessary for a fully functional GABAb receptor (14–19). δ- and κ-Opioid receptors form heterodimers with ligand binding and signaling properties resembling that of the k2 receptor (3). Finally, the somatostatin receptor (sst) sst3 and the D2 dopamine receptor heterodimerize to form a new receptor with enhanced activity (20).

The neuropeptide somatostatin (SS-14) is widely expressed throughout the central nervous system and periphery. SS-14 is involved in multiple functions including endocrine and exocrine hormone release, cognition, sleep, and motor activity. In addition, peptide derivatives of somatostatin have successfully been used in the treatment of neuroendocrine malignancies. The actions of SS-14 are mediated via five distinct somatostatin receptor subtypes, termed sst1–sst5, which belong to the superfamily of GPCRs. All somatostatin receptors bind SS-14 with high affinity and inhibit adenylyl cyclase. There is also

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1 The abbreviations used are: GPCR, G protein-coupled receptor; BS3, bis(sulfosuccinimidyl)suberate; DTT, dithiothreitol; ERK, extracellular signal-regulated kinase; GABA, γ-aminobutyric acid; HEK, human embryonic kidney; PAGE, polyacrylamide gel electrophoresis; PBS, phosphate-buffered saline; SS-14, somatostatin; sst, somatostatin receptor; GTPγS, guanosine 5′-3-O-(thio)triphosphate.
Somatostatin Receptor Heterodimerization

Evidence for different, although not mutually exclusive, pathways of intracellular signaling of somatostatin receptor subtypes, e.g., activation of extracellular signal-regulated kinases (ERK) via sst1, sst2, and sst3; activation of phosphotyrosine phosphatases via sst1, sst2, and sst3; activation of phosphatase A2 via sst2 and modulation of K+ channels via sst2 (21, 22). Individual target cells often express more than one somatostatin receptor, raising the possibility that the functional diversity of these receptors could be expanded by heterodimerization among somatostatin receptor subtypes.

Recently, Rocheville et al. (23) provided evidence, based on photobleaching fluorescence resonance energy transfer, for homodimerization of the sst1 somatostatin receptor. The sst1 receptor also appears to form heterodimers with sst2, but not sst3. In this study, we have examined dimerization of sst2 and sst3 somatostatin receptors. In coimmunoprecipitation studies using differentially epitope-tagged receptors, we show that sst2 and sst3 associate as dimers, both as homodimers and heterodimers. sst2/ssst3 heterodimerization resulted in a new receptor with enhanced sst2-like and diminished sst3-like activity.

**Experimental Procedures**

**Materials**—The sst-selective ligand L-779,976 and the sst2-selective ligand L-796,778 were kindly provided by Dr. Susan Rohrer (24) (Merck). The radioligand 3-[32P]Jiodotyrosyl-S14 (74 TBq/mmol) was from Amersham Pharmacia Biotech, and 3[S]GTPyS (46.25 TBq/mmol) was from PerkinElmer Life Sciences. Mouse monoclonal α-T7 tag antibody was obtained from Novagen (Madison, WI), and polyclonal antibodies against the somatostatin receptors. In coimmunoprecipitation studies using differentially epitope-tagged receptors, we show that sst2 and sst3 associate as dimers, both as homodimers and heterodimers. sst2/ssst3 heterodimerization resulted in a new receptor with enhanced sst2-like and diminished sst3-like activity.

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**Cell Culture and Transfections**—The wild-type rat sst2 and sst3 receptors were tagged at their amino termini either with the c-Myc epitope tag sequence MEQKLISEEDLLR or the T7 epitope tag sequence VQTVLQLAQYQIQY through polymerase chain reaction. Two more amino acids, KL, were added representing a HindIII cloning site encoded by the nucleotide sequence AACCT. The resulting fragments were then subcloned into pDNA31 expression vectors containing either a neomycin or a hygromycin resistance (Invitrogen, Groningen, The Netherlands). The integrity of all constructs was verified by dideoxy sequencing. Human embryonic kidney (HEK) 293 cells were obtained from ATCC and grown in Dulbecco’s modified Eagle’s medium supplemented with 10% fetal calf serum in a humidified atmosphere containing 10% CO2. Cells were first transfected with plasmids containing the neomycin resistance using the calcium phosphate precipitation method. Stable transfectants were selected in the presence of 500 μg/ml G418 (Life Technologies, Inc.). To generate lines coexpressing two differentially epitope-tagged receptors, cells were subjected to a second round of transfection using Effectene (Qiagen, Hilden, Germany) and selected in the presence of 500 μg/ml G418 and 300 μg/ml hygromycin B (Invitrogen). Three clones expressing sst2 alone, four clones expressing sst2 alone, and two clones coexpressing sst2 and sst3 were generated. Receptor expression was monitored using saturation ligand binding assays as described below. The Bmax values of all selected clones were in the range of 800–1200 fmol/mg of protein, and Kd values were in the range of 0.16–0.24 nm. In addition, quantitative Western blot analysis was carried out to ensure that clones coexpressing sst2 and sst3 were coexpressed within the same cells.

**Immunoprecipitation and Western Blot Analysis**—Cells were plated onto poly-L-lysine-coated 150-mm dishes and grown to 80% confluence. When indicated, cells were exposed to agonist, treated with reducing agents, or incubated with cross-linking agents. Agonist exposure was performed with either SS-14, L-779,976, or L-796,778 at a concentration of 1 μM for 30, 60, or 30 min at 37°C. Treatment with reducing agents was performed with 1 mM di-thiothreitol (DTT) for 30 min at 37°C. Incubation with cross-linking agents was performed with either 2 mM bisulfosuccinimidyl suberate (BS3) or 5 mM dithiobis(succinimidyl propionate) (both from Pierce) for 30 min in phosphate-buffered saline (PBS) at 4°C. The reaction was quenched by the addition of PBS (pH 7.5) for 15 min. Cells were then washed twice with PBS and harvested into ice-cold lysis buffer (10 mM Tris-HCl, pH 7.6, 5 mM EDTA, 3 mM EGTA, 250 mM sucrose, 10 mM iodoacetamide, and the following protein inhibitors: 0.2 mM phenylmethylsulfonyl fluoride, 10 μg/ml leupeptin, 1 μg/ml pepstatin, A, 1 μg/ml aprotinin, 10 μg/ml bacitracin). Iodoacetamide was included in each buffer used for protein preparation to prevent nonspecific disulfide linkages. Cells were swollen for 15 min on ice and homogenized. The homogenate was spun at 50,000 × g for 5 min at 4°C to remove unbroken cells and nuclei. Membranes were then pelleted at 20,000 × g for 30 min at 4°C, and pelleted membranes were lysed in detergent buffer (20 mM HEPES, pH 7.4, 150 mM NaCl, 5 mM EDTA, 3 mM EGTA, 4 mg/ml b-dodecylmaltoside, 10 mM iodoacetamide, and proteinase inhibitors as above) for 1 h on ice. The lysate was centrifuged at 20,000 × g for 30 min at 4°C. The protein content of the resulting supernatant was determined using the BCA protein assay (Pierce); samples containing equal amounts of protein (300 μg) were then subjected to immunoprecipitation, or glycoproteins were purified using wheat germ lectin.

For enrichment of glycoproteins, one ml of the supernatant was incubated with 100 μl wheat germ agglutinin–agarose beads (Amer sham Pharmacia Biotech) for 90 min at 4°C with continuous agitation. Beads were washed five times with detergent buffer, and adsorbed glycoproteins were either subjected to enzymatic deglycosylation or directly eluted into 200 μl of SDS-sample buffer (62.5 mM Tris-HCl, pH 6.8, 2% SDS, 20% glycerol, 150 mM di-thiothreitol, 0.005% bromphenol blue) at 60°C for 20 min. Deglycosylation experiments were performed using peptide N-glycosidase F according to the manufacturer’s protocol (New England Biolabs, Beverly, MA).

For immunoprecipitation, the lysates were preclarified with 50 μl of protein A-agarose beads (Calbiochem) for 2 h. After immunoprecipitation with 10 μg of either mouse monoclonal anti-T7, affinity-purified rabbit anti-c-Myc, affinity-purified rabbit anti-sst3 (6291), or affinity-purified rabbit anti-sst2A (6291), antibodies, immunocomplexes were collected using 100 μl of protein A-agarose beads. Beads were washed five times with detergent buffer, and immunoprecipitates were eluted from the beads with 200 μl of SDS-sample buffer at 60°C for 20 min. Equal amounts of protein of each sample were then loaded onto regular 6% SDS-polyacrylamide gels, which contain 0.1% SDS. When indicated gels containing 2-fold (0.2%) or 4-fold (0.4%) higher SDS concentrations were run to test the sensitivity of dimers to stronger denaturing conditions. After electrophoretic transfer, membranes were incubated with either mouse monoclonal anti-T7, affinity-purified rabbit anti-c-Myc, affinity-purified rabbit anti-sst3 (6291), or affinity-purified rabbit anti-sst2A (6291) antibodies at a concentration of 1 μg/ml for 12 h at 4°C, followed by horseradish peroxidase-conjugated anti-rabbit primary antibodies. Densitometric analysis of Western blots exposed in the linear range of the x-ray film was performed as described (31). The amount of immunoreactive material in each lane was quantified using NIH Image 1.57 software.

**Immunocytochemistry**—Cells were grown on polyl-lysine-treated coverslips overnight and then exposed to agonists. Cells were fixed with 4% paraformaldehyde and 0.2% picric acid in phosphate buffer, pH 6.9, for 40 min at room temperature and washed several times in 10 mM Tris, 10 mM phosphate buffer, 137 mM NaCl, and 0.05% thimerosal, pH 7.4 (TPBS). Specimens were then incubated for 3 min in 50% methanol and for 3 min in 100% methanol and subsequently washed several times in TPBS and preincubated with TPBS and 3% normal goat serum for 1 h at room temperature. For single immunofluorescence, cells were then incubated with either mouse monoclonal anti-T7, affinity-purified rabbit anti-c-Myc, affinity-purified rabbit anti-sst2A (6291), or affinity-purified rabbit anti-sst3 (7986) antibody at a concentration of 1 μg/ml in 12 h at 4°C, followed by goat-anti-rabbit IgG peroxidase-conjugated antibodies, and then incubated with either mouse monoclonal anti-T7, affinity-purified rabbit anti-c-Myc, affinity-purified rabbit anti-sst3 (7986) antibody at a concentration of 1 μg/ml in 12 h at 4°C, followed by goat-anti-rabbit IgG peroxidase-conjugated antibodies.
Immunosorbent beads were then resuspended in 0.5 ml PBS containing 0.1% Tween 20, membranes were incubated with mouse monoclonal anti-T7 antibody. Coimmunoprecipitation of the T7sst2A receptor with Myc-tagged sst2A receptors was used. Western blot analysis of membrane extracts from these cells with anti-sst2A antibody (6291) revealed a predominant receptor band migrating at 160 kDa, an additional band with a higher molecular mass migrating at 180 kDa, and a faint band migrating at 200 kDa. The positions of molecular mass markers are indicated on the left (in kDa). The arrows point to the dimeric and monomeric forms of the receptor. Three additional experiments gave similar results.

RESULTS

Homodimerization of the sst2A Receptor—To examine dimerization of the sst2A receptor, we used HEK 293 cells stably expressing either the T7-tagged sst2A alone or coexpressing Myc-tagged sst2A and T7sst2A were subjected to immunoprecipitation using rabbit anti-c-Myc antibody. Coimmunoprecipitates were immunoblotted using mouse monoclonal anti-T7 antibody. Coimmunoprecipitation of the T7sst2A can be seen only when Myc-tagged sst2A and T7-tagged sst2A are coexpressed (right lane). The positions of molecular mass markers are indicated on the left (in kDa). The arrows point to the dimeric and monomeric forms of the receptor. Three additional experiments gave similar results.

Data Analysis—Data from ligand binding, GTPγS, cAMP, and ERK assays were analyzed by nonlinear regression curve fitting using GraphPad Prism 3.0 software.

FIG. 1. The sst2A somatostatin receptor forms homodimers. A, HEK 293 cells expressing T7 epitope-tagged sst2A receptor were incubated in the absence (−) or presence (+) of 2 mM BS3, and membrane proteins were extracted and immunoblotted using the anti-sst2A antibody (6291) as described under “Experimental Procedures.” B, membrane proteins from T7sst2A-expressing cells were extracted and subjected to enzymatic deglycosylation using peptide N-glycosidase F (PNGase F). C, cells were incubated in the absence (−) or presence (+) of 1 μM SS-14 for 30 min. D, cells were incubated in the absence (−) or presence (+) of 1 mM DTT for 30 min, membrane proteins were extracted, and samples were run on regular SDS-polyacrylamide gels (lanes 1 and 2). Alternatively, samples were run on SDS-polyacrylamide gels containing a 4-fold higher concentration of SDS (lane 3). DTT was also present in the SDS-sample buffer. Note that the sst2A dimer is detectable without cross-linking and stable under reducing conditions; however, it is sensitive to higher concentrations of detergents. E, HEK 293 cells expressing either Myc-tagged sst2A alone or coexpressing Myc-tagged sst2A and T7sst2A were subjected to immunoprecipitation using anti-T7 antibody in the presence or absence of either SS-14, L-779,796, or L-796,778 in concentrations ranging from 10−12 to 10−6 M. The cells were incubated at 37 °C for 5 min at room temperature. The incubation was terminated by vacuum filtration through glass fiber filters presoaked in 0.1% polyethylenimine using an Inotech cell harvester (Ditikon, Switzerland). Filters were rinsed twice with washing buffer (50 mM Tris-HCl, pH 7.4) and air-dried. Bound radioactivity was determined using a γ-counter. Protein content was determined by the Lowry method.

GTPγS Binding Assays—Cells were harvested and lysed as described above except that a lysis buffer containing 50 mM Tris and 10 mM EDTA (pH 7.4) was used. The resulting pellet was resuspended in assay buffer (20 mM HEPES, 100 mM NaCl, 10 mM MgCl2, pH 7.4), and aliquots containing 25 μg of protein were incubated with 5 nM [35S]GTPγS in the presence or absence of either SS-14, L-779,796, or L-796,778 in concentrations ranging from 10−12 to 10−6 M. The experiment for each concentration was performed in triplicate. Assays were performed in 96-well polypropylene plates in a final volume of 200 μl for 45 min at room temperature. The incubation was terminated by vacuum filtration through glass fiber filters presoaked in 0.1% polyethylenimine in Dulbecco’s modified Eagle’s medium containing 0.5% fetal calf serum. Filters were rinsed twice with washing buffer (50 mM Tris−HCl, pH 7.4) and air-dried. Bound radioactivity was determined using a γ-counter. Protein content was determined by the Lowry method.

Confocal micrographs were taken by a person blinded to the treatments and 5.18 with 647-nm excitation and 665-nm long pass emission filters. Cyanine 3.18 was imaged with 568-nm excitation and 500–560-nm band pass emission filters, cyanine 2.18 was imaged with 488-nm excitation and 500–560-nm band pass emission filters, and cyanine 1.18 was imaged with 546-nm excitation and 500–560-nm band pass emission filters. Confocal micrographs were taken by a person blinded to the treatments and 5.18 with 647-nm excitation and 665-nm long pass emission filters. Cyanine 3.18 was imaged with 568-nm excitation and 500–560-nm band pass emission filters, cyanine 2.18 was imaged with 488-nm excitation and 500–560-nm band pass emission filters, and cyanine 1.18 was imaged with 546-nm excitation and 500–560-nm band pass emission filters.
kDa (Fig. 1A). These bands were not only detected with antibodies directed against the carboxyl terminus (6291, GP3) but also with antibodies directed against the amino-terminal added T7 or Myc tag (not shown). A similar ratio of these two immunoreactive bands was observed when the cells had been subjected to cross-linking using the cell-impermeable cross-linker BS3 prior to cell lysis (Fig. 1A). However, enzymatic deglycosylation reduced the size of the 80-kDa protein to 55 kDa and the size of the 160-kDa protein to 110 kDa, suggesting that the band with the higher molecular weight may consist of two sst2A receptor proteins (Fig. 1B). Exposure to SS-14 did not grossly modulate the dimer/monomer ratio (Fig. 1C).

The sst2A dimer was stable under reducing conditions. Similar levels of sst2A dimers were detected after incubation of the cells with 1 mM DTT for 30 min prior to cell lysis (Fig. 1D). The addition of DTT to the SDS-sample buffer also did not reduce the level of sst2A dimers detected on Western blots. sst2A dimers were also stable in the presence of 2% SDS (e.g., heating to 60 °C for 20 min in SDS-sample buffer and loading onto regular SDS-polyacrylamide gels). However, when these samples were run on SDS-polyacrylamide gels containing 2- or 4-fold higher SDS concentrations, the sst2A dimer was destabilized in a concentration-dependent manner, and only the monomeric form of the receptor was detectable under these conditions (Fig. 1D). This suggests that the sst2A dimer is formed by noncovalent interactions of two receptor proteins.

To directly examine the presence of sst2A receptor dimers, we used coimmunoprecipitation and Western blotting of differentially epitope-tagged sst2A receptors. HEK 293 cells coexpressing Myc-st2A and T7-st2A were lysed, and the receptors from these cells were immunoprecipitated using anti-c-Myc antibody. Immunoprecipitates were resolved by SDS-PAGE and immunoblotted with anti-T7 antibody. As depicted in Fig. 1E, anti-T7 antibody detected a single band migrating at 160 kDa, which represents the Myc-st2A/T7-st2A dimer. Receptor monomer was not detectable, suggesting that the coprecipitated sst2A dimers were stable during sample preparation and SDS-PAGE. The fact that T7-st2A and Myc-st2A were coimmunoprecipitated as part of a dimer complex from membrane extracts of untreated HEK 293 cells indicates that the sst2A receptor exists as a constitutive homodimer in these cells. No bands were detectable in immunoprecipitates prepared under identical conditions from cells expressing only Myc-st2A (Fig. 1E).

**Homodimerization of the sst3 Receptor**—To examine dimerization of the sst3 receptor, we used HEK 293 cells stably expressing the T7-tagged sst3 (Bmax 1182 ± 31 fmol/mg of membrane protein). Western blot analysis of membrane extracts from these cells with the anti-sst3 antibody (7896) revealed a predominant receptor band migrating at 80 kDa (Fig. 2A). We also observed an additional band with a higher molecular mass migrating at 160 kDa (Fig. 2A). These bands were also detected with antibodies directed against the amino-terminal added T7 tag (not shown). Treatment of cells with the cross-linker BS3 prior to cell lysis resulted in an increase of the 160-kDa band, suggesting that cross-linking can stabilize the sst3 dimer (Fig. 2A). However, no further increase in the intensity of the 160-kDa band was found when cells were exposed to SS-14 prior to cross-linking (Fig. 2A). Enzymatic deglycosylation reduced the size of the 80-kDa protein to 70 kDa and the size of the 160-kDa protein to 140 kDa, suggesting that the band with the higher molecular weight may consist of two sst3 receptor proteins (Fig. 2B).

Similar to that observed for the sst2A dimer, the sst3 dimer was stable under reducing conditions. The levels of sst3 dimers were unchanged after incubation of cells with 1 mM DTT for 30 min prior to cell lysis (Fig. 2C). The addition of DTT to the SDS-sample buffer also did not reduce the levels of sst3 dimers detected on Western blots. sst3 dimers were also stable in the presence of 2% SDS (e.g., heating to 60 °C for 20 min in SDS-sample buffer and loading onto regular SDS-polyacrylamide gels). However, when these samples were run on SDS-polyacrylamide gels containing 2- or 4-fold higher SDS concentrations, the sst3 dimer was destabilized in a concentration-dependent manner, and only the monomeric form of the receptor was detectable under reducing conditions; however, it is sensitive to higher concentrations of detergents. D, HEK 293 cells expressing either the wild-type sst3 receptor (T7sst3) or a C-terminal truncated sst3 receptor (T7sst3ΔC75) were immunoblotted using mouse monoclonal anti-T7 antibody. The positions of molecular mass markers are indicated on the left (in kDa). The arrows point to the dimeric and monomeric forms of the receptor. Four additional experiments gave similar results.

**FIG. 2. Characterization of sst3 somatostatin receptor homodimers by immunoblotting.** A, HEK 293 cells expressing T7 epitope-tagged sst3 receptor were incubated in the absence (−) or presence (+) of 2 mM BS3 and/or SS-14 (+). Membrane proteins were extracted and immunoblotted using the anti-sst3 antibody (7896) as described under *Experimental Procedures.* B, membrane proteins from T7sst3-expressing cells were extracted and subjected to enzymatic deglycosylation using peptide N-glycosidase F (PNGF), C, cells were incubated in the absence (−) or presence (+) of 1 mM DTT for 30 min. Membrane proteins were extracted, and samples were run on regular SDS-polyacrylamide gels (lanes 1 and 2). Alternatively, samples were run on SDS-polyacrylamide gels containing a 4-fold higher concentration of SDS (lane 3). DTT was also present in the SDS-sample buffer. Note that the sst3 dimer is detectable without cross-linking and stable under reducing conditions; however, it is sensitive to higher concentrations of detergents. D, HEK 293 cells expressing either the wild-type sst3 receptor (T7sst3) or a C-terminal truncated sst3 receptor (T7sst3ΔC75) were immunoblotted using mouse monoclonal anti-T7 antibody. The positions of molecular mass markers are indicated on the left (in kDa). The arrows point to the dimeric and monomeric forms of the receptor.
Heterodimerization of the sst2A and sst3 Receptors—First, we determined the levels of sst2A and sst3 receptor proteins in coexpressing HEK 293 cells using quantitative Western blot analysis. Since sst2A and sst3 were tagged with different epitopes, it was not possible to compare their expression levels directly using different antibodies. Therefore, equal amounts of membrane protein extracted from HEK 293 cells stably expressing the T7-tagged sst2A receptor alone (B_{max} 820 \pm 19 fmol/mg) or the T7-tagged sst3 receptor alone (B_{max} 1182 \pm 31 fmol/mg) or coexpressing T7-tagged sst2A and c-Myc-tagged sst3 receptors (B_{max} 1088 \pm 66 fmol/mg) were subjected to SDS-PAGE and subsequently probed with both anti-sst2A (6291) and anti-sst3 (7986) antibodies (Fig. 3A). Densitometric scanning of the resulting Western blots revealed that virtually identical levels of sst2A receptor protein were present in HEK 293 cells coexpressing sst2A and sst3 as compared with cells expressing sst2A alone (Fig. 3A, left panel). Conversely, virtually identical levels of sst3 receptor protein were present in HEK 293 cells coexpressing sst2A and sst3 as compared with cells expressing sst3 alone (Fig. 3A, right panel). Given the fact that T7sst2A and T7sst3 cells expressed similar numbers of somatostatin binding sites, we conclude that cotransfected cells expressed T7sst2A and Mycst3 receptor proteins in a ratio of ~1:1. However, it should be noted that the higher levels of somatostatin receptor proteins (sst2A plus sst3) present in coexpressing cells were not associated with an equivalent increase in the total number of somatostatin binding sites detected in saturation binding assays (B_{max} for T7sst2A-Mycsst3 1088 \pm 66 fmol/mg as compared with B_{max} for T7sst2A 820 \pm 19 fmol/mg and B_{max} for T7sst3 1182 \pm 31 fmol/mg).

We next examined the ability of sst2A and sst3 to assemble as heterodimers in coexpressing cells. As shown in Fig. 3B, the C-terminal anti-sst2A antibody (6291) detected a band migrating at 160 kDa in material immunoprecipitated using the C-terminal anti-sst3 antibody (7986), suggesting that this band represents a T7sst2A-Mycsst3 heterodimer. sst2A-sst3 Heterodimers can be immunoprecipitated under a variety of conditions (e.g. using the anti-c-Myc antibody or the anti-T7 antibody and immunoblotted vice versa) (Fig. 3C). All of these preparations revealed a single 160-kDa band, suggesting that stable sst2A-sst3 heterodimers were precipitated under these conditions and that sst2A-sst3 heterodimers were not subject to monomerization during sample preparation. However, when T7-tagged sst2A receptors were immunoprecipitated from T7sst2A-Mycsst3 cells using anti-sst2A antibody (6291) and then immunoblotted with anti-sst2A antibody (6291), both dimeric and monomeric forms of the receptor could be detected (Fig. 3B). Essentially identical results were obtained when Myc-tagged sst3 receptors were immunoprecipitated from T7sst2A-Mycsst3 cells using anti-sst3 antibody (7986) and then immunoblotted with anti-sst3 antibody (7986) (not shown). These results are in agreement with those seen on Western blots from membrane extracts of T7sst2A-Mycsst3 cells, suggesting that the receptor proteins existed in both monomeric and dimeric form in these cells (Fig. 3A).

As shown in Fig. 3C, anti-T7 antibody detected a band migrating at 160 kDa in material immunoprecipitated using anti-c-Myc antibodies specific for the sst3 receptor, suggesting that this band represents a T7sst2A-Mycsst3 heterodimer. In contrast, no bands were detectable in immunoprecipitates prepared under identical conditions from cells expressing only T7sst2A or T7sst3, or from a mixture of T7sst2A- and Mycst3-expressing cells (Fig. 3C). These data strongly suggest that sst2A-sst3 heterodimers preexisted in cells prior to cell lysis and were not artificially formed during sample preparation. Agonist exposure had little effect on the level of sst2A-sst3 heterodimer (Fig. 3D). However, we observed that after exposure of coexpressing cells, not only heterodimers but also traces of the monomeric receptor forms became detectable (Fig. 3D). The simplest explanation for this finding is that during agonist exposure a proportion of the sst2A-sst3 heterodimer was destabilized, presumably due to a change in the conformation and/or phosphorylation of the receptor that still allowed immunoprecipitation of sst2A-sst3 heterodimers but facilitated separation of the receptors during sample preparation and gel electrophoresis.

Endocytotic Trafficking of the sst2A\(\sim\)sst3 Heterodimer—We
next examined the effect of sst2A-sst3 heterodimerization on agonist-induced endocytosis using HEK 293 cells expressing either T7sst2A or T7sst3 or coexpressing both T7sst2A and Mycsst3. Cells were exposed to either 100 nM SS-14, 10 nM L-779,976 (sst2-selective agonist), or 100 nM L-796,778 (sst3-selective agonist) for 30 min. Cells were subsequently fixed and fluorescently labeled with either anti-sst2A antibody (6291) or anti-sst3 antibody (7986), and the subcellular distribution of receptor proteins was examined by confocal microscopy. Note that in untreated cells, both sst2A and sst3 were almost exclusively confined to the plasma membrane. SS-14 induced robust internalization of the sst2A as well as the sst3 receptor. While the sst3-selective agonist L-779,976 promoted endocytosis of sst2A but not sst3, the sst2-selective agonist L-796,778 promoted endocytosis of sst3 but not sst2A. Shown are representative results from one of three independent experiments performed in duplicate. Scale bar, 20 μm.

**Fig. 4. Comparison of agonist-induced endocytosis of sst2A and sst3 homodimers.** HEK 293 cells expressing either T7sst2A (upper panel) or T7sst3 (lower panel) were either not exposed (Control) or exposed to 100 nM SS-14 or 10 nM L-779,976 (sst2-selective agonist) or 100 nM L-796,778 (sst3-selective agonist) for 30 min. Cells were subsequently fixed and fluorescently labeled with either anti-sst2A antibody (6291) or anti-sst3 antibody (7986), and the subcellular distribution of receptor proteins was examined by confocal microscopy. Note that in untreated cells, both sst2A and sst3 were almost exclusively confined to the plasma membrane. SS-14 induced robust internalization of the sst2A as well as the sst3 receptor. While the sst3-selective agonist L-779,976 promoted endocytosis of sst2A but not sst3, the sst2-selective agonist L-796,778 promoted endocytosis of sst3 but not sst2A. Shown are representative results from one of three independent experiments performed in duplicate. Scale bar, 20 μm.

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**Fig. 5. Expression of sst2A-sst3 heterodimers results in a loss of robust effector response.** As shown in the upper panel, sst2A-like immunoreactivity was clearly confined to the plasma membrane in untreated cells. After 30 min of exposure to SS-14, a dramatic loss of sst2A-like immunoreactivity from the plasma membrane with a concomitant accumulation in vesicle-like structures within the cytoplasm was observed. In addition, treatment with the sst3-selective ligand L-779,976 but not with the sst2-selective ligand L-796,778 induced robust internalization of the sst2A receptor. Similarly, sst3-like immunoreactivity was present at the plasma membrane in the absence of agonist and was redistributed into vesicle-like structures in the presence of SS-14 (Fig. 4, lower panel). Treatment with the sst2-selective ligand L-779,976 but not with the sst3-selective ligand L-796,778 induced intracellular trafficking of the sst3 receptor.

In cells coexpressing T7sst2A and Mycsst3, both sst2A-like immunoreactivity and sst3-like immunoreactivity were seen at the cell surface, revealing extensive colocalization of sst2A and sst3 receptor proteins (Fig. 5, Control). Interestingly, after 30 min of SS-14 exposure, the sst2A receptor underwent robust internalization, whereas the sst3 receptor remained almost exclusively confined to plasma membrane (Fig. 5, SS-14). However, internalization of the sst2A receptor was not complete, which may explain the fact that sst2A-sst3 heterodimers could also be immunoprecipitated after treatment with SS-14 (Fig. 5, C). The sst3 receptor did not undergo endocytosis even after prolonged SS-14 exposure (up to 4 h, not shown). Similarly, after treatment with the sst3-selective ligand L-796,778, only the sst3 receptor and not the sst2A receptor was internalized (Fig. 5, L-796,778). In contrast, treatment with sst2-selective ligand L-779,776 did not induce substantial internalization of either sst2A or sst3 (Fig. 5, L-796,778).

**Ligand Binding Properties of the sst2A-sst3 Heterodimer—** We compared ligand-binding properties of sst2A-sst3 heterodimers with those of sst2A and sst3 homodimers and examined the ability of subtype-selective agonists to compete with [125I-Tyr1]SS-14 binding in membranes prepared from cells expressing either T7sst2A or T7sst3 or coexpressing both T7sst2A and Mycsst3 and Mycsst3. We found that T7sst2A cells had high affinities for SS-14 and the sst2-selective agonist L-779,976. T7sst3 cells had high affinities for SS-14 and the sst3-selective agonist L-796,778 as well as moderate affinities for the sst2-selective agonist L-796,778. However, T7ssst2A-Mycsst3 cells had high affinities only for SS-14 and the sst2-selective agonist L-779,976 but not for the sst3-selective agonist L-796,778 (Fig. 6, A–C). Specifically, the cells coexpressing sst2A and sst3 exhibited a 100-fold lower affinity for the sst3-selective agonist L-796,778 than cells expressing sst3 alone (Table I). These findings imply that sst2A-sst3 heterodimerization results in a new binding site with a pharmacological profile resembling that of the sst3A receptor. Although sst2A and sst3 receptor proteins were expressed in a 1:1 ratio, the sst2A-sst3 heterodimer showed no significant affinity for the sst3-selective ligand L-796,778.

**Stimulation of [35S]GTPγS Binding by sst2A-sst3 Heterodimers—** We next compared effector coupling efficiencies of sst2A-sst3 heterodimers with those of sst2A and sst3 homodimers and examined the ability of different agonists to stimulate [35S]GTPγS binding in membranes prepared from cells expressing either T7sst2A or T7sst3 or coexpressing both T7sst2A and Mycsst3. SS-14 induced a concentration-dependent stimulation of [35S]GTPγS binding in cells expressing either T7sst2A or T7sst3 as well as in cells coexpressing T7sst2A and Mycsst3 (Fig. 6, D–F). Notably, 1 μM SS-14 produced a more robust effector response in cells coexpressing T7sst2A and Mycsst3 (Table I) than in cells expressing only T7sst2A (Table II). The sst2-selective agonist L-779,976 stimulated strong [35S]GTPγS binding in cells expressing either T7sst2A or T7sst3 as well as in cells coexpressing T7sst2A and Mycsst3, indicating limited sst3 selectivity over sst2, of L-779,976 in concentrations exceeding 100 nM (Fig. 6E). In contrast to that seen in T7sst3 cells, the sst2-selective agonist L-796,778 produced no substantial [35S]GTPγS binding in cells expressing T7sst2A and Mycsst3. In addition, L-779,976 produced a similar stimulatory response as L-796,778 (sst2-selective agonist) in cells expressing T7sst3, indicating limited sst2 selectivity over sst3, of L-779,976 in concentrations exceeding 100 nM (Fig. 6F). The sst3-selective agonist L-796,778 produced no substantial [35S]GTPγS binding in cells coexpressing T7sst2A and Mycsst3 (Fig. 6, E and F).

**Inhibition of Adenylyl Cyclase by sst2A-sst3 Heterodimers—** The activation of somatostatin receptors by agonists results in decreased levels of intracellular cAMP. We therefore examined the ability of sst2A agonists to inhibit forskolin-stimulated cAMP accumulation in cells expressing either T7sst2A or T7sst3 or coexpressing both T7sst2A and Mycsst3. SS-14 induced an in-
hibition of adenylyl cyclase with half-maximal inhibitory concentrations (IC50) in the low nanomolar range in T7sst2A cells and T7sst3 cells as well as in cells coexpressing both T7sst2A and Mycsst3 (Fig. 6, G–I, Table I). The sst2-selective agonist L-779,976 produced similar robust responses in T7sst2A cells as in cells coexpressing T7sst2A and Mycsst3. In contrast, sst3-selective agonist L-779,778 inhibited forskolin-stimulated cAMP accumulation in T7sst3 cells only, but essentially no inhibition was found in cells coexpressing T7sst2A and Mycsst3 (Fig. 6, H and I, Table I). We also investigated the possibility of a partial agonism by L-779,776. If the sst3-selective ligand L-779,776 would be a partial agonist at the sst3 dimer or antagonist at the sst2A-sst3 heterodimer, it would be expected to block the SS-14-mediated inhibition of cAMP accumulation. However, SS-14-mediated responses were not attenuated in the presence of L-779,776 either on T7sst3 cells or on cells coexpressing both T7sst2A and Mycsst3, suggesting that L-779,776 is a pure sst3 agonist (data not shown).

ERK Activation by sst2A-sst3 Heterodimers—The activation of somatostatin receptors by agonists results in a rapid and transient stimulation of ERK1/2 phosphorylation. We next examined the ability of several agonists to increase the levels of phosphorylated ERK1/2 in cells expressing either T7sst2A or T7sst3 or coexpressing both T7sst2A and Mycsst3. As shown in Fig. 7, 5-min exposure to 100 nM SS-14 produced a robust increase in ERK1/2 phosphorylation in T7sst2A cells and T7sst3 as well as in cells coexpressing both T7sst2A and Mycsst3. The sst2-selective agonist L-779,976 (10 nM) promoted a similar response in T7sst2A cells as in cells coexpressing T7sst2A and Mycsst3. In contrast, the sst3-selective agonist L-796,778 (100 nM) stimulated ERK1/2 activity in T7sst3 cells only but not in cells coexpressing T7sst2A and Mycsst3 (Fig. 7, Table I). These findings imply that sst2A-sst3 heterodimerization results in a new receptor with a functional profile (e.g., [35S]GTPγS binding, inhibition of adenylyl cyclase, and ERK activation) resembling that of the sst2A receptor. Although sst2A and sst3 receptor proteins were expressed in a 1:1 ratio, the sst2A-sst3 heterodimer showed no significant functional response to the sst3-specific ligand L-796,778.

Desensitization of the sst2A-sst3 Heterodimer—Finally, we compared agonist-induced desensitization of sst2A dimer and sst2A-sst3 heterodimer. Cells expressing T7sst2A or coexpressing both T7sst2A and Mycsst3 were preincubated with 1 μM SS-14 for 0, 1, 2, 4, or 6 h. The medium was removed, and the ability of SS-14 to inhibit forskolin-stimulated cAMP accumulation was examined. The sst2A dimer underwent a rapid time-dependent loss of coupling to adenylyl cyclase with a maximum desensitization at 6 h (Fig. 8A). In contrast, the sst2A-sst3 heterodimer appeared to be more resistant to agonist-induced desensitization with a strong receptor response retained as long as 6 h (Fig. 8B). To test the possibility that the delayed desensitization of the sst2A-sst3 heterodimer may be related to agonist-induced monomerization and subsequent gain of function of the sst3 receptor, cells coexpressing T7sst2A and Mycsst3 were challenged with the sst3-selective ligand L-796,778 after extended exposure with SS-14. However, the sst3-selective ligand was not able to inhibit forskolin-induced cAMP accumulation in T7sst2A-Mycsst3 cells whether or not these cells had been preincubated with SS-14 (data not shown).

DISCUSSION

The Western blot and coimmunoprecipitation experiments carried out in the present study clearly demonstrate that the sst2A receptor as well as the sst3 receptor exist as constitutive homodimers at the plasma membrane when expressed alone and as heterodimers when coexpressed in HEK 293 cells. Both sst2A and sst3 dimers were resistant to reducing agents but sensitive to higher concentrations of detergents, suggesting...
that dimerization involves noncovalent hydrophobic interactions of the receptor proteins (Figs. 1D and 2C). Formation of homodimers between truncated sst3 receptors suggests that the cytoplasmic tail was not necessary for dimerization (Fig. 2D). Several dimerization interfaces have been proposed for other GPCRs such as the extracellular amino-terminal domain for the glutamate and calcium-sensing receptors, the intracellular third loop, and the VIth transmembrane region for the dopaminergic and β2-adrenergic receptor and the C-terminal tail for the δ-opioid and GABAB receptor (1, 2, 4, 5, 7, 14–19).

To study the sst2A-sst3 heterodimer directly, we performed coimmunoprecipitation experiments using HEK 293 cells coexpressing T7sst2A and Mycsst3. These studies clearly showed that the sst2A-sst3 heterodimers can be coimmunoprecipitated using a variety of antibody combinations (Fig. 3). To exclude the possibility that sst2A-sst3 heterodimers were artifactually formed during the preparation of cell lysates and sample processing, coimmunoprecipitation studies were also carried out using a mixture of cells expressing T7sst2A and Mycsst3 individually. Under these conditions, heterodimers could not be immunoprecipitated, strongly suggesting that sst2A-sst3 heterodimers were formed in vivo prior to cell lysis (Fig. 3A). Interestingly, when the material immunoprecipitated using an anti-sst2A antibody was probed with an anti-sst3 antibody, we detected only a single high molecular weight band corresponding to the sst2A-sst3 heterodimer. This suggests that heterodimers were stable during cell lysis and reducing SDS-PAGE. When material immunoprecipitated using an anti-sst2A antibody was probed with an anti-sst2A antibody, we detected an additional lower molecular weight band that corresponds to the sst2A monomer, indicating that, similar to the results seen on Western blots, both monomeric and heterodimeric forms of the receptor proteins were present at the plasma membrane. However, the possibility that sst2A and sst3 may exist as high molecular weight hetero-oligomeric arrays in coexpressing cells cannot be excluded.

Agonist treatment had little effect on the levels of sst2A and sst3 homo- and heterodimers detected on Western blots (Figs. 1C, 2A, and 3D). Interestingly, agonist exposure of the δ-opioid receptor was found to induce monomerization, which precedes receptor internalization (2). In contrast, recent studies using fluorescence resonance energy transfer suggest that dimers formed by the β2-adrenergic receptor and the sst5 somatostatin receptor are stabilized by agonist binding (1, 11, 20). On the other hand, agonist stimulation of the m3 muscarinic receptor and the calcium-sensing receptor neither promotes nor destabilizes receptor dimer formation (5, 6, 9, 10). It remains to be clarified whether the observed differences in agonist regulation of receptor dimerization reflect intrinsic differences in the structural properties of the studied receptor proteins or are due to variations in experimental conditions.

Although sst2A and sst3 receptor proteins were expressed...
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TABLE I
Ligand binding and functional properties of sst2A-sst3 heterodimers

| Ligand          | T7sst2A IC₅₀ (nm) | T7sst3 IC₅₀ (nm) | T7sst2A-Mycsst3 IC₅₀ (nm) |
|-----------------|------------------|-----------------|--------------------------|
| SS-14 (A)       | 4.03             | 16.4            | 1.98                     |
| L-779,976 (B)   | 1.68             | 393             | 0.67                     |
| L-796,778       | 3.109            | 24              | 2,502                    |

| L-779,976 (C)   | 1.68             | 393             | 0.67                     |
| L-796,778       | 3.109            | 24              | 2,502                    |

| cAMP accumulation IC₅₀ (nm) | T7sst2A | T7sst3 | T7sst2A-Mycsst3 |
|-----------------------------|--------|--------|-----------------|
| SS-14                       | 0.31   | 0.13   | 0.33            |
| L-779,976                   | 0.16   | 27     | 0.05            |
| L-796,778                   | >1,000 | 3.65   | >1,000          |

| ERK1/2 activation IC₅₀ (nm) | T7sst2A | T7sst3 | sst2A-T7sst3 |
|-----------------------------|--------|--------|-------------|
| SS-14                       | 0.92   | 1.7    | 0.75        |
| L-779,976                   | 0.32   | >100   | 0.15        |
| L-796,778                   | >1,000 | 16.5   | >1,000      |

Fig. 7. Activation of ERK1/2 by sst2A-sst3 heterodimers. HEK 293 cells expressing either T7sst2A (upper panel) or T7sst1, alone (middle panel) or cells coexpressing MycCst3 and T7sst1, (lower panel) were either not treated (−) or treated with 100 nM SS-14 (+) or 10 nM L-796,778 (+) (sst-selective agonist) for 5 min. Cells were lysed, equal amounts of protein were resolved by SDS-PAGE, and levels of phosphorylated ERK/2 were determined by immunoblotting as described under "Experimental Procedures." The arrows indicate the positions of phospho-ERK1/2. Three additional experiments gave similar results.

suggest that heterodimerization of sst₂A and sst₃ results in a new receptor with a pharmacological profile resembling that of the sst₂A receptor. However, the sst₂A-sst₃ heterodimer and the sst₂A homodimer differ in that the sst₂A-sst₃ heterodimer appears to be more resistant to agonist-induced desensitization of coupling to adenyl cyclase than the sst₂A homodimer (Fig. 8).

Several recent studies have reported on heterodimerization among GPCRs (e.g. the GABA-B heterodimers, κ-δ heterodimers, and D₂R-sst₃ heterodimers) (3, 12–20). In each case, heterodimerization results in a new receptor with enhanced functional activity. Here, we provide the first evidence for inactivation of a fully functional sst₃ receptor by heterodimerization with the sst₂A receptor.

The fact that the sst₃ receptor is rendered nonfunctional after heterodimerization with sst₂A may provide a plausible explanation for the unexpectedly low number of somatostatin binding sites on HEK 293 cells coexpressing sst₂A and sst₃. Comparative Western blot analysis revealed that virtually identical levels of sst₂A and sst₃ receptor protein were present in coexpressing cells as compared with cells expressing these receptors alone. Saturation binding assays revealed that SST₂₅₃ (Bₘₐₓ = 820 ± 19 fmol/mg) and SST₃₃ cells (Bₘₐₓ = 1182 ± 31 fmol/mg) exhibited similar densities of somatostatin binding sites. This suggests that sst₂A and sst₃ receptor proteins were expressed approximately in a 1:1 ratio in T7sst₂₅₃-Mycsst₃ cells. This also suggests that higher levels of total somatostatin receptor proteins (sst₂A plus sst₃) were present in coexpressing cells. However, this doubling of somatostatin receptor protein was not associated with an equivalent increase in the total number of detectable somatostatin binding sites in these cells (Bₘₐₓ = 1088 ± 66 fmol/mg).

The functional inactivation of sst₃ by heterodimerization with sst₂A may also explain the observed destabilization of the sst₂A-sst₃ heterodimer and selective internalization of sst₂A after SS-14 treatment. Agonist exposure may induce a conformational change of the sst₂A-sst₃ heterodimer that favors G protein-coupled receptor kinase-mediated phosphorylation of sst₂A while sst₃ remains in a nonphosphorylated state. Phosphorylation of intracellular domains of the sst₂A receptor would then be expected to facilitate binding of β-arrin and preferential targeting of this receptor to the endocytotic machinery. Nevertheless, internalization of the sst₂A receptor was not complete (Fig. 5), and sst₂A-sst₃ heterodimers could also be coimmunoprecipitated from membrane preparations of SS-14-treated cells (Fig. 3D). However, the agonist-induced conformational change appeared to destabilize the sst₂A-sst₃ heterodimer to an extent that still allowed coimmunoprecipitation of sst₂A-sst₃ heterodimers but facilitated separation of a proportion of heterodimers during sample preparation and gel electrophoresis and, hence, permitting detection of monomeric
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...receptor forms on Western blots (Fig. 3D).

sst₂A and sst₃ are widely distributed throughout the central nervous system and periphery (25–27). sst₂A-sst₃ heterodimerization may also occur in vivo, since colocalization of these receptors has been observed in several tissues including pancreatic islands and the anterior lobe of the pituitary. Interestingly, particularly high levels of sst₃ expression have been detected in the cerebellum; however, radioligand binding studies largely failed to detect sst₃ receptor binding sites in this region (27–29). Thus, functional inactivation of the sst₃ receptor by heterodimerization with sst₂A or other GPCRs may provide a possible explanation for some of the difficulties in detecting sst₃-specific binding and signaling in mammalian tissues.

The physical interaction between somatostatin receptors may have direct implications for the treatment of neuroendocrine malignancies that frequently overexpress several subtypes of somatostatin receptors (30, 31). Based on the present findings, a tumor coexpressing sst₂A and sst₃ would be expected to respond to treatment with sst₂A-selective but not with sst₃-selective agonists. In contrast, a tumor with isolated expression of sst₂A would be expected to respond to treatment with sst₂A-selective but not with sst₃-selective agonists. Evaluation of the somatostatin receptor status in a given tumor may therefore provide valuable predictive information for the treatment of human tumors with somatostatin receptor subtype-specific ligands (24, 31).

In conclusion, we provide biochemical and functional evidence for homo- and heterodimerization of the sst₂A and sst₃ somatostatin receptors. We show that heterodimerization results in a new receptor with a pharmacological and functional profile resembling that of the sst₂A receptor, however with a greater resistance to agonist-induced desensitization. The sst₂A-sst₃ receptor is the first heterodimer that results in activation of a fully functional receptor.

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Homo- and Heterodimerization of Somatostatin Receptor Subtypes:  
INACTIVATION OF sst3 RECEPTOR FUNCTION BY  
HETERODIMERIZATION WITH sst2A  
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