Subtypes of the Somatostatin Receptor Assemble as Functional Homo- and Heterodimers*

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The existence of receptor dimers has been proposed for several G protein-coupled receptors. However, the question of whether G protein-coupled receptor dimers are necessary for activating or modulating normal receptor function is unclear. We address this question with somatostatin receptors (SSTRs) of which there are five distinct subtypes. By using transfected mutant and wild type receptors, as well as endogenous receptors, we provide pharmacological, biochemical, and physical evidence, based on fluorescence resonance energy transfer analysis, that activation by ligand induces SSTR dimerization, both homo- and heterodimerization with other members of the SSTR family, and that dimerization alters the functional properties of the receptor such as ligand binding affinity and agonist-induced receptor internalization and up-regulation. Double label confocal fluorescence microscopy showed that when SSTR1 and SSTRs were coexpressed in Chinese hamster ovary-K1 cells and treated with agonist they underwent internalization and were colocalized in cytoplasmic vesicles. SSTR5 formed heterodimers with SSTR1 but not with SSTR4 suggesting that heterodimerization is a specific process that is restricted to some but not all receptor subtype combinations. Direct protein interaction between different members of the SSTR subfamily defines a new level of molecular cross-talk between subtypes of the SSTR and possibly related receptor families.

Many membrane proteins such as ion channels, receptor tyrosine kinases, and receptors for growth hormone and cytokines associate as functional oligomeric complexes (1–4). Although G protein-coupled receptors (GPCRs)1 are generally believed to operate as monomers, several recent lines of evidence based on thermodynamic, biochemical, and functional studies suggest that this class of membrane proteins may also associate as dimers (5–21). However, the question of whether dimerization is a general property of GPCRs and whether it is necessary for GPCR function remains controversial (9, 10, 15, 16, 21). The GABA-B receptor associates as a heterodimer via the cytoplasmic C-tail in the endoplasmic reticulum and is targeted to the plasma membrane as a preformed dimer, independent of agonist regulation (11–14, 21). Whether other GPCR dimers are similarly preformed or whether they undergo dimerization at the plasma membrane in response to agonist activation is unclear (9, 10, 15, 16, 21). Dopamine and muscarinic receptors have been postulated to exist on the membrane as preformed dimers that are stabilized by ligand binding (9, 19). The β-adrenergic receptor on the other hand undergoes ligand-dependent dimerization and activation, whereas agonists at the δ opioid receptor have been suggested to favor monomer formation that is required for agonist-induced internalization (10, 16). In the case of somatostatin (SST) receptors (SSTRs), there are five distinct subtypes that bind the two natural ligands, SST-14 and SST-28, with comparable low nanomolar affinity (22). The five subtypes also share common signaling pathways such as the ability to inhibit adenyl cyclase and to activate phosphotyrosine phosphatase (22–24). Furthermore, individual target cells typically express more than one SSTR subtype and often all five isoforms (25–28) raising the question of whether multiple SSTRs in the same cell are redundant or whether they interact for greater functional diversity. By using pharmacological, biochemical, and physical methods, here we show that SSTRs associate as dimers, both as homodimers or heterodimers with other members of the SSTR family, and that dimerization alters the functional properties of the receptor such as ligand binding affinity, signaling, and agonist-induced regulation. We provide the first direct evidence based on the sensitive fluorescence resonance energy transfer (FRET) analysis that hSSTR5 exists as a monomer in the basal state and undergoes dose-dependent increase in dimerization when treated with SST-14 suggesting that dimerization is induced by agonist binding.

EXPERIMENTAL PROCEDURES

Peptides and Antisera—Peptides and antisera were obtained as follows: SST-14, SST-28 (Bachem); Leu8-D-Trp22, Tyr25, SST-28 (LTT-SST-28, Leu8-D-Trp22, Tyr25, SST-28; SCH288, des-A_AA1,2,5[α-Trp3,1]AMP3[S]RIF, LTT-SST-28, Leu8-D-Trp22, Tyr25, SST-28; SCH288, des-A_AA1,2,5[α-Trp3,1]AMP3[S]RIF, SSTR, somatostatin receptor; wt hSSTR1, wild type human somatostatin receptor type 1; wt hSSTR4, wild type human somatostatin receptor type 4; HA-SSTR5, hemagglutinin-tagged somatostatin receptor type 5; ECL2, second extracellular loop segment; ECL3, third extracellular loop segment; C- tail, cytoplasmic carboxyl-terminal segment; CHO, Chinese hamster ovary; mAb, monoclonal antibody; phFRET, photobleaching fluorescence resonance energy transfer.

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Binding Assays, Internalization, and Up-regulation Experiments—Binding studies were carried out for 30 min at 37 °C with cell membrane protein or whole cells with 125I-labeled LTT-SST-28 radioligand or subtype-selective ligands as previously reported (29, 30, 32, 33). Receptor coupling to adenylyl cyclase was tested by incubating cells for 30 min with 1 mM forskolin with or without SST (10^{-10}–10^{-6} M) at 37 °C as described previously (30). Cells were then scraped in 0.1% HCl and assayed for cAMP by radioimmunoassay (30, 33). Internalization experiments were carried out by incubating cells overnight at 4 °C with radioligand with or without SST (0.1 mM) (30, 32, 33). After washing, cells were warmed to 37 °C for 15, 30, and 60 min to initiate internalization. At the end of each incubation, surface-bound radioligand was removed by acid wash, and internalized radioligand was measured as acid-resistant counts in 0.1 N NaOH extracts of acid-washed cells (30, 32, 33). The ability of long term treatment with SST to up-regulate surface SSTR binding was studied in cells cultured with 1 μM SST or SMS for 22 h as described previously (32, 33). After acid wash to remove surface-bound SST, whole cell binding assays were performed to determine total and nonspecific binding. Residual surface binding was calculated as the difference between control and experimental groups (32, 33).

Western Blots—CHO-K1 cells expressing HA-SSTR5 were analyzed for receptor protein by Western blots as reported previously (27). Membranes were incubated with or without SST-14 (10^{-6} M) for 30 min at 37 °C and then solubilized in sample buffer containing 62.5 mM/liter Tris-HCl, pH 6.8, 2% SDS, 10% glycerol, and 50 mM/liter dithiothreitol. 50-μg samples of membrane protein were fractionated by electrophoresis on 10% SDS-polyacrylamide gels as described by Laemmli (34). The fractionated proteins were transferred by electrophoresis to nitrocellulose membranes in a transfer buffer containing 0.025 mol/liter Tris, 0.192 mol/liter glycine, and 15% methanol. The membranes were then probed for HA-SSTR5 using the mouse monoclonal antibody and the lumilight Western blotting Kit (Roche Molecular Biochemicals) (27). Blots were analyzed semi-quantitatively using the computer scanning software Molecular Analyst.

Photobleaching (pb) FRET Microscopy—Generally, FRET efficiencies are determined indirectly by measuring changes in the quantum yield of any competitive donor deactivation process upon introduction of an acceptor molecule (35–39). Donor photobleaching represents such a competitive process that can be exploited in pbFRET microscopy. The effective FRET efficiency E is calculated from the photobleaching time constants of the donor (D) obtained in the absence (t_{D,A}) and presence (t_{D,A}) of acceptor (A) according to Equation 1.

\[
E = 1 - \frac{t_{D,A}}{t_{D,A}} \quad (\text{Eq. 1})
\]

In a two-state model, the minimal amount of receptor dimerization (α_{min}) is related to the fraction of acceptor labeled receptor (f_A) and E as shown in Equation 2.

\[
\alpha_{min} = \frac{SE}{f_A(2 + E)} \quad (\text{Eq. 2})
\]

where \( f_A \) is determined from the relative affinities of fluorescein- and rhodamine-conjugated mAbs and the concentration ratio used for incubation (39). pbFRET experiments were performed on CHO-K1 cells stably expressing HA-hSSTR5 using a Leica DMBL fluorescence microscope equipped with epi-illumination. An OSMR HBO 100-watt mercury lamp was used as excitation light source. In order to separate fluorescein excitation from emission as well as to optimize fluorescein excitation while simultaneously blocking rhodamine excitation, the following filters were used: Leitz BP 450–490 (excitation), RKP510 (dichroic mirror), and BP 515–535 (emission). Digital images (8-bit) were generated with an Electricon-1000U CCD camera with a spatial resolution of 1134 × 486 pixels of size 7.8 × 13.6 μm. Exposure as well as time delays were software controlled. IGOR Pro 3.13 (Wavemetrics, OR) was used for image analysis. Images were corrected for dark current, background, and flatness.

Immunocytochemistry—Expression of SSTRs in transfected CHO-K1 cells was determined by immunocytochemistry. Rabbit polyclonal anti-peptide antibodies directed against sequences in the amino-terminal segment of hSSTR1 (diluted 1:300) or mouse monoclonal anti-HA antibodies (diluted 1:300) were used as primary antibodies followed by reaction with rhodamine or fluorescein-conjugated secondary antibody as described previously (33). To demonstrate colocalization of hSSTR1 with hSSTR5, CHO cells stably cotransfected with wt hSSTR1 and HA-hSSTR5 were treated with 1 μM SMS for 12 h at 4 °C. For receptor localization on the plasma membrane, cells were fixed at 4 °C in 3.7% formalin for 15 min. For receptor localization in vesicles, cells were incubated for an additional 60 min at 37 °C to allow internalization, fixed, and permeabilized in methanol/acetone at −10 °C for 15 min. The fixed cells in both instances were processed for double label confocal fluorescence immunocytochemistry. Cells were mounted with immunfluor and viewed under a Zeiss LSM 410 confocal microscope. Images were obtained as single optical sections taken through the middle of cells and averaged over 32 scans/frame.

Statistical Analysis—Results are presented as mean ± S.E.

RESULTS

Agonist-dependent Homodimerization of hSSTR5—We first demonstrated SSTR homodimerization by functional complementation of two partially active mutants of human SSTR5 (hSSTR5) that we have previously described (29, 30) (Fig. 1). One is a conservative segment exchange mutant of the second extracellular loop, ECL2 hSSTR5 which fails to bind SST-14/ SSTR-28 but which is correctly targeted to the plasma membrane as shown by immunocytochemistry (29). The other is a cytoplasmic tail (C-tail) deletion mutant Δ318 hSSTR5 that displays complete loss of adenylyl cyclase coupling while retaining full agonist binding potency and the ability to undergo agonist-dependent internalization (30). We wondered whether loss of adenylyl cyclase coupling by the C-tail deletion mutant could be rescued by cotransfection, whereby the binding competent mutant would associate and signal through the C-tail of the binding-deficient mutant. The two mutants were stably cotransfected in CHO-K1 cells (B_{max} 119 ± 36 fmol/mg protein) and compared with individual Δ318 hSSTR5 (B_{max} 126 ± 43 fmol/mg protein) or ECL2 hSSTR5 (no binding) stable monotransfectants. Coupling to adenylyl cyclase was determined by the ability of SST-28 to inhibit forskolin-stimulated cAMP. In the cotransfectant, SST-28 produced dose-dependent inhibition of forskolin-stimulated cAMP (31 ± 2.5% at 1 μM agonist) which was completely abolished by pertussis toxin treatment (Fig. 2B). This suggests that the two mutant receptors assemble as homomers to constitute a functional G protein-linked effector complex. Competition analysis showed a significant 4-fold increase in the binding affinity of SST-14 for the mutant Δ318-hSSTR5/ECL2-hSSTR5 dimeric receptors (K_i 3.1 ± 1.9 nm) compared with Δ318 hSSTR5 alone (K_i 12.1 ± 2.5 nm) suggesting physical association leading to a change in receptor conformation (Fig. 2A). We next investigated the effect of expression of the mutant receptors on receptor internalization.
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Fig. 2. Homodimerization of hSSTR5. Effect of cotransfecting ECL2-hSSTR5 and Δ318-hSSTR5 mutants on ligand binding affinity, adenyl cyclase coupling, and internalization. A, displacement analysis of Δ318-hSSTR5 alone (○) (Kᵦ, 12.1 ± 2.5 nM) compared with that of cotransfectants (●) (Kᵦ, 3.1 ± 1.9 nM). B, SST-28 produces dose-dependent inhibition of forskolin-stimulated cAMP (△) that is abolished by 100 ng/ml pertussis toxin pretreatment (●). C, percent internalization of 125I-LTT-SST-28 by cells expressing Δ318-hSSTR5 alone (△) compared with Δ318-hSSTR5/ECL2 hSSTR5 cotransfectants (○). For comparative purposes, wt hSSTR5 monotransfected in CHO-K1 cells displayed a proportion of dimers (monomer:dimer ratio 54–65 M with 1-cyclase coupling, and internalization.

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Fig. 3. Representative Western blot of HA-hSSTR5 showing ligand-induced homodimerization. Membranes from nontransfected (control) or HA-hSSTR5 transfected CHO-K1 cells were incubated with different amounts of SST-14 for 30 min and analyzed by Western blots using HA monoclonal antibody. A, hSSTR5 exists as a mixture of broad monomeric 55–65 kDa and dimeric 105–115 kDa bands. Treatment with SST-14 results in a dose-dependent increase in the proportion of dimers. A sharp nonspecific band at 77 kDa is observed in control and transfected cells. B, semiquantitative analysis of the percent of monomer and dimer species using computer scanning software MasterScan. The nonspecific band was used as an internal standard for protein estimation. Mean ± S.E. of 3 independent experiments.

Agonist-dependent Homodimerization of hSSTR5 in Intact Cells by Photobleaching FRET Analysis—To obtain direct evidence for the association of SSTRs in intact cells, we probed for receptor homodimerization by pbFRET microscopy (35–39). HA hSSTR5 was visualized in CHO-K1 cells using fluorescein (donor)- and rhodamine (acceptor)-conjugated monoclonal antibody (mAb) against HA. Both fluorophore-tagged antibodies exhibited clear plasma membrane staining (Fig. 4A, a–c) as well as competitive antigen binding (Fig. 4A, d), from which their relative affinities could be determined. The decrease in donor fluorescence intensity due to photobleaching during prolonged exposure to excitation light was monitored in the absence (Fig. 4A, a) or presence (Fig. 4A, b) of acceptor, i.e. in the potential presence of an additional donor deactivation process, FRET. The photobleaching decay was analyzed for the plasma membrane regions, both on a pixel-by-pixel basis (Fig. 4, IIb and IIIb) as well as averaged over each image (Fig. 4II, c and d). We observed a significant slow down of the photobleaching process (as described by an increase in the photobleaching time constant) upon addition of rhodamine-labeled antibody to the cells suggesting that a large proportion of rhodamine molecules are in close enough proximity to fluorescein to act as acceptors for energy transfer. Given that the two fluorophores are associated with different receptor molecules, this finding suggests receptor association. In the basal state, we found effective FRET efficiencies of approximately 11 and 15% for donor:acceptor.

(Fig. 2C). CHO-K1 cells expressing either Δ318 hSSTR5 or Δ318 hSSTR5 and ECL2 hSSTR5 mutants were incubated at 37 °C for different times with 125I-labeled LTT-SST-28 (a nonselective radioligand for all five SSTR subtypes) with or without 0.1 μM SST-28 (30, 32). The Δ318 hSSTR5 mutant displayed time-dependent internalization with a maximum of 41 ± 2.8% at 60 min (Fig. 2C). Coexpression of the ECL2 mutant markedly inhibited internalization of the monomeric complex to only 0.9% at 60 min (Fig. 2C) upon addition of rhodamine-labeled antibody to the cells suggesting that a large proportion of rhodamine molecules are in close enough proximity to fluorescein to act as acceptors for energy transfer. Given that the two fluorophores are associated with different receptor molecules, this finding suggests receptor association. In the basal state, we found effective FRET efficiencies of approximately 11 and 15% for donor:acceptor.
FIG. 4. I, confocal microscope images showing fluorescently labeled monoclonal anti-HA-antibody bound to the plasma membrane of CHO-cells transfected with HA-hSSTR5. a, fluorescein-conjugated mAb; b, rhodamine-conjugated mAb; c, colocalization of fluorescein- and rhodamine-conjugated mAb (yellow); d, competitive binding of native-, fluorescein-, and rhodamine-conjugated mAb. Cells were incubated with 2.5 μg/ml mAb (total concentration) in various ratios of fluorescein/unlabeled (○) and rhodamine/unlabeled (●) mAb. The relative fluorescence intensity (averaged...
of cells analyzed (with an average number of... as they were measured on different days and were therefore affected by the decrease in excitation intensity of the UV lamp.

This relative affinity was... corresponding to higher levels of dimerization of at least 42 and 30%, respectively (39). Treatment with... (Equation 2).

TABLE I

| SST-14 concentration | D:A ratio | τ_{avg} | n | σ_{avg} | E | σ_{min} |
|----------------------|-----------|---------|---|---------|----|---------|
| 0 (basal state)      | 1:1       | D - A   | 35.6 ± 1.1 | 33 | 6.1 | 1 ± 4 | 3 ± 11 |
|                      | 1:2       | D + A   | 36.0 ± 1.0 | 32 | 5.5 | 6 ± 4 | 0 ± 10 |
|                      | 1:1       | D - A   | 35.6 ± 1.2 | 31 | 6.4 | 4.0 | 0 ± 10 |
| 10^{-10} M           | 1:1       | D - A   | 14.4 ± 0.2 | 40 | 1.2 | 5 ± 3 | 14 ± 8 |
|                      | 1:1       | D - A   | 31.6 ± 0.5 | 46 | 3.7 | 13 ± 3 | 33 ± 7 |
| 5 × 10^{-8} M        | 1:1       | D - A   | 30.4 ± 1.2 | 27 | 6.2 | 16 ± 4 | 40 ± 8 |
| 10^{-6} M            | 1:1       | D - A   | 36.8 ± 0.8 | 21 | 3.6 | 4.0 | 20 ± 3 | 48 ± 6 |
|                      | 1:2       | D - A   | 33.6 ± 0.8 | 36 | 4.8 | 20 ± 3 | 48 ± 6 |
| High expression cell line | 1:3 | D - A   | 28.8 ± 1.0 | 30 | 5.6 | 23 ± 5 | 45 ± 8 | 33 ± 7 |
| 0 (basal state)      | 2:1       | D - A   | 21.0 ± 0.7 | 37 | 4.5 | 3 ± 3 | 11 ± 3 | 24 ± 6 |
|                      | 1:1       | D + A   | 22.4 ± 0.5 | 46 | 3.7 | 15 ± 3 | 30 ± 5 |
|                      | 1:1       | D + A   | 26.3 ± 0.8 | 34 | 4.5 | 15 ± 3 | 30 ± 5 |
| 10^{-6} M            | 1:2       | D - A   | 19.9 ± 0.6 | 22 | 2.7 | 21 ± 3 | 42 ± 5 |
|                      | 1:3       | D - A   | 20.1 ± 0.4 | 54 | 3.1 | 27 ± 2 | 48 ± 3 |
|                      | 1:1       | D + A   | 27.4 ± 0.6 | 48 | 3.9 | 21 ± 3 | 42 ± 5 |

FIG. 5. Dose-dependent increase in effective FRET efficiency by SST-14 in CHO-K1 cells expressing relatively low density of HA-hSSTR5 (see Table I for D:A = 1:1).

hSSTR1—We next investigated SSTR heterodimerization and selected hSSTR1 and hSSTR5 to take advantage of their different pharmacological properties. Both receptors bind SST-14 and SST-28, but only SST5 and not SSTR1 binds the octapeptide SMS-(201–995) (SMS, Octreotide) (22). SSTR5 is internalized by acute agonist exposure, whereas hSSTR1 is resistant to internalization and is instead up-regulated at the membrane by prolonged agonist treatment (30, 32, 33). As previously over 25 cells) was plotted against the proportion of fluorescently labeled mAb. Binding affinity of the rhodamine-labeled mAb was identical to that of the unlabeled mAb (solid line), whereas it was reduced by a factor of 0.44 for the fluorescein-labeled mAb (dotted line). This relative affinity was used for determining the minimum level of receptor dimerization as function of FRET efficiency (Equation 2). II, photobleaching of fluorescein (donor) in absence of rhodamine (acceptor). In this example, cells were treated with 1 μM SST-14, and the ratio of donor labeled to unlabeled mAb was 1:2. a, during donor photobleaching, a sequence of 20 images was acquired, one image every 4 s with exposure time 3 s (only selection shown). For analysis of the photobleaching decay, only the high intensity membrane region was considered; the low intensity background and intracellular regions were masked (black). Leftmost, unmasked image of initial donor fluorescence. b, the decrease of fluorescence intensity was analyzed for each pixel of the unmasked region and fitted to a single exponential. The resulting time constants were plotted in the histograms shown. The average time constant of 18.0 s (black bar) was taken as τ_{D-A} (see Equation 1). c, histograms of fluorescence intensities for the selection of images in a, d, average fluorescence intensity of each image versus exposure time to excitation light. The monoeXponential fit (red) as well as the residue (green) demonstrate the good approximation of the photobleaching decay by a single exponential. III, a, photobleaching of fluorescein presence of rhodamine. The protocol was the same as in B, except that rhodamine-conjugated mAb was used in place of unlabeled mAb. b, the presence of rhodamine led to larger donor photobleaching time constants, with an average, τ_{D-R}, of 27.6 s, reflecting FRET between fluorescein and rhodamine.
Agonist-dependent Internalization of hSSTR1 through Heterodimerization with hSSTR5—We further investigated agonist-induced up-regulation of hSSTR1 through heterodimerization with Δ318 hSSTR5. hSSTR1 is up-regulated at the membrane by prolonged (22 h) exposure to SST-14 (33). SMS does not bind and therefore does not up-regulate this receptor (Fig. 8A). The Δ318 hSSTR5 mutant bound both SST-14 and SMS, but neither induced up-regulation. Treatment of the coexpressed receptors with SMS 1 μM for 22 h induced 11 ± 16% up-regulation of cell surface binding comparable with that obtained with 1 μM SST-14 (113 ± 23%). Pharmacological analysis of the up-regulated receptors with radioligand selective for SSTR1 (125I-SCH288) or SSTR5 (125I-Tyr3-SMS) showed 92 ± 12.5% increase in 125I-SCH288 binding without any change in 125I-Tyr3 SMS binding, thereby identifying hSSTR1 as the receptor subtype that was up-regulated at the cell surface by chronic SMS treatment (Fig. 8B). Since SMS does not bind hSSTR1, its ability to up-regulate this receptor must be through binding to Δ318 hSSTR5 and association with hSSTR1. Although cross-talk between the receptors at the level of signaling cannot be entirely excluded, this appears unlikely since deletion of the C-tail of hSSTR5 blocks signaling, at least via its receptor to undergo agonist-induced internalization as a monotransfectant (32, 33). hSSTR1 cotransfected with Δ318 hSSTR5, however, displayed 15 ± 5% internalization of 125I-SCH288 at 60 min. Since hSSTR1 alone cannot internalize 125I-SCH288, the presence of this radioligand intracellularly must reflect internalization of hSSTR1/Δ318 hSSTR5 heterodimers. This was further demonstrated by confocal fluorescence immunocytochemistry using CHO-K1 cells cotransfected with wt hSSTR1 and HA-hSSTR5. Both receptors were colocalized on the plasma membrane of nonpermeabilized cotransfected cells (Figs. 7, a–c). As previously reported, hSSTR1 was predominantly localized over the cell surface when expressed alone in CHO-K1 cells (Fig. 7g) (33). The hSSTR1 cells permeabilized after 60 min treatment at 37 °C with agonist SST-14 (1 μM) showed very poor labeling of cytoplasmic vesicular structures (Fig. 7h) (33). In contrast, when hSSTR1 was cotransfected with HA-hSSTR5, it underwent internalization in the presence of agonist and was indeed colocalized with hSSTR5 in cytoplasmic vesicles of permeabilized cells (Fig. 7, d–f). These results suggest that although hSSTR1 does not internalize when expressed alone, it does so when coexpressed with an appropriate partner, in this case hSSTR5.
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The density of endogenous SSTR expression in receptor-rich tissues such as the brain, pituitary, pancreas, and adrenals in the rat measured with a nonspecific radioligand such as 125I-LT3-SST-28 (which detects all five SSTR subtypes) ranges between 220 and 360 fmol/mg protein (40). In addition, because these tissues express all five SSTR isoforms, the concentration of individual subtypes is likely to be a fraction of this amount. To determine whether the level of receptor expression influencing dimerization, we initially studied recombinant hSSTR5 overexpressed in CHO-K1 cells and found significant dimerization of this receptor in the basal state, both by Western blots and FRET analysis. At lower levels of transfection corresponding to endogenous SSTR concentrations, however, hSSTR5 oc-

**DISCUSSION**

The existence of receptor dimers has been proposed for several GPCRs, based on studies of cross-linked or solubilized receptors or on functional complementation of mutant and chimeric receptors (5–10, 19–21). However, the question of whether GPCR dimers are necessary for activating or modulating normal receptor function has remained unclear (16, 21). By using FRET to monitor dimerization directly, as well as pharmacological and biochemical studies of both mutant and wild type receptors, here we provide strong evidence that members of the SSTR family, undergo agonist-dependent homo- and heterodimerization and that dimeric association alters SSTR functions such as ligand binding affinity, internalization, and up-regulation. We show that hSSTR5 forms heterodimers with hSSTR1 but not with hSSTR4 suggesting that heterodimerization of SSTRs is a specific process that is restricted to some but not all receptor subtype combinations.

**FIG. 7.** Confocal immunofluorescence analysis of wt hSSTR1 and HA-hSSTR5 stably cotransfected in CHO-K1 cells demonstrating receptor distribution on plasma membrane of nonpermeabilized cells (a–c) and in cytoplasmic vesicles in permeabilized cells after treatment with SMS 1 μM (d–f). a, fluorescein immunofluorescent images showing HA-hSSTR5 localized on the plasma membrane (green). b, rhodamine immunofluorescent images of wt hSSTR1 localized on the plasma membrane (red). c, merged image to show colocalization of the two receptors on the plasma membrane (yellow). d–f, in permeabilized cells, hSSTR5 (d, green label) and hSSTR1 (e, red label) are colocalized (f, yellow image) in cytoplasmic vesicular structures. g and h, rhodamine fluorescence of hSSTR1 expressed alone in CHO-K1 cells. hSSTR1 is distributed intracellularly with hSSTR5 when coexpressed but not alone.

**FIG. 8.** Agonist-induced up-regulation of hSSTR1 through heterodimerization. Cells expressing either wt hSSTR1, Δ318 hSSTR5, or both receptors together were incubated with control medium (open bars) or medium containing 1 μM SMS (black bars) or SST-14 (hatched bars) for 22 h and subjected to acid wash followed by whole cell binding with different radioligands. A, binding of 125I-LT3-SST-28 to mono- and cotransfected cells. B, binding of 125I-SCH288 and 125I-Tyr3 SMS to cotransfectants. C, up-regulation of endogenous hSSTR1 in MCF7 cells by treatment with SMS assessed by 125I-LTT-SST-28 and 125I-SCH-288 radioligands. Mean ± S.E. of 3 experiments.
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