To elucidate the signaling events mediated by specific somatostatin receptor (SSTR) subtypes, we expressed SSTR1 and SSTR2 individually in rat pituitary GH-2C1, and F10C1 cells, which lack endogenous somatostatin receptors. In transfected GH-2C1 cells, both SSTR1 and SSTR2 coupled to inhibition of Ca^{2+} influx and hyperpolarization of membrane potential via a pertussis toxin (PTx)-sensitive mechanism. These effects reflected modulation of ion channel activities, which are important for regulation of hormone secretion. Somatostatin analogs MK678 and CH275 acted as subtype selective agonists as expected. In transfected F10C1 cells, both SSTR1 and SSTR2 mediated somatostatin-induced inhibition of adenyly cyclase via a PTx-sensitive pathway. In addition, activation of SSTR2 in F10C1 cells, but not SSTR1, stimulated phospholipase C (PLC) activity and an increase in [Ca^{2+}]_{i} due to release of Ca^{2+} from intracellular stores. Unlike adenyly cyclase inhibition, the PLC-mediated response was only partially sensitive to PTx. To determine the structural determinants in SSTR2 necessary for activation of PLC, we constructed chimeric receptors in which domains of SSTR2 were introduced into SSTR1. Chimeric receptors containing only the third intracellular loop, or all three intracellular loops from SSTR2, mediated inhibition of adenyly cyclase, but failed to stimulate PLC activity as did wild-type SSTR2. Furthermore, the C-terminal tail of SSTR2 was not required for coupling to PLC. Thus, by expressing individual somatostatin receptor subtypes in pituitary cells, we have identified both overlapping and distinct signaling pathways for SSTR1 and SSTR2, and have shown that sequences other than simply the intracellular domains are required for SSTR2 to couple to the PLC signaling pathway.

Somatostatin (SS) is a tetradecapeptide which has diverse physiological actions (1, 39). It was first isolated and identified as an inhibitor of growth hormone (GH) secretion from the anterior pituitary gland (2). It also inhibits secretion of insulin, glucagon, gastrin, and secretin (1, 39). In the central nervous system, SS acts as a neuromodulator, regulating neuronal firing and facilitating release of neurotransmitters such as dopamine, norepinephrine, and serotonin (3). SS acts by binding to specific membrane receptors (4). Five SS receptor (SSTR) subtypes have been cloned and they belong to a family of receptors which couple via G proteins to cellular effector systems (5–9). In the pituitary gland, mRNAs for all five SSTRs are present (10). In the past, rat pituitary GH and GH-C1 cells have been used extensively to characterize endogenously expressed somatostatin receptors (11, 12). Somatostatin was shown to activate K+ channels and inhibit voltage-dependent Ca^{2+} channels (VDCC) leading to inhibition of Ca^{2+} influx (12). The secretion of GH is regulated mainly by changes in the cytosolic Ca^{2+} level; thus, the action of SS on ion channel activity is critically relevant to the physiological role of SS as an inhibitor of secretion. Since both SSTR1 and SSTR2 are present in GH and GH-C1 cells (13), the functional importance of each subtype is not known. Expression of a single SSTR subtype in heterologous cells such as COS and CHO cells does not allow studies of coupling of receptors to those specific ion channels that are characteristic of pituitary cells. In the first part of this report, we describe the expression of either SSTR1 or SSTR2 individually in pituitary GH-2C1 cells, which do not contain endogenous SSTRs, to study their independent roles in regulation of membrane ion channel activity.

Expression of SSTR1 and SSTR2 in heterologous systems such as COS and CHO cells has given inconsistent results as to whether both subtypes couple to inhibition of adenyly cyclase (14–16). It is possible that coupling of SSTRs to a particular G protein is affected by the specific cellular environment in which the receptors are expressed. Therefore, it is essential to study SSTR signaling pathways in a cell type in which SSTRs are expressed physiologically. We describe in this report that SSTR1 and SSTR2 have both overlapping and distinct patterns of signaling in pituitary cells. In addition, we examined the structural basis for the difference in signaling using a chimeric receptor approach.

### EXPERIMENTAL PROCEDURES

**Materials—** Pertussis toxin was purchased from List Biologicals (Campbell, CA). MK678 was obtained from Merck Laboratories (West Point, PA). Des-AA^{2-5}-[In-Trp{1}AMP]-SS (CH275) was synthesized by Drs. Carl Heoger and Jean Rivier at Salk Institute. myo-[125I]Insitol (80–120 Ci/mmol) and [3H]-[2-Tyr{1}SS (2000 Ci/mmol) were from American Inc. (Arlington Heights, IL). Cyclic AMP radioimmunoassay kits were purchased from NEN Life Products Inc. (Boston, MA). Fura-2/AM and bisoxonol were purchased from Molecular Probes (Eugene, OR).
Lipofectin was purchased from Life Technologies, Inc. (Grand Island, NY). Muta-gene kits and AG1–8X Dowex columns were from Bio-Rad. Somatostatin and other reagents were from Sigma.

**Cell Culture**—GH12C1 and F4C1 cells are clonal pituitary cell strains established from rat pituitary tumors (17). Both strains lack expression of the endogenous SSTR (4). Cells were grown in Ham’s F-10 nutrient mixture supplemented with 15% horse serum and 2.5% bovine serum (F10 10) at 37°C in a humidified atmosphere of 5% CO2, 95% air.

**Transient and Stable Transfection**—The cDNAs for SSTR1 from either the mouse (18) or rat (6) were used in this study, and no differences in SS binding and receptor-mediated signaling were detected. The cDNAs for the mouse SSTR2A isoform were used throughout this study (referred to as SSTR2A). SSTR1 and SSTR2 cDNAs cloned in the pcDNA3 vector (Invitrogen, Carlsbad, CA) were transfected into GH12C1 and F4C1 cells by the Lipofectin method according to the supplier’s protocol, or by electroporation with a Bio-Rad electroporator. Briefly, 106 cells were suspended in 0.4 ml of F10 medium containing 16 µg of plasmid DNA, and electroporation was performed at 260 V and 980 microfarads capacitance. In cotransfection experiments, 8 µg of human parathyroid hormone receptor cDNA and 8 µg of SSTR cDNA were used. For stable transfection, cells were selected in growth medium containing 500 µg/ml G418 beginning 48 h after transfection. After several weeks of culture in selection medium, single clones were isolated and screened using a radioligand binding assay for SSTRs (see below). Because these transgene-containing cell lines arose from primers from the vector and coding region, and solution RNAse protection assay using RNAs from different clones were used to confirm the expression of only the specific transfected SSTR isoform.

**Somatostatin Radioligand Binding Assay**—Cells were plated and grown in F10 medium in 24-well dishes for at least 24 h. Cells were incubated with minimal essential medium containing various concentrations (30 pM to 1 nM) of [125I]-labeled SS and/or 0.1% bovine serum albumin, 0.1% bacitracin, and 2 µg/ml aprotinin for 2 h at 37°C. The dishes were then washed rapidly 3 times with ice-cold phosphate-buffered saline. Cells were lysed in 0.1 N NaOH and the lysates counted in a γ-counter.

**Spectrofluorometric Measurement of Cytosolic Free Calcium Concentration ([Ca2+])**—For [Ca2+]i, measurement, cells were harvested using a HEPES-buffered salt solution (HBSS, 118 mM NaCl, 4.6 mM KC1, 10 mM N-tris(2-carboxyethyl) methyl ester (fura-2/AM) for 30 min at 37°C. Cells were then washed 3 times in HBSS containing 1 mM CaCl2. Fluorescence was measured using a Spex Fluorolog FLIIA spectrofluorometer at excitation wavelength of 342 nm and emission wavelength of 349 nm. The excitation and emission slit widths were 5 nm. [Ca2+]i, was calibrated from the fluorescence signal as described previously (19). Changes in [Ca2+]i were monitored using an anionic fluorescent dye bis-1,3-diethylthiobarbituric acid/tri-methine oxonol (bisoxonol) (12). Cells were harvested as described above. About 5 × 104 cells were used for each run in the presence of 40 mM bismalonic. Fluorescence was measured at excitation wavelength of 540 nm and emission wavelength of 580 nm. The excitation and emission slit widths were 5 and 10 nm, respectively.

**Electrophysiological Measurements**—The whole cell voltage clamp technique (20) was used to measure activity of VDCC in GH12C1 cells. The micropipet solution contained: 110 mM CsCl, 20 mM tetraethylammonium chloride–Cl-, 5 mM MgCl2, 5 mM NaATP, 4 mM BAPTA, 10 mM HEPES, pH 7.2, adjusted with Tris. The extracellular solution contained: 130 mM CsCl, 5 mM MgCl2, 10 mM N-tris(glu)con solution (HBSS, 118 mM NaCl, 4.6 mM KC1, 10 mM N-tris(2-carboxyethyl) methyl ester (fura-2/AM) for 30 min at 37°C. Cells were then washed 3 times in HBSS containing 1 mM CaCl2. Fluorescence was measured using a Spex Fluorolog FLIIA spectrofluorometer at excitation wavelength of 342 nm and emission wavelength of 349 nm. The excitation and emission slit widths were 5 nm. [Ca2+]i, was calibrated from the fluorescence signal as described previously (19). Changes in [Ca2+]i were monitored using an anionic fluorescent dye bis-1,3-diethylthiobarbituric acid/tri-methine oxonol (bisoxonol) (12). Cells were harvested as described above. About 5 × 104 cells were used for each run in the presence of 40 mM bismalonic. Fluorescence was measured at excitation wavelength of 540 nm and emission wavelength of 580 nm. The excitation and emission slit widths were 5 and 10 nm, respectively.

**Measurement of cAMP**—F4C1 cells were plated in 24-well dishes after transfection. After 48 h, cells were incubated with test reagents in the presence of 0.2 mM isobutylmethylxanthine for 15 min at 37°C. Intracellular cAMP was extracted with 50 mM HCl and quantitated by radioimmunoassay (21).

**Measurement of Total Inositol Polyphosphates (IPs)**—At 24 h after transfection, F4C1 cells were split into 24-well dishes at a density of 2 × 105 cells/well and incubated in F10 medium containing [3H]-inositol (2 µCi/ml) for 20 h. Cells were then washed with 0.5 ml of assay medium (20 mM HEPES-buffered minimal essential medium without sodium bicarbonate) with 5 mM LiCl for 10 min and then incubated with 0.5 ml of assay medium containing 5 mM LiCl and somatostatin for 1 h. Cells were then homogenized in 50 mM formic acid (0.5 ml, 20 mM) and added to each well to lyse the cells. IP fractions were separated by AG1–8X Dowex column chromatography (22). Inositol polyphosphates were eluted with 2 N ammonium formate, 20 mM formic acid and counted in a scintillation counter.

**Construction of Chimeric SSTR1/SSTR2 Receptors**—Chimeric receptors were constructed from parental mSSTR1 and mSSTR2 by the polymerase chain reaction and Kunkel’s method (23). The junctions for ICS in CH1 and CH2 (see “Results”) were Leu233 and Val276. In CH2, the residues in ICS and IC2 of SSTR1 were mutated one by one to the corresponding residues of SSTR2. In CH3, the junction is Val202. DNA sequencing was performed by the dyeoxy method (24) to verify the structural integrity.

**RESULTS**

SSTR1 and SSTR2 Each Coupled to Inhibition of Ca2+ Influx through VDCC and Hyperpolarization of Membrane Potential in GH12C1 Cells—In control untransfected GH12C1 cells or GH12C1 cells transfected with the pcDNA3 vector alone, no specific high affinity receptors for SS were detected by radioligand binding assays, and SS had no effect on [Ca2+]i. (Fig. 1, top panel) or membrane potential (see below). Several stable clones of GH12C1 expressing rat SSTR1 were obtained, and a single clone expressing 4,600 receptors/cell was used for further study. To obtain stable clones expressing SSTR2, more than 200 clones were screened, and no clone with >1,000 receptors/cell was obtained. However, several clones expressed the transfected SSTR2 mRNA, and one of them was used in subsequent functional experiments. In those GH12C1 clones expressing either SSTR1 or SSTR2, SS caused a decrease in [Ca2+]i, 2 J. Bruno and L. Chen, unpublished results.
SSTR2. Top, addition of 1 μM SS alone; middle, addition of 1.5 mM EGTA before 1 μM SS; bottom, addition of 10 μM nifedipine before 1 μM SS. The SS-induced decrease in [Ca\textsuperscript{2+}] was completely abolished by either EGTA or nifedipine. The traces are representative of at least three independent experiments.

(Fig. 1, middle panels). Pretreatment of the cells with PTx completely abolished the decreases in [Ca\textsuperscript{2+}], mediated by either SSTR1 or SSTR2 (Fig. 1, bottom panels). The decreases in [Ca\textsuperscript{2+}], mediated by SSTR1 and SSTR2 (Fig. 2, top panel) were abolished by either chelating extracellular Ca\textsuperscript{2+} with EGTA (Fig. 2, middle panels) or by incubating the cells with the l-type Ca\textsuperscript{2+} channel blocker nifedipine (Fig. 2, bottom panels). Thus, the decreases in [Ca\textsuperscript{2+}], induced by SS acting via SSTR1 and SSTR2 were mediated by inhibition of Ca\textsuperscript{2+} influx through VDCC. To verify this conclusion, the action of SS on Ca\textsuperscript{2+} channel activity was studied directly using the whole cell voltage clamp technique. Consistent with the effects of SS on [Ca\textsuperscript{2+}]\textsubscript{i}, the inward Ca\textsuperscript{2+} currents were inhibited acutely by SS in cells expressing either SSTR1 (Fig. 3B) or SSTR2 (Fig. 3C). The inhibition was reversed by washing out SS. No inhibition by SS was observed in GH12C1 cells transfected with vector alone (Fig. 3A).

We next examined the ligand specificity of the action of SS on [Ca\textsuperscript{2+}]\textsubscript{i}, using receptor subtype selective SS analogs. MK678, which binds preferentially to SSTR2 (18), had no effect on [Ca\textsuperscript{2+}]\textsubscript{i} in cells expressing only SSTR1, but it induced a decrease in [Ca\textsuperscript{2+}]\textsubscript{i} in cells expressing SSTR2 (Fig. 4). SS induced a decrease in [Ca\textsuperscript{2+}]\textsubscript{i} in cells expressing SSTR1 but not in cells expressing SSTR2 (Fig. 4). These results are the first demonstration that CH275 acts as an agonist to activate SSTR1 selectively.

Bisoxonol fluorescence has been used to measure changes in membrane potential in GH4C1 cells, and hyperpolarization of Vm induced by SS in GH4C1 cells was caused by activation of K\textsuperscript{+} channels (12). Using this technique, SS caused a decrease in cell (determined by saturation binding assays) were used for the study of signaling properties. Inhibition of adenyl cyclase activity by SS was determined by measuring the decrease in adenylyl cyclase activity in rat pituitary F4C1 cells via a PTx-sensitive G protein, probably G\textsubscript{i} or G\textsubscript{o}.

Both SSTR1 and SSTR2 Mediated Inhibition of Adenylyl Cyclase Activity in F4C1 Cells via a Pertussis Toxin-sensitive Mechanism—We next examined the coupling of SSTR1 and SSTR2 to adenyl cyclase activity in rat pituitary F4C1 cells, which also lack high affinity binding sites for SS. Stable clones of F4C1 cells expressing 17,200 rat SSTR1/cell or 5,400 SSTR2/cell were generated. The clones were used for the study of signaling properties. Inhibition of adenyl cyclase activity by SS was determined by measuring the decrease in adenylyl cyclase activity in GH12C1 cells expressing either SSTR1 or SSTR2 (Fig. 5, middle panels), indicating that activation of each receptor subtype caused hyperpolarization of membrane potential. These responses were inhibited completely by preincubation with PTx (Fig. 5, bottom panels). Thus, both SSTR1 and SSTR2 mediate modulation of ion channel activity in GH12C1 cells, via a PTx-sensitive G protein, probably G\textsubscript{i} or G\textsubscript{o}.

**Fig. 3.** Effects of SS on Ca\textsuperscript{2+} currents in GH4C1 cells. Whole cell Ca\textsuperscript{2+} currents were recorded under voltage- clamp conditions by depolarizing pulses from −80 mV to −10 mV. Representative traces are shown. A, lack of effect of SS in control GH2C1 cells transfected with vector alone; B, effect of SS in GH4C1 cells expressing SSTR1; C, effect of SS in GH4C1 cells expressing SSTR2. Control indicates current before addition of SS; SS indicates currents during superfusion of the cells with 1 μM SS; and Washout indicates currents after removal of SS.

**Fig. 4.** Subtype selective SS analogs induced [Ca\textsuperscript{2+}]\textsubscript{i} responses in appropriate GH2C1 cell clones. [Ca\textsuperscript{2+}]\textsubscript{i} was measured in GH2C1 cells stably expressing SSTR1 (left) or SSTR2 (right). Arrows indicate application of 100 nM MK678 or CH275. MK678 or CH275 caused no changes in [Ca\textsuperscript{2+}]\textsubscript{i} in control GH2C1 cells lacking SSTRs (data not shown). Each trace is representative of at least three independent experiments.
forskolin (Fsk)-stimulated cAMP accumulation. SS had no effect on basal or forskolin-stimulated cAMP levels in wild-type F4C1 cells not transfected with SSTRs (Fig. 6, left panel). However, SS acting via either SSTR1 or SSTR2 inhibited forskolin-stimulated cAMP accumulation, and this process was reversed completely by pretreatment with pertussis toxin (Fig. 6, middle and right panels). These results demonstrate that both SSTR1 and SSTR2 can mediate inhibition of adenyl cyclase activity via a PTx-sensitive G protein (possibly G_i/G_o) in F4C1 cells. In GH2C1 clones expressing either SSTR1 or SSTR2, SS did not inhibit vasoactive intestinal peptide-stimulated cAMP accumulation, while in the same cells carbachol, acting via endogenous acetylcholine receptors, did inhibit the vasoactive intestinal peptide effect (data not shown).

Only SSTR2 Coupled to the Inositol Lipid/[Ca^{2+}]_i Pathway in F4C1 Cells—Because several G_i-coupled receptors can activate more than one intracellular signaling pathway (26, 27), we examined the coupling of SSTR1 and SSTR2 to the inositol lipid pathway. In F4C1 cells stably expressing SSTR2, SS stimulated total inositol polyphosphate production about 5-fold over the basal level (Fig. 7). Stimulation was reduced about 35% by pretreatment with PTx. In F4C1 cells expressing an even higher level of SSTR1, no stimulation of IP production was induced by SS (Fig. 7).

The functional consequences of IP production were determined by measuring changes in [Ca^{2+}]. In control F4C1 cells or F4C1 cells expressing only SSTR1, SS had no effect on [Ca^{2+}], whereas in cells expressing SSTR2, SS induced an acute increase in [Ca^{2+}], (Fig. 8, top). The increase in [Ca^{2+}], in cells expressing SSTR2 was not abolished by chelation of extracellular Ca^{2+} with EGTA (Fig. 8, middle panel). Pretreatment of cells with 1 μM thapsigargin for 10 min, to deplete intracellular Ca^{2+} pools (28), abolished the increase of [Ca^{2+}], induced by SS (Fig. 8, bottom panel), supporting the conclusion that the increase in [Ca^{2+}], was due to release of Ca^{2+} from IP_3-sensitive intracellular Ca^{2+} stores. These results demonstrate that SSTR1 and SSTR2 differ in their ability to couple to the inositol lipid/[Ca^{2+}]_i pathway.
receptor was tested for its ability to express on the cell surface, to stimulate PLC activity, and to inhibit adenylyl cyclase activity in transient transfection assays. When transiently transfected into F4C1 cells, CH1, CH2, and CH3 expressed at levels comparable to wild-type SSTR1 and SSTR2 as determined by ligand binding experiments (Fig. 10). However, CH1 and CH2 did not couple to the inositol lipid pathway in response to SS as determined by the lack of rise in IP production. In F4C1 cells expressing CH3, SS induced formation of IPs as it did in cells expressing the intact SSTR2 (Fig. 11). Therefore, the three intracellular loops of SSTR2 are not sufficient for coupling to the inositol lipid pathway, and the C-terminal domain of SSTR2 is not required for this pathway. To exclude the possibility that CH1 and CH2 are functionally impaired to couple to any G protein, we studied their ability to inhibit adenylyl cyclase activity. To evaluate the action of SS on ligand-stimulated cAMP production, the human PTH receptor, a receptor known to couple to Gs (21), was transiently co-expressed with SSTR constructs in F4C1 cells. F4C1 cells lack functional PTH receptors, and PTH does not stimulate cAMP accumulation in untransfected F4C1 cells (data not shown). SS had no effect on PTH-stimulated cAMP accumulation in F4C1 cells (data not shown). SS had no effect on PTH-stimulated cAMP accumulation in F4C1 cells transfected with only the human parathyroid hormone receptor. All three chimeric receptors as well as wild-type SSTR1 and SSTR2 mediated inhibition of PTH-stimulated cAMP accumulation by SS (Fig. 12). Thus, the lack of coupling to PLC in CH1 and CH2 was not due to global nonfunctionality of these constructs. The finding that replacement of all three intracellular loops of SSTR1 with those of SSTR2 was not sufficient to confer SSTR2-specific signaling suggests that additional domains of SSTR2, possibly the transmembrane helices, are involved in determining the specificity of coupling of SSTR2 to the inositol lipid signaling pathway.

**DISCUSSION**

SS exerts its physiological and pharmacological actions by binding to one or more subtypes of cell surface receptors. Different SSTR isoforms have overlapping but distinct expression patterns (16), raising the possibility that some of the diverse actions of SS are mediated by different receptor subtypes. Alternatively, it is possible that a single receptor subtype can couple to more than one effector system and that such coupling may vary among cell types. It has been reported that SSTR1 and SSTR2 do not couple to inhibition of adenylyl cyclase in CHO cells (14); however, it has also been shown that SSTR1 and SSTR2 can couple, via Gi, to inhibition of adenylyl cyclase in other cell types (15). One possible explanation for these differences is that the G protein and effector components differ between the cell types. In the present study, SS did not inhibit adenylyl cyclase activity stimulated by vasoactive intestinal peptide in GH2C1 clones expressing either SSTR1 or SSTR2, while SS modulated ion channel activity in these cells. Both SSTR1 and SSTR2 mediated inhibition of adenylyl cyclase activity in F4C1 cells. Although we cannot rule out the possibility that the lack of effect of SS on adenylyl cyclase in GH2C1...
cells was due to a lower level of receptor expression, it is likely that SSTR coupling patterns to adenyl cyclase depend on the specific cellular environment.

Fujii et al. (31) reported, that in rat insulinoma RINm5F cells, SSTR2, but not SSTR1, coupled to inhibition of VDCC, suggesting that there are subtype specific differences in regulation of ion channel activity. On the other hand, we have demonstrated that in GH2C1 cells, both SSTR1 and SSTR2 mediate inhibition of voltage-gated Ca^{2+} channels. These results again demonstrate that SSTR isoforms couple to specific signal transduction pathways depending on the specific type of cells in which they are expressed.

Different signaling through the same receptor can be transduced by different G proteins (27). Specifically, the a1, b1, and y3 subunits of Go have been shown to mediate inhibition of Ca^{2+} channels by SSTRs in GH1 cells (32). In a GH2C1 cell strain in which Gi was knocked out by overexpressing antisense mRNA (33), the effect of SS on Ca^{2+} influx was no longer observed; however, the effect of SS on membrane potential was not altered in these knockout cells. Thus, membrane hyperpolarization which is due to activation of K+ channels is not dependent on Gi and may be mediated by Gi subunits. It is likely that in GH2C1 cells, SSTR1 and SSTR2 couple to their cognate G proteins with similar specificity.

In human embryonic kidney 293 cells expressing SSTR2, we have reported that SS activates PLC and increases [Ca^{2+}]i (38). Tomura et al. (34) reported, that in COS cells expressing SSTR2, SS stimulated PLC/[Ca^{2+}]i, while in cells expressing SSTR1, SS had little or no effect. In the present report, we have shown that in pituitary F4C1 cells expressing about 5,400 SSTR2 receptors/cell, that SS clearly activates the PLC/[Ca^{2+}]i pathway. Because this response was only partially blocked by PTx, it was mediated, at least in part, by a PTx-insensitive G protein, possibly Gq/11. SS induced increases in [Ca^{2+}]i have also been observed in normal cells, including astrocytes (35) and intestinal smooth muscle cells (36). Therefore, coupling to multiple pathways by SSTR2 cannot be attributed to an artifact of the transfected cell systems.

The intracellular domains of a receptor serve as contact sites for interacting with G proteins and, in many cases, determine the specificity of coupling (30). Therefore, it was somewhat unexpected that the introduction of the putative intracellular domains of SSTR2 into SSTR1 did not confer SSTR2-like PLC activation in response to SS under conditions in which the chimeric receptors were expressed as wild-type receptors. Thus, the specificity of G protein coupling by SSTR2 is not determined solely by its intracellular domains. Additional structural determinants, probably from the transmembrane domains of SSTR2, are needed to enable coupling to the inositol lipid signaling pathway. These findings are consistent with the concept that the transmembrane domains of a G protein-coupled receptor act in concert with the intracellular loops to affect the conformation of the contact regions for the G protein (37). In an attempt to define the transmembrane sequences required, we tested a series of SSTR1/SSTR2 chimeras in which both transmembrane and intracellular domains were swapped. Unfortunately, these chimeras all expressed poorly on the cell surface, suggesting that there are subtype specific differences in regulation of ion channel activity.

3 L. Chen, unpublished data.
making it impossible to study their signaling properties. Further studies, using chimeras with more subtle exchanges and adequate receptor expression will be necessary to identify the critical transmembrane and intracellular sequences needed to confer SSTR2-specific signaling.

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