A novel system that reports the G-proteins linked to a given receptor: a study of type 3 somatostatin receptor

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Abstract SSTR3, a somatostatin (SST) receptor, is an adenylyl cyclase (AC)-inhibiting receptor. To assign the G-protein α-subunit (Gα) linked to this receptor, we created a novel reporter system which utilizes the well-established facts that the C-terminal 5 residues of Gα are the receptor contact site and $G\alpha_s$ stimulates all subtypes of AC. We constructed chimeric $G\alpha_s$ the C-terminal 5 residues of which were replaced with the corresponding C-terminus of each known Gα, and examined which chimera confers SSTR3-induced activation of AC. Cellular transfection of SSTR3 and measurement of SST-dependent AC activity through co-transfected chimeric $G\alpha_s$ revealed that SSTR3 recognizes the C-termini of $G\alpha_{i1/2}$ but not of $G\alpha_o$ or $G\alpha_z$, and those of $G\alpha_{14}$ and $G\alpha_{16}$, but not of $G\alpha_q$ or $G\alpha_{11}$. As predicted by the chimeric $G\alpha_s$, SST-bound SSTR3 stimulated polyphosphoinositide turnover only when $G\alpha_{16}$ or $G\alpha_{14}$ was co-transfected. We conclude that the chimeric $G\alpha_s$ system provides a new approach towards the assignment of G-proteins linked to a given receptor.

Key words: G-protein; Subtype specificity; Somatostatin receptor; Reporting system; Adenylyl cyclase; G_s chimera

1. Introduction

Somatostatin (SST) is a 14-amino acid cyclic peptide which is the major physiological inhibitor of GH secretion from the pituitary [1] and involved in the regulation of insulin and glucagon secretion from the pancreatic islets [2]. In addition, SST has a role in modulating motor activity and cognitive processes in the central nervous system [3,4]. The diverse biological actions of SST are mediated by specific receptors. SST receptors consist of at least five subtypes, all of which possess a heptahelical architecture, suggesting that they are the receptors coupled to G-proteins. It has been inferred that they all suppress AC (adenylyl cyclase); however, there has been some dispute about several of them [5]. Among these receptors, SSTR3 (the type 3 receptor) is the best characterized receptor that can definitely inhibit AC. However, it remains unclear which member of the G_i family ($G\alpha_i$, $G\alpha_o$, $G\alpha_z$) is involved in SSTR3-mediated inhibition of AC or what other Gα family may couple to this SST receptor.

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G-proteins link cell surface receptors to various biological functions which include hormone action, neurotransmission, and chemotaxis, as well as perception of light, smell, and taste [6]. This transducer family consists of various Gα gene products. Their members are classified into two categories: one group is a family of sensory organ-specific G-proteins ($G\alpha_t$, $G\alpha_{olf}$, and $G\alpha_{gust}$), and the other is a less tissue-specific class ($G\alpha_s$, three $G\alpha_i$, two $G\alpha_o$, $G\alpha_z$, $G\alpha_q$, $G\alpha_{11}$, $G\alpha_{12}$, $G\alpha_{13}$, $G\alpha_{14}$, and $G\alpha_{16}$). As for the generic G-coupled receptors, determining the linked Gα of the latter subclass is most important in assessing the signal. However, the Gα assignment has not been simple, because (i) there are many Gα proteins inside the cell, each similar but distinct; (ii) only a few biochemical effects are known as the outputs of Gα; (iii) those known outputs are shared by many Gα; and (iv) most receptors couple to multiple Gα.

Among the few established outputs of Gα, stimulation of AC has been established as the direct effect of the G_s family; no other Gα can directly activate AC; and either type of AC is stimulated by $G\alpha_s$. These features allow the monitoring of $G\alpha_s$ activity in vivo by measuring the cAMP production. However, quite a few receptors do not activate $G\alpha_s$; assessment of the G-protein coupling of receptors based upon AC stimulation can only be made infrequently.

On the other hand, it has been accepted that the C-terminus of Gα is a site contacted by receptors. Conklin et al. [7] have shown that C-terminal 4–5 residues of $G\alpha_i$ are the minimal requirement for specific recognition by G_i-coupled receptors. Voyno-Yasenetskaya et al. [8] converted the coupling of the $G\alpha_i$-specific D_2 receptor to $G\alpha_{13}$ coupling by expressing the $G\alpha_{13}$ chimera having the C-terminal 5 residues of $G\alpha_i$. Liu et al. [9] also concluded that the C-terminal 5 residue region of Gα is an essential site for receptor contact. In contrast, the effector contact sites have been located more N-terminally in $G\alpha_s$ [10,11]. Conclusions made by these earlier studies suggest that a $G\alpha_s$ chimera ($G\alpha_s/\alpha_x$) having the C-terminal 5 residues of a Gα subunit ($G\alpha_x$, x = any) alters the output of $G\alpha_x$-coupled receptors to stimulate AC.

We created various chimeric $G\alpha_s$, referred to as $G\alpha_s/\alpha_x$, the C-terminal 5 residues of which were replaced with the corresponding C-terminus of each known Gα. We hypothesized that the G-protein coupling specificity of any AC-non-stimulating receptor is precisely assigned by expressing each of the chimeras to observe cAMP increase by the receptor ligand. If a receptor of interest is coupled to $G\alpha_x$, the ligand-bound receptor should recognize and activate the co-transfected $G\alpha_s/\alpha_x$ and cause paradoxical stimulation of AC. Therefore,

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one could know that the receptor has recognized the C-terminus of a particular $G\alpha_x$, by measuring the agonist-dependent increase in cAMP in cells expressing the receptor and the cognate $G\alpha_s/\alpha_x$.

In this study, we tested this hypothesis and the utility of these chimeras. As described above, SSTR3 is an established G_i-linked receptor. Therefore, we examined whether SSTR3 stimulates AC through the mediation by $G\alpha_s/\alpha_i$, as expected, and whether SSTR3 recognizes the chimera with $G\alpha_o$ or $G\alpha_z$, which is another Gα capable of inhibiting AC. We also examined whether other constructs of these chimeras can predict the novel G-coupling capacity of SSTR3. Our chimeric $G\alpha_s$ system correctly indicated the $G\alpha_i$-coupling function of SSTR3 and correctly predicted its so far unknown ability coupling to two non-$G\alpha_q$ members of the G_q family G-protein. Here, by extension of well-established G-protein properties, we have established a novel system that reports the G-protein specificity of a given receptor.

2. Material and methods

Human SSTR3 DNA encoded in the cytomegalovirus promoter-driven expression vector pCMV6 have been described [16]. The construction of $G\alpha_s$ chimeras using PCR was described previously [12]. Some of the nucleotides used for the construction of $G\alpha_s$ chimeras were described in [12]. Several which were not included are:

for $G\alpha_s/\alpha_i$ TTAAGAGATTGCGGCTTATTTTAAT
CTAGATTAAAATAAGCCGCAATCTC
for $G\alpha_s/\alpha_q$ TTAAGAGAGTACAACCTCGTTTAAT
CTAGATTAAACGAGGTTGTACTCTC
for $G\alpha_s/\alpha_{12}$ TTAAGAGATATCATGCTTCAATAAT
CTAGATTATTGAAGCATGATATCTC
for $G\alpha_s/\alpha_{13}$ TTAAGACAACTCATGCTTGAATAAT
CTAGATTATTCAAGCATGAGTTGTC
for $G\alpha_s/\alpha_{14}$ TTAAGAGAATTCAACTTAGTTTAAT
CTAGATTAAACTAAGTTGAATTCTC
for $G\alpha_s/\alpha_{16}$ TTAAGAGAGATCAATTTGTTGTAAT
CTAGATTACAACAAATTGATCTCTC

The final products were verified by sequencing. Rat PTHR (PTH/PTHRP receptor) cDNA was provided by Dr. Gino V. Segre. SST-14, referred to as SST here, and PTH 1-34 were from Sigma.

The COS cells were described previously [13]. Transient transfection was performed by the standard lipofection method [13]. Cells (2×10^4) were seeded onto a 24-well plate and cultured in complete growth media for 24 h, transfected with 0.25 μg cDNA (0.125 μg SSTR DNA and 0.125 μg chimera cDNA) and 1 μl of LipofectAMINE for another 24 h in serum-free DMEM, and cultured in complete growth media for an additional 24 h. The total amount of DNAs was adjusted by an empty plasmid to be 0.25 μg. As G-protein signals inhibit the transcriptional activity of various promoters that have been used for standardization [14], we did not employ the standardization with the reference genes. However, the reproducibility of the cAMP results in SSTR-/chimera-transfected cells between assays and between transfections was excellent, as described in Section 3.

AC activity was assessed by measuring cAMP formation, as described [13]. Twenty-four hours after transfection, cells were labeled with 6 μCi/ml of [^{3}H]adenine (Du Pont-NEN) for 24 h, and then treated with agonists and 1 mM IBMX for 30 min. The cAMP produced was assayed using two-step ion-exchange columns, and specific accumulation of cAMP was expressed as $[cAMP/(ADP+ATP)]\times10^3$, which represent intact-cell AC activity. PI (polyphosphoinositide) turnover was assessed by measuring IP production. Cells were seeded at 4×10^4 onto a 24-well plate, cultured in complete growth medium for 24 h, and transfected with plasmids for 24 h. The culture medium was changed to the labeling medium [inositol-free RPMI + dialyzed fetal calf serum and 10 μCi/ml of [^{3}H]myo-inositol (Du Pont-NEN)]; and cells were incubated with this medium for 12 h at 37°C. Cells were then washed 4 times with inositol-free RPMI and treated with 1 μl of SST in inositol-free RPMI for 5 min at 37°C. Cells in 0.2 ml media were lysed by adding 0.8 ml of ice-cold 12.5% (final 10%) TCA, and the sample was then put on ice for 20 min. The supernatant was mixed well with 1 ml of saturated diethyl ether to extract acid. After repeating extraction 5 times, the collected sample was neutralized with 1:100 dilution of concentrated ammonia, and then added to 4 ml of water. These samples were analyzed by the method [15] using Dowex columns. The quantity of PI turnover was assessed with the production of inositol phosphates. Statistical analysis was performed with Student's t test with $P<0.05$ accepted as statistically significant.

3. Results and discussion

We constructed $G\alpha_s$ chimeras consisting of the $G\alpha_s$ residues 1–389, which lacks the original five residues of $G\alpha_s$ at the C-terminus, and the C-terminal 5 residues of each known Gα (Fig. 1A). The C-terminal 5 residues are identical between $G\alpha_{i1}$ and $G\alpha_{i2}$, between $G\alpha_{o1}$ and $G\alpha_{o2}$, and between $G\alpha_q$ and $G\alpha_{11}$. They were designated $G\alpha_s/\alpha_i$, $G\alpha_s/\alpha_o$, $G\alpha_s/\alpha_z$, $G\alpha_s/\alpha_q$, $G\alpha_s/\alpha_{12}$, $G\alpha_s/\alpha_{13}$, $G\alpha_s/\alpha_{14}$, and $G\alpha_s/\alpha_{16}$, respectively. Each of the $G\alpha_s$ chimeras was expressed as a 52-kDa protein to similar degrees in COS cells (Fig. 1B), the expression level of which was incomparably higher than the endogenous $G\alpha_s$. SSTR3 was expressed in COS cells, as described previously [16]. In chimera-transfected cells without SSTR3 transfection, SST had no effect on AC up to 1 μM. Basal AC activity in cells transfected with an empty plasmid or SSTR3 cDNA was 1.4 ± 0.1 or 1.6 ± 0.3 [cAMP/(ADP+ATP)$\times10^3$, mean ± SE of four independent transfections], respectively. Thus, significant increases in basal AC were observed in cells transfected with every $G\alpha_s/\alpha_x$ chimera (see Figs. 2 and 3 for the AC activity in cells transfected with chimeras). However, similar increases in basal effector activity have been reported for comparable $G\alpha_q/\alpha_i$ chimeras [7]. CTX-stimulated values of cAMP formation were severalfold higher than these elevated basal activities, allowing for further stim-

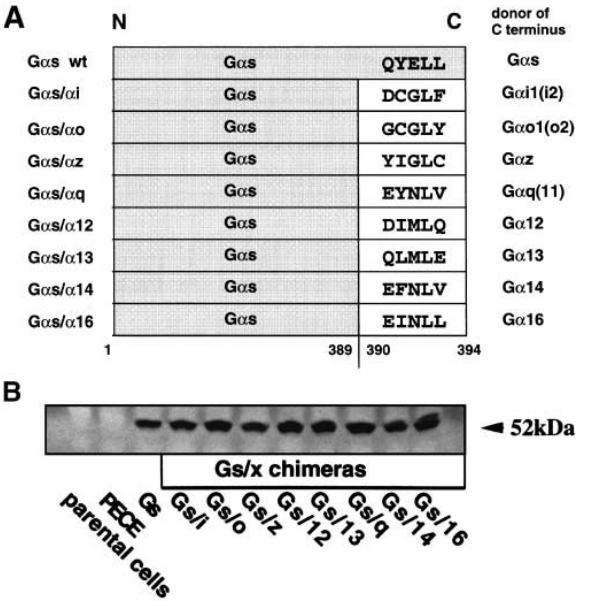

Fig. 1. Construction and expression of the $G\alpha_s$ chimeras. Constructed $G\alpha_s/\alpha_x$ and their C-terminal 5 residues are illustrated in (A). $G\alpha_s$ wt represents wild-type $G\alpha_s$. (B) Expression of the transfected $G\alpha_s$ chimeras. Cells were transfected with 3 μg/10^6 cells of either $G\alpha_s$ chimera cDNA and expression of each corresponding 52-kDa protein was analyzed by the standard immunoblotting with anti-common Gα antibody (Du Pont-NEN). Under the condition employed, the endogenous 45-kDa $G\alpha_s$ was neither detected.

ulation of AC in chimera-transfected cells in response to linked receptor stimulation.

With SSTR3, we transfected chimeras derived from the G_i family and examined SST-dependent increase in cAMP. SSTR3 has been shown to be an AC-inhibiting receptor in various cell systems [16–20]. In our COS cells transfected with SSTR3, SST treatment resulted in significant inhibition of AC (Table 1), whereas SST did not suppress AC without receptor transfection (data not shown). As in the literature, SSTR3 thus acted as an AC-inhibitory receptor in our system. In contrast to the suppression of AC in cells expressing SSTR3 alone, SST paradoxically augmented AC activity in cells transfected with SSTR3 and $G\alpha_s/\alpha_i$ (Fig. 2A). Because no stimulation was induced by SST in cells transfected with any chimera alone (Fig. 2B), SSTR3 was responsible for this action of SST. In cells transfected with SSTR3 and either $G\alpha_s/\alpha_o$ or $G\alpha_s/\alpha_z$, no stimulation of AC was induced by SST (Fig. 2A). We previously showed that our $G\alpha_s/\alpha_o$ is a functional construct because this chimera augmented cAMP activity in response to a stimulated G_o-coupled receptor [12]. The $G\alpha_s/\alpha_z$ construct was also functional because SST stimulation augmented AC activity when $G\alpha_s/\alpha_z$ was co-expressed with SSTR1 (Fig. 2C). Since only two or three residues, out of 394, are different between $G\alpha_s/\alpha_i$ and $G\alpha_s/\alpha_o$ or $G\alpha_s/\alpha_z$ (Fig. 1A), our data suggest that SSTR3 has an extremely selective potency to discriminate $G\alpha_i$ against other members of the G_i family.

We thus conclude that SSTR3 recognizes the C-terminus of $G\alpha_i$ but not of either $G\alpha_o$ or $G\alpha_z$, suggesting that SSTR3 inhibits AC through $G\alpha_i$, not through $G\alpha_o$ or $G\alpha_z$. This is partly consistent with the study of Murray-Whelan and Schlegel [21] arguing for a lack of SSTR association with $G\alpha_o$ in the brain, although the involved SSTR subtype was not specified by their study. The possibility is remote that in $G\alpha_s/\alpha_i$-expressing cells, SSTR3 activates endogenous G_i and the released $G\beta\gamma$ promotes AC activity, because (i) $G\beta\gamma$ requires the elevated $G\alpha_s$ activity to stimulate AC [22]; (ii) $G\alpha_s/\alpha_o$ and $G\alpha_s/\alpha_z$ exhibited basal $G\alpha_s$ activities as high as those of $G\alpha_s/\alpha_i$ chimeras; and (iii) co-expression of $G\alpha_s/\alpha_o$ or $G\alpha_s/\alpha_z$ did not assist SSTR3 stimulation of AC. Thus, AC activation by SST should be through the direct activation of $G\alpha_s/\alpha_i$ by SSTR3.

Whether the coupling of SSTR3 to other $G\alpha$ family could be predicted by our chimeras? We co-transfected SSTR3 with either of $G\alpha_s/\alpha_q$, $G\alpha_s/\alpha_{12}$, $G\alpha_s/\alpha_{13}$, $G\alpha_s/\alpha_{14}$, or $G\alpha_s/\alpha_{16}$ and measured AC activity in response to SST in a similar manner. In cells expressing SSTR3 and either $G\alpha_s/\alpha_{14}$ or $G\alpha_s/\alpha_{16}$, SST significantly stimulated AC (Fig. 3A). In contrast, in cells expressing SSTR3 and $G\alpha_s/\alpha_q$, no stimulation of AC was induced by SST (Fig. 3A). This was also the case with $G\alpha_s/\alpha_{12}$ or $G\alpha_s/\alpha_{13}$ (the AC activity in the presence of 1 μM SST was $72.6 \pm 6.2\%$ or $92.9 \pm 5.0\%$ of AC activity in the absence of SST, respectively). Either of $G\alpha_s/\alpha_q$, $G\alpha_s/\alpha_{12}$ or $G\alpha_s/\alpha_{13}$ was expressed to the extent comparable to those of $G\alpha_s/\alpha_{14}$ and $G\alpha_s/\alpha_{16}$ under the conditions employed (Fig. 1B). Again, no stimulation was observed without SSTR3 transfection

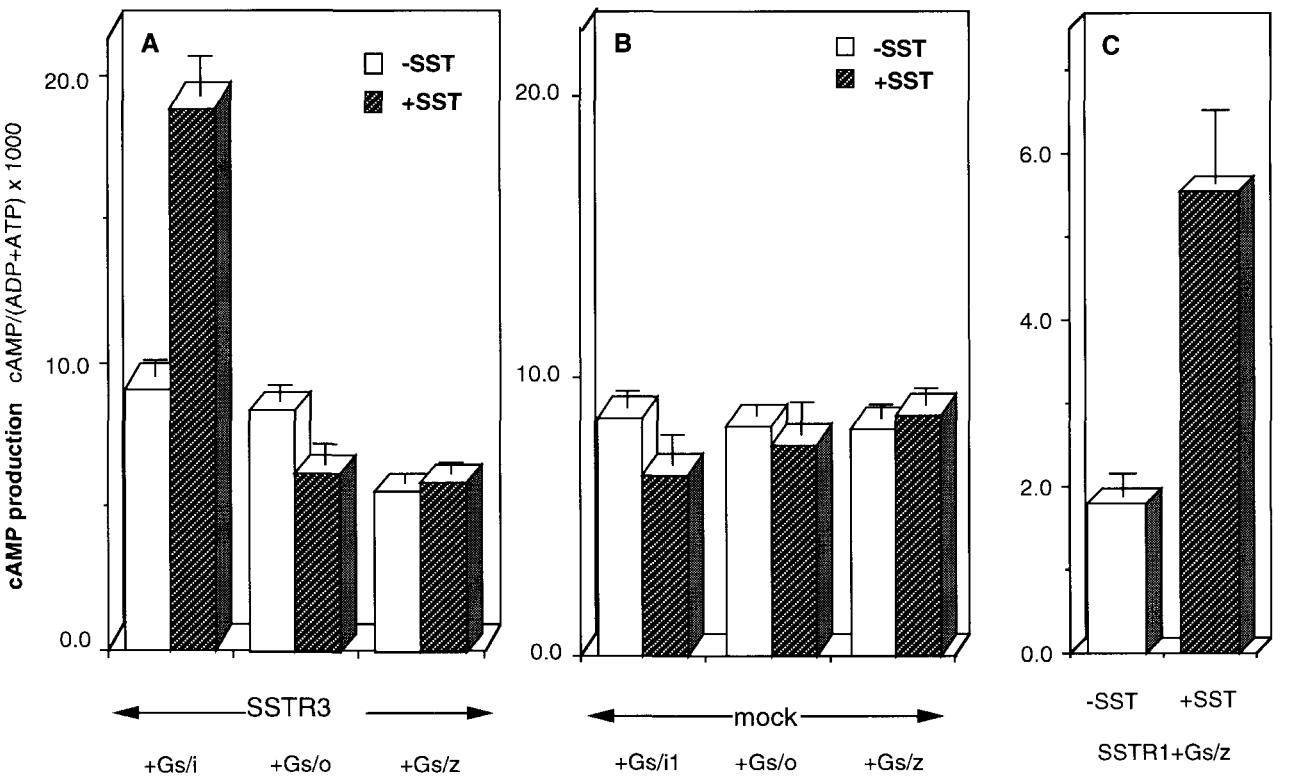

Fig. 2. Effects of the G_i family-$G\alpha_s$ chimeras to convert the SSTR3 action on AC. Effects of SST on cAMP formation in cells expressing either $G\alpha_s/\alpha_i$, $G\alpha_s/\alpha_o$, or $G\alpha_s/\alpha_z$ with (A) or without (B) SSTR3. Cells were transfected with 0.125 μg of either $G\alpha_s$ chimera and 0.125 μg of SSTR3 DNA (A) or an empty plasmid (B). C: cells were similarly transfected with $G\alpha_s/\alpha_z$ and SSTR1. Twenty-four hours after transfection, cells were stimulated for 30 min with or without 1 μM SST in the presence of 1 mM IBMX, and cAMP formation was measured. Similar results were found three more times for each chimera. The results represent means ± SE of four independent transfections. The effects of SST observed in co-expression of $G\alpha_s/\alpha_i$ with SSTR3 or co-expression of $G\alpha_s/\alpha_z$ with SSTR1 were statistically significant ($P < 0.05$ vs. no SST by Student's t test). There was a tendency that co-transfection of SSTR1 resulted in decreased production of cAMP.

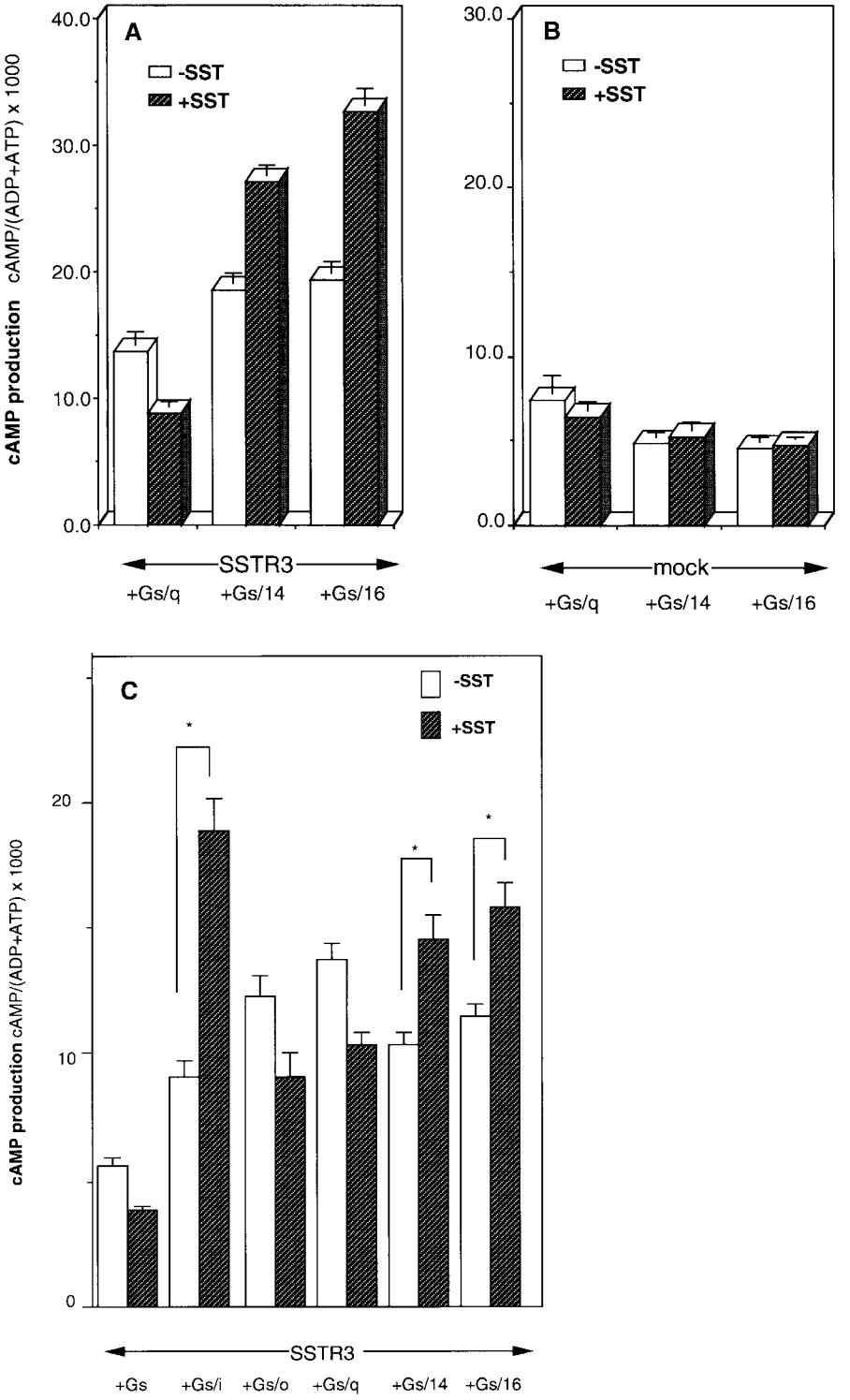

Fig. 3. Effects of the G_q family-$G\alpha_s$ chimeras to convert the SSTR3 action on AC. A,B: Effect of SST on cAMP formation in cells expressing either $G\alpha_s/\alpha_q$, $G\alpha_s/\alpha_{14}$, or $G\alpha_s/\alpha_{16}$ with (A) or without (B) SSTR3. Cells were transfected with 0.125 μg of either chimera and 0.125 μg of SSTR3 DNA (A) or an empty plasmid (B). Twenty-four hours after transfection, cells were stimulated with 1 μM SST, and cAMP production was measured. The results represent means ± SE of four independent transfections. Similar results were found three more times for each chimera. The effects of SST observed in co-expression of $G\alpha_s/\alpha_{14}$ and $G\alpha_s/\alpha_{16}$ were statistically significant ($P < 0.05$ vs. no SST). C: Quantitative comparison of the effects of SST on cAMP production in cells expressing $G\alpha_s$ chimera derived from the G_i and G_q family. Experiments were done as in other figures, but all the chimeras indicated were tested in parallel. The results represent means ± SE of four independent transfections. $^*P < 0.05$ vs. no SST.

(Fig. 3B), confirming the involvement of SSTR3 in the activation of $G\alpha_s/\alpha_{14}$ or $G\alpha_s/\alpha_{16}$. Although the observed SST-induced increase in AC might be small in quantity, this augmentation was statistically significant. Note that SST *reduced* cAMP formation when SSTR3 was transfected without $G\alpha_s/\alpha_{14}$ or $G\alpha_s/\alpha_{16}$. Therefore, the net effect of SSTR3 on these

Table 1
SSTR3 is coupled to AC suppression in an SST-dependent manner

	SST-	SST+
CTX−	0.73 ± 0.05	0.58 ± 0.02*
CTX+	25.9 ± 1.9	18.9 ± 0.4*

Effects of SST on basal and CTX (cholera toxin)-stimulated formation of cAMP in SSTR3-transfected cells. Cells were transfected with 0.125 μg SSTR3 DNA, as described in Section 2. Forty-eight hours after transfection, cells were treated for 30 min with or without 1 μM SST in the presence of 1 mM IBMX with or without 250 ng/ml CTX, and cAMP formation was measured and indicated as cAMP/(ADP+ATP)×10^3. All values are means ± SE of four independent transfections.
*$P < 0.05$ vs. no SST.

chimeras was larger than what the percent increase of the SST effect represented. We thus considered statistically significant effects in this assay as positive.

However, Lee et al. [23] constructed chimeras consisting of $G\alpha_{11}$ and $G\alpha_{16}$ and found that the C-terminal 155 residues of $G\alpha_{16}$, particularly residues 220–240, are required to confer C5a-induced activation of $G\alpha_{11}$. In contrast, our data suggest that the C-terminal 5 residues of $G\alpha_{16}$ may confer efficient SSTR3 coupling to $G\alpha_s$. For this reason, we repeated the G_q family chimera experiments in a way different from that just described, and performed quantitative comparison. In Fig. 3C, various chimeras derived from the G_i and G_q family were simultaneously tested in the presence of SSTR3 expression. The SST-dependent fold increase of either chimera activity was totally reproducible, in terms of the stimulation quantity as well as the relative ratio between the G_i and G_q families (Fig. 3C). These data confirm the coupling of SSTR3 to $G\alpha_s/\alpha_{14}$ and $G\alpha_s/\alpha_{16}$. The discrepancy from the study of Lee et al. [23] suggests that some other portions in $G\alpha_{16}$, conserved by $G\alpha_s$ but not by $G\alpha_{11}$, are also required for efficient coupling to receptors.

PLC (phospholipase C) stimulation is the established, specific output of the G_q family ($G\alpha_{q/11}$, $G\alpha_{14}$, $G\alpha_{16}$). We next examined whether SSTR3 can activate PLC through intact $G\alpha$ of this family. When both $G\alpha_{16}$ and SSTR3 were expressed in COS cells, SST robustly augmented IP (inositol phosphate) production (Fig. 4A). Single expression of either gene ($G\alpha_{16}$ or SSTR3) did not allow SST to stimulate PI

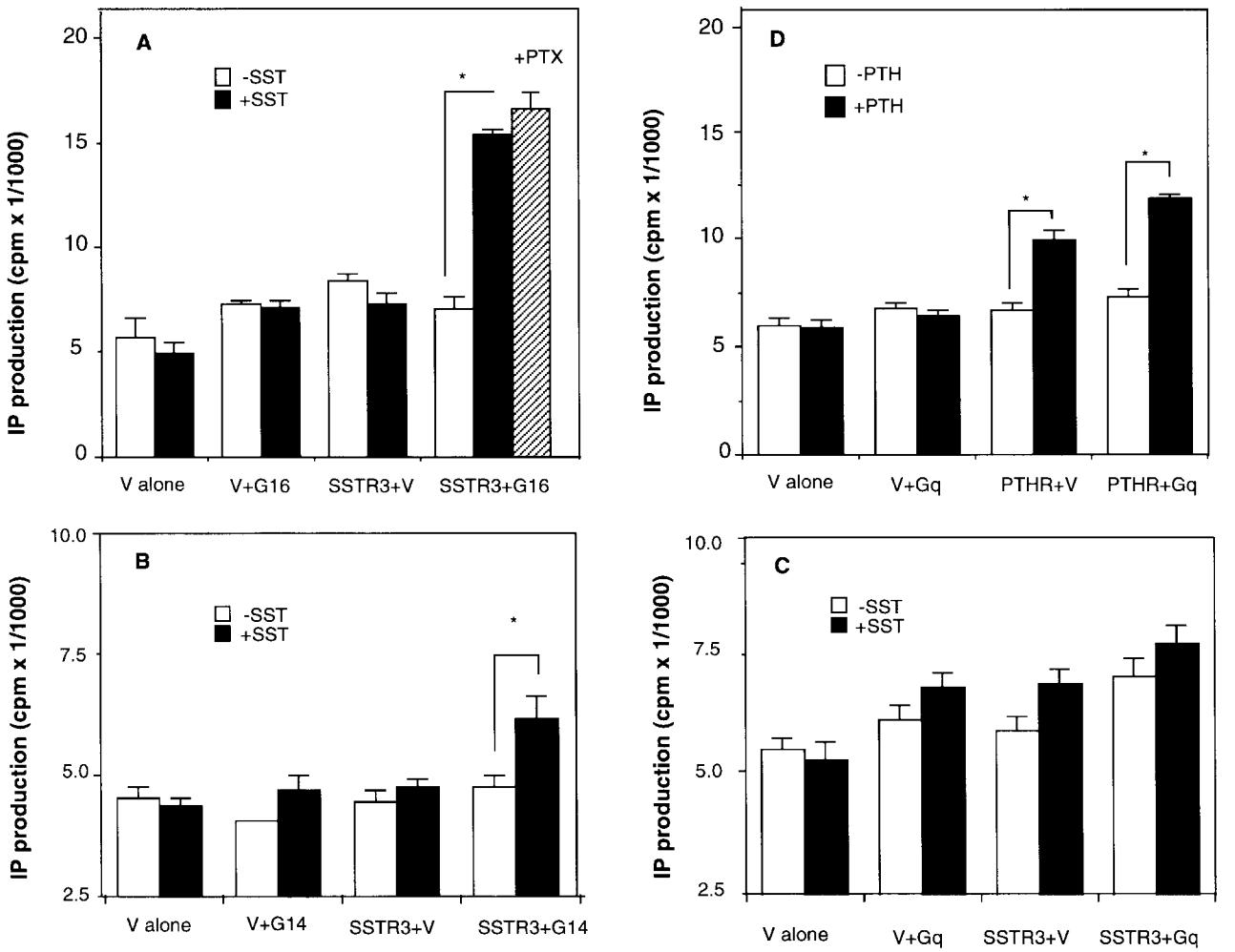

Fig. 4. Stimulation of PI turnover by SSTR3 through the $G\alpha_q$ class of G-proteins. Cells were transfected with 0.125 μg SSTR3 DNA (A,B,C) or PTHR cDNA (D) with 0.125 μg intact $G\alpha_{16}$ cDNA (A), $G\alpha_{14}$ cDNA (B), or $G\alpha_q$ cDNA (C,D). Thirty-six hours after transfection, cells were treated for 5 min with or without 1 μM SST or 1 μM PTH, and IP production was measured. The total amount of plasmids used for transfection was adjusted to 0.25 μg with a vector plasmid (indicated as V). For treatment with PTX, 24 h after transfection, cells were treated with 10 ng/ml PTX for 3 h and SST was similarly treated. The results represent means ± SE of three independent transfections. *$P < 0.05$ vs. no ligand.

turnover. Consistent with the PTX resistance of $G\alpha_{16}$, PTX failed to affect this stimulation (Fig. 4A), confirming that SSTR3-induced PI turnover is mediated by the transfected $G\alpha_{16}$ and not by $G\beta\gamma$ derived from endogenous G_i. Positive coupling with intact $G\alpha$ was also the case with $G\alpha_{14}$. Although the coupling between SSTR3 and $G\alpha_{14}$ seemed less efficient than that between SSTR3 and $G\alpha_{16}$, the effect of SST was significant in cells co-expressing SSTR3 and $G\alpha_{14}$ (Fig. 4B). Supporting the validity of the prediction by the chimeras, this observation suggests that certain N-terminal regions in $G\alpha_{14}$ are also required for its effective receptor coupling.

In contrast, SST had no effect on IP production in cells expressing SSTR3 with $G\alpha_q$ (Fig. 4C). Transfection of PTHR cDNA with or without $G\alpha_q$ resulted in significant stimulation of IP production in response to 1 μM PTH 1–34 (Fig. 4D). Transfection of $G\alpha_q$ further augmented the efficacy of PTHR to activate PLC activity. These observations are consistent with the reports that (i) PTHR causes PI turnover in a PTX-insensitive manner [24] and (ii) COS cells endogenously express $G\alpha_q$ and $G\alpha_{11}$ [25]. Note that the PTHR/$G\alpha_q$-induced PLC activity was exceeded by SSTR3/$G\alpha_{16}$-induced activity, indicating that SSTR3 coupling to $G\alpha_{16}$ was more than or equally as efficient as the PTHR-$G\alpha_q$ coupling. Taken together, our data indicate that SSTR3 specifically couples to non-$G\alpha_q$ members of the G_q family and induces PI turnover in intact cells.

We have shown herein that SSTR3 recognizes the C-terminus of $G\alpha_i$ but not of $G\alpha_o$ or $G\alpha_z$. Although the C-terminal recognition of $G\alpha_i$ by SSTR3 was expected by previous studies, inability of SSTR3 to recognize the C-termini of $G\alpha_o$ and $G\alpha_z$ was unexpected, as they are extremely homologous. No receptors have so far coupled to $G\alpha$ without recognizing its C-terminus [7–9]. Therefore, the present study indicates that SSTR3 has no molecular function to directly recognize $G\alpha_o$ or $G\alpha_z$; thus, it inhibits AC through G_i but not through G_o or G_z.

The chimeric $G\alpha_s$ system also served as an essential probe in further characterizing the G-protein-coupling function of SSTR3. It is shown here that SSTR3 directly couples to $G\alpha_{14}$ and $G\alpha_{16}$, but not to $G\alpha_q$. Until this study, little has been known about the coupling of this receptor with these G_q subclass of G-proteins. In theory, this approach can be applied to unlimited numbers of G-coupled receptors, including both muti-spanning and single-spanning ones. We however emphasize that the C-terminal residues of $G\alpha$ may not be the sole site for receptor contact, although they are necessary [7]. Therefore, positive data from our chimeras may not always signify the coupling to intact G-proteins, while negative data are able to exclude it. This notion is consistent with the report of Law et al. [19] showing that SSTR3 selectively couples to $G\alpha_{i1}$ but not $G\alpha_{i2}$ in CHO cells, despite the identical C-termini of these $G\alpha$. The observed quantitative difference of SSTR3 coupling between $G\alpha_{14}$ and $G\alpha_{16}$ also supports this idea.

The precise assignment of G-proteins linked to a given receptor has been very difficult. Among several approaches aiming at the assessment of the involved G-proteins in receptor signals, including those with antibodies for and peptides of $G\alpha$, only the approach with microinjection of anti-sense oligonucleotides has been successful [26]. However, there are critical limitations in that approach; for instance, that is only applicable to cellular responses detectable in a single cell. Our present study proposes a more accessible and comprehensive approach for the assignment of G-proteins coupled to any given receptor.

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