Vol. 204, No. 1, 1994 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
October 14, 1994 Pages 176-186

MULTIPLE EFFECTOR COUPLING OF SOMATOSTATIN RECEPTOR SUBTYPE SSTR1

Akira Kubota[1+], Yuichiro Yamada[1], Shinji Kagimoto[1], Koichiro Yasuda[1], Yoshimichi Someya[1], Yu Ihara[1], Yoshimasa Okamoto[1], Tohru Kozasa[2], Susumu Seino[3], and Yutaka Seino[1]

[1]*Department of Metabolism and Clinical Nutrition, Kyoto University Faculty of Medicine, Kyoto 606, Japan*

[2]*Department of Pharmacology, The University of Texas, Southwestern Medical Center at Dallas, Texas 75235-9041*

[3]*Division of Molecular Medicine, Center for Biomedical Science, Chiba University School of Medicine, Chiba 260, Japan*

Received August 28, 1994

SUMMARY: The signal transduction pathways of a cloned human somatostatin receptor subtype, SSTR1, have been investigated in CHO cells stably expressing this receptor. In SSTR1-expressing CHO cells, somatostatin-14 inhibits forskolin-stimulated cAMP formation in a dose-dependent manner with an ED_{50} of 1.0×10^{-9} M. Somatostatin-14 also stimulates inositol 1,4,5-trisphosphate formation in a dose-dependent manner with an ED_{50} of 4.0×10^{-8} M. Somatostatin-14 inhibitory action on adenylyl cyclase and stimulatory action on inositol 1,4,5-trisphosphate formation are both blocked by pertussis toxin, indicating that these effects of SSTR1 are mediated by pertussis toxin-sensitive G protein(s). Antiserum against Gi α3 blocked the inhibitory effects of somatostatin-14 on forskolin-stimulated adenylyl cyclase, but antiserum against Gi α1/Gi α2 did not, indicating that Gi α3 dominantly couples SSTR1 to adenylyl cyclase. These results demonstrate that SSTR1 can be coupled to different signaling pathways to exert multiple biological effects, one of which is mediated by Gi α3.

Somatostatin is a tetradecapeptide that was first isolated from hypothalamus as a growth hormone releasing inhibiting factor (1). It exerts diverse biological functions including inhibition of secretory and proliferative processes in many target organs, such as gastrointestinal tract and exocrine and endocrine pancreas (2). It also acts as a

[+]To whom correspondence should be addressed.

The abbreviations used are: SS-14, somatostatin-14; SS-28, somatostatin-28; PLC, phospholipase C; PTX, pertussis toxin; G protein, GTP-binding protein; CHO, Chinese hamster ovary; IP_3, inositol 1,4,5-trisphosphate; PI, phosphatidylinositol; IBMX, 3-Isobutyl-1-methylxanthine.

0006-291X/94 $5.00

neurotransmitter in the central nervous system (2). Moreover long-acting somatostatin analogs have been developed and shown to inhibit the hypersecretion and the proliferation of various hormone-producing tumors (3,4). These actions of somatostatin are mediated through its specific high-affinity receptors. Somatostatin receptors have been reported to be coupled to several intracellular effector systems (5-7), including adenylyl cyclase (8) and ion channels (9). Moreover the GTP-binding (G) proteins which couple somatostatin receptors to the effector systems have also been investigated (10,11). Recently, we have cloned five subtypes of human somatostatin receptors (SSTR1-SSTR5) whose tissue distributions and pharmacological properties are different (12-14), indicating that the various actions of somatostatin are mediated by a family of structurally-related proteins encoded by distinct genes. Among the five subtypes of human somatostatin receptors, SSTR1 shows wide distribution in the body, including brain *, stomach, colon, and pancreatic islets (12) and in various tumor tissues (15). In our previous report, the effector which was mediated by SSTR 1 was not made clear (16). In the present study, we have further investigated the intracellular effectors which are coupled to SSTR1 and characterized the signal transduction pathways using a reconstituted system. We show here that SSTR1 can be coupled to phospholipase C as well as to adenylyl cyclase.

MATERIALS AND METHODS

Materials: Somatostatin-14 (SS-14) from Peptide Institute (Osaka); somatostatin-28, carbachol, and pertussis toxin (PTX) from Sigma; RC 160 from Peninsula; SMS 201-995 from Sandoz; ^{125}I-[Tyr^{11}] somatostatin-14, IP_3 Radioimmunoassay kit, and [5,6,8,9,11,12,14,15-^{3}H] Arachidonic acid (150-230 μCi/ml) from Amersham; cAMP Radioimmunoassay kit from Yamasa (Chiba); astisera AS/7 and EC/2 from Du Pont/New England Nuclear; and rat Gi α 1, Gi α 2, and Gi α 3 cDNAs were provided by Dr. Y. Kaziro.

Establishment of CHO cells stably expressing SSTR1: A 1.5-Kb *Pst* I/*Xmn* I fragment of the human SSTR1 gene was inserted into the mammalian expression vector pCMV6b (12) and the resulting construct was cotransfected with pSV2neo into the CHO cells using Lipofectin reagent. Stable transfectants were selected in α MEM containing 400 μg/ml of G418. From these cell populations, clonal cell lines were isolated by single-cell cloning.

Measurements of cAMP formation in whole cells: CHO cells were grown to confluency in 12-well plates. The cells were incubated with the buffer containing 50 mM Tris (pH 7.4), 200mM sucrose, 5 mM $MgCl_2$, 1 mg/ml bacitracin, 10 mg/ml bovine serum albumin (buffer A), and 1 mM 3-Isobutyl-1-methylxanthine (IBMX) with or without test reagents at 37 °C for 30 min. The reaction was stopped by adding 30% trichloroacetic acid and cAMP levels were determined by radioimmunassay.

Binding assays: Binding assays were performed essentially as previously described using buffer A (12).

Measurements of inositol 1,4,5-trisphosphate (IP_3) formation: Measurement of IP_3 formation was carried out essentially as previously described (17). CHO cells were grown to confluency in the 10 cm culture dishes. The cells were preincubated with buffer A

* A. Kubota, unpublished observation.

containing 10 mM LiCl for 30 min and then the reaction was started by replacing the buffer with the same buffer containing the indicated reagents. After the incubation for 30 sec at 37 °C, the reaction was terminated by adding ice-cold 7.5% trichloroacetic acid. The amount of IP_3 in the sample was determined by radioimmunoassay.

Measurements of arachidonic acid release: [^{3}H] arachidonic acid release was measured essentially as described (18).

RNA blotting analysis: RNA blotting analysis was performed as described previously (12). The probes were: a 1.4 kbp *Xba* I-*EcoR* I fragment of the rat Gi α 1 cDNA ; a 1.0 kbp *Xba* I-*Pst* I fragment of the rat Gi α 2 cDNA ; and a 1.3 kbp *EcoR* I fragment of the rat Gi α 3 cDNA.

Adenylyl cyclase assays using cell membrane preparations: Adenylyl cyclase assay was performed essentially as described previously (11). Cells were homogenized in the buffer containing 50 mM Tris (pH 7.4), 1 mM EDTA, 5 mM $MgCl_2$, 10 μg/ml leupeptin, 10 μg/ml pepstatin, 200 μg/ml bacitracin, and 500 KIU/ml aprotinin and centrifuged at 1000 x g for 10 min at 4 °C. The supernatant was centrifuged at 140000 x g for 1 h at 4 °C and the pellet was homogenized in the buffer. Membranes were incubated with normal rabbit serum, or with Gi antisera (each 1/100 dilution) at 4 °C for 1 h before the assays. The reaction was started by adding the preincubated membranes (45 μg/tube) to the buffer containing 50 mM Tris (pH7.4), 100 mM NaCl, 3 mM $MgCl_2$, 1 mM IBMX, 0.3 mM ATP, 5.0 mM phosphocreatine, 30 unit/ml creatine phosphokinase, and 10 μM GTP. After incubation for 5 min at 30 °C with or without test reagents, the reaction was stopped by boiling at 95 °C for 3 min and the cAMP product was analyzed by radioimmunoassay.

RESULTS

CHO cells were permanently transfected with SSTR1 gene-containing expression vector and pSV2neo. Twelve clonal cell lines were isolated by selection with G418, and cell lines expressing high levels of SSTR1 were screened by measuring their binding properties for ^{125}I-[Tyr^{11}] somatostatin-14. Three cell lines were found to exhibit high affinity for ^{125}I-[Tyr^{11}] somatostatin-14, and among them a cell line which showed the highest inhibition of forskolin-stimulated cAMP formation by SS-14 was used for furhter examinations (CHO-SR1). No specific binding of ^{125}I-[Tyr^{11}] somatostatin-14 was observed in nontransfected CHO cells. Fig. 1 shows the effects of somatostatin on forskolin-stimulated cAMP formation in nontransfected CHO cells and CHO-SR1. In nontransfected CHO cells, SS-14 did not affect forskolin-stimulated cAMP formation. In CHO-SR1, SS-14 caused a 66 % inhibition of forskolin-stimulated cAMP formation. SS-14 inhibited forskolin-stimulated cAMP formation in a dose dependent manner and half-maximal inhibition (ED_{50}) of forskolin-stimulated cAMP formation occurred at 1.0 x 10^{-9} M SS-14 (Fig. 2). To characterize the potencies and relative efficacies of the agonists, we determined the effects of 1μM SS-14, somatostatin-28 (SS-28), RC 160, and SMS 201-995 on forskolin-stimulated cAMP formation (Fig. 3A). All these peptides also caused significant inhibition of forskolin-stimulated cAMP formation. The rank of the potency of these agonists was SS-14= SS-28>RC 160>SMS 201-995. We also examined the binding properties of SSTR1 to

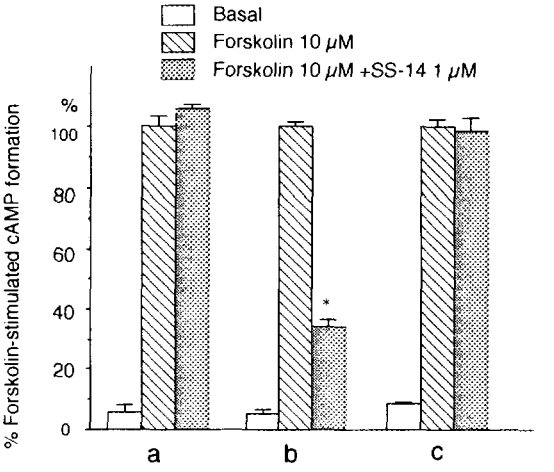

Figure 1. Effects of SS-14 on forskolin-stimulated cAMP formation. Nontransfected (a) or SSTR1-expressing (b, c) CHO cells were incubated with 10 μM forskolin and with or without 1 μM SS-1 4 for 30 min. (c) SSTR1-expressing CHO cells were incubated with 100 ng/ml of PTX for 24h before the assay. Values are means ± S.E. of triplicate determinations. * P< 0.05 compared with forskolin-stimulated levels.

these agonists in CHO-SR1 (Fig. 3B). SS-14 inhibited the binding of ^{125}I-[Tyr^{11}] somatostatin-14 in a dose dependent manner with an IC_{50} of 2.0 x 10^{-9} M, which is similar to the ED_{50} of SS-14 in inhibiting cAMP formation. The rank of the potency of these agonists in inhibiting the binding of radiolabeled somatostatin-14 was in reasonable agreement

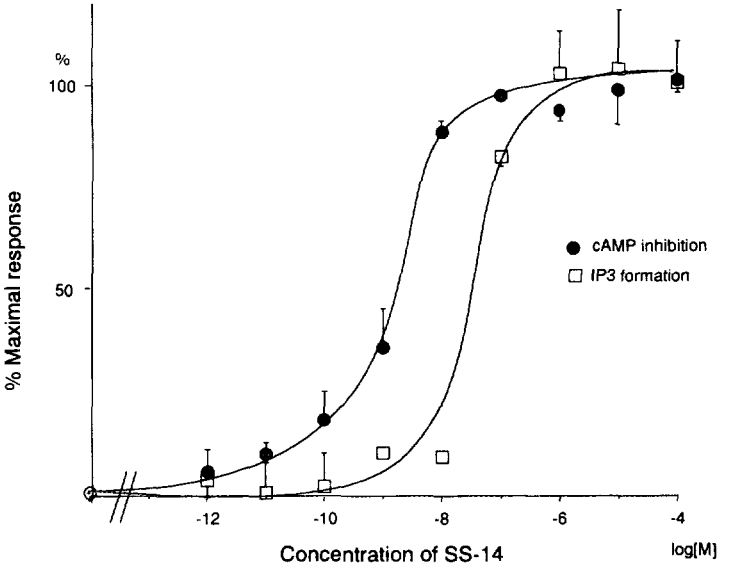

Figure 2. Dose-response analyses of somatostatin-induced cAMP inhibition and PI hydrolysis in CHO-SR1. Inhibition of forskolin-stimulatsd cAMP formation (●) and stimulation of PI hydrolysis (□) by varying concentrations of SS-14 were presented. Values are means ± S.E. of triplicate determinations.

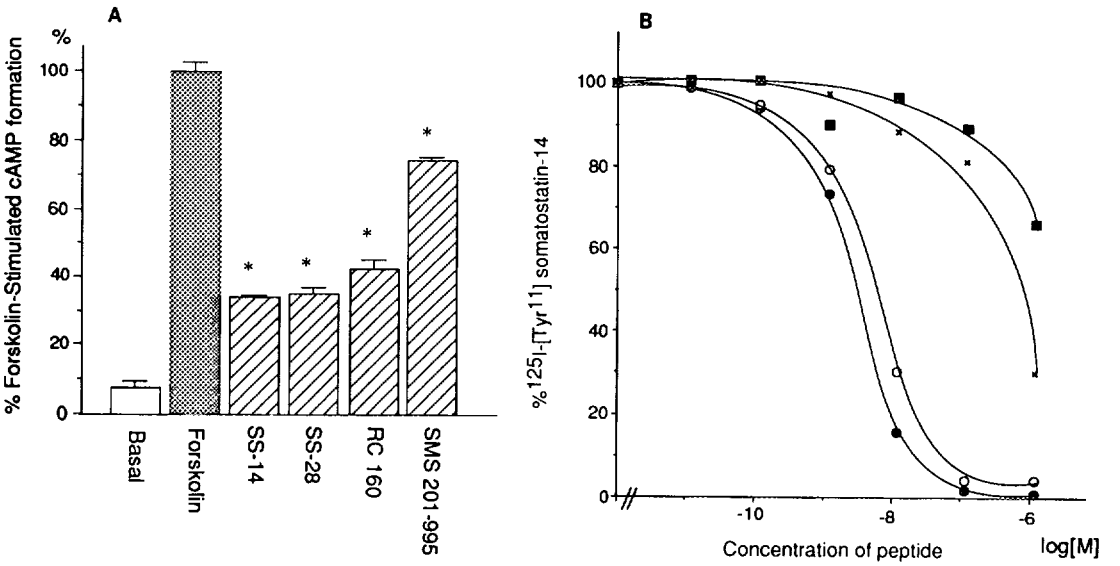

Figure 3. Effects of various agonists on forskolin-stimulated cAMP formation (A) and binding properties of SSTR1 to these agonists (B) in CHO-SR1. (A) CHO-SR1 cells were incubated with 10 μM forskolin and with or without 1μM SS-14, SS-28, RC 160, and SMS 201-995. Values are means ± S.E. of triplicate determinations. * $P< 0.05$ compared with forskolin-stimulated levels. (B) The ability of somatostatin-14 (●), somatostatin-28 (○), RC 160 (×), and SMS 201-995 (■) to inhibit the binding of [^{125}I-Tyr^{11}] somatostatin-14 was examined. Values are means of triplicate determinations.

with that of the agonists in inhibiting the cAMP formation. We examined the effects of PTX on the inhibition of cAMP formation by SS-14, which resulted in the complete blockage of the effects of SS-14 (Fig. 1).

In order to determine the possible linkage of SSTR1 to multiple signaling pathways, we examined the ability of SSTR1 to stimulate or inhibit PI hydrolysis in CHO-SR1. SS-14 caused a significant increase in the formation of IP_3, the primary products of PLC-catalyzed hydrolysis of PI (Fig. 4). Such stimulation of IP_3 formation by SS-14 was not observed in nontransfected CHO cells. As shown in figure 2, SS-14 stimulated IP_3 formation in a dose-dependent manner with an ED_{50} value of 4.0×10^{-8} M. We also examined the effects of SS-28 and the two somatostatin analogs on IP_3 formation. All the peptides significantly stimulated IP_3 formation and the rank of the potency was similar to that in inhibiting cAMP formation (Fig. 5). We next examined the effect of SS-14 on carbachol-stimulated IP_3 formation. Carbachol activated IP_3 formation in CHO cells and SS-14 showed an additive effect on the carbachol-stimulated IP_3 formation (Fig. 4). Pretreatment of the cells with PTX caused different effects on SS-14- and carbachol- stimulated IP_3 formation. It abolished the SS-14- stimulated IP_3 formation, but did not affect the carbachol-stimulated IP_3 formation.

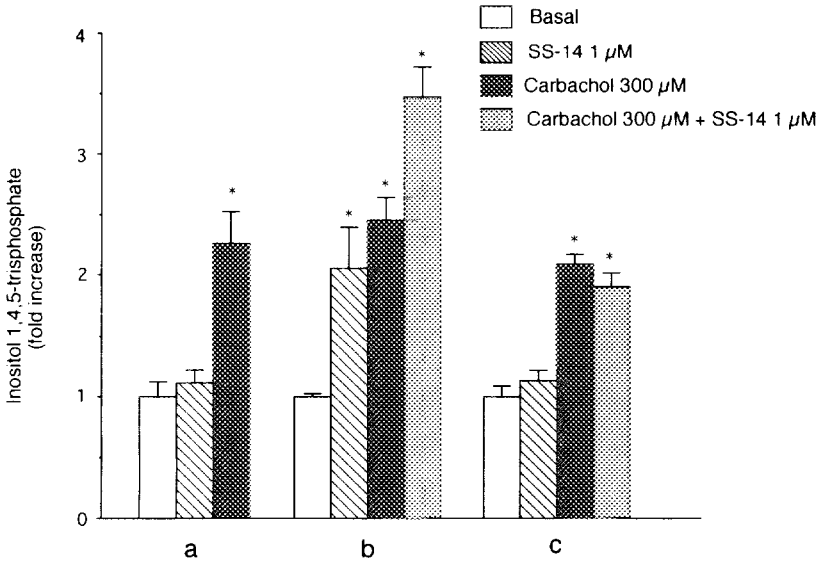

Figure 4. Effect of SS-14 on IP_3 formation in nontransfected and SSTR1-expressing CHO cells. Nontransfected (a) or SSTR1-expressing (b,c) CHO cells were incubated with 1 μM SS-14 and/or 300 μM carbachol for 30 sec and IP_3 content was determined. (c) SSTR1-expressing CHO cells were incubated with 100 ng/ml of PTX for 24h before the assay. Values are means ± S.E. of triplicate determinations. * $P< 0.05$ compared with respective controls.

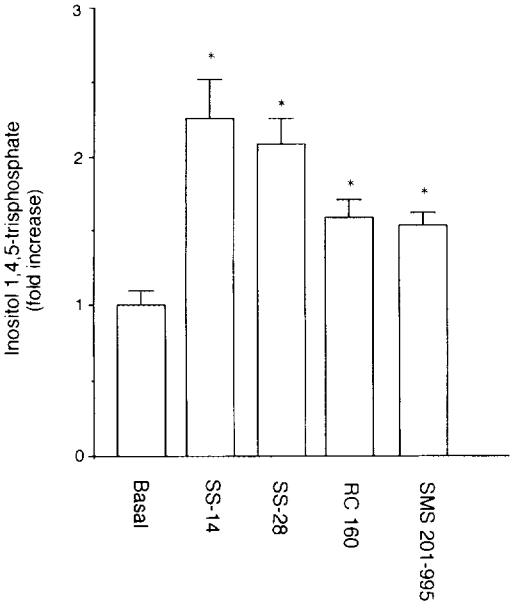

Figure 5. Effects of various agonists on IP_3 formation in CHO-SR1. CHO-SR1 cells were incubated with 1μM of each agonist and IP_3 formation was determined. Values are means ± S.E. of triplicate determinations. * $P< 0.05$ compared with control.

Since there is a report that somatostatin stimulates phospholipaseA_2 in striatal astrocytes (7), we examined the effect of somatostatin on arachidonic acid release in CHO-SR 1. Carbachol remarkably stimulated arachidonic acid release, while SS-14 had no effect (Table 1).

The inhibition of forskolin-stimulated cAMP formation by SS-14 was blocked by PTX, indicating that SSTR1 is coupled to adenylyl cyclase via PTX-sensitive Gi protein when expressed in CHO cells. Because there are three known subtypes of the α subunit of Gi protein, which are termed Gi α 1, Gi α 2, and Gi α 3 (19,20), we examined the expression of Gi α subtypes in CHO cells by RNA blotting analysis. In CHO cells, Gi α 2 and Gi α 3 mRNAs were detected, but Gi α 1 mRNA was not (Fig. 6). In order to determine which subtype of Gi α is coupled to SSTR1, we have performed adenylyl cyclase assays using membrane preparations of CHO-SR1 cells and examined the effects of antisera directed against Gi α subtypes on the actions of somatostatin (Fig. 7). AS/7 and EC/2 are the antisera directed against Gi α 1/Gi α 2 and Gi α 3, respectively (21). In these assays, when the membrane preparations were preincubated with normal rabbit serum, 1μM SS-14 caused a 16 % inhibition of forskolin-stimulated adenyly cyclase activity. Preincubation of the membranes with Gi α 1/Gi α 2 antiserum had no effect on the ability of SS-14 to inhibit forskolin-stimulated adenyly cyclase activity, but that with Gi α 3 antiserum blocked the inhibitory effect of SS-14.

DISCUSSION

Signal transduction pathways and effectors which are mediated by somatostatin receptors have been studied mainly using tissues and cell lines (5-9). These studies,

Table 1
Effects of SS-14 and carbachol on arachidonic acid release in CHO-SR1

Drugs	Arachidonic acid release (cpm/well)
None	658.3 ± 30.9
SS-14 1 μM	659.0 ± 19.4
Carbachol 300 μM	6462.3 ± 254.1
SS-14 1 μM+ carbachol 300 μM	6646.7 ± 195.7

CHO-SR1 cells were incubated with 1 μM SS-14 and/or 300 μM carbachol and arachidonic acid release was determined. Values are means ± S.E. of triplicate determinations.

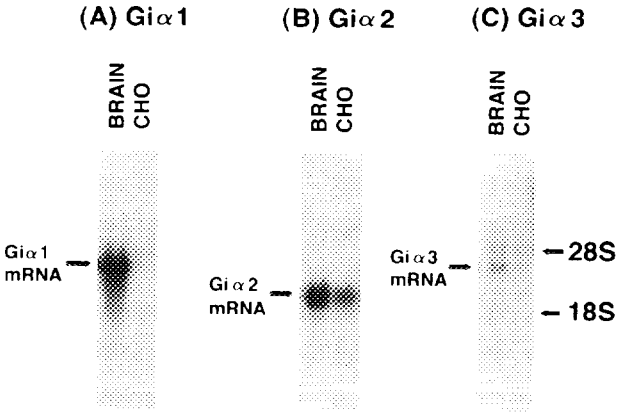

Figure 6. RNA blotting analysis of the expression of Gi α subtypes in CHO cells. Twenty micrograms of total RNA from rat brain and CHO cells was denatured with glyoxal and located on a 1% agarose gel, transferred to nylon membrane, and hybridyzed to ^{32}P-labeled Gi α 1 or -2 or-3 cDNA probes.

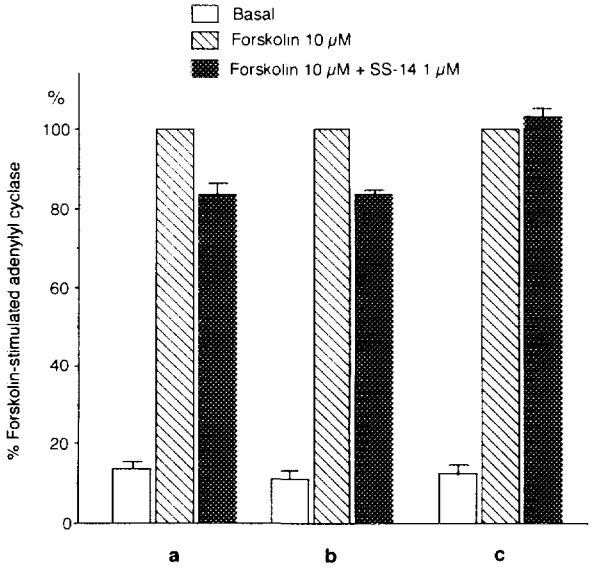

Figure 7. Effect of Gi α 1/Gi α 2 and Gi α 3 antisera on the inhibitory action of SS-14 on forskolin-stimulated adenylyl cyclase activity. Membrane preparations from CHO-SR1 cells were preincubated with normal rabbit serum (a), Gi α 1/Gi α 2 antiserum (b), or Gi α 3 antiserum (c) and effect of 1 μM SS-14 on forskolin-stimulated cAMP formation was examined. One μM SS-14 inhibited forskolin-stimulated adenylyl cyclase activity by 16.4± 2.8%, when the membranes were preincubated with normal serum (in a representative experiment with normal rabbit serum, adenylyl cyclase activity was as follows: basal; 18.4 ± 2.3, forskolin-stimulated; 153 ± 4.5, and forskolin with SS-14; 129.4 ± 5.4 pmol/mg/min). Data are expressed as percent of forskolin-stimulated levels. Data are means ± S.E. of three independent experiments done in triplicate or quadruplicate determinations.

however, were limited in their characterization of the properties of somatostatin receptors because of the possible existence of multiple receptor subtypes in tissue or cell preparations. In addition, it cannot be ascertained whether an unidentified subtype of the receptor induces intracellular signals in response to agonists. Recently, we have identified five subtypes of the human somatostatin receptor (12-14), a superfamily of G protein-coupled receptors. Among the five subtypes, SSTR1 has been shown to be widely distributed in the body (12), and moreover, we have found that SSTR1 mRNA is expressed in various endocrine tumors (15). In the previous study, we showed that CHO cells stably expressing SSTR1 exhibited a specific binding for SS-14. However, the effector coupled to SSTR1 in these cells was not made clear (16). Since the CHO cells expressing SSTR1 used in the previous study were polyclonal, we reestablished a clonal CHO cell line stably expressing SSTR1 (CHO-SR1) in this study and reexamined the possible effector which is coupled to SSTR1. Our results in this study demonstrate that in CHO-SR1, SSTR1 is coupled to dual intracellular effector systems, inhibition of adenylyl cyclase and stimulation of IP_3 formation. Dose-response analysis revealed that the inhibition of forskolin-stimulated cAMP formation and the stimulation of IP_3 formation by SS-14 are both in a dose-dependent manner in CHO-SR1. However, the ED_{50} value for the stimulation of IP_3 formation is much higher than that for the inhibition of cAMP formation. We determined the ability of SS-14, SS-28, and two somatostatin analogs to inhibit cAMP formation and to stimulate IP_3 formation in CHO-SR1. All these peptides significantly inhibits cAMP formation and stimulates IP_3 formation and the ranks of potency for them are both SS-14= SS-28>RC 160>SMS 201-995, which accord reasonably the binding potency of SSTR1 to these agonists.

Little is known of the effect of somatostatin on arachidonic acid metabolism. Marin *et al.* reported that somatostatin stimulates phospholipaseA_2 in striatal astrocytes (7). In the present study, SSTR1 does not affect arachidonic acid release in CHO-SR1.

The pretreatment of CHO-SR1 with PTX completely abolished the inhibitory effect of SS-14 on adenylyl cyclase, indicating that SSTR1 is coupled to adenylyl cyclase via PTX-sensitive G protein in CHO cells. We have demonstrated that among Gi subtypes, Gi α 2 and Gi α 3 are expressed in CHO cells and Gi α 1 is not. In order to identify which subtype of Gi protein couples SSTR1 to adenylyl cyclase, we have examined the effect of antisera directed against Gi α 1/Gi α 2 and Gi α 3 on the inhibition of adenylyl cyclase by SSTR1. The results show that Gi α 3 dominantly mediates the signal transduction from SSTR1 to adenylyl cyclase.

PLC-stimulation by SS-14 also was blocked by PTX, indicating that PTX-sensitive G protein mediates the PLC activation by SSTR1. Recently, a family of Gq proteins which activate PLC have been cloned (22-25), but all of them lack a ADP-ribosylation site by PTX.

There is increasing evidence that $\beta\gamma$ dimers derived from activated G protein can activate PLC (26,27). Katz *et al.* reported that in the M2 muscarinic acetylcholine receptor, which inhibits adenylyl cyclase, $\beta\gamma$ dimers derived from Gi2 or Gi3 activate PLC in a PTX-sensitive manner (26). It is possible, therefore, that this mechanism also is involved in the PLC-activation by SSTR1, although it remains to be determined whether $\beta\gamma$ dimers coupled to SSTR1 are derived from Gi3 or from other G proteins.

In summary, we demonstrate that SSTR1 mediates two intracellular signalings, the inhibition of cAMP formation and the stimulation of PI hydrolysis with considerably different ED_{50} in a reconstituted system. Although whether the signal transduction mechanisms of SSTR1 in a reconstituted system operate similarly in cells natively expressing SSTR1 remains to be determined, the present study provides a basis for the development of selective analogs for SSTR1, as well as for the understanding of the mechanism of somatostatin action.

ACKNOWLEDGMENTS: This study was supported in part by Grants-in-Aids for Scientific Research from the Ministry of Education, Science and Culture, Japan, by Grants-in-Aids from the Ministry of Health and Welfare, Japan, by Sandoz Pharmaceutical, Tokyo, Japan, by Ono Pharmaceutical, Osaka, Japan, by Takeda Research Foundation, Japan, and by grants for diabetes research from Otsuka Pharmaceutical Co., Ltd., Japan. We thank Dr. Y. Kaziro, DNAX Research Institute of Molecular and Cellular Biology, CA for providing rat Giα1, Giα2, and Giα3 cDNAs, and Dr. J. Pless, Sandoz, Basel for providing SMS 201-995.

REFERENCES

1. Brazeau, P., Vale, W., Burgus, R.,Ling, N., Butcher, M., Rivier, J., and Guillemin, R. (1973) Science 179, 77-79.
2. Reichlin, S. (1983) N. Engl. J. Med. 309, 1495-1501, 1556-1563.
3. Lamberts, S.W.J., Krenning, E.P., and Reubi, J.C. (1991) Endocrine Rev. 12, 450-482.
4. Bauer, W., Briner, U., Doepfner, W., Haller, R., Huguenin, R., Marbach, P., Pechter, T.J., and Pless, J. (1982) Life Sci. 31, 1133-1140.
5. Malm, D., Gievaer, A., Vonen, B., Burhol, P.G., and Florholmen, J. (1991) Acta Physiol Scand. 143, 413-419.
6. Moeller, I., Bunn, S.J., and Marley, P.D. (1989) Brain Research 484, 192-202.
7. Marin, P., Delumeau, J.C., Tence, M., Cordier, J., Glowinski, J., and Premont, J. (1991) Proc Natl Acad Sci USA. 88, 9016-9020.
8. Jakobs, K.H., Aktories, K., and Schultz, G. (1983) Nature 303, 177-178.
9. Wang H.L., Bogen C., Reisine T., and Dichter M. (1989) Proc Natl Acad Sci USA. 86, 9616-9620.
10. Luthin D.R., Eppler C.M., and Linden J. (1993) J. Biol. Chem. 268, 5990-5996.
11. Tallent M., and Reisine T. (1992) Mol. Pharmacol. 41, 452-45516.
12. Yamada Y., Post S.R., Wang K.,Tager H.S., Bell G.I., and Seino S (1992) Proc. Natl. Acad. Sci.USA. 89, 251-255.
13. Yamada, Y., Reisine, T., Law, S.F., Ihara, Y., Kubota, A., Kagimoto, S., Seino, M., Seino, Y., Bell, G.I., and Seino, S. (1992) Mol. Endocrinol. 6:2136-2142.
14. Yamada, Y., Kagimoto, S., Kubota, A., Yasuda, K., Masuda, K., Someya, Y., Ihara, Y., Li, Q., Imura, H., Seino, S., and Seino, Y. (1993) Biochem. Biophys. Res. Commun. 195, 844-852.

15. Kubota A., Yamada Y., Kagimoto S., Shimatsu A., Imamura M., Tsuda K., Imura H., Seino S., and Seino Y. (1994) J. Clin. Invest. 93, 1321-1325.
16. Rens-Domiano, S., Law, S.F., Yamada, Y., Seino, S., Bell, G.I., and Reisine, T. (1992) Mol. Pharmacol. 42, 28-34.
17. Futami, K., and Takenawa, T. (1989) J. Biol. Chem. 264, 14985-14989.
18. Felder, C.C., Kanterman, R.Y., Ma, A.L., and Axelrod, J. (1989) J. Biol. Chem. 264, 20356 -20362.
19. Itoh, H., Kozasa, T., Nagata, S., Nakamura, S., Katada, T., Ui, M., Iwai, S., Otsuka, E., Kawasaki, H., Suzuki, K., and Kaziro, Y. (1986) Proc Natl Acad Sci USA. 83, 3776-3780.
20. Itoh, H., Toyama, R., Kozasa, T., Tsukamoto, T., Matsuoka, M., and Kaziro, Y. (1988) J. Biol. Chem. 263, 6656-6664.
21. Simmonds, W.F., Goldsmith, P.K., Codina, J., Unson, C.G., and Spiegel, A.M. (1989) Proc Natl Acad Sci USA. 86, 7809-7813.
22. Strathmann, M., Winkle, T.M., and Simon, M.I. (1989) Proc Natl Acad Sci USA. 86, 7407-7409.
23. Strathmann, M. P., and Simon, M.I. (1990) Proc Natl Acad Sci USA. 87, 9113-9117.
24. Winkle, T.M., Scherle, P.A., Strathmann, M. P., Slepak, V.Z., and Simon, M.I. (1991) Proc Natl Acad Sci USA. 88,10049-10053.
25. Lee, H. C., Park, D., Wu, D., Rhee, S.G., and Simon, M.I. (1992) J. Biol. Chem. 267, 16044-16047.
26. Katz A., Wu D., and Simon M.I. (1992) Nature 360, 686-689.
27. Wu, D., LaRosa, G.J., and Simon, M.I. (1993) Science 261, 101-103.