Somatostatin Receptor Subtypes: Specific Expression and Signaling Properties

A. Schonbrunn, Y.-Z. Gu, P. Dournard, A. Beaudet, G.S. Tannenbaum, and P.J. Brown

The five cloned somatostatin (SRIF) receptors (ssts) are presumed to subserve unique biological roles by virtue of their tissue-specific expression and particular signal transduction mechanisms. However, the function of any individual sst subtype in its normal physiological milieu is not understood, because tissues and cells often express multiple ssts and, in the absence of receptor-specific SRIF analogs, the actions of individual receptors cannot be identified. To unravel the physiological role and signaling mechanism of the ssts, we have generated receptor subtype-specific antibodies and used these antibodies to determine the distribution of the receptor proteins and to identify the signal-transducing molecules with which particular sst subtypes interact.

SINCE THE ORIGINAL demonstration that the biological actions of somatostatin (SRIF) are initiated by binding to high-affinity plasma membrane receptors,[1] five SRIF receptor (sst) genes have been identified and named sst_1 through sst_5.[2] The sst_2 gene product is alternatively spliced to encode two receptor proteins, sst_2A and sst_2B, differing in their carboxy-terminal sequence, whereas the other genes each generate a single receptor protein.[2,3] Although all six sst subtypes bind the native peptides SRIF-14 and SRIF-28 with high affinity, they are believed to serve distinct biological functions. Therefore, studies are presently directed toward defining the physiological actions and the intracellular signal transduction mechanisms triggered by each receptor subtype. However, such investigations have been hindered in two ways. First, many target cells express mRNA for multiple ssts, but the lack of receptor subtype-specific ligands often makes it impossible to stimulate only one of the ssts present. Therefore, it is difficult to unambiguously identify the sst subtype producing a particular biological or biochemical response. Unraveling the signal transduction pathways triggered by individual ssts has been further complicated by a second factor. In cells expressing ssts endogenously, SRIF has been shown to regulate a variety of effectors, depending on the signaling pathways present in the cells under study.[4,5] For example, SRIF inhibits adenylyl cyclase in most target cells examined, but will regulate calcium and potassium channels only in electrically excitable secretory or neuronal cells.[6-8] Therefore, it is not surprising that the signaling mechanisms triggered by specific ssts have also differed among the various host cells in which ssts have been expressed by transfection.[4,5] Because the mechanism of action of endogenously expressed ssts has not been characterized in any normal target cell, the validity of the various expression systems used to examine sst signaling is at present unknown.

To circumvent some of these problems, we have developed specific antibodies to individual ssts and have begun to use these antibodies both to examine the distribution of the sst proteins and to isolate these receptors biochemically to characterize the signaling molecules with which they associate.

From the Department of Pharmacology, University of Texas Medical School, Houston, TX; and the Departments of Neurology and Neurosurgery, McGill University, Montreal, Quebec, Canada.

Address reprint requests to A. Schonbrunn, MD, Department of Pharmacology, University of Texas Medical School, PO Box 20708, Houston, TX 77225.

0026-0495/96/4508-1009$03.00/0

PREPARATION AND CHARACTERIZATION OF sst SUBTYPE-SPECIFIC ANTIBODIES

To generate receptor-specific antibodies, we identified unique sequences in the COOH-terminal region of individual ssts, synthesized synthetic peptides corresponding to these sequences, and used keyhole limpet hemocyanin-coupled polypeptides as immunogens.[5,9] The ability of the resulting rabbit polyclonal antibodies to specifically recognize individual sst proteins was tested in several different assays. First, the antibodies were each shown to efficiently immunoprecipitate ssts from Chinese hamster ovary (CHO) cells expressing the targeted receptor, but not from cells expressing any of the heterologous receptor subtypes. For example, the sst_1 antibody routinely precipitated 70% of the soluble [^{125}I-Tyr^{11}]ss-receptor complex prepared from CHO cells expressing this receptor, but precipitated less than 1% of the complex from cells expressing other ssts.[9] Preimmune serum was ineffective. Furthermore, addition of antigen peptide during the incubation with the corresponding antiserum completely inhibited immunoprecipitation of the receptor, whereas unrelated peptides had no effect. To characterize the nature of the receptor protein recognized by the antibodies, we prepared membranes from nontransfected CHO cells, as well as CHO cells expressing individual ssts and covalently labeled the receptors with the photoaffinity analog [^{125}I-Tyr^{11}-ANB-Lys^4]SRIF (^{125}I-azido-SRIF).[10] ^{125}I-azido-SRIF labeled broad bands at 60 and 85 kd in sst_1- and sst_2A-expressing CHO cells, respectively, but there was no specific labeling in the parental CHO cell line.[9] When photoaffinity-labeled receptors were immunoprecipitated, each antiserum precipitated only the corresponding receptor subtype. For example, using sst_1 antiserum, no specifically labeled bands were observed following sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis (PAGE) of immunoprecipitates from photoaffinity-labeled parental CHO cells or sst_2A-expressing CHO cells.[9] However, a broad 60-kd band was evident in the immunoprecipitate from sst_1-expressing cells, and immunoprecipitation of this protein was completely blocked by antigen peptide.[9] We also determined that both sst_1 and sst_2A antisera specifically recognized the cognate receptor on

Western blots, but did not cross-react with other sst subtypes[11] (Gu and Schonbrunn, unpublished observations). Again, both preimmune serum and antigen-blocked immune serum were inactive. Finally, we showed that sst_2A was specifically detected by immunocytochemical staining with the corresponding antiserum.[11] Together, these studies demonstrate that the generated antibodies can specifically recognize and immunopurify individual sst proteins.

sst DISTRIBUTION

The receptor subtype-specific antibodies permitted the distribution of individual sst proteins to be determined both in differentiated cell lines used as model systems for mechanistic studies of SRIF action and in tissues. The GH_4C_1 mammosomatotroph cell strain has been widely used to examine SRIF receptor signaling and regulation because SRIF rapidly and specifically inhibits growth hormone (GH) and prolactin (PRL) secretion by these cells in a manner that mimics its effects in estrogen-treated normal pituitary cells.[1,12] Both sst_1 and sst_2A proteins can be immunoprecipitated from these cells by specific receptor antibodies, consistent with the presence of these mRNAs.[9] However, although expression of sst_1 mRNA has been reported in RINm5F insulinoma cells,[13] Ar4-2J pancreatic acinar cells,[14] and AtT20 pituitary cells,[15] we found that only 6% of the occupied receptors from RINm5F cells could be precipitated by the sst_1 antibody and we did not detect any functional sst_1 protein in either the AR4-2J or the AtT20 cell lines.[9] In contrast, these cell lines all express sst_2A protein (Gu and Schonbrunn, unpublished observations). Therefore, the receptor-specific antibodies have permitted cells endogenously expressing various sst proteins to be identified. These studies clearly showed that mRNA measurements, especially as detected by the very sensitive reverse-transcriptase polymerase chain reaction assay, do not always accurately reflect the level of sst proteins present.

Although the distribution of mRNAs encoding ssts has been characterized in the human and rodent CNS,[16] the relative abundance and localization of the receptor proteins is unknown. Therefore, receptor-specific antibodies were used to determine the regional and cellular localization of sst_2A in rat brain.[11] As previously, antiserum adsorbed with an excess of antigen peptide and preimmune serum was used to provide appropriate negative controls.

The regional distribution of sst_2A immunoreactivity in rat brain sections was similar to that of SRIF-binding sites detected by receptor autoradiography with [^{125}I-Tyr^0, D-Trp^8]SRIF-14.[11] Because the analog used in the binding studies did not differentiate between ssts, the general correspondence between binding and sst_2A immunoreactivity indicated that the sst_2A was coexpressed with other ssts either in the same cells or in different subsets of neurons in the same regions. The sst_2A was distributed in nerve cell bodies and dendrites, as well as in axonal terminals, indicating that this receptor is in a position to transduce both postsynaptic and presynaptic effects of SRIF in mammalian brain. Interestingly, however, the distribution of the receptor between somatodendritic and axonal arbors varied dramatically between different brain regions.[11] In particular, immunoreactive terminals were most abundantly found in regions showing a high density of [^{125}I-Tyr^0, D-Trp^8]SRIF-binding sites, which probably reflects the high surface receptor levels in terminal fields. The regions containing immunoreactive cell bodies corresponded for the most part with those containing sst_2 mRNA as determined by in situ hybridization. However, there were areas previously reported to express high levels of sst_2 mRNA that showed either no or only low numbers of immunoreactive cells in our study.[11] Because our antibody is specific for the sst_2A splice variant, whereas the probes used in published in situ hybridization studies do not distinguish between sst_2A and sst_2B, these results raise the intriguing possibility that different splice variants of sst_2 have different neural functions.

sst SIGNALING

Based on early studies showing that hormone binding to endogenous ssts was inhibited both by guanine nucleotides

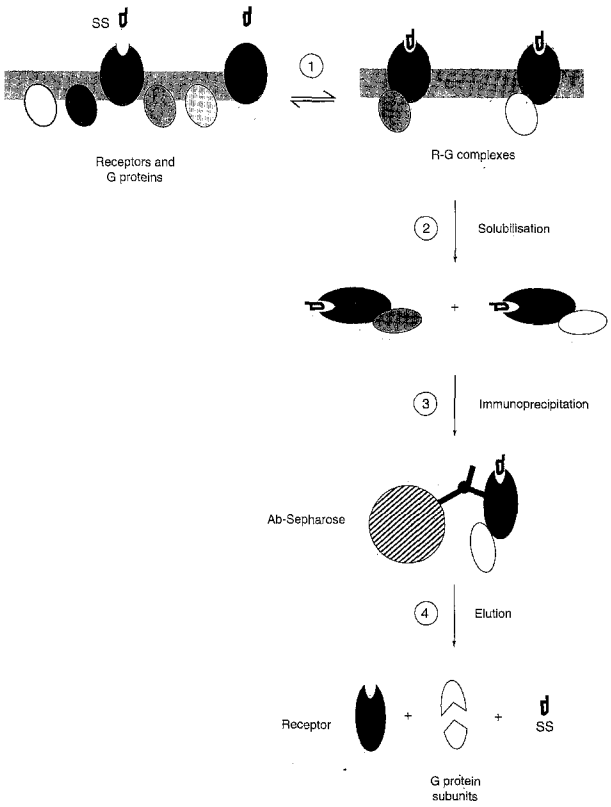

Fig 1. Immunoaffinity purification of the sst–G protein complex. Step 1: Membranes (shown to contain multiple ssts) are incubated with SRIF. Hormone binding to receptors promotes interaction with specific G proteins to produce a stable ternary complex.[21] Step 2: The intact ligand-receptor–G protein complex is solubilized with detergent. Step 3: Receptor subtype-specific antibodies are used to immunoprecipitate individual ssts with associated G proteins. Step 4: The receptors and G-protein subunits are eluted from the antibody-Protein A Sepharose beads with denaturing detergent. The released proteins are subjected to SDS-PAGE and the G-protein subunits coprecipitated with the receptor are identified by immunoblotting with specific G-protein antibodies.

and by pertussis toxin pretreatment, these receptors were proposed to act via G proteins belonging to the pertussis toxin–sensitive G_i/G_o family.[6,17] This conclusion was subsequently supported by experiments showing that pertussis toxin blocked SRIF inhibition of adenylyl cyclase and calcium channels, as well as SRIF activation of potassium channels and phosphatases.[7,8,17-19] However, when different biological end points were examined, less consistent results were obtained. For example, although pertussis toxin blocked SRIF inhibition of hormone secretion,[17] it did not affect its inhibition of pancreatic cell growth.[20] Hence, this latter effect was proposed to occur via pertussis toxin–insensitive G proteins or independently of G-protein coupling. These findings suggested that ssts were coupled to different effector systems by distinct signaling pathways. Clearly then, the critical step determining the intracellular signal transduction pathways triggered by SRIF in a target cell depends on the specificity of the interaction between the sst subtype activated and guanosine triphosphate (GTP)-binding transducing proteins or other effectors. To characterize the specificity of sst–G protein interactions, we developed a procedure to immunoprecipitate individual ssts complexed with G proteins (Fig 1).

We initially developed conditions the permitted the efficient solubilization of membrane ssts in a stable ternary complex with hormone and G proteins.[21] To determine whether the receptor antibodies coprecipitated receptor-associated G proteins, we took advantage of the ability of GTP analogs to uncouple the SRIF receptor–G protein complex and thereby decrease receptor affinity for agonists.[21] Membranes from CHO-K1 cells expressing sst_1 were prelabeled with [^{125}I-Tyr^{11}]SRIF, solubilized, and immunoprecipitated. Subsequently, the immunoprecipitate was resuspended in buffer and incubated at 25°C in the presence and absence of GTPγS. The observation that the guanine nucleotide-stimulated hormone dissociation from the immunoprecipitated receptor clearly demonstrated that the receptor remained functionally associated with G proteins.[9]

To identify the G protein subunits coprecipitated with ssts (Fig 1), membrane receptors were first incubated with SRIF to generate a stable hormone receptor–G protein complex. This complex was then solubilized with detergent and immunoprecipitated with the specific receptor antibodies.[9] The immunoprecipitated proteins were subjected to SDS-PAGE and immunoblotting with specific G-protein subunit antibodies (Gu and Schonbrunn, unpublished observations). We found that the sst_1 expressed in CHO cells was coprecipitated with both α and β G-protein subunits. Moreover, the receptor selectively associated with pertussis toxin–sensitive α_i subunits (Gu and Schonbrunn, unpublished observations). These results are consistent with our finding that when the endogenous sst_1 proteins are immunoprecipitated from GH_4C_1 pituitary cells, hormone binding to the precipitated receptors is markedly decreased by both GTPγS and pertussis toxin.[9] Thus, the observation that these receptors appear not to be G-protein–coupled or capable of inhibiting adenylyl cyclase in some expression systems[22] probably reflects the foreign environment provided by those hosts, rather than the normal signaling properties of this receptor subtype. Our studies are consistent with previous reports that SRIF can inhibit adenylyl cyclase and activate K^+ channels in GH_4C_1 cells through $G\alpha_i$ subunits,[7,23,24] although the ssts involved in these effects were not identified in previous studies. Current work is aimed at identifying the G protein subunit specificity of the individual ssts in detail. The approach we have developed to isolate individual ssts with specific receptor antibodies promises to be particularly valuable for investigating the effect of the cellular environment on receptor–G protein coupling selectivity in different SRIF-responsive cells.

REFERENCES

1. Schonbrunn A, Tashjian AH Jr: Characterization of functional receptors for somatostatin in rat pituitary cells in culture. J Biol Chem 253:6473-6783, 1978

2. Hoyer D, Bell GI, Berelowitz M, et al: Classification and nomenclature of somatostatin receptors. Trends Pharmacol Sci 16:86-88, 1995

3. Vanetti M, Kouba M, Wang X, et al: Cloning and expression of a novel mouse somatostatin receptor (SSTR-2B). FEBS Lett 311:290-294, 1992

4. Schonbrunn A: Signal transduction by somatostatin receptors, in Melmed S (ed): Molecular and Clinical Advances in Pituitary Disorders. Los Angeles, CA, Endocrine Research and Education, 1993, pp 305-310

5. Schonbrunn A, Gu Y-Z, Brown PJ, et al: Function and regulation of somatostatin receptor subtypes, in Somatostatin and Its Receptors. Ciba Foundation Symposium 190. New York, NY, Wiley, 1995, pp 204-221

6. Koch BD, Schonbrunn A: The somatostatin receptor is directly coupled to adenylate cyclase in GH_4C_4 pituitary cell membranes. Endocrinology 114:1784-1790, 1984

7. Yatani A, Codina J, Sekura RD, et al: Reconstitution of somatostatin and muscarinic receptor mediated stimulation of K^+ channels by isolated protein in clonal rat anterior pituitary cell membranes. Mol Endocrinol 1:283-289, 1987

8. White RE, Schonbrunn A, Armstrong DL: Somatostatin stimulates Ca^{2+}-activated K^+ channels through protein dephosphorylation. Nature 351:570-573, 1991

9. Gu Y-Z, Brown PJ, Loose-Mitchell DS, et al: Development and use of a receptor antibody to characterize the interaction between somatostatin receptor SSTR1 and G proteins. Mol Pharmacol 48:1004-1014, 1995

10. Brown PJ, Lee AB, Norman MG, et al: Identification of somatostatin receptors by covalent labeling with a novel, photoreactive somatostatin analog. J Biol Chem 265:17995-18004, 1990

11. Dournaud P, Gu Y-Z, Schonbrunn A, et al: Localization of the somatostatin receptor SSTR2A in rat brain using a specific antipeptide antibody. J Neuroscience (in press)

12. Schonbrunn A: Somatostatin action in pituitary cells involves two independent transduction mechanisms. Metabolism 39 2:96-100, 1990 (suppl)

13. Garcia PD, Myers RM: Pituitary cell line GH_3 expresses two somatostatin receptor subtypes that inhibit adenylyl cyclase: Functional expression of rat somatostatin receptor subtypes 1 and 2 in human embryonic kidney 293 cells. Mol Pharmacol 45:402-409, 1994

14. Buscail L, Delesque N, Estève J-P, et al: Stimulation of tyrosine phosphatase and inhibition of cell proliferation by somatostatin analogues: Mediation by human somatostatin receptor subtypes SSTR1 and SSTR2. Proc Natl Acad Sci USA 91:2315-2319, 1994

15. Patel YC, Panetta R, Escher E, et al: Expression of multiple somatostatin receptor genes in AtT-20 cells. Evidence for a novel somatostatin-28 selective receptor subtype. J Biol Chem 269:1506-1509, 1994

16. Hoyer D, Lübbert H, Bruns C: Molecular pharmacology of somatostatin receptors. Naunyn Schmiedebergs Arch Pharmacol 350:441-453, 1994

17. Koch BD, Dorflinger LJ, Schonbrunn A: Pertussis toxin blocks both cyclic AMP-mediated and cyclic AMP-independent actions of somatostatin: Evidence for coupling of Ni to decreases in intracellular free calcium. J Biol Chem 260:13138-13145, 1984

18. Kleuss C, Hescheler J, Ewel C, et al: Assignment of G protein subtypes to specific receptors inducing inhibition of calcium currents. Nature 353:43-48, 1991

19. Pan MG, Florio T, Stork PJS: G protein activation of a hormone-stimulated phosphatase in human tumor cells. Science 256:1215-1217, 1992

20. Viguerie N, Tahiri-Jouti N, Ayral AM, et al: Direct inhibitory effects of a somatostatin analog, SMS201-995, on AR4-2J cell proliferation via pertussis toxin-sensitive guanosine triphosphate-binding protein-independent mechanisms. Endocrinology 124:1017-1025, 1989

21. Brown PJ, Schonbrunn A: Affinity purification of a somatostatin receptor-G protein complex demonstrates specificity in receptor-G protein coupling. J Biol Chem 268:6668-6676, 1993

22. Bell GI, Reisine T: Molecular biology of somatostatin receptors. Trends Neurosci 16:34-38, 1993

23. Liu YF, Jakobs KH, Rasenick MM, et al: G protein specificity in receptor-effector coupling. Analysis of the roles of G_o and G_i2 in GH4C1 pituitary cells. J Biol Chem 269:13880-13886, 1994

24. Senogles SE: The D2 dopamine receptor isoforms signal through distinct $G_i\alpha$ proteins to inhibit adenylyl cyclase: A study with site-directed mutant $G_i\alpha$ proteins. J Biol Chem 269:23120-23127, 1994