Identification of Somatostatin Receptor Subtypes and an Implication for the Efficacy of Somatostatin Analogue SMS 201-995 in Treatment of Human Endocrine Tumors

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Abstract

The presence of somatostatin receptors has been demonstrated in various endocrine tumors as well as in normal tissues. We recently have cloned five human somatostatin receptor subtypes (SSTR1–SSTR5). These mRNAs are expressed in a tissue-specific manner. In this study, we have determined the somatostatin receptor subtypes expressed in various endocrine tumors using a reverse transcriptase polymerase chain reaction method. In two cases of glucagonoma and its metastatic lymph nodes in one case, all the SSTR subtype mRNAs except SSTR5 mRNA were expressed. In four cases of insulinoma, SSTR1 and SSTR4 mRNAs were detected, but SSTR2 mRNA was not detected in one case and SSTR3 mRNA was not detected in two cases, indicating a heterogeneous expression of SSTR subtypes in insulinomas. Interestingly, SSTR3 mRNA, which is highly expressed in rat pancreatic islets, is not expressed in normal human pancreatic islets, while SSTR1, SSTR2, and SSTR4 mRNAs are expressed. In three cases of pheochromocytoma, SSTR1 and SSTR2 mRNAs were detected, showing an expression pattern identical to that of normal adrenal gland. In a carcinoid, SSTR1 and SSTR4 mRNAs were detected. We have also found that human SSTR2 shows a high affinity for SMS 201-995, which has been used clinically for the treatment of endocrine tumors. Since SMS 201-995 was effective in the treatment of a patient with glucagonoma in which SSTR2 mRNA was present, but had no effect in a patient with carcinoid in which SSTR2 mRNA was not detected, this study suggests that the efficacy of SMS 201-995 may depend, at least in part, on the expression of SSTR2 in tumors. (*J. Clin. Invest.* 1994. 93:1321–1325.) Key words: reverse transcriptase polymerase chain reaction • glucagonoma • insulinoma • pheochromocytoma • carcinoid

Introduction

Somatostatin, a tetradecapeptide originally isolated from hypothalamus as a growth hormone–releasing inhibiting factor, has been found in many other tissues (1–2a). Somatostatin has diverse biological effects on cellular function including inhibition of secretory and proliferative processes (2–4). The various actions of somatostatin are mediated through its specific high affinity receptors (2, 2a, 5). The presence of somatostatin receptors has been demonstrated in many tumors as well as in normal tissues by classical biochemical binding techniques (3, 6–8).

Using the antiproliferative action of somatostatin, various somatostatin analogues have been developed for clinical applications (9, 10). One of the analogues, octreotide (SMS 201-995), has been approved for the treatment of endocrine tumors such as pituitary adenoma, pancreatic endocrine tumors, and carcinoid (3, 4, 11, 12). In addition, in vivo autoradiography by intravenous administration of radiolabeled Tyr^3-octreotide (SMS 204-090) has made it possible to localize somatostatin receptor–positive tumors and their metastases (3). Although these somatostatin analogues are useful in the diagnosis and treatment of endocrine tumors, some tumors have been shown to bear octreotide- or Tyr^3-octreotide–insensitive somatostatin receptors, which suggests the presence of different somatostatin receptor subtypes in endocrine tumors (3, 6). We recently have cloned five human somatostatin receptor subtypes (SSTR1–5),[1] whose mRNAs are expressed in a tissue-specific manner, indicating that various somatostatin actions are mediated by a family of structurally related proteins encoded by distinct genes (13–15). It is, therefore, important to identify the somatostatin receptor subtypes expressed in various endocrine tumors with regard to diagnosis, prognosis, and prediction of somatostatin analogue efficacy in treatment.

In this study we have determined the subtypes of somatostatin receptor expressed in various endocrine tumors using a reverse transcriptase PCR (RT-PCR) method. We show that SMS 201-995 exerts its biological effects through SSTR2 and suggest that the expression of SSTR2 may determine the efficacy of SMS 201-995 in the treatment of endocrine tumors.

Methods

Tissue samples. Two glucagonomas and metastatic lymph nodes, four insulinomas, three pheochromocytomas, and one carcinoid were studied. Tissue samples were obtained from patients at surgery, quick frozen in liquid nitrogen, and stored at −70°C.

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Received for publication 13 September 1993 and in revised form 7 December 1993.

J. Clin. Invest.

0021-9738/94/03/1321/05 $2.00
Volume 93, March 1994, 1321–1325

1. *Abbreviations used in this paper:* 5-HIAA, 5-hydroxyindole acetic acid; IC_{50}, inhibitory concentration, 50%; RT-PCR, reverse transcriptase PCR; SSTR, somatostatin receptor.

Table I. Primer Pairs for Amplifying Human SSTR Subtypes

Subtype		Size of PCR product
		bp
SSTR1	5'-GGAACTCTATGGTCATCTAC-3' (225–244)	233
	5'-GCTGAGCACAGTCAGACAGT-3' (438–457)	
SSTR2	5'-TGACAGTCATGAGCATCGAC-3' (399–418)	284
	5'-GCAAAGACAGATCATGGTGA-3' (663–682)	
SSTR3	5'-TCATCTGCCTCTGCTACCTG-3' (663–682)	222
	5'-GAGCCCAAAGAAGGCAGGCT-3' (865–884)	
SSTR4	5'-ATCTTCGCAGACACCAGACC-3' (548–567)	321
	5'-ATCAAGGCTGGTCACGACGA-3' (849–868)	
SSTR5	5'-CGTCTTCATCATCTACACGG-3' (598–617)	223
	5'-GGCCAGGTTGACGATGTTGA-3' (801–820)	

Numbers in parentheses indicate the location of the primer in the sequence with regard to translation start site.

RT-PCR. RNA was isolated from frozen tissue samples using the guanidium thiocyanate/cesium chloride procedure (16).

cDNA was synthesized using 1 μg of total RNA from above-mentioned tissues and normal human islets and 100 ng of oligo dT primer in a 20-μl solution that contained 50 mM Tris-HCl (pH 8.3), 75 mM KCl, 3 mM $MgCl_2$, 10 mM DTT, 500 μM each deoxynucleotide triphosphate, and 200 U Moloney murine leukemia virus reverse transcriptase (Superscript; GIBCO BRL, Gaithersburg, MD). After incubation of 1 h at 37°C followed by 30 min at 42°C, the reaction mixture was extracted with a solution of phenol-$CHCl_3$-isoamyl alcohol (25:24:1), and precipitated with ethanol. The amplification of RNA sequence was performed using PCR (17). cDNA derived from 50 ng of total RNA was combined with 1 μM oligonucleotide primers specific for each SSTR subtype in a 100-μl solution that contained 50 mM KCl, 10 mM Tris-HCl (pH 8.3), 1.5 mM $MgCl_2$, 200 μM of each deoxynucleotide triphosphate, and 2.5 U of Taq DNA polymerase. The reaction was carried out in a DNA thermal cycler (Perkin-Elmer Cetus Instruments, Norwalk, CT). After an initial denaturation at 94°C for 5 min, the samples were submitted to 37 reaction cycles for SSTR1 and 40 cycles for SSTR2–5 under the following conditions: annealing and extension for 2 min at 67°C for SSTR1, 62°C for SSTR2, and 65°C for SSTR3-5, and denaturation at 94°C for 1 min. PCR products were analyzed by electrophoresis on 2% agarose gels. The PCR products also were subcloned in M13mp18 and sequenced to confirm their identity. To exclude the possibility that PCR products might result from the amplification of genomic DNA that could contaminate the RNA preparation, 50 ng of the RNA preparation was used directly for PCR.

Transfection of human SSTR2 into COS cells and binding assays. SMS 201-995 has been used for the treatment of human endocrine tumors (3, 4, 11, 12). Our previous study has indicated that mouse SSTR2 has a high affinity for SMS 201-995 (18). To ascertain whether human SSTR2 also binds SMS 201-995 with a high affinity, we have characterized the pharmacological properties of human SSTR2.

A BamHI/XbaI fragment of human SSTR2 gene was inserted into the mammalian expression vector pCMV6b (13). The resulting construct was transfected into COS-1 cells by the DEAE-dextran method (15). 48 h after transfection, cells were washed twice in PBS, scraped, suspended in 20 mM Tris-HCl, 1 mM EDTA, and 225 mM sucrose (pH 7.5), sonicated, and centrifuged at 1,000 *g* for 10 min at 4°C. The supernatant was ultracentrifuged at 250,000 *g* for 30 min at 4°C. The pellets were resuspended in 10 mM Hepes buffer (pH 7.5) containing 500 KIU/ml aprotinin.

Binding properties of human SSTR2 to two kinds of somatostatin analogues, SMS 201-995 (provided by Dr. J. Pless, Sandoz AG, Basel, Switzerland) and RC-160 (Peninsula Laboratories, Inc., Belmont, CA) were examined according to the method described previously (15).

Treatment of patients with endocrine tumors with SMS 201-995. We previously reported a case of glucagonoma in which treatment with dacarbazine was very effective (19). Because the plasma glucagon levels gradually increased in spite of the treatment with dacarbazine, we have treated the patient with subcutaneous injection of SMS 201-995 at a daily dose of 150 μg. A patient with carcinoid has also been treated with subcutaneous injection of SMS 201-995 at a daily dose of 100–200 μg.

Results

The sequences of oligonucleotide primers used for PCR are listed in Table I. Fig. 1 shows the amplification of the plasmid DNAs containing each SSTR subtype gene, indicating that a specific amplification of each SSTR subtype was obtained under the conditions used. Sequence analysis confirmed that the DNA sequences of these PCR products were identical to those of SSTR subtypes previously described (13–15). Fig. 2, *a–d* shows the amplification of SSTR subtypes from cDNA preparations of various kinds of endocrine tumors, and the results are summarized in Table II. No PCR product was detected using any of the primer pairs specific for SSTR1–SSTR5 when RNA preparation was directly amplified, indicating that the products detected were not due to the amplification of geno-

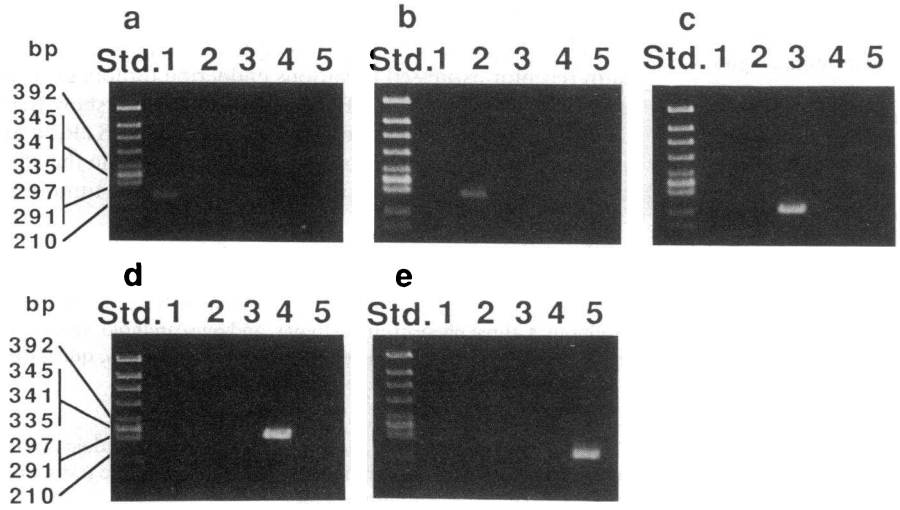
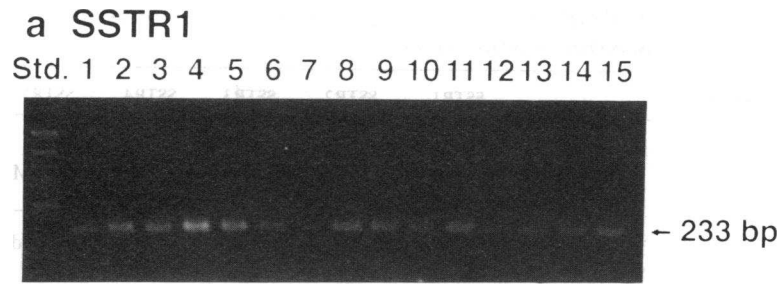
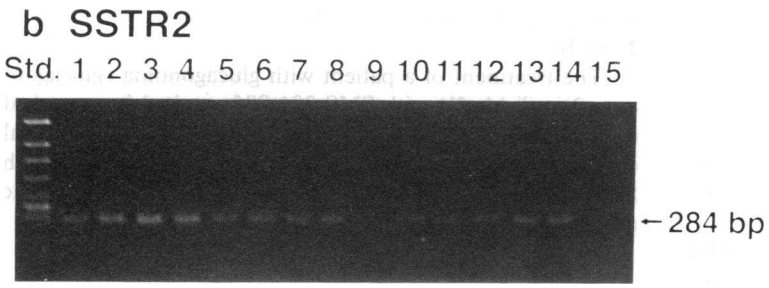
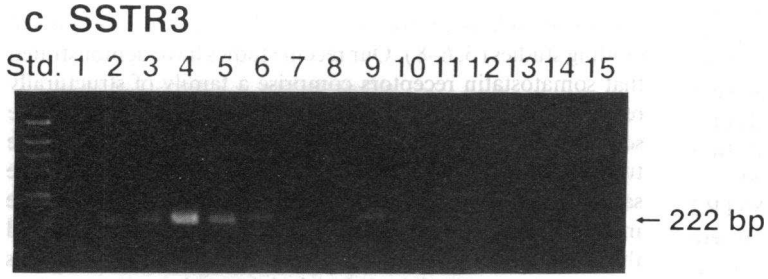
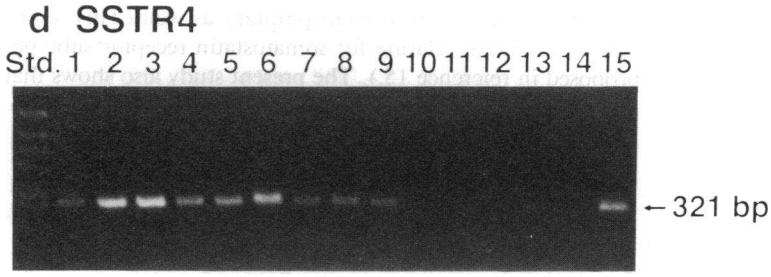


Figure 1. Amplification of the plasmid DNAs containing each SSTR subtype gene by PCR. Photographs of the ethidium bromide–stained agarose gels of PCR products are shown. The amplified plasmid in each lane is as follows: *1*, SSTR1; *2*, SSTR2; *3*, SSTR3; *4*, SSTR4; and *5*, SSTR5. *Std.*, lane containing HincII digest of ϕX 174 DNA. Primer pairs used for PCR are specific for: *a*, SSTR1; *b*, SSTR2; *c*, SSTR3; *d*, SSTR4; and *e*, SSTR5.









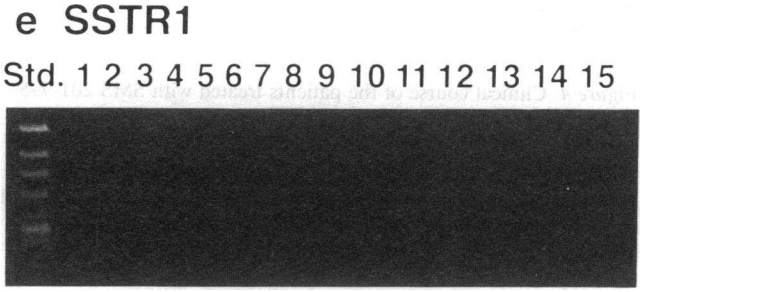


Figure 2. Amplification of SSTR subtypes from cDNAs (*a–d*) and RNAs (*e*) of endocrine tumors. Primer pairs used for PCR are specific for: *a*, SSTR1; *b*, SSTR2; *c*, SSTR3; and *d*, SSTR4. No PCR product is detected by direct amplification of RNA preparation using any of the primer pairs specific for SSTR1–SSTR5. A typical example of negative control for SSTR1 amplification is presented (*e*). The amplified cDNA or RNA in each lane is as follows: *1*, normal human pancreatic islets; *2*, glucagonoma 1; *3*, glucagonoma 2; *4*, pancreaticolienal lymph node metastasis of glucagonoma 2; *5*, lienal lymph node metastasis of glucagonoma 2; *6*, insulinoma 1; *7*, insulinoma 2; *8*, insulinoma 3; *9*, insulinoma 4; *10*, normal adrenal gland; *11*, pheochromocytoma 1; *12*, pheochromocytoma 2 found in the right adrenal gland; *13*, pheochromocytoma 2 found in the left adrenal gland; *14*, pheochromocytoma 3; and *15*, carcinoid. *Std.*, lane containing HincII digest of ϕX174 DNA.

Table II. Summary of the Expression of SSTR Subtypes Analyzed by RT-PCR

	SSTR1	SSTR2	SSTR3	SSTR4	SSTR5
Normal human islets	+	+	−	+	−
Glucagonoma 1	+	+	+	+	−
Glucagonoma 2					
Main tumor	+	+	+	+	−
Metastatic lymph nodes	+	+	+	+	−
Insulinoma 1	+	+	+	+	−
Insulinoma 2	+	+	−	+	−
Insulinoma 3	+	+	−	+	−
Insulinoma 4	+	−	+	+	−
Normal adrenal gland	+	+	−	−	−
Pheochromocytoma 1	+	+	−	−	−
Pheochromocytoma 2	+	+	−	−	−
Pheochromocytoma 3	+	+	−	−	−
Carcinoid	+	−	−	+	−

mic DNA (a typical example is shown in Fig. 2 *e*). The SSTR5 mRNA was not detected in any endocrine tumors examined (data not shown). In two cases of glucagonoma and its metastatic lymph nodes in one case, all the SSTR subtype mRNAs except the SSTR5 mRNA were detected. The SSTR1 and SSTR4 mRNAs were present in all cases of insulinoma. The SSTR2 mRNA was found in three cases of insulinoma and the SSTR3 mRNA was found only in two cases. In normal human pancreatic islets, SSTR1, SSTR2, and SSTR4 mRNAs were present. In three cases of pheochromocytoma and normal adrenal gland, the SSTR1 and SSTR2 mRNAs were found. In a carcinoid, the SSTR1 and SSTR4 mRNAs were detected.

The pharmacological characteristics of the human SSTR2 were examined in COS cells transiently expressing this receptor. SSTR2 exhibited specific and high affinity binding of [^{125}I-Tyr^{11}] somatostatin-14. SMS 201-995 and RC-160 potently inhibited [^{125}I-Tyr^{11}] somatostatin binding, with a half maximal inhibitory concentration (IC_{50}) of 4.0×10^{-10}M and 9.0×10^{-11}M, respectively (Fig. 3). The IC_{50} values of five somatostatin receptor subtypes for SMS 201-995 are summarized in Table III.

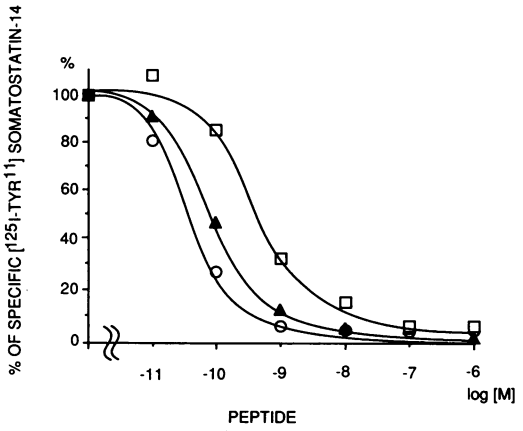


Figure 3. Binding properties of human SSTR2. The ability of somatostatin-14 (*open circle*), SMS 201-995 (*open box*), and RC-160 (*closed triangle*) to inhibit the binding of [^{125}I-Tyr^{11}] somatostatin-14 were examined. Total [^{125}I-Tyr^{11}] somatostatin-14 binding was 2,981 cpm, and nonspecific binding (binding remaining in the presence of 1 μM somatostatin) was 508 cpm. Data are the means of duplicate determination.

Table III. IC_{50} of the Five Human Somatostatin Receptor Subtypes for SMS 201-995

	SSTR1	SSTR2	SSTR3	SSTR4	SSTR5
IC_{50} for SMS 201-995	>1 μM*	0.4 nM	35 nM	>1 μM	7.0 nM

IC_{50} values are taken from references 14 (SSTR3) and 15 (SSTR4 and SSTR5). * Kubota, A., Y. Yamada, S. Kagimoto, S. Seino, and Y. Seino, unpublished observations.

The treatment of a patient with glucagonoma (glucagonoma 2 in Table II) with SMS 201-995 resulted in a marked reduction of plasma glucagon levels from 977 to 96–324 pg/ml (Fig. 4). In contrast, the treatment of a case of carcinoid with SMS 201-995 failed to reduce plasma 5-hydroxyindole acetic acid (5-HIAA) levels (Fig. 4).

Discussion

Somatostatin receptors have been found in various endocrine tumors as well as in normal tissues by classical biochemical binding studies (3, 6–8). Our recent studies have demonstrated that somatostatin receptors comprise a family of structurally related proteins (13–15). In this study we have identified the somatostatin receptor subtypes expressed in human endocrine tumors. Because of the limited availability of human tissue samples, we have used an RT-PCR method to determine the mRNA expression of each subtype. We recently have found that among the five human somatostatin receptor subtypes (SSTR1–5), no SSTR5 mRNA is detected in normal peripheral tissues examined (15). However, since a rat homologue of human SSTR5 has been shown to be expressed at high levels in rat pituitary (20), the possibility cannot be ruled out that SSTR5 is expressed in human pituitary as well (This report follows the nomenclature for somatostatin receptor subtypes proposed in reference 15.). The present study also shows that

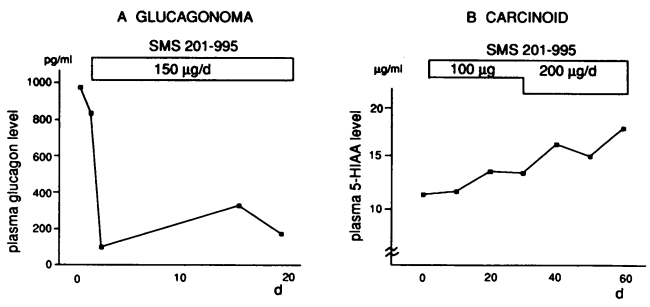


Figure 4. Clinical course of the patients treated with SMS 201-995. (*A*) A 47-yr-old male patient with glucagonoma 2 underwent the treatment with SMS 201-995 injected subcutaneously at a daily dose of 150 μg, which resulted in a marked reduction of plasma glucagon levels from 977 to 96–324 pg/ml and an improvement of glossitis and skin lesions. (*B*) A 75-yr-old male patient with carcinoid. In this case, the treatment with SMS 201-995 at a daily dose of 200 μg failed to reduce plasma 5-HIAA levels.

the SSTR5 mRNA is not present in any endocrine tumors examined.

In two cases of glucagonoma and its metastatic lymph nodes in one case, all the SSTR subtype mRNAs except the SSTR5 mRNA were detected. In insulinomas, the SSTR1 and SSTR4 mRNAs were present in all cases. The SSTR2 mRNA was found in three cases of insulinoma, and the SSTR3 mRNA was found only in two cases. These results indicate a heterogeneous expression of the human somatostatin receptor subtypes in insulinoma. We have previously found that SSTR3 mRNA is expressed at moderate levels in human brain and at high levels in rat pancreatic islets (14). Interestingly, the SSTR3 mRNA was not detected in normal human pancreatic islets while the SSTR1, SSTR2, and SSTR4 mRNAs were detected in the islets. Our preliminary study has also indicated that the expression pattern of SSTR1, SSTR2, and SSTR3 mRNAs is different in various tissues including brain, kidney, pancreas, and gastrointestinal tract among mouse, rat, monkey, and human (Kagimoto, S., Y. Yamada, A. Kubota, S. Seino, and Y. Seino, unpublished observations). This suggests that the expression pattern of somatostatin receptor subtypes is different in several tissues among different species. The SSTR1 and SSTR2 mRNAs were expressed in the pheochromocytomas examined. The expression pattern of SSTR subtype mRNAs in pheochromocytomas is identical to that in normal adrenal gland. In a carcinoid, the SSTR1 and SSTR4 mRNAs were present.

The somatostatin analogue SMS 201-995 has been used for the treatment of various endocrine tumors (3, 4, 11, 12). We have confirmed in our study that, as does mouse SSTR2 (18), human SSTR2 also binds SMS 201-995 with a high affinity. Moreover, among the five human somatostatin receptor subtypes, SSTR2 possesses the highest affinity for SMS 201-995. Since SMS 201-995 has been shown to have potent effects of antiproliferation as well as of inhibition on hormone secretion (3, 4, 11), SSTR2 could mediate these effects in endocrine tumor cells. In clinical use, it is known that the efficacy of SMS 201-995 varies, depending upon the histology of the endocrine tumors and other individual differences (3, 12). The present finding that SSTR2 mRNAs are not expressed in all the endocrine tumors studied suggests that the efficacy of SMS 201-995 may depend, at least in part, on the expression of the SSTR2. In fact, when one of the patients with glucagonoma in whom the SSTR2 mRNA was present in the tumor tissue was treated with SMS 201-995, a marked reduction of plasma glucagon levels was observed, and clinical symptoms including glossitis and skin lesions improved. In contrast, in the case of a patient with carcinoid in which the SSTR2 mRNA was not expressed, the treatment with SMS 201-995 failed to reduce plasma 5-HIAA levels. These two clinical experiences also support our suggestion that the expression of the SSTR2 subtype in endocrine tumors may determine the efficacy of SMS 201-995 in treatment, although more clinical cases should be examined.

Cloning of the five human somatostatin receptor subtypes and identification of the receptor subtypes expressed in various tumors should provide valuable information in regard to tumor diagnosis, prognosis, and prediction of somatostatin analog efficacy in treatment as well as for the development of selective analogues (3).

Acknowledgments

We thank Dr. J. Pless, Sandoz AG, Basel, Switzerland for providing SMS 201-995.

This study was supported in part by grants-in-aid for Scientific Research, for Cancer Research, and for Special Project Research on Cancer Bio-Science from the Ministry of Education, Science and Culture, Japan; grants-in-aid from the Ministry of Health and Welfare, Japan; by Sandoz Pharmaceutical, Tokyo, Japan; by Takeda Research Foundation, Japan; and by grants for diabetes research from Otsuka Pharmaceutical Co., Ltd., Osaka, Japan.

References

1. Brazeau, P., W. Vale, R. Burgus, N. Ling, M. Butcher, J. Rivier, and R. Guillemin. 1973. Hypothalamic peptide that inhibits the secretion of immunoreactive pituitary growth hormone. *Science (Wash. DC)*. 179:77–79.

2. Reichlin, S. 1983. Somatostatin. *N. Engl. J. Med.* 309:1495–1501.

2a. Reichlin, S. 1983. Somatostatin. *N. Engl. J. Med.* 309:1556–1563.

3. Lamberts, S. W. J., E. P. Krenning, and J. C. Reubi. 1991. The role of somatostatin and its analogs in the diagnosis and treatment of tumors. *Endocr. Rev.* 12:450–482.

4. Evers, B. M., D. Parekh, C. M. Townsend, Jr., and J. C. Thompson. 1991. Somatostatin and analogues in the treatment of cancer. *Ann. Surg.* 213:190–198.

5. Reisine, T., H. T. He, S. Rens-Domino, J. C. Martin, K. Raynor, S. Borislow, and K. Thermos. 1990. Biochemical properties of somatostatin receptors. *Metab. Clin. Exp.* 39(Suppl. 2):70–73.

6. Reubi, J. C., W. H. Häcki, and S. W. J. Lamberts. 1987. Hormone-producing gastrointestinal tumors contain a high density of somatostatin receptors. *J. Clin. Endocrinol. & Metab.* 65:1127–1134.

7. Reubi, J. C., R. Mauer, K. von Weder, J. Torhorst, G. M. Klijn, and S. W. J. Lamberts. 1987. Somatostatin receptors in human endocrine tumors. *Cancer Res.* 47:551–558.

8. Reubi, J. C., E. Krenning, S. W. J. Lamberts, and L. Kvols. 1990. Somatostatin receptors in malignant tissues. *J. Steroid Biochem. Mol. Biol.* 37:1073–1077.

9. Bauer, W., U. Briner, W. Doepfner, R. Haller, R. Huguenin, P. Marbach, T. J. Pechter, and J. Pless. 1982. SMS-201-995: a very potent and selective octapeptide analogue of somatostatin with prolonged action. *Life Sci.* 31:1133–1140.

10. Cai, R. Z., B. Szoke, R. Lu, D. Fu, T. W. Redding, and A. V. Schally. 1986. Synthesis and biological activity of highly potent octapeptide analogs of somatostatin. *Proc. Natl. Acad. Sci. USA.* 83:1896–1900.

11. Schally, A. V. 1988. Oncological applications of somatostatin analogues. *Cancer Res.* 48:6977–6985.

12. Wynick, D., and S. R. Bloom. 1991. The use of long-acting somatostatin analog octreotide in the treatment of gut neuroendocrine tumors. *J. Clin. Endocrinol. & Metab.* 73:1–3.

13. Yamada, Y., S. R. Post, K. Wang, H. S. Tager, G. I. Bell, and S. Seino. 1992. Cloning and functional characterization of a family of human and mouse somatostatin receptors expressed in brain, gastrointestinal tract and kidney. *Proc. Natl. Acad. Sci. USA.* 89:251–255.

14. Yamada, Y., S. Reisine, S. F. Law, Y. Ihara, A. Kubota, S. Kagimoto, M. Seino, Y. Seino, G. I. Bell, and S. Seino. 1992. Somatostatin receptors, an expanding gene family: cloning and functional characterization of human SSTR3, a protein coupled to adenylyl cyclase. *Mol. Endocrinol.* 6:2136–2142.

15. Yamada, Y., S. Kagimoto, A. Kubota, K. Yasuda, K. Masuda, Y. Someya, Y. Ihara, Q. Li, H. Imura, S. Seino, and Y. Seino. 1993. Cloning, functional expression and pharmacological characterization of a fourth (hSSTR4) and a fifth (hSSTR5) human somatostatin receptor subtypes. *Biochem. Biophys. Res. Commun.* 195:844–852.

16. Chirgwin, J. M., A. E. Przybyla, R. J. MacDonald, and W. J. Rutter. 1979. Isolation of biologically active ribonucleic acid from sucrose enriched in ribonuclease. *Biochemistry.* 18:5294–5299.

17. Saiki, R. K., D. H. Gelfand, S. Stoffel, S. J. Scharf, R. Higuchi, G. T. Horn, K. B. Mullis, and H. A. Erlich. 1988. Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase. *Science (Wash. DC)*. 239:487–491.

18. Rens-Domiano, S., S. F. Law, Y. Yamada, S. Seino, G. I. Bell, and T. Reisine. 1992. Pharmacological properties of two cloned somatostatin receptors. *Mol. Pharmacol.* 42:28–34.

19. Kurose, T., Y. Seino, H. Ishida, J. Fujita, T. Taminato, M. Matsukura, and H. Imura. 1984. Successful treatment of metastatic glucagonoma with dacarbazine. *Lancet.* i:621–622.

20. O'Carroll, A. M., S. J. Lolait, M. Konig, and L. C. Mahan. 1992. Molecular cloning and expression of a pituitary somatostatin receptor with preferential affinity for somatostatin-28. *Mol. Pharmacol.* 42:939–946.