Biological Activity and Receptor Binding Characteristics to Various Human Tumors of Acetylated Somatostatin Analogs (43393)

JACEK PINSKI, SLOBODAN MILOVANOVIC, TETSU YANO, ABRAHAM HAMAOUI, SINISA RADULOVIC, REN-ZHI CAI, AND ANDREW V. SCHALLY[1]

Endocrine, Polypeptide, and Cancer Institute, Veterans Affairs Medical Center, New Orleans, Louisiana 70146 and Section of Experimental Medicine, Tulane University School of Medicine, New Orleans, Louisiana 70112

Abstract. **Several somatostatin analogs with recently synthesized acetylated N terminus were assayed *in vivo* for their effects on sodium pentobarbital-stimulated growth hormone (GH) levels in fed male rats and gastrin-releasing peptide (14-27)-stimulated gastrin levels in fasted male rats. The binding characteristics of these analogs to somatostatin receptors were also examined in various human tumors and normal tissues. The analog RC-101-I, injected at a dose of 0.1 μg/100 g body wt, significantly suppressed GH release ($P < 0.01$) for at least 2 hr. Analog RC-160-II caused the longest inhibition of GH release, greater than that induced by nonacetylated parent analog RC-160, with GH levels showing significant suppression ($P < 0.01$) for more than 3 hr. Analogs RC-160-II and RC-101-I and RC-160, injected at a dose of 1.0 μg/100 g body wt, significantly ($P < 0.01$) suppressed gastrin-releasing peptide (14-27)-stimulated serum gastrin. Analog RC-101-I was active in this test at a dose of 0.1 μg/100 g body wt. RC-160-II showed significant binding to somatostatin-14 receptors in all investigated tissues (human colon, human colon cancer, breast cancer, human pancreas and pancreatic cancer, human prostate and prostate cancer, and rat cerebral cortex), but there were marked variations in binding affinities among various normal and cancerous tissues. The highest affinity was found in membranes of colon cancer (K_a = 18.4 nM^{-1}) and breast cancer (K_a = 12.46 nM^{-1}). The binding affinity of RC-160-II to somatostatin receptors in membranes of the breast cancer was similar to that of RC-160. RC-101-I showed higher binding affinity to somatostatin-14 receptors than RC-160 in human breast, pancreatic, and prostate cancer. With the exception of breast cancer tissue, the binding affinity of RC-101-I was significantly lower than that of RC-160-II in membranes of all investigated tissues. It can be concluded that acetylated somatostatin analogs RC-101-I and RC-160-II possess prolonged and enhanced biological activities in suppressing serum GH and gastrin in rats. Significant variations in binding affinities for these analogs in different tissues and various tumors suggest that differences may exist between somatostatin receptors in normal versus malignant tissues. This raises the possibility that some of these analogs could be used more selectively in the treatment of various neoplasms.** [P.S.E.B.M. 1992, Vol 200]

The cyclic tetradecapeptide somatostatin (SS)-14 is widely distributed in the body (1, 2), has multiple actions, the majority of which are inhibitory, and functions as an endocrine and paracrine hormone as well as a neurotransmitter (1, 2). Somatostatin is a physiological growth inhibitor (1, 3, 4), and exerts suppressive effects on a large variety of cells (1–10). In addition to its action on the pituitary and pancreas, SS-14 inhibits the secretion of gastric acid, serum levels of gastrin, and the release of other gastrointestinal hormones (1, 2, 8, 11). In clinical trials, SS-14 induced hormonal suppression in patients with acromegaly, endocrine pancreatic tumors such as insulinomas and glucagonomas, and ectopic tumors such as gastrinomas and vasoactive intestinal peptide-producing tumors (1, 2, 8–10).

[1] To whom requests for reprints should be addressed at 151, VA Medical Center, 1601 Perdido Street, New Orleans, LA 70146.

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However, the very short half-life of SS-14 makes its clinical use impractical (1, 12). A large number of somatostatin analogs with more selective and prolonged activities have been synthesized in various laboratories (1, 8, 13–15). More than 300 octapeptide analogs of SS-14 have been developed in our laboratory (14, 15). Some of these analogs were selective for suppressing growth hormone (GH) release, since the inhibitory potencies for insulin and glucagon release in rats are much smaller (14, 15). These analogs also suppressed gastric acid, gastrin, secretin, and cholecystokinin secretion (8, 11, 14, 15). Among these analogs, RC-160 was about 100 times more potent than SS-14 in tests for inhibition of growth hormone release *in vivo* in rats and possessed a prolonged duration of action (14, 16).

Among the hormones affected by somatostatin analogs and investigated in this study, GH and gastrin exert direct and indirect proliferative effects on various normal tissues and tumors, including normal breast, breast cancer, normal pancreas, and pancreatic cancer (17). Somatostatin analogs might inhibit the growth of these target tissues by suppressing the secretion of GH from the pituitary and gastrin from the stomach and through direct antiproliferative effects mediated by specific receptors for somatostatin located on cells of these tissues (1–4, 17–19).

Recently, we synthesized a series of somatostatin analogs with acetylated N terminus. The aim of the study reported here was to investigate the effect of some of these analogs on GH and gastrin secretion in rats and examine the binding affinities of these analogs to SS-14 receptors in various normal human tissues and in tumors, particularly those that might be affected by GH or gastrin. Normal pancreas and pancreatic cancers were also investigated in view of the established ability of the somatostatin analog RC-160 to inhibit the growth of pancreatic cancer (8, 17, 19).

Materials and Methods

Animals. Young, adult, male Sprague-Dawley rats, weighing 220–360 g, were used in all experiments. Animals were allowed standard diet and tap water *ad libitum*, and were maintained under controlled conditions (12:12-hr light:dark schedule, at 24 ± 2°C).

Peptides. Somatostatin-14 (SS-14), (Tyr^{11})SS-14, D-Phe-Cys-Tyr-D-Trp-Lys-Val-Cys-Trp-NH_2 (RC-160), *N*-acetyl-D-Phe-Cys-Tyr-D-Trp-Lys-Val-Cys-Trp-NH_2 (RC-160-II, corresponding to acetylated RC-160), *N*-acetyl-Phe-Cys-Tyr-D-Trp-Lys-Val-Cys-Trp-NH_2 (RC-160-I, having N-terminal Phe in the L-form, *N*-acetyl-Phe-Cys-Phe-D-Trp-Lys-Thr-Cys-Thr-NH_2 (RC-101-I), and GRP(14-27) were synthesized by solid phase methods and evaluated in our laboratory (14). RC-160-II and RC-101-I were made by classical synthesis by Novabiochem (Laufelingen, Switzerland) and supplied by Debiopharm S.A. (Lausanne, Switzerland). For the injection, the peptides were dissolved in 0.1 *M* acetic acid and diluted with the same solvent containing 0.2–0.5% bovine serum albumin.

GH Inhibition Time Course Assay. The rats were treated with sodium pentobarbital (6 mg/100 g body wt, ip) and, after 20 min, were injected subcutaneously with the somatostatin analogs at a dose of 0.1 μg/100 g body wt or saline. Sodium pentobarbital at half of the initial dosage was given at 60- to 90-min intervals to maintain anesthesia. Blood was collected from the jugular vein 15, 30, 60, 90, 120, 180, and 240 min after the injection of analogs.

Measurement of Inhibition of Gastrin Levels. Rats were deprived of food, but not water, for 18 hr prior to the experiment. For the experiment, the rats were anesthetized with 50 mg/kg body wt of sodium pentobarbital (Nembutal; Abbott Laboratories, North Chicago, IL). To test the inhibitory activity of the somatostatin analogs on gastrin secretion in rats, a bolus of the somatostatin analogs in doses of 0.1 and 1.0 μg/100 g body wt was injected 30 sec prior to the injection of gastrin-releasing peptide (GRP) (14-27) at a dose of 5.0 μg/100 g body wt. Blood samples were taken from the jugular vein before and at 3 and 6 min after GRP(14-27) stimulation.

Radioimmunoassay. All blood samples were centrifuged and kept at −70°C until assayed. Serum GH levels were measured by radioimmunoassay using materials supplied by the National Hormone and Pituitary Program of NIH (Bethesda, MD). The interassay variation was less than 15%, and the intra-assay was 10% or less. Serum gastrin in rats was determined by double antibody radioimmunoassay with a kit provided by Becton Dickinson and Co. (Orangeburg, NY). The interassay variation was less than 7.3%, and the intra-assay variation was less than 4.0%. Statistical significance was assessed by Duncan's new multiple range test (20).

Receptor Assay. *Buffers.* The homogenization buffer consisted of 0.3 *M* sucrose, 25 m*M* Tris base, 0.25 m*M* phenylmethylsulfonyl fluoride, 1 m*M* EGTA, 10 m*M* monothioglycerol, and Trasylol (aprotinin) at 10,000 kallikrein inactivator units/liter (pH 7.5). Receptor binding of SS-14 was carried out in 50 m*M* Tris base, 5 m*M* $MgCl_2$, 0.2% bovine serum albumin, Trasylol (100,000 units/liter), bacitracin (20 mg/liter), and 0.25 m*M* phenylmethylsulfonyl fluoride (pH 7.5). The wash buffer was the same as the assay buffers, but did not contain bovine serum albumin. The chemicals used for buffers were purchased from Sigma Chemical Co. (St. Louis, MO).

Preparation of membranes. Normal tissues from experimental animals were obtained from adult male Sprague-Dawley rats (Charles River Breeding Laboratories, Wilmington, MA). Normal human specimens were obtained from Laboratory Services of the Veterans

Affairs Medical Center (New Orleans, LA). Autopsies were performed 6–18 hr after death. Before completing the receptor assay, all autopsy specimens were carefully examined by our pathologist. Only those specimens that did not show signs of postmortem autolysis were selected for receptor studies. The ages of patients from whom the normal prostate specimens were obtained ranged from 58 to 62 years and, in cases of normal pancreas, 64 to 75 years. The specimen of human pancreatic adenocarcinoma was obtained from Dr. Jane Gurtler, East Jefferson Hospital, Metairie, LA. The patient was a 61-year-old woman with poorly differentiated adenocarcinoma of the pancreas. A sample of human prostate cancer was obtained after radical prostatectomy of a 61-year-old man with prostate cancer, Gleason Grade 7, and it was provided by Dr. T. G. Weilbaecher, VA Medical Center (New Orleans, LA). Two colon cancer specimens were obtained from Dr. D. L. Johnson (Veterans Affairs Hospital, Palo Alto, CA). One patient was a 69-year-old man with moderate differentiated rectosigmoid adenocarcinoma (Dukes C_2) and the second patient was a 74-year-old man with moderate differentiated sigmoid colon cancer (Dukes D). Somatostatin receptors were found on both colon cancer specimens, and membrane preparations were pooled and used for the binding test. Breast cancer specimens were obtained from Dr. J. L. Wittliff following biopsies performed in women, 28–82 years of age, suspected of having breast cancer. Breast biopsies were performed at James Graham Brown Cancer Center, University of Louisville, School of Medicine (Louisville, KY). Specimens from four patients, positive for somatostatin receptors, were pooled and used for testing somatostatin analogs.

Human cancer specimens were frozen and shipped on dry ice to the Endocrine, Polypeptide, and Cancer Institute (New Orleans, LA) for membrane receptor assays. The specimens were stored at −70°C until processed. Cerebral cortex tissue of male Sprague-Dawley rats was used as positive controls for SS-14 binding. Samples were cleaned of fat and connective tissue, cut into small slices, and homogenized in 5 vol of sucrose buffer using an Ultra-Turrax homogenizer (Tissumizer; Tekmar, Cincinnati, OH) at maximal speed 5 for 5-sec strokes at 0°C. The homogenate was centrifuged at 500*g* for 10 min at 4°C. The supernatant containing the crude membrane fractions was then ultracentrifuged at 70,000*g* for 45 min at 4°C (Beckman Preparative Ultracentrifuge; Beckman Instruments, Inc., Palo Alto, CA). The resulting pellet was resuspended in wash buffer and used for the receptor binding studies. Protein concentration was determined by the Bio-Rad (Richmond, CA) protein assay kit according to Bradford (21).

For the radioiodination of (Tyr[11])SS-14, a modification of the chloramine T method of Greenwood *et al.* (22) was used. The specific activity was 78–141.8 μgCi/μg. An aliquot of the peptide (5 μg in 5 μl of 0.01 *N* acetic acid) was mixed with 40 μl of 0.5 *M* phosphate buffer and 1 mCi of ^{125}INa in a volume of 2 μl. Ten μl of chloramine T were added. The reaction lasted 30 sec at room temperature and was stopped by adding 10 μl of cysteine (5.1 mg/ml in 0.5 *M* phosphate buffer) and diluting with 500 μl of 0.5 *M* phosphate buffer. The purification of the labeled peptide was carried out using high-performance liquid chromatography with a C-18 column (W-Porex 5C18, 250 × 4.6 mm i.d.; Phenomenex, Rancho Palos Verdes, CA).

The binding assay of SS-14 was conducted as described previously (23, 24). Binding reactions were performed in 12 × 75-mm polypropylene round-bottom culture tubes at 21°C using a competitive inhibition method. Displacement curves were obtained with 100–140 p*M* iodinated [^{125}I-Tyr[11]]SS-14 in the presence of increasing amounts of unlabeled SS-14 (1.5×10^{-11}–1.5×10^{-6} *M*). The procedure was as follows. Fifty microliters of unlabeled SS-14 in incubation buffer and 50 μl of labeled ligand were placed into the tube and vortexed, and 50 μl of the suspension containing the membrane receptor fraction were added. In each binding experiment, one set of tubes was used with 50 μl of wash buffer instead of membrane to determine the radioactivity bound to nonmembrane particles. Each assay point was performed in triplicate. The tubes were incubated for 120 min at 21°C. The reaction was terminated by rapid filtration through glass fiber filters (Filtermats-Receptor Binding; Skatron, Inc., Lier, Norway), prewetted in assay buffer, and presoaked for at least 3 hr in 0.5% polyethylenimine solution to minimize filter adsorption. A semiautomatic cell-harvesting system (Skatron) with 10-sec washing time was used. The radioactivity of filters was counted in an automatic gamma counter (Micromedic System, Inc., Huntsville, AL). The specifity of SS-14 binding to its receptors was investigated using competitive inhibition studies with labeled SS-14 and unlabeled D-Trp[6]-luteinizing hormone-releasing hormone (160 p*M* to 1.6 μm) and epidermal growth factor (0.1–100 ng).

Mathematical analysis of the binding data. The counts of radioactivity obtained from filters or tubes containing membrane particles were corrected by deducting the background count from assays in which no membranes were added. The Ligand-PC computerized curve-fitting program of Munson and Rodbard (25) was used to determine the types of receptor binding, the affinity constant (K_a), and the maximal binding capacity of receptors (B_{max}).

Results

GH Time Course Assay. The effects of the acetyl somatostatin analogs and of RC-160 on sodium pentobarbital-stimulated GH release in male rats are shown

in Figure 1. All four analogs (RC-160, RC-101-I, RC-160-II, and RC-160-I) exhibited significant inhibition of pentobarbital-induced GH release ($P < 0.01$), as compared with the control group, 15 min after the injection. RC-160-I (the L-Phe form of acetyl RC-160) showed the weakest suppressive effect on GH secretion, with serum GH returning to control levels 1 hr after administration of the L-form. In contrast, a more prolonged and significant ($P < 0.01$) inhibition of GH release for at least 90 min was observed after injection of RC-160, RC-101-I, and RC-160-II. The peak inhibition was seen at 30–60 min after administration of these analogs. Analog RC-160-II was the longest acting and induced significant suppression of GH secretion for at least 3 hr.

Gastrin Assay. The effects of the acetyl somatostatin analogs and of RC-160 on GRP(14-27)-stimulated serum gastrin release are seen in Figures 2 and 3.

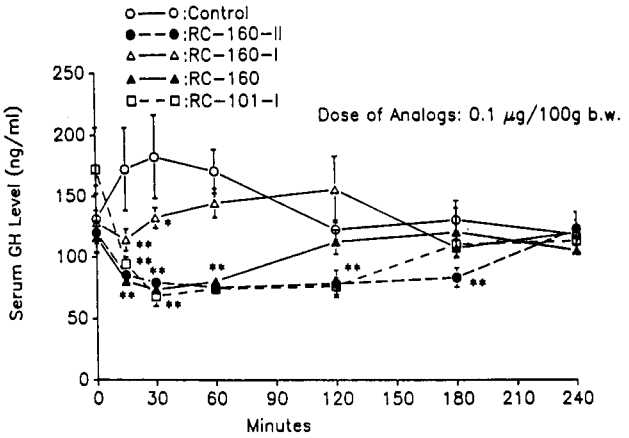

Figure 1. Time course of inhibition of sodium pentobarbital-stimulated GH secretion in rats by somatostatin analog RC-160 and various acetylated somatostatin analogs. The analogs were injected subcutaneously at a dose of 0.1 μg/100 g body wt. * $P < 0.05$ and ** $P < 0.01$ vs. control.

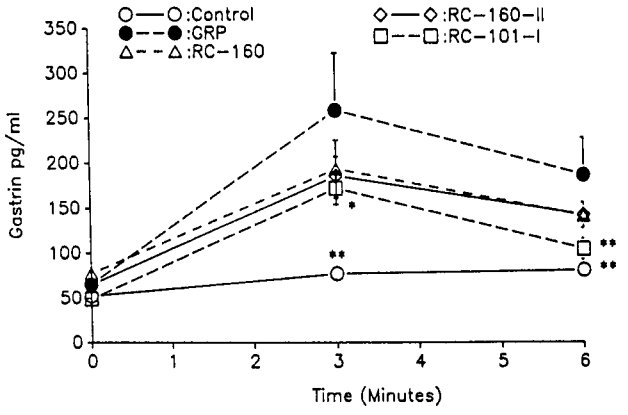

Figure 2. Effect of RC-160 and acetylated somatostatin analogs on GRP(14-27)-stimulated serum gastrin levels in rats. The analogs were injected intravenously at a dose of 0.1 μg/100 g body wt 30 sec before intravenous administration of GRP(14-27) at a dose of 5 μg/100 g body wt. * $P < 0.05$ and ** $P < 0.01$ vs the GRP(14-27) group.

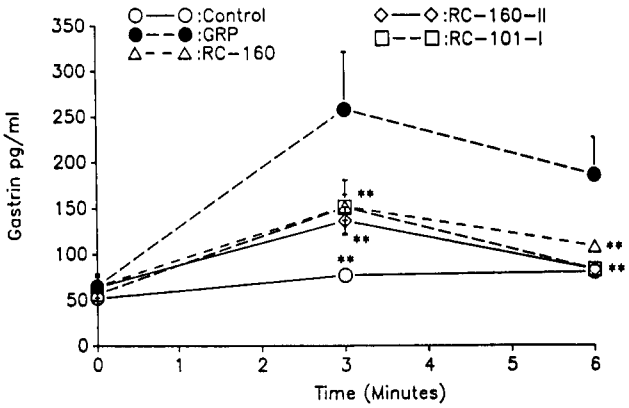

Figure 3. Effect of RC-160 acetylated somatostatin analogs on GRP(14-27)-stimulated serum gastrin levels in rats. The analogs were injected intravenously at a dose of 1.0 μg/100 g body wt 30 sec before intravenous administration of GRP(14-27) at a dose of 5 μg/100 g body wt. * $P < 0.05$ and ** $P < 0.01$ vs. the GRP(14-27) group.

Administration of the analogs RC-160 and RC-160-II at a dose of 0.1 μg/100 g body wt lowered serum gastrin levels 3 min after GRP(14-27) injection from 257.3 ± 63.5 pg/ml for the GRP(14-27)-stimulated group to 192.3 ± 32.0 pg/ml and to 184.6 ± 21.5 pg/ml for the RC-160 and RC-160-II treated groups, respectively, but this reduction was not statistically significant (Fig. 2). Only the analog RC-101-I at the dose of 0.1 μg/100 g body wt significantly suppressed gastrin levels 3 min ($P < 0.05$) and 6 min ($P < 0.01$) after GRP(14-27) administration. At a dose level of 1.0 μg/100 g body wt, all analogs significantly inhibited gastrin levels ($P < 0.01$) 3 and 6 min after GRP(14-27) stimulation (Fig. 3).

Receptor Assay. The binding affinities of somatostatin-14 and its analogs to membrane receptors in human pancreas, human pancreatic cancer, rat cerebral cortex, human prostate, human prostate cancer, human breast cancer, human colon, human colon cancer are illustrated in Table I. The displacement binding curves of these analogs in different tumor tissues are presented in Figure 4. The analysis of displacement curves of [^{125}I-Tyr^{11}]SS-14 by unlabeled SS-14 in all tissue specimens investigated, suggest that labeled SS-14 is bound to one class of noncooperative binding sites. Homologous displacement assay of [^{125}I-Tyr^{11}]SS-14 by unlabeled SS-14 in rat cerebral cortex suggest that the labeled peptide was bound to one class of receptors with high affinity (K_a = 38.11 nM^{-1}) and low capacity (B_{max} = 6.7 × 10^{-14} mole/mg of membrane protein). In heterogeneous displacement assay, RC-101-I showed 10 times and about five times higher affinity than RC-160-II and RC-160, respectively, to receptors for SS-14 in rat cerebral cortex membranes.

In normal human pancreas and human pancreatic cancer specimens, one class of low capacity binding sites was found with B_{max} values of 14.8 × 10^{-14} and 13.2 × 10^{-14} mole/mg of membrane protein, respec-

Table I. Displacement of [^{125}I-Tyr^{11}]SS-14 with SS-14 and Its Analogs from Membrane Receptors on Normal Human and Human Cancer Tissues[a]

Tissues	Peptides (unlabeled)	K_a (nM^{-1})
Rat cerebral cortex	SS-14	38.11
	RC-160	4.2
	RC-160-II	2.1
	RC-101-I	21.0
Normal human pancreas	SS-14	0.43
	RC-160	1.02
	RC-106-II	0.3
	RC-101-I	0.019
Human pancreatic cancer	SS-14	0.391
	RC-160	0.48
	RC-160-II	2.2
	RC-101-I	0.95
Normal human colon	SS-14	3.3
	RC-160	20.0
	RC-160-II	7.63
	RC-101-I	0.036
Human colon cancer	SS-14	1.54
	RC-160	16.6
	RC-160-II	18.36
	RC-101-I	0.0416
Normal human prostate	SS-14	0.14
	RC-160	No binding
	RC-160-II	0.032
	RC-101-I	0.27
Human prostate cancer	SS-14	2.4
	RC-160	0.12
	RC-160-II	3.1
	RC-101-I	0.36
Human breast cancer	SS-14	1.4
	RC-160	12.0
	RC-160-II	12.46
	RC-101-I	121.6

[a] The results of each experiment were calculated from data of triplicate tubes of a 10-point assay. The coefficients of variation of the three determinations of K_a values ranged from 8% to 24%. K_a, Affinity constant of ligand for membrane receptors.

tively. None of the analogs tested, except RC-160-II, showed high potency in displacing [^{125}I-Tyr^{11}]SS-14 from membranes of both normal and carcinomatous pancreatic tissues.

In membranes from normal human colon, RC-160 and RC-160-II showed the highest affinities for SS-14 receptors. Their binding was 6.0 and 2.2 times higher, respectively, than that of SS-14. Among acetylated analogs, RC-101-I displayed the lowest binding to somatostatin receptors in normal colon and colon cancer. Acetylated analog RC-160-II appeared to be the most potent in displacing [^{125}I-Tyr^{11}]SS-14 somatostatin from receptor membranes of colon cancer tissue (K_a = 18.36 nM^{-1}). Likewise, RC-160 was shown to have a high affinity for somatostatin receptors in normal colon and colon cancer.

Analysis of binding data suggested the presence of somatostatin receptors with low affinity and high capacity (B_{max} = 1.5 × 10^{-12} mole/mg of membrane protein) and high affinity and low capacity (B_{max} 1.4 × 10^{-14} mole/mg of membrane protein) in normal prostate and prostate cancer, respectively. In contrast to RC-160, acetylated analogs showed binding to somatostatin receptors in normal prostate. Affinity of RC-160-II for prostate SS-14 receptors was similar in range to SS-14.

In membranes of human breast cancer specimens, [^{125}I-Tyr^{11}]SS-14 showed binding to one class of high affinity and low capacity binding sites (K_a = 1.4 nM^{-1}, B_{max} = 7.5 × 10^{-14} mole/mg of membrane protein). The analysis of the heterologous displacement curve demonstrated that RC-101-I had extremely high affinity to somatostatin receptors on breast cancer membranes. Analog RC-160-II also showed high affinity to somatostatin binding sites in breast cancer.

Discussion

The measurements of serum GH levels indicate that the acetylated somatostatin analogs RC-160-II and RC101-I are at least as active in suppressing of sodium pentobarbital-stimulated serum GH as the potent analog RC-160. Both acetylated analogs showed increased duration of action for the inhibition of GH secretion as compared with RC-160. Somatostatin analog RC-160-II significantly lowered serum GH levels for more than 3 hr, whereas serum GH returned to control levels 2 hr after administration of RC-160. This prolonged activity could be attributed to attenuated degradation of acetylated somatostatin analogs. Therefore, the N-terminal modification of this analog by an acetyl group may have a protective effect against inactivation by proteases.

The fall in GH levels induced by somatostatin analogs could, through mechanisms involving endogenous growth factors such as insulin-like growth factors I and II, be of major importance for the inhibition of tumor growth (4, 19). Cell differentiation can be stimulated directly by GH and indirectly through GH-stimulated local production of insulin-like growth factor I (26–29).

A number of somatostatin analogs have been used for the treatment of acromegaly and endocrine tumors of the gastroenteropancreatic system such as carcinoid tumors, insulinomas, glucagonomas, gastrinomas, and vasoactive intestinal peptide-secreting tumors (17, 30–32). Experimental studies in animal models of breast cancer, prostate cancer, and carcinoma of exocrine pancreas suggest that somatostatin analogs, including RC-160, might inhibit the growth of these malignancies, and clinical trials are in progress (4, 17, 33–35).

The inhibition of serum gastrin levels by somatostatin analogs RC-160, RC-101-I, and RC-160-II is in agreement with the well-known suppressive effect of SS-14, both on gastric acid secretion and serum gastrin

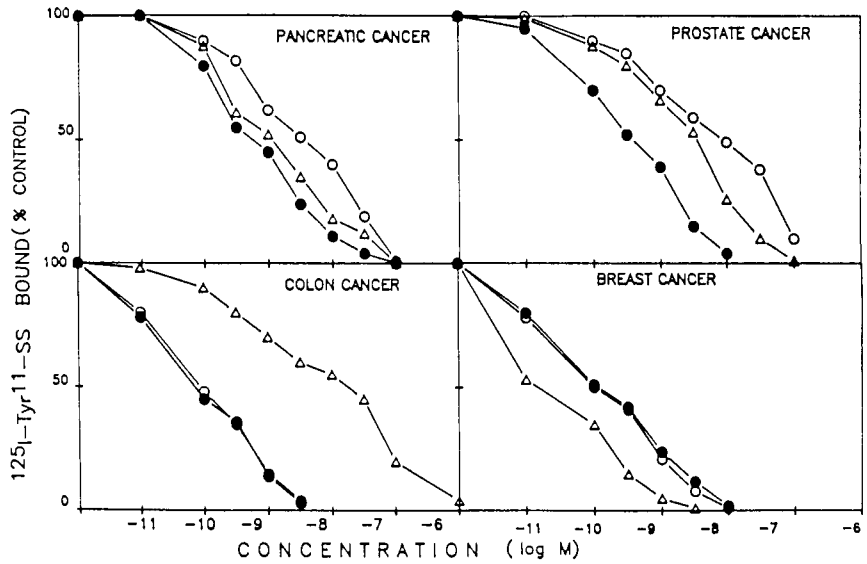

Figure 4. Ability of three *N*-acetyl octapeptide somatostatin analogs (○—○, RC-160; ●—●, RC-160-II, △—△, RC-101-I) to inhibit binding of [^{125}I-Tyr^{11}]SS-14 to membrane preparations from various tumors. The total binding was between 3% and 8% of the radioactivity added, with 50–70% specific binding. Results are expressed as the percentage of the saturable binding in the absence of the analog. In every experiment, each value was determined in triplicate.

(36). Somatostatin analog RC-101-I showed the activity in the suppression of serum gastrin levels at a dose of 0.1 μg/100 g body wt. These somatostatin analogs may interfere with the cholinergic transmission (11), the release of gastrin from antral G cells, and the action of gastrin on gastrinic receptors of the parietal cells (37). Various experimental findings suggest that gastrointestinal hormones might influence the growth of the malignant cells of the pancreas and phenotypic transformations (38). Cell culture experiments (reviewed in Ref. 39) have shown that gastrin, cholecystokinin, and secretin can stimulate the growth of pancreatic adenocarcinoma cells. A hormonal therapy of exocrine pancreatic cancer could be based on the new acetylated somatostatin analogs, alone or in combination with luteinizing hormone-releasing hormone analogs or other drugs (19, 40, 41). In *N*-nitrosobis(2-oxopropyl)amine-induced pancreatic cancer in hamsters, RC-160-II had a similar tumor growth inhibitory effect as RC-160 (42). On the basis of our receptor assay results, which indicate the presence of somatostatin receptors on different tumors, somatostatin analogs could also inhibit tumor cells directly. These results are in agreement with those previously reported from our and other groups, demonstrating low and high affinity binding sites for somatostatin and its analogs in normal tissues and in tumors (30, 41, 43). In the MIA PaCa-2 human pancreatic cancer cell line, somatostatin and its analog RC-160 reversed the stimulatory effect of epidermal growth factor on the phosphorylation of the tyrosine kinase portion of the epidermal growth factor receptor and on cell growth (44). These observations suggest that somatostatin analogs can act as endogenous growth inhibitors in cancer cells through the activation of tyrosine phosphatase (44). Another possible mechanism by which somatostatin analogs might inhibit tumor growth could be the interference with transmission of intracellular signals that regulate cell growth (3, 29) and with the synthesis of autocrine growth factors by tumor cells (35). Acetylated somatostatin analogs RC-160-II and RC-101-I showed powerful binding to various tumors. The presence of an acetyl group on the N-terminal of analog RC-160 seems to increase the binding to somatostatin receptors in human colon and pancreatic cancer. Analog RC-101-I showed the highest binding affinity to human breast cancer. RC-160-I demonstrated a higher binding affinity to rat cerebral cortex than did RC-160, but RC-160-II had only about half of the affinity of the nonacetylated analog. Present findings indicate that the binding affinities of various somatostatin analogs are different for diverse tumors. These observations support the concept of SS-14 receptor subpopulations (44, 45). It is possible that various somatostatin analogs could be used as selective agents for the treatment of tumors originating from different tissues.

The present results demonstrate a high effectiveness of our acetylated somatostatin analogs RC-101-I and RC-160-II in suppression of serum GH and gastrin levels in a rat model. A prolonged activity may be beneficial in clinical use of these analogs. In addition, our results demonstrate different binding affinities of these analogs to somatostatin receptors in various tumors, raising the intriguing possibility of using these analogs more selectively for the treatment of various neoplasms.

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