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Stimulation of tyrosine phosphatase and inhibition of cell proliferation by somatostatin analogues: Mediation by human somatostatin receptor subtypes SSTR1 and SSTR2

(somatostatin analogue RC-160/SMS-201-995/decreased cell growth/cancer)

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ABSTRACT **The effects of somatostatin analogues RC-160 and SMS-201-995 on tyrosine phosphatase and cell proliferation were investigated in COS-7 and NIH 3T3 cells expressing human somatostatin receptor subtype 1 or 2 (SSTR1 or SSTR2). Binding experiments were performed on membranes from COS-7 cells expressing human SSTR1 or SSTR2 using ^{125}I-labeled [Tyr^{11}]S-14 or [Tyr^{3}]SMS-201-995, respectively. The somatostatin analogues RC-160 and SMS-201-995 exhibited low affinity for SSTR1 (IC_{50} of 0.43 and 1.5 μM, respectively) and high affinity for SSTR2 (IC_{50} of 0.27 and 0.19 nM). Addition of these analogues to cells expressing either SSTR1 or SSTR2 did not result in an inhibition of adenylate cyclase activity. In SSTR2-expressing cells, both analogues induced a rapid stimulation of a tyrosine phosphatase activity (EC_{50}: RC-160, 2 pM; SMS-201-995, 6 pM) and an inhibition of serum-stimulated proliferation (EC_{50}: RC-160, 6.3 pM; SMS-201-995, 12 pM). In SSTR1-expressing cells, only RC-160 induced stimulation of a tyrosine phosphatase activity. Both analogues caused an inhibition of cell proliferation at a concentration higher than 10 nM in accordance with their affinities for the SSTR1 receptor subtype. A good correlation between the affinities of RC-160 and SMS-201-995 for each receptor subtype and their potencies to inhibit cell proliferation suggests the involvement of these receptors in cell growth regulation. Tyrosine phosphatase was stimulated by both these analogues in SSTR2 and by RC-160 in SSTR1 at affinities similar to their ability to inhibit growth and bind to receptors, implicating tyrosine phosphatase as a transducer of the growth inhibition signal. We also found that mRNAs of receptor subtypes were variably expressed in different pancreatic and colon cancer cell lines, indicating the necessity of a precise analysis of receptor subtypes in target tissues before therapy with analogues.**

Somatostatin is a tetradecapeptide that is widely distributed in the body. It participates in a variety of biological processes including neurotransmission, inhibition of hormonal secretion, and cell proliferation. This neuropeptide induces its biological effects by interacting with specific receptors that are coupled to a variety of signal transduction pathways including adenylate cyclase, ionic conductance channels, and protein dephosphorylation on serine/threonine and tyrosine residues (1, 2). Recently, five somatostatin receptor subtypes have been cloned (3–7). They belong to the guanine nucleotide-binding regulatory protein (G protein)-linked receptor family (3–7).

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The antiproliferative effect of somatostatin has been observed *in vitro* and *in vivo*, and this peptide may thus participate in the regulation of cell growth both in normal and tumoral cells (8). Somatostatin and its stable analogues may affect tumor growth *in vivo* by inhibiting the action of hormones and growth factors (8), although much evidence exists for directly mediated responses as we have previously shown in pancreatic cancer cells of human or rat origin (9–11). Analogues RC-121 (D-Phe-Cys-Tyr-D-Trp-Lys-Val-Cys-Thr-NH_2), RC-160 (D-Phe-Cys-Tyr-D-Trp-Lys-Val-Cys-Trp-NH_2), and SMS-201-995 [D-Phe-Cys-Phe-D-Trp-Lys-Thr-Cys-Thr(ol); hereafter referred to as SMS] antagonize the mitogenic effect of growth factors acting on tyrosine kinase receptors such as epidermal growth factor (EGF) and basic fibroblast growth factor (bFGF) (9–11). Furthermore, these analogues have been found to stimulate tyrosine phosphatase activity in normal and tumoral pancreatic cells (9, 12–15) and to activate the dephosphorylation of EGF receptor (9, 16). The ability of somatostatin analogues to stimulate tyrosine phosphatase correlates with their inhibitory effect on pancreatic cell growth, and this correlation supports the hypothesis that the growth inhibition is mediated by dephosphorylation of tyrosine protein signals. Somatostatin analogues might suppress tumor growth by reversing the stimulatory effect of EGF on phosphorylation of EGF receptor tyrosine kinase and EGF-phosphorylated proteins (17). We also observed that a membrane tyrosine phosphatase is coeluted with somatostatin receptor, suggesting that tyrosine phosphatase may be a part of the signal transduction pathway promoted by somatostatin receptor occupancy (16).

The somatostatin receptor subtypes and the molecular mechanism involved in the tyrosine phosphatase stimulation have been, until now, unknown (18, 19). We must better understand what physiological response every subtype elicits, how their signals are processed in the cell, and in what normal and/or pathological tissues each is expressed to choose the appropriate analogue for targeting to specific cells for therapeutic use.

In the present study, we examined the effects of two somatostatin analogues, RC-160 and SMS, on binding and signal transduction pathways of the two human cloned somatostatin receptor subtypes hSSTR1 and hSSTR2 transiently expressed in COS-7 cells. We also investigated the

Abbreviations: S-14, somatostatin-14; hSSTR1 and hSSTR2, human cloned somatostatin receptor subtypes 1 and 2; SMS, SMS-201-995; EGF, epidermal growth factor; bFGF, basic fibroblast growth factor; RT, reverse transcription; G protein, guanine nucleotide-binding protein; FCS, fetal calf serum; GTP[γ-S], guanosine 5′-[γ-thio]triphosphate.

long-term effect of the two analogues on the proliferation of NIH 3T3 cells stably expressing SSTR1 and SSTR2. In addition, we reported the distribution of these two receptor subtypes in various normal and tumoral cells.

MATERIALS AND METHODS

Peptides. Somatostatin analogue SMS was from Sandoz. The analogue RC-160 was synthesized and purified as described (20). Recombinant bFGF was purified using heparin-Sepharose chromatography (11).

Construction of Expression Vector and Transfections. The 1.38-kb *Eco*RI–*Nar* I and 1.35-kb *Sma* I–*Xba*I fragments, encompassing the open reading frames of the hSSTR1 and hSSTR2A cDNA, respectively (3), were subcloned into recombinant dicistronic mammalian expression vectors containing the simian virus 40 replication origin, the cytomegalovirus promoter, the encephalomyocarditis virus leader, and the Geneticin-resistance gene. The two resulting vectors, pRS1 and pRS2, were separately transiently expressed in monkey kidney COS-7 cells by the DEAE-dextran chloroquine method (21). For stable expression, mouse fibroblast NIH 3T3 cells were separately transfected with pRS1 and pRS2, using the calcium phosphate precipitation method (22). Stable transfectants were selected in Dulbecco's modified Eagle's medium (DMEM) containing Geneticin (G418; GIBCO) at 750 μg/ml. For each vector transfected, two clones expressing somatostatin receptor were selected for subsequent studies (pRS1: clones 1/11 and 1/18; pRS2: clones 2/5 and 2/8). NIH 3T3 cells were concomitantly transfected with a mock dicistronic vector devoid of SSTR cDNA (clone 712 was used as the control clone).

Binding Studies. [Tyr^{11}]Somatostatin-14 ([Tyr^{11}]S-14) and [Tyr^3]SMS were radioiodinated and purified by HPLC as described (23). COS-7 cells were grown in 10-cm diameter dishes for 48 hr after transfection (1.2×10^6 cells per dish), washed, and collected in 50 mM Tris (pH 7.8) containing soybean trypsin inhibitor at 0.3 mg/ml and 0.2 mM $CaCl_2$. The cells were then lysed and centrifuged at $600 \times g$ for 10 min at 4°C. The resultant supernatant was centrifuged at $26,000 \times g$ for 30 min at 4°C, and the pellet was resuspended in the same buffer and immediately analyzed for protein concentration using the Bradford protein assay (24). Binding for the Scatchard analysis was performed at 25°C for 90 min with ^{125}I-labeled [Tyr^{11}]S-14 (20 pM to 1 nM; specific activity of 1000 Ci/mmol; 1 Ci = 37 GBq) for SSTR1 receptor and ^{125}I-labeled [Tyr^3]SMS (20 pM to 1 nM; specific activity of 900 Ci/mmol) for SSTR2 receptor, in the above Tris buffer containing bovine serum albumin at 1 mg/ml and bacitracin at 0.5 mg/ml with 2–5 μg of membrane protein per assay according to published methods (23). Competitive inhibition experiments were carried out under the same conditions except that the incubates contained 30 pM ^{125}I-labeled [Tyr^{11}]S-14 (^{125}I-[Tyr^{11}]S-14) for the SSTR1 experiments or ^{125}I-labeled [Tyr^3]SMS (^{125}I-[Tyr^3]SMS) for the SSTR2 experiments and various concentrations of unlabeled analogues. Nonspecific binding was determined in the presence of 1 μM S-14 or SMS.

For clone selection, binding studies were performed using NIH 3T3 cells stably transfected with SSTR1 and SSTR2. Cells were cultured in 35-mm diameter dishes, in DMEM containing 10% fetal calf serum (FCS), until confluency (4×10^5 cells per dish). After DMEM was removed, cells were washed twice with cold Krebs/Hepes buffer (pH 7.4). Binding was performed at 25°C for 120 min in a final volume of 1.5 ml of Krebs/Hepes buffer (pH 7.4) containing bovine serum albumin at 15 mg/ml, soybean trypsin inhibitor at 0.3 mg/ml, bacitracin at 0.5 mg/ml, and 30 pM of ^{125}I-[Tyr^{11}]S-14 or ^{125}I-[Tyr^3]SMS (for hSSTR1 and hSSTR2 expressing cells, respectively). The cells were then washed and collected after a 10-min incubation in 0.1 M NaOH for determination of bound radioactivity.

Coupling of receptor subtypes with G protein was investigated by incubating cell membranes with 100 μM guanosine 5′-[γ-thio]triphosphate (GTP[γ-S]) or by treating cells for 16 hr with pertussis toxin (Sigma) at 50 ng/ml before the binding assay.

Adenylate Cyclase Assay. The conversion of [α-^{32}P]ATP into [^{32}P]AMP was determined according to the procedure of Salomon *et al.* (25). Assays were performed on COS-7 and NIH 3T3 cell crude membranes prepared as described (26), using 10 μg of protein per assay.

Tyrosine Phosphatase Assay. The substrate poly(Glu,Tyr) was phosphorylated with [γ-^{33}P]ATP (Amersham) as described (12, 14). COS-7 cells were grown in 10-cm-diameter dishes for 48 hr after transfection (1.2×10^6 cells per dish). Cell samples (in duplicate) were then washed with cold DMEM and incubated in medium containing the analogues for 15 min at 25°C in DMEM. Untreated cells were used as controls. Cells were collected and quickly lysed in liquid N_2 in a 50 mM Tris (pH 7.4) containing soybean trypsin inhibitor (0.3 mg/ml), 0.1 mM phenylmethylsulfonyl fluoride, and 50 mM sodium fluoride. After thawing and centrifugation at $500 \times g$ for 5 min, the homogenate was assayed for tyrosine phosphatase activity as described (12, 14), in a 100-μl reaction mixture containing 50 mM Tris (pH 7), bovine serum albumin at 1 mg/ml, 30,000 cpm of ^{33}P-labeled poly(Glu,Tyr), 1 μg of protein, 5 mM dithiothreitol, and 50 mM sodium fluoride. One unit of tyrosine phosphatase activity was defined as the amount of the enzyme that released 1 nmol of phosphate per min at 30°C from radiolabeled substrate.

Cell Growth Assay. NIH 3T3 transfected cells cultured in DMEM containing 10% FCS were plated in 35-mm-diameter dishes at 80×10^3 cells per ml (2 ml per dish). After an overnight attachment phase, the medium was changed to DMEM containing either 10% FCS or 1 nM bFGF with or without the analogues tested (added daily). Cell growth was measured after a 24- to 72-hr treatment by cell counting. Briefly, the culture medium was removed from the dish, and the cells were treated with 0.05% trypsin and 0.02% EDTA for 5 min at 25°C (1 ml per dish). A 100-μl aliquot of cell suspension was then taken and diluted to 20 ml with phosphate-buffered saline for cell counting, which was performed with an Coulter Counter model 2M (Coulter).

Reverse Transcription (RT)-PCR of SSTR1 and SSTR2. Total RNA was extracted by the modified procedure of Chomczynski and Sacchi (27). Cells or tissues were lyzed in RNAzol B (Bioprobe, Montreuil sous Bois, France) for 10 min at 4°C, and RNA was extracted as described (28). For RT, 1 μg of total RNA was first denatured at 94°C for 10 min and immediately chilled on ice. First-strand cDNA synthesis was then carried out for 10 min at 23°C followed by 2 hr at 39°C with total RNA in 50 mM Tris·HCl (pH 8.3) containing 200 units of Moloney murine leukemia virus reverse transcriptase (GIBCO), 26.5 μM oligo(dT), 1 mM dNTPs, 20 units of RNasin (Promega), 10 mM dithiothreitol, 75 mM KCl, and 3 mM $MgCl_2$ in a final volume of 20 μl. The RT mixture was then chilled on ice and diluted 2-fold with sterile water. PCR was then performed in a final volume of 50 μl containing 5 μl of reverse transcript total RNA, 2.5 units of *Taq* polymerase (Beckman), specific sense and antisense primers (1 μM for SSTR1 and SSTR2, 0.3 μM for β-actin), and 250 μM dNTPs in 10 mM Tris·HCl (pH 9) containing 1.5 mM $MgCl_2$, 50 mM KCl, and 0.01% Triton X-100. After denaturation of the sample at 94°C for 10 min, PCR was carried out on a DNA thermal cycler (Techne Laboratories, Princeton) for 35 cycles consisting of denaturation at 94°C for 1 min, annealing for 1 min (54°C for hSSTR1 and β-actin, 56°C for rat SSTR1, 62°C for hSSTR2 and rat SSTR2), and extension at 72°C for 1.5 min. The amplification was terminated by a final exten-

sion step at 72°C for 10 min. The following primers pairs were used: hSSTR1-specific oligonucleotide sense primer (5'-GCAACATGCTCATGCC-3') and antisense primer (5'-GCGTTATCCGTCCAGC-3'), hSSTR2A-specific sense primer (5'-ATGGACATGGCGGATGAGCCACT-3') and antisense primer (5'-TACTGGTTTGGAGGTCTCCATTGA-3'), rat SSTR1-specific sense primer (5'-GCAACATGCT-CATGCC-3') and antisense primer (5'-GCGTTATCCATC-CAGC-3'), and rat SSTR2A-specific sense primer (5'-GGGCGAATCCGGGGCA-3') and antisense primer (5'-GTTTGGAGGTCTCCATTG-3').

RESULTS

Functional Expression of hSSTR1 and hSSTR2. To determine the pharmacological properties of hSSTR1 and hSSTR2, we performed binding experiments with membranes prepared from COS-7 cells transiently expressing either receptor subtype. ^{125}I-[Tyr^{11}]S-14 specifically bound to hSSTR1-transfected COS-7 cell membranes. The Scatchard plot of the saturation curve revealed the presence of one class of high-affinity binding sites with a K_d of 0.1 ± 0.08 nM and a maximal binding capacity (B_{max}) of 1.05 ± 0.4 pmol/mg of protein from two experiments in duplicate (data not shown). Competitive inhibition experiments in the presence of unlabeled analogues revealed that the concentrations giving half maximal inhibition of binding (IC_{50}) were 0.5 ± 0.2 nM for S-14, 1.4 ± 0.3 nM for [Nle^8]somatostatin-28, 0.43 ± 0.07 μM for RC-160, and 1.5 ± 0.2 μM for SMS (Fig. 1A). As shown in Fig. 2, a high concentration of nonhydrolyzable GTP analogue GTP[γ-S] did not affect ^{125}I-[Tyr^{11}]S-14 binding to hSSTR1 receptors.

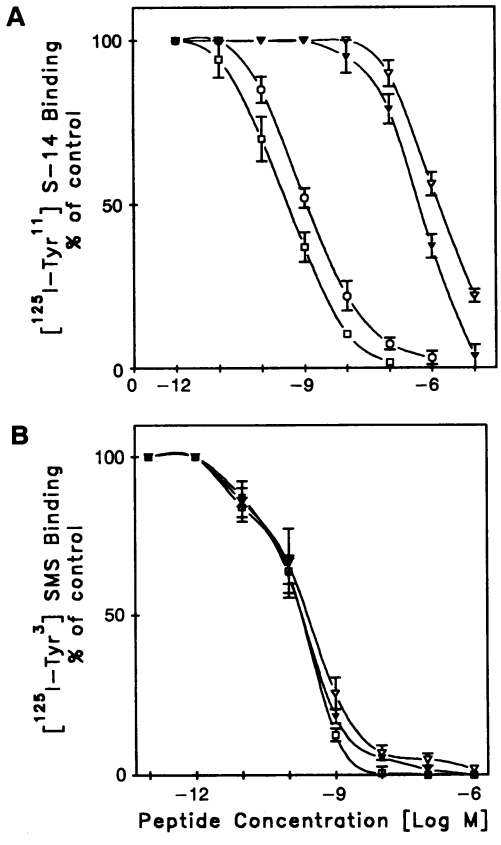

FIG. 1. (A) Concentration dependence of the inhibition of ^{125}I-[Tyr^{11}]S-14 binding by somatostatin analogues. Membranes from COS-7 cells transiently expressing hSSTR1 were incubated with ^{125}I-[Tyr^{11}]S-14 and the indicated concentrations of S-14 (□), [Nle^8]somatostatin-28 (○), RC-160 (▼), and SMS-201-995 (▽). (B) Similar experiment as in A with membranes from COS-7 cells transiently expressing hSSTR2 incubated with ^{125}I-[Tyr^3]SMS and the indicated concentrations of S-14 (□), SMS-201-995 (▽), and RC-160 (▼). Results are expressed as a percentage of the maximal specific binding observed in the absence of competitor and are the mean ± SEM of three different experiments performed in duplicate.

For binding studies on SSTR2, we used the more stable radioiodinated analogue [Tyr^3]SMS, previously shown useful for characterization of rat SSTR2 (4). ^{125}I-[Tyr^3]SMS specifically bound to hSSTR2-transfected COS-7 cell membranes. The Scatchard plot of the saturation curve revealed the presence of one class of high-affinity binding sites with a K_d of 57 ± 12 pM and a B_{max} of 1.1 ± 0.2 pmol/mg of protein, from two experiments in duplicate (data not shown). Displacement experiments in the presence of the unlabeled analogues revealed that the affinity of SMS to hSSTR2 (IC_{50} = 0.19 ± 0.06 nM) was similar to that of RC-160 (IC_{50} = 0.27 ± 0.12 nM) and S-14 (IC_{50} = 0.1 ± 0.05 nM) (Fig. 1B). Incubation of membranes with 100 μM GTP[γ-S] or pretreatment of cells with pertussis toxin inhibited the specific binding of ^{125}I-[Tyr^3]SMS to hSSTR2 by 95% and 70%, respectively (Fig. 2). In transfected NIH 3T3 cells, the analogues displayed similar affinities for both receptor subtypes as observed in transfected COS-7 cells (data not shown).

Adenylate Cyclase Activity. In NIH 3T3 cell membranes from clones expressing SSTR1 (clone 1/11) or SSTR2 (clone 2/8), adenylate cyclase basal activity (0.4 and 0.9 pmol per min per mg of protein, respectively) was found to be stimulated by 10 μM forskolin (52.6 and 62.5 pmol per min per mg of protein, respectively), 0.1 mM guanosine 5'-[β,γ-imido]triphosphate (10.4 and 20.9 pmol per min per mg of protein, respectively), and 0.1 mM isoproterenol (14.6 and 16.1 pmol per min per mg of protein, respectively). Under these conditions, no effect of S-14 or SMS (from 1 nM to 1 μM) was detected either on basal or stimulated adenylate cyclase activity in cells expressing either human SSTR receptor subtype (data not shown). Similar results were observed in COS-7 cells transiently expressing hSSTR2 (data not shown).

Stimulation of Tyrosine Phosphatase Activity by Somatostatin Analogues. Treatment of COS-7 cells transiently expressing hSSTR1 with somatostatin analogues revealed that RC-160 stimulated a tyrosine phosphatase activity in a time-

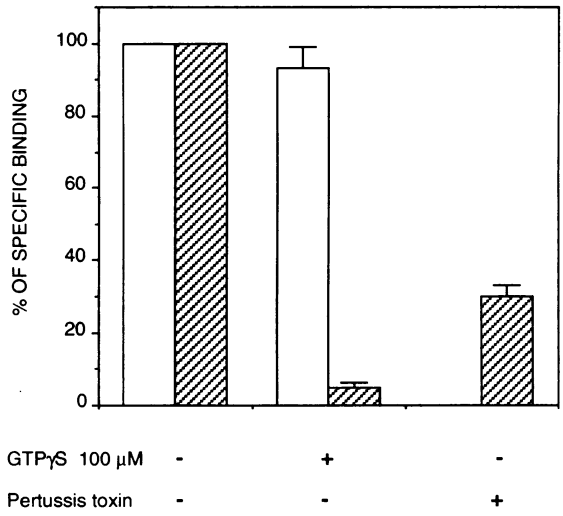

FIG. 2. Effect of GTP[γ-S] and pretreatment by pertussis toxin on the binding of ^{125}I-[Tyr^{11}]S-14 to hSSTR1 receptor (open bars) and ^{125}I-[Tyr^3]SMS to hSSTR2 receptor (hatched bars) transiently expressed in COS-7 cells. Total and nonspecific ^{125}I-[Tyr^{11}]S-14 binding in untreated membranes were 4800 and 641 cpm, respectively. Total and nonspecific ^{125}I-[Tyr^3]SMS binding in untreated membranes were 1342 and 28 cpm, respectively. Data are the mean ± SEM of two separate experiments performed in triplicate.

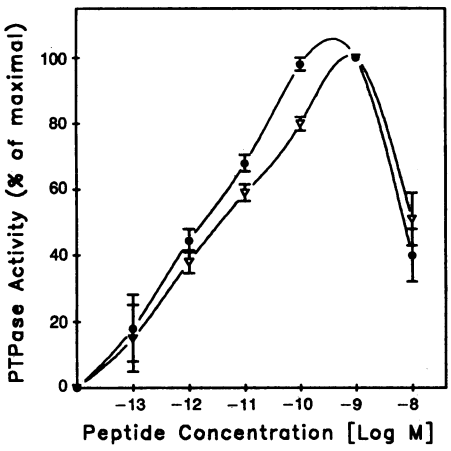

FIG. 3. Effects of somatostatin analogues on tyrosine phosphatase (PTPase) activity in COS-7 cells transiently expressing hSSTR2. Concentration-dependent RC-160 (●) and SMS-201-995 (▽) stimulation of tyrosine phosphatase activity are shown. Results are expressed as the percentage of maximal stimulation over basal value observed at 1 nM RC-160 and SMS (mean ± SEM of three separate experiments performed in triplicate).

and dose-dependent manner. This stimulation was maximal after 10 min and stable for 30 min of RC-160 treatment (data not shown). The basal value of tyrosine phosphatase activity averaged 0.16 ± 0.09 unit/mg of protein; treatment of cells with RC-160 at 0.1 and 1 μM for 15 min induced an increase in tyrosine phosphatase activity of 10% ± 3% and 28% ± 7% over basal value, respectively (mean ± SEM of three experiments performed in triplicate). At 0.1 and 1 μM SMS no stimulation of tyrosine phosphatase activity was found.

Incubation of COS-7 cells transiently expressing hSSTR2 with somatostatin analogues also induced an increase in tyrosine phosphatase activity in a time-dependent manner. Maximal stimulation was obtained after 15 min and was stable for 30 min (data not shown). Treatment of cells with analogues for 15 min induced a concentration-dependent stimulation of tyrosine phosphatase activity: the basal value averaged 0.44 ± 0.08 unit/mg of protein, and the maximal stimulation was observed with 1 nM RC-160 (44% ± 4% over basal value) and 1 nM SMS (56% ± 2% over basal value).

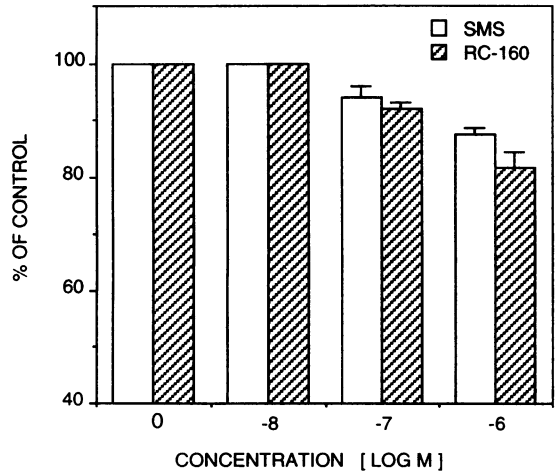

FIG. 4. Concentration-dependent inhibition of 1 nM bFGF-stimulated cell proliferation in NIH 3T3 cells stably expressing hSSTR1 (clone 1/11) by somatostatin analogues. Cells were untreated (bar 0) or treated with increasing concentrations of SMS-201-995 or RC-160 for 72 hr. Results are expressed as a percentage of the control value obtained with untreated cells (mean ± SEM of three separate experiments in quadruplicate).

Half-maximal stimulation was observed at 2 ± 1 pM for RC-160 and 6 ± 3 pM for SMS, respectively (Fig. 3). This stimulation was not sensitive to a 16-hr pretreatment of cells with pertussis toxin at 50 ng/ml (data not shown).

Inhibition of Cell Growth by Somatostatin Analogues. To study the effects of somatostatin analogues on growth of NIH 3T3 cells stably expressing hSSTR1 and hSSTR2, cell proliferation was induced by 10% serum or 1 nM bFGF. Under these conditions, RC-160 and SMS slightly inhibited for 72 hr the bFGF-induced cell proliferation of NIH 3T3 cells stably expressing hSSTR1 (Fig. 4). Similar results were obtained with two different clones (clones 1/11 and 1/18). This inhibition was observed only at 0.1 and 1 μM concentration of analogues, and RC-160 was more efficacious than SMS. These analogues had no effect on serum-induced cell proliferation regardless of the time of treatment (data not shown). Both RC-160 and SMS inhibited serum-induced proliferation of NIH 3T3 cells stably expressing hSSTR2. The effect was maximal after a 24-hr treatment, remained significant for 2 days (data not shown), and was dose dependent. Maximal inhibition was observed at 1 nM; half-maximal inhibition was produced by 6.3 ± 2.2 pM RC-160 and 12 ± 2 pM SMS, respectively (Fig. 5). Similar results were obtained in two different clones stably expressing hSSTR2 (clone 2/5 and 2/8). Similar results were also observed in bFGF-stimulated NIH 3T3 SSTR2-expressing cells (data not shown). The two somatostatin analogues had no effect on NIH 3T3-712 control clone cell proliferation (data not shown).

Expression of mRNA Encoding hSSTR1 and hSSTR2 in Pancreatic and Colon Cancer Cell Lines and Human Tissues. To compare the distinct expression of the two somatostatin receptor subtypes, we performed RT-PCR experiments. As shown in Fig. 6, SSTR1 mRNA was expressed in pancreatic cancer cell lines BxPC-3, CAPAN-1, CAPAN-2, Mia Paca-2, and AR4-2J and in normal human adult pancreas. SSTR1 mRNA was also expressed in the human colon carcinoma cell line HT-29 and weakly in normal adult human colon. The SSTR2 mRNA was clearly detected in pancreatic cancer cell lines PANC-1 and AR4-2J. A weak expression was also found in normal adult pancreas and in two other human pancreatic cancer cell lines, AsPC-1 and Mia Paca-2. No expression was found in normal human adult colon and in a colon cancer cell line.

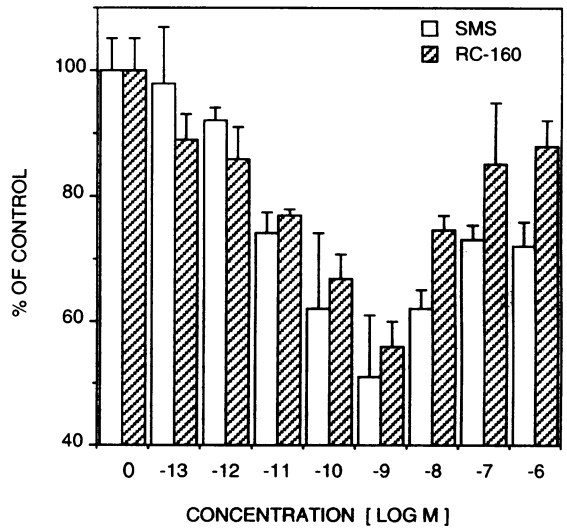

FIG. 5. Concentration-dependent inhibition by somatostatin analogues of FCS-stimulated cell proliferation in NIH 3T3 cells stably expressing hSSTR2 (clone 2/5). Cells were untreated (bar 0) or treated with increasing concentrations of SMS-201-995 or RC-160 for 24 hr. Results are expressed as a percentage of the control value obtained with untreated cells (mean ± SEM of three separate experiments performed in quadruplicate).

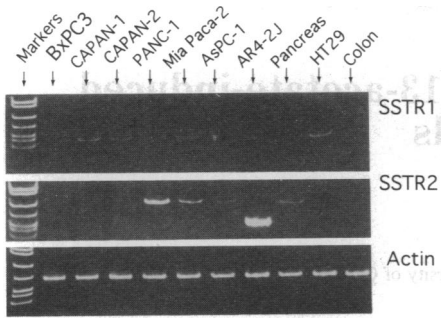

FIG. 6. RT-PCR analysis of SSTR1 and SSTR2 gene expression performed with primers specific for hSSTR1, rat SSTR1, hSSTR2, and rat SSTR2 and using 1 μg of total RNA from human (BxPC-3, CAPAN-1, CAPAN-2, PANC-1, Mia PaCa-2, AsPC-1) and rat (AR4-2J) pancreatic cancer cell lines, human normal adult pancreas, human colon cancer cell line HT29, and human normal adult colon. PCR products were separated by 7.5% PAGE and stained with ethidium bromide. The expected lengths of PCR products were 415 bp (hSSTR1 and rat SSTR1), 1105 bp (hSSTR2), 507 bp (rat SSTR2), and 517 bp (β-actin) (markers are pGEM markers from Promega).

DISCUSSION

The expression of hSSTR1 and hSSTR2 somatostatin receptor subtypes in COS-7 and NIH 3T3 cells allowed us to demonstrate two important functions linked to these receptors: stimulation of tyrosine phosphatase activity and inhibition of cell proliferation. The functional properties of hSSTR receptors transiently expressed in COS-7 cells were in agreement with previous studies performed with mouse and rat SSTR1 and SSTR2 (3, 18, 19). hSSTR1 bound S-14 with high affinity but had low affinity for structurally stable analogues; RC-160 was 3 times more potent than SMS. hSSTR2 exhibited a high affinity for S-14 and the two analogues and was coupled to a pertussis toxin-sensitive G protein while SSTR1 was not.

As reported for hSSTR1 and mouse SSTR2 receptors expressed in COS-1 and CHO-DG44 cells (3, 18, 19), these two receptors, expressed in COS-7 and NIH 3T3 cells, were not coupled to adenylate cyclase. These results differ from those recently observed in CHO-K1 cells expressing rat SSTR2 where somatostatin has been shown to inhibit cAMP accumulation (29). The expression of different G proteins and/or different relative amount of G proteins in transfected cell types could explain these discrepancies observed with SSTR2.

We demonstrated the biological effect mediated by hSSTR1 and hSSTR2 receptor subtypes—that is, a rapid stimulation of tyrosine phosphatase activity. The ability of somatostatin analogues to stimulate the enzymatic activity is well correlated with their affinity for receptor occupancy: in SSTR1-transfected cells RC-160 stimulated tyrosine phosphatase activity at high concentrations, whereas SMS, which exhibited a lower affinity for the receptor, had no effect. The high affinity of somatostatin analogues for hSSTR2 allowed a potent stimulation of tyrosine phosphatase activity at concentrations similar to those necessary to occupy the receptor.

In NIH 3T3 cells expressing hSSTR1 and hSSTR2, the analogues RC-160 and SMS inhibited stimulated cell proliferation. This effect occurred at concentrations related to the affinities for the receptors.

We found a good correlation between the ability of the analogues to inhibit radioligand binding, to stimulate tyrosine phosphatase activity, and to inhibit cell proliferation. This represents an additional argument for the involvement of a membrane tyrosine phosphatase in the signal transduction pathway and in the negative growth signal promoted by the somatostatin receptor (10, 12, 13).

RT-PCR analysis revealed that SSTR1 and SSTR2 are variably expressed in pancreatic and colon cancer cell lines. The high expression of SSTR2 observed in AR4-2J cells explains our previous findings on the effect of SMS on both stimulation of tyrosine phosphatase activity and cell proliferation (10, 11, 12, 16). In Mia Paca-2 cells, the two receptor subtypes are poorly expressed. This fact may suggest that another receptor subtype, more selective for RC-160 than that we observed herein for hSSTR1, may be better expressed in this cell line (9, 13).

SSTR1 and SSTR2, which mediate the antiproliferative effect of somatostatin, are unequally expressed in various cancer cell lines. The determination of receptor subtypes expressed in distinct tumors may allow a better selection and targeting of somatostatin analogues for therapy. The development of new analogues with still higher affinity and greater selectivity for various somatostatin receptor subtypes could improve their antitumoral properties.

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