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Inhibition of cell proliferation by the somatostatin analogue RC-160 is mediated by somatostatin receptor subtypes SSTR2 and SSTR5 through different mechanisms

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ABSTRACT **Effects of the stable somatostatin analogue RC-160 on cell proliferation, tyrosine phosphatase activity, and intracellular calcium concentration were investigated in CHO cells expressing the five somatostatin receptor subtypes SSTR1 to -5. Binding experiments were performed on crude membranes by using [^{125}I-labeled Tyr^{11}]somatostatin-14; RC-160 exhibited moderate-to-high affinities for SSTR2, -3, and -5 (IC_{50}, 0.17, 0.1, and 21 nM, respectively) and low affinity for SSTR1 and -4 (IC_{50}, 200 and 620 nM, respectively). Cell proliferation was induced in CHO cells by 10% (vol/vol) fetal calf serum, 1 μM insulin, or 0.1 μM cholecystokinin (CCK)-8; RC-160 inhibited serum-induced proliferation of CHO cells expressing SSTR2 and SSTR5 (EC_{50}, 53 and 150 pM, respectively) but had no effect on growth of cells expressing SSTR1, -3, or -4. In SSTR2-expressing cells, orthovanadate suppressed the growth inhibitory effect of RC-160. This analogue inhibited insulin-induced proliferation and rapidly stimulated the activity of a tyrosine phosphatase in only this cellular clone. This latter effect was observed at doses of RC-160 (EC_{50}, 4.6 pM) similar to those required to inhibit growth (EC_{50}, 53 pM) and binding to the receptor (IC_{50}, 170 pM), implicating tyrosine phosphatase as a transducer of the growth inhibition signal in SSTR2-expressing cells. In SSTR5-expressing cells, the phosphatase pathway was not involved in the inhibitory effect of RC-160 on cell growth, since this action was not influenced by tyrosine and serine/threonine phosphatase inhibitors. In addition, in SSTR5-expressing cells, RC-160 inhibited CCK-stimulated intracellular calcium mobilization at doses (EC_{50}, 0.35 nM) similar to those necessary to inhibit somatostatin-14 binding (IC_{50}, 21 nM) and CCK-induced cell proliferation (EC_{50}, 1.1 nM). This suggests that the inositol phospholipid/calcium pathway could be involved in the antiproliferative effect of RC-160 mediated by SSTR5 in these cells. RC-160 had no effect on the basal or carbachol-stimulated calcium concentration in cells expressing SSTR1 to -4. Thus, we conclude that SSTR2 and SSTR5 bind RC-160 with high affinity and mediate the RC-160-induced inhibition of cell growth by distinct mechanisms.**

Somatostatin is a tetradecapeptide with diverse biological effects on cellular function, including inhibition of secretory and proliferative processes (1, 2). These effects are mediated by specific receptors that belong to the guanine nucleotide binding protein (G-protein)-linked receptor family (3–7).

Five somatostatin receptor subtypes SSTR1 to -5 and one splice variant have been cloned from human (h), murine (m), and rat (r) sources (3–7). After expression of SSTR gene clones in mammalian cell lines, distinct profiles for binding somatostatin analogues and functional coupling to adenylate cyclase via a pertussis-toxin-sensitive G proteins have been observed for these receptors. However, this coupling seems to depend on the amount and the species of G-protein subunits expressed in the cell types examined (8).

mRNAs for the five receptor subtypes are expressed in a tissue- and species-specific manner. hSSTR1 to -4 mRNAs are variably expressed in tumors or cancer cell lines from central nervous system, colon, liver, pancreas, lung, breast, and skin (3, 9–12). Among them, SSTR2 seems to be the most frequently represented especially in central nervous system tumors, pituitary adenoma, carcinoid tumors, and islet cell carcinomas (10–12). The more recently cloned hSSTR5 appeared to be predominantly expressed in the anterior pituitary but was also present in stomach, jejunum, colon, pancreas, heart, and smooth muscle (7).

A number of somatostatin anologues have been used for the treatment of acromegaly and endocrine tumors of the gastroenteropancreatic system (such as carcinoid tumors, insulinomas, glucagonomas, gastrinomas, and VIPomas) (13). Experimental studies in animal models of mammary, prostatic, gastric, and pancreatic carcinomas suggest that somatostatin analogues, including RC-160 (D-Phe-Cys-Tyr-D-Trp-Lys-Val-Cys-Thr-NH_2), might inhibit the growth of these malignancies (13–15). We have demonstrated (10) that hSSTR1 and -2 transfected into COS-7 and NIH 3T3 cells mediate the stimulation of a membrane tyrosine phosphatase activity induced by the stable analogues RC-160 and SMS 201-995 [D-Phe-Cys-Phe-D-Trp-Lys-Thr-Cys-Thr(ol)]. Moreover, tyrosine phosphatase was stimulated at doses of analogues similar to those required to inhibit growth and binding to the receptors, implicating this enzyme as a transducer of the direct growth inhibition signal mediated by SSTR1 and SSTR2 (10).

Because SSTRs are differentially expressed in various tissues and are pharmacologically distinct, it is necessary to establish whether SSTR3, -4, and -5 are also implicated in the regulation of cell proliferation. In the present study, the five receptor subtypes stably expressed in CHO cells were characterized with respect to the mediation of growth inhibition, stimulation of tyrosine phosphatase activity, and modulation of intracellular calcium concentration ($[Ca^{2+}]_i$). We report evidence that only SSTR2 and -5 mediate the inhibition of cell proliferation induced by RC-160 and that these two receptor subtypes are coupled to distinct signal transduction pathways. The activation of a tyrosine phosphatase and the inhibition of calcium mobilization mediated, respectively, by SSTR2 and

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Abbreviations: S-14, somatostatin-14; SSTR1 to -5, somatostatin receptor subtypes 1 to 5; FCS, fetal calf serum; $[Ca^{2+}]_i$, intracellular Ca^{2+} concentration; CCK, cholecystokinin; G protein, guanine nucleotide binding protein; h, human; m, murine; r, rat; SHPTP1, sarc homology 2-containing tyrosine phosphatase 1.

SSTR5 could be important steps in the negative mitogenic signal induced by somatostatin.

MATERIALS AND METHODS

Materials. RC-160 was synthesized and purified as described (16). Somatostatin-14 (S-14) was a gift from L. Moroder (Max Planck Institute, Munich). Insulin was from Novo-Nordisk (Boulogne-Billancourt, France). The cholecystokinin (CCK) analogue CCK-8 was from Bachem. Pertussis toxin, 8-bromo-cAMP, soybean trypsin inhibitor, thrombin, okadaic acid, orthovanadate, and carbachol were from Sigma.

Cell Culture. hSSTR1, mSSTR2A, mSSTR3, mSSTR3 with G protein $G_{i\alpha1}$, hSSTR4, hSSTR5, and rSSTR5 were stably expressed in CHO cells (CHO-DG44 strain for SSTR1 to -4 and CHO-K1 strain for SSTR5) as described (4, 5, 7, 17–19). Cells were cultured in α-minimal essential medium (αMEM; GIBCO) containing 10% (vol/vol) fetal calf serum (FCS) and G418 (200 μg/ml) (GIBCO).

Binding Studies. [Tyr^{11}]S-14 was radioiodinated and purified by HPLC as described (20). CHO cells were grown in 75-cm^2 flasks for 48 h, washed twice with 20 mM sodium phosphate (pH 7.8), scraped off, and collected in 50 mM Tris·HCl (pH 7.8) containing soybean trypsin inhibitor (0.3 mg/ml). The cells were then lysed by freezing in liquid nitrogen in the same buffer for 10 min. After thawing, the lysate was centrifuged at 26,000 × *g* for 30 min at 4°C, and the pellet was resuspended in the same buffer and immediately analyzed for protein concentration by the Bradford method (21). Binding studies on the resultant crude membranes were as described (10). Briefly, in ligand competition experiments, 10–20 μg of membrane protein was incubated with 30 pM [^{125}I-labeled Tyr^{11}]S-14 at 25°C for 90 min in 50 mM Tris·HCl (pH 7.8) containing bovine serum albumin (1 mg/ml), soybean trypsin inhibitor (0.3 mg/ml), bacitracin (0.5 mg/ml), 5 mM Mg^{2+}, and unlabeled peptide analogue. Nonspecific binding was determined in the presence of 1 μM S-14.

Cell Growth Assay. CHO cells were cultured in αMEM containing 10% FCS and plated in 35-mm dishes at 55 × 10^3 cells per ml (2 ml per dish). After overnight attachment, the medium was changed to αMEM containing either 10% FCS or insulin with and without RC-160. Cell growth was measured after 24 h by counting cells with a Coulter counter model ZM as described (10).

Tyrosine Phosphatase Assay. The substrate poly(Glu, Tyr) was phosphorylated with [γ-^{33}P]ATP (Amersham) as described (22). After the CHO cells were grown in 75-cm^2 flasks for 48 h, the cells were washed with ice-cold αMEM, scraped off, and resuspended in αMEM containing bovine serum albumin (1 mg/ml) and soybean trypsin inhibitor (0.3 mg/ml). Cells were incubated in the same medium with RC-160 or S-14 for 15 min at 25°C (10^6 cells per assay). Untreated cells were used as controls. After centrifugation at 10,000 × *g* for 30 sec, αMEM was removed and cells were quickly lysed in liquid nitrogen in 50 mM Tris·HCl (pH 7.4) containing soybean trypsin inhibitor (0.3 mg/ml) and 0.1 mM phenylmethylsulfonyl fluoride. After thawing, the homogenate was assayed for the tyrosine phosphatase activity in 100 μl containing 50 mM Tris·HCl (pH 7.0), bovine serum albumin (1 mg/ml), 30,000 cpm of ^{33}P-labeled poly(Glu, Tyr), 1 μg of protein, and 5 mM dithiothreitol as described (10, 22). 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.

$[Ca^{2+}]_i$ Measurement. CHO cells were cultured on sterile glass coverslips in 10-cm diameter dishes for 48 h in αMEM containing 10% FCS, and $[Ca^{2+}]_i$ was measured as described (23) by using fura-2/AM (Molecular Probes) (14 μM for 1 h at 37°C). In all studies, the fluorescence ratio was converted to $[Ca^{2+}]_i$ by using the equation of Grynkiewicz *et al.* (24) (R_{min}, 0.78; R_{max}, 29.6; F_o/F_s, 13.2; K_d, 225 nM).

RESULTS

Functional Expression of SSTR1 to -5. In Table 1, the potencies of S-14 and RC-160 in the inhibition of [^{125}I-labeled Tyr^{11}]S-14 binding to CHO cells expressing SSTR1 to -5 are compared. Ligand competition analysis showed that all five expressed SSTRs bound S-14 with high affinity (IC_{50}, 0.13–2.3 nM) and in the following order of potency: SSTR2 > SSTR3 > SSTR1 > SSTR5 > SSTR4. RC-160 displayed high affinity for SSTR2, -3, and -5 and low affinity for SSTR1 and -4. The rank of potency was as follows: SSTR3 > SSTR2 > SSTR5 >> SSTR1 > SSTR4. The affinity of RC-160 was higher for rSSTR5 than for hSSTR5 with IC_{50} values of 0.2 and 21 nM, respectively.

Inhibition of Cell Growth by RC-160. To study the effect of RC-160 on growth of CHO cells stably expressing SSTR1 to -5, cell proliferation was induced by serum or by mitogenic peptides acting through tyrosine kinase (insulin) or the phosphatidylinositol pathway (CCK), known to be involved in growth stimulatory signals in CHO cells (25, 26). RC-160 inhibited serum-induced proliferation of CHO cells stably expressing SSTR2, rSSTR5, and hSSTR5 (Fig. 1). The effect was dose-dependent; half-maximal inhibition was produced by 53 ± 26 pM, 51 ± 40 pM, and 150 ± 70 pM RC-160 for SSTR2-, rSSTR5-, and hSSTR5-expressing cells, respectively. Regardless of the concentration used, RC-160 had no effect on SSTR1-, SSTR3-, and SSTR4-expressing cells (data from three experiments in triplicate, not shown). Since pertussis toxin alone inhibited FCS-induced cell proliferation of CHO cells (data not shown), we could not evaluate the effect of this toxin on the inhibitory action of RC-160 on SSTR2- and SSTR5-expressing cells. When cell growth was stimulated by 1 μM insulin, 1 nM RC-160 inhibited cell proliferation only in the SSTR2-expressing cells (Fig. 2) and had no effect on cells expressing SSTR1, -3, -4, or -5.

In SSTR5-expressing CHO-K1 cells, cell proliferation could be induced by 0.1 μM CCK-8. In these conditions, RC-160 inhibited CCK-induced cell proliferation in a concentration-dependent manner from 10^{-11} to 10^{-7} M (EC_{50}, 1.1 ± 0.9 nM) (Fig. 3). In CHO-DG44 cells expressing SSTR2, neither carbachol (0.1 mM) nor CCK-8 (0.1 μM) induced cell proliferation (data not shown).

Mechanisms Involved in SSTR2- and SSTR5-Mediated Inhibition of Cell Growth. To further characterize the mechanisms by which SSTR2 and -5 mediated the inhibition of cell proliferation, we investigated cellular effector pathways potentially modulated by SSTRs that might be involved in the antimitogenic effect of somatostatin (1, 2, 10, 13).

Role of cAMP. It is unlikely that the inhibition of cAMP formation was implicated in the antimitogenic effect of RC-160. The inhibition of cell proliferation by RC-160 in SSTR2-expressing cells was observed in spite of the absence of cyclase coupling in this system (17–19). In CHO cells coexpressing

Table 1. Inhibition of the binding of [^{125}I-labeled Tyr^{11}]S-14 to cloned SSTR1 to -5

	IC_{50}, nM	
Receptor	S-14	RC-160
hSSTR1	0.57 ± 0.4	200 ± 40
mSSTR2A	0.13 ± 0.02	0.17 ± 0.07
mSSTR3	0.2 ± 0.07	0.1 ± 0.01
hSSTR4	2.3 ± 0.7	620 ± 37
rSSTR5	0.6 ± 0.2	0.2 ± 0.1
hSSTR5	0.9 ± 0.1	21 ± 1

Results are mean ± SEM of two experiments done in duplicate.

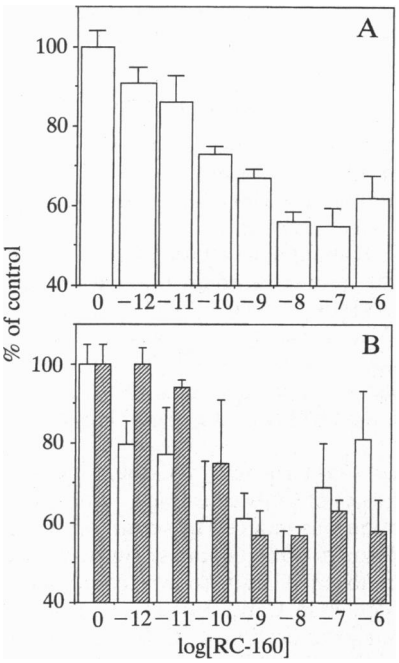

FIG. 1. Concentration-dependent inhibition by RC-160 of FCS-stimulated cell proliferation in CHO cells stably expressing SSTR2 and SSTR5. Cell proliferation was induced by 10% FCS and CHO cells were treated with RC-160 as indicated for 24 h. (*A*) mSSTR2-expressing cells. (*B*) rSSTR5 (open bars) and hSSTR5 (hatched bars). Results are expressed as the percentage of control values obtained with untreated cells. In these conditions, 10% FCS induced a stimulation of 91 ± 7.6% in mSSTR2-expressing cells, 73 ± 10% in rSSTR5-expressing cells, and 64 ±3% in hSSTR5-expressing cells above basal values observed for cells grown in FCS-free medium (mean ± SEM of three experiments done in quadruplicate).

SSTR3 and Giα1, SSTR3 is coupled to adenylate cyclase (18), but RC-160 had no effect on cell proliferation (data not shown). r- and hSSTR5s mediate the inhibition of cAMP production by S-14 and RC-160 in CHO-K1 cells (ref. 5 and unpublished results). However, we observed that 8-bromo-cAMP (1 mM) inhibited proliferation of CHO-K1 cells expressing SSTR5 (data not shown) as described in wild-type CHO-K1 cells (27). These results preclude the involvement of cAMP in SSTR5-mediated inhibition of proliferation in this cell system.

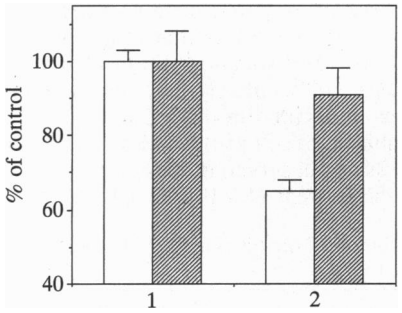

FIG. 2. Effect of RC-160 on insulin-stimulated cell proliferation in CHO cells stably expressing SSTR2 or SSTR5. Cell proliferation was induced by 1 μM insulin in CHO cells expressing mSSTR2 (open bars) or rSSTR5 (hatched bars), and cells were treated with 1 nM RC-160 (bars 2) for 24 h or not treated (bars 1). Results are expressed as the percentage of control value obtained with untreated cells. Under these conditions, 1 μM insulin stimulated cell proliferation by 32 ± 1% in SSTR2-expressing cells and 29 ± 5% in SSTR5-expressing cells, above basal values observed for cells grown in FCS-free medium (mean ± SEM of three experiments done in triplicate).

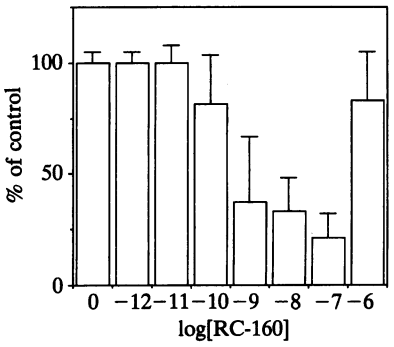

FIG. 3. Concentration-dependent inhibition by RC-160 of CCK-stimulated cell proliferation in CHO cells stably expressing SSTR5. Cell proliferation was induced with 0.1 μM CCK-8 and CHO cells expressing hSSTR5 were treated with RC-160 as indicated for 24 h. Results are the percentage of the control value obtained with untreated cells. Under these conditions, 0.1 μM CCK-8 stimulated cell proliferation by 35 ± 8%, above basal value observed for cells grown in FCS-free medium (mean ± SEM of three experiments done in quadruplicate).

Role of protein phosphatases. To elucidate the role of tyrosine phosphatase stimulation in the RC-160-induced inhibition of cell growth, we first investigated the effect of this analogue on tyrosine phosphatase activity. In CHO cells stably expressing SSTR2, RC-160 stimulated tyrosine phosphatase activity (Fig. 4), as we observed in NIH 3T3 and COS-7 cells transfected with hSSTR2 (10). This stimulatory effect was maximal at 0.1–1 nM; half-maximal stimulation was observed at 4.6 ± 2.7 pM RC-160. This stimulation was totally suppressed by pretreatment of cells with pertussis toxin (100 ng/ml) for 18 h (data from three experiments, not shown), indicating that a pertussis-toxin-sensitive G protein is involved in this effect. Pertussis toxin had no effect on basal tyrosine phosphatase activity.

In contrast, RC-160 had no effect on tyrosine phosphatase activity of cells expressing SSTR3 to -5 (data from three experiments performed in triplicate for each clone, not shown). In SSTR1-expressing cells, RC-160 increased the tyrosine phosphatase activity by 30 ± 7% but only at 1 μM (data not shown).

The good correlation in the potency of RC-160 to inhibit both binding and cell proliferation and to stimulate tyrosine phosphatase activity suggests that this enzyme is involved in the growth inhibition mediated by SSTR2. This hypothesis is strengthened by the observation that orthovanadate (1 μM), a

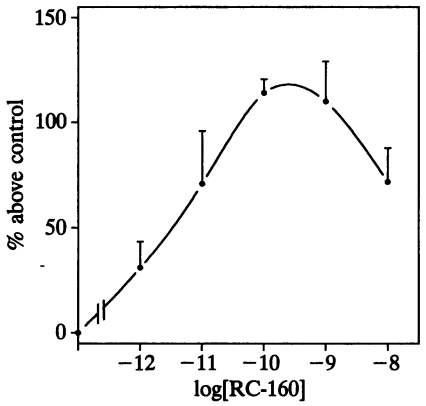

FIG. 4. Effects of RC-160 on tyrosine phosphatase activity in CHO cells stably expressing SSTR2. Concentration-dependent stimulation of tyrosine phosphatase activity by RC-160 is shown. Results are the percentage above basal values, which averaged 0.32 ± 0.05 unit/mg of protein (mean ± SEM of three experiments done in triplicate).

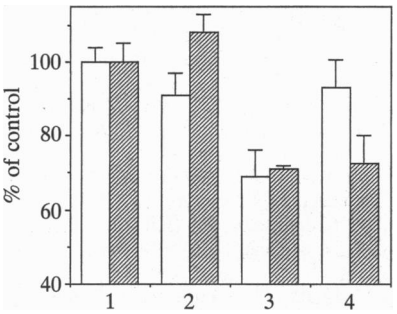

FIG. 5. Effect of sodium orthovanadate on RC-160-induced inhibition of cell proliferation in CHO cells stably expressing SSTR2 and SSTR5. Cell proliferation was induced by 10% FCS and CHO cells expressing mSSTR2 (open bars) or rSSTR5 (hatched bars) were treated (bars 3 and 4) or not (bars 1 and 2) with 1 nM RC-160 for 24 h in the presence (bars 2 and 4) and absence (bars 1 and 3) of 1 μM orthovanadate. Results are the percentage of control value obtained with untreated cells (mean ± SEM of three experiments done in triplicate).

potent inhibitor of tyrosine phosphatases, totally suppressed both the stimulatory effect of RC-160 on tyrosine phosphatase activity (data not shown) and the inhibitory effect of RC-160 on FCS-induced cell growth in SSTR2-expressing cells (Fig. 5).

In contrast, orthovanadate and okadaic acid (10 nM), the inhibitor of serine/threonine phosphatases (28), had no effect on the RC-160-induced inhibition of serum-induced proliferation of cells expressing SSTR5 (data not shown and Fig. 5).

Role of $[Ca^{2+}]_i$. In pituitary cells, somatostatin decreases basal $[Ca^{2+}]_i$ by modulating K^+ currents and inhibiting voltage-dependent calcium channels (1, 2, 29). In cells expressing SSTR1 to -5, RC-160 at 0.1 nM to 1 μM had no effect on basal $[Ca^{2+}]_i$. Moreover, a 50 mM K^+ depolarizing solution had no effect on $[Ca^{2+}]_i$ in all cellular clones, indicating the lack of voltage-dependent Ca^{2+} channels in these cells.

We then studied the effect of RC-160 on the inhibition of inositol phospholipid/Ca^{2+} pathway, as shown with S-14 in rat pancreas (30) and clonal hamster β cells (31). To increase $[Ca^{2+}]_i$, CCK-8 and carbachol, respectively, were used in CHO-K1 and CHO-DG44 cells. In hSSTR5-expressing CHO-K1 cells, CCK-8 (0.1 μM) induced a rapid and steep increase in $[Ca^{2+}]_i$ (+94 ± 5.4% over basal $[Ca^{2+}]_i$, which averaged 114.4 ± 9.8 nM; mean ± SEM of 15 determinations; Fig. 6*A*). In these conditions, RC-160 inhibited the CCK-induced increase in $[Ca^{2+}]_i$ in a dose-dependent manner. Maximal inhibition was observed at 0.1 μM RC-160; half-maximal inhibition was observed at 0.35 ± 0.05 nM RC-160 (Fig. 6*B*). Pretreatment of cells with pertussis toxin (100 ng/ml) for 18 h had no effect on basal $[Ca^{2+}]_i$ or on CCK-induced stimulation of $[Ca^{2+}]_i$ but suppressed the inhibitory effect of 0.1 μM RC-160 by 48 ± 1% (data from two experiments, not shown).

In CHO-DG44 cells expressing SSTR1 to -4, neither CCK (0.1 μM) nor thrombin (1 unit/ml) induced the mobilization of intracellular calcium, but carbachol (0.1 mM) induced a rapid and transient increase of $[Ca^{2+}]_i$ (+60 ± 9.2% over basal $[Ca^{2+}]_i$, which averaged 202 ± 28 nM; mean ± SEM of 15 determinations; data not shown). RC-160 did not modify the effect of carbachol on $[Ca^{2+}]_i$ in cells expressing SSTR1 to -4 (data not shown).

DISCUSSION

The expression of the five SSTRs in CHO cells permitted us to demonstrate that SSTR2 and SSTR5 mediate the inhibition of cell proliferation induced by the stable analogue RC-160. The functional studies of the five SSTRs showed that this analogue had a high affinity for SSTR2, -3, and -5. As previously observed for the other stable analogues SMS 201-995 and BIM 23014, the affinity of RC-160 for hSSTR5 was 100-fold lower than that observed for rSSTR5. This species variation in pharmacological properties might be explained by divergences in predicted amino acid sequences in the extracellular N-terminal domains but this view remains to be confirmed.

As demonstrated with hSSTR2 in COS-7 and NIH 3T3 cells (10), mSSTR2 expressed in CHO-DG44 cells mediated the RC-160-induced inhibition of cell proliferation stimulated by serum and insulin. This receptor is not coupled to adenylate cyclase when expressed in this cell line (17), but it mediates the stimulation of a tyrosine phosphatase activity involving a pertussis-toxin-sensitive G protein. This activation occurred at concentrations of analogue similar to those necessary to occupy the receptor and to inhibit cell proliferation. The fact that the RC-160-mediated inhibition of cell proliferation and activation of tyrosine phosphatase were blocked by orthovanadate, the inhibitor of tyrosine phosphatases, suggests that tyrosine phosphatase plays a role in somatostatin-mediated growth inhibition. We have found (32) that in rat pancreatic acinar membranes expressing SSTR2, SHPTP1 (sarc homology 2-containing protein tyrosine phosphatase 1), or a SHPTP1-related tyrosine phosphatase copurify with the somatostatin receptor and that this enzyme is associated with the somatostatin receptor at the membrane level. Recent studies on cells overexpressing tyrosine phosphatases suggest that these enzymes act as counterregulators of mitogenic tyrosine kinases (33). Furthermore, somatostatin can reverse the stimulatory effect of the epidermal growth factor on the phosphorylation of epidermal growth factor receptor and endogenous proteins (34). Thus, a possible role for SHPTP1 or a SHPTP1-related tyrosine phosphatase in an early signal transduction pathway mediated by SSTR2 and involved in the antiproliferative effect of somatostatin and its analogues is suggested.

We also demonstrated that RC-160 inhibited proliferation of cells expressing SSTR5. The fact that exogenous cAMP inhibited growth of these cells rules out the inhibition of

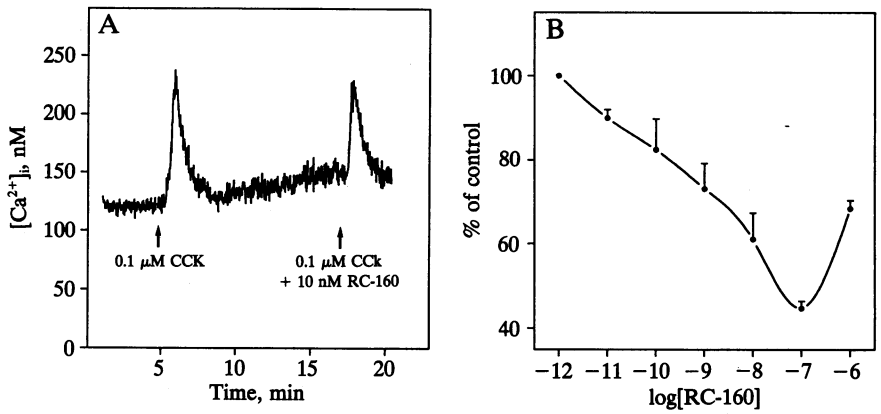

FIG. 6. Effect of RC-160 on $[Ca^{2+}]_i$ in SSTR5-expressing cells. (*A*) $[Ca^{2+}]_i$ changes in hSSTR5-expressing cells after addition of CCK-8 (0.1 μM) or CCK (0.1 μM)/RC-160 (10 nM). (*B*) Dose-dependent inhibition by RC-160 of CCK-induced (0.1 μM) increase in $[Ca^{2+}]_i$ in hSSTR5-expressing cells. Results are expressed as percentage of control value obtained with untreated cells, which averaged 222 ± 6 nM (mean ± SEM of three experiments).

adenylate cyclase in this effect. Furthermore, RC-160 neither modified the mitogenic signal induced by insulin acting through a tyrosine kinase (25) nor activated a tyrosine phosphatase in this clone. The inhibitory effect of RC-160 was not abolished by orthovanadate or by okadaic acid, a specific inhibitor of serine/threonine phosphatases 1 and 2A at the concentration used (28). Thus a protein phosphatase does not seem to be involved in the negative growth signal coupled to SSTR5. In contrast, in SSTR5-expressing cells, RC-160 suppressed CCK-induced calcium mobilization by a mechanism partially dependent on a pertussis-toxin-sensitive G protein and at concentrations similar to those necessary to inhibit both binding to the receptor and CCK-induced cell proliferation. Since CCK stimulates phospholipase C and inositol phospholipid breakdown pathways in various cells including CHO cells (35), the transient increase in $[Ca^{2+}]_i$ induced by CCK in CHO-K1 cells could represent the mobilization of intracellular Ca^{2+} through phospholipase C activation. Moreover, the phospholipase C/inositol lipid system is known to be involved in cell growth (26, 36) and this pathway could be implicated in the proliferative effect of CCK observed in CHO-K1 cells expressing SSTR5. In these cells, RC-160 inhibits both CCK-stimulated calcium mobilization and cell growth. This supports the coupling of SSTR5 to the phospholipase C/inositol phospholipid/Ca^{2+} inhibitory pathway.

As demonstrated in NIH 3T3 or COS-7 cells expressing human SSTR1 (10), we observed that RC-160 induced a stimulation of tyrosine phosphatase only at a high concentration, reflecting the low affinity of the analogue for this receptor subtype. However, this activation was certainly too weak to mediate an inhibition of serum- or insulin-induced cell proliferation in this cellular clone. Finally, in our system SSTR3 and -4 did not appear to be implicated in the mediation of cell growth inhibition.

In conclusion, our study demonstrates that the two receptor subtypes SSTR2 and SSTR5 are involved in the mediation of the antiproliferative effect of RC-160 that is clinically used. Many primary tumors and tumor cell lines express SSTR2 (9, 10, 12) and mRNAs for SSTR5 are also present in both central nervous system and gastroenteropancreatic tumors (unpublished data). All these results suggest a potential therapeutic use of somatostatin analogues for tumor growth inhibition.

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