We have previously reported in Chinese hamster ovary (CHO) cells expressing sst2 that activation of the sst2 somatostatin receptor inhibits insulin-induced cell proliferation by a mechanism involving stimulation of a tyrosine phosphatase activity. Here we show that the tyrosine phosphatase SHP-1 was associated with the insulin receptor (IR) at the basal level. Activation of IR by insulin resulted in a rapid and transient increase of tyrosine phosphorylation of IR, its substrates IRS-1 and Shc, and also of SHP-1. This was then followed by a rapid dephosphorylation of these molecules, which was related to the insulin-induced increase of SHP-1 association to IR and of SHP-1 activity. On the other hand, addition to insulin of the somatostatin analogue, RC160, resulted in a higher and faster increase of SHP-1 association to IR directly correlated with an inhibition of phosphorylation of IR and its substrates, IRS-1 and Shc. RC160 also induced a higher and more sustained increase in SHP-1 activity. Furthermore, RC160 completely suppressed the effect of insulin on SHP-1 phosphorylation. Finally, in CHO cells coexpressing sst2 and a catalytically inactive mutant SHP-1, insulin as well as RC160 could no longer stimulate SHP-1 activity. Overexpression of the SHP-1 mutant prevented the insulin-induced signaling to be terminated by dephosphorylation of IR, suppressed the inhibitory effect of RC160 on insulin-induced IR phosphorylation, and abolished the cell proliferation modulation by insulin and RC160. Our results suggest that SHP-1 plays a role in negatively modulating insulin signaling by association with IR. Furthermore, somatostatin inhibits the insulin-induced mitogenic signal by accelerating and amplifying the effect of SHP-1 on the termination of the insulin signaling pathway.

Somatostatin is a neuropeptide localized in numerous mammalian tissues. It exerts pleiotropic biological processes including neurotransmission, inhibition of hormonal and hydroelectrolytic secretions, and cell proliferation. This neuropeptide induces its biological effects by interacting with specific receptors that belong to the seven-transmembrane domain receptor superfamily. Five receptors have been recently cloned (sst1–5) and shown to mediate a variety of signal transduction pathways as inhibition of adenylate cyclase and guanylate cyclase, modulation of ionic conductance channels, and protein dephosphorylation (1–4). The five receptors bind to the natural peptides, somatostatin 14 and somatostatin 28, with high affinity, whereas somatostatin analogues selectively interact with sst2, sst3 and, sst5 (4–8).

Somatostatin and its stable analogues promote growth inhibition of various normal and tumor cells (9, 10). In pancreatic tumors cells, we demonstrated that somatostatin and analogues antagonize the mitogenic effect of growth factors acting on tyrosine kinase receptors such as epidermal growth factor (11). Somatostatin peptides also cause a rapid stimulation of a membrane protein tyrosine phosphatase (PTPase) activity and dephosphorylate epidermal growth factor receptors (12, 13). Among the five somatostatin receptors, it has recently been shown that sst2 selectively mediates the inhibitory effect of somatostatin analogues on serum- or insulin-induced cell proliferation by a mechanism involving stimulation of a PTPase in NIH 3T3 and Chinese hamster ovary (CHO) cells expressing sst2 (14, 15). We further demonstrated that a PTPase, identified as SHP-1, co-purified with somatostatin receptors in pancreatic acinar cells that highly expressed sst2 (16). Recent studies have shown that sst2 associates with sst2 and becomes activated in response to somatostatin, suggesting that SHP-1 may be part of the antiproliferative signal promoted by sst2 (17).

SHP-1, a Src homology 2 (SH2) domain containing intracellular PTPase, is predominantly expressed in multiple hematopoietic lineages and to a lesser degree in epithelial cells (18–21). This enzyme has been described to interact with numerous activated growth factor, cytokine, and antigen receptors. It is also implicated in the negative regulation of various immunoreceptor transduction pathways by dephosphorylating and inactivating these receptors or their cognate substrates (22–25). The essential role of SHP-1 as a negative regulator of hematopoietic cell signal transduction is consistent with the multiple defects in hematopoietic cells observed in motheaten mice characterized by mutations in the SHP-1 gene and loss of SHP-1 activity (26).

The identification of substrates of SHP-1 is essential for understanding the negative growth signal promoted by sst2. Because activated sst2 inhibits the mitogenic signal promoted by insulin, the insulin receptor is a potential candidate. Thus, we analyzed the early steps occurring downstream of activation...
of insulin receptor and sst2 in CHO cells expressing sst2. Our data suggest that SHP-1 is one negative regulator of the insulin signaling, and that upon activation, sst2 negatively modulates insulin signaling by accelerating and amplifying the regulatory functions of SHP-1.

MATERIALS AND METHODS

Reagents—Monoclonal anti-SHP-1 and anti-Shc antibodies and polyclonal anti-IRβ β subunit of insulin receptor (IR)) antibodies were purchased from Transduction Laboratories. Anti-IRS-1 antibodies were from Upstate Biotechnology Inc. Monoclonal anti-phosphotyrosine (PY-20) antibodies were from Santa Cruz Biotechnology, RC-160 was a kind gift from Dr. A. V. Schally (Tulane University, New Orleans, LA).

Construction and Expression of the sst2-SHP-1 Mutant in CHO Cells—The 1.2-kilobase XbaI fragment of mouse sst2a cDNA subcloned into pCMV6b vector was stably co-transfected in CHO (DG44 variant) cells using Lipofectin reagent as described previously (Dr. G. I. Bell, Howard Hughes Medical Institute, University of Chicago, and Dr. T. Reisine, University of Pennsylvania, School of Medicine, Philadelphia). (4). Stable transfectants were selected in Dulbecco’s modified Eagle’s medium containing geneticin at 600 μg/ml. Geneticin-resistant clones expressing sst2 (CHO/sst2) were screened for somatostatin binding using [125I]-Tyr3](1-27)somatostatin as tracer as described previously (14). The 2.1-kilobase HindIII/BamHI fragment of human SHP-1 cDNA (Dr. M. L. Thomas, Howard Hughes Medical Institute, Washington University, St Louis, MO) was subcloned into the expression vector pCDNA I neo vector (Invitrogen). The SHP-1(C453S) mutant was constructed with the oligonucleotide primer 5'-GAT GCC AGC GCT GGA ATG CAC AAT-3' by using the method of Kunkel et al. (27).

The mutation was confirmed by dideoxynucleotide sequencing. The mouse sst2 gene in the pCMV6c vector was stably co-transfected in CHO cells using Lipofectin reagent with the SHP-1(C453S) mutant in pCDNA I neo. Stable colonies obtained by selection with G418 (600 μg/ml) were screened for somatostatin binding and cellular clones expressing somatostatin binding sites at similar levels to CHO/sst2 clones were screened for the presence of SHP-1 using Western blot analysis as described below.

Cell Culture and Growth Assay—CHO-DG44 stably expressing the cloned mouse sst2 (CHO/sst2) or sst2 and SHP-1(C453S) (CHO/sst2-SHP-1(C453S)) were cultured in α-minimal essential medium (αMEM) containing 10% fetal calf serum and G418 (200 μg/ml) as described previously (14). For cell treatment, cells were plated in 100-mm diameter dishes (75 x 103 cells/dish). After an overnight attachment phase, cells were cultured in αMEM supplemented with 10% fetal calf serum and plated in 35-mm diameter dishes at 75 x 103 cells/ml (2 ml/dish). After an attachment phase, cells were cultured in serum-free medium overnight (time 0), and then medium was replaced by fresh αMEM containing 100 nM insulin with or without different concentrations of RC-160. Cell growth was measured after a 24-h treatment by cell counting with a Coulter counter model ZM (Coulter Electronics) as described previously (15).

Immunoprecipitations and Immunoblotting—Cells were washed with phosphate-buffered saline (pH 7.4) and then with lysis buffer (50 mM HEPES, 150 mM NaCl, 10 mM EDTA, 10 mM Na3P2O7, 100 mM NaF, 2 mM sodium orthovanadate, pH 7.4). Cells were lysed in 500 μl of lysis buffer containing 1% Triton X-100, 1% phenylmethylsulfonyl fluoride, 2 μg/ml aprotinin, 20 μg/ml leupeptin. After a 15-min incubation at 4 °C, the lysate was collected and centrifuged at 13,000 x g for 10 min at 4 °C to remove insoluble material. For immunodetection of association of SHP-1 with the β subunit of insulin receptor, cells were washed in phosphate-buffered saline and homogenized in a Dounce homogenizer with 50 mM Tris-HCl containing 1 mM EDTA, 5 mM MgCl2, 0.5 mM bacitracin, 0.03% soybean trypsin inhibitor, 5 mM sodium orthovanadate (pH 7.8). The homogenate was centrifuged at 15,000 x g for 30 min at 4 °C. The pellet was solubilized in 500 μl of lysis buffer for 1 h at 4 °C, and the lysate was then collected and centrifuged at 13,000 x g for 10 min at 4 °C.

Proteins of interest were immunoprecipitated with either monoclonal anti-SHP-1 or polyclonal ant-Shc antibodies. The immunoprecipitates were then resolved on 10-15% SDS-PAGE gels and electroblotted onto polyvinylidene difluoride or nitrocellulose membranes. Membranes were incubated overnight at 4 °C in saline buffer (1 mM Tris-HCl, 14 mM NaCl, pH 7.4) containing 5% non-fat dry milk and blotted with specific antibodies for 3 h at room temperature. Immunoreactive bands were detected by ECL immunodetection system (Amersham Corp.).

Tyrosine Phosphatase Assay—Cells were plated in 100-mm-diameter dishes for 48 h, then treated with 1 nM RC-160 for various times. The cells were then washed once with phosphate-buffered saline and lysed in lysis buffer (50 mM Tris-HCl buffer (pH 7.8) containing 2.5% CHAPS, 0.5 mg/ml cholesterol hemisuccinate, 0.05% soybean trypsin inhibitor, 140 mM NaCl, 5 mM MgCl2, 15 mM sodium orthovanadate) for 1 h at 4 °C. The lysate was clarified by centrifugation at 15,000 x g for 10 min at 4 °C. Soluble proteins (300 μl) were incubated with anti-SHP-1 antibody prebound to protein A-Sepharose for 2 h at 4 °C. The immune complexes were collected by centrifugation, washed once with a phosphate-buffered saline and lysed in lysis buffer (50 mM Tris-HCl buffer with 0.05% CHAPS, 0.05 mg/ml cholesterol hemisuccinate, 0.05% soybean trypsin inhibitor, 0.3% bovine serum albumin), and once with PTPase buffer (50 mM Tris, pH 7, 0.1% bovine serum albumin, 0.5 mg/ml bacitracin, 5 m g/ml dithiothreitol). The immune complexes were then collected for PTPase activity in 300 μl of PTPase buffer. The reaction was initiated by addition of 30,000 cpm of [32P-Poly(Glu, Tyr) prepared as described in Buscall et al. (15). One unit of PTPase activity was defined as the amount of the enzyme that released 1 pmol of phosphate/min at 30 °C from the radiolabeled substrate.

RESULTS

SomatostatinActivates SHP-1 Activity and Dephosphorylates Insulin-mediated Tyrosine-phosphorylated SHP-1—We previously reported that, in CHO cells expressing sst2, RC160 inhibited the mitogenic effect of insulin by a mechanism involving the stimulation of a PTPase (15). To assess whether SHP-1 plays a role in somatostatin-induced inhibition of insulin signaling, CHO/sst2 cells were incubated in the presence of 100 nM insulin with or without 1 nM RC160 for various times. SHP-1 was immunoprecipitated with anti-SHP-1 antibodies, and then the PTPase activity was examined in SHP-1 immunoprecipitates. As previously reported on the CCL39 fibroblast cell line (28), insulin caused a stimulation of SHP-1 activity in CHO/sst2 cells (Fig. 1A). A slight increase (20%, p < 0.02) was detected after 1 and 5 min of insulin treatment, with a maximal increase (70%) being observed at 10 min. Addition of RC160 in the incubation medium also resulted in an increase of SHP-1 activity, but the intensity and the time course of the stimulation were different from those observed with insulin alone. The stimulation of SHP-1 activity reached a maximum of about 170% of control as early as 1 min after RC160 addition and remained elevated for at least 10 min. The RC160-induced stimulation of SHP-1 activity was dose-dependent (Fig. 1B). It was observed at concentrations of RC160 in relation to its affinity for the sst2 receptor (15). Indeed, half-maximal and maximal stimulation occurred at 23 ± 4.9 pM and 1 nM RC160, respectively. As reported previously for the effect of analogues on tyrosine phosphatase activity (13–15), the stimulation of SHP-1 was decreased at 10 nM RC160. This could account for the coupling of sst2 to phospholipase C at concentrations higher than 1 nM (29). These results indicate that SHP-1 is a common target for insulin- as well as somatostatin-activated receptors and that activation of sst2 by RC160 leads to a rapid stimulation of SHP-1, which was more sustained and more efficient than that observed with insulin alone.

Several growth factors are known to induce the tyrosine phosphorylation of SHP-1, and insulin has been shown to phosphorylate SHP-1 on Ty246 in a lymphoma cell line (50). To determine the effect of insulin and somatostatin on the level of tyrosine phosphorylation of SHP-1, we performed Western blotting of SHP-1 immunoprecipitates with either anti-phosphotyrosine or anti-SHP-1 antibodies. Our immunoblots revealed that SHP-1 was rapidly tyrosine-phosphorylated in response to insulin (Fig. 2). Maximal stimulation of SHP-1 tyrosine phosphorylation by insulin (2-fold increase) was reached at 1 min after stimulation.
and decreased up to 10 min in our time course. Addition of RC160 to insulin-stimulated cells completely suppressed the stimulatory effect of insulin on SHP-1 tyrosine phosphorylation. A maximal inhibitory effect of RC160 was observed at 3 \( \mu M \) concentrations of RC160, or were not treated (hatched bar). Basal value of SHP-1 activity was 0.37 \( \pm 0.05 \) pmol/min/mg of solubilized protein. Results are means \( \pm \) S.E. of three experiments in duplicate. (Statistical comparison by Student's t test, treated versus untreated cells, \( p < 0.05 \).)

FIG. 2. Effect of insulin and RC160 on tyrosine phosphorylation of SHP-1 in CHO/sst2 cells. A, serum-starved CHO/sst2 cells were incubated at 37 °C for the indicated times with 100 nM insulin with (I+R) or without (I) 1 \( \mu M \) RC160 or were not treated (Cont, control) prior to solubilization. Cell lysates were immunoprecipitated with anti-SHP-1 (Ip: SHP-1) antibodies, subjected to SDS-PAGE, and analyzed by immunoblotting with anti-phosphotyrosine antibodies (blot: P-Tyr). The same filter was then reprobed with anti-SHP-1 antibodies (blot: SHP-1). Arrows indicate the position of SHP-1. B, immunoblots were densitometrically analyzed, and data were plotted as percentage of maximal values that were obtained from cells treated for 1 min with insulin and were taken as 100%. Data from four separate experiments are presented as means \( \pm \) S.E. (Statistical comparison by Student's t test, insulin versus control, \( p < 0.05 \) at 1 and 3 min; insulin + RC160 versus control, \( p < 0.02 \) at 3 min; insulin + RC160 versus insulin, \( p < 0.05 \) at 1 and 3 min.)

**FIG. 1.** A, time course of insulin and RC160-induced stimulation of SHP-1 activity in CHO/sst2 cells. CHO/sst2 cells were cultured for 24 h in αMEM containing 10% fetal calf serum and in serum-free αMEM overnight. Cells were then incubated at 37 °C for the indicated times with 100 nM insulin in the presence (hatched bar) or absence (hollow bar) of 1 \( \mu M \) RC160 prior to solubilization. B, concentration-dependent stimulation of SHP-1 activity by RC160. Cells were treated for 3 min at 37 °C with 100 nM insulin in the presence or absence (Ins) of increasing concentrations of RC160, or were not treated (C). Cells were then solubilized and immunoprecipitated with anti-SHP-1 antibodies. Immunoprecipitates were assayed for PTPase activity in the presence of \( ^{32}P \)-poly(Glu, Tyr) for 10 min at 30 °C. Results are expressed as percent of control PTPase activity obtained from cells at time 0 (A) or untreated cells (B). Basal value of SHP-1 activity was 0.37 \( \pm 0.05 \) pmol/min/mg of solubilized protein. Results are means \( \pm \) S.E. of experiments in duplicate. (Statistical comparison by Student's t test, treated versus untreated cells, \( p < 0.05 \).)

Somatostatin Dephosphorylates Autophosphorylated IR—Since autophosphorylation of IR\( \beta \) is one of the initial signaling events after binding of insulin to its receptor and activation of its intrinsic tyrosine kinase, we examined whether the phosphorylated IR\( \beta \) could be a target of SHP-1. Insulin-stimulated cells were treated or not with 1 \( \mu M \) RC160, and cell lysates were immunoprecipitated with the anti-\( \beta \) subunit of IR antibody and then immunoblotted with either the polyclonal anti-IR\( \beta \) or the monoclonal anti-phosphotyrosine antibody. Tyrosine phosphorylation of the 95-kDa \( \beta \) subunit of insulin receptor was barely detectable in control CHO/sst2 cells but in response to insulin action are believed to be crucial for the propagation of the insulin signal. Since SHP-1 appears to be a substrate of insulin receptor tyrosine kinase, we examined whether SHP-1 interacts with IR. The possible association of SHP-1 with IR was assessed by looking for the presence of IR\( \beta \) in SHP-1 immunoprecipitates from insulin-stimulated cells cultured with or without 1 \( \mu M \) RC160 for various periods of time. As seen in Fig. 3, in resting cells, there was a detectable association of SHP-1 with IR\( \beta \). Following insulin stimulation of the cells, the amount of SHP-1 associated with IR\( \beta \) did not change at 1 min of insulin treatment but increased by 2-fold above control levels (\( p < 0.02 \)) within 3–10 min of treatment. When insulin-stimulated cells were treated with RC160, we observed different kinetics of the association of SHP-1 with IR\( \beta \). Within 1 min of RC160 treatment, the amount of SHP-1 associated with IR\( \beta \) was 3-fold higher than control (\( p < 0.002 \)) and remained elevated up to 10 min. Thus, insulin induces an association of SHP-1 with IR\( \beta \), and the major effect of RC160 is a change in the kinetics and the magnitude of insulin-induced SHP-1-IR\( \beta \) interaction, which is induced more rapidly and efficiently.

Somatostatin Induced the Association of SHP-1 with IR—The IR is a heterotetrameric transmembrane tyrosine kinase. Binding of insulin to the extracellular receptor α subunit results in the rapid autophosphorylation of tyrosine residues within the IR\( \beta \), activating receptor tyrosine kinase activity toward endogenous substrates (31–33). These early steps of
stimulation, IRβ became highly tyrosine phosphorylated with a maximal level at 1 min (Fig. 4). Tyrosine phosphorylation of IRβ was transient and rapidly decreased by 60–70% at 3 and 10 min (p < 0.001). Thus the recruitment of SHP-1 to IRβ was followed by the dephosphorylation of IRβ. Addition of 1 nM RC160 to insulin-stimulated cells strikingly decreased the effect of insulin on IRβ tyrosine phosphorylation. The time course for RC160-induced IRβ dephosphorylation correlates well with that for RC160-induced SHP-1-IRβ association. Indeed, the phosphorylation of IRβ was maximally reduced by 80% (p < 0.001) at 1 min of RC160 treatment and the inhibitory effect of RC160 was sustained up to 10 min. The effect of RC160 was dose-dependent. Half-maximal and maximal effects were observed with 16 ± 6 μM and 1 nM RC160, respectively, the effect of RC160 being reversed at higher concentration. Furthermore, RC160 inhibition of insulin-stimulated IRβ tyrosine phosphorylation was reversed by pretreatment of cells with the tyrosine phosphatase inhibitor, orthovanadate, at 1 μM for 15 min (data not shown). Our results indicate that, first, the dephosphorylation of the activated IR is correlated with the association of SHP-1 with IR. Second, the RC160-mediated prevention of insulin-induced tyrosine phosphorylation of IR is time-related with the RC160-induced increase of SHP-1-IRβ association. This suggests that SHP-1 is a common target of insulin and somatostatin receptor-2, and acts as a negative regulator of IR.

Somatostatin Dephosphorylates Insulin-induced Phosphorylated IRS-1 and Shc—The two best characterized substrates for IRβ kinase have been identified as the 185-kDa protein termed IRS-1 (insulin receptor substrate-1) and the adaptor protein Shc (Src homology 2α-collagen related), which are required for the recruitment of SH2 domain-containing proteins (reviewed in Refs. 34 and 35). We also next investigated whether RC160-activated SHP-1 could affect the phosphorylation levels of these two IRβ target proteins. Anti-phosphotyrosine immunoblots on either IRS-1 or Shc immunoprecipitates revealed IRS-1 to be hyperphosphorylated on tyrosine by insulin treatment (Fig. 5). The time course of insulin-induced phosphorylation of IRS-1 was comparable to that of IRβ phosphorylation seen in Fig. 4. IRS-1 phosphorylation peaked at 1 min and then decreased by about 50% (p < 0.001) up to 10 min. Cells treated with insulin in the presence of 1 nM RC160 exhibited reduced IRS-1 phosphorylation in comparison with insulin alone, RC160 inducing an inhibition of about 50% of the level of insulin-stimulated IRS-1 phosphorylation, throughout the 10-min time course. When cells were preincubated with 1 μM vanadate for 15 min prior RC160 treatment, the inhibitory effect of RC160 was completely blocked and the level of IRS-1 tyrosine phosphorylation was similar to that observed with insulin alone (data not shown). The decrease of IRS-1 phosphorylation in response to increasing concentrations of RC160 indicated that half-maximal and maximal effects were obtained with 14 ± 8 μM and 1 nM RC160, respectively, the effects of RC160 being decreased at 10 nM RC160 (data not shown).

We then examined whether the phosphorylation of Shc was also altered by insulin and RC160. Shc is an SH2 domain-containing protein found as two dominant, widely expressed forms of 46 and 52 kDa as well as a 66-kDa form with a more restricted expression. Shc proteins act as adaptator proteins in signaling pathways generated from diverse stimuli. Shc phosphorylation was detected in the basal state and insulin stimulated by about 2-fold the tyrosine phosphorylation of Shc with a slower time course compared with that of IRβ and IRS-1 as previously reported (36, 37). Maximal phosphorylation occurred at 3 min and was decreased at 10 min (p < 0.001) (Fig. 6). Addition of RC160 to the culture medium resulted in a decrease of insulin-stimulated Shc tyrosine phosphorylation.
which reached 50% (p < 0.001) at 3 min. RC160-induced inhibition of Shc phosphorylation was suppressed by preincubation of cells for 15 min with 1 μM orthovanadate before RC160 treatment. The RC160-induced dephosphorylation of Shc was dose-dependent with half-maximal and maximal effects observed with 23 ± 2.5 px and 1 nx, respectively. At higher concentration of RC160, the effect of RC160 was reversed (data not shown). All these results showed that RC160 negatively regulates insulin signaling by dephosphorylating the IRβ and some of its key downstream targets. Furthermore, these last events may involve the activation of a tyrosine phosphatase.

Overexpression of Negative SHP-1(C453S) Mutant Prevents the Effect of Insulin and Somatostatin on SHP-1—To confirm whether SHP-1 is critical for insulin- as well as somatostatin-mediated decrease of IR signaling pathway, we generated a mutated SHP-1 cDNA in which the active cysteine at position 453 was mutated to serine. This mutation results in a catalytically inactive enzyme as observed by transient expression of the SHP-1 mutant in COS-7 cells.2 When CHO/sst2-SHP-1(C453S) cell lysates were immunoprecipitated with the anti-IR antibody and immunoblotted with anti-phosphotyrosine antibodies (blot: P-Tyr), the phosphatase was not further phosphorylated upon insulin stimulation. Remarkably, addition of RC160 did not cause any dephosphorylation of SHP-1, indicating that the blockade of SHP-1 catalytic activity renders the enzyme refractory to its regulation by insulin or RC160 (Fig. 8).

Overexpression of Negative SHP-1(C453S) Mutant Prevents the Dephosphorylation of Insulin Receptor and Suppresses the Effect of Somatostatin.—When CHO/sst2-SHP-1(C453S) cell lysates were immunoprecipitated with the anti-IRβ antibody and then immunoblotted with either the polyclonal anti-IRβ or the monoclonal anti-phosphotyrosine antibody, we observed that tyrosine phosphorylation of the β subunit of IR was barely detectable in control CHO/sst2-SHP-1(C453S) cells as readily observed in CHO/sst2 cells. Treatment of CHO/sst2-SHP-1(C453S) cells with insulin induced tyrosine phosphorylation of IRβ. However, this effect was not transient and increased up to 10 min of the time course (Fig. 9). These data indicate that the expression of the catalytically inactive SHP-1 blocks the negative autoregulation of insulin signaling. Furthermore, expression of the SHP-1 mutant abrogated the inhibitory effect of RC160 on insulin-induced IRβ phosphorylation, the level of IRβ phosphorylation being not modified by addition of RC160 at 1 and 3 min. At 10 min, RC160 decreased the level of IRβ phosphorylation by about 50%, suggesting that another tyrosine phosphatase that negatively regulated insulin signaling could be activated as well by RC160 in cells expressing the SHP-1 mutant.

Overexpression of Negative SHP-1(C453S) Mutant Abolishes the Antiproliferative Effect of Somatostatin.—Finally, we tested the effect of insulin and RC160 on the proliferation of CHO/
sst2-SHP-1(C453S) cells after incubation for 24 h with 1 μM insulin in the presence or not of 1 nM RC160. As observed in Fig. 10, in cells expressing the SHP-1 mutant and sst2, the basal proliferation was increased by about 50% \((p, 0.01)\) compared with control CHO cells or CHO/sst2 cells. Furthermore, insulin stimulated the proliferation of control cells or CHO/sst2 cells but had no more effect in CHO/sst2-SHP-1(C453S) cells. Finally, the inhibitory effect of RC160 was observed in CHO/sst2 cells \((-64%, p < 0.02)\) but was never seen in CHO/sst2-SHP-1(C453S) cells. These results clearly demonstrate that SHP-1 may play a role in the negative regul-
lation of insulin signaling and is critical for the transduction of the negative growth signal promoted by activation of sst2.

DISCUSSION

Over the past several years much progress has been made in elucidating intracellular signaling events mediating positive growth signal by tyrosine kinase receptor activation. The processes that mediate the inactivation of these events through activation of tyrosine dephosphorylation by extracellular stimuli remain less defined. We previously demonstrated that stimulation of a membrane tyrosine phosphatase activity is an early event in the negative control of insulin-induced mitogenic signal initiated by somatostatin-activated sst2 (14). We have shown here that SHP-1 is one negative regulator of the insulin signaling pathway. Somatostatin further promotes the activation of SHP-1, leading to an earlier and more amplified dephosphorylation of IR and its downstream substrates than that compared with insulin alone.

SHP-1 has been identified as a critical negative regulator of interleukin-3, erythropoietin, interferon-α/β, and growth hormone cytokine, as well as epidermal growth factor signaling, the recruitment of the enzyme to activated membrane receptors causing dephosphorylation of the receptors or/and of downstream signaling molecules (38). Insulin binding to its receptor results in autophosphorylation of tyrosine residues within the β subunit of the receptor, activating intrinsic tyrosine kinase activity toward endogenous substrates. These tyrosine phosphorylation events are rapidly reversed, and evidence from a variety of experimental approaches has been provided that intracellular PTPase PTP1B and the transmembrane PTPases variety of experimental approaches has been provided that stimulation of a membrane tyrosine phosphatase activity is an early event in the negative control of insulin-induced mitogenic signal initiated by somatostatin-activated sst2 (14). We have shown here that SHP-1 is one negative regulator of the insulin signaling pathway. Somatostatin further promotes the activation of SHP-1, leading to an earlier and more amplified dephosphorylation of IR and its downstream substrates than that compared with insulin alone.

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hypothesis that somatostatin negatively regulates insulin signal transduction by controlling first the recruitment of SHP-1 to IR and its activation, causing then a dephosphorylation and an inactivation of IR and its substrates. These events lead to an inhibition of the insulin downstream signaling and the mitogenic response initiated by insulin.

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sst2 Somatostatin Receptor Mediates Negative Regulation of Insulin Receptor Signaling through the Tyrosine Phosphatase SHP-1
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