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# Inhibition of Human Pancreatic Islet Insulin Release by Receptor-Selective Somatostatin Analogs Directed to Somatostatin Receptor Subtype 5

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**ABSTRACT.** Somatostatin (SS)-14 and SS28 are produced by pancreatic D cells and gut mucosa and inhibit pancreatic islet insulin and glucagon release. There are five distinct SS receptor (SSTR) subtypes, namely SSTR1–5, which show different affinities for SS14 and SS28. In order to identify the subtype responsible for inhibition of insulin release by human B cells, SSTR-selective SS analogs were tested in isolated human islets. Glucose-stimulated insulin secretion in human islets incubated for 1 hr at 20 mM glucose, and in islets cultured for 24 hr at a near-physiological (6.1 mM) glucose concentration, was inhibited (<50% of the control) by SSTR5-specific analogs and by SS14 and SS28. SS14, SS28, and different SSTR5 preferential analogs also inhibited islet amyloid polypeptide release during the 24-hr culture. On the other hand, a group of SSTR2-selective analogs failed to inhibit insulin release. Analysis by reverse transcription-polymerase chain reaction indicated that human islets express similar amounts of SSTR2 and SSTR5 mRNAs, while human pancreatic ductal cells express much lower levels of these mRNAs. In conclusion, our data suggest that SSTR5 is an important mediator of the insulin inhibitory action of SS in cultured human islets. BIOCHEM PHARMACOL **57**;10:1159–1164, 1999. © 1999 Elsevier Science Inc.

**KEY WORDS.** somatostatin; somatostatin receptor; insulin release; pancreatic islets; somatostatin analogs

SS[‖] is a 14-amino acid peptide, originally isolated from hypothalamic tissue as the neuroendocrine suppressor of growth hormone secretion [1]. The two naturally occurring peptides, SS14 and the N-terminally extended SS28, are widely distributed in the central nervous system, endocrine and peripheral tissue [2]. Both peptides have been found to inhibit the secretory activity of pancreatic B cells [3–6]. They can therefore be considered as therapeutic agents in cases of excessive insulin production [7], or in attempts to induce B cell "rest" as a way of decreasing the autoimmune assault in early type 1 diabetes mellitus [8]. Their clinical use is, however, hampered by the widespread distribution of SS receptors [SSTR; 9]. Recently, the structures of five SS receptor subtypes were elucidated [10–14]. This information led to the synthesis of subtype-selective analogs which are expected to exhibit a narrower range of pharmacological actions than the parent tetradecapeptide [15]. These analogs can now be used to identify the presence and involvement of particular receptor subtypes in different physiological functions. It was thus shown that SSTR2 and SSTR5 play a differential role in the regulation of growth hormone, thyroid-stimulating hormone, and prolactin release [15]. In rat pancreatic islets, SSTR2 appears to mediate inhibition of glucagon secretion and SSTR5 inhibition of insulin release [16]. Using immunohistochemistry, SSTR2 was found in most rat pancreatic acinar-, glucagon- and pancreatic polypeptide-containing cells, but in only a few insulin-containing cells [17]. Little information is available on the SSTRs present in human islets, but two recent studies have suggested that SSTR2 is the main mediator of SS-induced inhibition of insulin release [18, 19].

The present study examines the effects of SS receptor subtype analogs on the secretory activity of human B cells *in vitro*. It also assesses the expression of mRNAs encoding for the SSTR2 and SSTR5 in these cells. In some experiments, IAPP release was determined in parallel with insulin. Our results suggest that SSTR5 is an important mediator of SS-induced inhibition of human pancreatic B cells.

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‖ *Abbreviations:* SS, somatostatin; SSTR, somatostatin receptor subtype; IAPP, islet amyloid polypeptide; RT-PCR, reverse transcription-polymerase chain reaction; EH, Earle's Hepes medium; and GAPDH, glyceraldehyde-3-phosphate dehydrogenase.

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**TABLE 1. Binding affinities of SS and SS analogs**

| | SSTR binding affinity ($IC_{50}$ nM) | | | | |
|---|---|---|---|---|---|
| Peptide | SSTR1 | SSTR2 | SSTR3 | SSTR4 | SSTR5 |
| SS14 | 2.26 | 0.23 | 1.43 | 1.77 | 0.88 |
| SS28 | 2.38 | 0.30 | 1.02 | ND | 0.38 |
| BIM23268 | 18.4 | 15.1 | 61.6 | 16.3 | **0.37** |
| BIM23313 | 129 | 4.8 | 28.4 | 28.9 | **0.16** |
| BIM23190 | 4577 | **0.34** | 217 | >1000 | 11.1 |
| BIM23197 | 5547 | **0.19** | 26.8 | >1000 | 9.81 |
| BIM23014 | 2330 | **0.75** | 107 | 2100 | 5.21 |
| BIM23023 | 7170 | **0.42** | 89.1 | 2700 | 4.18 |

From Ref. 15; and Culler M, Woon C-W, Taylor JE and Coy DH, unpublished data. ND, not determined. Bold indicates the preferential binding affinity of each analog.

## MATERIALS AND METHODS

### *Somatostatin Analogs*

SS14, SS28, and the SS analogs BIM 23268, BIM 23313, BIM 23014, BIM 23023, BIM 23190, and BIM 23197 were provided by Biomeasure Inc. The synthesis, expression in Chinese hamster ovary K1 cells, and the radioligand receptor binding assays to determine the specific binding affinities of these analogs to different human SSTR subtypes have been previously described [15]. Table 1 summarizes the binding affinities of the analogs used in the present study [15*].

### *Human Islet Cells*

Human islets were isolated from pancreatic segments obtained from 20 heart-beating cadaveric organ donors [20]. The organs were sent by European hospitals affiliated with the β Cell Transplant or with the Eurotransplant Foundation (Leiden, The Netherlands) [20]. The age of the donors (mean ± SEM; N = 20) was 42 ± 2.3 years (range 23–59 years). Isolated islets were cultured for 4–20 days in Ham's F10 (GIBCO) containing 1% BSA (Boehringer Mannheim), 7.5 mM glucose, 0.075 mg/mL penicillin and 0.1 mg/mL streptomycin [21]. After culture, the islet preparations contained 49 ± 2% B cells and 15 ± 2% A cells, as determined by immunocytochemistry and electron microscopy [20, 22]. Messenger RNA was extracted from whole islets and islet ductal cells. Islet ductal cells are usual contaminants of human islet preparations, representing around 30% of the total islet cell population [20, 23, 24]. Ductal cell preparations (>90% pure) were obtained as previously described [23, 24].

For studies on glucose-induced insulin release, the islets were retrieved following culture and washed with glucose-free EH medium [22] containing 0.5% BSA. Groups of islet cells (average $1.2 \times 10^5$ cells/mL) were distributed as 0.1 mL samples over 5 mL plastic tubes (Falcon, Becton Dickinson and Co.) containing 0.9 mL EH without glucose. After 30-min sedimentation at room temperature, 0.5 mL of medium was removed and assayed for insulin content (basal insulin discharge). The remaining 0.5 mL, containing the sedimented islets, was mixed with an additional volume of 0.5 mL EH medium containing different concentrations of glucose and SS analogs. The cells were then incubated for 1 hr at 37°. At the end of this incubation, the tubes were centrifuged, the supernatant fractions aspirated and analyzed for their insulin content, and the cell pellets extracted in 2M acetic acid containing 0.25% BSA. Insulin in the medium and islet cell extracts was measured by radioimmunoassay [22].

In some experiments, the islet cells (around $1.3 \times 10^5$ islet cells/mL) were cultured for 24 hr in the Ham's F10 culture medium containing 1% BSA, 6.1 mM glucose, and the different SS analogs. After this period, the culture medium was assayed for insulin (as described above) and IAPP. The IAPP radioimmunoassay was performed in a two-step incubation. IAPP standards or samples were first incubated with antiserum (RIN 7321; Peninsula) for 24 hr and $^{125}$I-IAPP was added for 6 hr at 4°. Bound and free IAPP were separated by Pharmacia decanting suspension no. 3 (Pharmacia). The interassay and intra-assay coefficients of variation were 8 and 7%, respectively.

### *RT-PCR*

Poly(A)+ RNA was isolated from cell aggregates (approximately $10^5$ cells) using oligo$(dT)_{25}$-coated polystyrene Dynabeads (DYNAL). The reverse transcription mixture was prepared with the GeneAmp RNA PCR Kit (Perkin-Elmer) and the reaction solution (per 10 μL) was composed of mRNA equivalent to $3 \times 10^3$ cells, 10 mM Tris–HCl (pH 8.3), 50 mM KCl, 5 mM $MgCl_2$, 1 mM each deoxynucleotide triphosphate, 2.5 μM random hexamer primers, 1U/μL RNase inhibitor, and 2.5 U/μL Moloney murine leukemia virus reverse transcriptase. Each reaction was sequentially incubated for 10 min at room temperature, for 60 min at 42°, and then for 5 min at 99°. In preliminary experiments (data not shown), the specificity of each primer pair was optimized by adjusting magnesium ion concentration and primer annealing temperature. Amplification efficiency was checked by comparing the yields of PCR products at different cycles. The PCR mixture (final volume 25 μL) contained 5 μL cDNA, 10 mM Tris–HCl (pH 8.3), 50 mM KCl, 2 mM $MgCl_2$, 0.4 μM of each forward and reverse primer, 0.2 mM of each deoxynucleotide triphosphate, and 0.625 U AmpliTaq Gold DNA polymerase (Perkin Elmer-Cetus). The thermal conditions used for the "hot start" PCR [25] reactions were: initial denaturation of 12 min at 95°, then cycles of denaturation at 94° for 45 sec, ramp −1.8°/sec, annealing at 58° (SSTR2) and 65° (SSTR5) for 45 sec, ramp +1.3°/sec and extension at 72° for 90 sec, ramp + 1.3°/sec. The last step was extension at 72° for 10 min. All PCR reactions were performed in PTC-200 DNA thermal cycler (MJ Research) using the following primers, specific for each gene:

* Culler M, Woon C-W, Taylor JE and Coy DH, unpublished data.

hSSTR2-F: 5′-TGGTGGTCCTCACCTATG-3′,

hSSTR2-R: 5′-CGTAGGTAATGCCTATACAG-3′;

hSSTR5-F: 5′-CGTCTTCATCATCTACACGG-3′,

hSSTR5-R: 5′-GTGCAACCTCCGCTCCTGGGG-3′;

GAPDH-F: 5′-CATGTTCGTCATGGGTGTGA-3′,

GAPDH-R: 5′AGTGAGCTTCCCGTTCAGCT-3′.

To enable comparisons between the relative amounts of cellular mRNA for SSTR subtypes 2 and 5, external standards for each target cDNA and for the housekeeping gene GAPDH were used in the PCR reactions. In order to prepare these standards, PCR products of specific bands amplified from cDNA were detected on the ethidium bromide-stained agarose gel and their concentrations estimated by comparison against known amounts of molecular weight markers run in the same gel. Dilution series containing decreasing amounts of target cDNA templates were then prepared and amplified simultaneously with islet or ductal cell cDNA samples. The PCR amplification efficiencies for cDNA samples and their respective standards were then compared by densitometry (see below). For each cDNA sample, aliquots were used to amplify SSTR2, SSTR5, and GAPDH cDNAs. In order to exclude contamination by genomic DNA, similar amounts of non-reverse-transcribed RNA were used in the PCR (negative control). Following amplification, five microliters of PCR products were subjected to electrophoresis in a 2.5% agarose gel. The ethidium bromide-stained gels were photographed under UV transillumination using the Kodak Digital Science DC40 camera (Kodak). The PCR band intensities on the image were quantified by Biomax 1D Image Analysis Software (Kodak) and expressed in pixel intensities. The molar amounts of target cDNA present in each cDNA preparation were measured by comparing their band intensities with that generated by the respective standard series. The housekeeping gene GAPDH was used to correct for eventual variations in cDNA loading. The PCR products were sequenced by automated DNA sequencing performed on the ABI PRISM 310 Genetic Analyzer using the Dye Terminator Cycle Sequencing kit (Perkin–Elmer).

### *Statistical Analysis*

The total B cell number was calculated in each preparation based on the islet DNA content and the percentage of insulin-positive cells identified by immunocytochemistry [20]. Net insulin release was calculated by subtracting the basal insulin discharge (during the 30-min incubation at room temperature in the absence of glucose) from the insulin release measured during the 1-hr incubation at 37° at 2.5 or 20 mM glucose (test condition). Insulin release was expressed as a percentage of the cellular insulin content, and the dose–response curves were calculated from the insulin release expressed as a percentage of the control (i.e. islets not exposed to SS or its analogs). $EC_{50}$ values for each peptide were calculated from these dose–response curves by using the Graph-Pad Prism computer program. Values are expressed as mean ± SEM of several independent experiments (i.e. different donor human islets studied on separate days), and the statistical analysis of the data was performed by paired *t*-test.

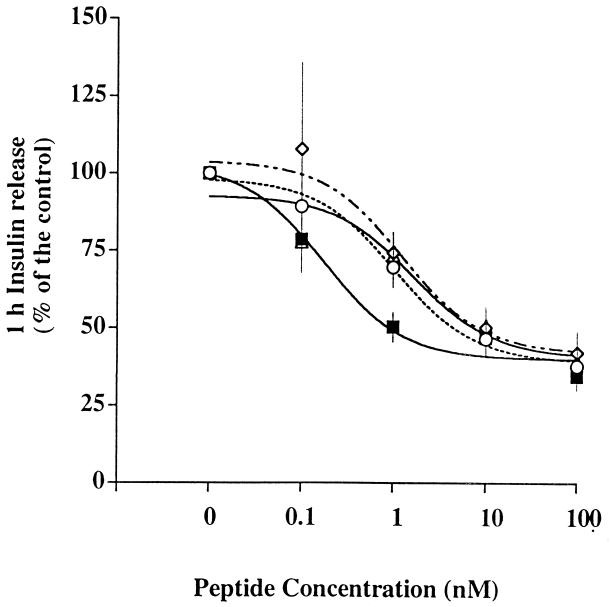


**FIG. 1. Effect of somatostatin and synthetic analogs on acute insulin release from cultured human islets. Islets were incubated for 1 hr in the presence of 20 mM glucose. Data represent means ± SEM for 5–7 independent experiments. The absolute insulin release under the control condition was 1.4 ± 0.48 ng/$10^3$ β cells/hr. SS14 (◇); SS28 (△); BIM 23268 (○); BIM 23313 (■).**

## RESULTS

The cultured human islet preparations contained 13.8 ± 2.2 ng insulin/$10^3$ B cells. When incubated for 1 hr, they released 2.5 ± 0.3% of their insulin content at 2.5 mM glucose and 7.9 ± 1.1% at 20 mM glucose (n = 20; $P <$ 0.0001 vs islets incubated at 2.5 mM glucose).

Glucose-stimulated insulin release was already significantly inhibited ($P < 0.01$ vs controls) by 1 nM SS14, SS28, and the synthetic analogs BIM 23268 and BIM 23313 (Fig. 1). At maximal concentration (100 nM), these agents inhibited insulin release by more than 50% ($P <$ 0.001). The $EC_{50}$ values for insulin release inhibition were 1.2, 1.6, 1.0, and 0.2 nM for SS14, SS28, BIM 23268, and BIM 23313, respectively. None of these agents suppressed basal insulin release at 2.5 mM glucose (data not shown). In another series of experiments, we observed that concentrations of up to 100 nM of BIM 23190, BIM 23197, BIM 23014, and BIM 23023, analogs which preferentially bind to SSTR 2, failed to inhibit glucose-induced insulin release. Thus, insulin release (% of control) was 94 ± 8, 109 ± 7, 88 ± 4, 98 ± 6, and 103 ± 9% for BIM 23190 and 93 ± 9, 98 ± 4, 95 ± 6, 100 ± 4, and 100 ± 9% for BIM 23197

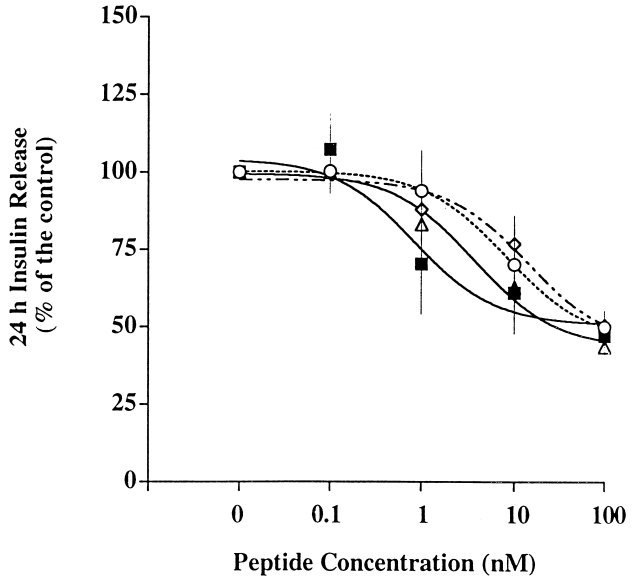


**FIG. 2. Effect of somatostatin and synthetic analogs on long-term insulin release from cultured human islets. Islets were cultured for 24 hr at 6.1 mM glucose. Data represent means ± SEM for 4–7 independent experiments. The absolute insulin release under the control condition was 3.18 ± 0.50 ng/$10^3$ β cells/24 hr. SS14 (◇); SS28 (△); BIM 23268 (○); BIM 23313 (■).**

(n = 4) at the peptide concentration of 0.01, 0.1, 1, 10, and 100 nM respectively. BIM 23014 and BIM 23023 showed similar results (N = 1, data not shown), while SS14 and BIM 23268 induced a marked inhibition of insulin release similar to Fig. 1 (data not shown).

In order to test whether the SS analogs inhibit insulin release during longer exposure times under near physiological glucose concentrations, human islets were cultured for 24 hr at 6.1 mM glucose in the presence of SS 14 and 28 or BIM 23268 and BIM 23313 (Fig. 2). The total amount of insulin released into the medium was significantly inhibited by the four peptides ($P < 0.05$ or $P < 0.01$) with the $EC_{50}$ values of 13.6 nM (SS14), 3.6 nM (SS28), 7.8 nM (BIM 23268), and 0.8 nM (BIM 23313) for insulin release (Fig. 2) and 14.5 nM (SS14), 12.4 nM (SS28), 10.2 nM (BIM 23268), and 3.19 nM (BIM 23313) for IAPP release, (Fig. 3B). The $EC_{50}$ values for the insulin release data indicated in Fig. 3A are similar to those discussed above for Fig. 2 (data not shown).

Both SSTR subtypes 2 and 5 mRNA are expressed in human islet cells (Fig. 4). In the three separate preparations tested, the amount of mRNA for SSTR5 was as abundant as that observed for SSTR2. In ductal cells, no SSTR2 receptor mRNA was detected, but there was a low expression of SSTR5 mRNA (Fig. 4). No genomic DNA contamination was detected in the mRNA preparations amplified in the same cycles, and the identity of the SSTR2 and SSTR5 PCR products identified in this study was confirmed by automated DNA sequencing (data not shown).

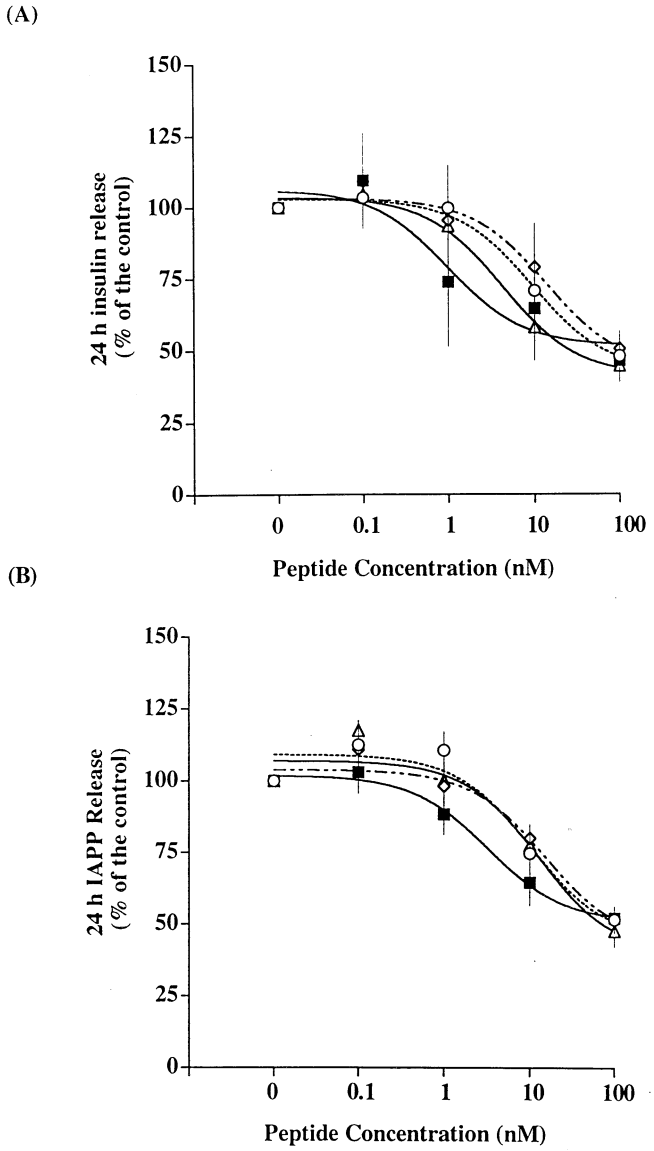


**FIG. 3. Effect of somatostatin and synthetic analogs on long-term insulin (A) and IAPP (B) release from cultured human islets. Islets were cultured for 24 hr at 6.1 mM glucose with or without somatostatin or synthetic analogs. Data represent means ± SEM for 3–4 independent experiments. The absolute insulin release under the control condition was 3.19 ± 0.58 ng/$10^3$ β cells/24 hr and that of IAPP was 33.3 pg/$10^3$ β cells/24 hr. SS14 (◇); SS28 (△); BIM 23268 (○); BIM 23313 (■).**

## DISCUSSION

Native SS14 and SS28 show high affinity for most SSTR subtypes, which are widely distributed over many organs and tissues. This feature limits their use for specific therapeutic purposes, such as inhibition of insulin release. In the present study, we used SSTR-selective analogs in order to identify the receptor(s) mediating the inhibitory effects of SS on insulin release by human islets. Analogs to SSTR5 induced a dose-dependent decrease in insulin secretion from human islets. These effects were observed at nM concentrations, similar to the effective concentrations re-

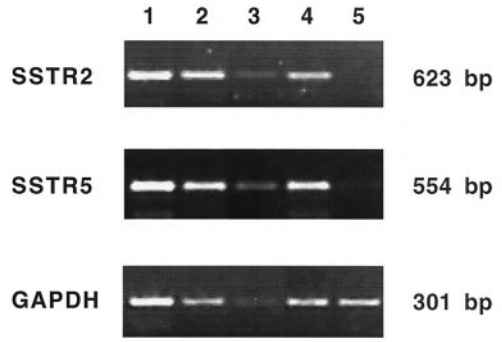


**FIG. 4. Expression of SSTR subtypes 2 and 5 in human pancreatic islet and ductal cells, as evaluated by RT-PCR. Aliquots of cDNA, each equivalent to $1.5 \times 10^3$ islet (lane 4) or ductal (lane 5) cells, were amplified using primer pairs specific for SSTR2, SSTR5, and GAPDH. Lanes 1–3 are diluted standards prepared as described in Materials and Methods, and containing template amounts equal to $4.5 \times 10^{-3}$, $4.5 \times 10^{-4}$, and $4.5 \times 10^{-5}$ attomoles for SSTR2 and SSTR5, respectively; and 1.44, 0.36, and 0.09 attomoles for GAPDH. The number of cycles used was 40, 40, and 31, respectively for SSTR2, SSTR5, and GAPDH. The figure is representative of three independent experiments.**

quired for the inhibitory effects of native SS. On the other hand, SSTR2-selective analogs failed to inhibit insulin release. While the utilized analogs were strongly preferential for either SSTR2 or 5, they also possessed varying degrees of affinity for the other SSTR subtypes. Comparing the observed insulin suppressing activity (Figs. 1 and 2) with the varying degrees of affinity of the different compounds for SSTR subtypes 1, 3, and 4, it seems unlikely that these receptor subtypes are major determinants of the suppression of insulin by SS. This, and the presence of mRNA for SSTR5 in human pancreatic islets, suggests that SSTR5 is an important mediator of the inhibitory effects of SS on human B cells. In rodent islets, SSTR5-specific analogs also inhibit insulin release [16]. SSTR2A was recently localized in rat islets, but was mostly confined to non-insulin-containing cells [17]. In line with this, there is evidence for the involvement of SSTR2 in the inhibition of glucagon release by rat islets [16].

We detected both SSTR2- and SSTR5-specific mRNAs in isolated human islets by RT-PCR. Since ductal cells are the most frequent non-endocrine cell type present in human islet preparations [20, 23, 24], the expression of both mRNAs was also examined in these cells. SSTR 2 and 5 mRNA expression was severalfold higher in islet than in ductal cells, indicating that these mRNAs are expressed by human islet endocrine cells. PCR is an enzymatic process where the amplification efficiency significantly affects the product yield. Thus, the intensities of detected bands may not reflect the real amount of cDNA present in the starting preparation. We attempted to improve the efficiency and specificity of each PCR amplification by using "hot start" PCR and dilution series containing known amounts of template included as a standard. It was thus found that the amplification efficiency of cDNA samples was similar to that observed in the template standard, enabling quantitative comparison of band intensities. Our RT-PCR data are contradictory to those reported by Kubata *et al.* [19]. These authors detected expression of SSTR2 in human islets, but not SSTR5. This discrepancy may be due to differences in human islet isolation and culture, cellular composition of the preparations, and the PCR conditions. Another study, using perfusion of human pancreata with different SS analogs, suggested that SSTR2 mediates SS-induced inhibition of insulin release [18]. Differences in study design and the high variability associated with the low number of human preparations utilized for the perfusions [18] prevent adequate comparison with our data. Clearly, a definitive answer as to the nature of the SSTR(s) expressed on human B cells will depend on future studies using immunohistochemistry and/or *in situ* hybridization.

The present observation that SSTR5-preferring compounds suppress both acute and 24-hr insulin secretion suggests that this class of compounds may have therapeutic potential. It is noteworthy that the $EC_{50}$ values for inhibition of insulin release at 24 hr were higher than that observed in the acute (1 hr) experiments. This may be due to either decreasing peptide activity during culture or the different glucose concentration utilized in these experiments, i.e. 20 mM in the acute experiments and 6.1 mM in the 24-hr experiments. Clinical use for this and other SSTR-selective analogs may be envisaged in states of excessive insulin release [7, 9] or in cases where a decrease in B cell function may contribute to reduce antigen expression and thus diminish an immune assault against these cells, as is the case in early type 1 diabetes mellitus or following islet transplantation [8, 26].

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