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Somatostatin Inhibits Insulin and Glucagon Secretion via Two Receptor Subtypes: An *in Vitro* Study of Pancreatic Islets from Somatostatin Receptor 2 Knockout Mice*

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ABSTRACT

Somatostatin (SST) potently inhibits insulin and glucagon release from pancreatic islets. Five distinct membrane receptors (SSTR1–5) for SST are known, and at least two (SSTR2 and SSTR5) have been proposed to regulate pancreatic endocrine function. Our current understanding of SST physiology is limited by the receptor subtype selectivity of peptidyl SST analogs, making it difficult to assign a physiological function to an identified SST receptor subtype. To better understand the physiology of SSTRs we studied the *in vitro* effects of potent subtype-selective nonpeptidyl SST analogs on the regulation of pancreatic glucagon and insulin secretion in wild-type (WT) and in somatostatin receptor 2 knockout (SSTR2KO) mice.

There was no difference in basal glucagon and insulin secretion between islets isolated from SSTR2KO and WT mice; however, potassium/arginine-stimulated glucagon secretion was approximately 2-fold higher in islets isolated from SSTR2KO mice. Neither SST nor any SSTR-selective agonist inhibited basal glucagon or insulin release. SST-14 potently inhibited stimulated glucagon secretion in islets from WT mice and much less effectively in islets from SSTR2KO mice. The SSTR2 selective analog L-779,976 inhibited glucagon secretion in islets from WT, but was inactive in islets from SSTR2KO mice. L-817,818, an SSTR5 selective analog, slightly reduced glucagon release in both animal groups, whereas SSTR1, -3, and -4 selective analogs were inactive. SST and L-817,818 inhibited glucose stimulated insulin release in islets from WT and SSTR2KO mice. L-779,976 much less potently reduced insulin secretion from WT islets.

In conclusion, our data demonstrate that SST inhibition of glucagon release in mouse islets is primarily mediated via SSTR2, whereas insulin secretion is regulated primarily via SSTR5. (*Endocrinology* **141:** 111–117, 2000)

SOMATOSTATIN (SST) is a peptide with a wide spectrum of biological action. Its effects are mediated via five somatostatin receptor subtypes (SSTR1–SSTR5) (1–13) that are heterogeneously distributed in various tissues. High levels of SSTR expression are found in the brain, gastrointestinal tract, pancreas, and pituitary gland (14–16). SSTRs play a role in different physiological processes, such as neurotransmission, inhibition of gastrointestinal motility, gastric acid flow, intestinal absorption, pancreatic enzyme secretion, GH release, as well as cell proliferation (17–24).

There are two major physiological ligands for SSTRs: SST-14 and SST-28, consisting of 14 and 28 amino acids, respectively. Radioligand binding studies have shown that SST-14 and SST-28 interact with SSTR1–4 with similar affinities, whereas SSTR5 preferentially binds SST-28 (13, 25). Both forms of SST play an important role in the regulation of glucagon and insulin release from the endocrine pancreas. SST-14 is considered to be a more potent inhibitor of glucagon secretion from the α-cells, whereas SST-28 predominantly inhibits insulin secretion from the β-cells (26–28). Because of the different potencies of SST-14 and SST-28 at the cloned receptors, it was suggested that the inhibition of insulin and glucagon release is mediated by different SSTRs (27).

As SST-14 and SST-28 bind with high affinity to all five SSTRs, it is not possible to distinguish between individual SSTRs using these peptides. Therefore, a number of SSTR selective agonists with distinct affinity profiles have been developed and used for pharmacological characterization of the individual SSTRs.

Our current understanding of the roles of SSTRs in the regulation of insulin and glucagon secretion derives from studies using SSTR selective peptide analogs. *In vivo* studies have demonstrated that SSTR5, but not SSTR2, selective SST analogs inhibit insulin secretion from rat endocrine islets (29). Recently, an *in vitro* study of mouse pancreatic islets showed that SSTR5, but not SSTR2, selective peptide analogs inhibit glucose-induced insulin release (30).

The development of SSTR-specific antibodies has allowed investigation of the morphological distribution of SSTRs in the endocrine pancreas (31). Thus, immunohistochemical studies of rat endocrine islets have demonstrated that SSTR2 is expressed on α-cells, whereas β-cells show only weak SSTR2 immunoreactivity (32), providing the morphological correlate to the pharmacological findings. Recently, a study of human pancreas demonstrated immunostaining of β- as well as α-cells with SSTR2 antibody (33), suggesting that species differences may exist.

Despite a number of pharmacological studies, the physiology of SSTRs in the endocrine islets of the pancreas has not

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been entirely defined, primarily due to the lack of the highly selective receptor ligands. An alternative approach for evaluation of the role of SSTRs is inactivation (knocking out) of a gene encoding a particular receptor subtype in an animal.

In the present study we used the SSTR2KO mice, developed in our laboratories (34), in combination with novel subtype-selective nonpeptidyl SST analogs (36) to evaluate the roles of SSTRs in regulation of insulin and glucagon secretion.

Materials and Methods

Materials

SST-14, Tyr-SST-28, Gey's balanced salt solution (GBSS), BSA, L-arginine, D-glucose, and potassium chloride were purchased from Sigma-Aldrich Corp. (St. Louis, MO). Collagenase type IV (186 U/mg) was obtained from Worthington Biochemical Corp. (Freehold, NJ). Tissue culture medium RPMI 1640, FBS, αMEM, penicillin, and streptomycin were obtained from Life Technologies, Inc. (Grand Island, NY). RIA kits for insulin and glucagon were purchased from Linco Research, Inc. (St. Louis, MO). The SSTR selective compounds, L-779,976, L-817,818, L-797,591, L-796,778, and L-803,087, were provided by Dr. S. P. Rohrer (Merck, Rahway, NJ). The identification and characterization of these compounds were previously reported (35).

Animals

Mice deficient in SSTR2 [homozygous (−/−) SSTR2KO] were generated by gene targeting in mouse embryonic stem cells as previously described (34). Corresponding wild-type adult C57BL/129 mice (20–35 g, 4–8 months of age) were used as controls. Mice were maintained under controlled conditions at 25 C, with food and water available *ad libitum*. All experimental protocols were approved by the Rahway Institutional Animal Care and Use Committee.

In vitro insulin/glucagon secretion

Mice were euthanized with CO_2, and blood was collected via intracardiac puncture. Islets were isolated by injection of 4 ml ice-cold collagenase solution (4 mg/ml GBSS supplemented with 1% BSA) into the bile-pancreatic duct (36). The distended pancreas was removed, trimmed from adjacent tissues, and incubated under static conditions for 15 min at 37 C. The collagenase action was stopped by the addition of 50 ml ice-cold GBSS. The digested tissue was washed three times with GBSS to remove collagenase, and islets were hand picked under a stereomicroscope. Islets were maintained for 24 h in tissue culture medium RPMI 1640 containing 11 mM glucose, 10% FBS, penicillin (100 U/ml), and streptomycin (0.1 mg/ml) at 37 C and 5% CO_2. Secretion experiments were carried out on islets that showed a round, smooth surface and no attached exocrine or connective tissue. There was no morphological difference between islets from WT and SSTR2KO mice as judged by inspecting the islets under the stereomicroscope. Staining with methylene blue indicated that approximately 95% of the isolated islets survived the 24-h incubation period in RPMI 1640 medium and GBSS. Approximately 80–160 islets/pancreas were isolated with no obvious quantitative differences between islets isolated from SSTR2KO and WT animals. Batch incubations were performed in GBSS (containing 5.5 mM glucose) supplemented with 0.2% BSA and antibiotics. Islets were washed three times in GBSS and incubated in GBSS at 37 C for a 45-min equilibration period. The 45-min preincubation followed by repeated washing (37) was sufficient to reverse the effects of the hyperglycemic RPMI 1640 on insulin secretion as assessed by the low basal level and the fold increase in insulin secretion during subsequent stimulation with 20 mM D-glucose. In addition, there was no indication for the presence of significant somatostatin levels in the serum-containing RPMI 1640 based on the short half-life of somatostatin in serum (38, 39) and its potent inhibitory effects on insulin and glucagon secretion. After the 45-min preincubation, islets were washed once with GBSS and transferred into a dish containing 4 ml GBSS. Islets in batches of 40 were then preincubated with somatostatin or analogs for 45 min. Islets were divided by size into three groups: small, medium, and large. Three islets from each group were combined into individual wells. Nine islets per 1 ml GBSS were divided into quadruplicates and incubated for an additional 2 h with 20 mM L-arginine plus 20 mM KCl or 20 mM D-glucose in the continued presence of SST or analogs. Basal glucagon and insulin secretions were determined from supernatants of islets incubated for 2 h without stimulators and SST or analogs. The incubation was terminated by brief centrifugation at 1000 × *g*. Supernatants were collected and assayed immediately for insulin and glucagon content by double antibody RIA.

Determination of glucagon and insulin contents in endocrine islets

Animals were fasted overnight, with water available *ad libitum* before experiments. Isolated islets in batches of 40 were cultivated for 24 h in RPMI 1640. Islets were washed three times in GBSS and preincubated for 45 min at 37 C, followed by an additional wash in GBSS and subsequent incubation for an additional 45 min at 37 C. Islets were divided into quadruplicates (9 islets/1 ml GBSS) and incubated for an additional 2 h at 37 C. Incubation medium was carefully removed, and islets were extracted overnight at −20 C in 1ml acid-ethanol (0.2 N HCl in 70% ethanol) (40). The extract was centrifuged 12,000 × *g* at 4 C, and the hormone content of the supernatant was measured by RIA.

Data analysis

All data represent the mean ± SEM and were obtained from four to six independent experiments unless otherwise stated. Data are presented as the percentage of maximal hormone release induced with 20 mM L-arginine plus 20 mM KCl or 20 mM D-glucose unless otherwise stated. Statistical comparisons between means within a group were made using Student's paired *t* test. Comparisons between means of different groups were analyzed by one-way ANOVA with a Neuman-Keuls multiple comparison posttest. Statistical comparisons and nonlinear regression analysis were performed with Prism 2.01 (GraphPad Software, Inc., San Diego, CA). Statistical significance was defined as $P < 0.05$.

Results

Effects of SST and analogs on glucagon secretion

Basal glucagon secretion was comparable between islets isolated from WT and SSTR2KO animals (WT, 14.4 ± 1.4 pg; SSTR2KO, 11.4 ± 1.0 pg) after 2 h of incubation in GBSS (Fig. 1). The estimated secretion rate was 0.8 ± 0.07 pg/h·islet from WT mice and 0.63 ± 0.06 pg/h·islet from SSTR2KO mice. Neither SST nor nonpeptidyl agonists altered the basal glucagon secretion (data not shown). Potassium/arginine (P/A)

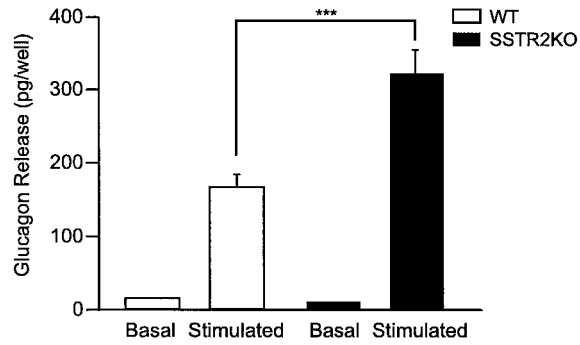

FIG. 1. Basal and P/A-stimulated glucagon secretion from islets isolated from WT and SSTR2KO mice. Islets of the same size were incubated for 2 h with 20 mM P/A. Basal glucagon secretion (WT islets, 10.54 ± 0.97; SSTR2KO islets, 9.47 ± 1.65; $P > 0.05$), P/A-stimulated glucagon secretion (STR2KO islets, 326 ± 32; WT islets, 169 ± 17; $P < 0.001$ *vs.* WT). *Bars* represent the mean (picograms of glucagon per 2 h/9 islets) ± SEM of 11 independent experiments. ***, $P < 0.001$.

at a concentration of 20 mM stimulated glucagon secretion from islets isolated from either WT or SSTR2KO mice (Fig. 1). As shown in Fig. 1, 20 mM P/A-stimulated glucagon secretion in islets isolated from SSTR2KO animals after 2 h of incubation was 326 ± 32 pg (18.1 ± 1.8 pg/h·islet), approximately 2-fold higher than that in islets from WT animals (169 ± 17 pg; 9.39 ± 0.9 pg/h·islet). Islets isolated from SSTR2KO mice showed an increased glucagon content; however, intracellular glucagon content was not statistically different compared with that in WT islets (SSTR2KO, 1.87 ± 0.42 ng/islet; WT, 1.27 ± 0.15 ng/islet; $P > 0.05$).

SST-14 potently inhibited P/A (20 mM)-stimulated glucagon secretion from WT islets with an IC_{50} value of 0.5 nM and a maximal inhibition of 84 ± 7% (Fig. 2). SST-14 inhibited glucagon release in islets isolated from SSTR2KO mice by only 27 ± 4% (Fig. 2).

SST-28 showed a similar concentration-dependent inhibition of glucagon secretion in islets from WT animals (Fig. 2). Compared with SST-14, SST-28 appeared less effective in reducing stimulated glucagon release; however, the difference was not statistically significant (IC_{50} =1.2 nM; $P > 0.05$ *vs.* SST-14). As shown in Fig. 2, the inhibitory potency of SST-28 on glucagon secretion from SSTR2KO islets was markedly reduced (100 nM SST-28, 36 ± 9% inhibition) compared with its effects on WT islets (100 nM SST-28, 73 ± 4% inhibition). The reduction of SST-14 and SST-28 effects on glucagon release in SSTR2KO islets indicates that these endogenous peptides act predominantly via SSTR2.

To confirm that the inhibition of glucagon release is mediated by SSTR2, we investigated effects of a novel nonpeptidyl SSTR2 selective compound, L-779,976 (35). A previous study demonstrated that L-779,976 displays over 10,000-fold selectivity to the human SSTR2 over SSTR1, -3, -4, and -5 (35). L-779,976 potently inhibits rat GH release *in vivo* and mouse glucagon secretion *in vitro* (35).

In our study, L-779,976 mimicked the inhibitory action of SST on P/A-stimulated glucagon release from WT islets in a concentration-dependent manner, with an IC_{50} value of 1.2 nM (Fig. 3A). The maximal inhibitory effect (72 ± 4%) was observed at the highest dose of 100 nM (Fig. 3A). L-779,976

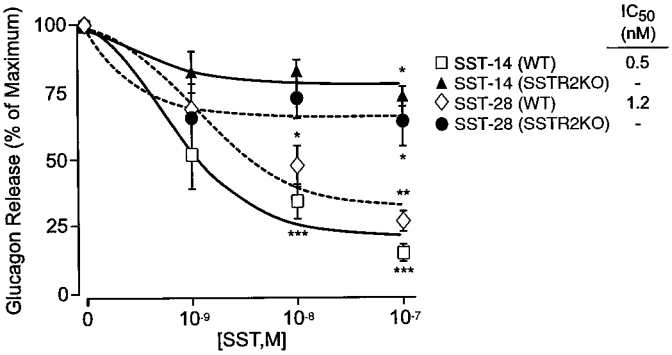

FIG. 2. Effects of SST-14 and SST-28 on P/A-stimulated glucagon secretion from WT (*squares*) and SSTR2KO (*triangles*) islets. Islets were incubated in the presence of 20 mM P/A and various concentrations of SST-14/-28 for 2 h. Data are expressed as a percentage of the maximal secretion. The maximal secretion (secretion in the presence of 20 mM P/A only) was defined as 100%. *Data points* represent the mean ± SEM of four to six independent experiments. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

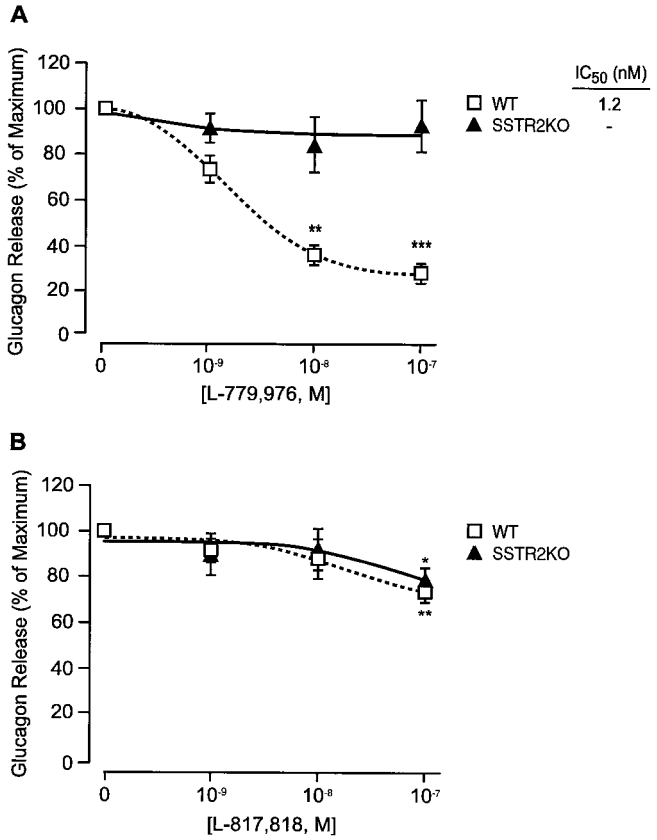

FIG. 3. Effects of L-779,976 on P/A-stimulated glucagon secretion from WT (*squares*) and SSTR2KO (*triangles*) islets (A). Effects of L-817,818 on P/A-induced glucagon secretion from WT (*squares*) and SSTR2KO (*triangles*) islets (B). Data are presented as a percentage of the maximal glucagon secretion, which was defined as 100%. Each *data point* represents the mean ± SEM of five or six independent experiments. ***, $P < 0.001$; **, $P < 0.01$; *, $P < 0.05$.

did not inhibit P/A-stimulated glucagon secretion from SSTR2KO islets (Fig. 3A). This finding provides convincing evidence that SST inhibits glucagon secretion in mouse pancreatic islets via SSTR2.

The roles of other SSTRs in regulating glucagon secretion remain unknown. In our study, SST-14 and SST-28 slightly decreased glucagon secretion from islets isolated from SSTR2KO animals, suggesting that other SSTRs are involved in this process. Previous studies in rodents have implicated SSTR5 in regulating the secretory activity of endocrine islets (30, 41). To address this question, we used a novel SSTR5 selective nonpetidyl analog, L-817,818.

L-817,818 displays highest selectivity for the human SSTR5 (35). In radioligand binding assays, L-817,818 was at least 100-fold selective for human SSTR5 compared with human SSTR2, -3, and -4, and 10-fold selective compared with human SSTR1 (35).

In the present study, L-817,818 was markedly less potent than SST-14, SST-28, or L-779,976 at reducing P/A-stimulated glucagon release (Fig. 3B). L-817,818 reduced glucagon secretion in islets from WT animals by 26 ± 5% at 100 nM, but was inactive at lower concentrations (Fig. 3B). Similar effects were observed in SSTR2KO islets (Fig. 3B).

Thus, it appears that SSTR5 may play a role in regulating

glucagon release from the murine pancreas, an idea reinforced by our results with WT and SSTR2KO islets and the SSTR5 selective compound L-817,818.

To determine whether other SSTRs are involved in the regulation of glucagon secretion, we studied the effects of the SSTR1, -3, and -4 selective SST analogs, L-797,591, L-796,778, and L-803,087 (35). None of the nonpeptidyl SST agonists showed any effect on P/A-stimulated glucagon secretion in islets isolated from WT or SSTR2KO animals (data not shown), suggesting that murine glucagon secretion is predominantly controlled by SSTR2.

Effects of SST and analogs on insulin secretion

We next investigated which SSTR regulates insulin secretion in the mouse pancreas. Islets isolated from WT mice showed insulin content comparable to that in islets from SSTR2KO animals (WT, 76 ± 5.1 ng/islet; SSTR2KO, 87 ± 5.3 ng/islet; $P > 0.05$). Basal insulin secretion from WT and SSTR2KO islets was similar after 2 h of incubation (WT, 29 ± 11 pg/h·islet; SSTR2KO, 42 ± 13 pg/h·islet; $P > 0.05$).

Basal insulin secretion was unaffected by either SST or the nonpeptidyl agonists after 2-h incubation (data not shown). We therefore investigated the effects of SST and analogs on D-glucose (20 mM)-stimulated insulin secretion. A 2-h incubation with 20 mM D-glucose led to an approximately 5-fold increase in insulin secretion from WT and SSTR2KO islets (WT, 150 ± 39 pg/h·islet; SSTR2KO, 210 ± 57 pg/h·islet; $P > 0.05$).

As shown in Fig. 4A, SST-28 inhibited glucose-stimulated insulin secretion from both WT and SSTR2KO islets. Maximal inhibition (55 ± 5%) of insulin release was observed at the highest tested concentration of 100 nM SST-28 (Fig. 4A). SST-14 inhibited glucose-induced insulin secretion with a similar potency as SST-28 in islets from WT and SSTR2KO mice (Fig. 4B). SST-14 and SST-28 also displayed similar effects on P/A (20 mM)-stimulated insulin secretion (data not shown).

The SSTR2 selective agonist L-779,976 slightly reduced glucose-induced insulin secretion from WT islets by approximately 16 ± 4% at the highest dose of 100 nM, an effect that was statistically significant compared with the value in untreated controls (Fig. 5A). However, L-779,976 did not statistically significant inhibit insulin secretion from SSTR2KO islets (Fig. 5A).

SSTR1, -3, and -4 selective agonists were inactive on stimulated insulin secretion in islets from WT and SSTR2KO animals (data not shown), indicating that SSTR1, -3, and -4 do not mediate SST inhibition of insulin secretion. We therefore investigated the effects of an SSTR5 selective agonist L-817,818 on insulin release. L-817,818 inhibited glucose-induced insulin secretion in WT islets by 42 ± 8% at 100 nM (Fig. 5B), an effect that was similar to that observed in SSTR2KO islets (Fig. 5B). Thus, our insulin secretion data suggest that SSTR5 is primarily responsible for SST inhibition of insulin release in mouse endocrine islets.

Taken together our data support the current hypothesis that SST regulates insulin secretion from β-cells via a different SSTR than the subtype that controls glucagon secretion from α-cells. Our results with SSTR2KO mice and receptor

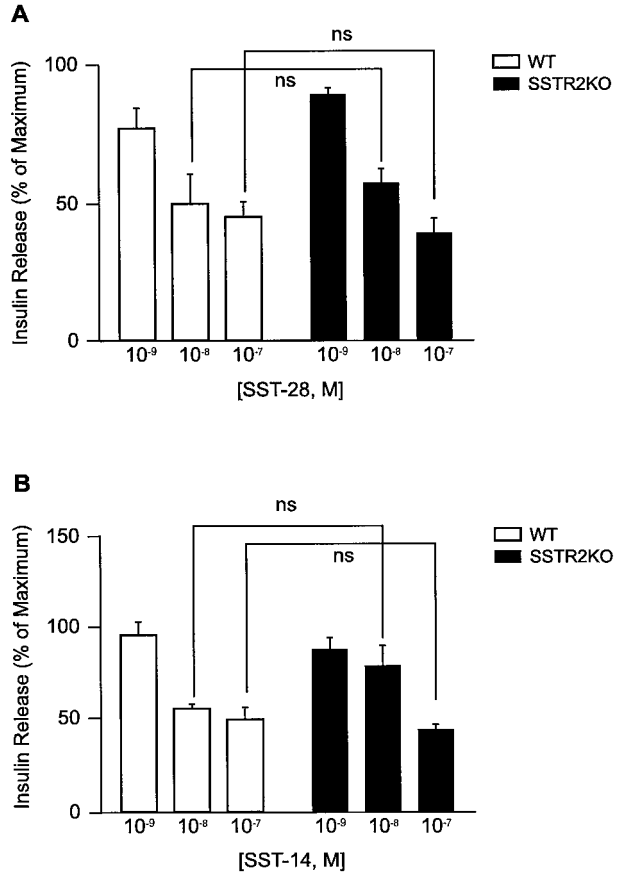

FIG. 4. A, Effects of SST-28 on glucose (20 mM)-stimulated insulin secretion from WT and SSTR2KO islets. B, Effects of SST-14 on glucose-stimulated insulin secretion from WT and SSTR2KO islets. Data are presented as a percentage of the maximal hormonal secretion, which was defined as 100%. *Bars* represent the mean ± SEM of four independent experiments. ns, Not significant ($P > 0.05$).

subtype selective SST agonists show that SSTR2 primarily mediates SST inhibition of glucagon, whereas SSTR5 is the principal subtype involved in the SST inhibition of insulin secretion.

Discussion

SST inhibits glucagon and insulin release in endocrine islets by interacting with membrane somatostatin receptors (28, 42, 43). The expression of three of the five known SSTRs, SSTR2 (16, 32, 33), SSTR3 (13, 15), and SSTR5 (15, 30, 41), in the endocrine pancreas was previously reported. SSTR2 has been identified in rat endocrine islets by immunohistochemistry, RT-PCR analysis, and Northern blot hybridization, whereas pharmacological studies have demonstrated its biological function (14, 16, 32). A recent immunohistochemical study in the rat demonstrated that SSTR2 is expressed at high levels on α-cells, whereas it is barely detectable on β-cells (32), suggesting a selective role for this receptor in regulating glucagon release. An *in vivo* study of the rat using SSTR2 selective peptidergic analogs implicated a role for SSTR2 in inhibition of glucagon release from pancreatic islets (29).

Our *in vitro* study, using a combination of islets from SSTR2 knockout animals and subtype selective SST analogs,

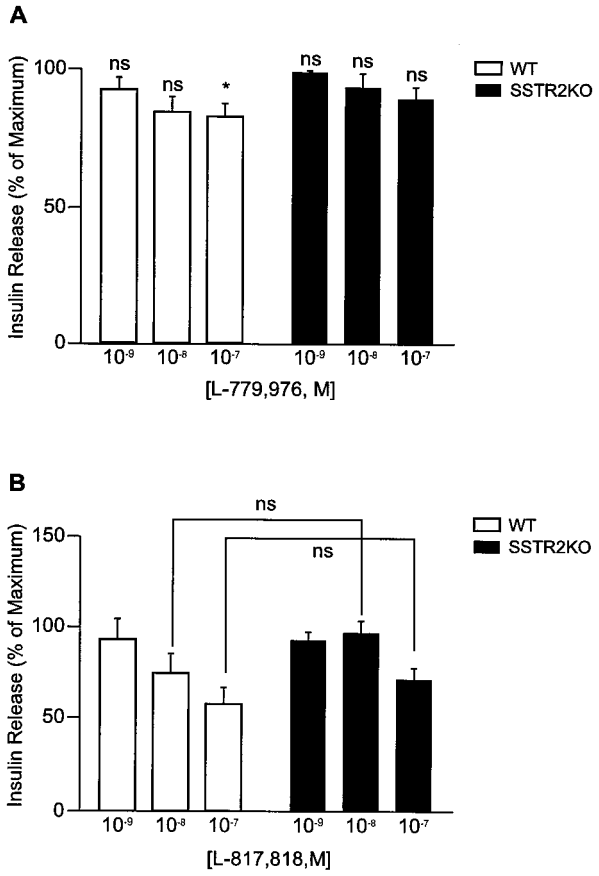

FIG. 5. A, Effects of L-779,976 on glucose (20 mM)-stimulated insulin release from WT and SSTR2KO islets. B, Effects of L-817,818 on glucose-stimulated insulin secretion from WT and SSTR2KO islets. Data are expressed as a percentage of the maximal insulin secretion. The maximal secretion (secretion in the presence of 20 mM glucose only) was defined as 100%. *Bars* represent the mean ± SEM of four independent experiments. *, $P < 0.05$; ns, not significant ($P > 0.05$).

demonstrates that SSTR2 mediates the inhibitory action of SST-14 on glucagon release in murine pancreatic islets. These findings are in concordance with previous pharmacological and morphological findings in rodents; however, earlier studies have relied on peptidyl analogs of SST-14, which may lack the desired specificity at a given receptor subtype (25, 44).

Several lines of evidence in our study support the idea that SSTR2 controls glucagon secretion in mouse endocrine islets. First, SST-14 potently inhibited glucagon release in islets isolated from WT animals up to 85%, whereas the effect was reduced to 27% in islets lacking SSTR2. Similarly, SST-28 decreased glucagon release in islets isolated from WT mice, but was much less potent in islets from SSTR2KO animals. The inhibitory role of SSTR2 on glucagon release in rodents is based merely on an *in vivo* study (29). It was demonstrated in rat pancreas that infusion of the SSTR2 selective peptide analog NC8–12 inhibited glucagon release (29). However, the inhibition of glucagon release did not correlate with the predicted potency that was observed in studies with cells heterologously expressing SSTR2. Moreover, NC8–12, although with reduced potency, also inhibited insulin release (29). In the same study another peptidyl analog, DC25–100, with approximately 10-fold higher affinity for SSTR5 than SSTR2, showed more potent inhibition of glucagon release than NC8–12 (29). Thus, it was not entirely clear from this study which receptor subtype plays a major role in the regulation of glucagon and insulin release.

In our study, however, SST potently inhibited glucagon release in islets isolated from WT animals, but its potency on glucagon secretion from SSTR2KO islets was dramatically reduced, indicating that SSTR2 plays the major role in this process. In addition, our data for SSTR2KO animals provide the first evidence that SSTR2 expressed on glucagon-producing cells mediates inhibition of glucagon release by the endogenous peptides SST-14 and SST-28.

An additional finding in our study was that P/A-stimulated glucagon secretion in SSTR2KO islets was higher than that in WT islets. As endocrine islets of the pancreas contain SST-14-producing δ-cells, we believe that this difference could be explained by the inhibitory action of intrapancreatic secreted SST (45) via SSTR2 in WT islets. In SSTR2KO islets, however, the inhibitory effect of intrapancreatic SST on stimulated glucagon secretion is abolished.

The structural organization of the rat pancreatic islets with glucagon-producing α-cells in proximity to somatostatin-producing δ-cells (46–48) makes a paracrine inhibitory action of SST via SSTR2 likely. However, as static incubation leads to accumulation of all secreted hormones in the incubation medium, more detailed studies, *e.g.* perfusion or perifusion of endocrine islets, would provide additional support for the hypothesis of paracrine action of SST on α-cells via SSTR2.

Recently, nonpeptidyl SST agonists with potent selectivity for all five human SSTRs have been developed (35, 49). However, their selectivity for rodent SSTRs has not yet been demonstrated. Recent studies on cells stably transfected with mouse SSTR2 show similar selectivity of SSTR2 and SSTR5 agonists as reported for human SSTRs (Strowski, M. Z., A. D. Blake, and J. M. Schaeffer, unpublished results). Whether this selectivity is retained for the mouse SSTR5 is currently under investigation (Strowski, M. Z., A. D. Blake, H. A. Wilkinson, M. P. Dashkevicz, M. Kohler, and J. M. Schaeffer, unpublished results).

In the present study the SSTR2 selective nonpeptidyl analog L-779,976 provides additional evidence that SSTR2 regulates glucagon release. L-779,976 inhibited glucagon secretion in WT islets, but was ineffective in SSTR2KO islets. The fact that L-779,976, as a potent and selective human SSTR2 agonist, was inactive in SSTR2KO islets suggests that L-779,976 has much higher selectivity for SSTR2 compared with other currently known peptidyl analogs.

Third, the SSTR1, SSTR3, and SSTR4 selective analogs did not show inhibitory effects on glucagon release despite potent activity in heterologous expression studies (35). This finding supports the hypothesis that SSTR1, SSTR3, and SSTR4 do not mediate the inhibitory action of SST on stimulated glucagon release in mouse endocrine islets, confirming data from an earlier *in vivo* study (29).

The inhibitory effect of L-817,818 suggests a minor role for this receptor in controlling glucagon secretion from α-cells. L-817,818 reduced stimulated glucagon release in mouse pancreatic islets, but the effect was substantially lower than

that observed with L-779,976. However, the present study does not rule out that SSTR5 might be expressed on rodent α-cells at low levels, but cannot be detected by conventional immunocytochemistry techniques (41) or in previous pharmacological studies (30). L-817,818 at the highest concentration of 100 nM may interact *in vivo* with SSTR2. However, as L-817,818 inhibited glucagon secretion in islets from WT and SSTR2KO mice at comparable potencies, it appears less likely that L-817,818 interacts with SSTR2. The possibility of interaction of L-817,818 with SSTR1 (L-817,818 displays only a 10-fold selectivity for hSSTR5 over hSSTR1) (35) was also ruled out, as we did not detect inhibition of the SSTR1 selective analog L-797,591 on glucagon secretion. However, as endocrine islets of the pancreas contain at least four distinct hormonal active cell types, we cannot rule out indirect effects of L-817,818 to reduce glucagon secretion.

Inhibition of insulin secretion by SST is believed to be mediated by SSTR5 (30, 50). SSTR5 mRNA was found in the whole pancreas (15), and a recent immunohistochemical study on rat endocrine islets detected presence of SSTR5 on β-cells (41). A pharmacological *in vitro* study of murine pancreatic islets demonstrated that an SSTR5 selective analog, DC32–92 (BIM23052), significantly reduced glucose-stimulated insulin release, whereas an SSTR2 selective analog, NC8–12, did not (30). An *in vivo* study in the rat demonstrated that a SSTR5 selective analog, DC 23–99, decreased insulin secretion, whereas a SSTR2 selective analog, NC8–12, did not (50). In our study, L-817,818, a SSTR5 selective SST analog, inhibited glucose-stimulated insulin secretion in islets from WT and SSTR2KO mice. These results support the role of SSTR5 in inhibiting rodent insulin release.

The present study shows that SSTR2 does not play a major role in inhibiting insulin release from mouse pancreatic islets despite a slight inhibitory effect of L-779,976 in WT islets. Our functional data are supported by immunological studies showing barely detectable SSTR2 expression on β-cells in the pancreatic islets in rodents (32). Both SST-14 and SST-28 inhibited insulin secretion from WT and SSTR2KO islets with comparable potencies in our study, providing additional support that SSTR2 does not play a crucial role in insulin secretion. However, data from human pancreatic islets suggest that species differences for SSTR expression may exist (33). Thus, further studies are necessary to clarify the role of the individual SSTRs in regulating hormonal secretion of the endocrine islets.

In conclusion, our data show for the first time that endogenous peptides SST-14 and SST-28 inhibit glucagon and insulin secretion from mouse pancreatic islets mainly via SSTR2 and SSTR5, respectively. We demonstrated an *in vitro* inhibition of glucagon secretion using nonpeptidyl SST analogs with potent selectivity for human SSTRs. Our data support the idea that SSTRs have distinct roles in the rodent pancreas (29, 30, 32, 50). Our study using a combination of somatostatin receptor subtype knockout animal and subtype selective SST agonists provides the first functional evidence for the crucial role of the SSTR2 in regulating glucagon secretion and of SSTR5 in mediating SST inhibition of insulin secretion.

Acknowledgments

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