Somatostatin secreted by islet δ-cells fulfils multiple roles as a paracrine regulator of islet function.

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ABSTRACT

Objective: Somatostatin (SST) is secreted by islet δ-cells and by extra-islet neuroendocrine cells. SST receptors have been identified on α- and β-cells, and exogenous SST inhibits insulin and glucagon secretion, consistent with a role for SST in regulating α- and β-cell function. However, the specific intra-islet function of δ-cell SST remains uncertain. We have used Sst^-/- mice to investigate the role of δ-cell SST in the regulation of insulin and glucagon secretion in vitro and in vivo.

Research Design and Methods: Islet morphology was assessed by histological analysis. Hormone levels were measured by radioimmunoassay in control and Sst^+/- mice in vivo and from isolated islets in vitro.

Results: Islet size and organisation did not differ between Sst^-/- and control islets, nor did islet glucagon or insulin content. Sst^-/- mice showed enhanced insulin and glucagon secretory responses in vivo. In vitro stimulus-induced insulin and glucagon secretion was enhanced from perifused Sst^-/- islets compared to control islets and was inhibited by exogenous SST in Sst^+/- but not control islets. No difference in the switch-off rate of glucose-stimulated insulin secretion was observed between genotypes but the cholinergic agonist carbamylicholine enhanced glucose-induced insulin secretion to a lesser extent in Sst^-/- islets compared to controls. Glucose suppressed glucagon secretion from control but not Sst^-/- islets.

Conclusions: We suggest that δ-cell SST exerts a tonic inhibitory influence on insulin and glucagon secretion which may facilitate the islet response to cholinergic activation. In addition, δ-cell SST is implicated in the nutrient-induced suppression of glucagon secretion.
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Islets of Langerhans are heterogeneous cell aggregates containing β, α, δ and PP cells which secrete insulin, glucagon, somatostatin (SST) and pancreatic polypeptide, respectively. The different cell types within the islet are affected by changes in the extracellular glucose concentration. Thus, elevations in circulating glucose stimulate insulin secretion from islet β-cells and inhibit glucagon secretion from α-cells as part of the reciprocal regulation of blood glucose by insulin and glucagon. SST secretion from islet δ-cells is also stimulated by increased extracellular glucose, although the threshold concentration for δ-cells to respond to glucose is lower than that for β-cells (1), and the ionic events in stimulus-response coupling differ between β- and δ-cells (2).

Rodent islets have a defined architecture with a β-cell core surrounded by a mantle of non-β-cells (3), whereas human islets do not show such pronounced anatomical subdivisions (4). The anatomical organization of islets is important for their correct functioning, and there is much evidence to suggest that cell-cell interactions within islets are crucial for normal function (5-9). Interactions between islet β-cells have been investigated extensively and we have previously demonstrated the importance of homotypic β-cell interactions in the regulation of insulin secretion (10;11). Similarly, there have been numerous studies of possible interactions between α- and β-cells within islets (9;12-15). Less attention has been paid to the possible intra-islet roles of δ-cell-derived SST. δ-cells comprise 5-10% of the islet endocrine cells (3) but the peptide hormone SST is also synthesized and secreted by neuroendocrine cells in the central nervous system and the gastrointestinal system, and the latter is the major contributor to circulating SST (16;17). SST often acts as an inhibitory regulator of endocrine systems, for example as a hypothalamic factor to suppress growth hormone secretion from the anterior pituitary (18), or as a local inhibitor of the release of gastrointestinal polypeptide hormones (19). SST receptors (SSTR1-5) have been identified on both α- and β-cells (20) and exogenously administered SST or SST analogues inhibit glucose-induced insulin secretion and arginine-induced glucagon secretion both in vitro and in vivo (21-24). In addition, studies using SSTR-deficient mice (SSTR1, 2 or 5) revealed changes in both basal and stimulated insulin secretion (21;25;26). However, studies using SSTR knock-out models are difficult to interpret since mouse islets express all five SSTRs (20) and reduced expression of one receptor sub-type may be compensated for by the over-expression of another. Therefore, although current data are consistent with a negative regulatory role for SST in islet secretory function it is unclear whether this effect can be ascribed to circulating SST or to locally released δ-cell SST.

The study of δ-cell function within islets is complicated by the possibility of multiple heterotypic interactions between islet cell types in experiments using intact primary islets. Studies of islet-SST have also been hampered by the lack of selective and potent SST receptor antagonists. To investigate the role of locally released δ-cell SST we have therefore used a mouse model in which disruption of the SST gene produced a SST-deficient phenotype (27). Our results suggest that locally released δ-cell SST exerts a tonic inhibitory influence on insulin and glucagon secretion and that...
this inhibitory effect of δ-cell SST may be involved in facilitating the insulin secretory response to cholinergic activation and the nutrient-induced suppression of glucagon secretion.

**RESEARCH DESIGN AND METHODS**

**Animals.** Sst\(^{-/-}\) mice on a C57BL/6J background were generated as described (27) and subsequently re-derived onto a CBA/Ca x C57BL/10 F1 background by back crossing to CBA/Ca x C57BL/10 F1 mice for 10 generations, followed by intercrossing to generate Sst\(^{-/-}\) mice on a mixed CBA/Ca x C57BL/10 F1 background. Age-matched CBA/Ca x C57BL/10 F1 mice served as controls.

**Islet isolation.** Islets from 8-12 week old female Sst\(^{-/-}\) or control mice were isolated by collagenase digestion (1 mg/ml, type XI, Sigma, UK) and separated from exocrine pancreatic tissue on a histopaque gradient (Sigma, UK). Islets were incubated overnight at 37°C (5% CO\(_2\)) in RPMI 1640 medium (10% FBS, 2% glutamine, 100 U/ml penicillin/0.1 mg/ml streptomycin, 11 mM glucose) prior to experiments.

**PCR.** RNA was extracted from islets using an RNAeasy kit (Qiagen, Sussex, UK). Reverse transcription to cDNA and RT-PCR amplification were performed as described previously (28) using the primer sequences shown in Table 1. All reactions were carried out for 40 cycles. Products were separated on agarose gels and visualised by staining with ethidium bromide (0.5 μg/ml).

**Immunohistochemistry and morphometric analysis.** An estimate of islet size and number per mm\(^2\) pancreas was obtained by morphometric analysis of fixed pancreatic sections labeled for insulin by immunohistochemistry. The entire paraffin-embedded pancreas from each animal was cut into 5μm sections onto microscope slides and sections selected for staining and morphometry. Sections were systematically sampled throughout the entire pancreas to give 20 sections per animal for analysis. Sections were de-waxed, hydrated and labeled with a mouse monoclonal antibody to insulin (Sigma, UK) and positive cells visualized with a peroxidase-conjugated secondary antibody to mouse IgG and diaminobenzidine as substrate. Sections were counter-stained with haematoxylin and digital images of sections used for image analysis. Diameters of islets and area of insulin- and glucagon-labelled cells within islets on each section were measured with the aid of image analysis software (ImageJ). Numbers of islets, defined as clusters of 5 or more β-cells on the section, were determined relative to pancreatic area. For assessment of the organization of α- and β-cells within the islets, 10 sections per animal were labeled with mouse monoclonal antibodies to glucagon (Sigma) or insulin, respectively, and positive cells visualized as described above.

**Insulin and glucagon secretion. In vivo studies.** 8-12 week old female mice were fasted overnight prior to the experiments. The animals were anesthetized with sodium pentobarbital (667 µg/100 µl/10g mouse i.p.) and a cannula was inserted into the jugular vein. Glucose (0.5g/kg) or arginine (0.25g/kg) was injected and blood samples were withdrawn at time points indicated in the figure legend. For insulin clearance tests non-fasted mice were injected i.v. with insulin (0.4 U/kg) and blood samples were withdrawn at time points indicated in the figure legend. Samples were centrifuged and blood glucose measured in the plasma using a glucose analyzer (Analox).

**In vitro studies.** For measuring the dynamics of insulin or glucagon release
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islets were transferred into chambers of a temperature-controlled multi-channel perifusion system as described previously (29) and perfused for 70 min at 37°C with a bicarbonate-buffered physiological salt solution (30) containing 2 mM glucose. The tissue was subsequently perfused (0.5 mI/min) with salt solution containing agents of interest and fractions were collected at 2 min intervals or as indicated on graphs. For static secretion experiments islets were pre-incubated for 60 min in buffer containing 2 mM glucose (or 1 mM for SST secretion experiments) after which batches of 15 islets were incubated for 60 min in 0.5 mI salt solution containing agents of interest. The somatostatin 14 isoform (SST-14, Bachem, UK) was used as an exogenous source of SST. For assessment of islet hormone content islets were pelleted by centrifugation, washed with PBS, lysed in acidified ethanol and sonicated.

Hormone immunoassays. Insulin and glucagon content of incubation medium and islet extracts, and insulin content of plasma samples were assessed by radioimmunoassay (RIA) using in-house assays, as described (31;32). SST and plasma glucagon levels were measured using commercially-available RIA kits (SST: Euro-Diagnostica, Sweden; Glucagon: Linco Ltd, St Charles, USA).

Data analysis. Data are expressed as means ± SEM and analyzed statistically using Student’s t test, one or two way ANOVA and Bonferroni’s multiple comparisons test, as appropriate. For statistical analysis of dynamic hormone release, data were expressed as the area under the curve (AUC) after subtraction of basal secretion values. Differences between treatments were considered significant at P<0.05.

RESULTS

Phenotypic characterisation of control and Sst⁺⁺ mice, pancreata and islets. There were no significant differences in growth or body weight in the Sst⁺⁺ female mice vs controls at the ages studied (20 ±0.5g vs 20.9 ±0.5g, Sst⁺⁺ vs control, n=6, P>0.2), nor did the Sst⁺⁺ animals show any overt behavioural dissimilarities or any differences in resting normoglycaemia (8.1±0.28 vs 7.4±0.14 mM glucose, Sst⁺⁺ vs control, n=36-41, P>0.2). Insulin tolerance tests showed no differences in sensitivity, or maximal glucose suppression between female Sst⁻⁻ and controls (see Fig. 2c, P>0.2). Histological analysis of pancreatic sections found no significant differences in the size distribution of islets in control and Sst⁺⁺ pancreata (control: 97.2±3.5 μm; Sst⁺⁺: 95.6±4.7, n=88-217 islets from 3 animals per group, P>0.2) nor in the number of islets per mm² pancreas (control: 0.739±0.054 islets/mm², Sst⁺⁺: 0.617±0.064, n=3, P>0.2). Furthermore, the ratio of α− to β−cell area (control: 0.267±0.031; Sst⁺⁺: 0.282±0.048, n=32-34 sections from 3 animals per group, P>0.2) and the cellular organisation of α− and β−cells within the islet was similar in islets from control and Sst⁺⁺ animals. Thus, in both control and Sst⁺⁺ islets glucagon-positive α-cells were located primarily at the periphery while β-cells formed a central core (Fig. 1).

In vivo islet hormone secretion from control and Sst⁺⁺ mice. To assess the importance of SST in regulating islet hormone release we initially examined the in vivo islet hormone secretory responses in female mice in the presence or absence of endogenous SST. No differences were detected in basal plasma levels of insulin and glucagon (Fig. 2, P>0.2), but plasma levels of both hormones were significantly elevated in Sst⁺⁺ mice when compared to control.
mice following intravenous administration of glucose (0.5 g/kg, Fig. 2a, P<0.01) or arginine (0.25 g/kg, Fig. 2b, P<0.001), respectively. In separate experiments plasma insulin levels were also enhanced in male Sst⁻/⁻ mice compared to control animals in response to glucose (data not shown).

**In vitro hormone secretion from control and Sst⁻/⁻ islets.** To determine whether the enhanced secretory responses in Sst⁻/⁻ mice could be attributable to a specific lack of δ-cell SST rather than a global absence of circulating SST, *in vitro* secretion studies were carried out using islets isolated from female Sst⁻/⁻ and control mice, as shown in Figure 3. There was no significant difference between the basal rate of hormone secretion from control and Sst⁻/⁻ islets (Fig. 3 a-c, P>0.2), whereas stimulus-induced insulin and glucagon release was significantly enhanced in the Sst⁻/⁻ islets (Fig. 3a-d). Thus, Sst⁻/⁻ islets showed approximately two-fold greater insulin and glucagon secretory responses to elevations in glucose and arginine, respectively, compared to control islets (glucose: P<0.05, arginine: P<0.001). The augmented secretory responses did not reflect increased islet hormone content since the content of both insulin (control: 66.0±8.6 ng/islet; Sst⁻/⁻: 50.0±7.2, n=6, P>0.1) and glucagon (control: 3.5±0.5 ng/islet; Sst⁻/⁻: 2.8±0.5, n=7, P>0.1) did not differ significantly between genotypes. As a consequence, the differences in secretory responses between genotypes were observed whether secretion was expressed as a rate (Fig. 3a) or as a percentage of total insulin content (Fig. 3b). Enhanced insulin secretion from Sst⁻/⁻ islets was not dependent on a nutrient stimulus, since similar effects were observed when secretion was initiated by the sulphonylurea tolbutamide, which bypasses glucose transport and metabolism and stimulates insulin secretion by direct effects on plasma membrane ATP-sensitive K⁺ channels (Fig. 3d, Sst⁻/⁻ vs control: P<0.01). Thus the first-phase of tolbutamide-induced insulin secretion was improved in Sst⁻/⁻ islets compared to control islets. Parallel experiments using control islets demonstrated that δ-cells also responded to the secretagogues which stimulate insulin and glucagon secretion, as shown in Figure 3e. Accordingly, glucose, tolbutamide and arginine all caused significant increases in SST secretion from islets *in vitro* by 4, 4.5 and 2-fold, respectively.

RT-PCR measurements confirmed the expression of mRNAs for SST receptor subtypes 1-5 in both control and Sst⁻/⁻ islets (Table 1). Exogenous SST (1 µM) had no significant effect *in vitro* on stimulus-dependent insulin (Fig. 4a) or glucagon (Fig. 4b) secretion from control islets (P>0.2), but exerted a marked inhibition of secretion of both hormones from Sst⁻/⁻ islets (Fig. 4a, P<0.001). **Insulin secretion from control and Sst⁻/⁻ islets following removal of glucose stimulus.** To investigate whether δ-cell SST facilitates the rapid cessation of insulin secretion when normoglycaemia is achieved we measured the rate of insulin secretion from perifused islets *in vitro*. As shown in Figure 5, the rate at which insulin secretion declined to basal levels upon removal of glucose (20 mM) was not decreased in Sst⁻/⁻ islets compared to control islets (Fig. 5a). Similarly, no differences in the switch-off rate of insulin secretion were detected when insulin secretion was expressed as a percentage of stimulated response, to take into account the different magnitude of glucose-induced stimulation in the two genotypes (Fig. 5b).
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Parasympathetic stimulation of insulin secretion from control and Sst⁻/⁻ islets. Figure 3e shows that glucose, arginine and tolbutamide all stimulated SST secretion from δ-cells, consistent with enhanced insulin and glucagon release in response to these stimuli in the absence of δ-cell SST. However, as shown in Figure 6a-b, activation of islet cholinergic receptors had opposite effects on insulin and SST secretion, inducing the expected enhancement of glucose-induced insulin secretion (P<0.001, Fig. 6a), while causing a concomitant inhibition of glucose-induced SST secretion (P<0.01, Fig. 6b). These observations are consistent with the effects of carbamylcholine (CCh), an acetylcholine analogue, on insulin secretion from control and Sst⁻/⁻ islets, as shown in Figure 6c. Thus, both control and Sst⁻/⁻ islets showed an amplification of glucose-induced insulin secretion in response to CCh (Fig. 6c) with no detectable differences in the rate of onset of CCh-induced insulin secretion. However, the extra magnitude of the secretory response of Sst⁻/⁻ islets to CCh was less than that of control islets, when compared to the glucose-induced secretory response alone (Fig. 6d).

Lack of glucose-induced suppression of glucagon secretion in Sst⁻/⁻ islets. Our studies using Sst⁻/⁻ islets also revealed an important role for δ-cell SST in the inhibition of glucagon secretion in response to increases in plasma glucose. Thus, glucose-induced suppression of glucagon secretion in vitro was not detected in Sst⁻/⁻ islets (Fig. 7), although it was present in control islets at both high (Fig. 7a,c) and intermediate (Fig. 7c,d) glucose concentrations in both static and dynamic secretion experiments. Figure 7b shows the release of insulin in response to glucose in the same experiment as Figure 7a.

DISCUSSION Although SST is acknowledged as an inhibitor of insulin and glucagon secretion, the physiological relevance of δ-cell SST remains unknown. Our in vivo data showing that the global absence of SST in Sst⁻/⁻ mice resulted in increased glucagon and insulin secretion in response to nutrient stimuli confirmed the importance of SST as a negative regulator of islet α- and β-cell function but did not identify δ-cells as the source of that SST. However, in our in vitro studies the isolated islets were removed from neural, neuroendocrine and gastrointestinal sources of SST, and this enabled us to focus on the functional consequences of an absence of islet SST. Overall, the lack of any significant differences in islet size, hormone content or morphology between Sst⁻/⁻ and control islets suggested that the observed differences in hormone secretion are most likely due to the absence of SST. Consequently our in vitro studies demonstrated that the lack of locally-released δ-cell SST had a profound effect on both insulin and glucagon secretion, leading to enhanced levels of stimulus-induced release of both hormones from Sst⁻/⁻ islets compared to control islets, without affecting basal levels of hormone secretion. Thus, while we cannot rule out an effect of circulating SST of neuroendocrine and/or gastrointestinal origin on islet function in vivo, our in vivo and in vitro studies are consistent with an important role for islet SST in the regulation of β- and α-cell secretory function. Our direct measurements of SST secretion in vitro also confirmed previous reports that some insulin and/or glucagon secretagogues, including
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Glucose, arginine and tolbutamide, also stimulate the secretion of SST from δ-cells (1;33;34). Taken together, our data are consistent with a model in which SST released from islet δ-cells exerts direct, tonic inhibitory effects on glucagon and insulin secretion from neighbouring α- and β-cells. Under resting conditions the quiescent δ-cells exert little or no influence on the neighbouring α- and β-cells and no difference is observed between control and Sst -/- islets with regard to basal secretion, nor in the basal levels of plasma insulin and glucagon between control and Sst -/- mice. However, exposure of islets to glucose or sulfonylureas activates both β-cells and δ-cells (1;34), which initiates an insulin secretory response and simultaneously causes the local release of SST to limit that response. Similarly, the stimulation of glucagon secretion by arginine is accompanied by both the activation of δ-cells (33) and the intra-islet release of SST to limit the α-cell secretory response. In this way both in vitro and in vivo results are consistent with δ-cell SST exerting an inhibitory paracrine influence on islet α- and β-cells. The route through which δ-cell SST reaches α- and β-cells is uncertain – early reports on islet blood flow do not support a vascular route from δ-cells to α- and β-cells (35;36), although other data do implicate a direct vascular route from δ- to β-cells (37). Alternatively, SST delivery may be mediated by diffusion via interstitial compartments (38;39) independent of islet vasculature.

An extensive exploration of the mechanism of action of islet SST was beyond the scope of this study but it is of interest that the first phase of insulin release was enhanced in Sst -/- islets, perhaps suggesting a direct or indirect effect via the ATP-sensitive K+ channels.

The proposed model of paracrine regulation of islet function by δ-cell SST is further supported by our observations that exogenous SST had no significant effect in vitro on stimulus-dependent insulin or glucagon secretion from control islets, but exerted a marked inhibition of secretion of both hormones from Sst -/- islets. The lack of effect of exogenous SST on hormone secretion from control islets is consistent with a tonic inhibition by endogenous δ-cell SST of islet hormone secretion that cannot be further repressed by the provision of exogenous SST. In contrast, the absence of this inhibitory input by endogenous SST in Sst -/- islets reveals the ability of exogenous SST to inhibit both insulin and glucagon secretion. An experimental model in which δ-cell SST prevents further inhibition by exogenous SST would account for the reported (22) and anecdotal variability of the effects of SST on islet hormone secretion in studies using isolated islets, since the inhibition of hormone secretion by exogenous SST will be inversely related to the content of endogenous SST in islet δ-cells, which are easily lost or damaged during islet isolation because of their location on the periphery of the islets, particularly in rodents (40).

The intra-islet inhibitory input from δ-cells is likely to be involved in fine-tuning β- and α-cell responses to external signals: the regulation of homeostatic responses through a balance between excitatory and inhibitory inputs has many parallels in physiology, and would enable the precise degree of control required for the endocrine regulation of plasma glucose within strict limits. We considered first whether δ-cell SST was employed to ensure the rapid cessation of insulin secretion on achieving normoglycaemia (41). However, our in vitro measurements demonstrated that the rate of decline of
insulin secretion on removal of glucose was not decreased in Sst$^{-/-}$ islets compared to control islets, suggesting that δ-cell SST does not regulate the termination of insulin secretory responses to glucose.

Although many insulin secretagogues also stimulate SST release from δ-cells we found that the receptor-operated cholinergic agonist, carbamylcholine (CCh), enhanced glucose-induced insulin secretion but caused a marked inhibition of glucose-induced SST secretion from control islets, as has been suggested by some previous studies (42;43). The proposed model of paracrine regulation of insulin secretion by δ-cell SST would therefore predict that activation of islet cholinergic receptors in the presence of stimulatory concentrations of glucose enhances insulin secretion both by direct stimulatory effects on β-cells, and by relieving the inhibitory δ-cell input as a consequence of the CCh-mediated inhibition of glucose-induced SST secretion. In this model the effect of cholinergic activation to enhance a maximum glucose-induced insulin secretory response will be reduced in the absence of SST because the tonic inhibitory effect of SST on glucose-induced insulin secretion will be absent and will therefore not be alleviated by CCh. Our in vitro studies using Sst$^{-/-}$ islets concur with this prediction. Thus, both control and Sst$^{-/-}$ islets showed an amplification of glucose-induced insulin secretion in response to CCh to achieve similar maximum rates of secretion. However, the extent of the CCh-induced component of the secretory response in Sst$^{-/-}$ islets was much less than that of control islets, when compared to their maximum responses to glucose alone. The overall effect of the presence of δ-cell SST was therefore to enhance the amplitude of the CCh-induced component of the secretory response. We propose that one physiological function of the tonic inhibitory input of δ-cell SST on islet β-cells is to ensure that a relatively transient stimulatory input via parasympathetic activation results in large changes in the overall mass of insulin secreted for the duration of the cholinergic activation.

Finally, our in vitro experiments using Sst$^{-/-}$ islets revealed a further role for δ-cell SST in the inhibition of glucagon secretion in response to increases in extracellular glucose. Various mechanisms have been proposed for the inhibitory effects of glucose on α-cell secretory function, including direct effects of glucose on α-cells (1;44) or paracrine inhibitory effects of β-cell secretory products such as insulin (14), Zn$^{2+}$ (13) and γ-aminobutyric acid (GABA)(45). In our experiments using Sst$^{-/-}$ islets glucose-induced suppression of glucagon secretion was not detected, although it was observed in control islets over a range of glucose concentrations, suggesting that glucose-induced SST release is a major contributor to the inhibitory effects of glucose on islet α-cells. Consistent with this, it has been reported recently that the concentration dependency of glucose-induced inhibition of glucagon secretion correlates closely to that for the stimulation of SST secretion, but is dissociated from that for β-cell activation (1). Our results therefore suggest an important role for δ-cell SST in the regulation of glucagon secretion by glucose, but do not rule out the involvement of other regulatory mechanisms.

In summary, our studies using Sst$^{-/-}$ mice support important intra-islet paracrine roles for δ-cell-derived SST in the regulation of islet hormone secretion. Intra-islet SST exerts tonic inhibitory
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effects on stimulus-induced insulin and glucagon secretion, which may be important in regulating responses to receptor-operated stimuli, such as cholinergic agonists. Our studies also suggest that δ-cell SST also plays a significant role in the suppression of glucagon secretion by glucose. These observations highlight the importance of δ-cells in the normal function of the endocrine pancreas, and suggest that δ-cell dysfunction in diabetes (46) may have important consequences for hormone secretion from islet α- and β-cells.

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Table 1: SST and SST receptor 1-5 mRNA expression in control and Sst\(^{-/-}\) islets.
SST mRNA was detected by RT-PCR in control but not in Sst\(^{-/-}\) mouse islet extracts confirming the absence of endogenous δ-cell SST in Sst\(^{-/-}\) mice. SSTR1-5 mRNAs were identified in both control and Sst\(^{-/-}\) islet extracts. F: forward primer, R: reverse primer.

| Primer sequence            | Product (bp) | \(T_{ann}\) (°C) | mRNA expression |  |
|----------------------------|--------------|-----------------|----------------|---|
| **SST:**                   |              |                 |                |   |
| F: ccagactccgtcagtttc      | 124          | 56              | +              | - |
| R: gtcagggcatcattct        |              |                 |                |   |
| **SSTR1:**                 |              |                 |                |   |
| F: ttttgcatctgctggatgc     | 167          | 59.9            | +              | + |
| R: ggaagagcctgtaaagttg     |              |                 |                |   |
| **SSTR2:**                 |              |                 |                |   |
| F: acgccaagatgaagaccatc    | 197          | 55.7            | +              | + |
| R: catgaccgtaagcagaaga     |              |                 |                |   |
| **SSTR3:**                 |              |                 |                |   |
| F: tgggtatctacgtgcttctg    | 163          | 59.9            | +              | + |
| R: agacggcacagatggagatcc   |              |                 |                |   |
| **SSTR4:**                 |              |                 |                |   |
| F: tgctaatgctggcatgaag     | 154          | 59.9            | +              | + |
| R: ttcagcttgacacaccaggag   |              |                 |                |   |
| **SSTR5:**                 |              |                 |                |   |
| F: cgacttcgtacaggaatcca    | 213          | 58              | +              | + |
| R: ctcacagagggctgcaca      |              |                 |                |   |
**Figure 1: Intra-islet organisation of α- and β-cells from control and Sst⁻/⁻ islets.**
Consecutive sections of control (A-B) or Sst⁻/⁻ (C-D) mouse islets were stained for insulin (A,C) or glucagon (B,D), respectively, and are representative of sections from 3 different animals.
Figure 2: Insulin and glucagon secretion in vivo
(a) Plasma insulin levels in Sst\textsuperscript{-/-} mice (solid symbols) and control mice (open symbols) 0, 2, 5 and 15 min after an intravenous glucose challenge (0.5g/kg). Points show means±SEMs for 4-6 separate animals.
(b) Plasma glucagon levels in Sst\textsuperscript{-/-} mice (solid symbols) and control mice (open symbols) 0, 2 and 5 min after injection of intravenous arginine (0.25g/kg). Points show means±SEMs for 5 and 14 separate animals (Sst\textsuperscript{-/-} and control, respectively).
(c) Plasma glucose levels in Sst\textsuperscript{-/-} mice (solid symbols) and control mice (open symbols) 0, 15, 30, 45 and 60 min after injection of intravenous insulin (0.4 U/kg). Points show means±SEMs for 5 separate animals.
Figure 3: Hormone secretion from control and SST⁻/⁻ islets
(a) Insulin secretion from control (open symbols) and SST⁻/⁻ (solid symbols) mouse islets at a sub-stimulatory concentration of glucose (2mM G, 0-10min), and at a stimulatory concentration of glucose (bar, 20mM G). Points show means±SEM, n=7-8 separate perfusion channels in each experiment, typical of 10 separate experiments. In (b) the same results are expressed as a percentage of total insulin content.
(c) Arginine-induced (20mM, bar) glucagon secretion from SST⁻/⁻ islets (solid symbols) and control islets (open symbols). Points show means±SEM, n=4 perfusion channels. Where no error bars are shown they are smaller than the size of the symbols.
(d) Insulin secretory responses to the sulphonylurea tolbutamide (100µM, bar) in the presence of 2mM glucose from SST⁻/⁻ islets (solid symbols) and control islets (open symbols). Points show means±SEM, n=4 perfusion channels.
(e) SST secretion from control islets in response to glucose, tolbutamide and arginine. Bars represent means±SEM, n=8-9 in one experiment typical of three separate experiments. *P<0.05, ** P<0.01 vs 1 mM glucose alone.
Figure 4: Inhibition of insulin and glucagon secretion by exogenous SST
Effect of exogenous SST (1 μM) on (a) dynamic glucose-induced insulin secretion (20mM G, bar) and (b) static arginine-induced (20mM) glucagon secretion from control islets (open symbols or bars) and Sst⁻/⁻ islets (solid symbols or bars). Points show means±SEM, n=4 separate perfusion channels in each experiment, typical of 6 separate experiments. Bars represent means+SEM, n=6. *** P<0.001 vs arginine alone.
**Figure 5: Rate of decline in insulin secretion from control and Sst<sup>−/−</sup> islets following removal of glucose stimulus.**

(a) Glucose-induced (20mM G, bar) insulin secretion from Sst<sup>−/−</sup> islets (solid symbols) and control islets (open symbols) is reversed to basal levels upon removal of stimulus.

(b) The decline in insulin secretion from stimulated to basal levels is expressed as percentage stimulated insulin secretion prior to removal of glucose. Points show means±SEM, n=4 separate perifusion channels.
Figure 6: Parasympathetic stimulation of insulin secretion from control and Sst⁻/⁻ islets.
(a) Static insulin and (b) SST secretion from control islets in response to glucose (20 mM) and the cholinergic agonist carbamylcholine (CCh, 500µM). Bars represent means±SEM, n=8-9 in one experiment typical of three separate experiments. ** P<0.01 vs 1 mM glucose alone, ††P<0.01, †††P>0.001 vs 20 mM glucose. Data in (a) and (b) are from the same experiment.
(c) Effect of CCh (500µM) on dynamic glucose-induced (20mM G, bar) insulin secretion from control (open symbols) and Sst⁻/⁻ (closed symbols) islets. Fractions were collected every 1 min between 10-30 min and 50-60 min and every 30 s between 30-50 min. Points show means±SEM, n=3 perifusion channels in one experiment representative of 3 separate experiments. In (d) the effect of CCh is expressed as a percentage stimulation of the glucose-induced (20mM) secretory response (mean amplitude of secretion at time points 31-40 min expressed as a percentage of mean amplitude at 21-30 min).
Figure 7: Glucose-induced suppression of glucagon secretion requires SST
(a) Effect of high glucose concentration (20 mM) on glucagon secretion from control islets (open bars) and Sst<sup>−/−</sup> islets (solid bars) in static incubations. Bars represent means+SEM, n=5-7. *P<0.05 vs 2 mM glucose. (b) Glucose-induced insulin secretion from the same experiment. ** P<0.01, *** P<0.001 vs 2mM glucose. There was no significant increase in glucagon secretion from Sst<sup>−/−</sup> islets in response to glucose in 7 separate experiments, although in 3 of these secretion appeared higher (see a).
(c) Glucagon secretion from control islets (open bars) and Sst<sup>−/−</sup> islets (solid bars) in response to increasing glucose concentrations. Islets were pre-incubated in the absence of glucose for 1 h prior to exposure to the glucose concentrations shown in the figure. Bars represent means+SEM, n=5-6. * P<0.05 vs 0 mM glucose.
(d) Effect of 10mM glucose (G, bar) on dynamic glucagon secretion from control islets (open symbols) and Sst<sup>−/−</sup> islets (solid symbols) pre-incubated in the absence of glucose for 20 min. Points show means±SEM, n=4 perifusion channels. Controls: P<0.001 for 0 vs 10 mM glucose mean values.