Paracrine regulation of somatostatin secretion by insulin and glucagon in mouse pancreatic islets

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Abstract

Aims/hypothesis The endocrine pancreas comprises the islets of Langerhans, primarily consisting of beta cells, alpha cells and delta cells responsible for secretion of insulin, glucagon and somatostatin, respectively. A certain level of intra-islet communication is thought to exist, where the individual hormones may reach the other islet cells and regulate their secretion. Glucagon has been demonstrated to importantly regulate insulin secretion, while somatostatin powerfully inhibits both insulin and glucagon secretion. In this study we investigated how secretion of somatostatin is regulated by paracrine signalling from glucagon and insulin.

Methods Somatostatin secretion was measured from perfused mouse pancreases isolated from wild-type as well as diphtheria toxin-induced alpha cell knockdown, and global glucagon receptor knockout (*Gcgr*^{-/-}) mice. We studied the effects of varying glucose concentrations together with infusions of arginine, glucagon, insulin and somatostatin, as well as infusions of antagonists of insulin, somatostatin and glucagon-like peptide 1 (GLP-1) receptors.

Results A tonic inhibitory role of somatostatin was demonstrated with infusion of somatostatin receptor antagonists, which significantly increased glucagon secretion at low and high glucose, whereas insulin secretion was only increased at high glucose levels. Infusion of glucagon dose-dependently increased somatostatin secretion approximately twofold in control mice. Exogenous glucagon had no effect on somatostatin secretion in *Gcgr*^{-/-} mice, and a reduced effect when combined with the GLP-1 receptor antagonist exendin 9-39. Diphtheria toxin-induced knockdown of glucagon producing cells led to reduced somatostatin secretion in response to 12 mmol/l glucose and arginine infusions. In *Gcgr*^{-/-} mice (where glucagon levels are dramatically increased) overall somatostatin secretion was increased. However, infusion of exendin 9-39 in *Gcgr*^{-/-} mice completely abolished somatostatin secretion in response to glucose and arginine. Neither insulin nor an insulin receptor antagonist (S961) had any effect on somatostatin secretion.

Conclusions/interpretation Our findings demonstrate that somatostatin and glucagon secretion are linked in a reciprocal feedback cycle with somatostatin inhibiting glucagon secretion at low and high glucose levels, and glucagon stimulating somatostatin secretion via the glucagon and GLP-1 receptors.

Keywords Glucagon · Intra-islet communication · Perfused mouse pancreas · Somatostatin secretion

Abbreviations

DT Diphtheria toxin
DT-Gcg Diphtheria toxin-induced glucagon knockdown

Ex9 Exendin 9-39
GLP-1 Glucagon-like peptide-1

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Introduction

The endocrine pancreas comprises the islets of Langerhans, which are scattered in the exocrine pancreas. The islets are micro organs consisting of about a couple of hundred to a couple of thousand endocrine cells, including the beta cells, alpha cells, delta cells, pancreatic polypeptide cells and epsilon cells. These cells are responsible for the secretion of insulin, glucagon, somatostatin, pancreatic polypeptide and ghrelin, respectively.

Fig. 2 Exogenous glucagon stimulates somatostatin secretion through glucagon and GLP-1 receptors. Somatostatin secretion from perfused mouse pancreases during infusion of glucagon (0.1–10 nmol/l) at 3.5 mmol/l glucose (**a**, **b**) or 12 mmol/l glucose in control mice (**c**, **d**); control mice + Ex9 (1 μ mol/l; added 30 min before) (**e**, **f**); *Gcgr*^{-/-} mice (**g**, **h**); and *Gcgr*^{-/-} mice + Ex9 (**i**, **j**). (**b**, **d**, **f**, **h**, **j**) Mean somatostatin output averaged over 10 min before and during infusion of glucagon. Significance was tested by one-way ANOVA (repeated measures) comparing stimulated output vs respective basal output. Data are shown as mean $\pm$ SEM; $n = 6$ –12; * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. Gcg, glucagon; SST, somatostatin

insulin to perfused mouse pancreases. Exogenous insulin had no acute effect on somatostatin secretion at 3.5 or 12 mmol/l glucose (Fig. 3a). Addition of the insulin receptor antagonist S961 likewise had no effect on basal secretion, glucose- or arginine-stimulated somatostatin secretion (Fig. 3b,c). To verify that glucagon-stimulated somatostatin secretion does not depend on insulin signalling, we infused glucagon (as above) with continuous addition of S961 (Fig. 3d,e). However, the exogenous glucagon infusions stimulated somatostatin secretion to a similar degree as in control mice (Fig. 2c), supporting the likelihood that insulin is not the main driver behind glucagon-regulated somatostatin secretion. This suggests that the effect of glucagon on somatostatin secretion is not indirectly mediated via insulin (neither glucagon-stimulated insulin secretion nor any other acutely stimulated insulin secretion).

Endogenous glucagon signalling affects somatostatin secretion

In pancreases from control mice (Fig. 4), somatostatin secretion was significantly stimulated by arginine and 12 mmol/l glucose (1.4–1.6-fold; $p < 0.05$). In mice with DT-induced knockdown of glucagon secretion (Fig. 4c, [14]), the somatostatin response to 12 mmol/l glucose and arginine infusion was completely lost compared with control mice (Fig. 4a,b). Furthermore, total somatostatin output was reduced by DT treatment (total response 973 ± 143 fmol/75 min vs 1820 ± 359 in control mice; $p < 0.05$). Infusion of GLP-1 and a mix of amino acids (Arg, Gln, Glu, Ala) were able to significantly ($p < 0.01$) stimulate somatostatin secretion in DT-Gcg mice, indicating that the delta cells are still functional (Fig. 4d) and capable of responding to strong stimuli. The GLP-1 receptor antagonist Ex9 acutely reduced somatostatin secretion with 50% in wild-type mice (Fig. 4e) indicating that activation of the GLP-1 receptor is important for somatostatin secretion. This effect was lost in DT-Gcg mice where all proglucagon products are knocked down (Fig. 4e) indicating a direct effect mediated by GLP-1 receptor activation. Pancreases from *Gcgr*^{-/-} mice with dramatically increased glucagon levels (Fig. 4h, [14]), exhibited an almost twofold increased total somatostatin output compared with control mice (Fig. 4f,g). Addition of the GLP-1 receptor antagonist Ex9 to perfused *Gcgr*^{-/-} or control pancreases reduced glucose-stimulated secretion of somatostatin, and administration of Ex-9 to perfused *Gcgr*^{-/-} pancreases abolished all increments in somatostatin secretion.

Discussion

Regulation of secretion of the pancreatic hormones and the role of intra-islet communication has been investigated for

Fig. 3 Somatostatin secretion is not directly regulated by insulin. (**a–c**) Somatostatin secretion was measured from perfused mouse pancreases of wild-type mice during infusion of insulin at 3.5 and 12 mmol/l glucose ($n = 8–10$) (**a**), the insulin receptor antagonist S961 (1 μ mol/l) at 3.5 and 12 mmol/l glucose ($n = 5–7$) (**b**), and during stimulation with arginine (10 μ mol/l) at 12 mmol/l glucose with or without addition of S961 ($n =$

11–13) (**c**). (**d**) Somatostatin secretion during infusion of exogenous glucagon (0.1–10 nmol/l) and simultaneous infusion of S961 ($n = 8$). (**e**) Mean somatostatin output averaged over 10 min before and during addition of glucagon in (**d**). Significance was tested by one-way ANOVA with post hoc Tukey adjustment. Data are shown as mean $\pm$ SEM. * $p < 0.05$. Gcg, glucagon; Gluc, glucose; SST, somatostatin

many years, but several questions remain unanswered. The complex structure of the individual pancreatic islets, the confusing and species-specific arrangement of the microcirculation, and the three predominating hormones, each with a powerful regulating effect on the secretion of each other, complicate the design of experiments and interpretation. It is becoming increasingly clear that, by looking at single cells types separately or isolated islets, deprived of the natural microcirculation, one misses important paracrine effects that each hormone and cell type exert on its surrounding cells, because all substances reach the peripheral cells by uncontrolled diffusion.

In agreement with previous results [6, 12], we initially confirmed that a complete blockade of all islet somatostatin receptor subtypes increases the secretion of both glucagon and insulin. The effects on glucagon secretion are profound at both 3.5 and 12 mmol/l glucose, indicating that endogenous somatostatin is secreted and exerts paracrine effects at both low and high glucose, although somatostatin secretion is dependent on the prevailing glucose levels. Thus, at 12 mmol/l glucose, the proportionate effect of somatostatin receptor antagonism is more pronounced, but glucagon secretion is still lower compared with secretion at hypoglycaemia. This could indicate that glucose plays a direct role in regulating glucagon secretion, and somatostatin is only partly responsible for glucose-mediated glucagon secretion [29, 30]. Insulin secretion was only affected by somatostatin at 12 mmol/l glucose, demonstrating that a tonic inhibition by endogenous somatostatin does not contribute to the very low levels of insulin at low glucose levels, whereas a paracrine

inhibitory effect by local somatostatin plays a significant role in the regulation of insulin secretion at higher glucose levels. Having confirmed the profound influence of somatostatin on glucagon secretion, we wished to investigate whether glucagon also plays a role in somatostatin secretion.

Glucagon can directly stimulate somatostatin secretion, independently of insulin

Studies have demonstrated that glucagon plays a role in stimulating insulin secretion by a direct effect on glucagon and GLP-1 receptors expressed on the beta cells [12, 14, 17]. A similar effect of glucagon on somatostatin secretion has been suspected based on reduced somatostatin secretion in response to glucagon antibodies [2], in agreement with effects of exogenous glucagon effects in isolated islets [20, 31]. We here demonstrate that exogenous glucagon stimulates somatostatin secretion in the perfused mouse pancreas with preserved microcirculation. The effect is greater at high glucose levels, consistent with a stimulating effect of glucose on somatostatin secretion, similar to what is found for beta cells. We and others have previously demonstrated that both glucagon and GLP-1 receptors are expressed in delta cells, although at lower levels than in beta cells [12, 32]. In the present experiments, antagonising either the glucagon or GLP-1 receptor reduced or completely abolished the effect of glucagon on somatostatin secretion, suggesting that both receptors are involved in glucagon's regulation of the delta cell. The significant reduction in somatostatin secretion with addition of a GLP-1 receptor antagonist clearly demonstrated that activation of the GLP-

Fig. 4 Endogenous glucagon signalling affects somatostatin secretion from perfused mouse pancreases. (**a–b**) Somatostatin and (**c**) glucagon secretion from the pancreas of DT-Gcg and control mice perfused at 3.5 and 12 mmol/l glucose with infusion of arginine ($n = 7–8$; key in **a** also applies to **c**). (**d**) Somatostatin secretion from DT-Gcg mice in response to 10 nmol/l GLP-1 and amino acids (Arg, Glu, Gln, Ala) ($n = 6$). (**e**) Ex9 (1 μ mol/l) infused at 12 mmol/l glucose in wild-type and DT-Gcg mice ($n = 5–6$). (**f–h**) secretion from $Gcgr^{-/-}$ and $Gcgr^{+/+}$ mouse pancreases with or without addition of Ex9 ($n = 7–11$; in **f, g** $Gcgr^{+/+}$ black/circles;

$Gcgr^{-/-}$, purple/squares; $Gcgr^{+/+}$ +Ex9, red/inverted triangles; $Gcgr^{-/-}$ +Ex9, light blue/upright triangles). (**b, g**) Mean somatostatin output averaged over 5 min before and during addition of glucose and arginine. Significance was tested by one-way ANOVA with post hoc Tukey adjustment. Data are shown as mean $\pm$ SEM. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ comparing wild-type littermates vs $Gcgr^{-/-}$; † $p < 0.05$, †† $p < 0.001$ comparing control vs DT-Gcg or $Gcgr^{-/-}$ $\pm$ Ex9. Data on glucagon secretion (**c, h**) have been previously published elsewhere [14]. AA, amino acids; SST, somatostatin

1 receptor is important for regulating somatostatin secretion. Since glucagon can activate both glucagon and GLP-1 receptors [14, 28], this would indicate a stimulating paracrine role of endogenous glucagon/GLP-1 on somatostatin secretion. Considering that the level of islet GLP-1 secretion is minimal compared with glucagon [14], it is likely that this effect reflects the actions of glucagon. Furthermore, the effect of GLP-1 receptor antagonism was lost in DT-Gcg mice, indicating that the effect of Ex9 is directly mediated by proglucagon products. Other studies have suggested that insulin can stimulate somatostatin secretion [18, 19], which could mean that the effect of glucagon on somatostatin secretion could be indirect, mediated through glucagon-induced insulin secretion. However, addition of the insulin receptor antagonist S961 had no influence on the effect of glucagon on somatostatin secretion. Furthermore, addition of up to 1 μ mol/l

insulin to the perfusate did not have any effect on somatostatin secretion (Fig. 3) in line with previous studies [20, 33], and infusion of insulin receptor antagonist S961 neither affected basal nor glucose- or arginine-stimulated somatostatin secretion. This suggests that insulin does not play a direct role in paracrine regulation of somatostatin secretion in mice.

Somatostatin secretion requires normal intra-islet glucagon signalling

To investigate the intra-islet paracrine effects of endogenous glucagon on somatostatin secretion, we used transgenic mouse models to disrupt glucagon signalling. We have previously utilised DT-induced destruction of proglucagon cells in transgenic mice expressing the human DT receptor under control of the proglucagon promoter to acutely knock down

the alpha cells and thereby glucagon secretion. These mice also exhibited significantly reduced insulin secretion, indicating that normal glucagon secretion is necessary to maintain appropriate insulin secretion [14, 21]. We here report that somatostatin secretion in these mice was likewise reduced compared with control mice. They were still able to secrete somatostatin in response to GLP-1 showing that the delta cells are still functional although with a lower level of basal somatostatin secretion. The lack of an arginine-stimulated response may therefore reflect a need for a rise in glucagon (which is lost in DT-Gcg mice) for arginine-stimulated somatostatin secretion, whereas GLP-1 may directly stimulate somatostatin through GLP-1 receptors on the delta cells. However, it is also possible that a basic tone of glucagon stimulation is required to maintain normal somatostatin secretion, without which responses to other stimuli are lost.

Global *Gcgr*^{-/-} mice have alpha cell hyperplasia and 20-fold increased glucagon secretion, and to a lesser extent delta cell hyperplasia [14, 22]. In these islets, we also identified increased secretion of small amounts of active GLP-1 from the islets, presumably reflecting a general increase in proglucagon formation compared with control mice [14]. In the present experiments, somatostatin secretion from *Gcgr*^{-/-} mice was significantly increased compared with control mice. This could be explained by an increased GLP-1 receptor activation (from increased secretion of glucagon as well as GLP-1). Consistent with this notion, simultaneous blockade of glucagon and GLP-1 receptors terminated somatostatin secretion, similar to what has previously been shown for insulin secretion [14, 34]. In general, this would suggest that glucagon does exhibit a stimulating effect on somatostatin secretion and that alpha cells regulate the function of both delta- and beta cells. The constitutive knockout of glucagon receptors in the *Gcgr*^{-/-} model has limitations due to compensatory mechanisms taking place, and the hypersecretion of proglucagon products complicates the overall picture. However, combined with the other models, it demonstrates an important role for glucagon acting directly within the pancreas on the secretion of islet hormones. Experimental inhibition of glucagon actions requires a simultaneous inhibition of both glucagon and GLP-1 receptors. Likewise, the use of GLP-1 receptor antagonists not only blocks GLP-1 but also any potential action of glucagon on GLP-1 receptors.

The role of somatostatin is still largely unresolved but its strong ability to inhibit hormone secretion is indisputable. Studies to investigate intra-islet communication are complicated by the fact that we cannot directly see what happens in each islet. Glucagon seems to stimulate secretion insulin [14, 16, 34] and somatostatin [2, 31, 35], while somatostatin plays a strong inhibitory role on insulin and glucagon secretion, suggesting that the islets are locked in a push–pull feedback regulation. Considering the observed dysregulated insulin and glucagon secretion in type 2 diabetes [36, 37], it is possible that somatostatin could play a role in the development of type 2 diabetes

[32–34]. Moreover, it has been suggested that the use of somatostatin receptor antagonists could potentially be used to correct the impaired glucagon secretion observed in people with type 2 diabetes in response to hypoglycaemia, where somatostatin does not seem to affect insulin secretion, as demonstrated in a rat model of diabetes [38–40]. The potential use of somatostatin as a therapeutic tool makes it important to know more about the secretion and actions of somatostatin in the pancreas both in normal physiology and under diabetic conditions. However, it is notoriously difficult to measure somatostatin in plasma due to an almost immediate break down. In addition, any surviving somatostatin in peripheral plasma might be derived from all regions of the gastrointestinal tract or the pituitary, not specifically from the pancreas. Furthermore, plasma levels of somatostatin-14 or -28 are very low, challenging the sensitivity of the applied assays. Therefore, most studies have been performed on isolated cells/islets/pancreas. This would be consistent with notions that somatostatin does not function as a circulating hormone, but mainly acts as a paracrine regulator acting locally on neighbouring cells in pancreatic islets and the intestine. The potential importance of somatostatin as a paracrine regulator has been demonstrated in several studies, where stimulators of somatostatin secretion (e.g. GLP-1, ghrelin) reduce glucagon and/or insulin secretion in a somatostatin-dependent manner [11, 12, 32]. Furthermore, the strong inhibitory effect of somatostatin on glucagon secretion has been suggested to play a role in glucose-reduced glucagon secretion [13, 29]. Thus, in conditions with high glucose, somatostatin secretion is increased and glucagon secretion reduced. The current studies indicate that, not only is somatostatin likely to profoundly regulate secretion of the alpha and beta cells, but somatostatin secretion is itself regulated by glucagon secretion. This might be important for both basal and amino acid-stimulated somatostatin secretion, whereas insulin did not seem to influence somatostatin secretion. Our results reinforce the concept that the pancreatic islets function as a complex integrated endocrine organ with important paracrine feedback cycles, tying the secretion by the many cell types together.

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Data availability The datasets generated and analysed during the current study are available from the corresponding authors on reasonable request.

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