The somatostatin-secreting pancreatic δ-cell in health and disease

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Abstract

The somatostatin-secreting δ-cells comprise ~5% of the cells of the pancreatic islets. The δ-cells have complex morphology and might, via cellular processes, interact with many more islet cells than suggested by their low numbers. δ-cells contain ATP-sensitive potassium channels. These channels are open at low levels of glucose, but close in response to glucose stimulation. This results in membrane depolarization, initiation of electrical activity and increased somatostatin secretion. Factors released by neighbouring α-cells or β-cells amplify the glucose-induced effects on somatostatin secretion. Somatostatin secreted from δ-cells acts locally within the islets as a paracrine or autocrine inhibitor of insulin, glucagon and somatostatin secretion. The effects of somatostatin are mediated by activation of somatostatin receptors that are coupled to the inhibitory G protein, which culminates in suppression of the electrical activity and exocytosis of α-cells and β-cells. Somatostatin secretion is perturbed in animal models of diabetes mellitus, which might explain the loss of appropriate hypoglycaemia-induced glucagon secretion, a defect that could be mitigated by SSTR2 antagonists. It is proposed that somatostatin antagonists or agents that suppress somatostatin secretion could be considered as an adjunct to insulin therapy. In this Review, we summarize the cell physiology of somatostatin secretion, what might go wrong in diabetes and the therapeutic potential of agents targeting somatostatin secretion or action.

[H1] Introduction

A human pancreas contains 1–3 million pancreatic islets (also known as islets of Langerhans)[1,2]. The islets are complex micro-organs that consist of several types of endocrine cells that have a key role in the regulation of whole-body energy metabolism[3]. Insulin is secreted by the β-cells (55-75% of the islet cells, depending on species[4]), which is the only hormone that lowers blood levels of glucose. Glucagon, the principle hormone in plasma that increases glucose levels is secreted by the α-cells (20-40% of the islet cells). The δ-cells (~5% of the islet cells) secrete somatostatin, which inhibits the release of insulin and glucagon). In general, levels of insulin and glucagon vary reciprocally and the insulin:glucagon ratio determines the balance between anabolism (glucose and lipid storage) and catabolism (glycogen and lipid breakdown and gluconeogenesis)[3].

The combination of lack of insulin and excess glucagon results in the severe metabolic disturbances associated with diabetes mellitus that culminate in hyperglycaemia[5,6]. Most therapeutic interventions focus on insulin: either by stimulating its endogenous release (by administering sulphonylureas or glucagon-like peptide 1 (GLP-1) agonists); promoting insulin action (by administering insulin sensitizers like metformin); or administering exogenous insulin. A serious and potentially fatal complication of insulin therapy is hypoglycaemia. Up to 10% of insulin-treated patients with diabetes mellitus are estimated to die of 'iatrogenic hypoglycaemia' (*iatros* is Greek for healer or physician)[7].

During the past 30–40 years, α-cells and β-cells have been the focus of much research because of their key roles in diabetes mellitus and we now have a good understanding of the regulation of insulin secretion[8,9]. A consensus model for the regulation of

glucagon secretion remains to be formulated but there is extensive information on the α-cell gene expression profile, ultrastructure, electrophysiology and exocytosis[10,11]. In comparison, the δ-cells have not received as much attention from islet physiologists and our understanding of the cellular control of somatostatin secretion remains fragmentary. Recent findings implicate increased somatostatin signalling as a cause of the reduced counterregulatory glucagon secretion during insulin-induced hypoglycaemia in animals with diabetes mellitus[12-14]. Conversely, insulin and glucagon secretion are both tonically inhibited by somatostatin[15] and there is evidence that hyperglucagonaemia in patients with poorly controlled diabetes mellitus can be suppressed by exogenously administered somatostatin[16-18]. Indeed, somatostatin infusion in patients with type 1 or type 2 diabetes mellitus improves glycaemic control, despite the patients requiring less insulin, by reducing glucagon secretion. Accordingly, it was proposed that a long-acting somatostatin agonist might be useful as an adjunct to insulin therapy[19].

Collectively, these observations merit renewed interest in pancreatic δ-cells. A Review on δ-cell physiology and somatostatin secretion in health and disease is therefore timely. Here, we present a model for the cellular regulation of somatostatin secretion in δ-cells and discuss the crucial role they play in controlling the α-cells and β-cells via inhibitory paracrine crosstalk. We also discuss the impact of diabetes mellitus on somatostatin secretion and the role of somatostatin in the pathophysiology of the disease. Finally, we consider the δ-cell and somatostatin signalling as pharmacological targets for the treatment of diabetes mellitus.

[H1] Discovery of somatostatin and δ-cells

Somatostatin was originally isolated from the hypothalamus and was found to inhibit the release of growth hormone (GH) in the pituitary[20]. Initially named 'GH release-inhibiting hormone', it was subsequently renamed somatostatin to reflect this growth-inhibiting effect. Shortly after its discovery, somatostatin was found to also be produced and secreted in the islets of Langerhans[21].

The different endocrine cells of the islets of were first identified based on their histological staining properties. Thus, glucagon-containing 'A-cells' were identified by alcohol-based fixation methods whereas the insulin-producing 'B-cells' cells were stained by aqueous-based fixation methods. In addition, a third type of islet cell was found that was not stained by either fixation method and it was referred to as 'clear' or 'C-cells'. Later, using a different staining method (the Mallory-Heidenhain azan trichrome technique), a third type of granulated cell in addition to the A- and B-cells was identified and referred to as 'D-cells' (because the name 'C-cell' was already taken). However, it is now clear that the most 'C-cells' were in fact 'D-cells' but the nomenlclature has not been changed and the third most abundant cell type is therefore still referred to as the D-cell. The history of islet histology and how it was eventually possible to correlate histological staining properties to hormone content is the subject of a recent review[22], which readers interested in this fascinating topic are encouraged to consult. In this review we will refer to the different islet cells using the Greek letter nomenclature. Thus, the A-, B- and D-cells are referred to as α-, β- and δ-cells, respectively.

[H1] Somatostatin

There are two main forms of somatostatin - also known as somatotropin release inhibitory factor (SRIF) in the body: somatostatin 14 (or SRIF-14) and somatostatin

28) or SRIF-28)[23]. Both forms of somatostatin are derived from the precursor pre-prosomatostatin (116 amino acids in length), which is cleaved into prosomatostatin (92 amino acids). Prosomatostatin undergoes carboxyl terminal post-translational processing to generate somatostatin 14 and somatostatin 28. Both peptides are very short-lived and have a half-life of 1 min in circulation. Whereas somatostatin 28 is the dominant isoform elsewhere in the gastrointestinal tract, the pancreatic δ-cells secrete somatostatin 14, which is stored in secretory granules[24] and released by Ca^{2+}-dependent exocytosis.

[H1] The pancreatic δ-cells

The δ-cells comprise only 5% of the islet cell population[4]. In mouse islets, the α- and β-cells occupy the islet periphery and core, respectively. Most of the δ-cells are located in the islet 'cortex' with a few δ-cells found in the islet centre. Whereas most α-cells and β-cells are rounded or rhomboid, the δ-cells show a more complex cellular morphology and have long, neurite-like processes (FIG. 1A). These processes make close contact with α-cells, β-cells and other δ-cells located at some distance from the cell body, thereby enabling an extensive paracrine network. The neurite-like processes can be >20 μm long (one-third of the average islet diameter[25]) and might thus influence multiple α-cells and β-cells via paracrine effects. This might explain the stimulation of both glucagon and insulin secretion observed in the presence of somatostatin receptor antagonists[26-29]. However, paracrine signalling (via intercellular diffusion of hormones) is not the only means of communication between islet cells. The β-cells are electrically connected via gap junctions[30-32]. Thus, a δ-cell might influence β-cells that are not in direct physical contact with via changes in electrical activity in intermediary cells. There is also experimental evidence for electrical coupling between β- and δ-cells[33].

In human pancreatic islets, the cellular architecture is less defined than in mice, with δ-cells located throughout the islet. Direct δ-cell to α-cell contacts are increased in islets from patients with type 2 diabetes mellitus[24], raising the interesting possibility that the α-cells might be subjected to stronger somatostatin-mediated paracrine inhibition in such patients.

[H1] Gastric and enteric D-cells

Somatostatin is also expressed throughout the gastrointestinal tract; these somatostatin-secreting cells are referred to as D-cells. The gastrointestinal D-cells are estimated to be responsible for 65% of total body somatostatin, the pancreatic islets only account for 5% and the remaining is from the central nervous system[34]. Although the gastrointestinal D-cells are notoriously difficult to study as they are scattered among the enterocytes, it is now possible to isolate sufficient numbers of D-cells to perform more comprehensive transcriptomic analysis and physiological characterization[29]. However, the regulation of somatostatin secretion from the gastrointestinal D-cells is beyond the scope of this Review and interested readers are instead referred to two reviews that cover aspects of this topic[35,36].

[H1] Islet somatostatin secretion

The regulation of somatostatin secretion in response to nutrient intake, in general, resembles that of insulin secretion. This is perhaps not surprising as β-cells and δ-cells share an immediate common progenitor cell[37]. The regulation of somatostatin secretion by nutrient intake, pharmacological agents, hormones and neurotransmitters are shown in FIG. 1B. Further details about membrane receptors expressed in pancreatic δ-cells and their respective ligands are shown in TABLE 1. In the following sections, we

consider how δ-cells sense nutrients and correlate the regulation of glucagon secretion with the latest published δ-cell transcriptome data[38,39].

[H2] Glucose

In mouse islets, glucose-induced somatostatin secretion is initiated at glucose concentrations as low as 3 mM and stimulation is half-maximal at glucose concentrations of 5–6 mM (Fig. 1C). A similar – but not identical - glucose dependence is observed in human islets[40].

δ-cells express high levels of high-affinity glucose transporter type 1 (GLUT1; also known as solute carrier family 2, facilitated glucose transporter member 1, which is encoded by *Slc2a1*) and type 3 (GLUT3; encoded by *Slc2a3*). δ-cells also express high levels of glucokinase (encoded by *GCK*), which explains the inhibition of somatostatin secretion by the glucokinase inhibitor mannoheptulose[41,42]. As will be discussed below, it is likely that the stimulatory effects of glucose are – at least in part - mediated by an increase in the cytoplasmic ATP:ADP rati.

[H2] Amino acids

Somatostatin secretion can be stimulated by the amino acids leucine and arginine[43-46]. The ability of arginine to stimulate somatostatin secretion probably reflects the expression of the high affinity cationic amino acid transporters 1 (CAT1) and 2 (CAT2), which are encoded by *SLC7A1* and *SLC7A2*, respectively [38,39]. In β-cells, these transporters mediate electrogenic uptake of amino acids such as arginine and lysine[47], which causes membrane depolarization and initiates action potential firing when K_{ATP} channel activity is reduced (for example, in the presence of glucose). Arginine probably stimulates δ-cell electrical activity somatostatin secretion by the same mechanism.

Leucine is transported via the large neutral amino acid transporter small subunit 1 (SLC7A5), which is expressed in δ-cells[38,39]. Following deamidation and the formation of α-ketoisocaproic acid[48], leucine is metabolised in the Krebs cycle and probably stimulates somatostatin secretion by a glucose-like mechanism mediated by an increased cytoplasmic ATP:ADP ratio.

[H2] Fatty acids

The plasma concentration of non-esterified free fatty acids (mainly palmitate, oleate, stearate and lineoleate[49]) fluctuates between <0.1 mM after a meal and 0.5–1.0 mM in the fasted state[3]. Palmitate inhibits glucose-induced somatostatin secretion[50]. Mouse δ-cells express high levels of the free fatty acid receptor 4 (also known as G-protein coupled receptor 120, GPR120), which is encoded by *FFAR4*. Interestingly, GPR120-specific agonists inhibit somatostatin secretion and these effects are absent in islets from *Ffar4* knockout mice[51]. This selective inhibition of the δ-cells could be expected to result in the removal of the paracrine suppression of α-cells and β-cells exerted by somatostatin, which might contribute to the acute palmitate-induced stimulation of both insulin and glucagon secretion[50,52]. Conversely, free fatty acid-induced suppression of somatostatin secretion might ensure adequate glucagon secretion in the fasted state (when circulating free fatty acid levels are high).

[H1] δ-cell electrophysiology

Like α-cells and β-cells, δ-cells are electrically excitable and glucose-induced somatostatin secretion is associated with stimulation of action potential firing[28] (FIG. 2A). The K_{ATP} channels expressed in δ-cells are exactly the same as those found in α-cells and β-cells. Expression levels of the K_{ATP} subunits ATP-sensitive inward rectifier

K^+ channel 11 (Kir6.2, encoded by *KCNJ11*) and sulfonylurea receptor 1 (SUR1, which is encoded by *ABCC8*) in δ-cells are similar to those in β-cells[38,39]. The K_{ATP} channels are active (open) at low concentrations of glucose and this maintains a negative (hyperpolarized) membrane potential in the δ-cells. This makes the δ-cell membrane potential more positive (depolarization) and initiates action potential firing with resultant stimulation of somatostatin secretion[41,43,53-55]. Pharmacological blockers of the K_{ATP} channels (such as the sulphonylureas glbienclamide and tolbutamide) have the same effect. Conversely, the K_{ATP} channel activator diazoxide prevents depolarization and thereby inhibits somatostatin secretion[56,57]. The K_{ATP} channel activity is lower than in β-cells, which might explain why somatostatin secretion is initiated at lower glucose concentrations than insulin secretion[58].

The stimulus-secretion coupling outlined above for the δ-cell is similar to what has been described in β-cells[25]. It is therefore surprising that the non-metabolisable glucose analogue 3-O-methyl-D-glucose[59] (an effect unlikely to be mediated via an increase in the intracellular ATP:ADP ratio). Thus, it appears that glucose could stimulate somatostatin secretion by a mechanism not involving the closure of K_{ATP} channels. Some evidence suggests that there is low expression of sodium/glucose cotransporter 1 (SGLT1, encoded by *Slc5a1*) in δ-cells[38,39]. The ability of 3-O-methyl-D-glucose to stimulate somatostatin secretion might therefore be secondary to the electrogenic (that is, the ability to change the membrane potential) uptake of this glucose analogue and that this, in some δ-cells with particularly low K_{ATP} channel activity, suffices to trigger electrical activity and somatostatin secretion. . SGLT1 will also transport D-glucose, but its contribution to total glucose uptake in pancreatic δ-cells is likely to be small as its expression is low compared with that of GLUT1 and GLUT3.

In mouse δ-cells, the action potential originates from a membrane potential as low as –60 mV, peak at +30mV and last for only a few milliseconds[60] (FIG. 2B). The different voltage-gated ion channels contributing to the action potential in the mouse pancreatic δ-cell was determined by electrophysiology[60] and transcriptomic studies[38,39]. The action potential is initiated by the opening of 'low-threshold' T-type Ca^{2+} channels (specifically the voltage-dependent T-type Ca^{2+} channel subunit α_{1H}, also known as $Ca_V3.2$). The T-type Ca^{2+} channels depolarize the δ-cell to -45mV, which is the threshold for activation of voltage-gated Na^+ channels (Na^+ channel protein type 3 subunit α and/or type 9 subunit α, also known as $Na_V1.3$ and/or $Na_V1.7$, respectively), and their rapid activation underlies the rapid upstroke of the δ-cell action potential. During the action potential, voltage-gated L-type (specifically voltage-dependent L-type Ca^{2+} channel subunit α_{1D}, also known as $Ca_V1.3$) and voltage-gated P/Q-type (voltage-dependent P/Q-type Ca^{2+} channel subunit α_{1A}, also known as $Ca_V2.1$) Ca^{2+} channels. The repolarising phase of the action potential involves the opening of A-type K^+ currents (specifically K^+ voltage-gated channel subfamily D member 1 and 2, also known as $K_V4.1$ and $K_v4.2$, respectively) with some contribution from delayed rectifying K^+ channels (specifically K^+ voltage-gated channel subfamily A member 5 and subfamily B member 1, also known as $K_V1.5$ and $K_V2.1$, respectively). Membrane repolarization of the action potential also results from the inactivation of the voltage-gated Na^+ channels and T- and L-type Ca^{2+} channels. In the interval between two successive action potentials, the $K_V1.5$ and $K_V2.1$ channels that activated during the action potential deactivates slowly and this, together with the recovery from inactivation of Na^+ channels and T- and L-type Ca^{2+} channels explains the slow depolarization between two successive action potentials. Single-cell transcriptomic analysis[61] indicates a similar ion channel complement in human δ-cells.

[H1] Modulators of somatostatin secretion

In addition to the direct effects of glucose (and other nutrients) on δ-cells, somatostatin secretion is modulated by paracrine factors (released from neighbouring α-cells and β-cells), circulating hormones and neurotransmitters released by intra-islet nerve endings (FIG. 1B and TABLE 1).

[H2] Intra-islet factors

[H3] Insulin. Pancreatic δ-cells express insulin receptors (encoded by *INSR*), but the action of insulin on somatostatin secretion is unclear. Anterograde infusion (that is, in the direction of normal blood flow) of an anti-insulinantibody (to immuno-neutralise endogenous insulin) leads to a dramatic 20-fold increase in stimulation of somatostatin secretion[62]. Administration of exogenous insulin has variably been reported not to affect[63], inhibit[46] or stimulate somatostatin secretion[64]. These variable results might be a consequence of different experimental conditions (isolated islets vs perfused pancreas etc.). It should be remembered that the intra-islet interstitial insulin concentration is likely to be very high; a single insulin granule contains 1.6 x 10^{-18} mol insulin and releasing this is sufficient to increase the interstitital insulin concentration between the islet cells to ~10 nM, which is >100-fold higher than circulating levels of insulin[25].

[H3] Glucagon. The δ-cells also express low levels of glucagon receptor (encoded by *GCGR*) and respond to glucagon with increased somatostatin secretion[44,65], presumably by stimulation of cAMP production (see below).

[H3] Urocortin 3. Urocortin 3 (encoded by *UCN3*) is a 38 amnio acid peptide that is a member of the corticotropin-releasing hormone (CRH) family[66]. It is co-released with insulin from β-cells and stimulates somatostatin secretion[56]. Urocortin 3 activates the

α-isoform of the corticotropin-releasing factor receptor 2 (CRHR2, encoded by *Crhr2*), which is selectively expressed by δ-cells within the islet[56]. In mice, genetic ablation of *Crhr2* or *Ucn3* leads to a 50–60% reduction of glucose-induced somatostatin secretion, an effect that was paralleled by a corresponding decrease in islet somatostatin content. By activation of CRHR2 receptor, urocortin 3 stimulates production of cAMP, which probably accounts for the stimulation of somatostatin secretion (see below). Collectively, these observations indicate that islet somatostatin secretion is modulated by local release of urocortin 3 from β-cells.

[H3] γ-Aminobutyric acid. The neurotransmitter γ-aminobutyric acid (GABA is also co-released with insulin from β-cells and stimulates somatostatin secretion in human islets[67]. GABA co-released with insulin and urocortin 3 might contribute to glucose-induced somatostatin secretion. In addition, evidence suggests that GABA released from human δ-cells depolarizes the δ-cell and stimulates electrical activity in an autocrine fashion[67]. Expression of GABA receptor subunits is low in mouse δ-cells (TABLE 1) but electrophysiological measurements suggest expression in human δ-cells is high. Thus, large Cl^-currents are evoked by application of GABA to human δ-cells[67].

[H3] Acetylcholine. In mouse islets, acetylcholine is released by cholinergic nerve endings[68]. The reported actions of acetylcholine on somatostatin secretion are conflicting as it has variably been found to stimulate[41] or inhibit[51,54] somatostatin secretion. Mouse δ-cells express muscarinic acetylcholine receptors (encoded by *Chrm3*) and M4 (encoded by *Chrm4*). The M3 receptors are coupled to the guanine nucleotide-binding protein G_q subunit, which leads to Ca^{2+} mobilization and somatostatin exocytosis, whereas the M4 receptors are coupled to the guanine nucleotide-binding protein G_i subunit, which results in the suppression of somatostatin

secretion. Thus, expression of these two muscarinic receptors, coupled to different canonical signalling cascades, might explain the contradictory effects of acetylcholine action on somatostatin secretion.

[H2] Endocrine modulators

[H3] GLP1 and GIP. δ-cells express GLP1 receptors (encoded by *GLP1R*) and low levels of gastric inhibitory polypeptide (GIP) receptors (encoded by *GIPR*). Predictably, somatostatin secretion is stimulated by the incretin hormones GLP1 (and its agonists), GIP and agents that increase cAMP (such as forskolin)[63,69,70]. How cAMP stimulates somatostatin secretion is discussed below.

[H3] Adrenaline. Of the adrenergic receptors, only α_{2A} receptors (encoded by *Adra2a*) are expressed at notable levels in δ-cells. The α_{2A} receptor is also responsible for the direct inhibition of insulin secretion in response to direct adrenergic inputs to β-cells and probably accounts for the reported inhibitory effects of adrenaline on somatostatin secretion[54,71].

[H3] Ghrelin. The hunger hormone ghrelin is known to inhibit insulin secretion. Many different and sometimes conflicting mechanisms have been proposed to explain how the ghrelin receptor, which acts via the guanine nucleotide-binding protein G_q subunit α ($G_{\alpha q}$) and thus would be predicted to stimulate intracellular Ca^{2+} release and stimulate secretion, actually inhibits insulin release. This conundrum was resolved with the discovery that ghrelin receptors (encoded by *GHSR*) are selectively expressed in δ-cells, where they mediate robust and selective secretion of somatostatin from mouse and human islets in response to ghrelin, which inhibits insulin release by a paracrine mechanism[38,39].

[H1] Ca^{2+}-regulated somatostatin secretion

Glucose-induced somatostatin secretion is associated with an elevation of intracellular levels of Ca^{2+} ($[Ca^{2+}]_i$). In isolated mouse δ-cells, large $[Ca^{2+}]_i$ oscillations are observed at glucose levels as low as 3 mM[72]. Glucose-induced $[Ca^{2+}]_i$ oscillations are also observed in δ-cells in intact islets at 3 mM glucose[73]. These fluctuations were suppressed by lowering glucose concentrations to 0.5 mM or the addition of the K_{ATP} channel activator diazoxide[74].

Membrane depolarization, caused by supraphysiological levels of extracellular K^+ (BOX 1), also increases $[Ca^{2+}]_i$ and stimulates somatostatin secretion[39,41,72,75]. This effect is mediated by opening of the voltage-gated Ca^{2+} channels and is inhibited by Ca^{2+} channel blocker such as isradipine and SNX482[41,56].

The nature of the glucose-induced $[Ca^{2+}]_i$ oscillations has not been conclusively established, but it is possible that they involve in part the mobilization of intracellular Ca^{2+}. The strong inhibitory effects of thapsigargin, dantrolene and ryanodine on glucose-induced somatostatin secretion[41] indicate an important role intracellular Ca^{2+} stores in somatostatin secretion. Dantrolene and ryanodine are inhibitors of the intracellular ryanodine receptors (RyRs; encoded by *Ryr1, 2 and 3*), which is a Ca^{2+} release channel in the sarcoplasmic reticulum, and thapsigargin is an inhibitor of the sarcoplasmic-endoplasmic reticulum Ca^{2+} ATPase (SERCA). Collectively, these finding suggest a cross-talk between plasmalemmal and intracellular Ca^{2+} channels and that Ca^{2+} entry via the voltage-gated Ca^{2+} channels might result in an additional increase in $[Ca^{2+}]_i$ by activation of Ca^{2+}-induced Ca^{2+} release (CICR) mediated by RyR (FIG. 2C). In mouse islets, RyR expression is almost exclusively restricted to δ-cells and that

Ryr3 is the dominant subtype[38,39]. Thus, any effects of ryanodine on insulin and glucagon secretion might reflect a paracrine effect (FIG. 3A).

The CICR-dependent component of somatostatin secretion is small at low levels of glucose but increases with rising levels of glucose (being half-maximal at ~10 mM)[41]. Glucose-induced somatostatin release is nearly abolished by inhibitors of protein kinase A and the cAMP sensor exchange protein directly activated by cAMP 2 (Epac2; also known as RAPGEF4) (unpublished observations). cAMP might promote CICR by sensitising RyR3 to Ca^{2+} (REF [76]). How glucose increases intracellular levels of cAMP and promotes CICR is not known, but it might be the result of paracrine signalling within the islets. For example, release of urocortin 3 and glucagon from neighbouring β- and α-cells might activate adenylate cyclase and increase cAMP upon binding to the CRHR2 and GCGR receptors, respectively.

[H1] Somatostatin as a paracrine regulator

Once somatostatin has been released from δ-cells, it exerts local paracrine effects by activation of somatostatin receptors (SSTRs) in other islet cells. Whether somatostatin secreted by pancreatic islets also exerts other systemic effects is not known but quantitative considerations suggest that such effects are unlikely to be of major functional significance: First, only 5–10% of somatostatin in plasma is somatostatin 14 (the δ-cell variety of somatostatin)[77]. Second, pancreatectomy (i.e. the surgical removal with removal of all pancreatic δ-cells) does not lower plasma levels of somatostatin[78]. Studies in dogs indicate that a glucose-induced stimulation of somatostatin secretion could only be detected in the pancreatic vein (that drains the pancreas) and that the somatostatin concentration changes little (if at all) in the vena cava and mesenteric vein (that drain the gut)[79].

There are five different somatostatin receptors (SSTR1–5)[80]. All the SSTRs couple to an inhibitory G protein (G_i) and the effects of somatostatin could be prevented by pretreatment with pertussis toxin. Transcriptomic analyses using RNAseq on fluorescence-activated cell sorting (FACS)-purified islet cell populations confirm that SSTR2 and 3 are the predominant receptor subtypes in mouse and human α-cells whereas β-cells predominantly express SSTR3[38,39,81] (FIG. 3A). Somatostatin exerts a plethora of effects in both α-cells and β-cells, which collectively result in reduction of insulin and glucagon secretion (FIG. 3B). Thus, somatostatin reduces the activity of adenylate cyclase (AC), resulting in lower cytoplasmic cAMP ($[cAMP]_i$)[82] and less cAMP-induced exocytosis, inhibits voltage-gated Ca^{2+} channels (VGCC), activates G protein-activated inward rectifier K^+ channel (GIRK) which causes membrane repolarization and suppression of action potential furing[83,84]. In addition, somatostatin exerts a direct inhibitory effect on exocytosis independent of $[cAMP]_i$ that might involve activation of the protein phosphatase calcineurin[84,85]. Interestingly, mouse δ-cells express SSTR1 as well as SSTR3[38,39], possibly suggesting its release is under autocrine feedback control, which may account for the strong stimulation of somatostatin secretion in the presence of somatostatin receptor antagonists[69,86].

In addition to the inhibitory effects of somatostatin on hormone release, somatostatin also influences β-cell mass. Somatostatin agonists are potent suppressors of neuroendocrine tumour growth[87] and inhibit the proliferation of MIN6 insulinoma cells[88] as well as mouse and human β-cells[89]. These results are in agreement with the observation that G_i signalling inhibits β-cell proliferation[90]. However, mice with no somatostatin gene expression do not demonstrate increased β-cell mass[54] but it is possible that any stimulation of β-cell proliferation was offset by a reduced trans-differentiation of δ-cells to β-cells[91].

[H1] Somatostatin and diabetes mellitus

Diabetes mellitus (both type 1 and 2) is considered a 'bihormonal disorder' involving defects in both insulin and glucagon secretion[92]. Given that somatostatin inhibits insulin and glucagon secretion, the question arises as to whether diabetes mellitus might involve all three major islet hormones.

Studies in rats and dogs with diabetes mellitus indicate that somatostatin secretion at low levels of glucose is higher than in control animals with no diabetes mellitus and was not stimulated by high levels of glucose[93,94,95]. There is no published information on the impact of diabetes mellitus on somatostatin secretion in isolated human islets. Measurements of circulating somatostatin-like immunoreactivity indicate that whereas a mixed meal increases circulating somatostatin-like immunoreactivity by 10% in healthy individuals, it is increased by 20% in patients with type 2 diabetes mellitus[96] (but as discussed above, only a fraction of this reflects somatostatin 14 originatin from □□□□δ-cell.

[H1] Therapeutic implications

A most important question is whether the δ-cells and somatostatin secretion could be pharmacologically targeted to provide benefits to patients with diabetes mellitus. The risk of hypoglycaemia constitutes a barrier to good glycaemic control and many insulin-dependent patients with diabetes mellitus are treated less aggressively with insulin than would otherwise be the case in order to reduce the risk of hypoglycaemia[97]. In fact, a U-shaped relationship exists between plasma levels of glucose and mortality in patients with diabetes mellitus, with the lowest mortality in patients with diabetes mellitus who HbA_{1C} levels of 7.5% (a surrogate marker of long-term glycaemic control)[98], which is

well above that typically seen in individuals who do not have diabetes mellitus (≤5%)[99]. It is likely that at least part of the increase in mortality at HbA_{1C} values below 7.5% is caused by hypoglycaemia

As discussed above, experiments in rat models of diabetes mellitus are suggestive of impaired counter-regulatory glucagon secretion during insulin-induced hypoglycaemia due to increased somatostatin signalling[12-14]. The consequences of somatostatin over-secretion under hypoglycaemic conditions might be corrected by preventing its biological effects. Indeed, SSTR2 antagonists restore counterregulatory glucagon secretion during insulin-induced hypoglycaemia in rats with diabetes mellitus[12-14]. However, these receptors are widely expressed (for instance, in the stomach, adrenal medulla, cerebral cortex and hypothalamus)[100] therefore translation to human studies requires safety testing. A radiolabelled SSTR2 antagonist (JR11) is being used in clinical trials[101], which will provide information on safety and tolerability.

When considering the therapeutic implications and the risk of hypoglycaemia, it is worth remembering that insulin not only produces hypoglycaemia but also a long-lasting reduction of the plasma concentration of K^+ (hypokalaemia)[102]. Hypokalaemia is associated with increased mortality[103], possibly via cardiac effects[104]. In general, hypokalaemia decreases electrical excitability in cells. This is because lowered extracellular K^+ levels generally lead to membrane hyperpolarization (BOX 1), with resultant reduction of cellular activity (secretion, nerve activity and muscle contraction). It is therefore paradoxical that hypokalaemia stimulates rather than inhibits somatostatin secretion[105]. The stimulation of somatostatin secretion was attributed to inhibition of the Na^+/K^+ ATPase. The Na^+-K^+ ATPase is electrogenic and for every ATP hydrolysed three Na^+ and two K^+ are transported across the cell

membrane in opposing directions, leading to a net loss of a positive charge inside the cell. Thus, the normal operation of the Na^+-K^+ ATPase tends to repolarise the membrane potential. In addition, the activity of the pump might lower the submembrane ATP:ADP ratio, thus increasing K_{ATP} channel activity and repolarising the δ-cell. Inhibition of the Na^+-K^+ ATPase by lowering extracellular levels of K^+ may thus depolarise the δ–cell by dual effects: it removes the repolarising influence of the Na^+-K^+ ATPase and it inhibits K_{ATP} channel activity by exerting an ATP-sparing effect(FIG. 3C). It has been estimated that the Na^+-K^+ ATPase accounts for up to 50% of energy expenditure[106]. Colectively these effects stimulate somatostatin secretion. This may culminate in impaired the counterregulatory hepatic glucose production during acute insulin-induced hypoglycaemia by suppression of glucagon secretion.

[H1] Conclusion

In this Review, we have attempted to illustrate the important roles played by pancreatic δ-cells and somatostatin in health and disease. It is clear that the pancreatic islets are very complex structures and that via paracrine cross-talk the islets are much more than the 'sum of the parts'. The δ-cells are emerging as a master regulator within the islets and represent an interesting and novel pharmacological target through which dysregulated insulin and glucagon secretion in diabetes mellitus might be corrected. Indeed, by virtue of their capacity for restoring counter-regulatory glucagon secretion, SSTR2 antagonists should be considered as an adjunct to insulin therapy, thereby enabling more aggressive insulin treatment by minimising the risk of hypoglycaemia.

Acknowledgements

Work discussed in this review was supported by a Wellcome Trust Senior Investigator Award (095531), the Knut and Alice Wallenberg Foundation, the Swedish Research Council, the Hartwell Foundation for Biomedical Research (201500731), the Juvenile Diabetes Research Foundation (CDA-2-2013-54) and the National Institutes of Health (DK110276).

Competing interests

The authors declare no competing interests.

Publisher's note

Key points

- The δ-cells of the pancreatic islets secrete somatostatin, a powerful paracrine inhibitor of both insulin and glucagon secretion from islet α-cells and β-cells.
- δ-cells are electrically excitable and glucose stimulates action potential firing and somatostatin secretion by both metabolic and non-metabolic effects.
- Factors (like GABA and urocortin 3) released by the β-cells stimulate somatostatin secretion, thereby providing a mechanism for feedback control of insulin and glucagon secretion during hyperglycaemia.
- Diabetes mellitus is associated with impaired glucagon secretion in response to hypoglycaemia; this effect is corrected by somatostatin antagonists, suggesting that diabetes mellitus might involve hypersecretion of somatostatin during hypoglycaemia.
- Agents that inhibit somatostatin secretion or action might reduce the risk of insulin-induced hypoglycaemia and should be considered as an adjunct to insulin therapy.

[B1] BOX 1

K^+ and the membrane potential

The K^+ equilibrium potential (E_K) is given by the Nernst equation

E_K=-61 mV * $\log_{10}$ ($[K^+]_i/[K^+]_o$)

where $[K^+]_i$ and $[K^+]_o$ represent the intra- and extracellular K^+ concentrations, respectively.

Assuming that the $[K^+]_i$ and $[K^+]_o$ are normally 110 mM and 5 mM, E_K can be estimated to be -81 mV. Indeed, in most cells the resting membrane potential approximates E_K because of high resting K^+ channel activity.

Increasing $[K^+]_o$ to 50 mM (as commonly used experimentally) will change E_K to -21 mV (thus opening Ca^{2+} channels and stimulating somatostatin secretion).

Conversely, a drop in $[K^+]_o$ to 2.5 mM shifts E_K to -100 mV (thus inhibiting electrical activity and somatostatin secretion).

Legends to Figures and Tables

Figure 1| δ-cell histology and somatostatin secretion. **a**| An Immunofluorescence confocal image of δ-cells in mouse pancreatic islets. Some δ-cells possess processes (arrows) that extend for tens of microns (Methods as in REF [41]). **b**| Regulation (both stimulation and inhibition) of somatostatin secretion by nutrients, hormones or neurotransmitters and pharmacological agents. **c**| Parallel measurements of somatostatin (red line) and glucagon (black line) secretion in mouse pancreatic islets[40]. 100% and 0% correspond to the maximum and the minimum secretion, respectively. The grey rectangle highlights that glucose exerts most of its inhibitory effect on glucagon secretion at concentrations up to 5 mM and that this is associated with some modest stimulation of somatostatin secretion. GLP1, glucagon-like peptide 1; GPR120, G-protein coupled receptor 120; M3, muscarinic acetylcholine receptor 3; M4, muscarinic acetylcholine receptor 4; NEFA, non-esterified free fatty acids; SERCA, sarcoplasmic-endoplasmic reticulum Ca^{2+}-ATPase; SSTR, somatostatin receptor.

Figure 2 | Regulation of somatostatin secretion by δ-cell electrical activity. **a** | Membrane potential measurements of a δ-cell in a mouse pancreatic islet exposed to 1 mM and 20 mM glucose[28,60]. High glucose initiates action potential firing that is suppressed by tetrodotoxin (TTX). **b**| Schematic of the contribution of different ion channels to δ-cell electrical activity. **c**| Stimulus–secretion coupling in a mouse δ-cell. Glucose uptake via GLUT1 and GLUT3 stimulates glucose metabolism, increases cytoplasmic ATP:ADP ratio and inhibits the K_{ATP} channels. The resultant membrane depolarization triggers action potential firing and Ca^{2+} influx via voltage-gated Ca^{2+} channels (VGCC). Ca^{2+} entry via VGCCs leads to mobilization of intracellular Ca^{2+} levels ($[Ca^{2+}]_i$) by Ca^{2+}-induced Ca^{2+} release (CICR) from the sarcoplasmic-endoplasmic reticulum (sER) by the activation of ryanodine receptor 3 (RyR3) Ca^{2+} release channels. The resultant increase in $[Ca^{2+}]_i$ triggers somatostatin secretion. CICR is amplified by cAMP (generated in response to glucagon or urocortin, via binding to GCGR and CRHR2a). Glucose may also depolarise δ-cells by a non-metabolic SGLT-depednent mechanism. A-current, A-type K^+ currents; Ca_L, voltage-gated L-type; $Ca_{P/Q}$, voltage-gated P/Q-type; Ca_T, voltage-gated T-type Ca^{2+} channels; K_v, delayed rectifying K^+ channels; Na_v, voltage-gated Na^+ channels; ↑ indicates an increase in concentration.

Figure 3| Somatostatin signalling in pancreatic islets. **a**| Schematic of somatostatin signalling in the islet. **b**| Effects of somatostatin in α- and β-cells. Activation of SSTR3 (in β-cells) or SSTR2 (in α-cells) by somatostatin (green circles)] leads to: inhibition of adenylate cyclase (AC), resulting in lower cytoplasmic cAMP ($[cAMP]_i$)[82] and less cAMP-induced exocytosis; inhibition of voltage-gated Ca^{2+} channels (VGCC); activation of G protein-activated inward rectifier K^+ channel (GIRK) which causes membrane repolarization and inhibition of action potential firing[83,84]; and a direct inhibitory effect on exocytosis[84]. Cellular processes are shown in green boxes. **c**| Schematic of how hypokalaemia (induced by administration of insulin) might stimulate somatostatin secretion by inhibition of the Na^+-K^+ ATPase. The operation of the Na^+-K^+ ATPase exerts a hyperpolarizing effect on the δ-cell via by its electrogenic operation and ATP consumption (leading to a reduced ATP:ADP ratio). Inhibition of the Na^+-K^+ ATPase leads to the initiation of electrical activity and Ca^{2+} entry via VGCC and CICR, as well as stimulation of somatostatin release. Solid arrows indicate the direction ions are moving in their respective ion channels; dashed arrows represent the sequence cellular processes that are taking place; cellular processes are presented in green coloured boxes.

TABLE 1: Receptors expressed by mouse pancreatic δ-cells and their effect on somatostatin secretion

Ligand	Receptor name	Gene symbol	Expression level (RPKM)[a,b]	Effect on somatostatin secretion
Urocortin 3 (UCN3)	CRHR2α	*Crhr2*	5.14	stimulates
Ghrelin	GHSR	*Ghsr*	48.44	stimulates
Glucogon-like peptide 1 (GLP1)	GLP1R	*Glp1r*	39.91	stimulates
Gastric inhibitory polypeptide (GIP)	GIPR	*Gipr*	13.70	stimulates
γ-aminobutyric acid (GABA)	Ionotropic (GABAA) and metabotropic (GABAB)	*Gabra1–5, Gabrb1–3, Gabrd, Gabre, Gabrg1–3, Garbrp, Gabrq, Gabrr1, 2 subunits*	Multiple genes, expression invariably low.	stimulates
Acetylcholine	Muscarinic M3	*Chrm3*	2.71	stimulates
Acetylcholine	Muscarinic M4	*Chrm4*	22.05	inhibits
Adrenaline	α2a adrenergic receptor	*Adra2a*	9.72	inhibits
Somatostatin	SSTR1	*Sstr1*	18.28	inhibits
	SSTR3	*Sstr3*	42.69	inhibits
Palmitate or non-esterified fatty acids	GPR120	*Ffar4*	60.89	inhibits
Insulin	INSR	*Insr*	12.16	conflicting reports
Glucagon	GCGR	*Gcgr*	5.62	stimulates

[a] reads per kilobase of transcript per million mapped reads (RPKM) sequenced (data from REF [39]).

[b] RPKM values provide a useful approximation of actual receptor expression levels, but many post-transcriptional processes contribute to the actual cell-surface expression of receptor protein.

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