COMMENTARY

Cyclic AMP links glucose stimulation to somatostatin secretion in δ-cells

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Somatostatin is an inhibitory peptide produced by neuroendocrine cells in the nervous system, gastrointestinal tract and pancreatic islets. By activating specific G protein–coupled receptors, somatostatin suppresses electrical activity, secretion and proliferation of the target cells (Patel, 1999). Pancreatic islets are key regulators of energy homeostasis, primarily because they secrete the blood glucose–lowering hormone insulin from β-cells and glucose-elevating glucagon from α-cells. Somatostatin is released from pancreatic δ-cells, which constitute ∼5–10% of the islet cell population (Brissova et al., 2005). The δ-cells have neurite-like extensions that enable contacts with many other cells and are, despite their low number, efficient paracrine regulators of islet function (Cejvan et al., 2003; Hauge-Evans et al., 2009; Brereton et al., 2015; Li et al., 2017). Somatostatin potently inhibits the release of both glucagon and insulin and likely controls the phase relationship of the normal, pulsatile release pattern of the islet hormones, in which glucagon pulses occur in opposite phases to those of insulin and somatostatin (Gylfe and Tengholm, 2014). The secretion of somatostatin is stimulated by glucose and other nutrients (Rorsman and Huising, 2018), as well as paracrine factors (van der Meulen et al., 2015; Adriaenssens et al., 2016; Vergari et al., 2019), but because of the scarcity of δ-cells and difficulties of accessing them for physiological investigations the understanding of the control mechanisms for somatostatin secretion is still rather limited (see Rorsman and Huising [2018] for a review of δ-cell physiology). In this issue of the *Journal of General Physiology*, Denwood et al. (2019) from the Rorsman laboratory present an extensive experimental effort that provides new insights into the mechanisms by which glucose stimulates somatostatin secretion from δ-cells.

The glucose sensing machinery of δ-cells shares many features with that of β-cells. For example, glucose enters the cells mainly via the GLUT family of facilitative transporters (although δ-cells recently were reported to also be equipped with Na⁺-dependent glucose transporters [Vergari et al., 2019]). Glucose is also phosphorylated by glucokinase in both cell types (Rorsman and Huising, 2018) and subsequent metabolism of the sugar increases the intracellular ATP/ADP ratio. ATP-sensitive K⁺ (K_{ATP}) channels then translate this metabolic signal into an electrical one (Berts et al., 1996). Accordingly, glucose-induced closure of K_{ATP} channels results in membrane depolarization and triggering of action potentials. δ-cells are often electrically active at lower glucose concentrations than β-cells, however, and the set of channels responsible for action potential generation differs between the cell types. In δ-cells, there are low-voltage-activated T-type Ca^{2+} channels, which in turn activate voltage-dependent Na⁺ channels and several types of high-voltage-activated Ca^{2+} channels (Braun et al., 2009). Glucose stimulation of δ-cells thereby results in an increase in the cytoplasmic Ca^{2+} concentration ($[Ca^{2+}]_i$), often manifested as oscillations (Berts et al., 1996; Göpel et al., 2000), that trigger exocytosis of somatostatin-containing secretory granules (Göpel et al., 2004).

Although δ-cells are electrically excitable, it turns out that membrane depolarization by itself is a weak stimulus for somatostatin secretion. Previous work, also from the Rorsman laboratory (Zhang et al., 2007), demonstrated that as much as 75% of glucose-induced somatostatin secretion is due to a voltage-independent amplifying effect of the sugar. The depolarization-stimulated entry of Ca^{2+} through voltage-dependent R-type channels triggers CICR via RYRs and the resulting mobilization of Ca^{2+} from intracellular stores amplifies exocytosis (Zhang et al., 2007). CICR is enhanced by glucose but it has been unclear how the sugar acts in this process. The present study (Denwood et al., 2019) pinpoints the intracellular messenger cAMP as the amplifying factor in glucose-stimulated somatostatin secretion.

By expressing a FRET-based cAMP reporter in islets from mice with transgenic fluorescent labeling of δ-cells, the authors found that the cytoplasmic cAMP concentration ($[cAMP]_i$) in δ-cells increased upon glucose stimulation. A similar effect was

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also observed in the nonlabeled cell population dominated by β-cells, confirming previous studies in this cell type (Dyachok et al., 2008). In support of the cAMP response in δ-cells being functionally relevant, it was shown that the amplifying effect of glucose on somatostatin secretion was mimicked by the adenylyl cyclase (AC) activator forskolin and enhanced by the $[cAMP]_i$-elevating hormone GLP-1, and that both the effect of glucose and that of the cAMP-generating agents were suppressed by inhibitors of the cAMP effector proteins PKA and Epac2. Moreover, when exocytosis was quantified by recordings of δ-cell membrane capacitance, cAMP directly stimulated exocytosis in response to controlled depolarizations. Together, these results provide persuasive evidence that cAMP is a critical signal in glucose-stimulated somatostatin secretion.

How does cAMP amplify somatostatin secretion? Several observations indicate that the messenger enhances signaling via Ca^{2+}. The amplifying effects of forskolin and cAMP were abolished when ER Ca^{2+} stores were depleted using the SERCA inhibitor thapsigargin. Similarly, the amplifying effect of glucose was impaired by ryanodine, which interferes with the RYR Ca^{2+} release channels. Interestingly, the effects of ryanodine and the PKA and Epac2 inhibitors were overlapping rather than additive, indicating a common mechanism of action.

To further clarify the relationship between cAMP and Ca^{2+}, Denwood et al. (2019) recorded $[Ca^{2+}]_i$ in δ-cells in which the Ca^{2+} reporter protein GCaMP3 was transgenically expressed. When the cells were maintained at a substimulatory glucose concentration and the membrane was depolarized with an excessive K^+ concentration, there was only a transient $[Ca^{2+}]_i$ elevation. In contrast, depolarization in the presence of stimulatory glucose levels induced recurring $[Ca^{2+}]_i$ spikes that were sensitive to thapsigargin, implying involvement of intracellular Ca^{2+} mobilization. More quantitative recordings performed with the Ca^{2+} indicator indo-1 introduced into the cells via a patch pipette showed that $[cAMP]_i$ elevation by forskolin moderately increased basal $[Ca^{2+}]_i$, potentially reflecting increased activation of the RYR Ca^{2+} release channels. This $[Ca^{2+}]_i$ elevation was associated with a strongly enhanced exocytosis response to subsequent voltage-clamp depolarization, potentially reflecting stimulated secretory vesicle priming. cAMP also increased peak $[Ca^{2+}]_i$ in response to such depolarizations and extended the duration of the $[Ca^{2+}]_i$ elevations beyond the period of depolarization. These effects could not be explained by alterations of the plasma membrane Ca^{2+} channel currents, but consistent with the involvement of ER Ca^{2+} release, they were sensitive to thapsigargin.

If cAMP promotes CICR, one would expect to see $[Ca^{2+}]_i$ spikes that are unrelated to membrane potential. This was indeed what Denwood et al. (2019) observed. Increases in cAMP sometimes induced "spontaneous" $[Ca^{2+}]_i$ spikes even when the membrane potential was clamped at –70 mV. The authors went further and performed parallel recordings of $[Ca^{2+}]_i$ and electrical activity in glucose-stimulated δ-cells. They found that many $[Ca^{2+}]_i$ spikes were not preceded by action potentials, indicating that, under these conditions, the glucose-induced $[Ca^{2+}]_i$ increases cannot be explained by voltage-dependent Ca^{2+} entry alone. The presence of voltage-independent $[Ca^{2+}]_i$ increases
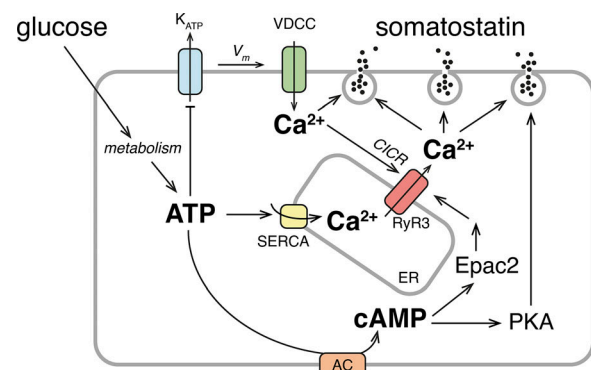

Figure 1. **Overview of glucose stimulus-secretion coupling in the pancreatic δ-cell.** Glucose enters the δ-cell and subsequent metabolism generates ATP at the expense of ADP. This change in energy status has several important effects. As depicted at the top, the increase in ATP (combined with reduced ADP) closes K_{ATP} channels, which cause the membrane potential (V_m) to become less negative and voltage-dependent Ca^{2+} channels (VDCC) to open. The resulting influx of Ca^{2+} triggers exocytosis of somatostatin-containing secretory granules. The Ca^{2+} signal is amplified by Ca^{2+} mobilization from the ER mediated by RYR3 and such CICR contributes to additional exocytosis. ATP generated by glucose metabolism amplifies the CICR process by fuelling the SERCA to sequester Ca^{2+} into the ER store, making it available for release when the RYR channels open. The principal novelty in the study by Denwood et al. (2019) is that glucose metabolism amplifies somatostatin secretion by promoting the generation of cAMP from ATP via adenylyl cyclases (AC). A main action of cAMP is to trigger CICR via Epac2, which somehow activates RYRs. cAMP concomitantly activates PKA, which exerts a potent stimulatory action directly on the exocytosis process.

may explain why the hyperpolarizing agent diazoxide sometimes fails to inhibit somatostatin secretion (Zhang et al., 2007).

cAMP regulates secretion via both PKA and the guanine nucleotide exchange factor Epac2 (Seino and Shibasaki, 2005). To elucidate cAMP effector protein involvement, Denwood et al. (2019) used both pharmacological activators and inhibitors as well as cells from Epac2-deficient mice. PKA and Epac2 inhibition had largely overlapping effects, but there were also differences. In general, the data indicate that Epac2 primarily influences Ca^{2+} signaling, whereas PKA mainly acts at the level of exocytosis. However, the precise mechanisms remain to be elucidated. In cardiomyocytes, Epac2 has been proposed to activate CICR by a mechanism involving phospholipase C-ε, which in turn activates PKCε and the Ca^{2+}/calmodulin-dependent protein kinase CaMKII, a known modulator of RYRs (Oestreich et al., 2009). A similar mechanism has been proposed in β-cells (Dzhura et al., 2010), but the functional importance of RYRs in β-cells is disputed and some studies indicate that cAMP-promoted CICR can be explained by PKA sensitization of inositol-1,4,5-trisphosphate receptors (Dyachok and Gylfe, 2004). In δ-cells, Epac2 activation of RYRs is unlikely to involve phospholipase C-ε, which shows very low expression (Adriaenssens et al., 2016). The most prominent RYR in δ-cells, RYR3, is a substrate for PKA phosphorylation—a modification that facilitates activation of the channel—but PKA inhibition did not influence Ca^{2+} release in δ-cells. An understanding of how Epac2 and PKA enhances CICR and somatostatin secretion clearly requires further investigation.

cAMP probably does not explain all of the amplifying effect of glucose on somatostatin secretion. Since PKA and Epac inhibitors abolished the effect of forskolin, but only partially inhibited that of glucose, additional mechanisms appear to be involved. It can be speculated that ATP and other metabolites exert amplifying effects on exocytosis, as is the case in β-cells (Prentki et al., 2013). Another important effect of glucose in β- and α-cells is to promote filling of ER Ca²⁺ stores (Tengholm et al., 1999; Liu et al., 2004), which by itself increases the magnitude of Ca²⁺ release. Denwood et al. (2019) considered this possibility, and as anticipated for such an effect of glucose in δ-cells, they found that release of ER Ca²⁺ by uncaging of inositol-1,4,5-trisphosphate evoked a larger $[Ca^{2+}]_i$ increase and more exocytosis at a high compared with a low glucose concentration.

The study by Denwood et al. (2019) does not clarify how glucose increases $[cAMP]_i$ in δ-cells. The mechanism may be similar to that in β-cells, in which glucose has been suggested to facilitate cAMP production by increasing the concentration of the AC substrate ATP (Dyachok et al., 2008). Because most ACs have a low K_m for ATP, they are perhaps not limited by substrate availability. It has also been suggested that the glucose effect on cAMP in β-cells involves the high-K_m soluble AC (Ramos et al., 2008), but this study was performed in a cell line and convincing evidence for a functionally important role of soluble AC in primary islet cells is yet awaited. Since AMP is an inhibitor of ACs, an alternative explanation for glucose-stimulated cAMP production is that it is mediated by the reduction of AMP, which occurs concomitantly with the glucose-induced increase of ATP (Peercy et al., 2015).

The available data do not exclude that the effect of glucose on cAMP is indirect and reflects paracrine signaling from e.g., glucagon or the corticotropin family member urocortin3, both of which act via Gαs-coupled receptors. Because glucose inhibits secretion from α-cells, the impact of glucagon should decrease with increasing glucose concentrations, but urocortin3 is co-eleased with insulin from glucose-stimulated β-cells and has been reported to be critical for glucose-stimulated somatostatin secretion (van der Meulen et al., 2015). Irrespective of the precise mechanism for glucose-stimulated cAMP formation, it is likely that the cAMP-dependent Ca²⁺ mobilization identified by Denwood et al. (2019) participates in amplification of somatostatin secretion by various cAMP-generating receptor agonists.

Fig. 1 summarizes stimulus-secretion coupling for glucose-induced somatostatin secretion from δ-cells. The study by Denwood et al. (2019) is entirely based on δ-cells from mice, and future studies are needed to clarify if human δ-cells are regulated by similar mechanisms. An intriguing possibility is that altered δ-cell signaling and somatostatin secretion underlie the aberrant islet hormone secretion patterns in diabetes. Apart from the well-documented insufficiency of insulin, the characteristic anti-phasic insulin and glucagon pulsatility is disrupted in type 2-diabetic patients, a disturbance that precedes manifestation of hyperglycemia and often is paralleled by hypersecretion of glucagon (Menge et al., 2011; Rohrer et al., 2012). Early clinical studies have indicated that infusion of somatostatin may be useful to reduce diabetes hyperglycemia (Gerich et al., 1977).

Another feature of both type 1 and type 2 diabetic patients is that the counter-regulatory glucagon response to hypoglycemia is reduced. It has been suggested from animal studies that this effect may be explained by hypersecretion of somatostatin (Yue et al., 2012). The δ-cell thus constitutes an interesting therapeutic target to improve glycemic control. Many questions remain and call for continued efforts to unravel the exciting physiology of the δ-cell and its important role as a paracrine regulator of islet function.

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