

REVIEW

Regulation of α -cell glucagon secretion: The role of second messengers

 Samuel Acreman^{1,2} | Quan Zhang¹

¹Oxford Centre for Diabetes, Endocrinology and Metabolism, Radcliffe Department of Medicine, University of Oxford, Oxford, OX3 7LE, UK

²Institute of Neuroscience and Physiology, Department of Physiology, Metabolic Research Unit, University of Goteborg, Box 430, S-405 30, Göteborg, Sweden

Correspondence

Quan Zhang, Oxford Centre for Diabetes, Endocrinology and Metabolism, Radcliffe Department of Medicine, University of Oxford, Oxford, OX3 7LE, UK,
Email: quan.zhang@ocdem.ox.ac.uk

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Abstract

Glucagon is a potent glucose-elevating hormone that is secreted by pancreatic α -cells. While well-controlled glucagon secretion plays an important role in maintaining systemic glucose homeostasis and preventing hypoglycaemia, it is increasingly apparent that defects in the regulation of glucagon secretion contribute to impaired counter-regulation and hyperglycaemia in diabetes. It has therefore been proposed that pharmacological interventions targeting glucagon secretion/signalling can have great potential in improving glycaemic control of patients with diabetes. However, despite decades of research, a consensus on the precise mechanisms of glucose regulation of glucagon secretion is yet to be reached. Second messengers are a group of small intracellular molecules that relay extracellular signals to the intracellular signalling cascade, modulating cellular functions. There is a growing body of evidence that second messengers, such as cAMP and Ca^{2+} , play critical roles in α -cell glucose-sensing and glucagon secretion. In this review, we discuss the impact of second messengers on α -cell electrical activity, intracellular Ca^{2+} dynamics and cell exocytosis. We highlight the possibility that the interaction between different second messengers may play a key role in the glucose-regulation of glucagon secretion.

KEYWORDS

glucagon, pancreatic islet, second messenger

1 | INTRODUCTION

Diabetes is a metabolic disorder stemming from a loss of proper blood glucose regulation by the islets of Langerhans. Located within the pancreas, these micro-organs are small cell clusters predominantly composed of insulin-secreting β -cells, glucagon-secreting α -cells, and somatostatin-secreting δ -cells.^{1–3} The fact that glucose-lowering insulin secretion and/or signalling is lost in diabetes has led it to be considered as an insulin-related mono-hormonal disease.⁴ However, over recent years, this β -cell centric view has been challenged and it has been proposed that diabetes, in fact, has a multi-hormonal aetiology, consisting of dysfunction in the secretion of both insulin and glucagon.^{5–7}

Glucagon is a potent glucose-elevating hormone and normally it is released by α -cells in response to a fall in plasma glucose. It constitutes part of the 'counter-regulatory' mechanism of the body by rapidly releasing glucose stored in the liver and stimulating gluconeogenesis, efficiently restoring normal blood glucose levels. The glucose-sensing function of α -cells and the consequent release of glucagon has been demonstrated to become dysregulated in diabetes.⁸ As a consequence, hyperglycaemia becomes exacerbated and the risk of severe, life-threatening hypoglycaemia increases dramatically. The importance of the role that glucagon plays in the pathophysiology of diabetes is further supported by studies in which application of glucagon receptor antagonists, thereby blocking glucagon function, was capable of restoring normoglycaemia in β -cell

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ablated animal models.^{9–12} Whereas efforts have already been made to apply glucagon receptor for the treatment of diabetes,^{13–15} it is undoubtedly clear that therapeutic approaches aiming to restore normal α -cell function could provide more optimal treatment options for diabetes, given its role in the prevention of hypoglycaemia.

Unlike the well-established β -cell stimulation-secretion coupling model, the precise mechanisms that regulate α -cell glucagon secretion have yet to reach a consensus. This knowledge gap hampers efforts to fully understand how dysfunction occurs in diabetes. The electrical excitability of α -cells naturally led to the hypotheses that high glucose inhibits glucagon secretion by modulating α -cell electrical activity and downstream Ca^{2+} -dependent exocytosis. To date, proposed mechanisms can be categorised as intrinsic and paracrine: intrinsic mechanisms suggest that glucose metabolism directly modulate activity of α -cells; while paracrine mechanisms involve factors released from neighbouring β - and δ -cells that mediate the inhibitory effects of glucose. These two types of mechanisms are not mutually exclusive and are likely complementary to each other; many of the effects have been proposed to be mediated through second messengers.

Second messengers are a group of small intracellular molecules (e.g., cAMP, Ca^{2+} , IP_3) that amplify/relay extracellular signals (or the ‘first messengers’) to execute biological functions. The effects of glucose on intracellular concentrations of second messengers in β -cells have been well documented,^{16,17} and recent reports indicate metabolic regulation of the same group of molecules also control non- β -cell activity and hormone secretion.^{18–21} In this review, we summarise the current research on glucose regulation of glucagon secretion, with a special focus on the involvement of the ubiquitous second messengers cAMP and Ca^{2+} .

2 | α -CELL ELECTRICAL ACTIVITY

Different from β -cells, α -cells are electrically active and exhibit Ca^{2+} oscillations at low glucose concentrations (<3 mmol/L). Therefore, for a long time, Ca^{2+} activity at low glucose was used as the identifying signature of α -cells in islets.²² Interestingly, many of the proteins involved in glucose sensing are similar to that of β -cells, for example, the ATP-sensitive potassium (K_{ATP}) channels are identical in both islet cell types.²³ Considering this, it is rather intriguing that the basal activity of the K_{ATP} channels is approximately ten-fold lower than that recorded in β -cells under the same conditions.²⁴ As a result, the high input resistance of α -cells enables the firing of action potentials even when the ambient glucose concentration is low (Figure 1A). The action potentials of α -cells are often overshooting (>+10 mV), suggesting the rapid activating and inactivating voltage-gated Na^+ (Na_v) channels are involved in the upstroke of

the action potentials. Indeed, $\text{Na}_v1.3$ (encoded by *Scn3a*) and 1.7 (encoded by *Scn9a*) are expressed in the α -cells with $\text{Na}_v1.3$ playing an important role in shaping the action potentials.²⁵ It is intriguing though that $\text{Na}_v1.7$, although contributing significantly to the Na^+ current in α -cells, provides little input to the action potential upstroke – attributable to its negative voltage-dependent inactivation.

Although most electrophysiological evidence showed that α -cells are electrically active at low glucose, there are conflicting reports regarding their electrical activity when challenged by high glucose (Figure 1B). Using the patch-clamp technique with intact islets, we demonstrated that glucose metabolism induced α -cell membrane depolarisation and increased firing frequency of action potentials. This coincides with a 20 mV reduction in the action potential amplitude.²⁴ We attributed this to reduction in α -cell K_{ATP} channel activity. Once transported into α -cells, glucose is phosphorylated by high affinity hexokinase 1 (HK1) and low affinity glucokinase (GCK) before being broken down to produce pyruvate, and entering the main ATP-generating citric acid cycle.²⁶ ADP is then converted to ATP, increasing the ATP:ADP ratio and closing the K_{ATP} channels. The consequent membrane depolarisation partially inactivates Na_v channels, thereby reducing action potential amplitude.^{24,27–29} While HK1 may control the basal glucose metabolic rate, metabolism of elevated glucose concentrations (≥ 5 mmol/L) is controlled by GCK. Conditional tissue specific ablation of GCK expression in α -cells resulted in a loss of glucose induced membrane depolarisation and inhibition of glucagon secretion in a mouse model.³⁰

It has also been proposed that high glucose induces membrane depolarisation through the process of glucose entry into the cell. Besides glucose transporters (GLUTs), glucose entry can also be facilitated via the activity of sodium-glucose co-transporters (SGLTs), utilising the Na^+ concentration gradient. Both SGLT 1 and 2 are expressed in α -cells, with respective K_m s of 2 and 5 mmol/L.^{31–33} These differing affinities between subtypes allows each to play a specific role in the cell. The low K_m SGLT1 transports glucose at low extracellular concentrations, whereas the high K_m SGLT2, functions at glucose concentrations that are maximally inhibitory of glucagon secretion. Mathematic modelling suggested the additional Na^+ conductance from the activation of SGLT2 contributes to α -cell membrane depolarisation at high glucose.³⁴ However, this cannot be the sole determinant of glucose-induced membrane potential change. If that were the case, glucose-induced depolarisation should occur almost immediately subsequent to elevation of extracellular glucose as it would bypasses glucose metabolism. This differs from experimental evidence demonstrating that membrane depolarisation often develops a few minutes after high glucose was applied.²⁴ It is therefore more likely that, rather than delivering a depolarising ion conductance, SGLT2

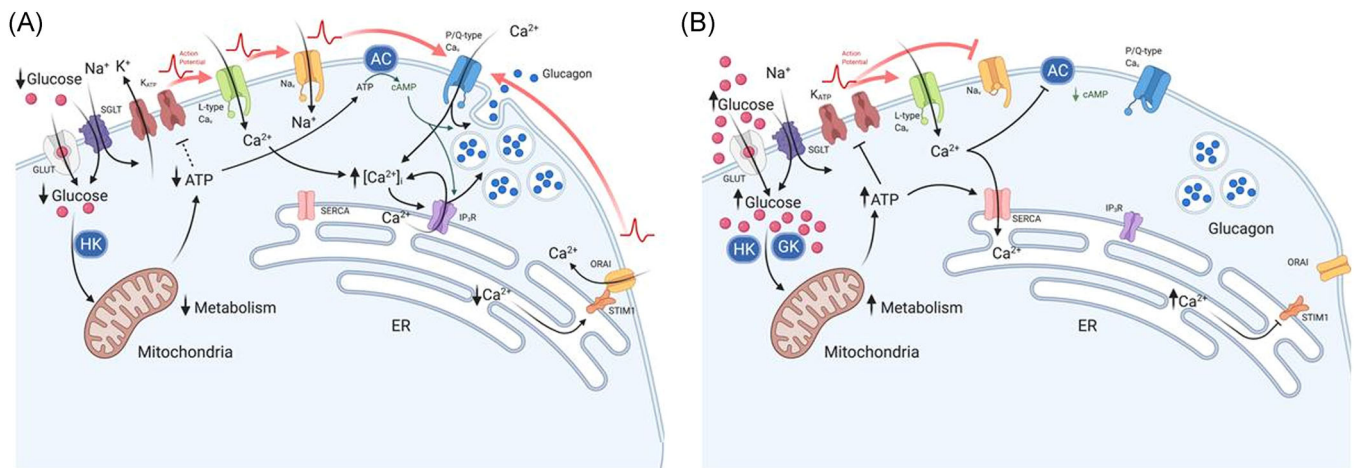


FIGURE 1 Effect of glucose on α -cell Ca^{2+} and cAMP at (A) low glucose and (B) high glucose concentrations, which result in changes to glucagon secretion. (A) At low glucose, there is a low rate of glucose transport across the membrane via GLUT and SGLT proteins. Glucose is then converted to glucose-6-phosphate, by hexokinase 1 (HK), to generate ATP via glycolysis. A modest increase in the ATP:ADP ratio, leads to partial closure of K_{ATP} -channels, maintaining the membrane potential at the point of allowing action potential (AP) generation: this enables the activation of L-type Ca_v and Na_v channels. The resulting high-amplitude action potentials activate P/Q-type channels, triggering exocytosis and glucagon release. In addition, adenyl cyclase (AC) production of cAMP primes vesicles for exocytosis and potentiates Ca^{2+} -induced Ca^{2+} release from the endoplasmic reticulum (ER). Consequently, exocytosis of glucagon-containing vesicles is high. The firing of action potentials can also be attributable to the activation of store-operated Ca^{2+} entry (SOCE) via ORAI and STIM1, which is triggered by ER Ca^{2+} depletion due to low SERCA activity. (B) At high glucose concentrations, there is a high rate of glucose transport into the cytosol, leading to membrane depolarisation. This occurs as a result of increased ATP production via glycolysis, due to substrate abundance: high K_m glucokinase (GCK) phosphorylates glucose to produce glucose-6-phosphate. High intracellular ATP results in complete closure of K_{ATP} -channels and, consequently, membrane depolarisation. Membrane depolarisation can also be induced by the process of glucose transport through sodium/glucose co-transporters (SGLTs): Na^+ is co-transported with glucose and thus provides a depolarising membrane conductance. Na_v channels, at this membrane potential, become inactivated, whereas L-type Ca_v channels remain activatable and contribute to action potential generation. However, action potential amplitude is reduced at high glucose concentrations (despite firing at a higher frequency) and are no longer able to activate P/Q-type Ca_v channels, the exocytosis-related Ca_v channel in α -cells. The effect of membrane depolarisation and higher action potential firing frequency may also lead to increased cytosolic Ca^{2+} within domains adjacent to Ca^{2+} -sensitive adenyl cyclase, inhibiting cAMP production and vesicle priming. However, higher ATP may also result in α -cell membrane repolarisation. High ATP-activated SERCA uptake of Ca^{2+} replenish ER Ca^{2+} , deactivating ORAI-mediated Ca^{2+} entry, resulting in suppression of α -cell excitability and glucagon secretion. Red arrows indicate action potential propagation. Figure created using BioRender.com

predominantly provides an entry route for glucose (which subsequently goes under metabolism, leading to the closure of K_{ATP} channels) parallel to GLUTs.

Differing from our observations, other groups observed α -cell membrane repolarisation in the presence of high glucose concentration.^{35,36} One of the ion channels suggested to mediate this response is the two-pore domain channel TASK-1.³⁶ TASK-1 channels are small conductance, outwardly rectifying K^+ channels active at physiological voltages.^{37,38} Inhibition of TASK-1 channels increases α -cell electrical activity and, intriguingly, glucose-inhibition of glucagon secretion. Therefore, these channels may function to limit excitability of α -cells at high glucose concentrations.³⁶ However, it remains to be established if/how the TASK-1 channel activity changes in response to glucose metabolism.

In addition to background leak K^+ channels, voltage-dependant K^+ (K_v) channels are involved in the excitability of α -cells. α -cell K_v channels open at the peak of the action potential, repolarising the membrane and shaping the downstroke of action potentials.³⁹ This hyperpolarising current, activated via K_v2 channels, enables reactivation of voltage-gated Ca^{2+} (Ca_v) and Na_v

channels, preventing depolarisation-induced inhibition of α -cell action potential firing.⁴⁰

K^+ and Na^+ are not the only cations involved in the electrical activity of the α -cell. Ca^{2+} currents, through Ca_v channels, also play an important role in the upstroke of the action potential. Blockade of L-type channels, which carry the bulk of the overall Ca^{2+} current in mice, eliminates action potential generation.⁴¹ It is possible that L-type Ca^{2+} channels function as the pace making channels in α -cells, initiating the action potential firing. The same Ca_v channel may play a similar role in human α -cell electrical activity.⁴² However, it is rather counterintuitive that dihydropyridines (e.g., nifedipine or isradipine), potent L-type Ca_v channel blockers, exert no apparent inhibitory effect on glucagon secretion at low glucose.

2.1 | Intracellular Ca^{2+} store in α -cell electrical activity

Transmembrane Ca^{2+} currents are not the sole determinants of cellular Ca^{2+} dynamics and electrical activity. The Ca^{2+} stored within the endoplasmic reticulum

(ER) has also been proposed to be part of the α -cell metabolic sensing machinery, regulating α -cell electrical activity (Figure 1). It was suggested that the low $[ATP]_i$ at low glucose restricts the sarco/endoplasmic Ca^{2+} ATPase (SERCA) activity, depleting the ER Ca^{2+} store. This, in turn, activates ER-bound Ca^{2+} sensing stromal interaction molecules (STIM), which undergo conformational changes, activating Orai1 Ca^{2+} channel on the plasma membrane. The Ca^{2+} influx through the Orai1 channels induces membrane depolarisation and α -cell electrical activity.⁴³ This is reversed in response to an increase in extracellular glucose, whereupon higher $[ATP]_i$ replenishes ER Ca^{2+} by the activation of SERCA and subsequently inactivates the store-operated channels (SOCs) to repolarise the membrane.^{44,45} However, we note here that expression of both Stim and Orai1 is fairly low in the α -cells and direct measurements of Orai1 current is technically challenging. Future studies using newly developed ER Ca^{2+} probes in conjunction with membrane potential recordings, would provide enlightenment to this intriguing hypothesis.

2.2 | Ca^{2+} in exocytosis

Electrical activity, or action potentials, provide signals for the release of hormones through exocytosis, a process where hormone containing granules fuse with the plasma membrane to release their cargo. As with most neural and exocrine cells, α -cell exocytosis is Ca^{2+} -dependent.^{46–48} α -cells are equipped with SNARE proteins (a group of proteins that form a complex required for exocytosis): VAMP2; SNAP-25; and Syntaxin-1.^{49,50} Furthermore, α -cells also express synaptotagmin VII (encoded by *Syt7*) a SNARE-related Ca^{2+} sensing protein.^{51,52} Ablating *Syt7* reduced glucose- and depolarisation-triggered cell exocytosis. It is interesting however, that residual exocytosis of *Syt7*^{-/-} α -cells is sensitive to the N-type Ca^{2+} channel blocker, ω -conotoxin. This suggests that: (1) α -cells express Ca^{2+} -sensing proteins other than *Syt7* and (2) α -cell granules are tightly coupled to a subtype of Ca_v (N-type) channel. These, together with observations that N- or P/Q-type Ca^{2+} channels are the exocytosis-relevant Ca_v channels,^{24,28} despite their relatively low contribution in transmembrane Ca^{2+} currents, raises the possibility that α -cells exhibit exocytosis ‘hotspots’. It is possible that in these areas N- and/or P/Q-type Ca^{2+} channels, SNARE complexes, and secretory granules are tightly coupled to form releasing sites for rapid secretion of glucagon through interaction with lipid rafts.⁵³ This is similar to that has been describe in L-type Ca^{2+} channels and insulin-containing granules in β -cells.⁵⁴ Interestingly, L-type Ca^{2+} channels, although conducting >50% of transmembrane Ca^{2+} currents, play an insignificant role in α -cell exocytosis and glucagon secretion further supporting the existence of specialised release ‘hotspots’ in α -cells.⁴¹

As discussed above, increase in intracellular Ca^{2+} triggers α -cell exocytosis and glucagon secretion.

However, interestingly, removal or lowering extracellular Ca^{2+} below physiological range paradoxically stimulates glucagon secretion.^{55–57} Perfusion of rat pancreas with solutions containing low Ca^{2+} (170 μ mol/L), transiently stimulated glucagon secretion at both low and high concentrations of glucose, an effect reversed by re-introduction of physiological concentrations of Ca^{2+} (1.94 mmol/L).⁴⁶ Furthermore, chelating extracellular Ca^{2+} by EGTA exerted similar effect on glucagon secretion.⁵⁵ However, with prolonged exposure to low Ca^{2+} , the initially high secretion of glucagon begins to decline, presumably due to depletion of intracellular Ca^{2+} stores.⁴⁶

Mediation of this response to extracellular Ca^{2+} can be attributed to the Ca^{2+} -sensing receptor (CaSR), a G_q -coupled GPCR, expressed in both α - and β -cells.^{58,59} CaSR may also be involved in the glucose-regulation of glucagon secretion and hyperactivity of these receptors (gain-of-function mutations) is correlated to impaired glucose-suppression of glucagon secretion and glucose tolerance.⁶⁰ However, it is interesting to note that loss-of-function CaSR mutations do not appear to influence glucose tolerance or insulin secretion.⁶¹

3 | PARACRINE REGULATION OF GLUCAGON

In addition to its intrinsic mechanisms, it has also been proposed that α -cell glucose sensing is, at least in part, mediated by the factors released by neighbouring β - and/or δ -cells, as well as incretins released from the gut.^{62–69} Because this has been discussed in a number of excellent reviews in the recent years, here we will only briefly review the literature relevant to the topic of this review. We categorise these external regulatory factors as inhibitory or stimulatory (Table 1).

3.1 | Inhibitory factors

Somatostatin (SST), produced by δ -cells, is secreted in response to high glucose and potentiated by both insulin and glucagon.^{21,70–72} It is a potent inhibitor of both glucagon and insulin, activating G_{α_i} -coupled somatostatin receptor (SSTR) 2 and 5, on α - and β - cells respectively.^{63,70,73–75} The effect of SSTRs activation is two-fold. On one hand, it inhibits adenylyl cyclases, lowering intracellular cAMP, a second messenger that stimulates glucagon secretion.⁷⁶ On the other hand, it also activates the G-protein-coupled inwardly-rectifying K^+ (GIRK) channels.^{62,73} Opening of the GIRK channels rapidly repolarises α -cells, abolishing α -cells electrical activity and subsequent glucagon secretion.

Beta cells may exert their effects on α -cells through insulin and co-released factors, such as Zn^{2+} and γ -Aminobutyric acid (GABA). Insulin has long been

TABLE 1 Non-nutrient factors regulating α -cell glucagon secretion

Factors	Origin	Effects on α -cells	Effect on glucagon secretion	References
Somatostatin	Islet δ -cells	Electrical activity/cAMP	Inhibitory	47,62,63,76
γ -Aminobutyric acid	Islet β -cells	Electrical activity	Inhibitory	66,67,77,81
Insulin	Islet β -cells	Electrical activity/cAMP	Inhibitory/no effect	76–78
Zn ²⁺	Islet β -cells	Electrical activity	Inhibitory/no effect	64,78
GLP-1	Intestinal L-cells, islet α -cells	Electrical activity, cAMP	Inhibitory	68,82–85
Adrenaline	Adrenal glands	Electrical activity, cAMP	Stimulatory	85,89,90
Glucagon	Islet α -cells	cAMP	Stimulatory	94
GIP	Intestinal L- or L/K-cells, islet α -cells	cAMP	Stimulatory	95,97,98

GLP-1: Glucagon-like peptide-1; GIP: glucose-dependent insulinotropic polypeptide

proposed as a negative regulator of glucagon secretion.⁷⁷ However, with conflicting effects of insulin on α -cell electrical activity,^{41,78} doubt has been cast on the idea that insulin acts as the primary factor in the suppression of glucagon secretion at high glucose. More recent evidence has demonstrated that the glucagonostatic effect of insulin is exerted by through stimulation of δ -cell SST secretion via SGLTs.⁷¹ Zn²⁺ is packed into insulin-containing granules through Zn²⁺ transporters and is required to stabilise the hexameric insulin crystals within.⁷⁹ Consequently, glucose-stimulated insulin secretion will greatly increase intra-islet Zn²⁺ concentration. It has been proposed that Zn²⁺ may activate α -cell K_{ATP} channels to inhibit α -cell electrical activity.⁷⁸ However, its impact on glucagon release from either islets or α TC1-9 cells was unclear.⁶⁴ GABA, meanwhile, is an inhibitory neurotransmitter released by β -cells in response to glucose.⁸⁰ It activates the A-type GABA receptor (GABA_AR), a Cl⁻ channel present on α -cells.^{66,67} This induces membrane hyperpolarization, inhibiting electrical activity and lowering of [Ca²⁺]_i.⁷⁷ Indeed, inhibition of GABA_AR increased glucagon secretion at low glucose and reduced the inhibitory effect of glucose on glucagon secretion.⁶⁷ It is interesting to note that the GABA_AR mediated effect can be enhanced in the presence of high glucose, where intra-islet insulin induces higher membrane insertion of the Cl⁻ channel.⁸¹

Glucagon-like peptide-1 (GLP-1), an incretin produced by intestinal L-cells, was shown to modulate glucagon secretion from α -cells.⁶⁸ Although, the low level of GLP-1 receptor expression in α -cells^{69,78} and the observation that GLP-1 inhibited glucagon secretion can be restored by inhibiting SSTR2, led to the hypothesis that GLP-1 effect is mediated by the stimulation of SST secretion.^{82,83} However, we showed that GLP-1 receptors are expressed in α -cells, albeit at low level,⁸⁴ and mediate the SST-independent effects of GLP-1 via the activation of PKA.⁸⁵

3.2 | Stimulatory factors

Adrenaline's stimulatory effect on glucagon secretion has been well established. Released in response to various physiological stresses, such as fight-or-flight, exercise, infection and hypoglycaemia.⁸⁶ Adrenaline rapidly elevates blood glucose by (1) direct stimulation of hepatic glucose production, (2) stimulation of glucagon and (3) inhibition of insulin secretion.^{87,88} It stimulates α -cells through the activation of α_1 - and β -adrenoceptors, which are G_{q/11} and G_s coupled receptors, respectively.^{85,89}

While activation of G_s-proteins exerts a strong direct stimulatory effect on α -cell exocytosis via increasing intracellular cAMP concentration,⁹⁰ the effect mediated by G_{q/11}-protein is through PKC, and, potentially, the release of ER Ca²⁺. The G_{q/11}-protein activates the Phospholipase C (PLC), which cleaves phosphatidylinositol 4, 5 biphosphate (PIP₂) into 1,4,5-inositol triphosphate (IP₃) and diacylglycerol (DAG). IP₃ subsequently activates the ligand-gated IP₃ receptor, a Ca²⁺-releasing channel located on the ER membrane, increasing cytosolic Ca²⁺ concentration.⁹¹ DAG binds to and activates protein kinase C (PKC), a serine/threonine kinase, on the plasma membrane. This process can be further potentiated by Ca²⁺-induced translocation of PKC to cell membrane.⁹² It has been shown that PKC activation by phorbol 12-myristate 12-acetate (PMA) stimulates glucagon secretion, without direct effect on α -cell electrical activity.^{85,93} It is therefore possible that PKC is able to potentiate IP₃R release ER Ca²⁺ and stimulate glucagon release.

Interestingly, glucagon itself can serve as a positive autocrine signal for α -cell glucagon secretion. α -cells are equipped with glucagon receptors (a G_s-coupled receptor) and glucagon was shown to elevate intracellular cAMP concentration, promoting exocytosis.⁹⁴ This mechanism can play an important role in glucagon secretion in response to hypoglycaemia, whereupon a large amount of glucagon is required within a short period.

Unlike GLP-1, another incretin produced by gut cells (K or L/K-cells), glucose-dependent insulinotropic polypeptide (GIP) potentiates glucagon secretion. Upon binding to its receptor, GIP stimulates α -cell cAMP production via G_{α_s} -dependent pathway,^{95–97} enhancing glucagon secretion at high glucose levels.⁹⁸ It is interesting to note that GIP is also produced by α -cells, albeit relatively low in quantity, possibly contributing to intraslet paracrine and autocrine regulation.⁹⁹

4 | cAMP

As discussed above, much of the physiological regulation of glucagon secretion is exerted via intracellular changes in α -cell cAMP concentrations. Downstream to the activation of G_s -coupled receptors, the predominant effect of cAMP is to potentiate glucagon secretion, and this can be replicated with the uncaging of cAMP or application of forskolin.^{37,100} Increases in cAMP concentration stimulate granules docking to the membrane, priming them for exocytosis, and forming the ready releasable pool of granules that can be rapidly released when proximal Ca^{2+} channels activated, thereby enhancing glucagon secretion.^{41,85,90} Therefore, it is possible that cAMP represents the key molecule for the glucose sensing in α -cells, as proposed by a recent study.¹⁸ Cyclic AMP is catalysed by adenylyl cyclases (ACs) and hydrolysed by phosphodiesterases (PDEs). Intracellular cAMP concentration can fluctuate rapidly depending on the activities of these two enzymes with opposing actions, resulting in oscillations similar to that seen with Ca^{2+} .¹⁹ Furthermore, the concentration of cAMP is not homogenous throughout the cytoplasm. It is likely to be compartmentalised, constrained by the localised distribution and activities of ACs and PDEs.¹⁰¹

4.1 | Production and degradation of cAMP

There are a 10 different AC subtypes,¹⁰² with AC3, AC5, AC6, and AC9 expressed in α -cells, AC6 being the most highly expressed.⁴⁹ Of these 4 subtypes, AC5 and AC6 have had their presence demonstrated at the protein level by immunohistochemistry.¹⁰³

The specific expression of PDEs, meanwhile, is yet to be fully elucidated in α -cells, in contrast to β -cells where their roles have been extensively studied.¹⁰⁴ Transcriptomic studies have revealed the expression of a large number of isoforms in α -cells.^{49,105} Inhibition of PDE3B and PDE4, with cilostamide and rolipram respectively, increases secretion of glucagon at high glucose, while blocking PDE4 also increased glucagon secretion at low glucose.⁷⁶ Both PDEs are activated by PKA-dependent phosphorylation and inhibited by cGMP and have been proposed to play a key role in

cAMP regulation in α -cells.⁷⁶ PDE1C, however, an isozyme expressed at a similar level in both α - and β -cells, may be of particular importance to α -cell function.¹⁰⁶ In β -cells, glucose enhances PDE1C responsiveness through the activity of Ca^{2+} /calmodulin. The higher responsiveness of the isozyme breaks down cAMP faster at high glucose to form a negative feedback pathway for insulin secretion.¹⁰⁷ It can be postulated that the same PDE may play a similar role in the downregulation of glucagon secretion in α -cells, preventing over-secretion of glucagon. Effectors of cAMP

4.2 | Effectors of cAMP

The cellular response to cAMP is mediated by two effectors: the serine/threonine kinase Protein kinase A (PKA) and exchange protein directly activated by cAMP 2 (EPAC2). PKA binds to four cAMP molecules on the two R subunits of the inactive holoenzyme. This induces a conformational change, releasing the two catalytic subunits,¹⁰⁸ which are then able to phosphorylate PDEs,¹⁰⁹ ACs, glucose transporters,¹¹⁰ and ion channels.¹¹¹

PKA activation potentiates glucagon secretion at high glucose in murine and human islets, however, this did not occur at low glucose concentrations.⁷⁶ It was suggested that PKA is maximally activated at low glucose, but activity at high glucose is curtailed by lower concentrations of cAMP. The main effect can be through the phosphorylation of SNARE proteins, particularly SNAP-25, which primes vesicles for exocytosis.¹¹² PKA is also able to phosphorylate the IP_3R , potentiating Ca^{2+} -induced Ca^{2+} release from the ER.¹¹³ Finally, PKA is able to self-regulate the levels of cAMP, providing a negative feedback loop, preventing overstimulation. This is performed by phosphorylation of several amino acid sites on ACs, particularly AC5 and AC6, reducing their enzymic activity, converting ATP to cAMP at a lower rate.¹¹⁴ They also are able to phosphorylate, and consequently activate, PDEs leading to enhanced degradation of cAMP.¹⁰⁹

The second, and less well studied, of the two major enzymes activated by cAMP binding is EPAC2. Upon binding of cAMP, EPAC2 undergoes a conformational change, freeing the catalytic lobe from the regulatory region.¹¹⁵ This allows it to act as a guanine-nucleotide-exchange factor (GEF) for a number of targets involved in exocytosis, such as Rap2B, RAP1, RAB3a, and RIM2.^{116–119}

EPAC2 is key to cAMP-stimulated glucagon secretion. Mice lacking EPAC2 expression have reduced, though not completely abolished, glucagon secretion in response to adrenaline.⁸⁵ This is similar to that seen in β -cells.¹¹⁷ Unlike PKA, EPAC2 agonists augment glucagon secretion independent of glucose concentration.⁷⁶ This is possibly because EPAC2 contains two different cAMP binding domains with different affinities.¹²⁰ The effect of EPAC2 on glucagon secretion may be exerted at the level of gene expression and protein production,¹²¹ as well as via

modulation of α -cell exocytosis. For example, SNAP-25 is a target for EPAC2,¹²² although these effects may be mediated indirectly via the scaffold-associated protein Rab-interacting molecule 2 (RIM2).^{119,123} RIM2 is key in the interactions within the SNARE complex, interacting with synaptotagmin, RAB3, Ca_v channels, munc13, and SNAP-25.¹²⁴ In β -cells, separation of RIM2 from the exocytotic machinery through deletion of the RAB3 binding domain, impairs exocytosis.¹²⁵ RIM2 is highly expressed in α -cells and glucagon secretion in response to insulin increased in *Rim2 α ^{-/-}* mice.^{49,126}

Cyclic AMP can therefore function as a molecular switch for α -cell exocytosis, the final step of glucagon secretion. Indeed, capacitance measurements with intracellular application of cAMP demonstrated a steep dependence of α -cell exocytosis to cAMP.⁹⁰ Given that α -cell intracellular cAMP concentration is glucose-dependent, it is highly likely to constitute part of the mechanism by which glucose regulates glucagon secretion.

4.3 | Effect of cAMP on electrical activity

In addition to their direct effect on the exocytotic machinery, it is possible that cAMP effectors are able to modulate α -cell electrical activity too. Hyperpolarisation-activated cyclic-nucleotide-gated (HCN) channels are members of a superfamily of voltage-gated cation channels that are permeable to both Na^+ and K^+ ions and produce a slowly activating inward current. The presence of HCN channels has been demonstrated in both primary islet and cells lines.¹²⁷ Unlike most voltage-dependent channels, HCN channels are activated by membrane hyperpolarisation as well as activation of GPCRs that elevate cAMP levels.¹²⁸ These greatly facilitate the activation of the channels by shifting their gating voltage by 10 mV or more to more positive potentials. Similarly, Na_v channels may be phosphorylated by PKA.¹²⁹ This acts to reduce the peak Na^+ current through the channel.^{130,131}

5 | INTERACTION BETWEEN Ca^{2+} AND cAMP

5.1 | cAMP effect on Ca^{2+}

As discussed above, both Ca^{2+} and cAMP play an important role in α -cell glucagon secretion. Their effects can be exerted on electrical activity and/or cell exocytosis. Furthermore, the interaction between the two molecules, is a key element of the regulatory mechanism.

The effect of cAMP on intracellular Ca^{2+} has been well documented. Cyclic AMP-dependent PKA phosphorylation of L-type Ca^{2+} channels increases channel activity.^{132,133} The increased conductance and role in secretion after GPCR stimulation has led to the assertion that L-type Ca_v channels become coupled to α -cells exocytotic machinery and influx through these channels directly triggers exocytosis.^{41,125}

Furthermore, α -cell Ca^{2+} conductance is also increased through phosphorylation of N-type or P/Q-type Ca_v s, though via EPAC2-dependant mechanisms,⁸⁵ and additionally enhanced by RIM2-mediated co-localisation of these with secretory granules.¹³⁴

In addition to the effects on transmembrane Ca^{2+} influx, α -cell cAMP is able to directly potentiate Ca^{2+} release from intracellular stores.¹³⁵ It was later identified that cAMP-induced ER Ca^{2+} release is mediated by the PKA/EPAC2-dependent activation of Tpc2 channels located at the lysosomal acidic stores.⁹⁰

5.2 | Ca^{2+} effect on cAMP

When compared with the well-established effect elicited by cAMP on intracellular Ca^{2+} , little is known about how Ca^{2+} may regulate intracellular cAMP in α -cells. However, all AC isoforms expressed in α -cells can be modulated by factors within Ca^{2+} -signalling pathway, such as PKA, Ca^{2+} /calmodulin-dependent protein kinase (CaMK), or Ca^{2+} itself. AC3 and AC9 are inhibited indirectly, with CaMKII inhibiting AC3, though only when previously stimulated by G_s ,^{136,137} while AC9 is inhibited by phosphorylation by calcineurin.¹³⁸ By comparison, AC5 and AC6 are directly inhibited by free Ca^{2+} through the competitive displacement of free Mg^{2+} ions key to the catalytic activity of the enzymes.^{139,140} It is therefore possible that Ca^{2+} plays a regulatory role in the α -cell intracellular cAMP concentration too. As an increase in glucose metabolism is stimulatory to α -cell electrical activity,²⁴ it is reasonable to postulate that this may lead to an increase in the cytosolic Ca^{2+} concentration (though the changes may be subtle and restricted within a certain subcellular compartment). This could, in principle, inhibit AC activity and lower the intracellular cAMP concentration, reducing α -cell exocytosis (Figure 1B). This idea was partially tested in a recent study¹⁸ using simultaneous imaging of cAMP and Ca^{2+} . While the authors concluded that Ca^{2+} had little effect on cAMP oscillations in α -cell, it should be noted the Ca^{2+} probe used in the study is a strong chelator of Ca^{2+} and therefore the experimental condition may not have been ideal to address this question. Future studies using less invasive methods may bring novel insights to this interesting interaction of the two second messengers.

6 | CODA

Considered to be an “emergency hormone”, glucagon prevents life-threatening hypoglycaemia by raising blood glucose levels when they begin to fall. Furthermore, glucagon is key to ensuring normal glucose tolerance.^{141,142} This is not only due to the potent glucose-elevating capacity of glucagon, but may also be attributed to other beneficial effects exerted on the β -cells (Reviewed by Stanojevic and Habener¹⁴³).

In diabetes, α -cell glucose sensing is often impaired, glucagon secretion is no longer stimulated by hypoglycaemia and often cannot be inhibited by hyperglycaemia. Not only does this lead to recurrent hypoglycaemia, a potentially life-threatening medical condition that is currently the limiting factor in glycaemic management of the disease,¹⁴⁴ but also exacerbates hyperglycaemia.¹⁴⁵ Therefore, pharmacological interventions targeting α -cell function will offer safer and better treatment for diabetes. However, despite extensive research on α -cell physiology, the exact mechanism by which glucose regulates glucagon secretion remains obscure. As discussed above, the two ubiquitous second messengers, Ca^{2+} and cAMP, are implicated in glucagon secretion, and importantly, can be effectively regulated by glucose metabolism. Therefore, it is highly likely that the synergistic action of these two signalling molecules is at the core of glucose regulation of glucagon secretion. The interaction of intracellular Ca^{2+} and cAMP is complex. Although there is currently no direct experimental evidence, the possible dual role of Ca^{2+} as both a triggering signal and limiting factor (through the inhibition of adenylyl cyclases, reducing cAMP) for cell exocytosis could potentially explain the inhibitory action of glucose (and sulfonylurea) on glucagon secretion. With the improvement of cAMP and Ca^{2+} sensors, future studies focusing on their interaction will undoubtedly shed light on α -cell physiology and pathophysiology in diabetes, opening novel avenues for developing better and safer treatment of the disease.

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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