THEMATIC REVIEW

α-cell electrophysiology and the regulation of glucagon secretion

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This paper forms part of a special collection marking 100 years since the Discovery of Glucagon. The guest editors for this section were James Cantley, Rebecca Hull and Vincent Poitout.

Abstract

Glucagon is the principal glucose-elevating hormone that forms the first-line defence against hypoglycaemia. Along with insulin, glucagon also plays a key role in maintaining systemic glucose homeostasis. The cells that secrete glucagon, pancreatic α-cells, are electrically excitable cells and use electrical activity to couple its hormone secretion to changes in ambient glucose levels. Exactly how glucose regulates α-cells has been a topic of debate for decades but it is clear that electrical signals generated by the cells play an important role in glucagon secretory response. Decades of studies have already revealed the key players involved in the generation of these electrical signals and possible mechanisms controlling them to tune glucagon release. This has offered the opportunity to fully understand the enigmatic α-cell physiology. In this review, we describe the current knowledge on cellular electrophysiology and factors regulating excitability, glucose sensing, and glucagon secretion. We also discuss α-cell pathophysiology and the perspective of addressing glucagon secretory defects in diabetes for developing better diabetes treatment, which bears the hope of eliminating hypoglycaemia as a clinical problem in diabetes care.

Key Words

- α-cell
- electrophysiology
- glucagon
- diabetes

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Introduction

In the body, blood glucose levels are kept within a narrow range by concerted action of glucagon and insulin, the glucose-regulating hormones secreted by α- and β-cells, respectively (Göke 2008). Whereas the islet α-cells were identified histologically already in 1907 (by then they were named 'A-cells') (Lane 1907), the hormone they produce, glucagon, was discovered in 1923 and described as a 'glucose agonist' that can rapidly increase blood glucose (Kimball & Murlin 1923), an effect opposite to that of insulin. Because of its potent glucose-elevating efficacy, glucagon plays a key role in glucose counter-regulation against hypoglycaemia and has been used as an emergency antidote for severe hypoglycaemia (Elrick *et al.* 1958), a life-threatening medical condition of dangerously low blood glucose that is often caused by iatrogenic use of insulin/insulin secretagogues.

Normally, glucagon secretion is stimulated by a fall in blood glucose but suppressed at euglycaemia and hyperglycaemia (Göke 2008). This secretory pattern becomes defective in diabetes and leads to glycaemic

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volatility: failure to secrete glucagon at low glucose contributes to the occurrence of hypoglycaemia (Cryer & Gerich 1985), while hyperglucagonemia at high glucose exacerbates hyperglycaemia (Menge *et al.* 2011). Therefore, therapies that can restore normal α-cell function/glucagon secretion would significantly improve diabetes treatment, particularly for better prevention of hypoglycaemia. However, exactly how α-cells function becomes dysregulated in diabetes remains unknown. This gap in the knowledge is mainly because the normal cellular mechanism that regulates α-cells remains incompletely understood, despite over five decades of research.

Like β-cells, α-cells are excitable cells and couple electrical activity to glucagon release; but different from β-cells, α-cells are electrically active at low glucose (Zhang *et al.* 2013). Although there are various (and sometimes opposite!) theories on how glucose metabolism regulates α-cell activity (Gylfe 2016), it is clear that the electrophysiological control of α-cells plays an important role in their nutrient sensing and glucagon secretion. The development of improved patch-clamping techniques (Hamill *et al.* 1981) has decisively boosted the understanding of the ion currents associated with α-cell excitability (Wesslen *et al.* 1987, Rorsman & Hellman 1988) and glucagon secretion (Gromada *et al.* 1997). The current state of the art on this interplay between α-cell electrophysiology and glucagon secretion is the scope of this review, where we attempt to provide an overview that may help to understand how these elusive cells function and, importantly, how these regulatory mechanisms may become defective in diabetes.

A brief history of α-cells

Although glucagon has not received as much attention as insulin, its history is almost as long (Fig. 1, a simplified timeline). The first description of a pancreatic factor that raised blood glucose concentration was made a century ago, in 1923, when Murlin and colleagues discovered a fraction in a pancreatic extract that had 'the power to act in just the opposite way to insulin; namely, to raise the blood sugar...' (Murlin *et al.* 1923), which later they named glucagon (Kimball & Murlin 1923). However, the actual isolation of the glucagon molecule only happened in 1948 (Sutherland & De Duve 1948) and its crystallisation in 1953 (Staub *et al.* 1953). This was quickly followed by its clinical applications in the 1950s to reverse hypoglycaemia (Elrick *et al.* 1958). The development of the indirect immunofluorescence technique by Coons *et al.* (Coons *et al.* 1955) and the production of reliable

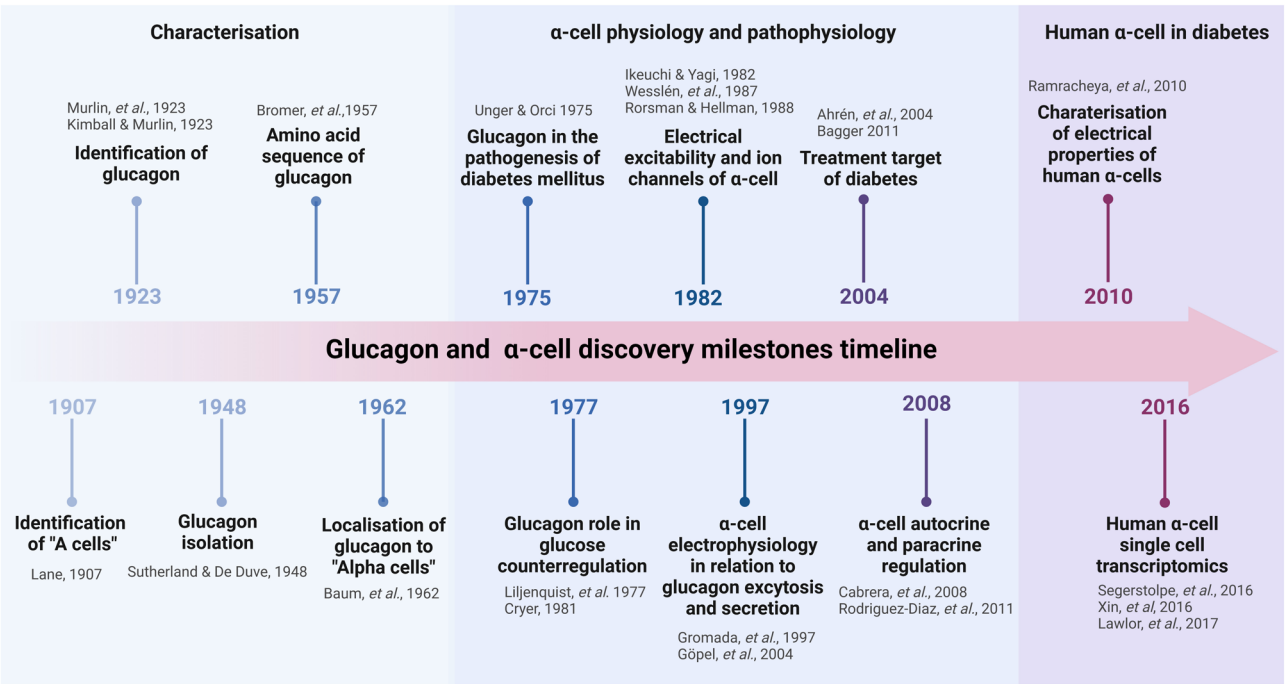

Figure 1
Glucagon and α-cell discovery milestone timeline. The three phases of glucagon and α-cell research history with bullet points referring to references in the text. Created with BioRender.com.

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glucagon antibodies by Unger *et al.* (Unger *et al.* 1959) were pivotal for Baum *et al.* to localise glucagon expression to 'alpha cells' (Baum *et al.* 1962), the cells that were histologically identified in 1907 by maintaining their staining properties upon 'alcohol' fixation ('A cells') (Lane 1907), comfiring the biochemical observation made by Sutherland and De Duve (Sutherland & De Duve 1948). The glucagon antibodies also led to the development of a radioimmunoassay (Unger *et al.* 1959) which measures glucagon, enabling accurate evaluation of glucagon secretory responses.

But how do α-cells work? They were shown to be electrically excitable (Ikeuchi & Yagi 1982), and historical milestones related to α-cell electrophysiology include the development of experimental approaches using intact islets to study α-cell ionic currents (Göpel *et al.* 2000), exocytosis (Göpel *et al.* 2004), and electrical activity and the electrophysiological characterisation of human α-cells (Ramracheya *et al.* 2010).

The increasing availability of human pancreatic islets for research from the beginning of the 21st century has revolutionised our understanding of the α-cell. As Dolenšek *et al.* pointed out, there are remarkable morphological differences between rodent and human endocrine pancreas that must have functional implications (Dolenšek *et al.* 2015). Indeed, studies in human islets have shown that glucagon is not the only molecule secreted by α-cells with intra-islet signalling properties. It was elegantly demonstrated that glutamate is also secreted by human α-cells, upon which it has a stimulatory autocrine effect via AMPA/kainate ionotropic receptors (Cabrera *et al.* 2008). It has been suggested that human α-cells also secrete acetylcholine, which has a positive paracrine effect on neighbouring β-cells (Rodriguez-Diaz *et al.* 2011*b*). However, in a more recent study, vesicular acetylcholine transporters were found to be absent in human α-cells (Tang *et al.* 2018), echoing the transcriptomic data acquired in human islets (Xin *et al.* 2016). It will be possible to address this discrepancy by direct amperometric detection of acetylcholine release (Keighron *et al.* 2015) from α-cells at single-cell resolution. In addition, glucagon-like peptide 1 (GLP-1) was also found in a subset of human α-cells (Marchetti *et al.* 2012) and not only in intestinal L-cells. Interestingly, whereas GLP-1 content in non-diabetic human islets is only a fraction of that of glucagon (<1%) (Galvin *et al.* 2021), its secretion was significantly higher in islets from type 2 diabetic (T2D) donors than from euglycaemic donors, indicating that there may be a switch not only in glucose sensitivity but also in α-cell qualitative hormonal output in diabetes. Clearly, there is much more to the α-cell than meets the (glucagon) eye!

Morphological considerations

In most living animals, hypoglycaemia is an uncommon and harmful condition that can be corrected by behavioural or hormonal regulation (counter-regulation). The release of counter-regulatory hormones (including glucagon) must be fast to avoid prolonged fuel deprivation for normal body function. Indeed, as a first-line defence against hypoglycaemia, glucagon secretion is rapid in response to hypoglycaemia (Schwartz *et al.* 1987) and is facilitated by several α-cell morphological features.

In many species (with the exception of guinea pig (Rorsman & Hellman 1988)), α-cells, compared to β-cells, are not only a relatively small population (~30–40%) but also smaller in size (diameter = 10 μm vs 15 μm in β-cells, in murine islets) (Barg *et al.* 2000). In electrophysiological terms, the small membrane area of α-cells determines their low membrane capacitance (~3 pF vs >5 pF in β-cells, conversion factor = 10 fF/μm^2). Together with their spherical morphology (which enables rapid distribution of charges), minute changes in α-cell ion channel activity can quickly alter its whole-cell membrane potential (e.g. the velocity of action potential upstroke, dV/dt, reaches up to 37 V/s) (Zhang *et al.* 2013) and subsequent glucagon release.

Glucagon is released from α-cells through Ca^{2+}-dependent exocytosis, a process where glucagon-containing granules fuse to the plasma membrane to release their cargo (Barg *et al.* 2000). α-cells are densely granulated with ~7000 granules per cell (i.e. the granular density is ~9 granules/μm^3, given the average α-cell volume of ~800 μm^3), occupying the majority of the cellular volume (average granule volume = 0.08 mm^3) (Barg *et al.* 2000). Therefore, many granules are docked at the plasma membrane (docked-granule density = 0.6 granules/μm^2 (Omar-Hmeadi *et al.* 2020), accounting for ~180 docked granules per cell at a given time) and can be readily released upon stimulation. A single depolarisation as short as 20 ms is sufficient to trigger the release of ~50 granules (Hamilton *et al.* 2018). The high granule density may also contribute to a rapid refilling of the 'readily-releasable pool' of granules, enabling continuous high-speed exocytosis under repetitive stimulation (Barg *et al.* 2000). In addition, α-cells have an extensive endoplasmic reticulum (ER) network (Pfeifer *et al.* 2015) and can provide additional Ca^{2+} through the process of Ca^{2+}-induced Ca^{2+} release (CICR) for high-volume exocytosis. This forms

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part of the adrenaline-stimulated glucagon secretion (Hamilton *et al.* 2018)

Together, these morphological features enable rapid and robust glucagon secretory response to hypoglycaemia, fulfilling its role as an emergency counter-regulatory hormone.

Ion channels in α-cell electrical excitability, glucose sensing and glucagon secretion

Voltage-gated ion channels

There is a plethora of ion channels expressed in α-cells, governing their cellular excitability and exocytosis (see illustration in Fig. 4). Like all excitable cells, α-cell electrical activity depends on the activity of its voltage-gated ion channels.

Voltage-gated Na^+ channels (Na_v channels) are Na^+-permeable pores that can be opened by membrane depolarisation. An influx of Na^+ ions via Na_v channels can rapidly charge the membrane, forming/accelerating the upstroke of action potentials (APs), often leading to overshooting APs (peak potential >0 mV). Once opened, Na_v channels quickly (~2 ms) become inactivated and are only reactivated when the cell returns to resting membrane potential (Milescu *et al.* 2008). In α-cells, the total Na^+ current is on average ~−450 pA (triggered by depolarisation from −70 mV to 0 mV) at the physiological range of membrane potential. Blocking Na_v channels with the broad-spectrum Na_v channel blocker tetrodotoxin reduces both α-cell AP amplitude and glucagon secretion at low glucose (Zhang *et al.* 2014).

α-cell Na^+ current mainly flows through two types of Na_v channels, with 70–90% of current flowing via $Na_v1.3$ and 10–20% via $Na_v1.7$ (Zhang *et al.* 2014). Therefore, the voltage-dependent inactivation of α-cell Na^+ currents is biphasic: ~25% (the $Na_v1.7$ component) inactivates half maximally ($V_{1/2}$) at ~−90 mV, while the $V_{1/2}$ of ~75% of the current (the $Na_v1.3$ component) is ~−50 mV (Fig. 2A). Importantly, the slope of the voltage-dependent inactivation of the latter component is fast (n_h=~12 mV), and as the membrane potential steps more positively, the number of activatable Na_v channels declines rapidly, reaching ~10% at ~−40 mV. Consequently, α-cell APs vary significantly at different membrane potentials, both in amplitude and in upstroke velocity (Fig. 2B). This has a direct impact on the activity of voltage-gated Ca^{2+} channels (Ca_v channels), the ion channels that provide exocytosis-triggering Ca^{2+} signals (Zhang *et al.* 2013).

Rodent α-cells are equipped with at least three types of high voltage-activated (HVA) Ca_v channels (L-, P/Q-, and N-type Ca_v channels) (Göpel *et al.* 2004, MacDonald *et al.* 2007, Zhang *et al.* 2013). Although the majority of the Ca^{2+} current flows through L-type Ca_v channels, blockade of these channels does not affect glucagon secretion/α-cell exocytosis in the absence of adrenergic activation, at least in mouse islets (Göpel *et al.* 2004). Instead, N- or P/Q-type Ca_v channels are the exocytosis-relevant Ca^{2+} channels in α-cells, despite their relatively low contribution to the transmembrane Ca^{2+} currents (~20% each) (De Marinis *et al.* 2010, Zhang *et al.* 2013). It is possible that α-cells are compartmentalised, and N- and/or P/Q-type Ca_v channels are tightly coupled

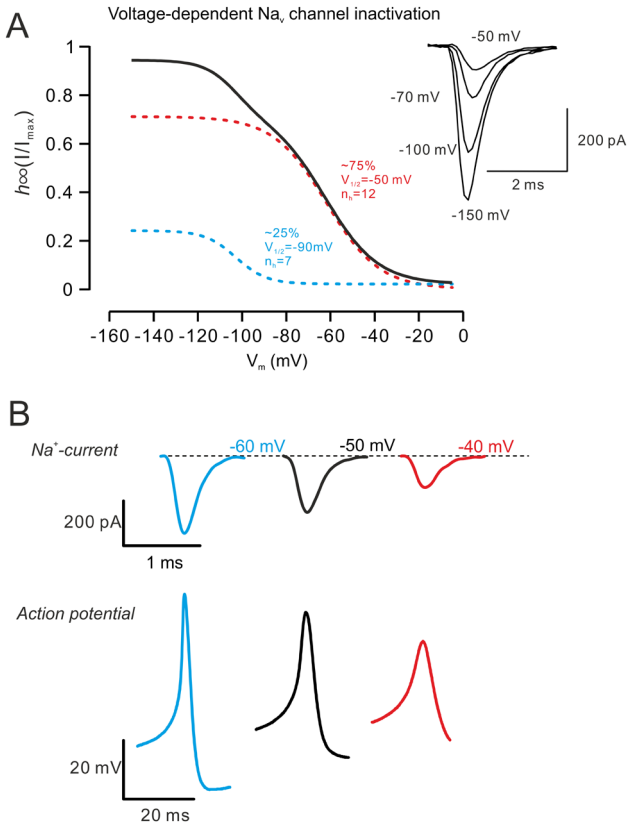

Figure 2

α-cell Na_v channels and action potentials. (A) Biphasic voltage-dependent inactivation of α-cell Na_v current (black), in which ~25% of the current (blue-dashed line) inactivates at negative voltage with a slow slope factor and ~75% (red-dashed line) inactivates at positive voltage with a fast slope factor. Examples of Na_v currents recorded at different membrane potentials are shown on the right. h_{∞}, voltage-dependent inactivation; I_{max}, total activatable Na^+ current; $V_{1/2}$, half maximal inactivation voltage; n_h, slow factor of voltage-dependent inactivation. (B) Na_v currents and action potentials at different membrane potentials. Large Na^+ current at negative membrane potential contributes to fast and high-amplitude α-cell action potential (blue traces), and small Na^+ current at more positive membrane potential leads to low-amplitude α-cell action potential (red traces).

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to the exocytotic machinery, forming efficient exocytosis 'hotspots' (Xia *et al.* 2007). L-type Ca_v channels, on the other hand, may not be associated with these 'hotspots,' and the Ca^{2+} influx through these channels may bear other functions. Interestingly, the relationship between Ca_v channels and α-cell exocytosis/glucagon secretion becomes different in the presence of adrenaline, which activates β-adrenergic receptor to increase intracellular cAMP levels. Blocking L-type Ca_v channels with isradipine abolishes adrenaline-stimulated glucagon secretion and α-cell exocytosis, which are resistant to pharmacological-inhibition of P/Q-type (with ω-agatoxin VIA) or N-type Ca_v channels (ω-conotoxin GVIA) (Gromada *et al.* 1997). It is possible that high levels of cAMP enhance L-type Ca_v channel activity and recruit more granules for exocytosis. Furthermore, these channels may be coupled to ER Ca^{2+} release promoted by cAMP-dependent signalling pathways: (i) they may be linked with ER Ca^{2+} loading when α-cells are electrically active – blocking L-type Ca_v channels prior to adrenaline application attenuated adrenaline-induced increase in cytosolic Ca^{2+} (Hamilton *et al.* 2018), while acute application of the blocker only modestly reduced the effect of adrenaline. This may be in synergy with the store-operated ER-filling mechanism that is independent of α-cell electrical activity, which was elegantly demonstrated by the Gylfe group (Liu *et al.* 2004) and (ii) larger L-type Ca^{2+} current may activate ER Ca^{2+}-releasing ryanodine receptors (Nordenskjöld *et al.* 2020) to directly induce ER Ca^{2+} release. Adrenaline can also stimulate Ca^{2+} release from α-cell ER via $InsP_3$ receptors ($InsP_3Rs$), activated by α1-adrenoceptor-generated $InsP_3$ (Vieira *et al.* 2004). Indeed, blockade of the $InsP_3Rs$ with xestospongin C also reduced adrenaline-evoked α-cell Ca^{2+} increase (Hamilton *et al.* 2018). However, it is important to point out that the above observations were made in rodent islets, and the ion channel composition is quite different in human α-cells. Human islet electrophysiology studies pioneered by Braun and colleagues found that the contribution of transmembrane Ca^{2+} in human α-cells is almost opposite to that of the rodent α-cells, with 70% and 21% of whole-cell Ca^{2+} charge influx flowing through the P/Q-type and L-type Ca_v channels, respectively (the N-type Ca_v channels are responsible for the remaining ~10%) (Ramracheya *et al.* 2010). It should be noted that this does not completely reflect the amplitude of the Ca^{2+} currents that flow through the two HVA Ca_v channels, since they have distinct channel kinetics (L-type Ca_v channels inactivate fast and the peak current is comparable to that of the P/Q-type Ca^{2+} current in human α-cells). Interestingly, the tight coupling between P/Q-type Ca_v channels and exocytosis is preserved in human α-cells, whereas the L-type Ca_v channels are involved in Ca^{2+} oscillations. It is possible that the latter contributes to the generation of human α-cell electrical activity. However, it is puzzling that the L-type Ca_v channel blocker isradipine only produced a 25% reduction in hypoglycaemia-induced glucagon secretion from human islets and did not abolish glucose sensing in α-cells. This is in stark contrast to the 75% inhibition exerted by the P/Q-type Ca_v channel blocker ω-agatoxin VIA, which also rendered glucagon secretion glucose blind. This raised an interesting perspective that glucose metabolism can directly regulate human α-cell exocytosis via effects on the P/Q-type Ca_v channels, which was later experimentally demonstrated by the MacDonald group (Dai *et al.* 2022). As such, glucose can control glucagon secretion at a level that is independent of α-cell electrical activity, preventing unwanted spontaneous glucagon release at high glucose (where electrical activity often persists). Apart from the HVA Ca_v channels (L-, N-, and P/Q-type), α-cells also express low-voltage-activated T-type Ca_v channels, which may function as the pacemaker for AP firing (Rorsman 1988). In addition to Na_v and Ca_v channels, voltage-gated K^+ channels (K_v channels) also participate in α-cell APs by forming their repolarising phase (downstroke) (Spigelman *et al.* 2010). α-cells possess large K^+ currents that flow through several types of K_v channels. Whereas the mRNA of $K_v2.1$, $K_v3.3$, $K_v4.1$, and Ca^{2+}-dependent voltage-sensitive BK channels is detected in mouse α-cells (DiGruccio *et al.* 2016), pharmacological analysis demonstrates that the majority of K^+ current is mediated by $K_v2.1$ and BK channels (Spigelman *et al.* 2010). α-cell K_v currents are comprised of two components: a rapid-activating and inactivating component (A-current) that is sensitive to $K_v4.x$-blocker heteropodatoxin-2 and a sustained component that can be blocked by stromatoxin (a $K_v2.1/2.2$-specific blocker) (Ramracheya *et al.* 2010). The large K^+ current enables the rapid repolarisation of α-cells, sometimes leading to post-depolarisation hyperpolarisation, essential for reactivating Na_v channels and regenerative AP firing. Blocking K_v channels leads to increased β-cell electrical activity and insulin secretion by broadening the AP duration (Atwater *et al.* 1979). However, tetraethylammonium (TEA, a broad-spectrum K_v channel blocker) inhibits glucagon secretion. This was attributed to TEA-dependent

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α-cell membrane depolarisation that inactivates Na_v and Ca_v channels, disabling AP regeneration (Spigelman *et al.* 2010). As such, K_v channels are positive regulators of α-cell electrical activity/glucagon secretion.

Are K_{ATP} channels key to α-cell glucose sensing?

As discussed above, the voltage-sensitive channels are essential apparatus for generating α-cell APs. Due to their electrophysiological properties, particularly the voltage-dependent inactivation of Na_v channels, the exact membrane potential of the α-cell is critical for AP firing and glucagon secretion. α-cell membrane potential is determined by its background ionic conductance, formed by several ion channels, including the ATP-sensitive K^+ channels (K_{ATP} channels).

Like β-cells, α-cells are equipped with K_{ATP} channels, an inwardly rectifying K^+ channel whose activity is controlled by the intracellular ATP/ADP ratio (Bokvist *et al.* 1999). In β-cells, a low ATP/ADP ratio at low glucose maximally opens the K_{ATP} channels (~2 nS) (Göpel *et al.* 1999), setting the membrane potential close to the K^+ reversal potential (~−70 mV), where no electrical activity is generated. Interestingly, although molecularly identical to that of β-cells, α-cell basal K_{ATP}-channel activity is much lower (~0.1 nS at 1 mM glucose) (Zhang *et al.* 2013). This is possibly because of a high intracellular ATP level in α-cells even when extracellular glucose is low. Indeed, washing out ATP from α-cells rapidly increases their K_{ATP} conductance (Zhang *et al.* 2013). The exact source of the ATP restricting basal K_{ATP}-channel activity in α-cells is unclear, but it may be due to high-level glucose transport and metabolism at low glucose levels. α-cells are equipped with GLUT1, a high-affinity glucose transporter (K_m = 1–2mM) (Heimberg *et al.* 1995), and a low-K_m sodium–glucose co-transporter SGLT1 (K_m = 0.5 mM) (Suga *et al.* 2019), which can import glucose at low ambient glucose levels. Moreover, unlike β-cells, α-cells express the high-affinity glycolytic enzyme hexokinase-1 (HK1, K_m = ~1 mM) (DiGruccio *et al.* 2016), which phosphorylates glucose to provide substrates for glycolysis. Together, these may explain how α-cells can utilise glucose to generate ATP at low ambient glucose levels.

Interestingly, α-cells remain active and glucagon secretion persists in the complete absence of glucose (Rorsman & Hellman 1988, Gromada *et al.* 1997, Vieira *et al.* 2007). It is unclear how they remain active under glucose deprivation, given AP firing is ATP demanding (Attwell & Laughlin 2001). One possibility is that the creatine/phosphocreatine ATP-buffer system can transfer phosphate to ADP to produce ATP, as it does in β-cells (Krippeit-Drews *et al.* 2003). Moreover, it was proposed that α-cells can generate ATP via fatty acid oxidation (Briant *et al.* 2018). In mice with α-cells lacking CPT1, an enzyme that shuttles fatty acids into the mitochondria, fasting blood glucose and glucagon are reduced (Briant *et al.* 2018). This echoes the observation that reducing lipogenesis in α-cells by knocking out acetyl-CoA-carboxylase 1 dampens glucose sensitivity, an effect linked to an impaired K_{ATP}-channel activity (Veprik *et al.* 2022).

The metabolic sensitivity of the K_{ATP} channel makes it a possible fuel sensor of α-cells, similar to its role in β-cells. In both human and mouse islets, increasing extracellular glucose (from 1 to 6 mM) reduces α-cell K_{ATP}-channel conductance by ~25% (Bokvist *et al.* 1999, Göpel *et al.* 2000, Zhang *et al.* 2013, Basco *et al.* 2018), an effect exerted by glucokinase (GCK)-dependent glucose metabolism. This depolarises the α-cell membrane to a potential (from ~−55 mV at 1 mM glucose to ~−45 mV at 6 mM glucose) where activatable Na_v channels are reduced (from >60% to ~25%) and thus the amplitude of the APs is significantly reduced (Göpel *et al.* 2000, MacDonald *et al.* 2007). The low-amplitude APs can only open a fraction of the exocytosis-related P/Q-type Ca_v channels; hence cell exocytosis is greatly reduced (~10% of that at 1 mM glucose). This is consistent with the observation that the K_{ATP}-channel inhibitors sulphonylureas can potently inhibit hypoglycaemia-stimulated glucagon secretion.

Interestingly, the effect of sulphonylureas on α-cell electrical activity is not unvarying when tested in intact islets. Tolbutamide, a sulphonylurea, strongly depolarises most α-cells (Fig. 3A), an effect similarly observed in β- and δ-cells (Fig. 3B and C). However, we noticed that, in a small fraction of α-cells, tolbutamide exerted a paradoxical effect on their membrane potential: it induced transient hyperpolarisation (to ~−80 mV) and suppressed AP firing in between depolarisations and continuous electrical activity (Fig. 3D). Whereas we attribute the depolarisation to a direct effect on α-cells, the hyperpolarisation is likely to be due to paracrine effect exerted by stimulated neighbouring δ-cells (Cheng-Xue *et al.* 2013). Indeed, tolbutamide-induced hyperpolarisation is completely absent in dispersed single α-cells (Gromada *et al.* 2004), where paracrine signalling is removed. This mixed effect on α-cell membrane potential was also observed in the presence of high glucose and hyperpolarisation could be reversed by blocking somatostatin receptors (Zhang *et al.* 2013). As such, sulphonylureas could inhibit glucagon secretion through a dual action: they depolarise α-cells to reduce

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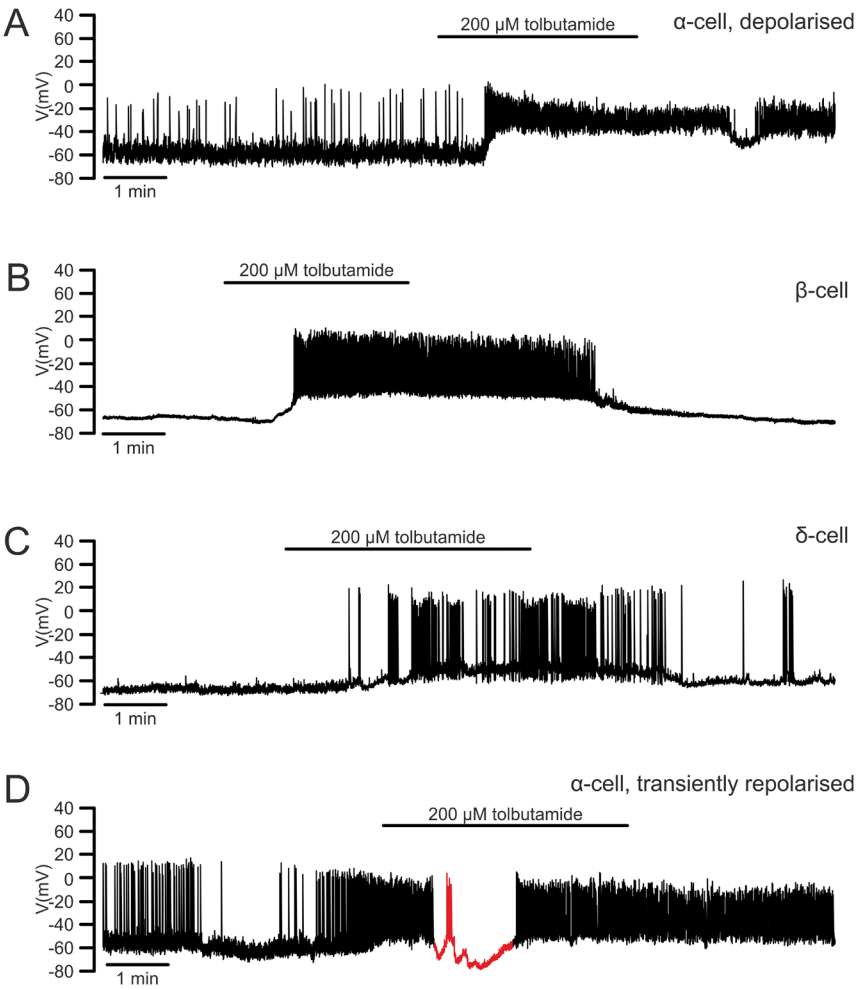

Figure 3
α-, β- and δ-cell membrane potential in response to the sulphonylurea tolbutamide. (A) An example of an α-cell depolarised by 200 μM tolbutamide. (B)–(C). As in A but shows the responses seen in β- and δ-cells. (D) An example of dual effect of tolbutamide on α-cell membrane potential; note the transient repolarisation (red) shortly after the application of tolbutamide.

AP and exocytosis and increase intra-islet paracrine tone to further suppress glucagon secretion. The importance of the sulphonylurea-mediated paracrine effect was highlighted by studies from the Gilon group, where a stimulatory effect of K_{ATP}-channel blockers on glucagon secretion was observed in somatostatin-deficient mice (Cheng-Xue *et al.* 2013, Singh *et al.* 2021). Clearly, future studies using α-cell-specific K_{ATP}-channel deficient mice could help address the precise role of K_{ATP} channels in α-cell intrinsic glucose sensing.

Glucose-induced depolarisation may not only be mediated by K_{ATP}-channel closure. It was reported that glucose can be co-transported with Na^+ through SGLT2 (at a 1:1 ratio) into α-cells (Bonner *et al.* 2015). This transport is theoretically electrogenic and should induce rapid depolarisation to reduce glucagon secretion. Indeed, the SGLT2 blocker dapagliflozin stimulates glucagon secretion at high glucose. However, there is evidence that the Na^+/glucose co-transport action alone does not produce a depolarisation sufficient to inhibit glucagon secretion. For instance, the non-metabolizable glucose analogue 3-*O*-methyl-D-glucose, which is co-transported with Na^+ through SGLT2, does not inhibit low-glucose-stimulated glucagon secretion (Cheng-Xue *et al.* 2013).

Apart from the depolarisation hypothesis for glucose-suppressed glucagon secretion, glucose-induced (intrinsic) membrane repolarisation has been observed in several laboratories. It was reported that activation of the two-pore K^+ channel TWIK (tandem of P domains in a weak inward rectifying K+ channel)-related acid-sensitive K^+ channel 1 (TASK1 channel) at high glucose contributed to reduced α-cell excitability. Blockade or genetic ablation of TASK1 depolarised membrane and stimulated AP firing at high glucose (Dadi *et al.* 2015). As such, TASK1 may be an additional α-cell glucose sensor. It was also suggested that α-cell electrical activity at low glucose is maintained by store-operated depolarising Ca^{2+} currents through Orai1 channels (Liu *et al.* 2004). At high glucose, activated sarco-endoplasmic reticulum Ca^{2+}-ATPase (SERCA) pumps Ca^{2+} into the ER, inactivating ER-bound Ca^{2+}-sensing stromal

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interaction molecules (STIMs). This closes Orai1 and the α-cell repolarises, suppressing AP firing and glucagon secretion. However, depleting ER Ca^{2+} using thapsigargin did not abolish glucose sensitivity of glucagon secretion, arguing against a strong involvement of STIM/Orai in α-cell excitability (Gromada *et al.* 2004).

Other mechanisms suggested to mediate glucose-induced α-cell hyperpolarisation are activation of the Na^+/K^+ pump (Bode *et al.* 1999) and glucose-induced cell swelling (Davies *et al.* 2007) with subsequent Cl^- influx through volume-regulated channels (Best *et al.* 2010). It is worth noting that the Na^+/K^+ pump was later proposed to maintain low α-cell membrane potential at low glucose by a CPT1/β-oxidation-dependent mechanism; at high glucose, α-cells switch to glucose metabolism, which produces membrane depolarisation via the closure of the K_{ATP} channels (Briant *et al.* 2018). An interesting Cl^- channel in the α-cells is the cAMP-activated cystic fibrosis transmembrane conductance regulator (CFTR). There is still much to learn on this topic, but we and others have detected CFTR on the cell surface of rodent and human α-cells (Edlund *et al.* 2017, Huang *et al.* 2017) and recorded CFTR currents in human α-cells (Edlund *et al.* 2017). RNA sequencing of sorted islets cells did reveal CFTR transcripts in the α-cell fraction but at low levels (Blodgett *et al.* 2015). Although it is still unclear what proportion of the α-cells express CFTR, or at what levels, both mathematical modelling and experimental evidence suggest that when it is present, CFTR exerts a glucagonostatic effect by repolarising α-cells (Edlund *et al.* 2017). Interestingly, Yu *et al.* also recently reported that glucose can directly regulate α-cell intracellular cAMP: low levels of glucose could induce cAMP elevation that is independent of paracrine signalling from insulin or somatostatin (Yu *et al.* 2019). This may have a direct impact on α-cell electrical activity and excoytosis (Omar-Hmeadi *et al.* 2020), effectively regulating glucagon secretion.

Glucagon secretion is not only controlled by glucose but also by other nutrients such as amino acids. Arginine is a strong glucagon secretagogue (Gerich *et al.* 1974). As a cationic amino acid, its transmembrane transport action is electrogenic (via CAT2 which is highly expressed in α-cells (DiGruccio *et al.* 2016)) and normally leads to a large increase in intracellular Ca^{2+} concentration (Le Marchand & Piston 2012). Interestingly, arginine's stimulatory effect is biphasic (Gerich *et al.* 1974). It is tempting to speculate that the first spike of glucagon secretion is caused by a large but transient membrane depolarisation (due to positive charge influx) and the second phase is due to arginine metabolism (Le Marchand & Piston 2012). Glycine, another amino acid, when bound to its receptor, a ligand-gated Cl^- channel, should in principle repolarise α-cells, reducing intracellular Ca^{2+} and glucagon secretion. However, it has been reported that glycine stimulates glucagon secretion and α-cell intracellular Ca^{2+} concentration in human islets (Li *et al.* 2013). It is possible that the intracellular Cl^-concentration in human α-cells is high and glycine could therefore exert a depolarising effect that is similar to that on human β-cells (Yan-Do *et al.* 2016). Exactly how the amino acids affect α-cell electrical activity requires more detailed electrophysiological analyses. Furthermore, a recent study identified that metabolites, including lactate and pyruvate, robustly inhibit human and mouse α-cell secretion without apparent effect on β- or δ-cells. Lactate entry into α-cells results in K_{ATP}-channel activation, membrane hyperpolarisation and reduced $[Ca^{2+}]_i$ under low glucose conditions (Zaborska *et al.* 2020).

Undoubtedly, the nutrient and metabolite control of α-cell electrical activity is complex and involves multiple ion channels and metabolic pathways (Fig. 4A and B). The theories discussed above are not mutually exclusive but exactly how α-cells sense environmental metabolic status and respond accordingly is likely to remain a hot topic of debate for years to come. Are K_{ATP} channels the key to intrinsic glucose sensing in α-cells? Clearly, their strong tonic inhibition sets a high membrane resistance of the cells at nearly all glucose concentrations. This enables the α-cell electrical activity/membrane potential and function to be significantly altered by minute changes to the activity of either the K_{ATP} channels or other ion channels.

Paracrine and neuronal control of α-cells

The theories regarding how glucose controls glucagon secretion are not limited to intrinsic mechanisms. Many have proposed that α-cell glucagon secretion is controlled by neighbouring cells and/or neuronal regulation (Fig. 5).

Control by β-cells

Insulin

Insulin has long been considered the regulator for glucagon secretion, and insulin receptors as well as proteins involved in insulin signalling are highly expressed in α-cells (DiGruccio *et al.* 2016). Indeed, knocking-out insulin receptors in α-cells led to hyperglucagonemia and mild glucose intolerance (Kawamori *et al.* 2009).

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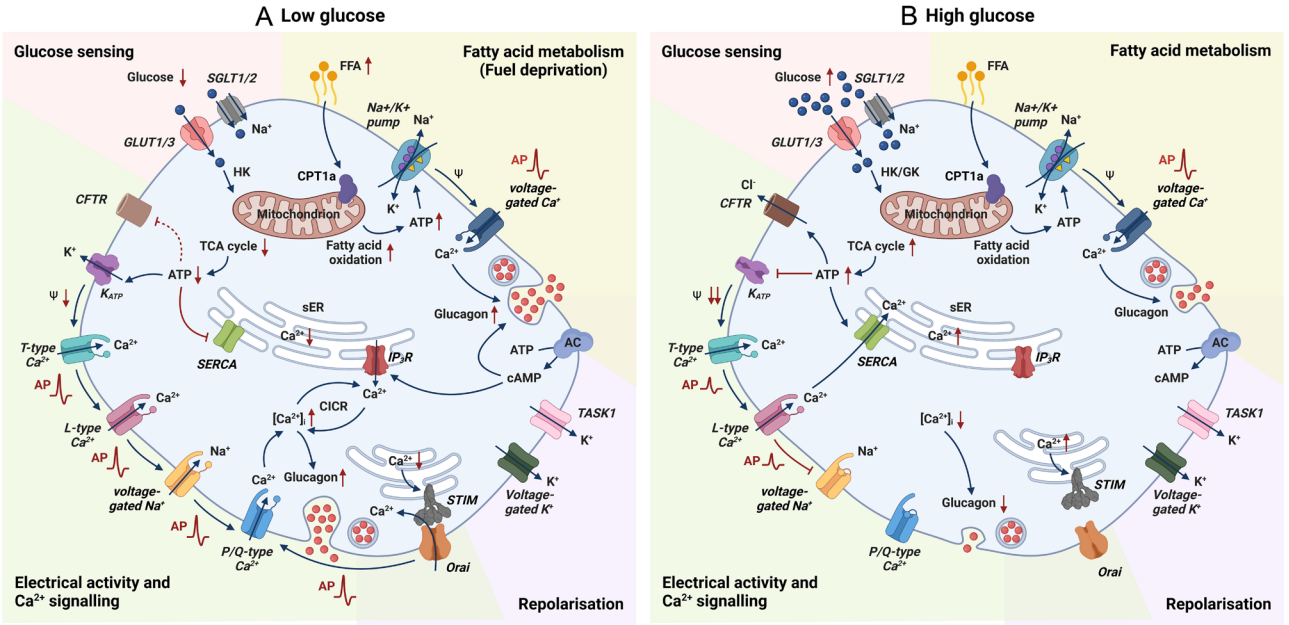

Figure 4

An atlas of ion channel activities in α-cells at (A) low glucose and (B) high glucose. (A) At low glucose, there is a low rate of glucose uptake via glucose transporters (GLUTs) and sodium-glucose linked transporters (SGLTs). Glucose is then metabolised by hexokinase (HK) to modestly increase cytoplasmic ATP/ADP ratio (red-shaded area), leading to partial closure of K_{ATP} channels. This maintains the α-cell membrane potential (Ψ) sufficiently depolarised to allow action potential (AP) firing initially driven by T-type Ca^{2+} channels while also preventing voltage-dependent inactivation of the voltage-gated Na^+ channels. The resulting high-amplitude AP activates P/Q-type Ca^{2+} channels, and the following Ca^{2+} influx amplifies $[Ca^{2+}]_i$ through potentiation of Ca^{2+} induced Ca^{2+} release (CICR) from the endoplasmic reticulum (ER), further triggering exocytosis of glucagon-containing secretory granules. The relatively low ATP level at low glucose also inhibits sarco/endoplasmic reticulum Ca^{2+}-ATPase (SERCA) activity. The subsequent ER Ca^{2+} depletion triggers the activation of store-operated Ca^{2+} current flowing through Orai channels, also contributing to the firing of AP (green-shaded area). Additionally, during the fuel deprivation state when glucose becomes low, fatty acids play a significant role in sustaining basal glucagon secretion. ATP generated by fatty acid oxidation energises the Na^+–K^+ pump, keeping the α-cell membrane potential sufficiently repolarised to prevent inactivation of ion channels involved in AP firing (yellow-shaded area). Moreover, two-pore K^+ channel TWIK-related acid-sensitive K^+ channel 1 (TASK1) and voltage-dependent K^+ channels are involved in the repolarisation of α-cells (purple-shaded area). (B) At high glucose, increased glucose transport and associated elevation in ATP/ADP (red-shaded area) trigger complete closure of K_{ATP} channels, strong membrane depolarisation, inactivation of voltage-gated Na^+ channels, reduced AP amplitude and less Ca^{2+} influx through P/Q-type Ca^{2+} channels. In parallel, ER Ca2+ stores are replenished by ATP-activated SERCA update of [Ca2+]i. This may inactivate Orai-mediated Ca2+ entry into the cytosol, reducing cell excitability.. The above two mechanisms together reduce $[Ca^{2+}]_i$ culminating in the suppression of glucagon secretion (green-shaded area). When glucose is elevated, cystic fibrosis transmembrane conductance regulator (CFTR) could be activated by intracellular metabolites (i.e. ATP and cAMP). CFTR-mediated Cl- efflux regulates the membrane potential through an intrinsic α-cell effect, also resulting in the inhibition of glucagon secretion. AC, adenylyl cyclase; FFA, free fatty acids; CPT1a, carnitine palmitoyltransferase 1a; GK, glucokinase; IP_3R, inositol trisphosphate receptor; STIM, stromal interaction molecule. Created with BioRender.com.

Upon binding to its receptor, insulin activates the phosphatidylinositol 3 kinase/Akt-dependent pathway (Kaneko *et al.* 1999), which reduces the K_{ATP}-channel ATP sensitivity in α-cells, dampening their excitability (Leung *et al.* 2006). Furthermore, insulin was reported to induce FoxO1 nuclear exclusion, subsequently reducing proglucagon gene transcription (McKinnon *et al.* 2006) with an impact on long-term glucagon maintenance.

γ-aminobutyric acid

γ-aminobutyric acid (GABA) is present in islets and has been located to insulin vesicles (Braun *et al.* 2007) and synaptic like microvesicles (Thomas-Reetz *et al.* 1993) in β-cells, from where it is released in a glucose- and Ca^{2+}-dependent manner (Braun *et al.* 2010, Braun *et al.* 2004). More recently, it was reported that GABA is also present in the β-cell cytosol and can be secreted via non-vesicular release mediated by volume-regulated anion channels (Menegaz *et al.* 2019).

In human α-cells, mRNA transcripts for both ionotropic $GABA_A$ receptors and metabotropic $GABA_B$ receptors are detectable (Blodgett *et al.* 2015). However, $GABA_B$ receptors probably play a limited role since the $GABA_B$ receptor antagonist CGP555845 does not affect glucagon secretion in human islets (Taneera *et al.* 2012). In contrast, ample evidence suggests that $GABA_A$ receptor activation in human and rodent islets reduces glucagon secretion (Rorsman *et al.* 1989, Wendt *et al.* 2004, Taneera *et al.* 2012). Interestingly, we found that

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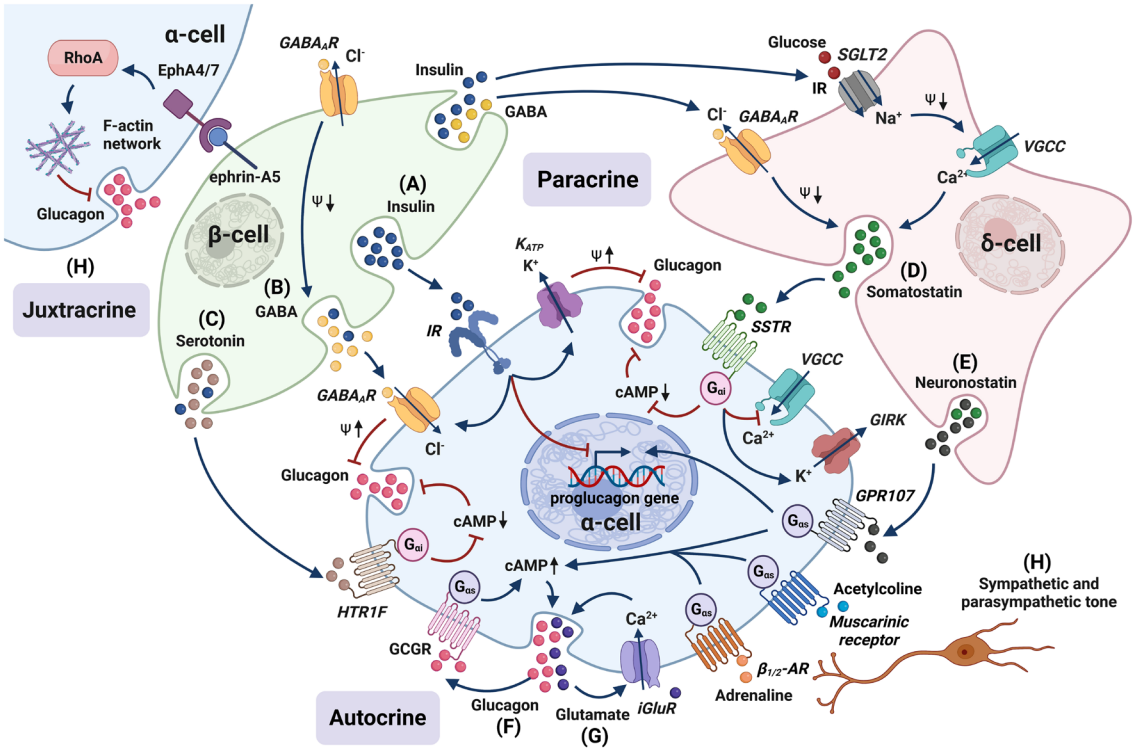

Figure 5

Paracrine, autocrine, and juxtracrine regulation of α-cell. In addition to glucose, numerous paracrine, autocrine, hormonal and nervous signals fine-tune glucose secretion under different physiological conditions. (A) Upon binding to insulin receptor (IR), insulin inhibits glucagon secretion via increasing K_{ATP}-channel activity, potentiating γ-aminobutyric acid (GABA) signalling by promoting the localisation of type A GABA receptors ($GABA_A$) to the plasma membrane, and inducing reduction in α-cell proglucagon gene transcription. Insulin could also indirectly decrease glucagon release by stimulation of intra-islet somatostatin secretion via δ-cell sodium-glucose linked transporter 2 (SGLT2). (B) While GABA on one hand has a direct inhibitory effect on glucagon secretion through $GABA_A$ receptor activation with a resulting hyperpolarising Cl^- current in α-cells, on the other hand it stimulates insulin and somatostatin release which further leads to α-cell inhibition. (C) Serotonin is co-secreted with insulin and inhibits glucagon release via activation of $G_{αi}$-coupled serotonin receptor 1F (HTR1F) resulting in decreased cAMP levels. (D) The downstream signalling of somatostatin by activation of somatostatin receptor (SSTR) on α-cells involves activation of $G_{αi}$ protein leading to decreased cytoplasmic levels of cAMP; hyperpolarisation and inhibition of action potential firing via G protein-coupled inwardly rectifying K^+ (GIRK) channels; and inhibition of Ca^{2+} influx through voltage-gated P/Q-type Ca^{2+} channels. (E) Neuronostatin-mediated increase in glucagon secretion by binding to GPR107, triggering cAMP-independent protein kinase A (PKA) phosphorylation and proglucagon mRNA accumulation in α-cells. (F) Glucagon released by α-cell could stimulate its own secretion as an autocrine regulator by binding to glucagon receptor (GCGR) and promoting downstream cAMP generation. (G) Glutamate is also a positive autocrine signal for glucagon release. By acting on ionotropic glutamate receptors (iGluRs) of the AMPA/kainate type, glutamate enhances glucagon release via membrane depolarisation and opening of voltage-gated Ca^{2+} channels (VGCC). (H) Sympathetic and parasympathetic tones also modulate α-cell glucagon secretion. (I) α- and β-cell juxtracrine signalling is mediated by EphA/ephrin-A pathway and downstream RhoA activity. $β_{1/2}$-AR, $β_{1/2}$ adrenergic receptor. Created with BioRender.com.

insulin potentiates GABA signalling by promoting membrane localisation of $GABA_A$ receptors, linking the two β-cell-derived factors in combined paracrine control of glucagon secretion (Xu *et al.* 2006). $GABA_A$ receptors are ligand-gated Cl^- channels and, in most cases, their activation hyperpolarises the cell, reducing AP generation. This is somewhat different in human islets. Immunostaining shows $GABA_A$ receptor expression in human α-cells (Taneera *et al.* 2012), and functional $GABA_A$ currents were recorded in the same cells. However, Braun *et al.* found $GABA_A$ currents from human α-cell to be relatively small and only detectable in a subset of α-cells (Braun *et al.* 2010); instead, large $GABA_A$ currents from both β- and δ-cells were detected. Surprisingly, $GABA_A$ receptor activation in the latter cells is depolarising (due to their high intracellular Cl^- concentration) and hence stimulatory for insulin and somatostatin secretion. This opens up two potential pathways for GABA to affect α-cells. GABA could have a direct inhibitory effect on glucagon secretion via $GABA_A$ receptor activation with a resulting hyperpolarising Cl^- current in the α-cells (in the case of low intracellular Cl^- concentration); and/or GABA stimulates insulin and somatostatin secretion to inhibit glucagon secretion. A better understanding of how/whether these two mechanisms synergistically function requires more detailed investigations.

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Interestingly, long-term exposure to high glucose (>1 h) promotes β-cell GABA catabolism by shunting GABA into the citric acid cycle via the 'GABA shunt' (Wang *et al.* 2006, Pizarro-Delgado *et al.* 2010). This reduces both the intracellular content and release of GABA when stimulated acutely with glucose. Therefore, prolonged hyperglycaemia can also affect the GABA-mediated β-cell paracrine control of glucagon secretion.

Zinc and serotonin

Zn^{2+} crystallises with insulin in large dense-core vesicles and is co-released with insulin from β-cells (Hardy *et al.* 2011). Zn^{2+} was shown to inhibit pyruvate-stimulated glucagon secretion in perfused rat pancreas (Ishihara *et al.* 2003), an effect that was confirmed by static secretion and electrophysiological experiments in purified rat α-cells (Franklin *et al.* 2005). Later studies, conducted in a hypoglycaemic state, found that switching off either free Zn^{2+} or Zn^{2+} bound to insulin, rather than insulin itself, represents the 'switch-off' signal from β-cells to α-cells that initiates glucagon secretion (Zhou *et al.* 2007). The mechanism by which Zn^{2+} reduces glucagon secretion involves the opening of α-cell K_{ATP} channels that dampens α-cell excitability and restricts the opening of Ca^{2+} channels (Franklin *et al.* 2005, Slucca *et al.* 2010). However, possibly due to different experimental settings and species differences, several groups have reported that Zn^{2+} does not suppress glucagon secretion or intracellular Ca^{2+} in human islets and mouse α-cells (Ravier & Rutter 2005, Quoix *et al.* 2009, Ramracheya *et al.* 2010). Furthermore, whole-body ZnT8 (Zn^{2+} transporter) deletion has no effect on glucagon secretion (Nicolson *et al.* 2009).

Serotonin is also co-secreted with insulin and activates $G_{\alpha i}$-coupled serotonin receptor 1F (HTR1F) on neighbouring α-cells, resulting in decreased cAMP levels and suppression of glucagon secretion (Almaca *et al.* 2016). This study also suggested that reduced serotonergic control of α-cells can be a contributing factor for glucagon dysregulation in diabetes.

Control by δ-cells

In mouse islets, somatostatin-releasing δ-cells are localised in islet periphery and close to α-cells, while in humans they are scattered throughout the islets (Brereton *et al.* 2015). δ-cells exhibit a neuron-like morphology with processes that can reach several cell layers (Arrojo *et al.* 2019). This enables the low-population δ-cells (~10% of the islet cells) to exert islet-wide paracrine regulation. Somatostatin is a powerful inhibitor of glucagon (Xu *et al.* 2020), and its effect on α-cells is primarily mediated by somatostatin receptor 2 (SSTR2) both in mouse and in human islets (Gromada *et al.* 2001, Kailey *et al.* 2012). SSTR2 is a $G_{\alpha i}$-coupled receptor, and its activation inhibits α-cells via (i) decreasing adenylyl cyclase activity and cytoplasmic cAMP levels (Elliott *et al.* 2015); (ii) activating G protein-coupled inwardly rectifying K^+ channels to reduce cellular excitability (Kailey *et al.* 2012); and (iii) inactivating Ca_v channels. In mice, glucagon release is increased across the full range of physiological glucose levels when somatostatin is knocked out (Cheng-Xue *et al.* 2013) or SSTR2 is blocked (Lai *et al.* 2018), suggesting tonic δ-cell inhibition of α-cells. It is well established that the paracrine action of somatostatin contributes to the glucagonostatic effect of high glucose, although the functions/mechanisms of somatostatin release at low glucose remain to be understood.

δ-cells also release neuronostatin, a peptide produced from pro-somatostatin (Samson *et al.* 2008). Neuronostatin was shown to increase glucagon secretion by binding to GPR107, resulting in cAMP-independent PKA phosphorylation and proglucagon mRNA accumulation in α-cells (Elrick *et al.* 2016). However, the physiological importance of neuronostatin in the control of glucose homeostasis remains to be determined.

α-cell autocrine and juxtracrine control

In addition to intra-islet paracrine signalling, α-cell autocrine control of glucagon secretion has also been reported. It was found that glucagon could stimulate α-cell exocytosis by binding to glucagon receptors (GCGR), promoting cAMP generation (Ma *et al.* 2005). Glucagon also regulates its own synthesis in α-cells by signalling through GCGR, protein kinase C (PKC), and PKA, suggesting a long-term autocrine effect on hormone synthesis (Leibiger *et al.* 2012). α-cells also produce and release glutamate, an excitatory neurotransmitter. Acting on ionotropic glutamate receptors (iGluRs) of the AMPA/kainate type, glutamate enhances glucagon release via membrane depolarisation and opening of Ca_v channels (Cabrera *et al.* 2008).

Juxtracrine signalling in α-cells comes primarily through direct contact with β-cells and work synergistically with paracrine and autocrine control of glucagon release (Hughes *et al.* 2018). In particular, the EphA/ephrin-A pathway has been shown to regulate glucagon secretion via α-cell EphA4 interactions with

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ephrin-A5 expressed on the surface of β-cells (Hutchens & Piston 2015). Stimulation of EphA4 suppresses glucagon secretion by modulating the activity of RhoA, a signalling hub that affects Ca^{2+} signalling, cortical F-actin density and exocytosis in α-cells (Ng *et al.* 2022).

Central nervous system control of α-cells

The central nervous system works in tandem with islets to maintain glucose homeostasis via direct autonomic innervation, indirect neuroendocrine mechanisms and glucose sensing to modulate glucose counter-regulation (Faber *et al.* 2020).

The autonomic tone on α-cells

Adrenaline and noradrenaline are released from both islet sympathetic innervation and adrenal medulla. Stress, including hypoglycaemia, triggers the release of these mediators. In human and rodent islets, adrenaline directly stimulates glucagon secretion via activation of β-adrenergic receptors. Possible mechanistic routes are mobilisation of Ca^{2+} from lysosomal acidic stores and ER (Hamilton *et al.* 2018) and increasing cAMP to activate Epac2 (De Marinis *et al.* 2010). Interestingly, it was reported that sympathetic innervation in human islets is restricted to blood vessels (Rodriguez-Diaz *et al.* 2011*a*), questioning whether neuronal modulation directly controls α-cells in human. This is different in type 1 diabetes (T1D) though, where a direct sympathetic innervation of α-cells was demonstrated (Campbell-Thompson *et al.* 2021).

Regarding parasympathetic control of glucagon secretion, acetylcholine stimulates glucagon secretion in rodent islets through binding to muscarinic receptors (reviewed in (Ahrén 2000)). Human α-cells, on the other hand, do not respond to acetylcholine (Molina *et al.* 2014), although they are suggested to be an important source of acetylcholine regulating other islet cells (Rodriguez-Diaz *et al.* 2011*b*).

The brain as glucose sensor for counter-regulation to hypoglycaemia

Hypoglycaemia-associated autonomic failure (HAAF) is a phenomenon first described by Simon Heller and Philip Cryer, in which insulin-induced hypoglycaemia (IHH) leads to reduced α-cell and sympathetic responses, creating a vicious cycle by increasing the susceptibility to future hypoglycaemic episodes (Heller & Cryer 1991). HAAF is of particular concern in people living with T1D, and a role for brain glucose-sensing neurons has been identified in its mechanism (Cryer 2006).

The glucose-excited and glucose-inhibited neurons in ventromedial nucleus of the hypothalamus (VMH) work in concert to stimulate or inhibit glucose counter-regulation (Sherwin 2008). Previous hypoglycaemia impairs VMH glucose sensing by multiple adaptations, including reduced VMH K_{ATP}-channel activity (McCrimmon *et al.* 2005), increased activation of inhibitory neuronal circuits (VMH GABA (Chan *et al.* 2008) or urocortin 3 input (Flanagan *et al.* 2003)), and suppressed VMH AMP kinase activity (Alquier *et al.* 2007). This could lead to insufficient glucagon and adrenaline release during hypoglycaemia. It is worth noticing that these responses are reversible from both clinical and preclinical perspectives; thus, therapies targeting these mechanisms have the potential to restore normal counter-regulation in people living with T1D.

α-cells in diabetes and targeted anti-diabetic treatments

People living with diabetes often present three types of defects in glucagon secretion: (i) impaired glucagon counter-regulation in response to hypoglycaemia, which is frequently seen in T1D (Cryer 2012); (ii) fasting hyperglucagonemia; and (iii) postprandial hyperglucagonemia, which often exacerbates hyperglycaemia in T2D (Shah *et al.* 2000, Dunning & Gerich 2007). Agents targeting glucagon signalling (glucagon receptor antagonists) can effectively attenuate hyperglycaemia in animal models of diabetes (Okamoto *et al.* 2017) and people living with diabetes (Pettus *et al.* 2018). Overall, the current data support the provocative glucagonocentric hypothesis proposed by Unger *et al.* that glucagon excess, rather insulin deficiency, is the *sine qua non* of diabetes (Unger & Cherrington 2012). However, the glucagonocentric hypothesis was later challenged by the observation that, in a streptozotocin (STZ) model of diabetes, α-cell ablation did not relieve the diabetic phenotype, although it is worth noting that removal of α-cells improved glucose tolerance in STZ-treated animals (Steenberg *et al.* 2016). Proposed mechanisms by which glucagon becomes defective in diabetes are illustrated in Fig. 6.

Mechanisms of defective glucagon counter-regulation

Hypoglycaemia, a common complication of T1D, is partly attributable to inadequate glucagon secretion at low glucose. From the perspective of insulin's inhibitory

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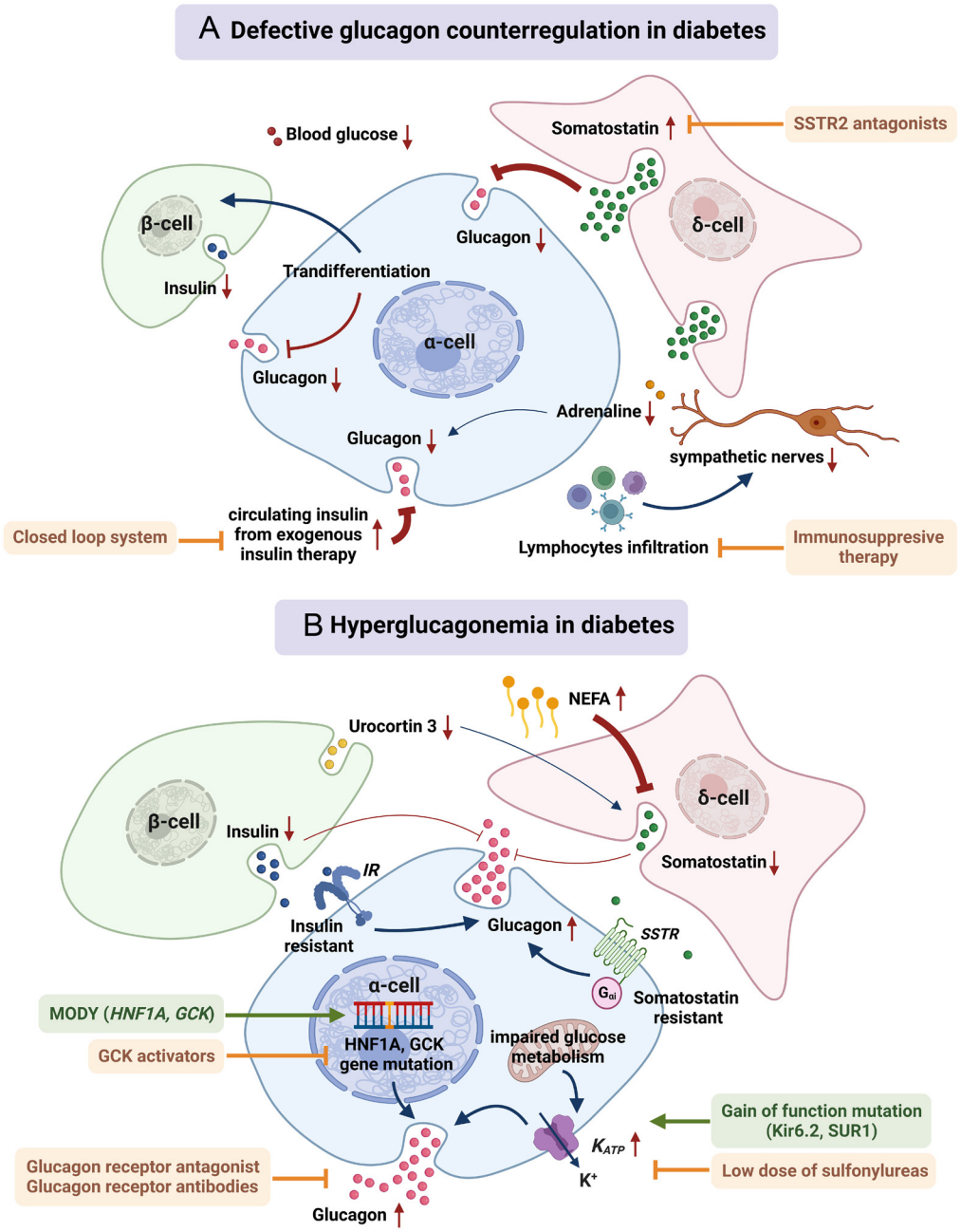

Figure 6

α-cell defects in diabetes and targeted anti-diabetic treatments. Therapeutic strategies are highlighted in orange/brown, while gene defects associated with inappropriate glucagon secretion are highlighted in green. (A) Mechanisms of defective glucagon counter-regulation in diabetes. Inadequate glucagon secretion of α-cell at low glucose could be attributed to intensive insulin regimen triggering strong insulin inhibitory effect on α-cell during hypoglycaemia, excessive somatostatin secretion, impairment of sympathetic tone-mediated glucagon secretion, and α-cell loss of identity by transdifferentiation into β-cells. The abovementioned mechanisms could be counteracted by targeting δ-cell tonic inhibition with somatostatin receptor 2 (SSTR2) antagonist, immunosuppressive therapy inhibiting lymphocytic activation and subsequent islet neuropathy, or the use of closed-loop system automatically modulating insulin pump dose according to continuous glucose monitoring. (B) Mechanisms of hyperglucagonemia in diabetes. α-cell intrinsic defects such as gain-of-function mutation of K_{ATP} channel in neonatal diabetes, *increased* K_{ATP} *channel activity* as a consequence of perturbed α-cell metabolism in type 2 diabetes (T2D), and *HNF1A* and *GCK* mutation in maturity-onset diabetes of the young (MODY) could trigger inadequate glucose-induced suppression of glucagon. Low dose of K_{ATP} channel blocker sulfonylureas, glucokinase (GCK) activators, and glucagon receptor antagonists are beneficial to counteract the hyperglucagonemia in this group of people living with diabetes. Paracrine defects can also lead to hyperglucagonemia through α-cell insensitivity to paracrine inhibition of insulin and somatostatin. β-cell loss at late stage of diabetes resulting in reduced insulin and urocortin 3-potentiated somatostatin secretion could cause subsequent hyperglucagonemia. Moreover, raised non-esterified fatty acid (NEFA) level in T2D can also inhibit somatostatin release. Created with BioRender.com.

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action on glucagon, intensive insulin treatment in T1D can cause high circulating insulin during hypoglycaemia, which could inhibit α-cell activity to disable the glucagon response (Raju & Cryer 2005). For δ-cell-related defects, it has been shown that excessive somatostatin secretion contributes to glucagon failure in hypoglycaemia (Yue *et al.* 2013), while SSTR2 antagonists restore hypoglycaemia-stimulated glucagon release, preventing hypoglycaemia in diabetic animals (Karimian *et al.* 2013). However, before translating into clinical application, the safety of SSTR2 antagonists needs to be carefully assessed since SSTR2 is widely expressed in the body (stomach, adrenal medulla, cerebral cortex, hypothalamus, and pituitary gland) (Taleb & Rabasa-Lhoret 2016).

The autonomic nervous system also contributes to glucagon response in IIH by modulating sympathetic and parasympathetic tones (Taborsky & Mundinger 2012). It was found that during the onset of T1D, the majority of islet sympathetic nerves are lost (termed as early sympathetic islet neuropathy) due to lymphocytic infiltration/activation. This results in defective sympathetically mediated glucagon secretion, aggravating IIH (Mundinger & Taborsky 2016). Thus, early-stage immunosuppressive therapy could potentially be beneficial for preventing this neuropathy.

The defective glucagon secretion could also be attributable to the loss of α-cell identity by adopting β-cell features when β-cells are depleted in diabetes (Bru-Tari *et al.* 2019, Furuyama *et al.* 2019). It is possible that this change in identity (α-cell to β-cell) could invert glucose-dependent glucagon secretory pattern if glucagon remains to be produced in transdifferentiated cells. It will be interesting to test to what extent this can affect glycaemic control in animal models and people living with diabetes.

Mechanisms of hyperglucagonemia

α-cell-intrinsic defects

In situ α-cell electrophysiology revealed that hyperglucagonemia is related to increased AP amplitude and firing frequency, higher Na_v current density, and reduced K_v current density in a STZ-induced diabetes model (Huang *et al.* 2013). These findings suggest that there is an intrinsic mechanism of glucagon dysregulation.

K_{ATP} channels are involved in the intrinsic glucose-sensing mechanism of α-cells. Gain-of-function mutations in the genes encoding the pore-forming (Kir6.2 and *KCNJ11*) and regulatory (SUR1, *ABCC8*) subunits of the K_{ATP}-channel cause neonatal diabetes due to loss of β-cell glucose sensing (Gloyn *et al.* 2004). A common variant (E23K; rs5219) in *KCNJ11* is associated with enhanced T2D risk (Gloyn *et al.* 2003) due to increased K_{ATP}-channel activity (Schwanstecher *et al.* 2002) and impaired glucose-induced suppression of glucagon secretion *in vivo* (Tschritter *et al.* 2002). We have suggested that, in T2D, dysregulation of glucagon secretion may be associated with slightly increased K_{ATP}-channel activity in α-cells, possibly as a consequence of impaired glucose metabolism (Zhang *et al.* 2013). Indeed, low concentration of K_{ATP}-channel blocker tolbutamide restores normal glucose regulation of glucagon release in metabolically compromised and T2D islets. This was then confirmed in clinical trials, where low-dose sulfonylureas (0.3 mg/day glibenclamide) was found to be useful in reducing fasting hyperglucagonemia in people living with T2D (Spiliotis *et al.* 2022).

Maturity-onset diabetes of the young (MODY) is an inherited autosomal dominant condition, most commonly caused by mutations in *HNF1A* (MODY 3) and *GCK* (MODY 2). Although its link to insulin secretory defects has been well investigated, less is known about α-cell pathophysiology in MODY patients. *HNF1A* can control glucagon secretion in α-cells through modulation of SGLT1, and $Hnf1a^{-/-}$ mice showed higher fasting glucagon levels and exhibited inadequate suppression of glucagon after glucose challenge (Sato *et al.* 2020, Saponaro *et al.* 2022). In *HNF1A*-MODY patients, low-dose gliclazide, a sulphonylurea, was found to improve hyperglucagonemia after a glucose challenge (Saponaro *et al.* 2022). However, whether long-term treatment with gliclazide affects α-cell function and the mechanism underlying the treatment response needs further investigation. α-cell GCK plays a central metabolic role in the suppression of glucagon secretion at euglycaemic and hyperglycaemic levels (Basco *et al.* 2018). In GCK-MODY patients, the threshold for glucose to suppress glucagon is higher than that in people without diabetes (Guenat *et al.* 2000). Thus, GCK activators could potentially normalise glucagon secretion by tuning glycolysis and α-cell K_{ATP}-channel activity (Nakamura & Terauchi 2015).

Chronic hyperglycaemia was also reported to induce α-cell dysregulation. Mechanistically, impaired ATP production in α-cells is triggered by increased Na^+ uptake through SGLTs, intracellular and mitochondrial acidification, and protein succination due to reduced fumarase activity (Knudsen *et al.* 2019). This defect can be corrected by low concentrations of tolbutamide and prevented by SGLT inhibitors.

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Paracrine defects

Considering that insulin inhibits glucagon release, β-cell loss and defects in intra-islet insulin-signalling pathway may contribute to the development of diabetic hyperglucagonemia. In T2D, the defective paracrine role of β-cells is supported by the loss of the inverse relationship between pulsatile insulin and glucagon secretion (Menge *et al.* 2011). However, the moderate insulin secretory defects at the initial stage of T2D does not fully support the idea that post-prandial hyperglucagonemia is only due to β-cell dysfunction. It is possible that α-cells develop insulin resistance, including blunted insulin-stimulated Akt phosphorylation, during chronic exposure to high glucose and insulin (Tsuchiyama *et al.* 2007).

Defective glucagon regulation can be caused by impaired paracrine control from δ-cells. It was reported that human T2D islet α-cells develop somatostatin resistance (Omar-Hmeadi *et al.* 2020). This could be responsible for the post-prandial hyperglucagonemia in T2D. In addition, long-term exposure to non-esterified fatty acid (NEFA, often elevated in T2D) reduces glucose-stimulated somatostatin secretion and correspondingly induces a 50% increase in glucagon release (Collins *et al.* 2008). Furthermore, in diabetes, β-cell urocortin-3, a stimulant of δ-cell secretion (van der Meulen *et al.* 2015), is greatly depleted and therefore could be associated with insufficient somatostatin secretion and hyperglucagonemia.

Conclusion

As the islet cells that produce glucagon, a principal counter-regulatory hormone, the α-cells play a vital role in the prevention of hypoglycaemia and the maintenance of systemic glucose homeostasis. However, although decades (a century if you count from the discovery of glucagon) of research have greatly developed our understanding of the cell, the exact regulatory mechanism(s) of the α-cells remain(s) enigmatic. The α-cell electrophysiology, as part of its physiology, is fascinating and has been the topic of studies into the fundamental aspects of how α-cells function, secrete glucagon, and sense glucose.

The role of glucagon dysregulation in diabetes is recognised, and glucagon receptor antagonists are already emerging as a promising new class of anti-diabetic drugs. It can be predicted that with the α-cells in the limelight of islet research, endeavours for understanding the pathophysiology of glucagon dysregulation will lead to the development of therapies that can restore normal α-cell function in diabetes. This will ultimately offer better diabetes care, particularly in the aspect of hypoglycaemia prevention, and will greatly improve the quality of life of people living with diabetes.

Declaration of interest
The authors declare that there is no conflict of interest.

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