REVIEW

ATP-regulated potassium channels and voltage-gated calcium channels in pancreatic alpha and beta cells: similar functions but reciprocal effects on secretion

Patrik Rorsman · Reshma Ramracheya ·
Nils J. G. Rorsman · Quan Zhang

Abstract Closure of ATP-regulated K^+ channels (K_{ATP} channels) plays a central role in glucose-stimulated insulin secretion in beta cells. K_{ATP} channels are also highly expressed in glucagon-producing alpha cells, where their function remains unresolved. Under hypoglycaemic conditions, K_{ATP} channels are open in alpha cells but their activity is low and only ~1% of that in beta cells. Like beta cells, alpha cells respond to hyperglycaemia with K_{ATP} channel closure, membrane depolarisation and stimulation of action potential firing. Yet, hyperglycaemia reciprocally regulates glucagon (inhibition) and insulin secretion (stimulation). Here we discuss how this conundrum can be resolved and how reduced K_{ATP} channel activity, via membrane depolarisation, paradoxically reduces alpha cell Ca^{2+} entry and glucagon exocytosis. Finally, we consider whether the glucagon secretory defects associated with diabetes can be attributed to impaired K_{ATP} channel regulation and discuss the potential for remedial pharmacological intervention using sulfonylureas.

Abbreviations
$[Ca^{2+}]_i$	Intracellular Ca^{2+} concentration
$[cAMP]_i$	Intracellular cAMP concentration
FACS	Fluorescence-activated cell sorting
GCK	Glucokinase
K_{ATP}	ATP-regulated K^+ (channel)
PKA	Protein kinase A
SERCA	Sarco-endoplasmic reticulum Ca^{2+}-ATPase

> **Notation**
> Ω, Ohm – unit of electrical resistance
> A, Ampere – unit of electrical current
> F, Farad – unit of capacitance, capacity to store electric charge
> S, Siemens – unit of electrical conductance, capacity to pass current

P. Rorsman (✉) · R. Ramracheya · N. J. G. Rorsman · Q. Zhang
Radcliffe Department of Medicine, Oxford Centre for Diabetes, Endocrinology and Metabolism, University of Oxford, Oxford OX3 7LJ, UK
e-mail: patrik.rorsman@drl.ox.ac.uk

Introduction

Glucagon, secreted by the pancreatic alpha cells of the pancreatic islets, is the body's principal hyperglycaemic hormone [1, 2]. Together with insulin, glucagon ensures that plasma glucose levels are kept within a narrow range by accelerating hepatic glucose production via stimulation of glycogenolysis [3], regardless of eating, exercise and fasting [4]. Glucagon secretion is triggered by amino acids, fatty acids, neurotransmitters (such as adrenaline [epinephrine] and gastric inhibitory polypeptide [GIP]) [2], but a fall in plasma glucose levels arguably represents the physiologically most important physiological stimulus.

Interest in the regulation of glucagon secretion has been renewed by the recent rediscovery of the central role of glucagon in diabetes. Thus, over-secretion of glucagon at high plasma glucose levels exacerbates the hyperglycaemia caused by the lack of insulin [5]. The finding that mice remain normoglycaemic even after complete destruction of their beta cells if glucagon action is prevented [6] provides a spectacular demonstration of the contribution of glucagon to the hyperglycaemia seen in diabetes.

Regulation of glucagon secretion: intrinsic, paracrine or both?

The metabolic regulation of glucagon secretion remains hotly debated. A central question is whether the alpha cells are capable of sensing changes in the plasma glucose levels by themselves ('intrinsic regulation') [7] or if they are regulated by factors released from neighbouring endocrine cells ('paracrine regulation') [8]. The fact that insulin and glucagon secretion are reciprocally regulated by glucose gave rise to the idea that glucagon secretion is under paracrine control by insulin (the 'switch-off' hypothesis) [9, 10]. The switch-off hypothesis is attractive as it provides a simple explanation for the hypersecretion of glucagon seen in diabetes (when insulin secretion is low) and why this is corrected by administration of exogenous insulin. Variations on this theme postulate that the inhibitory effect of high glucose on alpha cells is mediated by factors co-released with insulin from the beta cells (like γ-aminobutyric acid [GABA] [11], Zn^{2+} [12] or γ-hydroxybutyrate [GHB] [13]). In addition, there is strong evidence that glucagon secretion is under vagal regulation [14, 15].

However, a problem with the switch-off hypothesis is that glucagon secretion is almost maximally inhibited at glucose concentrations that do not stimulate insulin secretion [16]. This is highlighted by the data in Fig. 1, which summarises the time- and concentration-dependent effects of glucose on insulin and glucagon secretion measured in a perfused mouse pancreas preparation. Increasing the glucose concentration from 1 to 6 mmol/l reduced glucagon secretion by 60–70%. It is notable that glucagon secretion remained equally
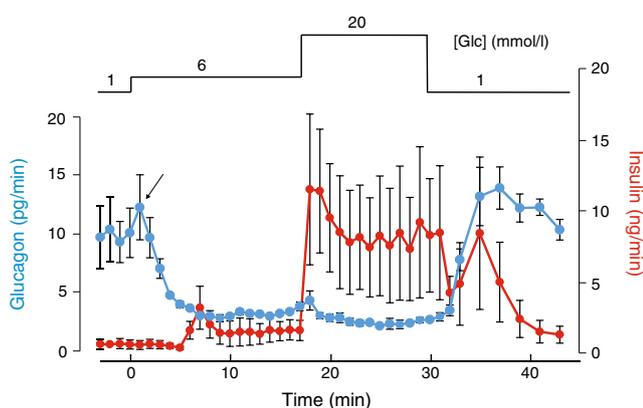

Fig. 1 Reciprocal effects of glucose on glucagon and insulin secretion. The pancreas was perfused via the aorta at physiological rate ~0.3 ml/min [84] and in the normal direction and the effluent collected via the portal vein. Insulin secretion (red) was stimulated and glucagon secretion (blue) suppressed when the extracellular glucose concentration ([Glc]) was increased from 1 to 6 and then 20 mmol/l. Note the transient stimulation of glucagon secretion when glucose was elevated (arrow), that inhibition of glucagon secretion is seen before any detectable stimulation of insulin secretion and prominent 'off response' in glucagon secretion when glucose was lowered from 20 to 1 mmol/l

suppressed during the first and second phases of insulin secretion and that inhibition peaked before any detectable stimulation of insulin secretion. Moreover, increasing the glucose level from 6 to 20 mmol/l resulted in much stronger stimulation of insulin secretion (approximately tenfold higher than steady-state release at 6 mmol/l) but this did not translate into further inhibition of glucagon secretion.

The dissociation between insulin signalling and glucagon secretion is also supported by the surprisingly mild effects on glucagon secretion observed following genetic ablation of the insulin receptors in the alpha cells [17, 18]. The findings that glucose remains capable of inhibiting glucagon secretion in somatostatin-deficient islets [19] and in the presence of somatostatin receptor antagonists [20] also suggest that somatostatin released by the delta cells does not function as a 'switch-off' signal for glucagon release. Collectively, these data provide little evidence of a paracrine regulation of glucagon secretion by insulin at glucose concentrations at or below the threshold for stimulation of insulin or somatostatin release (6 mmol/l). However, we acknowledge that paracrine regulation may become functionally more significant at glucose concentrations associated with stronger stimulation of insulin or somatostatin secretion (or other factors released by the beta and delta cells). It should also be noted that isolated islets (when innervation has been severed) remain capable of responding to low glucose concentrations with stimulation of glucagon secretion.

Pancreatic alpha cells are electrically excitable but regulation by glucose is obscure

Like beta cells, alpha cells are electrically excitable. However, unlike beta cells, alpha cells fire action potentials [21–23] and exhibit spontaneous oscillations in intracellular Ca^{2+} concentrations ($[Ca^{2+}]_i$) at low (0–3 mmol/l) glucose concentrations [24–26]. The occurrence of spontaneous electrical activity and $[Ca^{2+}]_i$ oscillations in alpha cells at low glucose levels is well established and explains why glucagon secretion is stimulated under hypoglycaemic conditions. However, the impact of higher glucose concentrations on glucagon secretion is more controversial. Some studies suggest that elevation of glucose levels hyperpolarises alpha cells [27] and lowers $[Ca^{2+}]_i$ [28–31]. Others have indicated that isolated alpha cells (obtained by fluorescence-activated cell sorting [FACS]) behave like beta cells, i.e. they are electrically silent with low $[Ca^{2+}]_i$ at low glucose levels, but elevation of glucose triggers depolarisation, electrical activity and elevation of $[Ca^{2+}]_i$ [32]. Finally, there are reports suggesting little effect of glucose on $[Ca^{2+}]_i$ in intact islets [24, 33]. The latter finding has led to the proposal that the regulation of glucagon granule release may be exerted at a late stage in the alpha cell stimulus–secretion coupling, distal to the elevation of $[Ca^{2+}]_i$ [34].

The above summary may not be a comprehensive review of the literature, but the intention here is rather to highlight the great variability of the reported responses to glucose.

We have developed techniques that allow patch-clamp studies of freshly isolated intact islets [35]. Fig. 2 shows measurements of $[Ca^{2+}]_i$ and electrical activity from alpha cells using this preparation. Bursts of increased $[Ca^{2+}]_i$ are present at 1 mmol/l glucose (when glucagon secretion is stimulated), but elevation of glucose to 6 mmol/l, a maximally inhibitory concentration (see Fig. 1), is not associated with any detectable reduction of $[Ca^{2+}]_i$ (Fig. 2a). Unexpectedly, elevation of glucose *depolarised* the alpha cell by ~10 mV and doubled the rate of action potential firing (Fig. 2b). In hormone release experiments in groups of isolated islets prepared the same way as those used for electrophysiology, 6 mmol/l glucose consistently inhibited glucagon secretion by ~50% [26]. This effect is quantitatively similar to those observed in the perfused pancreas, suggesting that islet function was not compromised during isolation. Our in situ measurements of alpha cell electrical activity and $[Ca^{2+}]_i$ therefore suggest a dissociation between these processes and glucagon secretion. A possible resolution of this conundrum is outlined below.

Metabolism–secretion coupling in alpha cells

Glucose metabolism in glucagon-secreting alpha cells differs from that in beta cells in several important respects. First, glucose uptake is mediated by GLUT1 rather than GLUT2 [36]. Second, although alpha cells (like beta cells) express high-K_m glucokinase (GCK; hexokinase IV), they also express the low-K_m hexokinase II, which accounts for ~50% of glucose phosphorylation in alpha cells vs <10% in beta cells [37]. Third, whereas the rates of glucose utilisation (glycolysis+Krebs cycle) are comparable in alpha and beta cells [36], the rate of glucose oxidation (oxidative phosphorylation alone) in alpha cells is only ~10% of that in beta cells [38]. Time-resolved but non-quantitative measurements of the cytoplasmic ATP concentration have revealed that elevation of glucose induces a prompt increase in ATP [26, 33]. Quantitative biochemical measurements of ATP and ADP in pure alpha and beta cell fractions further suggest that the ATP/ADP ratio in alpha cells at 1 mmol/l glucose is as high as that in beta cells exposed to 6 mmol/l glucose [38].

It is interesting to correlate this information on alpha cell metabolism with the effects of various metabolic inhibitors on glucagon secretion. Thus, 3-O-methyl-D-glucose, a glucose analogue that is transported into the cell but not metabolised, does not influence glucagon secretion [19]. Mannoheptulose, an inhibitor of GCK, prevents the glucose-induced suppression of glucagon secretion [26]. The effects of mitochondrial inhibitors (such as oligomycin [26] and dinitrophenol [39]) are complex; in the presence of mitochondrial inhibitors,
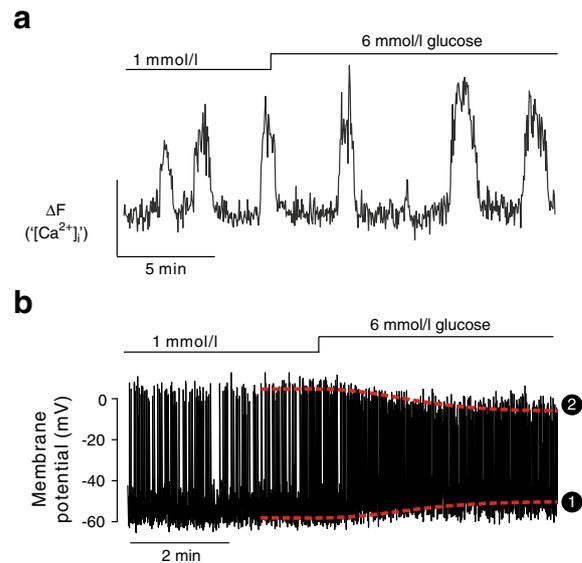

Fig. 2 Glucose inhibits glucagon release without affecting alpha cell electrical activity. (**a–b**) Effects of glucose on $[Ca^{2+}]_i$ (**a**) and electrical activity (**b**) in alpha cells in intact freshly isolated mouse pancreatic islets. Note in (**a**) the weak effect of glucose (6 mmol/l) on spontaneous $[Ca^{2+}]_i$ oscillations (reported as changes in fluo3 fluorescence [ΔF]; an upward deflection corresponds to an increase in $[Ca^{2+}]_i$). Note in (**b**) that glucose (6 mmol/l) depolarises the alpha cell by ~10 mV (dashed line ❶) and decreases the height of action potentials by ~10 mV (dashed line ❷). Also note that alpha cells are spontaneously active at 1 mmol/l glucose in both (**a**) and (**b**). Data based on findings reported in [26]

glucagon secretion is stimulated at high glucose levels but inhibited at low glucose concentrations. Thus, alpha cells in which mitochondrial metabolism has been inhibited retain the ability to respond to glucose, albeit in the 'wrong' direction, raising the interesting possibility that glycolysis plays a more prominent regulatory role in glucagon secretion than in insulin secretion.

Ion channels and islet cell electrical activity

The electrophysiological properties of any given cell are determined by its ion channel complement. In this section we briefly outline how the different ion channels contribute to electrical activity in mouse and human alpha and beta cells.

Pancreatic beta cells exposed to low glucose are hyperpolarised (−70 mV) and electrically silent. This is because the ATP-regulated K$^+$ (K$_{ATP}$) channel activity is very high (3 nS; corresponding to ~300 K$_{ATP}$ channels being simultaneously active). When glucose is elevated to >6 mmol/l, K$_{ATP}$ channel activity is reduced by >75% and the cells depolarise. Importantly, K$_{ATP}$ channel closure alone is not sufficient to produce membrane depolarisation; a depolarising membrane current is also required [35]. Possible candidates include members of transient receptor potential (TRP) channel family (for a review, see [40]). The

glucose-induced depolarisation leads to the activation of voltage-gated Ca^{2+} channels (principally dihydropyridine-sensitive L-type Ca^{2+} channels) [41]. Activation of the L-type Ca^{2+} channels during action potential firing produces a localised increase in $[Ca^{2+}]_i$ just below the plasma membrane and near the Ca^{2+} channels, which thereby triggers exocytosis of insulin granules, some of which may be physically tethered to the Ca^{2+} channels [42]. Although there are several important electrophysiological differences between mouse and human beta cells, the fundamental aspects of glucose sensing are the same [43].

As already discussed, mouse alpha cells are electrically active at 1 mmol/l glucose and fire action potentials from a membrane potential of approx. −55 mV [26, 44]. The alpha cells are equipped with K_{ATP} channels of exactly the same type as those found in beta cells but their net activity is limited to ~0.1 nS (approximately eight simultaneously active channels). As a consequence of the low K_{ATP} channel activity, alpha cells have a very high input resistance (3–5 GΩ) [26, 44]. This means currents as small as 1–2 pA (equivalent to the current associated with openings of individual ion channels) will change the membrane potential by 3–10 mV. Thus, small changes in ion channel activity may have dramatic effects on membrane potential, action potential firing and glucagon secretion.

The upstroke of the action potential in mouse alpha cells is due to the opening of voltage-gated Na^+ channels [44]. During the peak of the action potential, voltage-gated Ca^{2+} channels activate. The magnitude of the Ca^{2+} current is only ~15% of the Na^+ current [45, 46], and blocking the Na^+ channels exerts a strong inhibitory effect on both alpha cell electrical activity and glucagon secretion [26]. In mouse alpha cells, ~70% of the Ca^{2+} current flows through L-type Ca^{2+} channels and only ~20% is due to opening P/Q-type Ca^{2+} channels [26, 44]. Nevertheless, hormone release measurements using the P/Q-type Ca^{2+} channel inhibitor ω-agatoxin[1] indicate that the P/Q-type Ca^{2+} channels are particularly important for glucagon secretion evoked by low glucose concentrations [26]. In addition, mouse alpha cells express low-threshold T-type Ca^{2+} channels that may be involved in the initiation ('pace-making') of action potential firing [47, 48].

Increasing glucose to ≥6 mmol/l results in complete inhibition of the already low K_{ATP} channel activity. As discussed above, this leads to a membrane depolarisation of ~10 mV

[26]. As in beta cells, this depolarisation requires a depolarising membrane current. The identity of this current in alpha cells remains unknown but a store-operated membrane conductance discussed below could potentially be involved. Crucially, elevation of glucose is not only associated with an increased rate of action potential firing, there is also a reduction in action potential height (Fig. 2). The latter effect occurs because the voltage-gated Na^+ channels underlying the upstroke undergo voltage-dependent inactivation and thereby become non-functional. At the most negative membrane potential, observed at 1 mmol/l glucose, 75% of the Na^+ channels remain available for activation. This decreases to 30% in the presence of 6 mmol/l glucose. The reduction in the proportion of Na^+ channels available for spike generation explains the lowered action potential amplitude. This is important because the P/Q-type Ca^{2+} channels that mediate the Ca^{2+} influx triggering glucagon exocytosis principally activate at the peak of the action potential.

Reactivation of the Na^+ channels occurs during the interval between two successive action potentials and the inter-spike membrane potential, which in turn is influenced by the number of voltage-dependent K^+ channels activated during the action potential; the stronger the activation of K^+ channels, the more negative the inter-spike membrane potential and the greater the reactivation of Na^+ channels. This may explain the paradox that blocking voltage-gated K^+ channels in alpha cells is associated with inhibition of glucagon secretion and electrical activity rather than the stimulation seen in beta cells [49–51].

Electrophysiologically, human alpha cells are almost identical to human beta cells [51, 52]. Yet, glucose has opposite effects on insulin and glucagon secretion. Importantly, K_{ATP} channel activity in isolated human alpha cells exposed to 1 mmol/l glucose is 75% lower than in beta cells under the same experimental conditions [51] and is in fact comparable to that in human beta cells exposed to insulin-releasing glucose concentrations, which probably explains the occurrence of spontaneous action potential firing in alpha cells under hypoglycaemic conditions. The net K_{ATP} channel activity in human alpha cells (after subtraction of unspecific leak currents around the recording electrode) is equivalent to one K_{ATP} channel being active at any time in the entire alpha cell. Such low K_{ATP} channel activities are not easily measured experimentally. However, the finding that the K_{ATP} channel blocker tolbutamide depolarised human alpha cells by ~10 mV, reduced action potential height by ~10 mV [51] and inhibited glucagon secretion in human islets [16] indicates that even a K_{ATP} channel activity as low as this is sufficient to keep the alpha cell sufficiently repolarised to maintain action potential height and glucagon exocytosis. It remains to be demonstrated experimentally that glucose also reduces K_{ATP} channel activity in human alpha cells. Circumstantial evidence that glucose regulates glucagon secretion in human islets by

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[1] The non-L-type Ca^{2+} current component in mouse alpha cells was previously attributed to activation of ω-conotoxin-sensitive N-type Ca^{2+} channels. However, it was subsequently discovered that such channels are expressed at very low levels in alpha cells, whereas ω-agatoxin-sensitive P/Q-type Ca^{2+} channels are highly expressed. Pharmacological experiments revealed that ω-conotoxin had no effect on alpha cells Ca^{2+} currents in the presence of ω-agatoxin. We therefore conclude that ω-conotoxin unspecifically blocks P/Q-type Ca^{2+} channels in alpha cells. Here, non-L-type Ca^{2+} channels are therefore referred to as P/Q-type Ca^{2+} channels even when referred to as N-type in the original publications.

closing K_{ATP} channels has been provided by the observation that the inhibitory effect of glucose on glucagon secretion can be reversed by low concentrations of the K_{ATP} channel activator diazoxide (2–3 μmol/l) [16, 26].

The key role of the K_{ATP} channels in the regulation of glucagon secretion is further illustrated by the strong reduction of glucagon secretion at low glucose concentrations and the (nearly complete) loss of glucose-induced suppression following genetic ablation of the K_{ATP} channels [16, 19, 53–55]. It has previously been reported that incubation of isolated mouse [56] and human islets [57] at high glucose concentrations (>20 mmol/l) results in a less effective inhibition of glucagon secretion than lower (5–8 mmol/l) glucose concentrations. Interestingly, in K_{ATP} channel knockout mice, we found that increasing glucose stimulated glucagon secretion in a concentration-dependent manner. Thus, glucose appears to control glucagon secretion by both K_{ATP} channel-dependent (inhibition) and -independent (stimulation) processes (Fig. 3a). This is reminiscent of the situation in beta cells [58], with the important difference that the K_{ATP} channel-dependent ('triggering') and -independent ('amplifying') effects on insulin secretion are both stimulatory (Fig. 3b), whereas they have opposing effects on glucagon secretion. The nature of the K_{ATP} channel-independent stimulation of glucagon secretion remains to be elucidated, but it is noteworthy that glucose has been reported to amplify depolarisation-evoked exocytosis in voltage-clamped rat alpha cells [32].

In addition to the K_{ATP} channel-dependent mechanism for intrinsic glucose-induced inhibition of glucagon secretion we outline above, it has been postulated that glucose regulates glucagon secretion via a store-operated Ca^{2+} current flowing through Orai1 channels [28]. According to this hypothesis, elevation of glucose will activate the sarco-endoplasmic reticulum (sER) Ca^{2+}-ATPase (SERCA). When the Ca^{2+} stores are filled, the Orai1 channels will be turned off, leading to alpha cell hyperpolarisation, cessation of action potential firing and suppression of glucagon secretion. The Orai1-dependent mechanism has recently been reviewed elsewhere [59]. It is noteworthy that the K_{ATP} channel- and Orai1-dependent mechanisms for the intrinsic regulation of glucagon secretion predict opposite effects on the alpha cell membrane potential [60, 61]. Our data showing that glucose depolarises rather than repolarises the alpha cell are not consistent with a major role of Orai1 in glucagon secretion, but this does not exclude a modulatory role. Indeed, we have found that the SERCA inhibitor thapsigargin partially antagonises the inhibitory effect on glucagon secretion [62]. Further studies are required to determine the relative importance of the K_{ATP} channel and Orai1-dependent regulation of glucagon secretion.
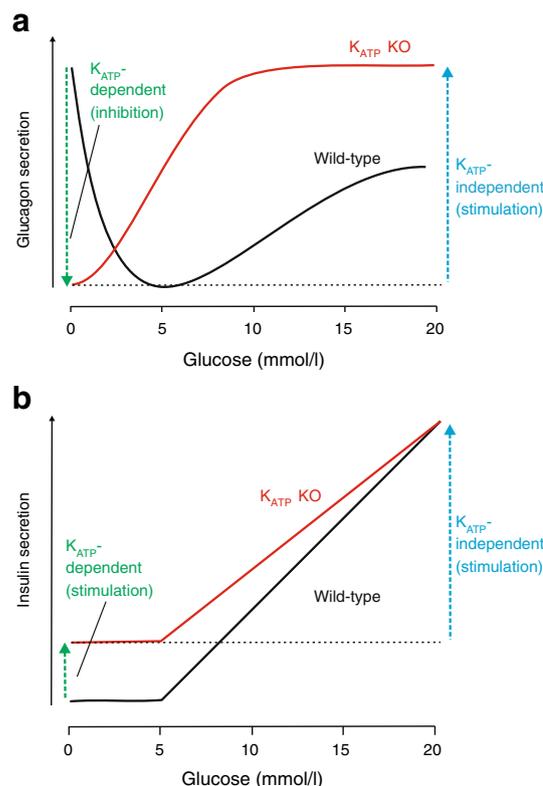

Fig. 3 K_{ATP} channel-dependent and -independent effects on insulin and glucagon secretion. (a) Effects of increasing glucose concentrations on glucagon secretion in wild-type and K_{ATP} channel knockout (Kir6.2$^{-/-}$) islets. In wild-type mice, increasing glucose from 0 to 5 mmol/l inhibits glucagon secretion, whereas higher glucose concentrations are less inhibitory. In islets lacking functional K_{ATP} channels (K_{ATP} KO), increasing glucose results in a concentration-dependent stimulation of glucagon secretion. Thus, K_{ATP} channels mediate the inhibitory effect (green arrow left) and K_{ATP} channel-independent processes produce a stimulation of glucagon secretion (blue arrow right). In wild-type islets, the K_{ATP} channel-dependent processes superimpose, giving rise to the V-shaped concentration dependence of glucagon secretion. (b) As in (a) but comparing glucose-induced insulin secretion in wild-type and K_{ATP} channel KO islets. In K_{ATP} channel KO islets, basal insulin secretion is increased but the amplification at glucose concentrations >5 mmol/l is intact. Note that the K_{ATP} channel-dependent and -independent effects (green and blue arrows) both stimulate insulin secretion. Curves are based on data reported in [16]. Secretion rates have been normalised to maximum rate in the respective mouse strains

Why does glucose stimulate insulin secretion but inhibit glucagon secretion?

In alpha cells and beta cells, elevation of glucose produces membrane depolarisation and increases action potential frequency. Why then does glucose inhibit glucagon secretion whilst stimulating insulin secretion? There is a simple explanation to this conundrum, illustrated schematically in Fig. 4.

In beta cells, action potential firing and insulin secretion at low glucose concentrations are virtually zero. When glucose is elevated, glucose-induced electrical activity initiates insulin secretion from a very low basal level. Although the action
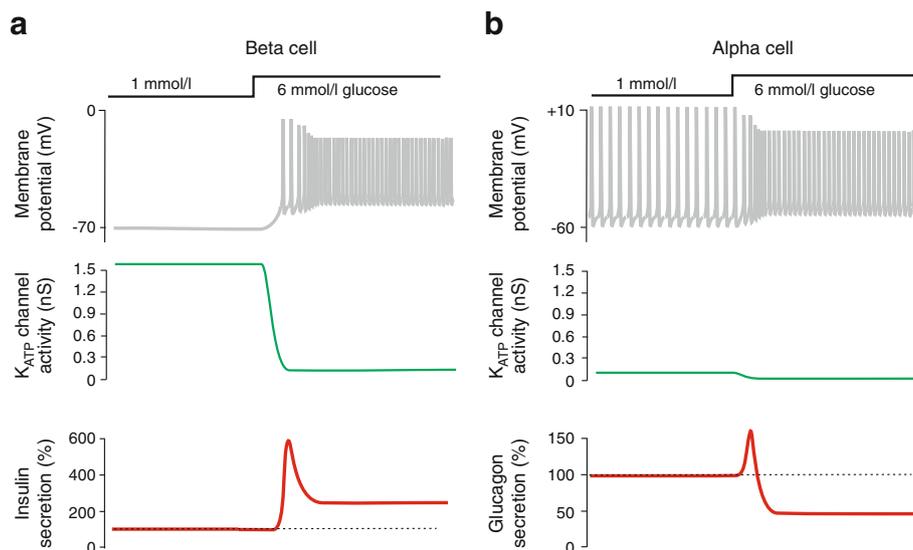
a

Beta cell

b

Alpha cell

Fig. 4 Schematic representation of glucose-dependent effects on membrane potential/action potential firing and secretion in beta and alpha cells. (**a**) At low glucose, beta cells are hyperpolarised and electrically silent, because of high K_{ATP} channel activity, and insulin secretion minimal. Increasing glucose results in K_{ATP} channel closure, membrane depolarisation and initiation of electrical activity and insulin secretion. During electrical activity, the amplitude of the action potentials declines. Because insulin granule exocytosis is steeply voltage-dependent, the decrease in action potential amplitude results in a reduction of insulin secretion. This may account for first (the initial bout of large-amplitude action potentials) and second phase insulin secretion (the steady-state firing of action potentials once the action potential amplitude has decreased to a new steady-state amplitude). Note that although insulin secretion declines as the action potential height is reduced, it remains

much higher than at low glucose (indicated by dashed line). (**b**) Because of low K_{ATP} channel active, alpha cells are electrically active at low glucose concentrations and the resulting action potential firing is associated with stimulation of glucagon secretion. As in beta cells, increasing glucose leads to membrane depolarisation and an increased action potential firing in alpha cells that transiently stimulates glucagon secretion (see Fig. 1). Membrane depolarisation also leads to a reduction in action potential height that (because of steep voltage dependence of exocytosis) results in reduced glucagon secretion and, at steady-state, glucagon secretion at high glucose is lower than at low glucose. Thus, the membrane potential-induced reduction in exocytosis will result in net inhibition of glucagon secretion compared with that at low glucose (indicated by dashed line)

potential amplitude often undergoes a time-dependent decrease (leading to a gradual reduction of Ca^{2+}-dependent exocytosis [43]), insulin secretion will remain much above basal levels even after this has occurred (Fig. 4a).

Under hypoglycaemic conditions, alpha cells generate large-amplitude Na^+-dependent action potentials and this, via Ca^{2+} entry through P/Q-type Ca^{2+} channels, accounts for the high basal rate of glucagon secretion. As in beta cells, elevation of glucose leads to membrane depolarisation and an increased rate of action potential firing. However, the increase in action potential firing is fairly moderate and limited to an increase of 100%. In addition to the effect on action potential frequency, as discussed above, glucose has another very important effect: it produces a reduction in action potential height, which results in diminished glucagon granule exocytosis. We estimate that the 10 mV decrease in action potential amplitude we observe (Fig. 2b), via reduced P/Q-type Ca^{2+} channel activation, results in 80% inhibition of glucagon exocytosis. The associated increase in action potential frequency is insufficient to compensate for the much greater reduction of exocytosis. Combining these two effects it is predicted that glucose should inhibit glucagon secretion by

~60% (Fig. 4b), which is very close to the inhibition observed experimentally (see Fig. 1).

This scenario would also explain the paradox that in alpha cells glucose (6 mmol/l) has little effect on $[Ca^{2+}]_i$ whilst inhibiting glucagon secretion; the increase in action potential frequency leads to increased Ca^{2+} influx via L-type Ca^{2+} channels that partially compensates for the reduced activation of P/Q-type Ca^{2+} channels because of the lower action potential height on $[Ca^{2+}]_i$ (but not on glucagon secretion). Interestingly, stronger inhibitory effects on alpha cell $[Ca^{2+}]_i$ in intact islets have been reported at higher glucose concentrations (11 mmol/l) [16, 30], possibly reflecting stronger paracrine suppression owing to greater stimulation of insulin and/or somatostatin release.

The capacity of glucagon-like peptide 1 (GLP-1) to inhibit glucagon secretion [63] has also been attributed to an intrinsic mechanism (but see [64] for an alternative paracrine viewpoint) that culminates in reduced activation of alpha cell P/Q-type Ca^{2+} channels [65]. The latter effect is mediated by direct protein kinase A (PKA)-dependent inhibition of alpha cell P/Q-type Ca^{2+} channels [65] that is not secondary to changes in K_{ATP} channel activity and membrane potential.

The scenario outlined in Fig. 4 emphasises the importance of a high rate of action potential firing and consequential stimulation of glucagon secretion in alpha cells exposed to low glucose concentrations resulting from low resting K_{ATP} channel activity. Importantly, the magnitude of the K_{ATP} current that can be activated by wash-out of intracellular ATP is, if anything, larger in alpha cells than in beta cells [66]. Thus, the low K_{ATP} channel activity in alpha cells is not due to low expression of the channel. Rather, K_{ATP} channel activity must be under very strong tonic inhibition, possibly reflecting the high ATP/ADP ratio in alpha cells [38].

Differential roles of L- and P/Q-type Ca²⁺ channels in mouse pancreatic alpha cells

Both human and mouse alpha cells contain P/Q as well as L-type voltage-gated Ca^{2+} channels [44, 51]. In mouse islets, blocking the L-type Ca^{2+} channels has little effect on glucagon secretion evoked by low glucose. By contrast, blocking the

P/Q-type Ca^{2+} channel inhibits glucagon secretion as strongly as a maximally inhibitory glucose concentration [26, 51]. Thus, it appears that glucagon secretion evoked by low glucose is selectively triggered by Ca^{2+} entry via P/Q-type Ca^{2+} channels (20% of the Ca^{2+} current) and that Ca^{2+} entry via L-type Ca^{2+} channels (70% of the Ca^{2+} current) is seemingly unimportant in this context. How can this paradox be explained?

To address this question we used high-resolution capacitance measurements of exocytosis (Fig. 5a) [67]. In agreement with the glucagon secretion measurements, depolarisation-evoked exocytosis in alpha cells is inhibited by ω-agatoxin [26]. We found that depolarisations with a duration as short as 10 ms, which is comparable to the duration of the alpha cell action potential, evoke significant exocytosis (Fig. 5b). Importantly, the intracellular diffusion of Ca^{2+} during such brief action potentials is very limited, so only glucagon granules situated in the immediate vicinity of the P/Q-type Ca^{2+} channels undergo exocytosis. This stimulation of exocytosis is mediated by Ca^{2+} binding
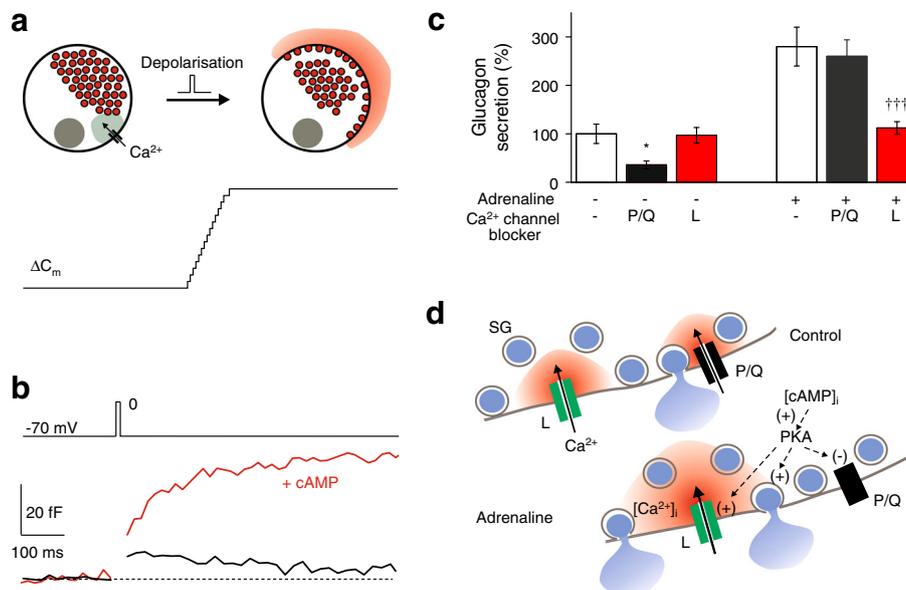

Fig. 5 Action potential height and Ca²⁺ channel activation in the control of glucagon exocytosis. (**a**) Schematic illustration of capacitance measurements. Exocytosis is associated with the addition of the granular membranes to the plasma membrane, resulting in an increased cell surface area (**a**). This can be detected as an increase in membrane capacitance (ΔC_m), which is proportionally related to the increase in cell surface area (ΔSA); thus, $\Delta C_m = \varepsilon \times \Delta SA$, where ε is the specific capacitance (10 fF/μm^2). Fusion of a glucagon granule (diameter: 0.2 μm) can be estimated to produce a capacitance increase of ~2 fF [85]. (**b**) Increase in membrane capacitance evoked by 10 ms depolarisation from −70 mV to 0 mV in the absence (black trace) and presence (red trace) of 0.1 mmol/l cAMP. Note the larger exocytotic response in the presence of cAMP and that ΔC_m continues to increase beyond the depolarisation. (**c**) Effects of blocking P/Q- and L-type (see footnote 1) Ca^{2+} channels on glucagon secretion at 1 mmol/l glucose in the absence (−) and presence (+) of 5 μmol/l

adrenaline as indicated. Data from [65]. **$p < 0.01$ vs 1 mmol/l glucose without adrenaline; ††† $p < 0.001$ vs 1 mmol/l glucose in the presence of adrenaline. Glucagon secretion rates are expressed as a per cent (%) of that at 1 mmol/l glucose in the absence of adrenaline (=100%). (**d**) Schematic representation of glucagon granule exocytosis evoked by low glucose alone and in the presence of adrenaline and the relationship to P/Q- and L-type Ca^{2+} channels. The shaded areas indicate 'active zones' around inner mouths of Ca^{2+} channels in which $[Ca^{2+}]_i$ rises to exocytotic levels. Via an increase in $[cAMP]_i$ and activation (+) of PKA, adrenaline increases exocytosis due to Ca^{2+} influx via L-type Ca^{2+} channels by (1) inhibiting (−) P/Q-type Ca^{2+} channels, (2) increasing Ca^{2+} entry via L-type Ca^{2+} channels and (3) sensitising the exocytotic machinery to $[Ca^{2+}]_i$, thereby expanding the 'active zones' (as indicated by dashed arrows)

to synaptotagmin-7, a Ca^{2+} sensor for exocytosis expressed in alpha cells [68].

What is the function of the L-type Ca^{2+} channels in mouse alpha cells? Clearly they are not involved in exocytosis evoked by action potential firing under hypoglycaemic conditions. However, when glucagon secretion is stimulated by adrenaline (epinephrine; acting via β-receptors), glucagon secretion becomes almost entirely reliant on L-type Ca^{2+} channels [21, 65] (Fig. 5c). Adrenaline depolarises the alpha cells by ~10 mV and reduces the action potential height by >10 mV [65]. Thus, the effects of adrenaline on electrical activity resemble those of glucose, and yet it stimulates rather than inhibits glucagon secretion. We believe that an adrenaline-induced increase in intracellular cAMP concentration ($[cAMP]_i$) [69] counteracts the impact of membrane depolarisation/reduced action potential height on glucagon secretion. Via activation of PKA, which is cAMP-dependent, and the cAMP-sensor known as exchange protein directly activated by cAMP 2 (EPAC2), this increase in $[cAMP]_i$ culminates in (1) inhibition of P/Q-type Ca^{2+} channel activity, (2) stimulation of L-type Ca^{2+} channel activity, (3) mobilisation of Ca^{2+} from intracellular Ca^{2+} stores [28] and (4) sensitisation of exocytosis to $[Ca^{2+}]_i$ [65]. The combination of these effects accounts for the suppression of the P/Q-type Ca^{2+} channel-dependent component of glucagon secretion and concomitant enhancement of the L-type Ca^{2+} channel-dependent component. The latter effect is a consequence of the increased Ca^{2+} sensitivity of exocytosis which means that granules not immediately adjacent to the P/Q-type Ca^{2+} channels may also undergo release (Fig. 5d), accounting for a component of exocytosis that outlasts the duration of the depolarisation by >100 ms (Fig. 5b).

Glucagon secretion defects of diabetes recapitulated by small increases in K_{ATP} channel activity

Diabetes has been referred to as a 'bihormonal disorder' [70, 71], involving both inadequate insulin secretion and defective glucagon secretion. The defects in glucagon secretion include over-secretion at high glucose (when it is not needed) and inadequate release at low glucose (when it is needed) [72, 73]. Of course, the concept of diabetes as a bihormonal disorder does not imply that the hormonal defects are limited to insulin and glucagon secretion. Defects of glucagon secretion, similar to those previously observed clinically in patients with type 2 diabetes, are also observed in isolated islets. Thus, glucagon secretion at low glucose levels tends to be reduced and at high glucose levels islets from diabetic donors release more glucagon than islets from non-diabetic donors (Fig. 6a). These fairly dramatic changes in glucagon secretion are correlated with a reduction of ~30% in glucose-induced insulin secretion (Fig. 6b).
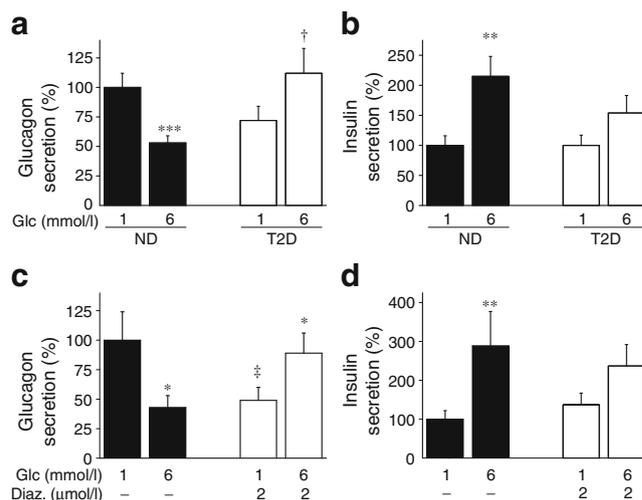

Fig. 6 Inverted regulation of glucagon secretion by glucose in diabetic islets recapitulated in non-diabetic islets following pharmacological activation of K_{ATP} channels. Effects of increasing glucose from 1 to 6 mmol/l in islets from non-diabetic donors (ND) and type 2 diabetic (T2D) patients on glucagon (**a**) and insulin secretion (**b**). Glucagon (**c**) and insulin secretion (**d**) from isolated human islets from ND at 1 and 6 mmol/l glucose in the absence and presence of 2 μmol/l diazoxide. Secretion data are expressed as a per cent (%) of that observed at 1 mmol/l glucose under control conditions (no test substances added; =100%) or in non-diabetic islets. *$p<0.05$; **$p<0.01$; ***$p<0.001$ vs 1 mmol/l glucose under the same experimental conditions. †$p<0.05$ vs 6 mmol/l glucose. ‡$p<0.05$ vs 1 mmol/l glucose under control conditions. Data in (**a**) and (**c**) taken from [26]

Interestingly, the glucagon secretion defect associated with type 2 diabetes can be recapitulated by treating islets from non-diabetic donors with a low (2 μmol/l) concentration of the pharmacological K_{ATP} channel activator diazoxide (Fig. 6c), an experimental condition that increases the whole-cell K_{ATP} channel activity by as little as ~100 pS [26]. At this concentration, diazoxide does not have much effect on glucose-induced insulin secretion (Fig. 6d and [16]) and somatostatin secretion [62]. In the presence of 2 μmol/l diazoxide, glucagon secretion is strongly reduced at low glucose concentrations and increasing the glucose concentration stimulates rather than inhibits glucagon secretion. These observations raise the exciting possibility that the inverted glucose regulation of glucagon secretion seen in type 2 diabetes may be a consequence of a minute increase in alpha cell K_{ATP} channel activity. In this context it is also of interest that it has previously been reported that carriers of the activating K_{ATP} channel variant E23K exhibit reduced suppression of glucagon secretion during hyperglycaemia [74].

An inverted glucagon secretion response to glucose was also seen in islets treated with oligomycin, an inhibitor of mitochondrial ATP synthase (Fig. 7a). In the presence of this mitochondrial inhibitor, glucagon secretion was reduced at low glucose concentrations and was stimulated rather than inhibited by elevated glucose levels.
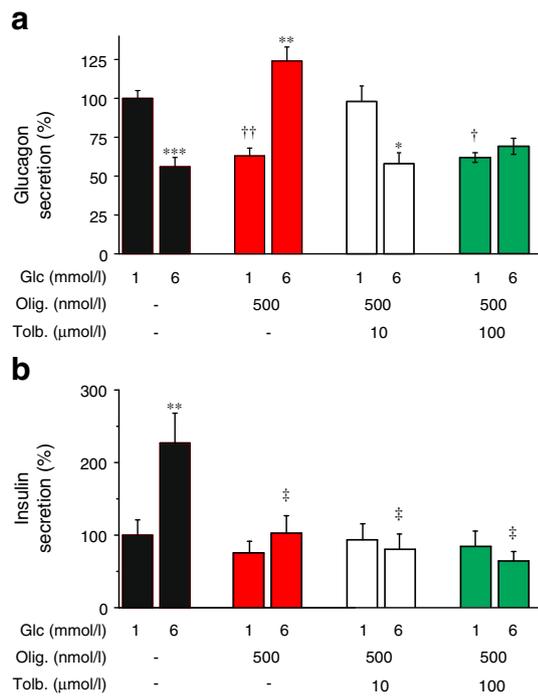
Fig. 7 Inverted regulation of glucagon secretion by glucose in metabolically compromised islets. Effects of glucose on glucagon (**a**) and insulin secretion (**b**) from isolated mouse islets at 1 and 6 mmol/l glucose (Glc) in the absence and presence of 500 nmol/l oligomycin (Olig.) and 10 or 100 μmol/l tolbutamide (Tolb.), as indicated. Secretion data are expressed as a per cent (%) of that observed at 1 mmol/l glucose in the absence of oligomycin (=100%). $*p<0.05$; $**p<0.01$; $***p<0.001$ vs 1 mmol/l glucose under the same experimental conditions with the different experimental conditions indicated by the same colour bar fill; $^{†}p<0.05$; $^{††}p<0.01$ vs 1 mmol/l glucose under control conditions; $^{‡}p<0.05$ vs 20 mmol/l glucose. Some of the data in (**a**) are taken from [26]

Importantly, normal glucose regulation of glucagon secretion in such metabolically compromised islets could be restored by a low tolbutamide concentration (10 μmol/l) (Fig. 7a). When a tenfold higher concentration of tolbutamide was used (100 μmol/l), glucagon secretion was strongly inhibited, regardless of the glucose concentration. In oligomycin-treated islets, glucose failed to stimulate insulin secretion and tolbutamide was without stimulatory effect (Fig. 7b). Thus, the normalisation of glucagon secretion produced by tolbutamide cannot be attributed to increased insulin release.

These data suggest that the glucagon secretion defects associated with type 2 diabetes may be a consequence of increased K_{ATP} channel activity in the alpha cell. If this is the case, then it should be possible to correct the glucagon secretion defect seen in islets from type 2 diabetic organ donors with tolbutamide. This is indeed the case, and we have recently reported that 10 μmol/l tolbutamide restores normal glucose regulation in islets from diabetic donors [26].

Understanding the glucagon secretion defects of diabetes

Figure 8 schematically summarises the regulation of electrical activity/glucagon secretion and how it depends on K_{ATP} channel activity. In healthy alpha cells (Fig. 8a), K_{ATP} channel activity at low glucose concentrations is very low but greater than 0. Under these conditions, the alpha cell membrane potential is sufficiently depolarised to allow action potential firing but negative enough to prevent inactivation of the voltage-gated Na^+ channels. Thus, large-amplitude action potentials, associated with activation of P/Q-type Ca^{2+} channels and glucagon exocytosis, are generated.

When glucose levels are elevated, K_{ATP} channels close and the alpha cell depolarises, which, as discussed above, results in voltage-dependent inactivation of the Na^+ channels, lowered action potential height and reduced activation of the P/Q-type Ca^{2+} channels with resultant suppression of glucagon exocytosis.

Based on the experiments using low concentrations of diazoxide, we postulate that K_{ATP} channel activity is slightly higher (+100%) in type 2 diabetic than in healthy alpha cells (Fig. 8b). The experiments with oligomycin suggest that this may be a consequence of impaired mitochondrial function. The increased K_{ATP} channel activity leads to a more negative alpha cell membrane potential, so that action potential firing at low glucose concentrations is either reduced or completely suppressed and glucagon secretion is correspondingly reduced. Despite the impaired mitochondrial metabolism, an increase in glucose remains capable of elevating the intracellular ATP/ADP ratio sufficiently to partially close the K_{ATP} channels. As a result, alpha cells depolarise, leading to increased action potential firing. This explains why glucagon secretion is stimulated rather than inhibited by glucose in type 2 diabetic islets. The electrophysiological and secretory responses shown here schematically have recently been substantiated by computational analyses [60].

It has been reported that the Na^+ current density in alpha cells from diabetic rats is twice as big as that in non-diabetic cells [75]. Such an effect might also contribute to the oversecretion of glucagon at high glucose in patients with type 2 diabetes. This is because even after the Na^+ channels have undergone partial voltage-dependent inactivation in response to glucose-induced depolarisation, remaining Na^+ channel activity would be sufficient to ensure continued generation of full size action potentials.

We have found that a low concentration of tolbutamide restores normal glucose regulation of glucagon secretion in islets from donors with type 2 diabetes. We used a concentration of 10 μmol/l, which is close to the level that reduces K_{ATP} channel activity by 50% [76]. Thus, K_{ATP} channel activity in type 2 diabetic alpha cells exposed to tolbutamide is comparable to that seen in non-diabetic cells under hypoglycaemic conditions (Fig. 8c). Under these conditions, a further
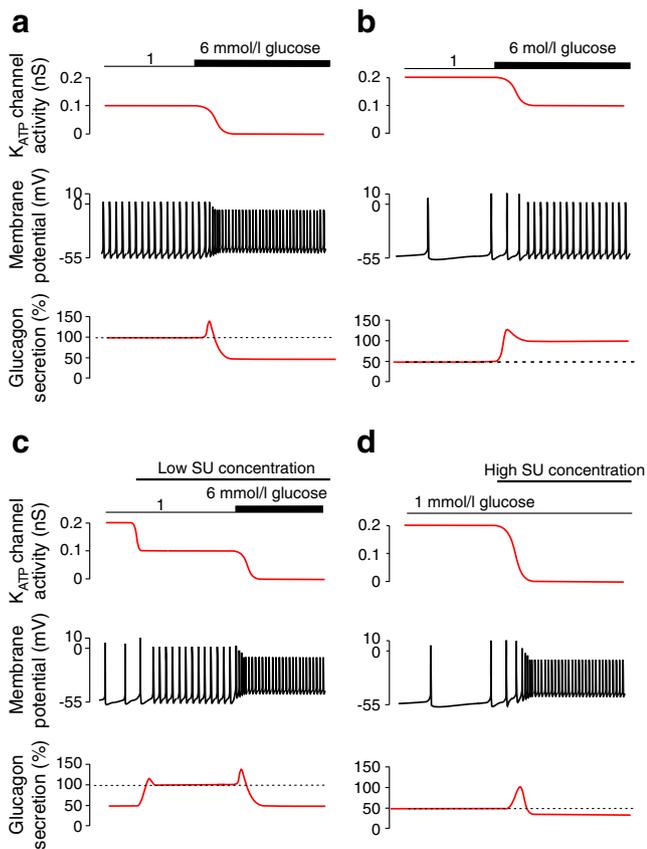
Fig. 8 Dual effects of K_{ATP} channel activity on alpha cell electrical activity and glucagon secretion. (**a**) Schematic representation of the relationship between K_{ATP} channel activity, electrical activity and glucagon secretion in non-diabetic alpha cell. Increasing glucose reduces net K_{ATP} channel activity from 0.1 nS (equivalent to eight K_{ATP} channels being simultaneously active in the entire alpha cell) to 0 nS. This results in membrane depolarisation, reduced action potential height and inhibition of glucagon secretion. Dashed line indicates glucagon secretion at 1 mmol/l glucose. (**b**) As in (**a**) but depicting a type 2 diabetic alpha cell with a K_{ATP} channel activity was twice that in ND alpha cells (i.e. 0.2 nS). Type 2 diabetic alpha cells are partially repolarised and action potential frequency is reduced. Increasing glucose inhibits K_{ATP} channel activity by 50%, resulting in slight membrane depolarisation and increased action potential firing. Unlike the situation in non-diabetic alpha cells, the membrane potential remains sufficiently repolarised to prevent a (major) reduction of action potential height, and glucagon secretion is therefore stimulated. (**c**) As in (**b**) but a low concentration of sulfonylureas (SU) was added before elevation of glucose to block 50% of the K_{ATP} channels. This results in increased action potential firing and stimulation of glucagon secretion. Subsequent elevation of glucose is further reduction of K_{ATP} channel activity (down to ~0 nS), strong membrane depolarisation, reduction of action potential height and inhibition of glucagon secretion. Dashed line indicates secretion at 1 mmol/l glucose in the presence of SU. (**d**) As in (**a**) but demonstrating effects of high SU concentrations. Addition of SU leads to complete inhibition of K_{ATP} channel activity (from 0.2 nS to 0 nS) with resultant strong depolarisation, reduction of action potential height and inhibition of glucagon secretion. Subsequent addition of glucose will have little (if any) further effect

reduction in K_{ATP} channel activity produced by glucose can be expected to result in strong membrane depolarisation, reduced action potential height/Ca^{2+} entry and inhibition of glucagon

secretion, similar to the responses in non-diabetic alpha cells (Fig. 8a).

These observations suggest that low concentrations of sulfonylureas may help to restore normal glucose regulation of glucagon secretion in diabetic patients—both an increase at low glucose and a reduction at high glucose. It is important, however, that the dose of sulfonylureas is carefully titrated. Figure 8d explains schematically why this is the case. A high concentration of sulfonylureas will completely block K_{ATP} channel activity in the diabetic alpha cell. This will be associated with strong membrane depolarisation, reduced action potential height and suppression of glucagon exocytosis even at low glucose concentrations, and subsequent elevations of glucose will exert no additive effect.

Sulfonylureas have previously been reported to inhibit glucagon secretion in vivo [77], and this was taken as an argument that glucagon secretion is suppressed by intra-islet insulin in a paracrine fashion. However, since sulfonylureas exert a direct effect on the alpha cells, it is equally likely that this effect reflects a direct effect mediated by closure of K_{ATP} channels in the alpha cells.

In type 1 diabetes, it has been reported that sulfonylureas stimulate glucagon secretion [78–80]. This is reminiscent of what is observed in isolated rat alpha cells obtained by FACS. In these cells, K_{ATP} channel activity is increased 200% above that observed in alpha cells in freshly isolated intact mouse islets [32]. There is also evidence that alpha cells are reprogrammed following destruction of the beta cells [81]. It is possible that isolated alpha cells, as a consequence of the deprivation of factors released from beta cells (including insulin), have an increased K_{ATP} channel activity. Indeed, nucleotides and Ca^{2+} co-released with insulin potentiate insulin secretion in an autocrine fashion mediated by G_q/G_{11}-coupled receptors [82]. When this signalling was prevented by genetic ablation of G_q/G_{11}, beta cell excitability was reduced via increased K_{ATP} channel activity. Similar mechanisms may become activated in alpha cells in type 1 diabetes or when alpha cells become separated from their beta cell neighbours, which would explain the observed increase in K_{ATP} channel activity that in turn explains why alpha cells adopt beta cell characteristics (such as being stimulated rather than inhibited by glucose or sulfonylureas).

Coda

Here we argue that glucagon secretion is determined by K_{ATP} channel activity and that glucose-induced closure of these channels translates into suppression of glucagon secretion. The concept that increased K_{ATP} channel activity may explain the inverted glucose response and the loss of appropriate counter-regulation in diabetic patients has obvious therapeutic implications. It should be relatively easy to conduct a clinical

study on the potential usefulness of low concentrations of sulfonylureas (lower than those required to stimulate insulin secretion [83]) with a view to restoring normal glucose regulation of glucagon secretion. Sulfonylurea-based therapy targeting glucagon secretion may therefore be particularly relevant in insulin-treated type 1 diabetic patients and in patients with type 2 diabetes managed by diet and/or insulin sensitisers.

Duality of interest The authors declare that there is no duality of interest associated with this manuscript.

Contribution statement All authors are responsible for and contributed to the conception and design of the study, as well as the writing and editing of preliminary versions of the paper. All authors have approved the final version.

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