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Review article

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Revisiting the role of glucagon in health, diabetes mellitus and other metabolic diseases

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Abstract

Insulin and glucagon exert opposing effects on glucose metabolism and, consequently, pancreatic islet β-cells and α-cells are considered functional antagonists. The intra-islet hypothesis has previously dominated the understanding of glucagon secretion, stating that insulin acts to inhibit the release of glucagon. By contrast, glucagon is a potent stimulator of insulin secretion and has been used to test β-cell function. Over the past decade, α-cells have received increasing attention due to their ability to stimulate insulin secretion from neighbouring β-cells, and α-cell–β-cell crosstalk has proven central for glucose homeostasis in vivo. Glucagon is not only the counter-regulatory hormone to insulin in glucose metabolism but also glucagon secretion is more susceptible to changes in the plasma concentration of certain amino acids than to changes in plasma concentrations of glucose. Thus, the actions of glucagon also include a central role in amino acid turnover and hepatic fat oxidation. This Review provides insights into glucagon secretion, with a focus on the local paracrine actions on glucagon and the importance of α-cell–β-cell crosstalk. We focus on dysregulated glucagon secretion in obesity, non-alcoholic fatty liver disease and type 2 diabetes mellitus. Lastly, the future potential of targeting hyperglucagonaemia and applying dual and triple receptor agonists with glucagon receptor-activating properties in combination with incretin hormone receptor agonism is discussed.

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Key points

- Glucagon is a 29-amino acid peptide hormone mainly secreted from pancreatic α-cells and has primarily been recognized for its role in glucose homeostasis.
- Glucagon secretion seems to be partly regulated by the direct effect of glucose on α-cells; however, paracrine regulation from neighbouring β-cells and δ-cells is also important.
- Several amino acids are glucagonotropic, and glucagon increases hepatic uptake and turnover of amino acids and stimulates ureagenesis — a feedback cycle referred to as the liver–α-cell axis.
- The importance of α-cell–β-cell crosstalk is increasingly recognized; studies suggest that α-cells are necessary for β-cell function (insulin secretion) and might preserve β-cell mass.
- Fasting hyperglucagonaemia in diabetes mellitus might be both a pathophysiological trait in glucose metabolism and a helpful metabolic adaptation in hepatic lipid and amino acid metabolism.
- Glucagon receptor antagonism improves glycaemic control in type 1 diabetes mellitus and type 2 diabetes mellitus but with adverse effects; future strategies targeting obesity and type 2 diabetes mellitus might involve glucagon co-agonism.

Introduction

Shortly after insulin was discovered by Banting and Best in 1921 (ref. [1]), Kimball and Murlin described pancreatic extract impurities that caused hyperglycaemia when infused into dogs[2]. The factor causing this effect, glucagon, was also initially referred to as the "hyperglycaemic glycogenolytic factor" or the "glucose antagonist". It was not until 1948 that pancreatic α-cells were established as the source of glucagon[3]. Since Unger and Orci introduced their bi-hormonal hypothesis of diabetes mellitus, which states that glucagon elevation is as important as insulin deficiency for hyperglycaemia[4], the understanding of the pathophysiology of type 2 diabetes mellitus (T2DM) has included the duality of relative hyperglucagonaemia and insulin resistance with relative hypoinsulinaemia. Thus, insulin and glucagon have been recognized for their opposing effects on glucose metabolism and consequently have been considered functional antagonists, with glucagon opposing the glucose-lowering effects of insulin by stimulating glycogenolysis and gluconeogenesis[5,6]. Additionally, the hormones themselves affect the secretion of one another. The intra-islet hypothesis states that glucagon secretion undergoes inhibition from insulin, leading to hyperglucagonaemia in conditions with decreased insulin secretion (for example, type 1 diabetes mellitus (T1DM), late-stage T2DM and pancreatectomy).

Knowledge of pancreatic islet morphology has shaped our understanding of intra-islet communication. Human islet morphology is variable both within a single pancreas and between different pancreata[7]. Islet organization has been proposed to be in a mantle-core system, with clusters of β-cells in the core surrounded by other cells, yet a more complex random distribution arrangement has also been suggested[7]. Studies on intra-islet vasculature also show a clear vascularization penetrating the β-cell core[7], enabling glucagon to have an active role in the paracrine and endocrine regulation of insulin secretion and overall islet function.

In this Review, we highlight current knowledge of glucagon secretion and α-cell–β-cell crosstalk in the context of the effects of glucagon on glucose metabolism, amino acid turnover and hepatic lipid oxidation. In addition, we review current knowledge on the role of glucagon for obesity, hepatic steatosis and T2DM. Finally, we discuss the potential of targeting hyperglucagonaemia or, conversely, applying glucagon agonists in combination with incretin hormones for future treatment of metabolic disease.

Challenges in glucagon measurements

Glucagon is a 29-amino acid peptide hormone that is thought it be primarily produced in the pancreas. This hormone circulates in low picomolar concentrations and consequently glucagon research has been challenged by the difficulty in making precise and accurate measurements of glucagon in plasma. The development of glucagon assays started with the first sensitive radioimmunoassay developed by Unger et al.[8] (reviewed in ref. [9]). This group made important discoveries on glucagon physiology, improved glucagon assays and demonstrated suppression of circulating glucagon by carbohydrates in normal physiology. Furthermore, they uncovered the lack of post-prandial suppression and hypersecretion of glucagon in people with T1DM or T2DM[10]. They also discovered a particularly sensitive and specific rabbit antiserum, which dominated the field of glucagon research for decades[9,11].

True glucagon 33–61 is post-translationally processed from the prohormone proglucagon 1–160 (Fig. 1). Processing of proglucagon is tissue specific and depends on the predominating prohormone convertase cleaving the prohormone into smaller entities. In the pancreas, the predominating convertase is prohormone convertase 2 (PC2), which cleaves proglucagon to the fully processed 29-amino acid glucagon peptide (glucagon 33–61), in parallel with glicentin-related pancreatic polypeptide (GRPP) and a minor amount of the longer glucagon 1–61 peptide[12] (Fig. 1). In enteroendocrine cells in the gut, PC1 (sometimes referred to as PC1/3) predominates and cleaves the prohormone, resulting in equimolar amounts of glicentin, glucagon-like peptide 1 (GLP1) and GLP2 being secreted. Gut-derived glicentin is further processed and released as oxyntomodulin and GRPP, in ratios that range between 1:2 and 1:3 in relation to glicentin[13]. Of note, glucagon is also secreted from intestinal L cells under certain circumstances[14,15]. Furthermore, cells containing predominantly PC2 might also produce PC1 and vice versa, or the cleavage sites of the prohormone convertases on proglucagon are not as specific as hitherto believed[16–20].

The closely related end-products of the post-translational processing of proglucagon challenge the specificity of antibodies used in glucagon assays[9]. Some glucagon assays cross-react with glucagon-like intestinal molecules (glicentin and oxyntomodulin)[21–23], which clearly shows that low cross-reactivity with glucagon-like substances is essential for the specificity of glucagon assays[9]. Currently, methods of glucagon measurement in plasma include radioimmunoassay, enzyme-linked immunosorbent assay (ELISA) and mass spectrometry-based methods. A glucagon radioimmunoassay requires an antibody with suitable affinity and specificity (reviewed in ref. [9]). C-terminal antibodies are preferred because they can cross-react exclusively with proglucagon 1–61 (a peptide with some glucagon bioactivity)[12], compared with mid-region antibodies (cross-reacting with glucagon 1–61, glicentin and oxyntomodulin) and N-terminal antibodies (cross-reacting with oxyntomodulin) (Fig. 1).

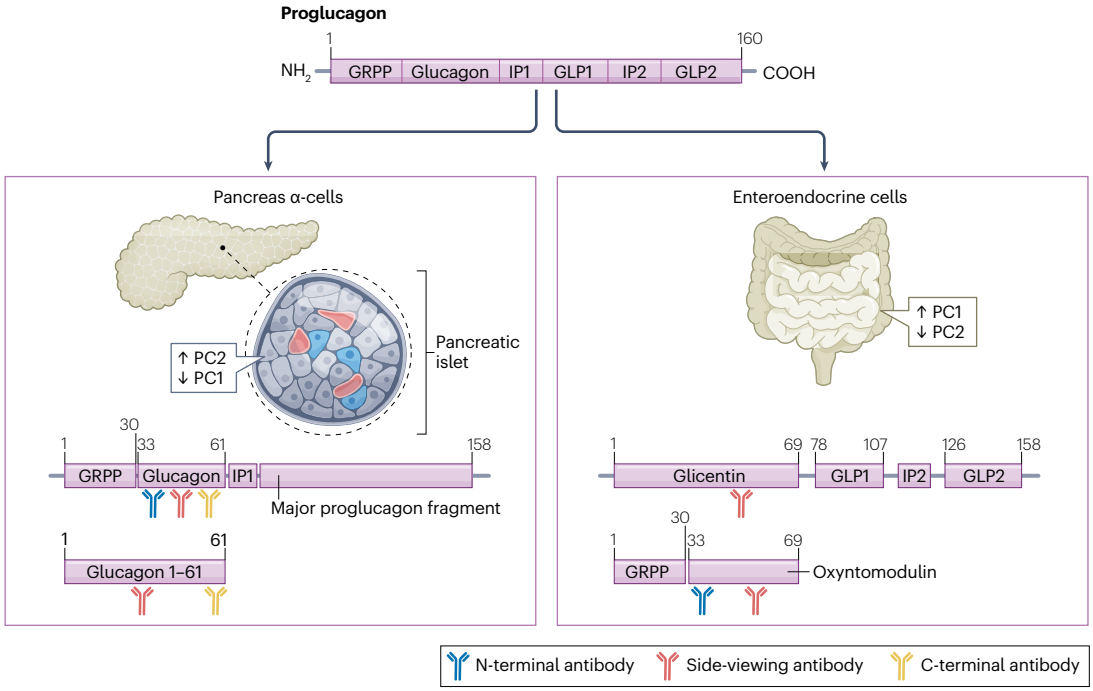

Fig. 1 | Tissue-specific proglucagon processing and measurement of glucagon by antibodies. In pancreatic α-cells, prohormone convertase 2 (PC2) predominates and PC1 (otherwise known as PC1/3) activity is low, leading to proglucagon processing and the release of the fragments of glicentin-related pancreatic polypeptide (GRPP), pure glucagon, the longer glucagon 1–61, intervening peptide 1 (IP1) and the major proglucagon fragment. In intestinal enteroendocrine cells (such as L cells), PC1 activity predominates over PC2 activity, leading to proglucagon processing and the release of glicentin, glucagon-like peptide 1 (GLP1), GLP2 and IP2. Fragments of GRPP and oxyntomodulin are also released during proglucagon processing in enteroendocrine cells, which is considered a further breakdown of glicentin. Glucagon-identifying antibodies can be subdivided into N-terminal antibodies (blue), side-viewing antibodies (red), and C-terminal or glucagon-specific antibodies (yellow). The figure illustrates the possible cross-reaction of N-terminal and side-viewing antibodies for glucagon with glucagon 1–61, glicentin and oxyntomodulin.

To solve the inadequate specificity of radioimmunoassays, the sandwich ELISA for glucagon measurement was developed. In the N-terminal and C-terminal sandwich ELISA, a pair of antisera binds to the free sites of the terminus of the glucagon molecule and any elongation or modification of the ends of the molecule leads to loss of binding in the assay. A high-quality sandwich ELISA is now commercially available[24]. Unfortunately, many immunoassays and ELISAs suffer from low sensitivity and specificity[24,25]; thus, the sensitivity and specificity of any new glucagon assay should always be assessed.

Mass spectrometry of non-abundant peptides is a powerful tool for the evaluation of proteins in plasma. However, for glucagon measurement, the technology is still challenged by sensitivity, especially due to recovery problems of the peptide of interest during chemical or physicochemical isolation of the peptide[9].

Glucagon in human physiology

The multiple effects of glucagon in several target tissues are reflected in the broad distribution of the glucagon receptor (GCGR), a G protein-coupled receptor (GPCR). GCGR is mainly found in the liver but is also present in the kidneys, heart, adipocytes, lymphoblasts, spleen, brain, adrenal glands, retina and gastrointestinal tract[26]. Knowledge of GCGR distribution is primarily based on rodent tissue[26–28] and identifying GCGR protein expression in vivo has been challenged by a lack of specific antibodies against the GCGR[29]. Nevertheless, glucagon has a broad range of physiological effects (Box 1).

Glucose metabolism

The effect of glucagon on hepatic glucose metabolism is well recognized. After binding to GCGR on hepatocytes, glucagon activates protein kinase A and initiates a chain of phosphorylation events that lead to the breakdown of glycogen (glycogenolysis) and the formation of glucose 6-phosphate (a substrate for endogenous glucose production)[30]. Thus, glucagon increases hepatic glucose production by stimulating glycogenolysis and gluconeogenesis while inhibiting glycolysis and glycogenesis[30,31] (Fig. 2). Accordingly, glucagon acts as a defence against hypoglycaemia.

Lipid metabolism

Effects of glucagon on peripheral lipolysis and β-oxidation have been questioned, as the GCGR has not been found in human adipocytes[32]. Glucagon allegedly activates hormone-sensitive lipase and lipolysis in adipose tissue of several species[32] as well as in human adipocytes in vitro under supraphysiological concentrations of 10^{-8} mmol/l (ref. [33]). However, under physiological concentrations of 19–64 pmol/l, glucagon has no lipolytic effect in clinical studies[34–37]. Furthermore, any lipolytic effect in human adipocytes under supraphysiological concentrations is easily abolished by insulin, which is known to inhibit lipolysis[32].

The involvement of glucagon in hepatic lipid metabolism is well established (Fig. 2a). Glucagon stimulates hepatic β-oxidation and inhibits hepatic lipogenesis via three major pathways (reviewed in ref. [32]).

First, by a cAMP response element-binding protein (CREB)-dependent pathway, carnitine acyl transferase 1 is stimulated[38], which facilitates the breakdown of long-chain fatty acids and provides substrates for β-oxidation[39]. Second, glucagon hinders the formation of malonyl-CoA and directs free fatty acids (FFAs) into β-oxidation instead of re-esterification into triglycerides. Third, glucagon increases the AMP-to-ATP ratio, which stimulates the transcription of peroxisome proliferator-activated receptor-α[38,40], increasing the transcription of genes involved in β-oxidation. Importantly, insulin reverses all the abovementioned steps and the hepatic insulin-to-glucagon ratio seems to determine the net effect of hepatic lipid metabolism[41,42].

Amino acid metabolism

Some amino acids, in particular alanine, are substrates for hepatic gluconeogenesis by their conversion into pyruvate. Glucagon stimulates gluconeogenesis from amino acids by controlling several rate-limiting steps of gluconeogenesis[31]. Glucagon markedly stimulates hepatic amino acid metabolism (ureagenesis)[43], reducing the circulating concentrations of amino acids and clearing potential harmful ammonia generated by transamination[44,45] (Fig. 2a). Glucose and glucagon exert opposing effects on ureagenesis, with glucagon being superior in the stimulation of ureagenesis[45]. Hepatic GCGR signalling enhances the transcription of genes via pyruvate kinase A-mediated phosphorylation of CREB[46,47]. CREB might be involved in the regulation of N-acetyl glutamate synthase, which is involved in the formation of urea[48]. The role of glucagon in the regulation of the enzymatic steps of ureagenesis is not fully understood[49], but the expression of two important enzymes (carbamoyl phosphate and ornithine transcarbamylase) is downregulated by hepatic steatosis[50]. Besides stimulating the enzymatic steps of ureagenesis, glucagon can also increase the formation of urea by increasing the transcription of hepatocyte plasma membrane-expressed amino acid transporters, thereby increasing substrate supply[51,52]. These mechanisms are corroborated by results from *Gcgr*-knockout mice and GCGR antagonism in clinical and preclinical studies (reviewed in ref. [53]).

Box 1

Physiological actions of glucagon

Actions identified in preclinical studies

- Brain
  - ↓ Appetite
- Heart
  - ↑ Cardiomyocyte survival
- Muscle
  - ↓ Glucose uptake
- Visceral adipose tissue
  - ↑ Lipolysis
- Brown adipose tissue
  - ↑ Thermogenesis
- Liver
  - ↑ Hepatocyte survival

Actions identified in clinical studies

- Brain
  - ↓ Food intake
- Heart
  - ↑ Heart rate
- Gastrointestinal tract
  - ↓ Gastric emptying
  - ↓ Peristaltic motility
- Brown adipose tissue
  - ↑ Resting energy expenditure
- Kidney
  - ↑ Glomerular filtration
- Liver
  - ↑ Hepatic glucose output
  - ↑ Ureagenesis
  - ↑ Lipid oxidation
  - ↓ Lipid synthesis

Energy homeostasis

Energy expenditure. In rats, injections of glucagon have been associated with increased oxygen consumption[54]. Furthermore, in humans, glucagon infusions result in increased resting energy expenditure when insulin levels are low[55]. Low insulin levels seem to be a prerequisite for glucagon-induced thermogenesis in humans[56]. The mechanisms of action of glucagon on energy expenditure are complex but studies implicate brown adipose tissue and the sympathetic nervous system[31]. This mechanism of action is substantiated by the increase in glucagon concentrations and changes in brown adipose tissue and the hypothalamus–pituitary–adrenal gland axis observed in rats after cold exposure[57,58].

Appetite, food intake and gastric emptying. Interestingly, exogenous glucagon decreases food intake and promotes body weight loss in several species, including humans[31,58–60]. The regulatory mechanisms controlling glucagon-induced satiety are poorly understood. However, data collectively suggest that glucagon-induced satiety is mediated via vagal afferent fibres in the hepatic branch transmitting signals to the central nervous system and the involvement of a liver–vagus–hypothalamus axis[31,58,59]. In rats, infusions of glucagon antibodies increase meal size[61,62]. Moreover, exogenously infused glucagon at physiological concentrations reduces meal size in humans[63]. In addition, gastrointestinal motility might be reduced by glucagon. A barium meal fluoroscopy showed that exogenous glucagon slows gastric emptying[64] and supraphysiological glucagon concentrations delay gastric emptying of liquids and inhibit motility in the gastrointestinal tract[65]. Thus, glucagon might be part of a physiological meal-induced satiety response, which fits well with the observation that glucagon concentrations increase during consumption of a mixed meal (that is, containing proteins, carbohydrates and fats)[66].

Haemodynamics

Gcgr-knockout mice have a lower intrinsic heart rate than wild-type mice (under conditions of no nervous system input on cardiac tissue)[67], supporting the notion that endogenous glucagon could play a part in the regulation of heart rate. Most studies investigating pharmacological glucagon administration (>1 mg) in humans find short-lasting (20 min) increased (by 25–30%) measures of cardiac inotropy (contractility), increased cardiac chronotropy (heart rate; by 5–25%) and increased mean arterial pressure (by 5–18%; reviewed in ref. [68]). In general, preclinical experiments report glucagon to have positive inotropic and chronotropic effects on the heart, whereas human data are inconsistent. The inconsistency could reflect large interindividual differences in patient cardiac reserves, with the greatest effect of glucagon in healthy volunteers or patients with mild heart failure, compared with patients with severe heart failure[68]. Studies indicate that a supraphysiological threshold might need to be reached before any haemodynamic effects of glucagon appear. In addition, the dose–response relationship of glucagon on haemodynamics in humans is not clear. Studies are warranted on the long-term effects of increased concentrations of glucagon in humans in the physiological range and

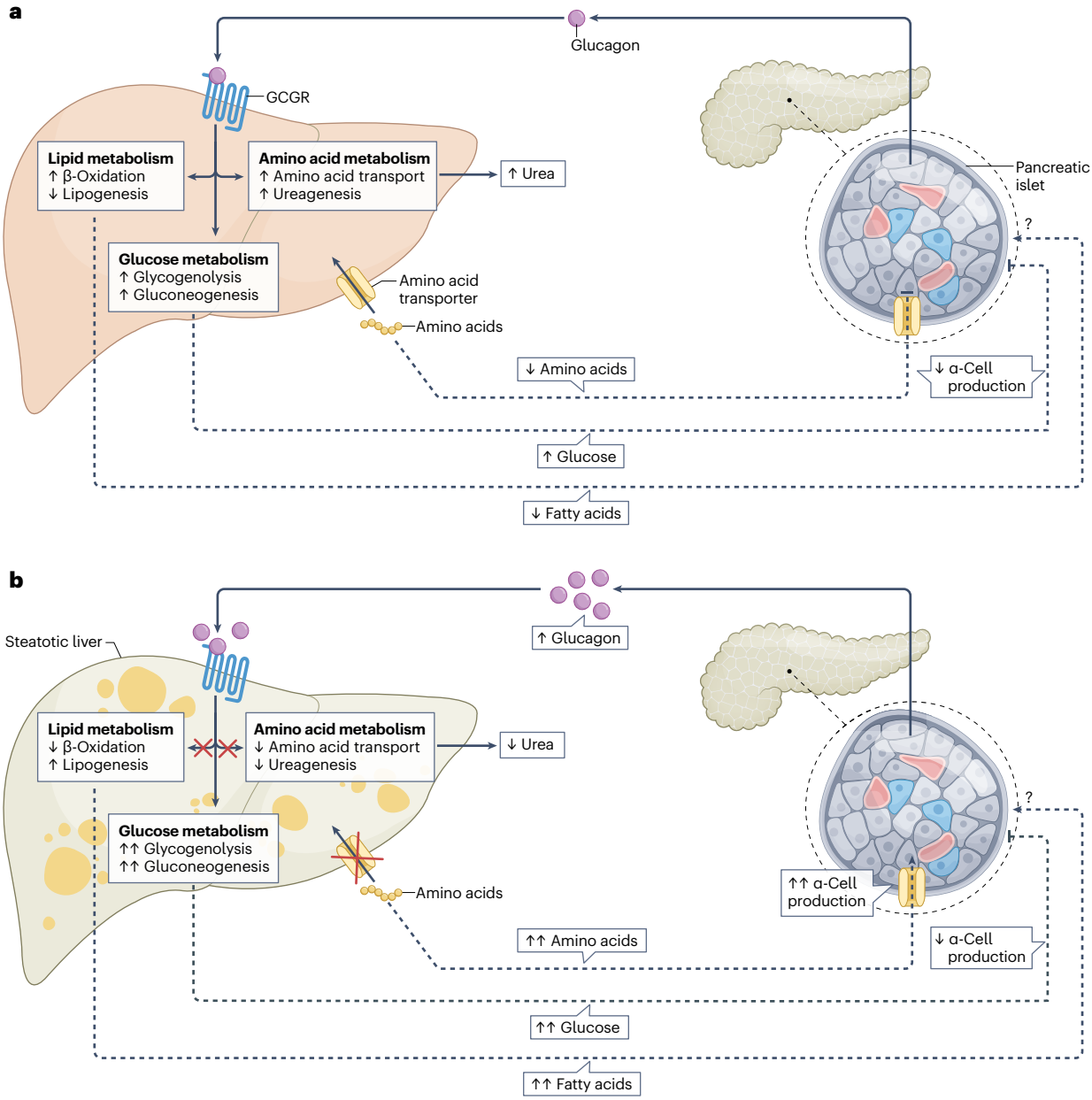

Fig. 2 | The liver–α-cell axis. a, Glucagon secreted from pancreatic α-cells act in a feedback axis with the liver on glucose, amino acid and lipid metabolism via the glucagon receptor (GCGR) on hepatocytes. In turn, glucose, amino acids and lipids modulate glucagon secretion from α-cells. Glucagon increases hepatic glycogenolysis and gluconeogenesis, which results in the net increased hepatic secretion of glucose; the consequently high circulating levels of plasma glucose inhibit glucagon secretion from α-cells. Glucagon stimulates hepatic amino acid transport and ureagenesis; the resulting decreased circulating concentration of amino acids reduces glucagon secretion, as amino acids stimulate glucagon secretion. Glucagon increases hepatic β-oxidation and decreases lipogenesis, lowering the circulating concentration of free fatty acids. How circulating fatty acids might affect glucagon secretion is not established. **b**, In metabolic disease, such as type 2 diabetes mellitus and/or non-alcoholic fatty liver disease, hepatic steatosis drives a resistance towards glucagon at the level of hepatic lipid and amino acid turnover but not of hepatic glucose metabolism. When amino acids do not enter the urea cycle due to glucagon resistance, circulating levels of amino acids increase. Additionally, hepatic steatosis leads to decreased expression of hepatic amino acid transporters, preventing hepatic amino acid uptake. Circulating amino acids serve as messengers to the α-cells of the pancreas and increase glucagon secretion via amino acid transporters on α-cells. Furthermore, hepatic lipid metabolism is affected by glucagon resistance, with decreased β-oxidation and increased lipogenesis resulting in increased hepatic lipid deposition and increased circulating levels of free fatty acids. How this increase affects α-cell secretion is not established. Glucagon resistance does not seem to affect glucose metabolism. Dashed arrows indicate feedback pathways from the liver to α-cells. Adapted from ref. [53], CC BY 4.0 (https://creativecommons.org/licenses/by/4.0/).

mildly increased above physiological values. To cloud the picture even further, the GCGR antagonist LY2409021 resulted in increased ambulatory blood pressure[69].

Glucagon secretion

The regulation of glucagon secretion is complex, with nutrients as well as paracrine, endocrine and autonomic regulation influencing the net production of α-cells (Fig. 3). Indeed, glucagon secretion is regulated by plasma concentrations of glucose. Traditionally, hypoglycaemia is considered the strongest stimulator of glucagon secretion, whereas hyperglycaemia inhibits glucagon secretion. However, islets isolated from individuals with T2DM have paradoxically increased glucagon secretion at increasing glucose concentrations[70,71] and the same is evident in people with either T1DM or T2DM after alimental glucose loads (discussed later).

The proposed mechanisms of how glucose regulates glucagon secretion involve direct, intrinsic regulation within the α-cells themselves as well as paracrine mechanisms. For example, mediators released from β-cells and δ-cells surrounding α-cells have been postulated as regulators[70]. Both types of regulation are probably involved: α-cells are

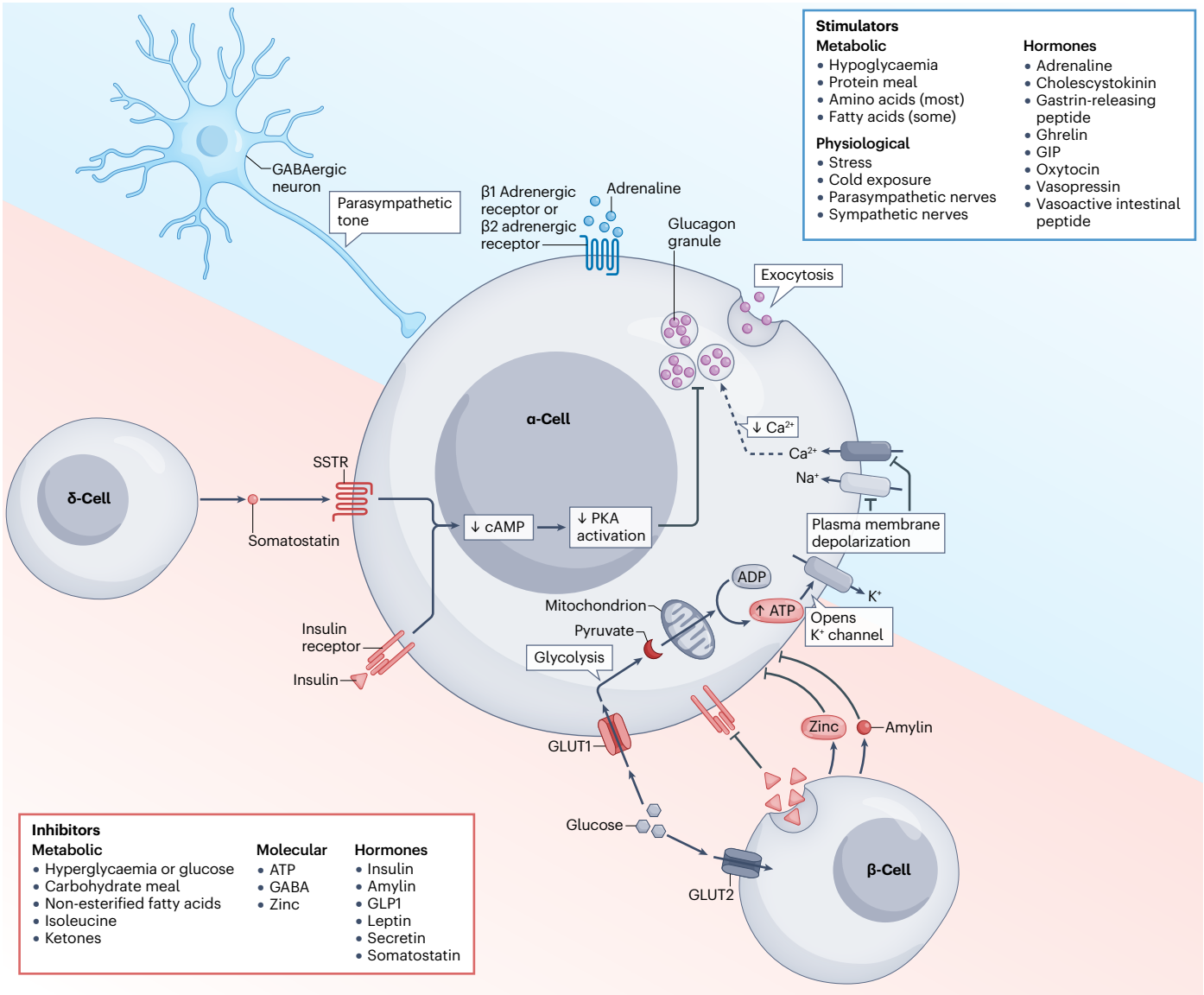

Fig. 3 | Classical direct and indirect stimulators and inhibitors of glucagon secretion. Summary of inhibitors and stimulators of glucagon secretion. Glucose from the circulation inhibits glucagon secretion directly from the α-cell through glucose transporter type 1 (GLUT1), and directly and indirectly via insulin, zinc and amylin secretion from the β-cell. During hyperglycaemia, the formation of ATP from glycolysis keeps the K_{ATP}-sensitive channels open, depolarizing the membrane that closes (inactivates) the Na^+ and Ca^{2+} channels and reduces intracellular levels of Ca^{2+}, thereby inhibiting glucagon exocytosis (dashed arrow). Conversely, during hypoglycaemia, consequent low intracellular levels of ATP lead to inactivation of K_{ATP} channels, resulting in opening of Na^+ and Ca^{2+} channels and exocytosis of glucagon. Somatostatin from δ-cells inhibits glucagon secretion via decreased intracellular levels of cAMP. GIP, glucose-dependent insulinotropic polypeptide; GLP1, glucagon-like peptide 1; PKA, protein kinase A; SSTR, somatostatin receptor.

capable of intrinsic regulation by glucose, which is supported by studies with single α-cells (isolated from pancreatic islets) responding with inhibited glucagon secretion to glucose stimulation[72,73], and α-cells are affected by products from surrounding islet cells (paracrine regulation).

When reviewing data on glucagon secretion, it must be remembered that the cell architecture and vasculature differ between human and rodent islets (discussed later). Additionally, the physiological setting (such as single cell versus intact islet studies), differences in glucose concentrations applied, availability of nutrients, or in vitro versus in vivo settings, amongst other factors, could affect the conclusions and should be considered[70].

Direct regulation by glucose

α-Cells are electrically excitable and show spontaneous oscillations in cytosolic levels of Ca^{2+} at low concentrations of glucose (<3 mM)[31,74,75]. Furthermore, increasing concentrations of glucose lead to decreased α-cell electrical activity[76] and decreased intracellular Ca^{2+} oscillations[77,78]. Many of the intrinsic pathways downstream to glucose stimulation in α-cells resemble those in β-cells, for instance, Ca^{2+} fluctuations[75]. An important difference between β-cells and α-cells could be related to their glucose-sensing ability[75]. Glucose is transported into α-cells by the glucose transporter type 1 (GLUT1), which has a high affinity for glucose and ensures a continuous and rapid uptake of glucose in case of increases in glucose concentration[75] (Fig. 3). By contrast, in β-cells, glucose uptake is facilitated by GLUT2, which senses glucose in the physiological range, while GLUT2 is largely absent in α-cells[79].

Both α-cells and β-cells are regulated by the ATP-sensitive K^+ (K_{ATP}) channels that translate external glucose concentrations to internal membrane potentials[31]. Moreover, both cell types contain a series of ion channels that modulate cell membrane potential in a glucose-dependent manner[80]. α-Cells require a low intracellular concentration of ATP to inhibit K_{ATP} channels. During hyperglycaemia, where ATP levels are high, K_{ATP} channels are thus open, which depolarizes the membrane and inactivates (closes) Na^+ channels. This inactivation hinders voltage-gated Ca^{2+} channels from opening and the reduced influx of Ca^{2+} limits exocytosis of glucagon granules[31,74,81,82] (Fig. 3). Conversely, when both blood concentrations of glucose and α-cell intracellular levels of ATP levels are low, K_{ATP} channels are only partly open, which results in the opening of Ca^{2+} channels, an influx of Ca^{2+} and exocytosis of glucagon granules[82] (Fig. 3). Thus, fluctuations in intracellular levels of Ca^{2+} are involved in glucose-dependent glucagon secretion. However, data on intracellular levels of Ca^{2+} are inconsistent and some studies describe the role of intracellular Ca^{2+} as being permissive but not necessary for glucagon release[83].

Several theories have been put forward for α-cell-intrinsic control of glucagon release and currently no consensus exists. An alternative mediator for the direct effect of glucose on glucagon secretion might be cAMP; one suggested mechanism by which glucose inhibits glucagon secretion is via a drop in cAMP levels, either by an indirect somatostatin pathway[84] or by a direct effect of glucose[85]. Additionally, α-cell Ca^{2+} channels, with L-type versus non-L-type channels and their different thresholds for depolarization, might be of importance for intrinsic control of glucagon secretion as well as for endoplasmic reticulum-dependent regulation of glucagon release[86].

Taken together, glucose directly affects glucagon secretion, but glucose-dependent regulation of glucagon secretion is also partly mediated via paracrine effects. For instance, glucagon secretion from isolated α-cells is affected by the concentration of glucose[87,88]. Furthermore, glucagon release is inhibited in intact rodent and human islets stimulated with high levels of glucose, including when paracrine γ-hydroxybutyrate (GABA) and zinc signals are blocked[81].

Paracrine regulation

Glucagon secretion is thought to be under paracrine control by insulin, GABA, amylin and zinc (β-cells) and somatostatin (δ-cells) from neighbouring islet cells. The morphological distribution of cells within the islets is therefore of importance for communication among cells. Rodent and human islet morphology differs, with human islets containing more α-cells than do rodent islets[89]. Furthermore, rodents are thought to have an islet structure with β-cells at the core and other cells in the mantle region, whereas β-cells in human islets are surrounded by α-cells, and the close contacts between these cells suggests that intra-islet crosstalk has an important role in human islets[89].

Insulin. Numerous studies have shown that insulin inhibits glucagon secretion[75] and α-cells express insulin receptors[90]. Furthermore, ablation of insulin receptors in *Insr*-knockout mice induces hyperglucagonaemia and hyperglycaemia in the fed state[91]. Likewise, immunoneutralization of insulin stimulates glucagon secretion[92] and chronic conditions of insulin depletion (such as T1DM or late T2DM) are characterized by hyperglucagonaemia and increased glucagon secretion[93–100]. Insulin might also be indirectly responsible for the glucagonostatic effect of glucose; however, studies indicate that insulin is not a prerequisite for the glucagonostatic effect of glucose[83,101].

Zinc. Present in considerable quantities in the pancreas[31], zinc co-crystallizes with insulin in the secretory granules of β-cells[102]. Zinc is secreted from β-cells during hyperglycaemia and targets α-cells to inhibit the release of glucagon via the opening of K_{ATP} channels and inhibition of electrical activity in α-cells[90].

Amylin. This peptide hormone is also co-released with insulin from β-cells in response to nutrients, especially glucose[31]. Amylin was initially identified as amyloid depositions in the pancreatic islets of individuals with diabetes mellitus[103–105]. Later, amylin was recognized to inhibit insulin secretion in the perfused rat pancreas under basal conditions and after glucose stimulation[106]. In rats, infusion of amylin supresses arginine-induced glucagon release[107] and amylin receptor blockage increases glucagon secretion[108]. The amylin analogue pramlintide improves glycaemic control in individuals with T1DM and T2DM[109] by delaying gastric emptying[110] and inhibiting postprandial glucagon secretion[111,112]. The regulation of glucagon secretion by amylin seems to be indirect as amylin does not affect glucagon secretion in isolated islets[113] or perfused rat pancreas[106,114].

GABA. This inhibitory neurotransmitter acts on $GABA_A$ and $GABA_B$ receptors in the brain. $GABA_A$ receptors are ligand-gated Cl^- channels and $GABA_B$ receptors are GPCRs. The overall understanding is that GABA from β-cells inhibits glucagon release[83,115–118]; however, a functional GABA receptor is not expressed (or expression levels are undetectable) in α-cells[83]. Moreover, some studies have not found effects of GABA on Ca^{2+} concentrations[119,120] or electrical membrane potentials of α-cells[71,121]. GABA might also inhibit glucagon secretion by facilitating glucose-mediated inhibition of glucagon secretion[117].

Somatostatin. The predominant form of somatostatin is somatostatin 14 (ref. [122]), which is secreted from pancreatic δ-cells and applies a potent, tonic inhibition of glucagon secretion[74,83,123]. α-Cells and δ-cells

are in close contact[124] and, if not in direct proximity, they communicate via extensions of the δ-cells (filopodia-like structures), enabling them to reach a large number of α-cells[125]. Somatostatin inhibits glucagon secretion by three known mechanisms[126]: (1) membrane hyperpolarization of α-cells through G protein-gated K^+ channels and inhibition of electrical activity[127]; (2) through inhibition of adenylate cyclase activity and reduction of intracellular cAMP in α-cells[128]; and (3) via inhibition of exocytosis in α-cells by activation of calcineurin[127].

Regulation by incretin hormones

The incretin effect is the phenomenon that orally ingested glucose causes a greater insulin secretion response than isoglycaemic intravenous glucose infusion. This effect is due to the release of the incretin hormones, that is, glucose-dependent insulinotropic polypeptide (GIP) from enteroendocrine K cells and GLP1 from enteroendocrine L cells after glucose stimulation of the gut[20,129]. GLP1 inhibits the release of glucagon in a glucose-dependent manner, but the mechanism behind the glucagonostatic effect of GLP1 remains debated; the prevailing theory is that it is mediated via an indirect inhibitory effect of insulin[130]. However, a direct effect via the GLP1 receptor (GLP1R) on α-cells has also been shown[131]. A possible alternative mechanism could be through paracrine somatostatin actions as shown in the perfused mouse pancreas model[132]. In vivo, GLP1 infusion inhibits glucagon secretion in humans[133–136]. Furthermore, the GLP1R antagonist exendin(9-39)NH_2 increases glucagon secretion in humans[137,138].

GIP modulates insulin and glucagon secretion in a glucose-dependent manner[139]. In the isolated perfused rat pancreas, GIP stimulates insulin secretion at glucose concentrations above 5.5 mmol/l and increases glucagon secretion at glucose concentrations below 5.5 mmol/l (ref. [140]). This mechanism seems to be preserved in people with T1DM or T2DM, where GIP counteracts insulin-induced hypoglycaemia[141,142] and increases postprandial glucagon concentrations[142–146]. GIP antagonism conversely decreases postprandial glucagon excursions in healthy individuals and in individuals with T2DM[147,148].

Regulation by amino acids

Several amino acids are glucagonotropic and seem equivalent to hypoglycaemia in stimulating glucagon secretion[31]. The glucagon-stimulating activity of amino acids seems to be most potent for alanine, arginine, cysteine and proline in rodents[149,150], asparagine in dogs[151], and alanine, glycine and serine in sheep[152]. However, the glucagon-stimulating potency of individual amino acids is not yet known in humans.

The glucagon-stimulating activity of amino acids is abolished by hyperglycaemia and is thus glucose dependent[153]. After prolonged fasting, glucagon concentrations fall to normal levels despite persistently low plasma levels of glucose[154] and α-cells might be more responsive to fluctuations in amino acid levels than to hypoglycaemia[155–157]. Alanine is a gluconeogenic precursor and could have a central role in glucagon secretion[158], whereas branched-chained amino acids have little effect[156]. The mechanism of how amino acids promote glucagon secretion is poorly understood. Studies indicate that amino acids trigger glucagon release via membrane receptors (amino acid transporters) and alter the membrane potential of α-cells following intracellular accumulation[155]. However, an alternative mechanism could be GPCR signalling or calcium-sensing receptors[155].

Regulation by fatty acids

Regulation of glucagon secretion by lipids remains controversial[32]. In early studies, increased concentrations of FFAs by infusion of triglycerides suppressed glucagon concentrations in dogs[159,160] and in humans[161]. However, when stimulated with FFAs, isolated duck and mouse α-cells respond oppositely, with increased glucagon secretion[162,163]. These findings could be due to the detection of other proglucagon products given the low specificity of glucagon assays. The presence of FFAs in many forms with varying stimulatory effects on glucagon secretion increases the complexity[32,164]. For illustration, glucagon secretion was not different between healthy individuals on either a high-fat diet or a low-fat diet for 2 weeks[165]. By contrast, individuals who ingested long-chain fatty acids (olive oil and C8) showed increased plasma concentrations of glucagon after 40 min but no increase was observed after ingestion of short-chain fatty acids (C4)[166]. However, circulating concentrations of GIP are increased in humans after ingestion of long-chain fatty acids, possibly explaining the increase in glucagon secretion[166].

Crosstalk between α-cells and β-cells

Intra-islet crosstalk has been studied for several decades[31,75,83]. The effect of insulin from β-cells on neighbouring α-cells has been studied and proposed in the intra-islet insulin hypothesis. This hypothesis states that a decrease in intra-islet insulin is a signal for glucagon secretion by releasing the tonic insulin-mediated inhibition on α-cells. The mechanism proposed is thus a defence signal to prevent hypoglycaemia; however, the intra-islet insulin hypothesis involves a 'one-way' signal from β-cells to α-cells and does not account for intra-islet crosstalk.

In the 2000s, the impact of α-cells on β-cell function was thought to be negligible, probably because studies were mainly based on rodent islets[167]. α-Cells are more abundant in human islets than in mouse islets[168], suggesting that α-cells have a key role in islet physiology in humans. Human β-cells are frequently surrounded by α-cells, enabling physical contact between cells and intra-islet crosstalk[89]. The close contacts allow the cells to use membrane-bound molecules for communication, thereby promoting cell function and survival[169]. Additionally, knowledge of islet microcirculation has expanded: from microcirculation flow from β-cells to α-cells in rodent islets[170], to a more sophisticated model of desegregated human islets with vascularization penetrating the β-cell core, bidirectional flow, and circulation integrated with the exocrine pancreas[7,171].

Receptors for glucagon and insulin are expressed on both β-cells and α-cells in rodents[172,173], and GCGRs are more abundant in β-cells[172]. Negative feedback regulation from products secreted from β-cells and δ-cells has been shown to regulate glucagon secretion[90,174]. Similarly, glucagon stimulates insulin secretion[175] and β-cells in close contact with α-cells release more glucose-stimulated insulin compared with β-cells deprived of these contacts[176]. The effect of glucagon on β-cells is mediated via GCGRs[177]. However, studies suggest that GLP1R on β-cells is the preferred pathway for glucagon-mediated insulin secretion[178–180] (Fig. 4), which has been confirmed in studies of *Glp1r*-knockout mice[181].

In addition to the paracrine effect of glucagon, acetylcholine released from α-cells also acts as a paracrine stimulator of insulin secretion[167] (Fig. 4). As proof of concept for this model, insulin secretion is amplified from isolated human islets in the presence of cholinesterase blockers[182]. People with T2DM show elevated α-cell-to-β-cell mass ratios[183], potentially because α-cells are necessary for β-cell insulin secretion. As confirmation, blocking proglucagon action on β-cells radically diminishes nutrient-stimulated insulin secretion[178–180].

Antidiabetic drugs affecting glucagon

T2DM is characterized by hyperglucagonaemia and clinical studies have focused on glucose-lowering drugs that limit glucagon secretion

or action as well as on the role of glucagon in their glucose-lowering effect (reviewed in ref. [184]). The role of glucagon in diabetic hyperglycaemia was substantiated when GCGR antagonists proved to lower fasting plasma glucose and HbA_{1c} in individuals with T2DM[185,186]. Older antidiabetic agents have not been as extensively studied regarding their effect on glucagon concentrations compared with newer agents. Clinical studies investigating metformin and sulfonylureas show varying effects on glucagon secretion (that is, a decrease, increase or no effect)[184]. Endogenous insulin suppresses glucagon secretion, but the effect of exogenous insulin on glucagon concentrations has not been thoroughly investigated. However, small studies in individuals with T2DM report either no effect or a decrease in glucagon concentration[184].

'Incretin enhancers', namely dipeptidyl peptidase 4 (DPP4) inhibitors, prevent the degradation of incretin hormones. These actions thus supress glucagon during hyperglycaemia (via GLP1-mediated effects) and increase glucagon secretion during hypoglycaemia (via GIP-mediated effects); thus, DPP4 inhibitors do not impair counter-regulatory glucagon responses during hypoglycaemia[187]. Clinical studies with DPP4 inhibitors in individuals with T2DM are consistent and report lower postprandial glucagon secretion compared with normal glucagon responses[184]. Likewise, studies with the DPP4 inhibitors linagliptin and vildagliptin during hypoglycaemic clamping in individuals with T2DM showed no disruption of the normal glucagon response to hypoglycaemia[187], which might even be increased[188]. The combination of a DPP4 inhibitor and a GCGR antagonist in individuals with T2DM additively lowers postprandial blood concentrations of glucose[189].

'Incretin mimetics', namely GLP1R agonists, decrease fasting and postprandial glucagon concentrations in individuals with T2DM and increase the insulin-to-glucagon ratio; this glucagonostatic effect of GLP1R agonists probably contributes to approximately one-third of their glucose-lowering effect[134,184]. Data on glucagon changes after long-term and chronic treatment with GLP1R agonists is limited; however, a study with 48 weeks of liraglutide treatment showed an increase in the circulating concentration of glucagon after oral glucose tolerance test (OGTT) in individuals with T2DM[190]. These data warrant additional studies on long-term effects with optimal glucagon assays.

Clinical studies in individuals with T2DM with the sodium–glucose cotransporter 2 (SGLT2) inhibitors dapagliflozin[191–194] and empagliflozin[195] have demonstrated increased fasting and postprandial circulating concentrations of glucagon. The increased blood concentrations of glucagon occurring with SGLT2 inhibitors have been associated with a rise in endogenous glucose production[194,195], although a smaller proof-of-concept study could not confirm increments in either glucagon concentrations or endogenous glucose production[196].

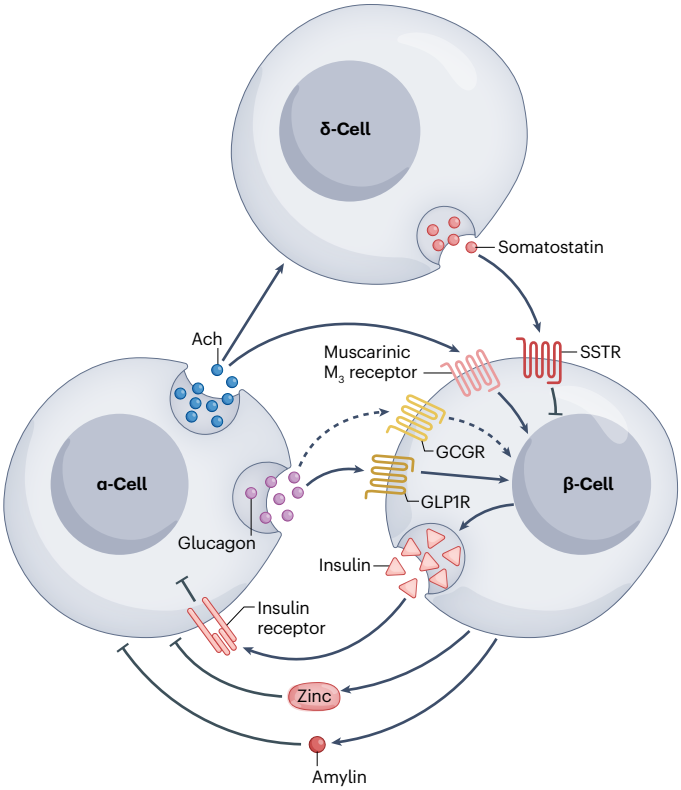

Fig. 4 | Intra-islet endocrine cell crosstalk. Paracrine regulation of insulin secretion from β-cells and glucagon secretion from α-cells in terms of crosstalk between cell types. Insulin and zinc co-secreted from β-cells as well as amylin suppress the secretion of glucagon from α-cells. This inhibition of insulin on α-cell secretion is part of the intra-islet insulin hypothesis, stating that falling circulating levels of glucose and subsequent lower insulin secretion are a signal to α-cells to increase glucagon secretion to prevent hypoglycaemia. Glucagon from α-cells signals via the glucagon receptor (GCGR) on β-cells to increase insulin secretion. However, glucagon prefers to signal via the glucagon-like peptide 1 receptor (GLP1R) on β-cells and this pathway is more insulinotropic than the GCGR pathway (indicated by the dashed arrow). Acetylcholine (Ach) from α-cells binds muscarinic M_3 receptors on β-cells, directly stimulates insulin secretion and indirectly inhibits insulin secretion by inducing somatostatin production from δ-cells. SSTR, somatostatin receptor.

Glucagon secretion in metabolic disease

Dysregulated glucagon secretion has been implicated in the pathophysiology of diabetes mellitus, but studies have revealed hyperglucagonaemia to be more closely related to obesity and liver fat content than to diabetes mellitus itself.

Hyperglucagonaemia in T2DM

T2DM is characterized by elevated fasting plasma concentrations of glucagon[197–200]. In combination with relative hypoinsulinaemia and insulin resistance, hyperglucagonaemia results in decreased glucose clearance and augmented endogenous glucose production[198,201]. Following ingestion of glucose, people with T2DM respond with an inappropriate initial increase in glucagon secretion followed by delayed suppression of glucagon[100]. However, the ability to suppress glucagon during isoglycaemic intravenous glucose infusions is preserved in T2DM[100,202].

The lack of suppression of glucagon postprandially has been implicated in postprandial hyperglycaemia in people with T2DM[203,204]. Traditionally, this hyperglucagonaemic response has been explained by α-cell resistance and decreased sensitivity to glucose and insulin[205]. However, the different glucagon responses observed after OGTT or isoglycaemic intravenous glucose infusion are incompatible with the notion that glucose and/or insulin are responsible for inappropriate glucagon secretion following oral glucose ingestion in T2DM[197,206]. Individuals without diabetes mellitus can also excrete glucagon in excess in response to OGTT with glucose loads of 75 g or more[197,207]. These findings suggest that post-absorptive hyperglucagonaemia could be due to secretion of glucagonotropic factors from the gut such as GIP[208].

Box 2

Clinical actions of glucagon agonism and antagonism

Glucagon agonism

- Glucose metabolism
  - ↑ Hepatic glucose output
- Amino acid metabolism
  - ↑ Amino acid catabolism
- Lipid metabolism
  - ↓ Liver fat content
  - ↑ Hepatic lipid oxidation
  - ↓ Hepatic lipogenesis
- Haemodynamics
  - ↑ Blood pressure
  - ↑ Cardiac inotropy and chronotropy
- Pancreas
  - ↑ Insulin secretion
- Energy homeostasis
  - ↓ Food intake
  - ↑ Energy expenditure
- Other effects
  - ↓ Gastric emptying

Glucagon antagonism

- Glucose metabolism
  - ↓ Hepatic glucose output
  - ↓ HbA1c
- Amino acid metabolism
  - ↓ Amino acid catabolism
- Lipid metabolism
  - ↑ Liver fat accumulation
  - ↑ Circulating LDL cholesterol
  - ↑ Cholesterol absorption
- Haemodynamics
  - ↑ Blood pressure
- Pancreas
  - α-Cell hyperplasia
  - β-Cell proliferation?
- Energy homeostasis
  - ↑ Body weight
- Other effects
  - ↑ Liver transaminases

An alternative mechanism could be due to secretion of gut-derived glucagon, consistent with the finding of glucagon in people who have undergone pancreatectomy[14,16,209]. Hyperglucagonaemia observed in people with T2DM could also be due to an altered α-cell-to-β-cell ratio caused by the greater susceptibility of β-cells to cellular stress and apoptosis[210]. Decreased clearance of glucagon could potentially contribute to hyperglucagonaemia in people with T2DM but recent data published in 2022 suggest normal glucagon clearance in T2DM[211].

Hyperglucagonaemia in obesity

Despite the well-established relationship between T2DM and fasting hyperglucagonaemia, the latter is not pathognomonic for T2DM and fasting hyperglucagonaemia also occurs in individuals with obesity and normal glucose tolerance[97,212]. As not all people with T2DM display fasting hyperglucagonaemia, the determining factor for the development of hyperglucagonaemia could be non-alcoholic fatty liver disease (NAFLD) (that is, hepatic steatosis)[213]. The hypothesis proposed is that NAFLD drives hepatic resistance to glucagon, which delivers a feedback mechanism via increased circulating levels of amino acids to pancreatic α-cells, resulting in hyperglucagonaemia[213]. A study conducted in 2020 showed increased glucagon resistance at the level of hepatic amino acid turnover in healthy individuals with obesity and NAFLD compared with healthy lean individuals (non-steatotic)[214]. This feedback loop between the liver and pancreas is named the liver–α-cell axis[99,215,216] and, when disrupted by, for example, steatosis-induced hepatic glucagon resistance, levels of circulating amino acids increase due to reduced glucagon-stimulated ureagenesis; this hyperaminoacidaemia could cause hyperglucagonaemia[212,213] (Fig. 2b). *Gcgr*-knockout mice have increased circulating concentrations of amino acids and α-cell hyperplasia[217]. Furthermore, antagonizing the GCGR in humans increases circulating concentrations of glucagon and amino acids, especially glucagonotropic amino acids[218], underlining that disruption of hepatic glucagon signalling disrupts the liver–α-cell axis. Pancreatic α-cell hyperplasia in GCGR-disrupted mice has been linked to amino acid-dependent processes via mechanistic target of rapamycin (mTOR)-dependent signalling[219]. In addition, research published in 2017 showed that amino acids could moderate glucagon secretion via amino acid transporters in α-cells[220,221].

Hyperglucagonaemia — friend or foe?

Whether hyperglucagonaemia in metabolic disease is a pathogenic response consistent with the development of the condition or represents a metabolically helpful adaptation remains unclear[156]. It has been argued that α-cell hyperplasia and hyperglucagonaemia drive and precipitate metabolic dysfunction[97,222]. However, studies indicate that α-cells might improve β-cell function and act to preserve β-cells, which to the contrary suggests a direct critical relationship between β-cells and α-cells in both mouse[178–180] and human[175,180] islets. People with T2DM have an increased α-cell-to-β-cell mass ratio[183] and mice fed a high-fat diet have increased α-cell mass compared with mice on regular chow diet[223]. In addition to increased glucagon secretion during metabolic stress, α-cell function is modified to express more PC1, leading to secretion of GLP1 (ref. [17]); thus, increased α-cell function as a metabolic adaptation increases glucagon and GLP1 secretion, which can act as insulin secretagogues. Of note, α-cells are more resistant than β-cells to metabolic stress such as palmitate-induced apoptosis and endoplasmatic reticulum stress[224]. These findings support a role for α-cells in islet cell preservation and endorse research that attempts to induce α-cell transdifferentiation into β-cells to create robust insulin-producing islet cells.

Modulation of glucagon secretion or action is a potential therapeutic target in metabolic disease. GCGR antagonists have proven efficient at reducing hyperglycaemia, without an increased risk of hypoglycaemia, in clinical trials in T2DM[186,225,226] and T1DM[227,228]. To date, however, all of the GCGR antagonists tested in clinical trials have presented adverse effects such as a rise in plasma levels of liver transaminases, increased risk of hepatic steatosis, alterations in circulating levels of cholesterol and/or lipid metabolism, increased blood pressure, and increased risk of α-cell hyperplasia[229] (Box 2), which makes them inappropriate for the treatment of T1DM or T2DM. Hence, pharmacological antagonism of glucagon action is currently not a treatment option in T1DM, T2DM or other metabolic conditions.

Pharmacological advances have led to the development of co-agonists (for example, glucagon and GLP1)[230–233] and tri-agonists (for example, glucagon, GLP1 and GIP)[234,235], which have been investigated as antidiabetic and anti-obesity therapies[156,234,236,237]. Several arguments have been put forward for the use of glucagon agonists in combination with GLP1R agonists (Box 2). Glucagon has a synergistic effect with GLP1 on insulin secretion[238] and co-infusion of glucagon and GLP1 could have a synergistic effect on reduced food intake[238]. Furthermore, glucagon might increase energy expenditure via an as-yet unclear mechanism[156] and might improve liver fat content due to increased hepatic β-oxidation[32]. The insulinotropic potential of glucagon and incretins on β-cells suggests that these could be ideal therapeutic agents in combination; however, dual-agonists and tri-agonists that target glucagon have not been approved for clinical use. The challenge in the development of these drugs is to balance the beneficial effects

of glucagon on body weight and lipid metabolism with the hyperglycaemic effects of glucagon. Thus, glucagon has evolved from being the culprit of diabetes mellitus to having a clear role in intra-islet signalling with importance for β-cell function. Moreover, dysfunctioning α-cells leading to hyperglucagonaemia in metabolic disease might represent a pathophysiological adaptation for the maintainance of energy balance and glucose homeostasis.

Conclusions

The counter-regulatory effects of glucagon in glucose homeostasis are well established in normal physiology. Increased glucagon secretion in metabolic disease is also increasingly recognized as an α-cell and possibly also gut-derived adaptation to the overflow of nutrients and β-cell stress. Thus, paracrine intra-islet communication between β-cells and α-cells might act to improve and preserve β-cell function and diminish β-cell loss, and hyperglucagonaemia in metabolic disease might be a helpful adaptation. Many antidiabetic drugs lower glucose, in part by lowering glucagon secretion or function. However, considering research on α-cell–β-cell crosstalk, the physiological effects of glucagon, including decreased hepatic fat storage combined with potentially decreased appetite and/or food intake and increased resting energy expenditure, are being exploited in the development of dual-agonists and tri-agonists of glucagon combined with incretin hormones. The adverse effects observed in clinical trials in individuals with T1DM or T2DM treated with GCGR antagonists, including transaminitis, increased hepatic fat content, increased blood pressure and dyslipidaemia, are worrying for these populations at high risk for cardiovascular events. These off-target results warrant further research within glucagon antagonism, whereas the beneficial effects of glucagon agonism in co-agonism with GLP1R agonists (and possibly GIP) are anticipated with optimism.

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Author contributions

S.H. and A.A. researched data for the article. All authors contributed substantially to discussion of the content. S.H. wrote the article. All authors reviewed and/or edited the manuscript before submission.

Competing interests

S.H. has served as a consultant for Novo Nordisk. F.K.K. has served on scientific advisory panels, been part of speakers bureaus, served as a consultant to and/or received research support from Amgen, AstraZeneca, Bayer, Boehringer Ingelheim, Carmot Therapeutics, Eli Lilly, Gubra, MedImmune, MSD/Merck, Mundipharma, Norgine, Novo Nordisk, Sanofi, ShouTi, Zealand Pharma and Zucara, and is a minority shareholder in Antag Therapeutics. T.V. has served on scientific advisory panels, been part of speakers bureaus, and served as a consultant to and/or received research support from Amgen, AstraZeneca, Boehringer Ingelheim, BMS, Eli Lilly, Gilead, GSK, Mundipharma, MSD/Merck, Novo Nordisk, Sanofi and Sun Pharmaceuticals. A.A. has no competing interests.

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