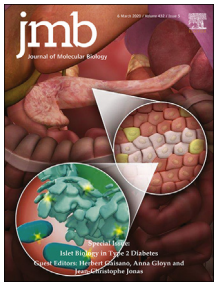

The Role of α-Cells in Islet Function and Glucose Homeostasis in Health and Type 2 Diabetes

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Abstract

Pancreatic α-cells are the major source of glucagon, a hormone that counteracts the hypoglycemic action of insulin and strongly contributes to the correction of acute hypoglycemia. The mechanisms by which glucose controls glucagon secretion are hotly debated, and it is still unclear to what extent this control results from a direct action of glucose on α-cells or is indirectly mediated by β- and/or δ-cells. Besides its hyperglycemic action, glucagon has many other effects, in particular on lipid and amino acid metabolism. Counterintuitively, glucagon seems also required for an optimal insulin secretion in response to glucose by acting on its cognate receptor and, even more importantly, on GLP-1 receptors. Patients with diabetes mellitus display two main alterations of glucagon secretion: a relative hyperglucagonemia that aggravates hyperglycemia, and an impaired glucagon response to hypoglycemia. Under metabolic stress states, such as diabetes, pancreatic α-cells also secrete GLP-1, a glucose-lowering hormone, whereas the gut can produce glucagon. The contribution of extrapancreatic glucagon to the abnormal glucose homeostasis is unclear. Here, I review the possible mechanisms of control of glucagon secretion and the role of α-cells on islet function in healthy state. I discuss the possible causes of the abnormal glucagonemia in diabetes, with particular emphasis on type 2 diabetes, and I briefly comment the current antidiabetic therapies affecting α-cells.
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Introduction

Pancreatic α-cells are the major source of glucagon which is the first line of defense against hypoglycemia. The stimulation of glucagon secretion by α-cells in response to hypoglycemia results from an activation of the autonomic nervous system and an action of glucose at the islet level [1,2]. The mechanisms by which glucose controls glucagon release at the islet level are still largely unknown. In particular, it is unclear whether the sugar controls glucagon secretion by a direct action on α-cells, or indirectly via β- and/or δ-cells (Fig. 1).

Diabetes is considered as a bihormonal disease with impaired insulin secretion/action and altered glucagon secretion. In both type 1 diabetes (T1D) and type 2 diabetes (T2D), the glucagon response to hypoglycemia (mainly iatrogenic) is impaired, and a hyperglucagonemia is observed, at least in the fasted and postprandial states, largely contributing to hyperglycemia. The causes of these defects could involve an altered control of α-cell secretion, a dysregulated liver-α-cell axis [3,4], and/or an atypical production of glucagon by the gut [5,6].

Recent findings have shown that α-cells could exert beneficial effects on glucose homeostasis, by optimizing insulin secretion in response to glucose and amino acids [7–11]. In addition, glucagon affects several facets of the metabolism, including a stimulation of hepatic lipid oxidation, satiety, and energy expenditure [12]. These effects are useful to combat obesity.

This article briefly reviews the physiology and pathophysiology of glucagon, mainly focusing on the control of glucagon secretion at the islet level, and discusses the potential causes of the defective glucagon secretion in diabetes.

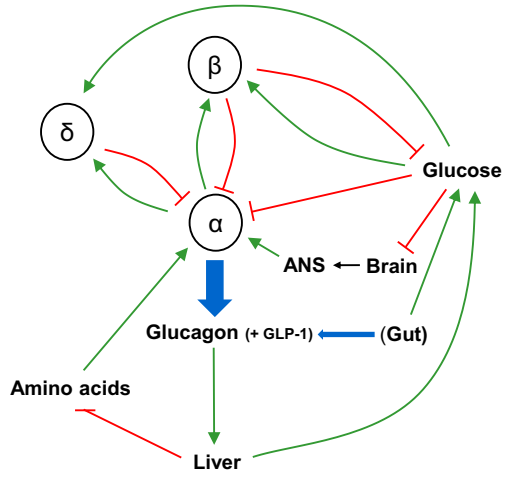

Fig. 1. (graphical abstract). Mechanisms by which glucose controls glucagonemia. Pancreatic α-cells are the major source of glucagon in healthy conditions. The sympathetic and parasympathetic branches of the autonomic nervous system (ANS) stimulate glucagon secretion in response to hypoglycemia. There is also a local control of glucagon release by glucose at the islet level. However, it is still unclear whether glucose controls glucagon secretion by a direct action on α-cells or indirectly via β- and/or δ-cells. The main target of glucagon is the liver which increases blood glucose and decreases aminoacidemia (via stimulation of gluconeogenesis). This latter effect attenuates glucagon release because amino acids are strong glucagonotropic agents. In diabetes, all levels of control of glucagon secretion and action can be disturbed, but no clear culprit mechanism emerges (see text for details). In some specific conditions of metabolic stress or adaptation (such as diabetes), L-cells from the gut also produce glucagon, whereas α-cells produce GLP-1. Green and red arrows represent stimulation and inhibition, respectively.

Islet Microanatomy

The islet microanatomy presents major interspecies differences, especially between rodents and humans [13,14]. Briefly, in mice and rats, α-cells represent ~20% of the endocrine islet cells and are localized at the periphery of the islet, except for some transgenic mouse models in which α-cells are also found in the core of the islet [15]. In humans, α-cells represent ~35% of the endocrine islet cells and are localized randomly in the islet [16,17]. In both humans and rodents, α-cells are more abundant in the body and the tail than in the head of the pancreas [18].

Within an islet, β-cells are electrically coupled via gap junctions made of connexin 36 [19]. It is commonly believed that there is no homologous (between the same cell type) or heterologous (between different cell types) coupling of α- and δ-cells [20,21]. However, some recent studies suggest that, in mice, δ-cells and, even, α-cells, might be partially coupled to β-cells [22–24], but this is not unanimously admitted (see below).

It is worth mentioning that the identity of α-cells is not irreversibly established for their whole life span. They can transdifferentiate into β-cells (see later). Interestingly, α-cells expressing glucagon and glucagon-negative cells expressing ARX (an α-cell-specific transcription factor) are among the most proliferative cells in human islets [25]. This illustrates the high plasticity potential of α-cells, even though the mass of α-cell mass remains unchanged across adult human life span [26].

Islets are much more vascularized than the exocrine pancreas. The organization of their vascularization (from mantle to core, from core to mantle, or randomly) is debated [14]. Paracrine interactions allow mutual crosstalk between cells. The classic paracrine/endocrine influences are the following ones: somatostatin very potently inhibits the secretion of all islet hormones, and glucagon stimulates that of somatostatin and insulin. The influence of insulin on the other islet cell types is still a matter of debate (see later). The endocrine islet cells can also exert paracrine and/or autocrine effects through other signals/peptides than their main hormones. For instance, ATP, GABA, serotonin, zinc, amylin, and urocortin 3 are secreted by β-cells. Neuronostatin (formed from preprosomatostatin) is secreted by δ-cells, and might affect α- and β-cell function [27]. Unlike mouse α-cells, human α-cells secrete acetylcholine which controls insulin and somatostatin secretion in a complex way [28]. They also secrete glutamate which is a positive autocrine signal for glucagon release [29].

The innervation of islets is species dependent. In mouse islets, parasympathetic and sympathetic axons densely innervate β-, α-, and δ-cells [30]. However, in human islets, endocrine cells are barely innervated [31]. Most axons innervating the islet cells are sympathetic and they preferentially contact smooth muscle cells of the vasculature. Very few parasympathetic axons innervate human islet cells.

Proglucagon-derived Peptides

Synthesis

Proglucagon is the precursor of several peptides whose presence in a cell type depends on the cell-specific expression of prohormone convertases [32,33]. Briefly, prohormone convertase 2 (PC2 encoded by *Pcsk2*), expressed by pancreatic α-cells, cleaves proglucagon into glucagon (= proglucagon 33–61) and various less physiologically relevant peptides. Prohormone convertase 1/3 (PC1/3 encoded by *Pcsk1*), expressed by L cells of the intestine, cleaves proglucagon into various peptides

which can undergo additional modifications. Among them, the most physiologically relevant peptides for glucose homeostasis are GLP-1 and oxyntomodulin.

The differential processing of proglucagon by PC2 in α-cells and by PC1/3 in L-cells is not a strict rule. α-Cells can also produce GLP-1 in some metabolic stress conditions [34], or after blockade or invalidation of glucagon receptors [35]. Whether α-cells are also able to produce biologically active GLP-1 in healthy conditions is a very controversial issue [5,8,34,36,37]. The very low expression of *Pcsk1* (PC1/3) in α-cells (~80 times lower than in β-cells [38]) suggests only a minor production of GLP-1.

Controversies about the existence of extrapancreatic glucagon have also been reported (reviewed in Ref. [5]). Detection of glucagon requires specific assays for true glucagon [39]. These new techniques revealed the existence of true extrapancreatic glucagon secreted by the gut in fully pancreatectomized patients with no residual C-peptide [40]. However, it is unclear whether true extrapancreatic glucagon exists in healthy conditions and to which extent it contributes to glucagonemia.

Effects of proglucagon-derived peptides

Glucagon and GLP-1 act on their own receptors, GcgR and GLP-1R, respectively, which are both coupled to Gs and, to a lesser extent, to Gq [41,42]. Recent studies suggest that glucagon acts also on GLP-1R, albeit with less efficacy than on GcgR [8,43,44]. Oxyntomodulin acts on both GcgR and GLP-1R, but with an efficiency ~100 times lower than that of their respective ligands [45].

Glucagon and GLP-1 exert a plethora of effects on metabolism and various organs (reviewed in Refs. [12,33,34,46,47]). At the islet level, glucagon, GLP-1, and oxyntomodulin stimulate insulin and somatostatin secretion from β- and δ-cells which express GcgR and GLP-1R [38,45,48]. Their effects on α-cells are less clear. α-Cells express very low levels of GcgR [38,48]. However, it was suggested that glucagon stimulates its own secretion from α-cells by an autocrine loop [49]. GLP-1 inhibits glucagon secretion via a glucose-dependent effect, decreasing in amplitude as the glucose concentration drops [41,50]. The underlying mechanisms are much debated [51]. It is unclear whether GLP-1R are fully absent [38,52,53], expressed in a subpopulation of α-cells [54], or only slightly expressed in most α-cells [55]. Some studies suggest a direct inhibitory effect of GLP-1 on α-cells resulting from a small elevation of cAMP which leads to PKA-dependent inhibition of P/Q-type Ca^{2+} channels [55,56]. Other reports suggest, on the contrary, a direct stimulation of a minor fraction of α-cells by GLP-1 [57]. Another theory is that the glucagonostatic effect of GLP-1 is indirect, mediated by somatostatin [58]. Inhibition of glucagon release by modulation of the autonomous nervous system by GLP-1 is also possible [59,60]. Surprisingly, oxyntomodulin stimulates glucagon secretion [45].

Influences of proglucagon-derived peptides secreted by α-cells on insulin secretion

Because the activity of α-cells is inhibited by lower glucose concentrations that the ones stimulating insulin secretion, it was long believed that glucagon and/or GLP-1 released from α-cells are not important for the control of insulin secretion, although differences between species have been reported [61]. However, recent studies have completely changed this view and have attributed to α-cells a major role in the control of insulin secretion by glucose. It was proposed that the glucagon concentration in the interstitial space around α-cells is high enough to efficiently activate both GcgR and GLP-1R, and that the insulinotropic effect of glucagon via GLP-1R is even more potent than that via GcgR [7,8,11]. This effect of glucagon has led to the counterintuitive suggestion that glucagon lowers glycemia when β-cells are active, and hence complements rather than opposes insulin action to maintain euglycemia [9,10]. Other studies found that, *in vivo*, factors can compensate for loss of signaling of proglucagon-derived peptides secreted by α-cells in healthy conditions, but that the influence of α-cells on β-cells becomes essential to maintain glucose tolerance during metabolic stress or aging [7,62]. GLP-1R activation by α-cell-derived GLP-1 is also possible, and it has been suggested that GLP-1 secreted by α-cells is even more important for glucose homeostasis than GLP-1 secreted from L-cells [37]. However, the role of GLP-1 secreted by α-cells in normal conditions is contested [8].

Physiological Control of Glucagon Secretion by Agents Other Than Glucose

Glucagon secretion is physiologically controlled by nutrients, neurotransmitters, and hormones. The mechanisms by which glucose controls glucagon secretion will be discussed in depth later.

Nutrients other than glucose

Contradictory data have been reported about the effects of fatty acids [46]. Old *in vivo* and *in vitro* studies, performed in animal species or humans, have reported clear inhibitory effects of free fatty acids (FFAs) on glucagon secretion [63,64]. However, more recent studies reported stimulatory effects of FFAs [65–67] which might be mediated by an activation of the free fatty acid receptor 1 (FFAR1/GPR40), or by a carnitine palmitoyltransferase 1a (CPT1a)-dependent

fatty acid oxidation, and involve a stimulation of L-type Ca^{2+} channels. The glucagonotropic effect of FFAs increases with the chain length of saturated FFAs and is stronger with saturated than unsaturated FFAs [68]. Ketone bodies (β-hydroxybutyrate and acetoacetate) inhibit glucagon secretion [63]. By contrast, most amino acids stimulate glucagon release, but with variable potencies [69]. Arginine is one of the most effective ones, and is often used in provocative tests of α-cell function [70]. The glucagonotropic effect of amino acids is physiologically useful to prevent hypoglycemia after protein intake because amino acids also stimulate insulin secretion [71].

Hormones and neurotransmitters

Many hormones and neurotransmitters affect glucagon secretion. Adrenaline and noradrenaline are among the most potent glucagonotropic agents. Acetylcholine, glutamate, GIP (gastric-inhibitory polypeptide or glucose-dependent insulinotropic polypeptide), CCK (cholecystokinin), GRP (gastrin-releasing peptide), PACAP (pituitary adenylate cyclase-activating polypeptide), VIP (vasoactive intestinal polypeptide), oxyntomodulin, oxytocin, and vasopressin also stimulate glucagon release [60,72,73]. By contrast, somatostatin is one of the most potent glucagonostatic agents. Insulin, GABA (γ-aminobutyric acid), GLP-1, leptin, ghrelin, and amylin have also been reported to inhibit glucagon release [2,60,72,72,74], but their effects are sometimes debated (see later). For several of them, it is unclear whether their glucagonostatic effect results from a direct action on α-cells and/or an indirect action via a paracrine factor. For instance, insulin, GLP-1, and ghrelin might inhibit glucagon release indirectly, via δ-cells [38,58,75].

Hypoglycemia is sensed by the central nervous system, mainly the hypothalamus, which activates the sympathetic and the parasympathetic nervous system to stimulate glucagon secretion, particularly in case of profound hypoglycemia. This involves acetylcholine, noradrenaline, and also, very likely, neuropeptides [1]. Many other stress conditions, such as hypoxia, hyperthermia, physical stress, sepsis, inflammation, trauma, or burns stimulate glucagon release, that is, in conditions under which a fuel mobilization is beneficial [76].

Control of Glucagon Secretion by Glucose

Glucagon secretion is stimulated by a drop in glycemia, whereas it is inhibited by a rise in glycemia. Glucose therefore attenuates glucagon release elicited by various secretagogues, such as arginine. The mechanisms by which glucose controls glucagon secretion at the islet levels are still largely unknown. In particular, it is unclear whether the sugar controls glucagon release by a direct action on α-cell or acts indirectly by stimulating the release of a paracrine signal from β- or δ-cells (Fig. 1). Many hypotheses have been reviewed [2,42,60,72,77–84] and some of them are discussed in the following sections.

What is the intracellular signal in α-cells controlling glucagon release in response to glucose?

Is it $[Ca^{2+}]_c$?

There is no doubt that a rise in $[Ca^{2+}]_c$ triggers glucagon release [85]. However, it is unclear whether glucose controls glucagon release by changing $[Ca^{2+}]_c$. The published $[Ca^{2+}]_c$ measurements in α-cells report very divergent results and show that glucose decreases $[Ca^{2+}]_c$ [19,86–88], does not affect it [82,89] or increases it [24,53,90–92]. To complicate matters further, it has been suggested that glucose induces local changes in $[Ca^{2+}]_c$ under the plasma membrane that cannot be detected by conventional whole cell $[Ca^{2+}]_c$ measurements [82]. In support of this hypothesis, the selective inhibition of non-L-type high-threshold voltage-dependent Ca^{2+} channels inhibits glucagon secretion without affecting $[Ca^{2+}]_c$ [93]. Other studies suggest that Ca^{2+} plays a permissive role for glucagon release and that glucose controls glucagon secretion independently of a change in $[Ca^{2+}]_c$ [24,84,91,92].

The existence of a heterologous coupling (between different cell types) is also controversial. Most studies do not support this theory [20,21,72,92], but some recent data show a coupling between α- and β-cells, at least, at high glucose [22,24]. This intriguing observation seems paradoxical because it has been suggested that pulsatile insulin and glucagon release are in opposite phase. However, it can be explained by the overriding of Ca^{2+} stimulation by paracrine inhibition by somatostatin [22,24]. A recent study has indeed documented a coupling between β- and δ-cells [22]. A heterologous coupling is consistent with the expression of connexins in α-, β-, and δ-cells. Interestingly, connexin 36 (*Gjd2*), the protein responsible for the coupling between β-cells [94] is also expressed in α- and δ-cells, although at a lower level [38].

Is it cAMP?

Intracellular cAMP is a potent stimulator of glucagon release. A rise in cAMP is one important mechanism by which adrenaline stimulates glucagon secretion. Two recent studies, using immunodetection of cAMP or cAMP-sensitive sensors, suggest that a drop in cAMP is the mechanisms by which glucose inhibits glucagon release [95,96]. One

study proposes that this effect is indirectly mediated by somatostatin which decreases cAMP production by inhibiting adenylyl cyclase via $G\alpha_i$ coupled to SSTR, and by insulin which binds to its receptor in α-cells and stimulates cAMP degradation by activating phosphodiesterase 3B [95]. Thus, the drop in cAMP results from an inhibition of the rate of its synthesis and a stimulation of the rate of its degradation. The resulting decrease in cAMP would inhibit PKA and glucagon release. The second study claims that the inhibitory effect of glucose on cAMP is independent of insulin and somatostatin, but results from a direct action of the sugar on α-cells [96].

Intrinsic control

It was reported that glucose directly inhibits or stimulates α-cells. The paragraphs below summarize the different hypotheses explaining these effects. It is important to keep in mind that conclusions from secretion experiments performed on isolated α-cells should be interpreted with caution. Indeed, recent studies suggested that isolated α-cells secrete glucagon in an uncontrolled fashion because of the loss of contact with neighboring cells [84,97,98]. Regulated glucagon secretion could depend on juxtacrine signals (see later) [97].

Role of α-cell K_{ATP} channels in the control of glucagon secretion by glucose: do they inhibit or stimulate glucagon release?

α-Cells express K_{ATP} channels [82,83,93]. Their role is highly debated contrary to the situation in β-cells. In β-cells, glucose accelerates cell metabolism, which increases the ATP/ADP ratio and closes K_{ATP} channels. The resulting decrease in K^+ conductance depolarizes the plasma membrane up to the threshold for the activation of high-threshold voltage-dependent channels (mainly L-type) which open and increase $[Ca^{2+}]_c$. In α-cells, K_{ATP} channels also transduce changes in cell metabolism into changes in electrical activity [82,83,93,99–102]. This is well illustrated by the large increase in K_{ATP} current (I_{KATP}) and drop in $[Ca^{2+}]_c$ induced by pharmacological blockade of cell metabolism with azide [101]. However, contrary to what is observed in β-cells, most α-cell K_{ATP} channels are already closed at low glucose [82,83,93,99–102]. The reasons are unclear. This might reflect a higher rate of metabolism at low glucose (because they express GLUT1 and hexokinases in addition to glucokinase [38,103,104]), a higher sensitivity of the K_{ATP} channels to ATP in α- than β-cells [105,106], and/or a different modulation of K_{ATP} channels by other factors such as PIP_2 which decreases the ATP sensitivity of the K_{ATP} channel [107,108]. In addition, α-cells have different low-threshold voltage-dependent channels (Na^+ and T-type Ca^{2+} channels) than those of β-cells [82,93,109]. These channels are important for the generation of action potentials in α-cells.

One much-cited model suggests that glucagon secretion occurs only in a narrow range of K_{ATP} channel activity. It has been built on the following key observations: (a) closure of K_{ATP} channels by tolbutamide applied to a medium containing a low glucose concentration inhibits glucagon secretion, (b) mild opening of the channels by low diazoxide concentrations reverses the glucagonostatic effect of glucose, whereas (c) strong opening of the channels by high diazoxide concentrations inhibits glucagon release [93,102,110,111]. Thus, the model suggests that, at low glucose, α-cell K_{ATP} channel activity is very low and keeps the plasma membrane slightly depolarized at the level of the threshold for the activation of low-threshold voltage-dependent channels. An increase in the glucose concentration or the addition of K_{ATP} channel blockers leads to strong inhibition of K_{ATP} channels. This depolarizes the plasma membrane and inactivates low-threshold voltage-dependent channels involved in action potential firing, which, via reduced action potential height, decreases Ca^{2+} influx and inhibits exocytosis. The glucagonostatic effect of glucose can be reversed by low concentrations of the K_{ATP} channel opener, diazoxide, which brings back the activity of K_{ATP} channels in the optimal range of the window (corresponding to a low glucose concentration). Of course, a large increase of I_{KATP} (with diazoxide concentrations $> 10\ \mu M$) hyperpolarizes the α-cell below the threshold for the activation of voltage-dependent Ca^{2+} channels and also inhibits glucagon release.

However, this model which predicts that glucose depolarizes α-cells is not unanimously admitted [86,89,112,113]. Indeed, some studies reported an opposite effect [114–117]. Several observations even indicate that glucose inhibits glucagon secretion independently of K_{ATP} channel closure. (a) The sugar inhibits glucagon secretion from islets lacking K_{ATP} channels (*Sur1*$^{-/-}$ or *Kir6.2*$^{-/-}$ mice) or in the presence of high concentrations of tolbutamide which maximally close K_{ATP} channels [86,101,112]. (b) We did not find that low diazoxide concentrations reverse the glucagonostatic effect of glucose as predicted by the K_{ATP} channel-based model [101]. (c) In the absence of paracrine influence of somatostatin, 7 mM glucose inhibits glucagon secretion whereas K_{ATP} channel blockers always stimulate glucagon release [101].

What is then the role of K_{ATP} channels in α-cells? Experiments comparing the effects of K_{ATP} channel blockers in the presence or absence of paracrine influence of somatostatin shed new light on this conundrum. The following observations suggest that closure of α-cell K_{ATP} channels stimulates exocytosis by the same mechanisms than in β-cells, whereas closure of δ-cell K_{ATP} channels stimulates

somatostatin secretion, which counteracts the direct stimulatory effect of K_{ATP} channel blockers on α-cells (see also the section "Stimulation by glucose"). (a) Tolbutamide increases $[Ca^{2+}]_c$ in isolated α-cells, which is rarely the case of glucose [86,89,91,118,119]. (b) In the presence of a low glucose concentration, that is, when the rate of glucagon secretion is high, K_{ATP} channel blockers inhibit or do not affect glucagon release of control islets. However, they always stimulate glucagon secretion of islets devoid of paracrine influence of somatostatin (islets preincubated with pertussis toxin, or islets or pancreas from *Sst*$^{-/-}$ mice) [101,120]. (c) In the presence of an inhibitory concentration of glucose, that is, when the rate of glucagon secretion is low, K_{ATP} channel blockers stimulate, but never inhibit, glucagon release of control islets [101,120]. The stimulation by K_{ATP} channel blockers is much larger in islets devoid of paracrine influence of somatostatin [101,120]. (d) The rate of glucagon secretion is lower in *Kir6.2*$^{-/-}$ or *Sur1*$^{-/-}$ islets than in control islets. However, the rate of glucagon release is much higher in *Sst*$^{-/-}$/*Kir6.2*$^{-/-}$ islets than in *Sst*$^{-/-}$ islets. Together, these observations suggest that sulfonylureas control glucagon secretion of normal islets by two mechanisms: a direct stimulation of α-cells by closure of their K_{ATP} channels (observed in the absence of paracrine influence of somatostatin), and an indirect inhibition via somatostatin released from δ-cells on closure of their K_{ATP} channels. The net effect of K_{ATP} channel blockers on glucagon secretion from control islets results from a balance between both effects (Figs. 2 and 3). It depends on the rate of glucagon release which is affected by the glucose concentration. When the rate is already low, the stimulation is apparent. This is why K_{ATP} channel blockers stimulate glucagon release in the presence of 7 mM glucose [101,101,120]. By contrast, when the rate is already high, the inhibition is more apparent. That explains why K_{ATP} channel closure/ablation tends to inhibit glucagon release at low glucose concentrations. The glucagonostatic effect of K_{ATP} channel blockers seen at low glucose is therefore *fully* mediated by somatostatin.

It should be pointed out that the hypothesis that the glucagonostatic effect of glucose is not mediated by the closure of K_{ATP} channels does not mean that these channels are not required to see the effect of glucose. Indeed, all studies agree that full K_{ATP} channel opening by high diazoxide concentrations strongly inhibits glucagon release and largely (but

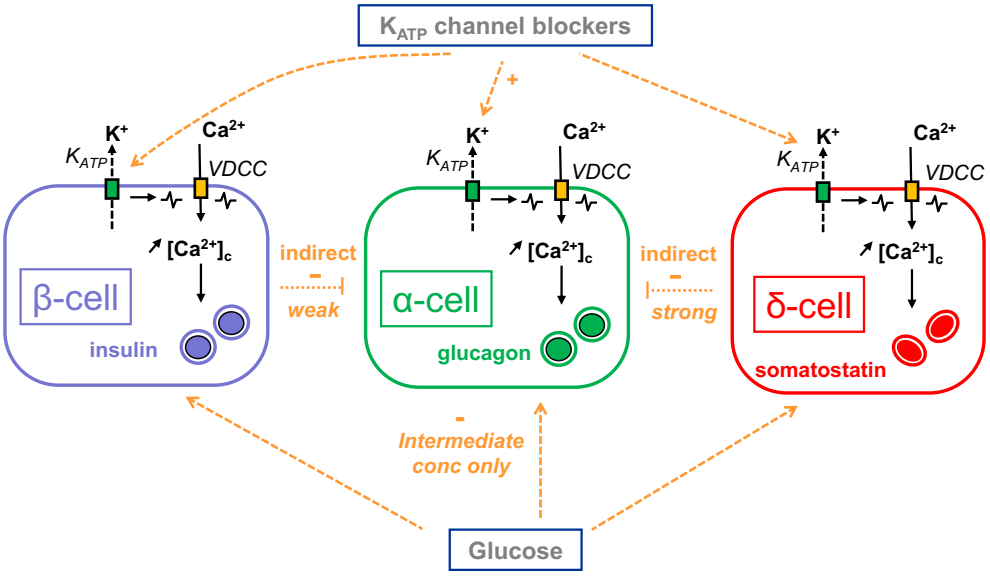

Fig. 2. Direct and indirect mechanisms by which K_{ATP} channel blockers and glucose control glucagon secretion at the islet level. These compounds affect glucagon release by a direct action on α-cells or by an indirect action via β- and δ-cells. Somatostatin exerts a potent glucagonostatic effect whereas insulin or other β-cell-derived factors inhibit only slightly glucagon secretion. The upper part illustrates the effect of K_{ATP} channel blockers. By directly closing K_{ATP} channels of each cell type, they stimulate exocytosis in each of them (without any paracrine influence). The net effect of K_{ATP} channel blockers (observed in intact islets, i.e. with paracrine interactions) results from a balance between the direct stimulatory effect on α-cells and the indirect inhibitory effect via β- and δ-cells. The lower part illustrates the effect of glucose on glucagon release. In the absence of paracrine influence from β- and δ-cells, the sugar inhibits glucagon secretion only at intermediate concentrations. Higher concentrations of glucose inhibit glucagon secretion via somatostatin (mainly) and, possibly also, via a β-cell-derived paracrine factor. See Figs. 3 and 4 for more details on the dose-dependence of the direct and indirect effects of glucose and K_{ATP} channel blockers on glucagon secretion.

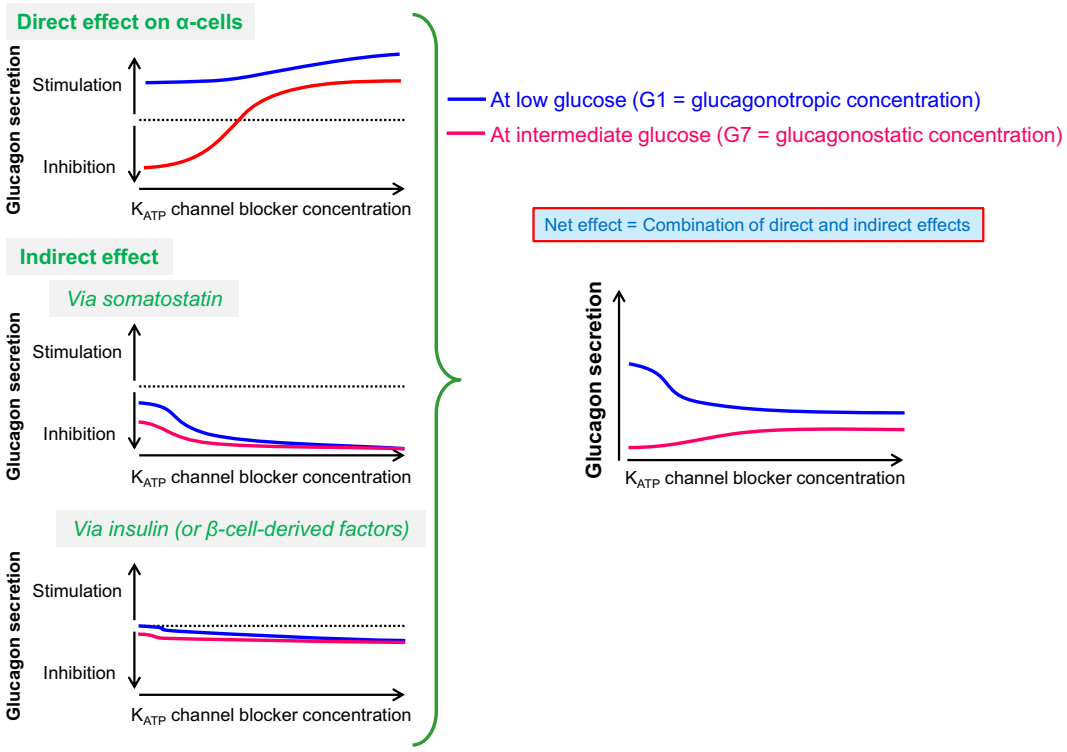

Fig. 3. Direct and indirect effects of K_{ATP} channel blockers on glucagon secretion. The left panels represent the effects of K_{ATP} channel blockers on glucagon secretion, either mediated by a direct action on α-cells (upper panel) or an indirect action via somatostatin (middle panel) or insulin (or β-cell-derived factors, lower panel). The effects are either a stimulation (upward arrow) or an inhibition (downward arrow) of glucagon secretion. The right panel illustrates the net effect on glucagon secretion which results from the combination of the direct and indirect effects. The effects of the K_{ATP} channel blockers are illustrated at two glucose concentrations, a low concentration associated with a high glucagon secretion (blue trace) or an intermediate glucose concentration associated with a low glucagon secretion (red trace). At low glucose (blue trace), glucagon secretion is intrinsically high. K_{ATP} channel blockers stimulate glucagon release by a direct effect on α-cells (illustrated by the upward arrow on top left panel). However, they also stimulate somatostatin secretion (represented by an inhibition of glucagon release, downward arrow on middle left panel) which strongly counteracts the direct glucagonotropic effect of K_{ATP} channel blockers. K_{ATP} channel blockers also stimulate insulin secretion (downward arrow on lower left panel) which only mildly inhibits glucagon release. The net effect is an inhibition of glucagon release (right panel: blue trace) mainly due to the indirect action of somatostatin. At an intermediate glucose concentration (red trace), K_{ATP} channel blockers stimulate glucagon, somatostatin, and insulin secretion. The direct effect on α-cells is dominant (upward arrow on top left panel) and only partially counteracted by the indirect glucagonostatic effect of somatostatin and insulin (downward arrows on middle and lower left panels). Thus, the net effect is a stimulation of glucagon release (right panel: red trace) mainly due to the direct stimulatory effect of K_{ATP} channel blockers on α-cells. This model suggests that any glucagonostatic effect of K_{ATP} channel blockers, as observed in low glucose, is *fully* mediated by somatostatin. This model is essentially based on experiments performed on mouse islets. It does not exclude the possibility that insulin- and somatostatin-independent effects also contribute to the so-called "direct" effects shown on the top left panel. It is still unclear whether this model is fully transposable or not to human islets.

not completely: see Refs. [101,121]) prevents the glucagonostatic effect of glucose.

Other models of intrinsic inhibition of glucagon secretion by glucose

There are several alternative models of inhibition of glucagon secretion by glucose. One of them suggests that glucose inhibits a depolarizing store-operated current (I_{SOC}) [115]. At low glucose levels, the activity of the ATP-requiring sarco-endoplasmic reticulum Ca^{2+}-ATPase (SERCA) is low, and a low Ca^{2+} concentration in the endoplasmic reticulum ($[Ca^{2+}]_{ER}$) activates I_{SOC} which carries Ca^{2+} and, possibly also, other ions. Several molecular partners might be involved: STIM, a Ca^{2+} sensor in the membrane of the endoplasmic reticulum (ER), which activates Orai, a channel in the plasma membrane [122]. By depolarizing the plasma membrane, I_{SOC} activates voltage-dependent Ca^{2+} channels,

allowing a rise in $[Ca^{2+}]_c$ and subsequent stimulation of glucagon secretion. Raising the glucose concentration increases the ATP concentration. By activating SERCAs, this increases $[Ca^{2+}]_{ER}$, reduces I_{SOC} and brings the membrane potential below the threshold for activation of voltage-dependent Ca^{2+} channels. This lowers $[Ca^{2+}]_c$ and inhibits glucagon secretion [86,115]. This hypothesis is based on several observations. A key observation is that inhibition of SERCAs by thapsigargin or cyclopiazonic acid, which depletes the ER in Ca^{2+}, increases α-cell $[Ca^{2+}]_c$ and prevents the lowering effect of glucose [86,115]. Moreover, these inhibitors, as well as low glucose, trigger the accumulation of STIM1 puncta in the subplasmalemmal ER where they cocluster with Orai1 in the plasma membrane [123].

Other models have been suggested: the activation by glucose of the hyperpolarizing Na^+/K^+ pump [124] or of the two-pore-domain K^+ channel (K2P), TASK1 [117], which would inhibit electrical activity [124], an inhibition by glucose of the AMP-dependent protein kinase, a modulation of Per-Arnt-Sim (PAS) domain-containing protein kinase [125] or a glucose-induced swelling of the α-cell [126] with subsequent activation of Cl^- influx through volume-regulated channels [127] and/or CFTR ([128,129], but contested in Ref. [130]).

Stimulation by glucose

A few studies suggest that glucose stimulates glucagon secretion from isolated α-cells by a mechanism similar to that observed in β-cells. Two key observations support this hypothesis. (a) Glucose and K_{ATP} channel blockers stimulate electrical activity and increase $[Ca^{2+}]_c$ in rat α-cells [53,90]. (b) They also directly stimulate glucagon release from rat and mouse α-cells [90,92]. Because these effects are observed in isolated α-cells, it is assumed that the inhibition of glucagon release by glucose from whole islets results from an indirect paracrine influence from non-α-cells within the islet that fully counteracts the direct stimulatory effect of glucose on α-cells. This interpretation is compatible with elegant experiments showing that the forced acceleration of α-cell metabolism stimulates glucagon release whereas the forced acceleration of both α- and β-cell metabolism inhibits glucagon release [131].

Another paradoxical stimulation of glucagon release which concerns whole islets has been documented. Several studies have tested the dose-dependent effect of glucose on glucagon secretion from whole islets or from the perfused pancreas. Most of them show that the maximal inhibition of glucagon secretion is reached at around ~7 mM glucose [83,86,93,111,120,121], and that this inhibition tends to be weaker at higher concentrations of the sugar. Sometimes, the dose-response curve displays a clear U-shape with a higher rate of glucagon release at very high glucose (25–30 mM) than in the absence of the sugar [79,121]. However, this loss of inhibition by high glucose was not observed by some groups [101,132]. The reasons of these discrepancies are unknown.

Paracrine control

Observations in support of a paracrine control

Several observations suggest that a paracrine factor physiologically controls glucagon release. Probably one of the most striking observations is that the control of glucagon secretion is lost in type 1 diabetic patients, which supports a requirement of β-cells for a proper control of glucagon secretion [133]. Other studies showed that the specific activation of β-cell metabolism inhibits glucagon release [131], or that glucagon release from pure human α-cells becomes regulated by glucose only if α-cells are reaggregated with β-cells [98]. Because somatostatin potently inhibits glucagon secretion, it has been suggested that it might also fulfill the role of paracrine factor.

The study of the pulsatility of insulin, somatostatin, and glucagon secretion is helpful to understand the potential existence of a paracrine control between β-, δ-, and α-cells. Experiments with the perfused rat, dog, monkey, and human pancreas [134–136] revealed insulin, glucagon, and somatostatin secretion oscillations (period of 8–10 min/cycle) during exposure to a constant glucose concentration. Two studies found that the three hormones oscillate independently of each other [134,135]. However, two other studies reported that all three hormones oscillate with a similar period (4–5 min/cycle), and that somatostatin secretion oscillates almost in phase with insulin secretion (with a delay of ~30 s) but in antiphase with glucagon secretion [136]. Interestingly, oscillations of the three hormones (period of 5–8 min/cycle) were also found in batches of 5–15 isolated perifused human or mouse islets, that is, in conditions where the only possible interactions between islets are of paracrine nature [137,138]. In fact, the secretion of all three hormones was stable at 3 mM glucose, but oscillated at 20 mM glucose with insulin and somatostatin secretion in phase, and glucagon secretion in antiphase. Despite the fact that glucagon secretion oscillated at 20 mM glucose, the average pulsatile glucagon secretion was lower at 20 mM glucose than the stable secretion at 3 mM of the sugar. A tentative explanation for the apparent stable glucagon secretion at low glucose is that $[Ca^{2+}]_c$ oscillations are random and nonsynchronized in α-cells [24]. Under these conditions, β-cells are silent and have a basal $[Ca^{2+}]_c$. The appearance of glucagon secretion oscillations at 20 mM would be due to a paracrine influence. Thus, at this glucose concentration, β-cells oscillate in phase. Their rhythmic activity would entrain δ-cells [22–24], either by electrical coupling

[22], or by releasing a somatostatin-stimulating factor. ATP, coreleased with insulin, might be this factor because Y2 purinergic agonists stimulate somatostatin secretion [139]. Insulin might be another candidate, although it is contested [75,140]. Paracrine factors released by β- and/or δ-cells during each ascending phase of insulin and somatostatin secretion would inhibit glucagon secretion, thereby generating antiparallel oscillations of insulin/somatostatin and glucagon secretion.

Paracrine factors

In this paragraph, I will briefly review the possible paracrine factors that might control glucagon secretion and I will discuss whether they are involved or not in the control of glucagon secretion by glucose (Fig. 1).

Insulin. Several observations suggest that insulin inhibits glucagon secretion. (a) α-Cells express insulin receptors [53] and their ablation specifically in α-cells induces a hyperglucagonemia and hyperglycemia in the fed state [141]. (b) Insulin inhibits glucagon secretion of the perfused pancreas of streptozotocin-treated rats [142], of pure human α-cell aggregates (but only at insulin concentration in the μM range) [98], and decreases glucagonemia in type 1 diabetic patients [143]. Some studies showed that the glucagonostatic effect of insulin is abolished by wortmannin, a phosphatidylinositol 3-kinase inhibitor [19,53,90,111,144]. (c) Insulin-deficient states are characterized by hyperglucagonemia and increased glucagon release [64,142,145,146]. (d) Immunoneutralization of insulin stimulates glucagon release [147].

Several mechanisms of inhibition of glucagon secretion have been suggested (although often contested). Insulin could increase the K_{ATP} current and decrease $[Ca^{2+}]_c$ in α-cells [53]. It could stimulate the translocation of $GABA_A$ receptors to the cell surface, thereby promoting the activation of a GABA-mediated hyperpolarizing Cl^- current [144]. It could decrease cAMP levels in α-cells by stimulating its degradation via phosphodiesterase 3B [95]. Insulin could also inhibit glucagon release by stimulating somatostatin secretion via a SGLT2-dependent mechanism [75]. An additional possibility is that some of the effects of insulin are actually mediated by urocortin 3 which is coreleased with insulin and stimulates somatostatin secretion [148].

An important question is whether insulin is indirectly responsible for the glucagonostatic effect of glucose. Some arguments are in favor of this suggestion. (a) There is an inverse relationship between the glucose-stimulated insulin secretion and the glucose-inhibited glucagon release. (b) The glucagon secretion in response to a drop of the glucose concentration is lost in type 1 diabetic patients [149], and in alloxan-treated pigs and dogs [150,151]. It is strongly reduced in the perfused pancreas or perifused islets from streptozotocin-treated rats [142,152]. Glucose even stimulates glucagon release of the perfused pancreas of streptozotocin-treated mice [153]. (c) Hyperinsulinemia attenuates the glucagon response to hypoglycemia [154]. (d) The glucagon response of rodent islets to low glucose is impaired by immunoneutralization of intraislet insulin or knockdown of insulin receptors by siRNA [53,155].

A variant is the "switch-off" hypothesis according to which insulin exerts a tonic inhibition on glucagon secretion, and removal of this brake in hypoglycemic state is necessary to trigger glucagon secretion [152,156]. Another hypothesis suggests that insulin exerts a permissive effect and is required for the suppression of glucagon release by high glucose [157].

The critical role of insulin in the control of glucagon secretion by glucose is however not supported by many other observations. (a) The most striking one is the distinct glucose dependences for the inhibition of glucagon secretion and the stimulation of insulin release. Several reports showed that glucagon secretion is already suppressed by glucose concentrations that are below the threshold for the stimulation of insulin secretion [19,83,86]. However, there might be species differences because a recent study reported a fully inverse glucose concentration-response relationship for insulin and glucagon secretion from human islets [61]. (b) Insulin failed to affect glucagon release from mouse islets [89] or from the alloxan-treated lobe of dog pancreas [157]. (c) High insulin concentrations did not prevent the glucagonostatic effect of glucose in mouse islets [89]. Likewise, hyperinsulinemic–hypoglycemic clamp experiments showed that hypoglycemia largely increased glucagonemia under supraphysiological hyperinsulinemia in mice expressing or not the insulin receptor in α-cells [158].

The take-home message of all these studies is that insulin is not required for the glucagonostatic effect of glucose. However, a contribution of insulin in the glucagonostatic effect of insulinotropic concentrations of glucose cannot be excluded (Figs. 2 and 4).

Zinc. Zn^{2+} is taken up by the ZnT8 transporter into the granules of islets cells [159]. In β-cells, two Zn^{2+} binds to six molecules of insulin to form insulin crystals in the secretory granules. On exocytosis, Zn^{2+} is coreleased with insulin and it is expected that relatively high concentrations of Zn^{2+} (μM) are reached in the interstitial space. Zn^{2+} has been proposed as a paracrine factor mediating the glucagonostatic effect of glucose [131,160]. A "switch-off" hypothesis involving Zn^{2+} has even been suggested [161].

The mechanisms by which Zn^{2+} would inhibit glucagon secretion are unclear. It might inhibit $[Ca^{2+}]_c$ oscillations [53,90,160,162], possibly by activating the K_{ATP} current in α-cells [53]. However, some studies report no effect [89] or a stimulatory effect of Zn^{2+} [19,86,111] on $[Ca^{2+}]_c$, and a lack of effect of Zn^{2+} on K_{ATP} channels of α-cells [162].

The studies suggesting that Zn^{2+} inhibits glucagon secretion are based on the observation that Zn^{2+} chelators prevent the glucagonostatic effect of glucose [53,131]. However, others failed to see such an effect [93]. A clearer picture of the role of Zn^{2+} in the control of glucagon secretion was obtained from experiments with *ZnT8*$^{-/-}$ mice. In these mice, stimulation of insulin secretion failed to release detectable Zn^{2+} because the ion does not accumulate in the granules [159]. However, the control of glucagon secretion by glucose remained normal in both global *ZnT8*$^{-/-}$ and β-cell-specific *ZnT8*$^{-/-}$ mice [101,159,160]. α-Cell-specific *ZnT8*$^{-/-}$ mice have also normal glucagon secretion [163]. These studies indicate that Zn^{2+} is not responsible for the glucagonostatic effect of glucose. However, they do not exclude the remote possibility that Zn^{2+} can affect glucagon release because small amounts of Zn^{2+} can still be accumulated in β-cells independently of ZnT8 [164].

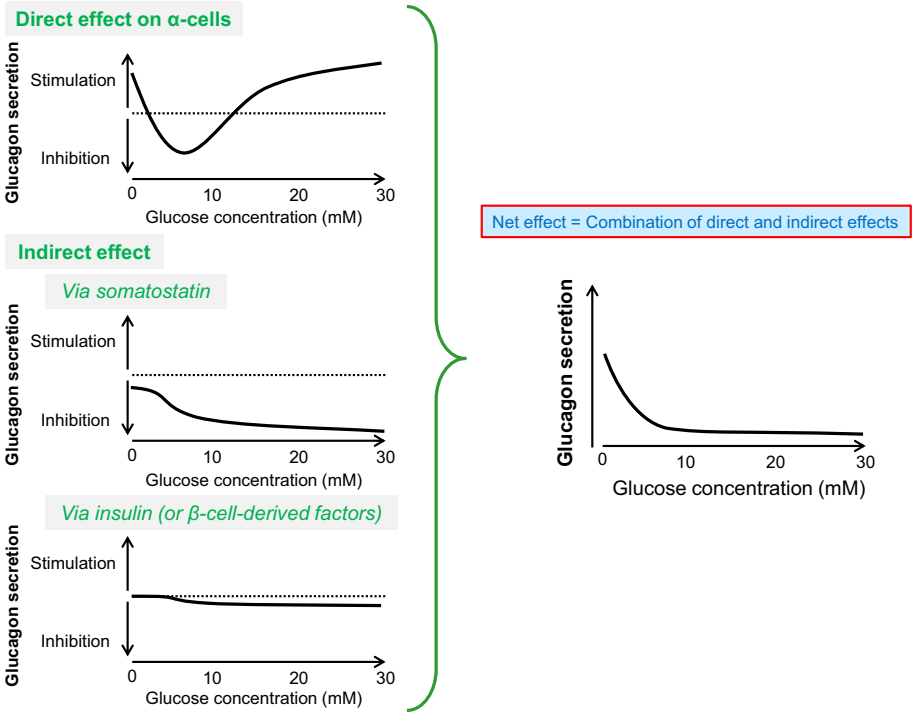

Fig. 4. Direct and indirect effects of glucose on glucagon secretion. The left panels represent the effects of glucose on glucagon secretion, either mediated by a direct action on α-cells (upper panel) or an indirect action via somatostatin (middle panel) or insulin (or β-cell-derived factors, lower panel). The effects are either a stimulation (upward arrow) or an inhibition (downward arrow) of glucagon secretion. The right panel illustrates the net effect on glucagon secretion which results from the combination of the direct and indirect effects. Glucose inhibits glucagon secretion by mechanisms that are differently recruited depending on the concentration of the sugar. Upper left panel, glucose, from low to ~7 mM, dose-dependently inhibits glucagon release by activating a mechanism that is independent of K_{ATP} channels and that is likely intrinsic to α-cells (possibly cAMP). The inhibition progressively wanes above ~7 mM glucose. Glucose, at 20 and 30 mM glucose, even fails to inhibit glucagon release in the absence of paracrine factor (mainly somatostatin). Middle and lower left panels, glucose dose-dependently stimulates the release of somatostatin and insulin that, respectively, strongly and weakly inhibit glucagon secretion (illustrated by the downward arrows). Right panel, in the absence of glucose or at low glucose, glucagon secretion is already restrained by a tonic inhibition of somatostatin. The net effect of glucose on the three mechanisms depicted on the left is a dose-dependent inhibition of glucagon secretion. It is mainly independent of somatostatin for low glucose concentrations but starts to involve somatostatin for high concentrations of the sugar. This suggests that the glucagonostatic effect of glucose only partially depends on somatostatin. This model is essentially based on experiments performed on mouse islets. It does not exclude the possibility that insulin- and somatostatin-independent effects also contribute to the so-called "direct" effects shown on the top left panel. It is still unclear whether this model is fully transposable or not to human islets.

GABA, γ-hydroxybutyrate, and serotonin. In the brain, GABA is a major inhibitory neurotransmitter that acts on two major types of receptors: $GABA_A$ and $GABA_B$ receptors. $GABA_A$ receptors are pentameric ligand-gated Cl^- channels composed of various subunit isoforms (6 α subunits Gabra1−6, 3 βs Gabrb1−3, 3 γs Gabrg1−3, 1 δ Gabrd, 1 ϵ Gabre, 1 π Garbrp, and 1 θ Gabrq). The most common pentamer comprises 2 α, 2 β, and 1 γ subunit [165]. The net effect of $GABA_A$ receptor activation depends on the reversal potential of Cl^- which varies between cell types and is very negative (and, hence, inhibitory) in many neurons in the brain. $GABA_B$ receptors are G protein-coupled receptors that can activate a K^+ conductance and, being coupled to $G_i/_0$, that can also decrease cAMP levels and inhibit Ca^{2+} channels. There exist 2 different subunits (*Gabbr1* and *Gabbr2*) [166].

Surprisingly, GABA is present in β-cells in similar high concentrations as in GABAergic neurons [167−170]. GABA can be released by two routes. The Ca^{2+}-dependent route involves the exocytosis of synaptic-like microvesicles (SLMVs, ~diameter of ~90 nm) in which GABA is strongly accumulated [167,168] and of insulin-containing vesicles (LDCVs, ~diameter of ~300 nm) which might also contain GABA [171]. The Ca^{2+}-independent route involves plasma membrane transporters.

α-Cells express very low mRNA levels of the different $GABA_A$ receptor subunits. Only one β isoform of one subunit (*Gabrb3*) is significantly expressed in mouse and human α-cells [38,48]. Because a functional $GABA_A$ receptor requires at least an α and a β subunit [165], this raises the question of the existence of functional $GABA_A$ receptors in α-cells. Several studies suggest that GABA inhibits glucagon secretion [90,170,172,173] by activating $GABA_A$ receptors [170,172,173]. However, other studies did not find any effect of GABA on $[Ca^{2+}]_c$ [86,89] or the membrane potential of mouse [116] and human [174] α-cells. Mouse and human α-cells also express low mRNA levels of the two $GABA_B$ receptor subunits (*Gabbr1* and *Gabbr2*). The role of $GABA_B$ receptors in α-cells is unclear because neither $GABA_B$ receptor agonists nor antagonists affected glucagon secretion [170].

It should be mentioned that GABA could also affect glucagon secretion via δ-cells. Interestingly, GABA was found to depolarize δ-cells, which has been explained by the activation of a Cl^- conductance by $GABA_A$ receptors and the existence a high reversal potential for Cl^- in δ-cells [174]. However, we did not see any effect of GABA on somatostatin release [170].

The contribution of GABA in the glucagonostatic effect of glucose has been suggested a long time ago [173]. Subsequent studies provided controversial results because $GABA_A$ receptor antagonists prevented [172] or not [93,170] the glucagonostatic effect of glucose. The glucose-dependence of GABA release does not support the hypothesis that GABA mediates the inhibitory effect of glucose on glucagon release. Indeed, the Ca^{2+}-dependent release of GABA requires that $[Ca^{2+}]_c$ is elevated in β-cells. However, many studies showed that glucose inhibits glucagon secretion by concentrations that do not yet increase $[Ca^{2+}]_c$ in β-cells. Moreover, glucose inhibits [175,176], stimulates [177], or does not affect GABA release [178], whereas it reduces the islet GABA content [176,179]. All these studies indicate that GABA is not required for the control of glucagon release by glucose. However, they do not exclude a modulating action.

GABA might have very interesting roles. It was suggested that long-term GABA administration induces β-cell neogenesis from α-cells [180]. However, this conclusion is not supported by another study [181]. Others suggested that GABA promotes β-cell proliferation [182]. These discrepancies might be explained by strain and species differences [182].

In the brain, GABA is degraded by GABA transaminase (GABA-T) into succinate semialdehyde (SSA) which can then be transformed by NADPH-dependent SSA reductase into γ-hydroxybutyrate (GHB). Like GABA, GHB is considered as an inhibitory neurotransmitter that would act on a specific GHB receptor [183] or on GABA receptors [184]. It has recently been suggested that GHB released from β-cells is responsible for the glucagonostatic effect of glucose [179]. However, our own experiments do not support this hypothesis (unpublished data).

Recent studies suggested that serotonin is released from human β-cells in response to glucose and inhibits glucagon secretion by decreasing cAMP levels in α-cells via 5-HT1F receptors [185] or acting on other types of 5HT receptors [186].

Somatostatin. α-Cells display signs of close contacts with δ-cells [187]. If they are not in the immediate vicinity of δ-cells, they can also receive filopodia from δ-cells that are several microns away from α-cells [188]. α-Cells are more susceptible than β-cells to a local paracrine influence of somatostatin [189]. Somatostatin-14, the predominant form secreted by pancreatic δ-cells [190], potently inhibits glucagon secretion. Both human and mouse α-cells strongly express SSTR2 and SSTR3 but barely express the other SSTRs [38,120,191]. SSTR2 is even considered as α-cell specific [38,192]. The high and α-cell-specific expression of SSTR2 has sometimes led to believe erroneously that SSTR2 is the only physiologically important SSTR receptor by which somatostatin controls glucagon release [193]. However, experiments using SSTR2/3-selective antagonists suggested also an important role of SSTR3, at least in mice [120]. An additional

contribution of the other weakly expressed SSTR subtypes remains possible [120].

Because all SSTRs are coupled to $G_{i/o}$ protein, the glucagonostatic effect of somatostatin is prevented by pretreatment with pertussis toxin [83,101,194]. Somatostatin inhibits glucagon release by at least three mechanisms [195]. (a) It activates a G protein-gated inwardly rectifying K^+ (GIRK) current (coupled to G_{i2}), leading to membrane hyperpolarization and inhibition of electrical activity [196]. (b) As expected from its coupling to $G_{i/o}$ protein, it inhibits adenylate cyclase activity, leading to a reduction of cAMP levels and PKA-stimulated glucagon release [189]. (c) It also inhibits exocytosis by activating (via G_{i2}) calcineurin, a serine/threonine protein phosphatase [196].

Somatostatin exerts a tonic inhibition on glucagon secretion [60,101,197]. This is supported by the strong stimulation of glucagon release induced by genetic invalidation of SSTR2 or somatostatin [101,120,198], blockade of SSTR2 or SSTR3 [86,120], immunoneutralization of somatostatin by antibodies [199], or treatment with pertussis toxin [101,120]. This tonic inhibition probably explains why it is difficult to see an acute effect of somatostatin applied exogenously [101]. By contrast, in $Sst^{-/-}$ mice, somatostatin strongly inhibits glucagon secretion even at very low concentrations [101,120].

Because glucose stimulates somatostatin secretion [86,197], it was suggested that somatostatin is responsible for the glucagonostatic effect of glucose. As mentioned previously, the observation that the oscillations of glucagon and somatostatin secretion are antiparallel supports this hypothesis. However, other observations speak against it. Indeed, the glucagonostatic effect of glucose persisted in the presence of SSTR2/3 antagonists, a somatostatin antibody [83,86,111,199] or after pretreatment with pertussis toxin [101,200]. We recently tested the involvement of somatostatin in the control of glucagon secretion by various glucose concentrations (0–30 mM) using two different experimental models, pertussis toxin treatment and genetic invalidation of somatostatin ($Sst^{-/-}$ mice) [120]. We found that, in control islets (with paracrine influence of somatostatin), glucose dose-dependently inhibits glucagon secretion, that the maximal inhibition is reached already at 7 mM glucose and that the amplitude of this inhibition remains stable at higher glucose concentrations. By contrast, the dose-response curve is different in mouse islets without paracrine influence of somatostatin and displays a U-shape. The inhibition is maximal at 7 mM glucose, but it progressively wanes at higher glucose concentration in such a way that, at 15 and 30 mM glucose, glucagon secretion is no longer inhibited and similar to glucagon release in the absence of the sugar (Fig. 4). These observations indicate that the glucagonostatic effect of glucose is mainly independent of somatostatin for low glucose concentrations (1–7 mM) but starts to involve somatostatin at high concentrations of the sugar ($\geq$10 mM) [120]. This suggests that the glucagonostatic effect of glucose only partially depends on somatostatin. The observation that somatostatin starts to be involved at glucose concentrations $\geq$10 mM is compatible with the observations that somatostatin is secreted at relatively high glucose concentrations, similar to those that stimulate insulin release [83,197], and that β-cells might entrain δ-cells by electrical coupling [22]. However, one study supports a similar sensitivity of somatostatin and glucagon secretion to glucose [86]. A stimulation of somatostatin secretion by insulin is another possible mechanism of control of glucagon secretion [75].

α- and δ-cells possess K_{ATP} channels [201]. Because K_{ATP} channel blockers strongly stimulate somatostatin release [101,201] but inhibit glucagon secretion when applied in a medium containing a low glucose concentration (i.e., in conditions where glucagon secretion is not inhibited by glucose) [93,101], it is possible that somatostatin is involved in the control of glucagon secretion by K_{ATP} channel blockers. We support this hypothesis because suppression of the somatostatin influence (using islets treated with pertussis toxin or islets of $Sst^{-/-}$ mice) transformed the glucagonostatic effect of tolbutamide into a strong glucagonotropic effect [120] (see, aforementioned, § on the role of K_{ATP} channels).

Combining intrinsic and paracrine influence?

As discussed previously, many mechanisms of control of glucagon secretion by glucose have been suggested. It is very likely that several of them coexist, that their respective contributions depend on the physiological context (such as the level of glycemia), and that, in some situations, glucagon secretion is controlled by redundant mechanisms. My personal view based on experiments on mice is that intrinsic or somatostatin- and insulin-independent mechanisms are more important at low glucose concentrations, and that paracrine influences (mostly from somatostatin) also operate at higher glucose concentrations to inhibit glucagon secretion (Figs. 2 and 4). It is still unclear whether this view is transposable to humans. This complexity of control would be the price to pay to avoid that, under physiological conditions, glycemia drops below a dangerous level or, inversely, increases too high.

Juxtacrine signaling between α- and β-cells

Juxtacrine signaling is a common type of signaling between adjacent cells [202]. Contrary to the endocrine or paracrine signaling, it requires a direct contact between cells. Many types of

communications by juxtacrine signaling exist. Recently, a direct communication between α- and β-cells has been documented. It involves ephrin receptor/ephrin signaling. Stimulation of Eph receptor A4/7 on α-cells by ephrins (possibly ephrinA5) on β-cells correlates with maintenance of a dense F-actin network and maintains glucagon secretion at a low level, which is essential for a proper regulation of glucagon release [203]. In support of this hypothesis, ablation of EphA receptors in α-cells leads to an abnormal and uncontrolled glucagon release. Moreover, isolated α-cells display an uncontrolled glucagon release that can be restored by activation of the EphA receptor signaling in α-cells [84,97,203]. Although this type of juxtacrine signaling seems required for a proper control of glucagon secretion, it is unknown to which extent glucose controls glucagon release by acutely modulating the juxtacrine signaling. It might be speculated that an altered ephrin receptor/ephrin signaling between α- and β-cells contributes to the hyperglucagonemia found in diabetes. A ~50% decrease in ephrinA5 was found in human islets from donors with T2 diabetes [84]. All these results are, however, at variance with the recent observation that the EphA activator ephrinA5 stimulates glucagon release from pure human α-cell aggregates [98].

Glucagon in Diabetes

Impaired glucagon secretion

Diabetic patients (T1D and T2D) exhibit three defects of glucagon secretion: an impaired glucagon response to hypoglycemia [156], a fasting hyperglucagonemia and a postprandial hyperglucagonemia [3,74,204].

The impaired glucagon response occurs often during iatrogenic hypoglycemia. It is frequent in type 1 diabetic patients, caused by an excess of insulin. It also occurs in type 2 diabetic patients, caused by antidiabetic agents, such as sulfonylureas or glinides.

Diabetic patients also suffer from a fasting hyperglucagonemia and a failure to suppress their postprandial glucagonemia adequately [204–207]. The hyperglucagonemia is absolute or relative (because plasma glucagon is often inappropriately high in the context of hyperglycemia). It maintains an inadequately high rate of hepatic glucose production in the fasting state [208], which contributes to up to ~50% of hyperglycemia in diabetes [206]. The impaired glucagon secretion is specific to glucose because diabetic patients display even a larger glucagon response to arginine than control individuals [209]. Interestingly, some, but not all type 2 diabetic patients, have hyperglucagonemia, suggesting that it is not strictly linked to T2D. It would rather be linked to nonalcoholic fatty liver disease (NAFLD) [4].

Many studies corroborate the contribution of hyperglucagonemia to hyperglycemia in diabetes because reduction of glucagon action very efficiently attenuates hyperglycemia in experimental models of diabetes [42,72,74,133,146,206,210–216]. Several studies from the group of Unger have even suggested the provocative glucagonocentric hypothesis according to which glucagon is the *sine qua none* condition of diabetes [74,214]. However, other subsequent studies have disproved this hypothesis. They showed that blockade of glucagon action prevents or reverses diabetes only if residual β-cells persist [211,213]. The lack of insulin remains thus the major factor causing hyperglycemia in β-cell-deficient diabetes [212].

Mechanisms of the altered glucagonemia

Many factors can alter glucagonemia in diabetes (Fig. 1). It is likely that there are distinct causes of impaired glucagon response to hypoglycemia, fasting hyperglucagonemia and postprandial hyperglucagonemia. They are briefly discussed in the following.

- Does the impaired glucagon secretion in response to hypoglycemia result from an impaired autonomous input?

The nervous system largely contributes to the glucagon response to moderate-to-marked hypoglycemia [1]. Hence, an impaired nervous control in long-term diabetic patients who suffer from neuropathy might contribute to the defective glucagon response [217]. An early neuropathy impairing the glucagon response to hypoglycemia has also been documented in T1D but not in T2D [1,218]. In addition, hyperglycemia induced by streptozotocin for only 7 days suppressed celiac ganglia neurotransmission and impaired the sympathetically mediated glucagon response [219].

- Does the impaired glucagon secretion result from an impaired influence from β-cells?

Given that insulin might inhibit glucagon secretion (although it is still debated, see the aforementioned), it has been suggested that the impaired glucagon response to glucose results from an impaired influence of β-cells. This concept is also part of the "switch-off" hypothesis. Diabetes has even been qualified as a disease of paracrinopathy [220]. The paracrine role of insulin is also supported by studies reporting a loss of antiparallel oscillations between insulin and glucagon in type 2 diabetic [221] and prediabetic individuals [222]. This latter observation even suggests that the loss of pulsatile insulin-glucagon crosstalk precedes hyperglycemia. However, based on the β-cell mass, the causal role of β-cells is uncertain at least for T2D. There is indeed a ~35% decrease in the β-cell mass in type 2 diabetic

patients [223–225], which has been estimated too small to explain the excessive glucagon secretion [224]. Another possibility is that hyperglucagonemia results from a resistance of the α-cell to insulin [226]. Thus, chronic glucose infusion in rats induced a hyperglucagonemia which preceded a decline in insulin secretion, suggesting that α-cell dysfunction occurs before any measurable deficit in insulin secretion [227]. However, the opposite situation was also reported in humans. Indeed, a 24 h-experimental hyperglycemia impaired pancreatic β-cell function but did not impair α-cell glucagon secretion in normal glucose-tolerant subjects [228]. Hence, the role of β-cells in the impaired glucagon secretion is still unclear.

- Does the impaired glucagon secretion result from an impaired influence from δ-cells?

Because δ-cells are partly involved in the control of glucagon secretion by glucose, at least in mice [120], it can be speculated that the impaired glucagon secretion in diabetes is due to an impaired paracrine influence from δ-cells. Analyses of the δ-cell mass in both T1D and T2D show that the number of δ-cells is relatively unaffected by diabetes [229,230], or, when the pancreatic somatostatin content is lower in T2 diabetic than in nondiabetic patients, the ratio somatostatin/glucagon content remains similar [224]. An alteration of the δ-cell function in diabetes is a possible alternative mechanism explaining the impaired glucagon release. In support of this hypothesis, long-term exposure of mouse islets to fatty acids (which are often elevated in T2D) induces a decreased somatostatin release associated with an oversecretion of glucagon [66]. Somatostatin secretion is impaired in the perfused pancreas of diabetic GK rats [231] and in high-fat-diet fed mice [188]. Urocortin 3 is one of the first β-cell markers to disappear in prediabetes [148]. Because urocortin 3 coreleased with insulin stimulates somatostatin secretion, it might be speculated that the lower expression of urocortin 3 is associated with a lower somatostatin release and hence hyperglucagonemia. All these data suggest that hyperglucagonemia in diabetes results from an attenuated glucagonostatic influence of somatostatin. By contrast, it was suggested that a defective glucagon response to low glucose results from an exacerbated somatostatin tone because SSTR2 antagonists improve the glucagon response to hypoglycemia in diabetic rats [81,232,233]. The discrepant conclusions of all these studies indicate that the role of δ-cells in the impaired glucagon secretion in diabetes remains enigmatic.

- Does the hyperglycagonemia result from an increased absolute number of α-cells?

Chronic hyperglycemia increases the proportion of α-/β-cells in mice [234]. The proportion of α-cells per islet is also increased in T2 diabetic patients because of a ~35% decrease in the β-cell mass [223,225,235]. However, there are controversies regarding the changes in the absolute α-cell mass. It was found to be higher in T2 diabetic than nondiabetic patients [225] whereas other studies reported that it was identical in both groups [224].

- Does the impaired glucagon secretion result from a defect intrinsic to α-cells, caused by hyperglycemia?

The observation that intravenous glucose infusion (but not oral administration, see later) decreases glucagonemia in diabetic patients and normoglycemic individuals suggest that α-cells are still glucose responsive in diabetes [205,236,237]. However, it is unclear whether they are less sensitive to glucose because the amount of glucose that was infused was larger in type 2 diabetic patients than in normal individuals. *In vitro* experiments showed that there might be an impaired sensitivity to glucose because a glucose concentration that was glucagonostatic on islets from control individuals was ineffective [238] or glucagonotropic [83] on islets from type 2 diabetic patients. This also shows that the defect persists in culture. A recent study suggests that the deleterious effect of chronic hyperglycemia results from a Na^+-dependent reduction of ATP production that increases protein succination because of reduced activity of fumarase, a key enzyme of the Krebs cycle [238]. An influence of non-α-cells in the establishment of this deleterious effect remains possible. Another study showed that α-cells of streptozotocin-induced diabetic mice with residual β-cell mass hypersecrete glucagon because of increased electrical activity associated with altered electrophysiological characteristics [239]. Surprisingly, RNA sequencing of single human α-cells did not reveal changes in the expression of genes implicated in glucose sensing in T2D [2].

- Is the impaired glucagon secretion secondary to hyperlipidemia?

As discussed previously, hyperglycemia is a probable culprit. Hyperlipidemia, as found in T2D, might also be a causal factor. Thus, long-term exposure to fatty acids (such as palmitate or oleate) increases glucagon secretion of mouse and rat islets and αTC1-cells [68], and abolishes the glucagonostatic effect of glucose [66]. The hypersecretion of glucagon after prolonged exposure to palmitate might be mediated by FFAR1/GPR40 activation and/or by a decreased paracrine influence of somatostatin [66,240]. Thus, elevated plasma FFA levels might exacerbate the hyperglucagonemia associated with T2D.

- Does the impaired glucagon secretion result from the appearance of α-cells with decreased identity?

Accumulating evidences suggest that β-cells can dedifferentiate in diabetic humans [241] and mice [234]. They might adopt α-cell characteristics. The opposite is also true: α-cells can adopt features of β-cells in animal models of β-cell depletion [242,243],

in diabetes [244], by manipulation of the histone methylation signature (epigenetic markers) [192], or in response to GABA [180]. Hyperglycemia could be a trigger for the loss of identity. Indeed, experimentally induced chronic hyperglycemia in mice produced a 20-fold increase in the number of bihormonal insulin/glucagon positive cells which is reversed when glycemia is normalized ([234] but see also Ref. [245]). This also occurs in humans. Indeed, a loss of β-cell glucose sensitivity in nondiabetic individuals is also correlated to the appearance of bihormonal insulin/glucagon positive cells suggesting that α-cells might transdifferentiate into β-cells in an attempt to cope with a higher demand of insulin secretion [246]. Bihormonal glucagon/insulin cells or double positive glucagon/Nkx6.1 (a β-cell-specific transcription factor) have also been observed in T2 diabetic patients [247]. Moreover, α-cells express much more frequently aldehyde dehydrogenase 1A3 (ALDH1A3), a progenitor cell marker, in islets from T2 diabetic patients than in control individuals [235]. Glucagon-positive cells with loss of α-cell identity could therefore secrete glucagon in an abnormal way. However, it is unknown to what extent these changes in identity really contribute to the impaired glucagon secretion.

- Does extrapancreatic glucagon contribute to hyperglucagonemia?

There are indications that extrapancreatic glucagon contributes to the postprandial hyperglucagonemia in diabetes. Indeed, oral or intravenous administration of glucose decreases glucagonemia in normal individuals. However, in type 2 diabetic patients, oral administration transiently increases glucagonemia before decreasing it, whereas intravenous glucose infusion mimicking the glycemic changes induced by oral glucose administration (isoglycemic infusion) does not increase glucagonemia but immediately decreases it [6,205,236,237]. The appearance of the transient increase in glucagonemia seen only after an oral glucose load suggests that it comes from the gut. Indeed, 50% more PC2-positive cells are found in the small intestine of T2 diabetic patients as compared with healthy controls [6,248]. They might correspond to L-cells which are stimulated by intestinal nutrients.

- Is the hyperglucagonemia secondary to glucagon resistance in the liver?

There are several arguments in favor of this hypothesis [3,4,34,36,249–251], at least when T2D is associated with liver steatosis. Indeed, a hyperaminoacidemia occurs in liver diseases [250], similarly to what is observed after blockade or disruption of the glucagon receptor [36]. Because amino acids (such as alanine, glutamine) stimulate glucagon release, the hyperaminoacidemia would be responsible for the hyperglucagonemia which may be viewed as a compensatory response helping to normalize amino acid turnover. The hyperaminoacidemia can be consecutive to a state of hepatic glucagon resistance which is reflected by a higher glucagon/alanine index. The glucagon resistance would reflect the inability of glucagon to stimulate amino acid metabolism, but not hepatic glucose production. This glucagon resistance might be caused by hepatic steatosis which is worsened by insulin resistance [4,249]. Moreover, fasting hyperglucagonemia can also occur independently of a diabetic state and is observed in other cases of liver diseases (associated to hepatic glucagon resistance) [6], suggesting that fasting hyperglucagonemia is not specific to diabetes. Interestingly, it has long ago been proposed that glucagon resistance contributes to hyperlipidemia [252]. The complexity of this hypothesis is that it is unclear how the hepatic glucagon resistance would inhibit amino acid metabolism without affecting hepatic glucose production.

In summary, many factors can impair glucagon secretion or alter glucagonemia. It is therefore difficult to figure out whether the alteration of glucagonemia in diabetes results from an alteration of the α-cell function itself and/or reflects an alteration of factors extrinsic to the α-cell disturbing glucagonemia. It is likely that the defective glucagon secretion in diabetes is multifactorial, and even includes other factors that were not listed previously, such as inflammation, reactive oxygen species, ….

Moreover, the recent demonstration that α-cell promotes insulin secretion particularly in conditions of metabolic stress [7,62] raises several questions. Does the impaired α-cell function in diabetes aggravate the impaired insulin secretion, or does the hyperglucagonemia represent a compensatory mechanism aiming at preserving insulin secretion in face of the metabolic demand of the β-cell [10]? In that context, it is noteworthy that α-cells are much more resistant than β-cells to metabolic stress [253].

Therapeutic Puzzle: Should We Inhibit or Stimulate Secretion of Proglucagon-derived Peptides From α-Cells in Diabetes?

On the sole basis of the hyperglycemic action of glucagon and the hyperglucagonemia in diabetes, there is a rationale to inhibit glucagon secretion and/or action. There is also a rationale to stimulate glucagon secretion and/or action. In T1D and T2D with β-cell failure, glucagon secretion is not adequately stimulated by hypoglycemia (most frequently caused by antidiabetic therapy) [156]. Therefore, it seems relevant to stimulate glucagon release only when glycemia drops. In that context, the ideal drug should exert a glucose-dependent action, which is

far from being an easy task: an inhibition of the hyperglucagonemia at high glucose and a stimulation of the glucagon response to low glucose.

On another hand, T2D is also characterized by insufficient insulin secretion (and/or action). Because proglucagon-derived peptides secreted by α-cells are essential for an optimal insulin secretion in response to glucose [7–11], it seems logical to stimulate secretion of these peptides from α-cells, but only when glycemia is high. Moreover, glucagon (at least at supraphysiological doses) exerts effects that might be useful to combat obesity and T2D, such as decreased lipidemia, stimulation of energy expenditure and decreased food intake and weight [12].

As can be seen from these considerations, it is currently impossible to normalize glycemia of all diabetic patients by targeting glucagon secretion/action with one single class of agents because the correction of one defect might aggravate another one. A wise solution is probably to adapt the therapeutic treatments to the characteristics and needs of the patients.

Effect of Common Antidiabetic Therapies on Glucagon

The antiglucagon treatments (antisense oligonucleotides, glucagon receptor antagonists, glucagon receptor antibodies) are very efficient to decrease glycemia [74,133,206,210,212,214,216,254] and several clinical trials have even been performed [255,256]. Part of these beneficial effects are the consequences of compensatory mechanisms occurring on glucagon action inhibition, such as increased production of GLP-1 by α-cells, of FGF-21, etc [10,257]. However, in the long term, a blockade of glucagon action induces undesired effects such as increased plasma LDL cholesterol levels, hepatic steatosis, increased frequency of hypoglycemia, α-cell hyperplasia that might potentially be malignant … [2,12,32,212,258,259]. α-Cell hyperplasia is also observed in patients with inactivating mutations of the GcgR (Mahvash disease) [260]. It is likely caused by hyperaminoacidemia due to the disruption of the liver-α-cell axis involving glucagon [34,251,261,262]. However, a recent study showed that the proliferative property of GcgR antagonists is severely restricted with advanced age [263]. Despite these side-effects, glucagon receptor inhibition is a very attractive way to normalize blood glucose in patients with severe insulin-resistant states such as inactivating mutations of the insulin receptor [216].

In the following paragraphs, I will not review in-depth the usefulness of antiglucagon or multiagonists therapies (including GLP-1, GIP and/or glucagon, or oxyntomodulin) in the treatment of T2D and obesity [264,265]. The rational for using such multiagonist strategies is to take the beneficial effects of glucagon on weight loss (through a decrease in food intake and an increase in energy expenditure) provided that the hyperglycemic effect of glucagon is mitigated by another hormone such as GLP-1. Extensive reviews can be found on that topic [12,266]. Thus, I will only discuss main therapies that affect glucagon secretion. Information about less-common therapies (such as amylin analogs) or potential therapeutic targets (such as GPR119 agonists) can be found elsewhere [267,268].

- ***Sulfonylureas***: These drugs stimulate insulin and somatostatin secretion. However, no clear effect of these compounds on glucagonemia is documented. Glimepiride did not affect the glucagon response to hypoglycemia whereas glyburide decreased it during a hyperinsulinemic hypoglycemic clamp [269,270]. Variable effects have also been reported *in vitro.* Namely, sulfonylureas stimulated [90], did not affect [271] or inhibited [110,272] glucagon secretion. Our experiments suggest that this variability might be explained by their two mechanisms of action, direct on α-cells and indirect through somatostatin secretion by δ-cells (see the aforementioned). An additional level of complexity is added given that sulfonylureas can also affect glucagon secretion indirectly, by acting on K_{ATP} channel-expressing neurons in the brain [112].
- ***GLP-1***: GLP-1 receptor agonists are commonly used to treat diabetes [47,273]. They stimulate insulin and somatostatin secretion and decrease glucagon release [199]. The glucagonostatic and insulinotropic effects of GLP-1R agonists, which contribute equally to their glucose-lowering action at pharmacological concentrations [274], both display the interesting property of being glucose-dependent. Thus, contrary to sulfonylureas that stimulate insulin release even at low glucose, GLP-1R agonists amplify insulin secretion and inhibit glucagon secretion only at normal or elevated glycemia [50], thereby reducing the risk of hypoglycemia.
- ***DPP-4 inhibitors***: Because dipeptidyl peptidase IV (DPP-4) rapidly degrades GLP-1, DPP-4 inhibitors are routinely used to treat diabetes [273,275]. Because DPP-4 also degrades GIP, its inhibition increases plasma levels of GIP, the other incretin with a glucagonotropic action [276]. The glucagonotropic effect of GIP is glucose-dependent, stronger at low than at high glucose [277], and is very suitable to stimulate glucagon secretion during hypoglycemic episodes. DPP-4 inhibition also prevents the transformation of GIP into GIP(3–30)NH_2, a potent antagonist of the GIP receptor [278]. Therefore, DPP-4 inhibitors combine two beneficial effects: a glucagonostatic

effect of GLP-1 during hyperglycemia and a glucagonotropic effect of GIP during hypoglycemia [279]. Although GIP effects are largely lost in patients with T2D [265,273,280,281], dual GLP-1/GIP receptor agonists exert very beneficial effects for treatment of diabetes and obesity [282]. Because α-cells could secrete GLP-1 in metabolic stress conditions, DPP-4 inhibitors could increase the paracrine influence of GLP-1 on β-cells, thereby improving glucose homeostasis [62]. Interestingly, DPP-4 is expressed in human islet α- and β-cells, and its expression is reduced in type 2 diabetic islets [283]. It is worth noting that DPP-4 inhibitors also prevent the degradation of other peptides with metabolic effects, such as PYY, oxyntomodulin, and PACAP [284].

- ***SGLT-2 inhibitors***: The sodium-dependent glucose transporter 2 (SGLT2 encoded by *Slc5a2*) is responsible for 90% of glucose reabsorption by the kidney tubules. Several studies in humans reported that the SGLT2 inhibitors (SGLT2i) dapagliflozin and empagliflozin increase glucagonemia and stimulate the hepatic production of glucose [285,286]. The mechanisms responsible for increased glucagonemia are highly contested [287,288]. Some studies suggest that it results from a direct effect of the gliflozins on α-cells which would express SGLT2 [289]. A side-action of SGLT2i on SGLT1 [290], or an indirect influence of the gliflozins via δ-cells have also been suggested [75]. However, other studies reported no expression of SGLT2 and low expression of SGLT1 in α-cells [38,48,288], and no glucagonotropic effects of the gliflozins, suggesting that the increase in glucagonemia induced by the gliflozins *in vivo* would simply result from their glucose-lowering effect [38,288]. To further complicate the story, one group suggested that the hyperglucagonemia induced by dapagliflozin results from an effect of the drug in the brain [291], whereas another group suggested the opposite, that is, that dapagliflozin lowers blood glucose levels in part by suppressing hepatic glucagon signaling [292]. All these discrepancies call for further investigations.

Conclusion

The field of glucagon is one of the most controversial. The reasons are multiple. (a) Proglucagon is the precursor of two peptides with opposite actions on blood glucose: glucagon which is hyperglycemic, and GLP-1 which exerts glucose-lowering effects. α- and L-cells are the major sources of glucagon and GLP-1, respectively. However, in specific situations (metabolic diseases or adaptations), α-cells can produce GLP-1, and L-cells can produce glucagon. Hormone secretion by these two cell types is controlled by glucose in an opposite way. (b) Glucagon acts on its own receptor but also on GLP-1 receptors. (c) Glucagon exerts metabolic effects that go beyond simply glucose homeostasis. All these effects, in particular, the regulation of aminoacidemia, in turn affect glucagonemia. (d) Glucagon seems essential for an optimal insulin secretion in response to glucose, and it enhances insulin-stimulated glucose disposal. These glucose-lowering effects are opposite to the classical hyperglycemic effects attributed to glucagon. (e) Glucagon, which used to be considered as a "bad guy" in diabetes and obesity because of its hyperglycemic effect, is now considered as a "good guy" thanks to its beneficial effects on lipid metabolism, satiety, and energy expenditure (at least at supraphysiological doses). (f) Glucagon secretion is controlled not only by the autonomic nervous system in case of hypoglycemia, but also by glucose itself at the islet level. As of today, there is no clear understanding of the mechanisms by which glucose controls glucagon release at the islet level. This makes it difficult to understand how glucagon secretion is altered in diabetes.

Compounding the story, it has been suggested that there are misconceptions about the α-cell physiology in diabetes, and that the α-cell response to glucose is not really impaired in T2D. Some arguments support this provocative hypothesis [3,4]. Briefly, not all type 2 diabetic patients exhibit fasting hyperglucagonemia. NAFLD rather than diabetes is associated with hyperglucagonemia. The cause of hyperglucagonemia would be the hyperaminoacidemia (because of hepatic glucagon resistance) that stimulates glucagon secretion in a normal way. On the other hand, the hyperglucagonemia in T2D after a meal rich in carbohydrates would be due to extrapancreatic glucagon. It would be transient and the glucagonemia would drop thereafter normally.

As can be seen from this review, many challenges still need to be addressed to fully understand the glucagon physiology.

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Duality of interests

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Keywords:
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Abbreviations used:
DPP-4, dipeptidyl peptidase IV; ER, endoplasmic reticulum; FFA, free fatty acid; GABA, γ-aminobutyric acid; GcgR, glucagon receptor; GHB, γ-hydroxybutyrate; GLP-1R, GLP-1 receptor; GIP, gastric-inhibitory polypeptide or glucose-dependent insulinotropic polypeptide; GLP-1, glucagon-like peptide 1; K_{ATP} channel, ATP-sensitive K^+ channel; NAFLD, non-alcoholic fatty liver disease; PC, prohormone convertase; SERCA, sarco-endoplasmic reticulum Ca^{2+}-ATPase; SGLT, sodium-dependent glucose transporter; SOC, store-operated current; SSTR, somatostatin receptor; T1D, type 1 diabetes; T2D, type 2 diabetes.

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