REVIEW

Physiology of the pancreatic α -cell and glucagon secretion: role in glucose homeostasis and diabetes

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Abstract

The secretion of glucagon by pancreatic α -cells plays a critical role in the regulation of glycaemia. This hormone counteracts hypoglycaemia and opposes insulin actions by stimulating hepatic glucose synthesis and mobilization, thereby increasing blood glucose concentrations. During the last decade, knowledge of α -cell physiology has greatly improved, especially concerning molecular and cellular mechanisms. In this review, we have addressed recent findings on α -cell physiology and the regulation of ion channels, electrical activity, calcium signals and glucagon release. Our focus in this review has been the multiple control levels that modulate

glucagon secretion from glucose and nutrients to paracrine and neural inputs. Additionally, we have described the glucagon actions on glycaemia and energy metabolism, and discussed their involvement in the pathophysiology of diabetes. Finally, some of the present approaches for diabetes therapy related to α -cell function are also discussed in this review. A better understanding of the α -cell physiology is necessary for an integral comprehension of the regulation of glucose homeostasis and the development of diabetes.

Journal of Endocrinology (2008) **199**, 5–19

Introduction

The principal level of control on glycaemia by the islet of Langerhans depends largely on the coordinated secretion of glucagon and insulin by α - and β -cells respectively. Both cell types respond oppositely to changes in blood glucose concentration: while hypoglycaemic conditions induce α -cell secretion, β -cells release insulin when glucose levels increase (Nadal *et al.* 1999, Quesada *et al.* 2006a). Insulin and glucagon have opposite effects on glycaemia as well as on the metabolism of nutrients. Insulin acts mainly on muscle, liver and adipose tissue with an anabolic effect, inducing the incorporation of glucose into these tissues and its accumulation as glycogen and fat. By contrast, glucagon induces a catabolic effect, mainly by activating liver glycogenolysis and gluconeogenesis, which results in the release of glucose to the bloodstream. An abnormal function of these cells can generate failures in the control of glycaemia, which can lead to the development of diabetes (Dunning *et al.* 2005). Actually, diabetes is associated with disorders in the normal levels of both insulin and glucagon. An excess of glucagon plasma levels relative

to those of insulin can be determinant in the higher rate of hepatic glucose output, which seems to be critical in maintaining hyperglycaemia in diabetic patients (Dunning *et al.* 2005).

Despite the importance of the α -cell and glucagon secretion in the regulation of glycaemia and nutrient homeostasis, little is known about the physiology of these cells compared with the overwhelming information about β -cells. Several factors may explain this lack of information about glucagon secretion. First, the scarcity of this cell population in islets of animal models such as mice and rats along with several technical limitations of conventional methods have made it more difficult to study α -cells than β -cells (Quoix *et al.* 2007). Second, the lack of functional identification patterns has also been an important limitation in α -cell research. However, in recent years notable progress has been made in the study of α -cell function at the cellular and molecular levels. This review attempts to describe recent advances in α -cell physiology and the regulation of glucagon secretion. Additionally, it focuses on the pathophysiology of these cells, their role in diabetes, as well as potential therapeutic strategies.

Islet of Langerhans: cell architecture and function

Glucagon-secreting α -cells are one of the main endocrine cell populations that coexist in the islet of Langerhans along with insulin-secreting β -cells. The islet is further composed by other scarce secretory populations such as δ - and polypeptide releasing (PP)-cells, which release somatostatin and pancreatic polypeptide respectively. This multicellular structure constitutes the endocrine unit of the pancreas and is responsible for the regulation of blood glucose homeostasis. Approximately one million islets are distributed throughout a healthy adult human pancreas, representing 1 and 2% of the total mass of the organ. Each islet, with sizes varying from 100 to 500 μm , is made up of 1000–3000 cells. In mouse and rat islets, β -cells are the main population accounting for 60–80% of the total number of cells, while 15–20% are α -cells, <10% are δ -cells and less than 1% correspond to the PP-cell population (Brelje *et al.* 1989, Brissova *et al.* 2005). The architecture of rodent islets is characterized by the location of β -cells in the core and the non- β cells distributed in a mantle around the insulin-secreting cell population. This cellular distribution along with several studies on microcirculation within the islet suggests that the order of paracrine interactions is from β - to α - and δ -cells (Bonner-Weir 1991). The rich vascularization within the islet ensures a rapid sensing of plasma glucose levels by these endocrine cells, allowing an appropriate secretory response. In human islets, however, there are important differences in composition and spatial organization compared with rodents (Cabrera *et al.* 2006). While the proportion of δ - and PP-cells are similar in the human islet, β -cells are less abundant (48–59%) and the α -cell population reaches a 33–46%, suggesting that glucagon secretion plays a major role in humans (Cabrera *et al.* 2006). These islet cell populations show a random distribution pattern, where the majority of β -cells are in contact with non- β -cells, suggesting that paracrine interactions among different populations may be more active (Cabrera *et al.* 2006). Another divergence between human and rodent islets is the intercellular communication among the different populations. In mice, β -cells work as a syncytium in terms of electrical activity and Ca^{2+} signalling due to the high level of coupling mediated by gap junctions of connexin36 (Gopel *et al.* 1999, Nadal *et al.* 1999, Quesada *et al.* 2003). This coupling favours a more vigorous insulin secretion (Vozzi *et al.* 1995). By contrast, coupling can be found between several human β -cells in clusters within the same islet but not in the whole β -cell population (Quesada *et al.* 2006b). This kind of intercellular communication is probably the result of the human islet cytoarchitecture and its functional meaning is still unknown (Cabrera *et al.* 2006). Unlike β -cells, α - and δ -cells from rodents and humans are not functionally coupled and work as independent units. In addition to nutrients and paracrine signals, islet function is further regulated by sympathetic, parasympathetic and sensory nerves that go

deeply into the islet (Ahren 2000). Thus, multiple regulation levels determine hormone release from pancreatic islets.

Glucagon secretion by pancreatic α -cells

Stimulus-secretion coupling in α -cells: from ion channel activity to exocytosis

Pancreatic α -cells are equipped with a specific set of channels that generate action potentials of Na^+ and Ca^{2+} in the absence or at low levels of glucose (Gromada *et al.* 1997). This electrical activity triggers Ca^{2+} signals and glucagon secretion. Elevated glucose concentrations inhibit all these events. ATP-dependent K^+ (K_{ATP}) channels play a fundamental role in α -cells, such as they do in β -cells, since they couple variations in extracellular glucose concentrations to changes in membrane potential and electrical activity. In intact rat α -cells, K_{ATP} channels have a lower ATP sensitivity ($K_i = 0.94 \text{ mM}$) than the one observed in excised patches ($K_i = 16 \mu\text{M}$; Bokvist *et al.* 1999, Gromada *et al.* 2007), but a very similar one to the values recorded in mouse and rat intact β -cells. However, K_{ATP} channels exhibit a higher ATP sensitivity in intact mouse α -cells ($K_i = 0.16 \text{ mM}$; Leung *et al.* 2005). Consequently, lower ATP concentrations are required to obtain the maximal inhibition of K_{ATP} conductance compared with mouse β -cells. Recent evidence has indicated that the densities of these channels are similar in mouse α - and β -cells (Leung *et al.* 2005). The repolarization of action potentials is mediated by voltage-dependent K^+ channels. While delayed rectifying K^+ channels have been demonstrated in rat, mouse and guinea pig α -cells, a tetraethylammonium-resistant voltage-dependent K^+ current (A-current) has only been identified in mice (Barg *et al.* 2000, Leung *et al.* 2005). Furthermore, tetrodotoxin-sensitive Na^+ currents are fundamental for the generation of action potentials in these cells. Na^+ channels are activated at voltages above -30 to -20 mV (Gopel *et al.* 2000), and their blockade by tetrodotoxin leads to the inhibition of glucagon secretion (Gromada *et al.* 2004, Olsen *et al.* 2005, MacDonald *et al.* 2007). Additionally, α -cells have a heterogeneous presence of Ca^{2+} channel subtypes with different roles. While L and N channels have been reported in rat α -cells (Gromada *et al.* 1997), mouse α -cells express L-, T-, N- and probably R-type Ca^{2+} channels (Gopel *et al.* 2000, Gromada *et al.* 2004, Pereverzev *et al.* 2005, MacDonald *et al.* 2007). The low voltage-activated T-type channels work as pacemakers in the initiation of action potentials in mice (Gopel *et al.* 2000). They open around -60 mV , the action potential initiation threshold in α -cells. The high voltage-activated L and N channels open during action potentials when the membrane potential exceeds -40 to -30 mV . Although most of the Ca^{2+} current goes through L-type channels in α -cells, the Ca^{2+} required for exocytosis at low-glucose levels is mediated by N-type channels, and their blockade by ω -conotoxin-GVIA inhibits glucagon secretion in these

conditions (Gromada *et al.* 1997, 2004, Olsen *et al.* 2005, MacDonald *et al.* 2007). However, in the presence of cAMP-elevating agents, L channels are the major conduit for Ca^{2+} (Gromada *et al.* 1997).

A model to explain the glucose regulation of electrical activity in mouse α -cells has been postulated in the light of recent studies (Fig. 1). At low-glucose levels, the activity of K_{ATP} channels renders a membrane potential of about -60 mV. At this voltage, T-type channels open, which depolarize the membrane potential to levels where Na^+ and N-type Ca^{2+} channels are activated, leading to regenerative action potentials (Gromada *et al.* 2004, MacDonald *et al.* 2007). Ca^{2+} entry through N-type channels induces glucagon secretion. The repolarization of action potentials is mediated by the flowing of K^+ A-currents. At low-glucose concentrations, this electrical activity triggers oscillatory Ca^{2+} signals in both human and mouse α -cells in intact islets (Nadal *et al.* 1999, Quesada *et al.* 1999, 2006b; Fig. 2).

Figure 1 Schematic model for glucose-dependent regulation of glucagon secretion in the mouse α -cell. Glucose is incorporated into the α -cell by the transporter SLC2A1. At low-glucose concentrations, the moderate activity of K_{ATP} channels situates the α -cell membrane potential in a range that allows the opening of voltage-dependent T- and N-type Ca^{2+} channels and voltage-dependent Na^+ channels. Their activation triggers action potentials, Ca^{2+} influx and exocytosis of glucagon granules. The opening of A-type K^+ channels is necessary for action potential repolarization. However, high-glucose concentrations elevate the intracellular ATP/ADP ratio, blocking K_{ATP} channels and depolarizing the membrane potential to a range where the inactivation of voltage-dependent channels takes place. This results in the inhibition of electrical activity, Ca^{2+} influx and glucagon secretion. The function of L-type channels predominates when cAMP levels are elevated. See text for further details.

Figure 2 Opposite Ca^{2+} signalling patterns in α - and β -cells in response to glucose. At low-glucose concentrations (0.5 mM), electrical activity triggers oscillatory Ca^{2+} signals in α -cells that lead to glucagon release. Elevation of glucose levels (11 mM) inhibits all these events. By contrast, 11 mM glucose stimulate Ca^{2+} signalling and insulin secretion in β -cells. Both fluorescence records were obtained by confocal microscopy from two cells within an intact mouse islet. Inset shows a thin optical section (~ 6 μm) of a mouse islet loaded with the Ca^{2+} -sensitive fluorescent probe Fluo-3.

However, the increase in extracellular glucose levels rises the cytosolic ATP/ADP ratio which blocks K_{ATP} channels, depolarizing α -cells to a membrane potential range where the channels involved in action potentials become inactivated (Gromada *et al.* 2004, MacDonald *et al.* 2007). As a consequence, electrical activity, Ca^{2+} signals and glucagon secretion are inhibited (Figs 1 and 2). Thus, glucagon release from α -cells is mainly supported by an intermediate K_{ATP} channel activity that maintains a membrane potential range able to sustain regenerative electrical activity (MacDonald *et al.* 2007). A similar model has been also proposed for human α -cells (MacDonald *et al.* 2007). Nevertheless, this scheme has been argued by some reports indicating that glucose may be hyperpolarizing rather than depolarizing (Liu *et al.* 2004, Manning Fox *et al.* 2006). It has also been proposed that glucose would inhibit glucagon secretion by suppressing a depolarizing Ca^{2+} store-operated current independent of K_{ATP} channels (Liu *et al.* 2004, Vieira *et al.* 2007).

In rat α -cells, the activity of K_{ATP} channels at low-glucose concentrations also keeps the membrane potential at about -60 mV, where spontaneous Na^+ and Ca^{2+} action potentials are produced (Gromada *et al.* 1997). However, in contrast to the situation in mice, the stimulus-secretion coupling in rat α -cells is similar to that of β -cells. That is, elevations of extracellular glucose levels increase the intracellular ATP/ADP ratio, blocking K_{ATP} channels and depolarizing the membrane potential, which stimulates Ca^{2+}

influx through N channels and glucagon secretion (Franklin *et al.* 2005, Olsen *et al.* 2005). Accordingly, the pharmacological inhibition of glucose metabolism increases K_{ATP} channel activity in rat α -cells (Olsen *et al.* 2005). This model indicating a β -cell-like stimulus-secretion coupling is based on recent studies that have used isolated rat α -cells. However, these results contrast with the observations showing that glucose inhibits α -cell electrical activity and glucagon secretion in intact rat islets (Franklin *et al.* 2005, Manning Fox *et al.* 2006). Therefore, the blocking effect observed in rat islets at high-glucose concentrations is most likely the result of paracrine signalling by β -cell activation (Wendt *et al.* 2004).

Regulation of α -cell function by glucose: direct or paracrine effect?

Whether glucose inhibits α -cells directly or by paracrine mechanisms has been a matter of debate, and, probably, the predominant level of control may depend on the physiological situation. Part of this controversy is also due to the divergences found in the stimulus-secretion coupling of different animal models. Although paracrine signalling may be critical for the glucose inhibition of glucagon secretion in rats (Wendt *et al.* 2004, Franklin *et al.* 2005, Olsen *et al.* 2005), a direct effect has been observed in mice and humans (Asplin *et al.* 1983, MacDonald *et al.* 2007). In mice and humans, a glucose direct action on α -cells has been proven in isolated cells under conditions where paracrine effects are negligible, and in intact islets incubated with different paracrine signalling inhibitors (Gromada *et al.* 2004, Ravier & Rutter 2005, Shiota *et al.* 2005, MacDonald *et al.* 2007, Vieira *et al.* 2007). Moreover, secretion studies prove that glucose inhibits glucagon release at concentrations below the threshold for β -cell activation and insulin release (MacDonald *et al.* 2007, Vieira *et al.* 2007).

Several reports on experiments using genetic mouse models support the role of glucose-modulated K_{ATP} channels in α -cell function. The regulation of glucagon secretion by glucose is impaired in ABCC8-deficient mice lacking functional K_{ATP} channels (Gromada *et al.* 2004, Munoz *et al.* 2005). A similar situation occurs in KCNJ11Y12X mouse with a KCNJ11 mutation in the K_{ATP} channel (MacDonald *et al.* 2007). In humans, the Glu23Lys polymorphism in the KCNJ11 subunit of these channels is associated with diminished suppression of glucagon release in response to hyperglycaemia (Tschritter *et al.* 2002). Nevertheless, since K_{ATP} channels seem to be essential for the α -cell regulation in the proposed models, some considerations on glucose metabolism should be taken into account. Although α -cells possess the high-affinity, low-capacity glucose transporter SLC2A1, instead of the high-capacity SLC2A2 present in the β -cell, it has been demonstrated that glucose transport is not a limiting factor in α -cell glucose metabolism (Gorus *et al.* 1984, Heimberg *et al.* 1995, 1996). However, several studies indicate that important biochemical differences exist between both cell types. While the ratio of lactate dehydrogenase/mitochondrial glycerol phosphate dehydrogenase is low in the β -cell, this

ratio is higher in non- β cells (Sekine *et al.* 1994). Additionally, α -cells may express higher levels of the lactate/monocarboxylate transporter than β -cells but lower ones of pyruvate carboxylase (Sekine *et al.* 1994, Zhao *et al.* 2001). These biochemical differences indicate that β -cells are more efficient in the mitochondrial oxidation of glucose, while α -cells rely more on anaerobic glycolysis (Schuit *et al.* 1997, Quesada *et al.* 2006a). This lower coupling between glycolytic events in the cytosol and ATP synthesis in mitochondrial respiration of α -cells would explain the fact that, in response to glucose, cytosolic ATP increases are small in these cells (Ishihara *et al.* 2003, Ravier & Rutter 2005) and that ATP/ADP changes are almost invariable (Detimary *et al.* 1998). Therefore, some aspects at the above-mentioned models for α -cell stimulus-secretion coupling deserve more attention, especially those concerning the modulation of K_{ATP} channel activity by glucose metabolism and ATP production. Other mechanisms regulating K_{ATP} channels may also have an important role.

Regulation of glucagon secretion by fatty acids and amino acids

Although the lipotoxicity theory and its role in obesity-induced diabetes have increased the interest in the interactions between fatty acids and islet functions, little is known about their effect on the regulation of the α -cell compared with those on β -cells. While initial studies suggested an inhibitory effect on glucagon secretion (Andrews *et al.* 1975), more recent investigations have indicated that short-term exposure to fatty acids stimulates the release of this hormone (Bollheimer *et al.* 2004, Olofsson *et al.* 2004, Hong *et al.* 2005). The short-term stimulatory action depends on the chain length, spatial configuration and degree of saturation of the fatty acid (Hong *et al.* 2005). The action of palmitate has been studied in mice at the cell level. This fatty acid increases α -cell exocytosis by enhancing Ca^{2+} entry through L-type Ca^{2+} channels and also, by relief of the inhibitory paracrine action of the somatostatin secreted from δ -cells (Olofsson *et al.* 2004). A study using clonal α -cells on the long-term effect of palmitate and oleate concluded that they also enhance glucagon secretion and triglyceride accumulation in a time- and dose-dependent manner but inhibit cell proliferation (Hong *et al.* 2007). In agreement with this, the long-term exposure of rat islets to fatty acids induces a marked increase in glucagon release, a decrease in glucagon content and no changes in glucagon gene expression (Gremlich *et al.* 1997, Dumonteil *et al.* 2000). In addition to fatty acids, amino acids are also relevant in the modulation of the α -cell function. Amino acids such as arginine, alanine and glutamine are potent stimulators of glucagon secretion (Pipeleers *et al.* 1985, Kuhara *et al.* 1991, Dumonteil *et al.* 2000). However, a few amino acids such as isoleucine can also inhibit α -cell secretion while leucine has a dual effect: it is a positive stimulus at physiological concentrations but becomes a negative one at elevated levels (15 mM; Leclercq-Meyer *et al.* 1985). In any case, the function of amino acids and fatty acids in the α -cell requires further investigation at the cellular and molecular levels.

Autocrine, paracrine, endocrine and neural regulation of glucagon secretion

Autocrine, paracrine and endocrine signalling The spatial distribution of α -cells and the vascular organization within the islet sustain an important intercellular communication through autocrine and paracrine mechanisms (Fig. 3). In addition to insulin, glucagon or somatostatin, secretory granules from islet cells contain other molecules with biological activity, which are released to the extracellular space by exocytosis, activating surface receptors in the same cell, in neighbouring islet cells, or in distant cells within the islet via the vascular system. Several paracrine mechanisms are activated at high-glucose concentrations as a result of β - and δ -cell stimulations, and thus, they may participate in the glucose-induced inhibition of glucagon release.

Insulin and zinc. One of the most important paracrine mechanisms responsible for inhibiting glucagon release is conducted by insulin, acting via several pathways. An appropriate expression of the insulin receptor in mouse α -cells seems to be essential for glucose-regulated glucagon secretion (Diao *et al.* 2005). In INR1-G9 clonal α -cells, insulin has been found to inhibit glucagon release through the activation of phosphatidylinositol 3-kinase (PIK3; Kaneko *et al.* 1999). The insulin receptor–PIK3 signalling pathway is also involved in the modification of the sensitivity of K_{ATP} channels to ATP in mouse α -cells, which may affect the secretory response (Leung *et al.* 2006). Furthermore, insulin increases K_{ATP} channel activity in isolated rat α -cells, inducing an inhibitory effect on glucagon release via membrane hyperpolarization (Franklin *et al.* 2005). In addition to the effects on K_{ATP} channels, insulin can translocate A-type GABA receptors to the cell membrane,

Figure 3 Paracrine signalling in the α -cell. See text for details. ADCY, adenylyate cyclase; AMPA-R, α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor; GABA, γ -aminobutyric acid; GLP1, glucagon-like peptide-1; GRM, metabotropic glutamate receptor; PKA, protein kinase A; SSTR2, somatostatin receptor-2.

which increases the response to GABA secreted by β -cells, favouring membrane hyperpolarization and suppression of glucagon secretion (Xu *et al.* 2006). Since α -cell Ca^{2+} signals rely on electrical activity, insulin also inhibits Ca^{2+} signals induced by low-glucose concentrations (Ravier & Rutter 2005). Therefore, several pieces of evidence indicate that insulin inhibits glucagon release mainly by altering α -cell membrane potential.

Insulin is stored within the secretory granule forming stable hexamers around two atoms of Zn^{2+} . After exocytosis, these hexameric crystals are exposed to a change in pH from 5.5 to 7.4, become dissociated and release both atoms of Zn^{2+} . Recent studies have claimed that zinc atoms can also work as modulators of the α -cell function (Gyulkhandanyan *et al.* 2008), although their role remains controversial. On one hand, it has been found that Zn^{2+} activates K_{ATP} channels and decreases glucagon release in isolated rat α -cells (Ishihara *et al.* 2003, Franklin *et al.* 2005). Zn^{2+} seems to be the switch-off signal to initiate glucagon secretion during hypoglycaemia in streptozotocin-induced diabetic rats (Zhou *et al.* 2007a). However, these results contrast with the absence of effects on mouse α -cells (Ravier & Rutter 2005).

Somatostatin and glucagon Somatostatin is produced and secreted by several tissues in addition to the δ -cell population of the islet and works as an inhibitor of both glucagon and insulin release (Fehmann *et al.* 1995). Immunocytochemical studies in human islets have demonstrated that, among the five identified somatostatin receptor (SSTR) subtypes, SSTR2 is highly expressed in α -cells while SSTR1 and SSTR5 are expressed in β -cells (Kumar *et al.* 1999). In mice and rats, SSTR2 also predominates in the α -cell and SSTR5 in the β -cell population (Hunyady *et al.* 1997, Strowski *et al.* 2000). These receptors are coupled to G-proteins and induce multiple intracellular effects. Electrophysiological studies have shown that somatostatin activates K^{+} channels in α -cells, inducing membrane hyperpolarization and suppressing electrical activity, which affects Ca^{2+} -dependent exocytosis (Yoshimoto *et al.* 1999, Gromada *et al.* 2001). Capacitance measurements have further elucidated that somatostatin directly decreases exocytosis by depriving secretory granules through the activation of the serine/threonine protein phosphatase calcineurin pathway (Gromada *et al.* 2001). Also, a negative interaction of somatostatin with adenylate cyclase and cAMP levels has been reported in rat α -cells (Schuit *et al.* 1989). In addition to the effects of insulin and somatostatin on α -cells, glucagon itself works as an extracellular messenger. It exerts an autocrine positive feedback that stimulates secretion in both isolated rat and mouse α -cells by an increase in exocytosis associated to a rise in cAMP levels (Ma *et al.* 2005).

GLP1 The incretin hormone glucagon-like peptide 1 (GLP1) is released from the L-cells of the small intestine after food intake, stimulating insulin production and inhibiting glucagon release. Because of this dual effect, GLP1 is a potential therapeutic agent in the treatment of diabetic patients that manifest insulin

deficiency as well as hyperglucagonaemia (Dunning *et al.* 2005). The observed suppressing effect of GLP1 on glucagon secretion *in vivo* and in perfused pancreas contrasts with those effects found in single α -cells (Dunning *et al.* 2005). In isolated rat α -cells, GLP1 stimulates glucagon secretion by interacting with specific receptors coupled to G-proteins that activate adenylate cyclase, which increases cAMP levels (Ding *et al.* 1997, Ma *et al.* 2005). Thus, it seems that paracrine mechanisms may be responsible for the GLP1 suppressing action (Dunning *et al.* 2005). This possibility has been underscored by the findings in experiments using β -cell-specific knock-out mice for the transcription factor Pdx1. In these mice, the lack of effect of GLP1 on β -cells is also accompanied by its inability to induce an inhibitory action on glucagon plasma levels (Li *et al.* 2005). Moreover, GLP1 may also affect the α -cell function by interacting with the autonomic nervous system (Balkan & Li 2000).

Other extracellular messengers The neurotransmitter γ -aminobutyric acid (GABA) is another α -cell modulator. GABA accumulates in β -cell vesicles and is released by Ca^{2+} -dependent exocytosis, stimulating A-type GABA receptors in neighbouring α -cells. Activation of these receptors is coupled to inward Cl^{-} currents that hyperpolarize the α -cell plasma membrane, decreasing glucagon release in rats and guinea pigs (Rorsman *et al.* 1989, Wendt *et al.* 2004). Similar conclusions were obtained in mouse islets and clonal α TC1-9 cells (Xu *et al.* 2006, Bailey *et al.* 2007). The neurotransmitter L-glutamate also accumulates in the α -cell secretory granules because of vesicular glutamate transporters 1 and 2 found in these cells (Hayashi *et al.* 2003a). In low-glucose conditions, L-glutamate is cosecreted with glucagon, triggering GABA release from neighbouring β -cells and, subsequently, inhibiting the α -cell function as previously described (Hayashi *et al.* 2003b). Additionally, glutamate can activate autocrine signalling pathways in α -cells through the multiple glutamate receptors expressed in these cells, which include ionotropic AMPA and kainate subtypes and metabotropic receptors (Inagaki *et al.* 1995, Uehara *et al.* 2004, Cabrera *et al.* 2008). Although activation of ionotropic receptors may stimulate glucagon release (Bertrand *et al.* 1993), metabotropic glutamate receptors inhibit rat glucagon secretion at low-glucose concentrations through a down-regulation of cAMP levels (Uehara *et al.* 2004). Another α -cell regulator is amylin or islet amyloid pancreatic polypeptide (Iapp). This polypeptide is a 37 amino acid hormone mainly synthesized in β -cells, although it can be produced in δ -cells as well. This peptide is cosecreted with insulin by exocytosis and has an inhibitory effect on glucagon basal concentrations as well as on those levels observed after arginine stimulation (Akesson *et al.* 2003, Gedulin *et al.* 2006). This glucagonostatic effect has been reported in the plasma levels of mice and rats as well as in perfused pancreas or intact islets. Since amylin also reduces somatostatin and insulin release, some authors have proposed that endogen amylin within the islet may establish a negative feedback to avoid excessive secretion from α -, β - and δ -cells (Wang *et al.* 1999). Also, the purinergic messenger ATP is highly accumulated in β -cell secretory granules and in nerve terminals.

It has recently been reported that ATP inhibits Ca^{2+} signalling and glucagon secretion in mouse α -cells, indicating that purinergic receptors are involved in α -cell function (Tuduri *et al.* 2008). Purinergic regulation of glucagon release has also been described in rat islets (Grapengiesser *et al.* 2006).

Neural regulation As previously stated, the islet of Langerhans is highly innervated by parasympathetic and sympathetic nerves that ensure a rapid response to hypoglycaemia and protection from potential brain damage (Ahren 2000). Some terminals of these nerves store and release classical neurotransmitters, such as acetylcholine and noradrenaline, as well as several neuropeptides, which stimulate or inhibit glucagon secretion depending on the neural messenger released. Cholinergic stimulation involving muscarinic receptors and intracellular Ca^{2+} mobilization enhances α -cell function both *in vivo* and in isolated cells (Ahren & Lundquist 1982, Berts *et al.* 1997). Noradrenaline increases glucagon secretion as well (Ahren *et al.* 1987). Sympathetic activation can also induce adrenaline release from the adrenal medulla, which potently stimulates glucagon secretion by enhancing Ca^{2+} influx through L-type Ca^{2+} channels and accelerating granule mobilization (Gromada *et al.* 1997). In addition to classical neurotransmitters, several neuropeptides such as vasoactive intestinal polypeptide, pituitary adenylate cyclase-activating polypeptide and gastrin-releasing peptide, which may stimulate glucagon release from pancreas, can be accumulated in parasympathetic nerves, while galanin and neuropeptide Y can be stored in sympathetic nerve terminals (Ahren 2000). Multiple actions have been reported for the latter neuropeptides. The effects and mechanisms involved in neural regulation of α -cells have yet to be established at the cellular and molecular levels. These systems are mainly regulated by glucose-sensing neurons of the ventromedial hypothalamus, which respond to plasma glucose levels with mechanisms very similar to those of the β -cell, including the activity of glucose-regulated K_{ATP} channels (Borg *et al.* 1994, Miki *et al.* 2001, Song *et al.* 2001). Actually, it has been observed that the α -cell response to hypoglycaemia is also impaired in KCNJ11-deficient mice whose neurons of the ventromedial hypothalamus lack functional K_{ATP} channels and glucose responsiveness (Miki *et al.* 2001).

Glucagon physiological and pathophysiological actions and its role in diabetes

Glucagon synthesis

The proglucagon-derived peptides glucagon, GLP1 and GLP2, are encoded by the proglucagon gene, which is expressed in the central nervous system, intestinal L-cells and pancreatic α -cells. A post-translational cleavage by prohormone convertases (PC) is responsible for the maturation of the proglucagon hormone that generates all these peptides (Mojsov *et al.* 1986). The different expression of PC subtypes

in each tissue mediates the production of each different peptide. In α -cells, the predominance of PCSK2 leads to a major production of glucagon together with the products glicentin, glicentin-related pancreatic polypeptide, intervening peptide 1 and the major proglucagon fragment (Dey *et al.* 2005). The absence of PCSK2 in knock-out mice leads to a lack of mature glucagon (Furuta *et al.* 2001). In enteroendocrine cells, PCSK1/3 enzymes cleave the proglucagon hormone to generate GLP1 and GLP2 along with glicentin, intervening peptide 2 and oxyntomodulin (Mojsov *et al.* 1986).

The regulation of glucagon gene expression has not been studied as extensively as the insulin gene. The inhibitory effect of insulin on glucagon secretion has also been confirmed in gene expression and it occurs at the transcriptional level (Philippe *et al.* 1995). In diabetic rats, glucagon gene expression is augmented and is accompanied by hyperglucagonaemia in conditions of hyperglycaemia and insulin deficiency. Insulin treatment normalized glucagon expression and plasma levels in these rats, an effect that was not attributed to the restoration of normal glucose levels (Dumonteil *et al.* 1998). It was concluded that insulin, unlike glucose, modulates glucagon expression. The lack of response to glucose was further confirmed in isolated rat islets (Gremlich *et al.* 1997, Dumonteil *et al.* 2000), contrasting with the up-regulation of glucagon expression observed in clonal α -cells (Dumonteil *et al.* 1999, McGirr *et al.* 2005). The effect of amino acids on glucagon gene regulation has also been studied. While arginine increases glucagon expression in isolated rat islets: a process that is mediated by protein kinase C (PKA; Yamato *et al.* 1990, Dumonteil *et al.* 2000), histidine plays a fundamental role in clonal $\alpha\text{TC1-6}$ cells (Paul *et al.* 1998). Other nutrients, such as the fatty acid palmitate, produces a down-regulated glucagon expression at short term in rat islets in a dose-dependent manner (Bollheimer *et al.* 2004). By contrast, no effect with palmitate has been observed in other long-term studies (Gremlich *et al.* 1997, Dumonteil *et al.* 2000). Like insulin, somatostatin also inhibits glucagon expression. It has been reported that somatostatin down-regulates glucagon expression basal levels as well as those produced by forskolin stimulation in clonal INR1G9 cells (Fehmann *et al.* 1995, Kendall *et al.* 1995).

Glucagon receptor

The rat and mouse glucagon receptor is a 485 amino acid protein, belonging to the secretin–glucagon receptor II class family of G protein-coupled receptors (Mayo *et al.* 2003). Glucagon binding to this receptor is coupled to GTP-binding heterotrimeric G proteins of the G_{α_s} type that leads to the activation of adenylate cyclase, cAMP production and PKA. This receptor can also activate the phospholipase C/inositol phosphate pathway via G_q proteins, resulting in Ca^{2+} release from intracellular stores (Fig. 4; Wakelam *et al.* 1986, Mayo *et al.* 2003). The glucagon receptor is present in multiple tissues including the liver, pancreas, heart, kidney, brain and

Figure 4 The role of glucagon and the glucagon receptor in the liver. Glucagon signalling regulates positively (+) or negatively (–) the multiple steps of the hepatic glucose metabolism. This modulation affects the expression and/or the activity of several enzymes of glucose metabolism. See text for details. ADCY, Adenylate cyclase; CREB, cAMP response element binding; F(1,6)P2, fructose-1,6-bisphosphate; F(2,6)P2, fructose-2,6-bisphosphate; F-6-P, fructose 6-phosphate; FBP1, fructose-1,6-bisphosphatase; FBP2, fructose-2,6-bisphosphatase; G-1-P, glucose 1-phosphate; G-6-P, glucose 6-phosphate; G6PC, glucose-6-phosphatase; GP, glycogen phosphorylase; GS, glycogen synthase; IP3, inositol 1,4,5-trisphosphate; OAA, oxaloacetate; PC, pyruvate carboxylase; PEP, phosphoenolpyruvate; PCK2, phosphoenolpyruvate carboxykinase; PFKM, phosphofructokinase-1; PPARGC1A, peroxisome proliferators-activated receptor- γ coactivator-1; PIP2, phosphatidylinositol 4,5-bisphosphate; PKLR, pyruvate kinase; PLC, phospholipase C; Pyr, pyruvate. Dashed lines: red, inhibition; blue, stimulation.

smooth muscle. Thus, it modulates multiple responses in these tissues, including effects on ion transport and glomerular filtration rate in kidney among others (Ahloulay *et al.* 1992). In any case, the regulation of glucose homeostasis is the major function of glucagon and its receptor. This role will be described in the next paragraph.

Glucagon control of glucose homeostasis and metabolism

Several lines of defence protect the organism against hypoglycaemia and its potential damaging effects, especially in the brain, which depends on a continuous supply of glucose, its principal metabolic fuel. These defences include decreased insulin release and increased secretion of adrenaline and glucagon. Additionally, glucose-sensing neurons of the ventromedial hypothalamus further control responses to

glycaemia changes, as previously mentioned. Among all these regulatory systems, glucagon plays a central role in the response to hypoglycaemia and also opposes to insulin effects. The main action of glucagon occurs in the liver where the insulin/glucagon ratio controls multiple steps of hepatic metabolism. Glucagon stimulates gluconeogenesis and glycogenolysis, which increases hepatic glucose output, ensuring an appropriate supply of glucose to body and brain, and at the same time, it decreases glycogenesis and glycolysis. The glucagon receptor in the liver is highly selective for glucagon, but it exhibits a modest affinity for glucagon-like peptides (Hjorth *et al.* 1994). Its main action on the liver is mediated by the activation of adenylyl cyclase and the PKA pathway. Glucagon regulates gluconeogenesis mainly by the up-regulation of key enzymes such as glucose-6-phosphatase (G6PC) and phosphoenolpyruvate carboxykinase (PCK2) through the

activation of the cAMP response element-binding protein (CREB) and peroxisome proliferator-activated receptor γ -coactivator-1 (PPARGC1A; Herzig *et al.* 2001, Yoon *et al.* 2001; Fig. 4). PCK2 and G6PC, along with fructose-1,6-biphosphatase (FBP1) have a key role in the rate of gluconeogenesis (Fig. 4). PCK2 mediates the conversion of oxalacetate into phosphoenolpyruvate while G6PC regulates glucose production from glucose-6-phosphate. FBP1 is responsible for the conversion of fructose-1,6-biphosphate (F(1,6)P₂) into fructose-6-phosphate (F6P). Its activity is regulated by glucagon since this hormone decreases the intracellular levels of fructose-2,6-biphosphate (F(2,6)P₂), an allosteric inhibitor of FBP1 (Kurland & Pilkis 1995). Additionally, this decrease in F(2,6)P₂ also reduces the activity of phosphofructokinase-1 (PFKM), down-regulating glycolysis. The glycolytic pathway is further inhibited by glucagon at the pyruvate kinase (PKLR) level (Slavin *et al.* 1994). Glycogen metabolism is mainly determined by the activity of glycogen synthase (GS) and glycogen phosphorylase (GP). While glucagon is important for GP phosphorylation and activation, it inhibits GS function by phosphorylation and its conversion into an inactive form of the enzyme (Band & Jones 1980, Ciudad *et al.* 1984, Andersen *et al.* 1999).

Glucagon can also stimulate the uptake of amino acids for gluconeogenesis in the liver. Indeed, subjects with hyperglucagonaemia can develop plasma hypoaminoacidaemia, especially of amino acids involved in gluconeogenesis, such as alanine, glycine and proline (Cynober 2002). Glucagon is also involved in the regulation of fatty acids in adipocytes. Hormone-sensitive lipase mediates the lipolysis of triacylglycerol into the non-esterified fatty acids and glycerol, which are released from adipocytes. It has been reported that although glucagon does not modify the transcriptional levels of this enzyme, it increases the release of glycerol from adipocytes (Slavin *et al.* 1994). This mobilization of glycerol from adipose tissue can further be used in the liver during gluconeogenesis. However, the existence of a lipolytic action of glucagon observed in several animal models is still controversial in humans. While a positive effect of glucagon on lipolysis has been reported in human subjects (Carlson *et al.* 1993), several recent studies have indicated that it lacks a role in a physiological context (Gravholt *et al.* 2001). An elevated glucagon to insulin ratio accelerates gluconeogenesis as well as fatty acid β -oxidation and ketone bodies formation (Vons *et al.* 1991). Thus, glucagon may also be involved in diabetic ketoacidosis, a medical complication in diabetes derived from the overproduction of ketone bodies (Eledrisi *et al.* 2006).

The role of α -cell function in diabetes

More than 30 years ago, Unger & Orci (1975) proposed the bihormonal hypothesis to explain the pathophysiology of diabetes. According to this hypothesis, this metabolic disease is the result of an insulin deficiency or resistance along with an absolute or relative excess of glucagon, which can cause a

higher rate of hepatic glucose production than glucose utilization, favouring hyperglycaemia. At present, there exists multiple clinical and experimental evidence that support this hypothesis. The rate of hepatic glucose output has been correlated with the hyperglycaemia found in animal models of diabetes as well as in human diabetes, and the maintenance of this abnormality has also been associated with hyperglucagonaemia (Baron *et al.* 1987, Consoli *et al.* 1989, Gastaldelli *et al.* 2000, Dunning & Gerich 2007, Li *et al.* 2008). In type 2 diabetes, the impairment of insulin release and development of insulin resistance is often accompanied by absolute or relative increased levels of glucagon in the fasting and postprandial states (Reaven *et al.* 1987, Larsson & Ahren 2000). In this situation, insulin is not effective as a negative feedback for hepatic glucose output while glucagon potentiates glucose mobilization from the liver, thus contributing to hyperglycaemia. Another malfunction reported in diabetic patients is the lack of suppression of glucagon release in hyperglycaemic conditions, which would contribute further to postprandial hyperglycaemia in both type 1 and type 2 diabetes (Dinneen *et al.* 1995, Shah *et al.* 2000). However, this irregular α -cell behaviour does not occur when insulin levels are adequate, suggesting that abnormalities in glucagon release are relevant for hyperglycaemia in the context of diabetes or impairment of insulin secretion or action (Shah *et al.* 1999). Hyperglucagonaemia is also responsible for the development of hyperglycaemia and diabetes in patients with the glucagonoma syndrome, a paraneoplastic phenomenon characterized by an islet α -cell pancreatic tumour (Chastain 2001).

Another defect in normal glucagon secretion has important consequences in the management of hypoglycaemia. The secretory response of α -cells to low-glucose concentrations is impaired in type 1 and long-lasting type 2 diabetes, increasing the risk of episodes of severe hypoglycaemia, especially in patients treated with insulin (Cryer 2002). In this regard, iatrogenic hypoglycaemia is a situation that implies insulin excess and compromised glucose counter-regulation, and it is responsible for a major complication in diabetes treatment, increasing the morbidity and mortality of this disease (Cryer 2002). This lack of glucagon response to hypoglycaemia has been associated with multiple failures in α -cell regulation; yet, the mechanisms are still under study (Bolli *et al.* 1984, Cryer 2002, Zhou *et al.* 2007b). Even though islet allotransplantation can provide prolonged insulin independence in patients with type 1 diabetes, the lack of α -cell response to hypoglycaemia usually persists after transplantation, indicating that this procedure does not restore the physiological behaviour of α -cells (Paty *et al.* 2002).

All these problems in the glucagon secretory response observed in diabetes have been attributed to several defects in α -cell regulation including defective glucose sensing, loss of β -cell function, insulin resistance or autonomic malfunction. However, the mechanisms involved in α -cell pathophysiology still remain largely unknown and deserve more investigation for better design of therapeutic strategies. In this regard,

although direct therapeutic approaches to correct the lack of α -cell response to hypoglycaemia are missing, several proposals have been developed to amend glucagon excess, as we will see in the next section.

Molecular pharmacology of glucagon release and action: therapeutic potential in diabetes treatment

Given that absolute or relative glucagon excess seems to be critical in the development and/or maintenance of hyperglycaemia in diabetes by increasing hepatic glucose output, the strategies targeted to correct this malfunction are suitable for the improvement of glucose levels. In this respect, several experimental and therapeutic approaches have been developed (for a further review, see Dunning & Gerich 2007). The specific control of glucagon secretion by pharmacological modulation is complex since several components of the α -cell stimulus-secretion coupling are also present in β - and δ -cells. Thus, the manipulation of glucagon action by modulating the glucagon receptor signalling seems to be an effective alternative (Li *et al.* 2008). This strategy has been supported by several studies. Glucagon receptor knock-out mice have hyperglucagonaemia and α -cell hyperplasia, but their glucose tolerance is improved and they develop only a mild fasting hypoglycaemia (Gelling *et al.* 2003). These mice have a normal body weight, food intake and energy expenditure although less adiposity and lower leptin levels. These results are consistent with the experiments with anti-sense oligonucleotides for the glucagon receptor. Diabetic db/db mice treated with these oligonucleotides had lower glucose, triglyceride and free fatty acids blood levels, as well as improved glucose tolerance, and they developed hyperglucagonaemia without apparent effects on α -cell size or number (Liang *et al.* 2004). This approach is also accompanied by an increase in GLP1 and insulin levels in Zucker diabetic fatty rats and db/db and ob/ob mice (Sloop *et al.* 2004). Furthermore, the use of high affinity, neutralizing glucagon monoclonal antibodies improved glycaemic control and reduced hepatic glucose production in diabetic ob/ob mice (Sorensen *et al.* 2006). Therefore, these experimental results are a further support that glucagon antagonism may be beneficial for diabetes treatment.

Modulation of glucagon secretion

Sulphonylureas Sulphonylureas are efficient K_{ATP} channel blockers that have been extensively used for the clinical treatment of diabetes. In rat α -cells, sulphonylureas stimulate electrical activity, Ca^{2+} signalling and glucagon release (Franklin *et al.* 2005). In mice, however, tolbutamide produces membrane depolarization, but a decrease in Ca^{2+} signalling and glucagon release (Gromada *et al.* 2004). Recent experiments in mouse α -cells have shown that, in the absence of glucose, this drug increases glucagon secretion at

concentrations up to 1 μ M, but higher doses are inhibitory (MacDonald *et al.* 2007). This biphasic effect is due to the mouse α -cell electrical behaviour (Fig. 1): glucagon release takes place within a narrow window of intermediate K_{ATP} channel activity (and membrane potential), and thus it is inhibited when the cell is hyperpolarized or depolarized beyond this membrane potential range (MacDonald *et al.* 2007). Accordingly, with this scheme, the K_{ATP} channel opener diazoxide can also have a biphasic effect on glucagon secretion. These effects will change depending on the extracellular glucose concentrations that necessarily influence K_{ATP} channel activity (MacDonald *et al.* 2007). This biphasic behaviour may explain the disparity of effects found for sulphonylureas (Loubatieres *et al.* 1974, Ostenson *et al.* 1986). In humans, sulphonylureas are associated to a glucagon secretion decrease in healthy and type 2 diabetic subjects (Landstedt-Hallin *et al.* 1999), while they stimulate glucagon levels in type 1 diabetic patients (Bohannon *et al.* 1982). Since sulphonylureas also induce insulin and somatostatin secretion, which affect α -cells, these drugs offer a poor specific control of glucagon secretion.

GLP1 mimetics and DPP4 inhibitors In addition to stimulating insulin release, GLP1 can suppress glucagon secretion in humans, perfused rat pancreas and isolated rat islets in a glucose-dependent manner (Guenifi *et al.* 2001, Nauck *et al.* 2002). Because GLP1 is rapidly cleaved and inactivated by the enzyme dipeptidyl peptidase-IV (DPP4), a good alternative would be to design either GLP1 derivatives with higher resistance to DPP4 or agents that increase GLP1 endogenous levels. Among the GLP1 mimetics, exenatide is a synthetic polypeptide with high resistance to DPP4 cleavage that decreases glucagon levels in normal and diabetic subjects (Degn *et al.* 2004). Liraglutide, another GLP1 derivative with long-lasting actions, can reduce glucagon release after a meal in patients with type 2 diabetes (Juhl *et al.* 2002). Alternatively, DPP4 inhibitors like sitagliptin and vildagliptin increase the endogenous effects of GLP1, reducing glucagon plasma concentrations in diabetic individuals (Rosenstock *et al.* 2007). Since all these alternatives produce opposing actions on insulin and glucagon, they generate promising expectations for diabetes treatment.

Imidazolines The insulinotropic effects of imidazoline compounds are mediated by K_{ATP} channel blockade, which leads to depolarization, Ca^{2+} influx and secretion, and by direct interactions with the exocytotic machinery (Zaitsev *et al.* 1996). Remarkably, imidazoline compounds such as phentolamine also suppress glucagon secretion in both rat and mouse islets, an action mediated by a direct effect on α -cell exocytosis via activation of phosphatase calcineurin proteins, and independent of K_{ATP} channels or Ca^{2+} currents (Hoy *et al.* 2001). Given that imidazoline compounds stimulate insulin release while inhibiting glucagon secretion, these drugs are potentially valuable in diabetes.

Somatostatin analogues Because of the different expression of SSTTR in the islet (Kumar *et al.* 1999), several studies have explored the modulation of glucagon secretion by subtype-specific somatostatin analogues (Strowski *et al.* 2006). It has been shown that SSTTR2 is the subtype receptor predominantly expressed in rodent α -cells, and that SSTTR2-deficient mice develop hyperglycaemia and non-fasting hyperglucagonaemia (Singh *et al.* 2007). In mice, the use of a highly SSTTR2-selective non-peptide agonist inhibited glucagon release without affecting insulin release (Strowski *et al.* 2006). However, there is some overlapping in human islets between the different SSTTR subtypes in α - and β -cells that limit, at present, the use of subtype-specific somatostatin analogues (Singh *et al.* 2007).

Amylin and pramlintide Amylin, which is cosecreted with insulin from β -cells, inhibits glucagon secretion stimulated by amino acids but does not affect hypoglycaemia-induced glucagon release (Young 2005). Since α -cell response to amino acids is often exaggerated in diabetic patients, amylin or amylinomimetic compounds such as pramlintide are used as an effective alternative for the treatment of postprandial and amino acid-induced excess of glucagon secretion (Dunning *et al.* 2005, Young 2005).

Modulation of glucagon action and glucagon receptor signalling

Peptide-based glucagon receptor antagonists Several linear and cyclic glucagon analogues have been developed to work as glucagon receptor antagonists. Essentially, they impair the ability of glucagon to stimulate adenylate cyclase activity in liver, thus reducing hepatic glucose output and improving plasma glucose levels. This is the case of [des-His¹, des-Phe⁶, Glu⁹] glucagon-NH₂, which reduces glucose levels in streptozotocin-induced diabetic rats (Van Tine *et al.* 1996). Recent investigations have demonstrated that the antagonist des-His-glucagon binds preferentially to the hepatic glucagon receptor *in vivo*, and this correlates with the glucose lowering effects (Dallas-Yang *et al.* 2004).

Non-peptide glucagon receptor antagonists Multiple, competitive and non-competitive, non-peptide antagonists have been reported to act on glucagon binding and/or function. For instance, a novel competitive antagonist (N-[3-cyano-6-(1, 1-dimethylpropyl)-4, 5, 6, 7-tetrahydro-1-benzothien-2-yl]-2-ethylbutanamide) was recently shown to inhibit glucagon-mediated glycogenolysis in primary human hepatocytes and to block the increase in glucose levels after the administration of exogenous glucagon in mice (Qureshi *et al.* 2004). The information about the effect of these antagonists on humans is, however, scarce. In this respect, Bay 27-9955 is an oral glucagon receptor antagonist that has been tested in humans, demonstrating its efficacy in reducing glucose levels induced by exogenous glucagon (Petersen & Sullivan 2001).

Despite the success of several approaches to modulate glucagon secretion or action and improve glucose control in animal models or in humans, more information is still required. Long-standing studies should address whether the utilization of these agents could lead to undesired hypoglycaemia in humans, accumulation of lipids or compensatory mechanisms that decrease the benefits of these therapies in the long term. In this aspect, the results obtained in animal models are positive: although the glucagon receptor knock-out mouse develops hyperglucagonaemia, it is not hypoglycaemic and does not have an abnormal accumulation of lipids (Gelling *et al.* 2003). Additionally, recent long-term studies in mice further prove the viability of glucagon antagonism (Winzell *et al.* 2007). Thus, present data are promising and indicate that several therapeutic agents targeted to glucagon signalling and α -cell secretion may be useful for the management of diabetes.

Conclusions

Pancreatic α -cells and glucagon secretion are fundamental components of the regulatory mechanisms that control glucose homeostasis. However, α -cell physiology has remained elusive compared with the overwhelming information about insulin secretion and the β -cell. In recent years, however, several groups have initiated intensive efforts to understand α -cell physiology and identified essential pieces of its stimulus-secretion coupling. Additionally, important aspects of the regulation of α -cell metabolism and the control of glucagon expression are being elucidated. All of this information will favour an overall comprehension of the α -cell function and its role in glucose homeostasis. Nevertheless, more research is required to understand the α -cell behaviour, not only in healthy subjects but in pathological conditions as well. In conclusion, since the malfunction of the glucagon secretory response is involved in diabetes and its complications, a complete understanding of the α -cell will allow for a better design of therapeutic approaches for the treatment of this disease.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

Funding

This work was supported by grants from the Ministerio de Educación y Ciencia (BFU2007-67607 and PCI2005-A7-0131 to I Q; BFU2005-01052 to A N). CIBERDEM is an initiative of the Instituto de Salud Carlos III.

References

- Ahloulay M, Bouby N, Machet F, Kubrusly M, Coutaud C & Bankir L 1992 Effects of glucagon on glomerular filtration rate and urea and water excretion. *American Journal of Physiology. Renal Physiology* **263** F24-F36.

- Ahren B 2000 Autonomic regulation of islet hormone secretion – implications for health and disease. *Diabetologia* **43** 393–410.
- Ahren B & Lundquist I 1982 Influences of gastro-intestinal polypeptides and glucose on glucagon secretion induced by cholinergic stimulation. *Hormone and Metabolic Research* **14** 529–532.
- Ahren B, Veith RC & Taborsky GJ Jr 1987 Sympathetic nerve stimulation versus pancreatic norepinephrine infusion in the dog: 1). Effects on basal release of insulin and glucagon. *Endocrinology* **121** 323–331.
- Akesson B, Panagiotidis G, Westermark P & Lundquist I 2003 Islet amyloid polypeptide inhibits glucagon release and exerts a dual action on insulin release from isolated islets. *Regulatory Peptides* **111** 55–60.
- Andersen B, Rassov A, Westergaard N & Lundgren K 1999 Inhibition of glycogenolysis in primary rat hepatocytes by 1, 4-dideoxy-1,4-imino-D-arabinitol. *Biochemical Journal* **342** 545–550.
- Andrews SS, Lopez-S A & Blackard WG 1975 Effect of lipids on glucagon secretion in man. *Metabolism* **24** 35–44.
- Asplin C, Raghu P, Dorman T & Palmer JP 1983 Glucose regulation of glucagon secretion independent of B cell activity. *Metabolism* **32** 292–295.
- Bailey SJ, Ravier MA & Rutter GA 2007 Glucose-dependent regulation of gamma-aminobutyric acid (GABA A) receptor expression in mouse pancreatic islet alpha-cells. *Diabetes* **56** 320–327.
- Balkan B & Li X 2000 Portal GLP-1 administration in rats augments the insulin response to glucose via neuronal mechanisms. *American Journal of Physiology. Regulatory, Integrative and Comparative Physiology* **279** R1449–R1454.
- Band GC & Jones CT 1980 Functional activation by glucagon of glucose 6-phosphatase and gluconeogenesis in the perfused liver of the fetal guinea pig. *FEBS Letters* **119** 190–194.
- Barg S, Galvanovskis J, Gopel SO, Rorsman P & Eliasson L 2000 Tight coupling between electrical activity and exocytosis in mouse glucagon-secreting alpha-cells. *Diabetes* **49** 1500–1510.
- Baron AD, Schaeffer L, Shragg P & Kolterman OG 1987 Role of hyperglucagonemia in maintenance of increased rates of hepatic glucose output in type II diabetics. *Diabetes* **36** 274–283.
- Bertrand G, Gross R, Puech R, Loubatieres-Mariani MM & Bockaert J 1993 Glutamate stimulates glucagon secretion via an excitatory amino acid receptor of the AMPA subtype in rat pancreas. *European Journal of Pharmacology* **237** 45–50.
- Berts A, Gylfe E & Hellman B 1997 Cytoplasmic Ca^{2+} in glucagon-producing pancreatic alpha-cells exposed to carbachol and agents affecting Na^{+} fluxes. *Endocrine* **6** 79–83.
- Bohannon NV, Lorenzi M, Grodsky GM & Karam JH 1982 Stimulatory effects of tolbutamide infusion on plasma glucagon in insulin-dependent diabetic subjects. *Journal of Clinical Endocrinology and Metabolism* **54** 459–462.
- Bokvist K, Olsen HL, Hoy M, Gotfredsen CF, Holmes WF, Buschard K, Rorsman P & Gromada J 1999 Characterisation of sulphonylurea and ATP-regulated K^{+} channels in rat pancreatic A-cells. *Pflugers Archiv: European Journal of Physiology* **438** 428–436.
- Bollheimer LC, Landauer HC, Troll S, Schweimer J, Wrede CE, Scholmerich J & Buettner R 2004 Stimulatory short-term effects of free fatty acids on glucagon secretion at low to normal glucose concentrations. *Metabolism* **53** 1443–1448.
- Bolli GB, Tsalikian E, Haymond MW, Cryer PE & Gerich JE 1984 Defective glucose counterregulation after subcutaneous insulin in noninsulin-dependent diabetes mellitus. Paradoxical suppression of glucose utilization and lack of compensatory increase in glucose production, roles of insulin resistance, abnormal neuroendocrine responses, and islet paracrine interactions. *Journal of Clinical Investigation* **73** 1532–1541.
- Bonner-Weir S 1991 Anatomy of the islet of Langerhans. In *The Endocrine Pancreas*, pp 15–28. Ed. E Samols. New York: Raven Press.
- Borg WP, Daring MJ, Sherwin RS, Borg MA, Brines ML & Shulman GI 1994 Ventromedial hypothalamic lesions in rats suppress counterregulatory responses to hypoglycemia. *Journal of Clinical Investigation* **93** 1677–1682.
- Breije TC, Scharp DW & Sorenson RL 1989 Three-dimensional imaging of intact isolated islets of Langerhans with confocal microscopy. *Diabetes* **38** 808–814.
- Brissova M, Fowler MJ, Nicholson WE, Chu A, Hirshberg B, Harlan DM & Powers AC 2005 Assessment of human pancreatic islet architecture and composition by laser scanning confocal microscopy. *Journal of Histochemistry and Cytochemistry* **53** 1087–1097.
- Cabrera O, Berman DM, Kenyon NS, Ricordi C, Berggren PO & Caicedo A 2006 The unique cytoarchitecture of human pancreatic islets has implications for islet cell function. *PNAS* **103** 2334–2339.
- Cabrera O, Jacques-Silva MC, Speier S, Yang SN, Köhler M, Fachado A, Vieira E, Zierath JR, Kibbey R, Berman DM *et al.* 2008 Glutamate is a positive autocrine signal for glucagon release. *Cell Metabolism* **7** 545–554.
- Carlson MG, Snead WL & Campbell PJ 1993 Regulation of free fatty acid metabolism by glucagon. *Journal of Clinical Endocrinology and Metabolism* **77** 11–15.
- Chastain MA 2001 The glucagonoma syndrome: a review of its features and discussion of new perspectives. *American Journal of the Medical Sciences* **321** 306–320.
- Ciudad C, Camici M, Ahmad Z, Wang Y, DePaoli-Roach AA & Roach PJ 1984 Control of glycogen synthase phosphorylation in isolated rat hepatocytes by epinephrine, vasopressin and glucagon. *European Journal of Biochemistry* **142** 511–520.
- Consoli A, Nurjhan N, Capani F & Gerich J 1989 Predominant role of gluconeogenesis in increased hepatic glucose production in NIDDM. *Diabetes* **38** 550–557.
- Cryer PE 2002 Hypoglycaemia: the limiting factor in the glycaemic management of Type I and Type II diabetes. *Diabetologia* **45** 937–948.
- Cynober LA 2002 Plasma amino acid levels with a note on membrane transport: characteristics, regulation, and metabolic significance. *Nutrition* **18** 761–766.
- Dallas-Yang Q, Shen X, Strowski M, Brady E, Saperstein R, Gibson RE, Szalkowski D, Qureshi SA, Candelore MR, Fenyk-Melody JE *et al.* 2004 Hepatic glucagon receptor binding and glucose-lowering *in vivo* by peptidyl and non-peptidyl glucagon receptor antagonists. *European Journal of Pharmacology* **501** 225–234.
- Degn KB, Brock B, Juhl CB, Djurhuus CB, Grubert J, Kim D, Han J, Taylor K, Fineman M & Schmitz O 2004 Effect of intravenous infusion of exenatide (synthetic exendin-4) on glucose-dependent insulin secretion and counterregulation during hypoglycemia. *Diabetes* **53** 2397–2403.
- Detimary P, Dejonghe S, Ling Z, Pipeleers D, Schuit F & Henquin JC 1998 The changes in adenine nucleotides measured in glucose-stimulated rodent islets occur in beta cells but not in alpha cells and are also observed in human islets. *Journal of Biological Chemistry* **273** 33905–33908.
- Dey A, Lipkind GM, Rouille Y, Norrbom C, Stein J, Zhang C, Carroll R & Steiner DF 2005 Significance of prohormone convertase 2, PC2, mediated initial cleavage at the proglucagon interdomain site, Lys70–Arg71, to generate glucagon. *Endocrinology* **146** 713–727.
- Diao J, Asghar Z, Chan CB & Wheeler MB 2005 Glucose-regulated glucagon secretion requires insulin receptor expression in pancreatic α -cells. *Journal of Biological Chemistry* **280** 33487–33496.
- Ding WG, Renstrom E, Rorsman P, Buschard K & Gromada J 1997 Glucagon-like peptide I and glucose-dependent insulinotropic polypeptide stimulate Ca^{2+} -induced secretion in rat alpha-cells by a protein kinase A-mediated mechanism. *Diabetes* **46** 792–800.
- Dinneen S, Alzaid A, Turk D & Rizza R 1995 Failure of glucagon suppression contributes to postprandial hyperglycaemia in IDDM. *Diabetologia* **38** 337–343.
- Dumonteil E, Magnan C, Ritz-Laser B, Meda P, Dussoix P, Gilbert M, Ktorza A & Philippe J 1998 Insulin, but not glucose lowering corrects the hyperglucagonemia and increased proglucagon messenger ribonucleic acid levels observed in insulinopenic diabetes. *Endocrinology* **139** 4540–4546.
- Dumonteil E, Ritz-Laser B, Magnan C, Grigorescu I, Ktorza A & Philippe J 1999 Chronic exposure to high glucose concentrations increases proglucagon messenger ribonucleic acid levels and glucagon release from InR1G9 cells. *Endocrinology* **140** 4644–4650.
- Dumonteil E, Magnan C, Ritz-Laser B, Ktorza A, Meda P & Philippe J 2000 Glucose regulates proinsulin and prosomatostatin but not proglucagon messenger ribonucleic acid levels in rat pancreatic islets. *Endocrinology* **141** 174–180.

- Dunning BE & Gerich JE 2007 The role of alpha-cell dysregulation in fasting and postprandial hyperglycemia in type 2 diabetes and therapeutic implications. *Endocrine Reviews* **28** 253–283.
- Dunning BE, Foley JE & Ahren B 2005 Alpha cell function in health and disease: influence of glucagon-like peptide-1. *Diabetologia* **48** 1700–1713.
- Eledrisi MS, Alshanti MS, Shah MF, Brolosy B & Jaha N 2006 Overview of the diagnosis and management of diabetic ketoacidosis. *American Journal of the Medical Sciences* **331** 243–251.
- Fehmann HC, Strowski M & Goke B 1995 Functional characterization of somatostatin receptors expressed on hamster glucagonoma cells. *American Journal of Physiology. Endocrinology and Metabolism* **268** E40–E47.
- Franklin I, Gromada J, Gjinovci A, Theander S & Wollheim CB 2005 β -cell secretory products activate α -cell ATP-dependent potassium channels to inhibit glucagon release. *Diabetes* **54** 1808–1815.
- Furuta M, Zhou A, Webb G, Carroll R, Ravazzola M, Orci L & Steiner DF 2001 Severe defect in proglucagon processing in islet A-cells of prohormone convertase 2 null mice. *Journal of Biological Chemistry* **276** 27197–27202.
- Gastaldelli A, Baldi S, Pettiti M, Toschi E, Camastra S, Natali A, Landau BR & Ferrannini E 2000 Influence of obesity and type 2 diabetes on gluconeogenesis and glucose output in humans: a quantitative study. *Diabetes* **49** 1367–1373.
- Gedulin BR, Jodka CM, Herrmann K & Young AA 2006 Role of endogenous amylin in glucagon secretion and gastric emptying in rats demonstrated with the selective antagonist, AC187. *Regulatory Peptides* **137** 121–127.
- Gelling RW, Du XQ, Dichmann DS, Romer J, Huang H, Cui L, Obici S, Tang B, Holst JJ, Fledelius C *et al.* 2003 Lower blood glucose, hyperglucagonemia, and pancreatic alpha cell hyperplasia in glucagon receptor knockout mice. *PNAS* **100** 1438–1443.
- Gopel S, Kanno T, Barg S, Galvanovskis J & Rorsman P 1999 Voltage-gated and resting membrane currents recorded from β -cells in intact mouse pancreatic islets. *Journal of Physiology* **521** 717–728.
- Gopel SO, Kanno T, Barg S & Rorsman P 2000 Patch-clamp characterisation of somatostatin-secreting delta-cells in intact mouse pancreatic islets. *Journal of Physiology* **528** 497–507.
- Gorus FK, Malaisse WJ & Pipeleers DG 1984 Differences in glucose handling by pancreatic A- and B-cells. *Journal of Biological Chemistry* **259** 1196–1200.
- Grapengiesser E, Salehi A, Qader SS & Hellman B 2006 Glucose induces glucagon release pulses antisynchronous with insulin and sensitive to purinoceptor inhibition. *Endocrinology* **147** 3472–3477.
- Gravholt CH, Moller N, Jensen MD, Christiansen JS & Schmitz O 2001 Physiological levels of glucagon do not influence lipolysis in abdominal adipose tissue as assessed by microdialysis. *Journal of Clinical Endocrinology and Metabolism* **86** 2085–2089.
- Gremlich S, Bonny C, Waeber G & Thorens B 1997 Fatty acids decrease IDX-1 expression in rat pancreatic islets and reduce GLUT2, glucokinase, insulin, and somatostatin levels. *Journal of Biological Chemistry* **272** 30261–30269.
- Gromada J, Bokvist K, Ding WG, Barg S, Buschard K, Renstrom E & Rorsman P 1997 Adrenaline stimulates glucagon secretion in pancreatic A-cells by increasing the Ca^{2+} current and the number of granules close to the L-type Ca^{2+} channels. *Journal of General Physiology* **110** 217–228.
- Gromada J, Hoy M, Buschard K, Salehi A & Rorsman P 2001 Somatostatin inhibits exocytosis in rat pancreatic α -cells by Gi2-dependent activation of calcineurin and depriming of secretory granules. *Journal of Physiology* **535** 519–532.
- Gromada J, Ma X, Hoy M, Bokvist K, Salehi A, Berggren PO & Rorsman P 2004 ATP-sensitive K^{+} channel-dependent regulation of glucagon release and electrical activity by glucose in wild-type and SUR1 $^{-/-}$ mouse alpha-cells. *Diabetes* **53** (Suppl 3) S181–S189.
- Gromada J, Franklin I & Wollheim CB 2007 Alpha-cells of the endocrine pancreas: 35 years of research but the enigma remains. *Endocrine Reviews* **28** 84–116.
- Guenifi A, Ahren B & Abdel-Halim SM 2001 Differential effects of glucagon-like peptide-1 (7–36)amide versus cholecystokinin on arginine-induced islet hormone release *in vivo* and *in vitro*. *Pancreas* **22** 58–64.
- Gyulkhandanyan AV, Lu H, Lee SC, Bhattacharjee A, Wijesekera N, Fox JE, MacDonald PE, Chimienti F, Dai FF & Wheeler MB 2008 Investigation of transport mechanisms and regulation of intracellular Zn^{2+} in pancreatic α -cells. *Journal of Biological Chemistry* **283** 10184–10197.
- Hayashi M, Otsuka M, Morimoto R, Muroyama A, Uehara S, Yamamoto A & Moriyama Y 2003a Vesicular inhibitory amino acid transporter is present in glucagon-containing secretory granules in alphaTC6 cells, mouse clonal alpha-cells, and alpha-cells of islets of Langerhans. *Diabetes* **52** 2066–2074.
- Hayashi M, Yamada H, Uehara S, Morimoto R, Muroyama A, Yatsushiro S, Takeda J, Yamamoto A & Moriyama Y 2003b Secretory granule-mediated co-secretion of L-glutamate and glucagon triggers glutamatergic signal transmission in islets of Langerhans. *Journal of Biological Chemistry* **278** 1966–1974.
- Heimberg H, De Vos A, Pipeleers D, Thorens B & Schuit F 1995 Differences in glucose transporter gene expression between rat pancreatic alpha- and beta-cells are correlated to differences in glucose transport but not in glucose utilization. *Journal of Biological Chemistry* **270** 8971–8975.
- Heimberg H, De Vos A, Moens K, Quartier E, Bouwens L, Pipeleers D, Van Schaftingen E, Madsen O & Schuit F 1996 The glucose sensor protein glucokinase is expressed in glucagon-producing alpha α -cells. *PNAS* **93** 7036–7041.
- Herzig S, Long F, Jhala US, Hedrick S, Quinn R, Bauer A, Rudolph D, Schutz G, Yoon C, Puigserver P *et al.* 2001 CREB regulates hepatic gluconeogenesis through the coactivator PGC-1. *Nature* **413** 179–183.
- Hjorth SA, Adelhorst K, Pedersen BB, Kirk O & Schwartz TW 1994 Glucagon and glucagon-like peptide 1: selective receptor recognition via distinct peptide epitopes. *Journal of Biological Chemistry* **269** 30121–30124.
- Hong J, Abudula R, Chen J, Jeppesen PB, Dyrskog SEU, Xiao J, Colombo M & Hermansen K 2005 The short-term effect of fatty acids on glucagon secretion is influenced by their chain length, spatial configuration, and degree of unsaturation: studies *in vitro*. *Metabolism* **54** 1329–1336.
- Hong J, Jeppesen PB, Nordentoft I & Hermansen K 2007 Fatty acid-induced effect on glucagon secretion is mediated via fatty acid oxidation. *Diabetes/Metabolism Research and Reviews* **23** 202–210.
- Hoy M, Bokvist K, Xiao-Gang W, Hansen J, Juhl K, Berggren PO, Buschard K & Gromada J 2001 Phentolamine inhibits exocytosis of glucagon by Gi2 protein-dependent activation of calcineurin in rat pancreatic alpha-cells. *Journal of Biological Chemistry* **276** 924–930.
- Hunyady B, Hipkin RW, Schonbrunn A & Mezey E 1997 Immunohistochemical localization of somatostatin receptor SST2A in the rat pancreas. *Endocrinology* **138** 2632–2635.
- Inagaki N, Kuromi H, Gono T, Okamoto Y, Ishida H, Seino Y, Kaneko T, Iwanaga T & Seino S 1995 Expression and role of ionotropic glutamate receptors in pancreatic islet cells. *FASEB Journal* **9** 686–691.
- Ishihara H, Maechler P, Gjinovci A, Herrera PL & Wollheim CB 2003 Islet beta-cell secretion determines glucagon release from neighbouring alpha-cells. *Nature Cell Biology* **5** 330–335.
- Juhl CB, Hollingdal M, Sturis J, Jakobsen G, Agerso H, Veldhuis J, Porsen N & Schmitz O 2002 Bedtime administration of NN2211, a long-acting GLP-1 derivative, substantially reduces fasting and postprandial glycemia in type 2 diabetes. *Diabetes* **51** 424–429.
- Kaneko K, Shirotani T, Araki E, Matsumoto K, Taguchi T, Motoshima H, Yoshizato K, Kishikawa H & Shichiri M 1999 Insulin inhibits glucagon secretion by the activation of PI3-kinase in In-R1-G9 cells. *Diabetes Research and Clinical Practice* **44** 83–92.
- Kendall DM, Poitout V, Olson LK, Sorenson RL & Robertson RP 1995 Somatostatin coordinately regulates glucagon gene expression and exocytosis in HIT-T15 cells. *Journal of Clinical Investigation* **96** 2496–2502.
- Kuhara T, Ikeda S, Ohneda A & Sasaki Y 1991 Effects of intravenous infusion of 17 amino acids on the secretion of GH, glucagon, and insulin in sheep. *American Journal of Physiology. Endocrinology and Metabolism* **260** E21–E26.
- Kumar U, Sasi R, Suresh S, Patel A, Thangaraju M, Metrakos P, Patel SC & Patel YC 1999 Subtype-selective expression of the five somatostatin receptors (hSSTR1–5) in human pancreatic islet cells: a quantitative double-label immunohistochemical analysis. *Diabetes* **48** 77–85.

- Kurland IJ & Pilkis SJ 1995 Covalent control of 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase: insights into autoregulation of a bifunctional enzyme. *Protein Science* **4** 1023–1037.
- Landstedt-Hallin L, Adamson U & Lins PE 1999 Oral glibenclamide suppresses glucagon secretion during insulin-induced hypoglycemia in patients with type 2 diabetes. *Journal of Clinical Endocrinology and Metabolism* **84** 3140–3145.
- Larsson H & Ahren B 2000 Islet dysfunction in insulin resistance involves impaired insulin secretion and increased glucagon secretion in postmenopausal women with impaired glucose tolerance. *Diabetes Care* **23** 650–657.
- Leclercq-Meyer V, Marchand J, Woussen-Colle MC, Giroix MH & Malaisse WJ 1985 Multiple effects of leucine on glucagon, insulin, and somatostatin secretion from the perfused rat pancreas. *Endocrinology* **116** 1168–1174.
- Leung YM, Ahmed I, Sheu L, Tsushima RG, Diamant NE, Hara M & Gaisano HY 2005 Electrophysiological characterization of pancreatic islet cells in the mouse insulin promoter-green fluorescent protein mouse. *Endocrinology* **146** 4766–4775.
- Leung YM, Ahmed I, Sheu L, Gao X, Hara M, Tsushima RG, Diamant NE & Gaisano HY 2006 Insulin regulates islet α -cell function by reducing KATP channel sensitivity to adenosine 5'-triphosphate inhibition. *Endocrinology* **147** 2155–2162.
- Li Y, Cao X, Li LX, Brubaker PL, Edlund H & Drucker DJ 2005 Beta-cell Pdx1 expression is essential for the glucoregulatory, proliferative, and cytoprotective actions of glucagon-like peptide-1. *Diabetes* **54** 482–491.
- Li XC, Liao TD & Zhuo JL 2008 Long-term hyperglucagonaemia induces early metabolic and renal phenotypes of Type 2 diabetes in mice. *Clinical Science* **114** 591–601.
- Liang Y, Osborne MC, Monia BP, Bhanot S, Gaarde WA, Reed C, She P, Jetton TL & Demarest KT 2004 Reduction in glucagon receptor expression by an antisense oligonucleotide ameliorates diabetic syndrome in db/db mice. *Diabetes* **53** 410–417.
- Liu YJ, Vieira E & Gylfe E 2004 A store-operated mechanism determines the activity of the electrically excitable glucagon-secreting pancreatic α -cell. *Cell Calcium* **35** 357–365.
- Loubatieres AL, Loubatieres-Mariani MM, Alric R & Ribes G 1974 Tolbutamide and glucagon secretion. *Diabetologia* **10** 271–276.
- Ma X, Zhang Y, Gromada J, Sewing S, Berggren PO, Buschard K, Salehi A, Vikman J, Rorsman P & Eliasson L 2005 Glucagon stimulates exocytosis in mouse and rat pancreatic α -cells by binding to glucagon receptors. *Molecular Endocrinology* **19** 198–212.
- MacDonald PE, De Marinis YZ, Ramracheya R, Salehi A, Ma X, Johnson PR, Cox R, Eliasson L & Rorsman P 2007 A K ATP channel-dependent pathway within α cells regulates glucagon release from both rodent and human islets of Langerhans. *PLoS Biology* **5** e143.
- Manning Fox JE, Gyulchandanyan AV, Satin LS & Wheeler MB 2006 Oscillatory membrane potential response to glucose in islet beta-cells: a comparison of islet-cell electrical activity in mouse and rat. *Endocrinology* **147** 4655–4663.
- Mayo KE, Miller LJ, Bataille D, Dalle S, Goke B, Thorens B & Drucker DJ 2003 International Union of Pharmacology. XXXV. The glucagon receptor family. *Pharmacological Reviews* **55** 167–194.
- McGirr R, Ejlbick CE, Carter DE, Andrews JD, Nie Y, Friedman TC & Dhanvantari S 2005 Glucose dependence of the regulated secretory pathway in α TC1-6 cells. *Endocrinology* **146** 4514–4523.
- Miki T, Liss B, Minami K, Shiuchi T, Saraya A, Kashima Y, Horiuchi M, Ashcroft F, Minokoshi Y, Roeper J *et al.* 2001 ATP-sensitive K⁺ channels in the hypothalamus are essential for the maintenance of glucose homeostasis. *Nature Neuroscience* **4** 507–512.
- Mojsov S, Heinrich G, Wilson IB, Ravazzola M, Orci L & Habener JF 1986 Preproglucagon gene expression in pancreas and intestine diversifies at the level of post-translational processing. *Journal of Biological Chemistry* **261** 11880–11889.
- Munoz A, Hu M, Hussain K, Bryan J, Aguilar-Bryan L & Rajan AS 2005 Regulation of glucagon secretion at low glucose concentrations: evidence for adenosine triphosphate-sensitive potassium channel involvement. *Endocrinology* **146** 5514–5521.
- Nadal A, Quesada I & Soria B 1999 Homologous and heterologous asynchronicity between identified α -, β - and δ -cells within intact islets of Langerhans in the mouse. *Journal of Physiology* **517** 85–93.
- Nauck MA, Heimesaat MM, Behle K, Holst JJ, Nauck MS, Ritzel R, Hufner M & Schmigel WH 2002 Effects of glucagon-like peptide 1 on counterregulatory hormone responses, cognitive functions, and insulin secretion during hyperinsulinemic, stepped hypoglycemic clamp experiments in healthy volunteers. *Journal of Clinical Endocrinology and Metabolism* **87** 1239–1246.
- Olofsson CS, Salehi A, Gopel SO, Holm C & Rorsman P 2004 Palmitate stimulation of glucagon secretion in mouse pancreatic α -cells results from activation of L-type calcium channels and elevation of cytoplasmic calcium. *Diabetes* **53** 2836–2843.
- Olsen HL, Theander S, Bokvist K, Buschard K, Wollheim CB & Gromada J 2005 Glucose stimulates glucagon release in single rat α -cells by mechanisms that mirror the stimulus-secretion coupling in β -cells. *Endocrinology* **146** 4861–4870.
- Ostenson CG, Nylen A, Grill V, Gutniak M & Efendic S 1986 Sulfonyleurea-induced inhibition of glucagon secretion from the perfused rat pancreas: evidence for a direct, non-paracrine effect. *Diabetologia* **29** 861–867.
- Paty BW, Ryan EA, Shapiro AM, Lakey JR & Robertson RP 2002 Intrahepatic islet transplantation in type 1 diabetic patients does not restore hypoglycemic hormonal counterregulation or symptom recognition after insulin independence. *Diabetes* **51** 3428–3434.
- Paul GL, Waegner A, Gaskins HR & Shay NF 1998 Histidine availability alters glucagon gene expression in murine α TC6 cells. *Journal of Nutrition* **128** 973–976.
- Pereverzev A, Salehi A, Mikhna M, Renstrom E, Hescheler J, Weiergraber M, Smyth N & Schneider T 2005 The ablation of the Ca(v)2.3/E-type voltage-gated Ca²⁺ channel causes a mild phenotype despite an altered glucose induced glucagon response in isolated islets of Langerhans. *European Journal of Pharmacology* **511** 65–72.
- Petersen KF & Sullivan JT 2001 Effects of a novel glucagon receptor antagonist (Bay 27-9955) on glucagon-stimulated glucose production in humans. *Diabetologia* **44** 2018–2024.
- Philippe J, Morel C & Cordier-Bussat M 1995 Islet-specific proteins interact with the insulin-response element of the glucagon gene. *Journal of Biological Chemistry* **270** 3039–3045.
- Pipeleers DG, Schuit FC, Van Schravendijk CF & Van de Winkel M 1985 Interplay of nutrients and hormones in the regulation of glucagon release. *Endocrinology* **117** 817–823.
- Quesada I, Nadal A & Soria B 1999 Different effects of tolbutamide and diazoxide in α , β -, and δ -cells within intact islets of Langerhans. *Diabetes* **48** 2390–2397.
- Quesada I, Fuentes E, Andreu E, Meda P, Nadal A & Soria B 2003 On-line analysis of gap junctions reveals more efficient electrical than dye coupling between islet cells. *American Journal of Physiology: Endocrinology and Metabolism* **284** E980–E987.
- Quesada I, Todorova MG & Soria B 2006a Different metabolic responses in α -, β -, and δ -cells of the islet of Langerhans monitored by redox confocal microscopy. *Biophysical Journal* **90** 2641–2650.
- Quesada I, Todorova MG, Alonso-Magdalena P, Beltra M, Carneiro EM, Martin F, Nadal A & Soria B 2006b Glucose induces opposite intracellular Ca²⁺ concentration oscillatory patterns in identified α - and β -cells within intact human islets of Langerhans. *Diabetes* **55** 2463–2469.
- Quoix N, Cheng-Xue R, Guiot Y, Herrera PL, Henquin JC & Gilon P 2007 The GluCre-ROSA26EYFP mouse: a new model for easy identification of living pancreatic α -cells. *FEBS Letters* **581** 4235–4240.
- Qureshi SA, Rios CM, Xie D, Yang X, Tota LM, Ding VD, Li Z, Bansal A, Miller C, Cohen SM *et al.* 2004 A novel glucagon receptor antagonist inhibits glucagon-mediated biological effects. *Diabetes* **53** 3267–3273.
- Ravier MA & Rutter GA 2005 Glucose or insulin, but not zinc ions, inhibit glucagon secretion from mouse pancreatic α -cells. *Diabetes* **54** 1789–1797.
- Reaven GM, Chen YD, Golay A, Swislocki AL & Jaspan JB 1987 Documentation of hyperglucagonemia throughout the day in nonobese and obese patients with noninsulin-dependent diabetes mellitus. *Journal of Clinical Endocrinology and Metabolism* **64** 106–110.

- Rorsman P, Berggren PO, Bokvist K, Ericson H, Mohler H, Ostenson CG & Smith PA 1989 Glucose-inhibition of glucagon secretion involves activation of GABAA-receptor chloride channels. *Nature* **341** 233–236.
- Rosenstock J, Baron MA, Dejager S, Mills D & Schweizer A 2007 Comparison of vildagliptin and rosiglitazone monotherapy in patients with type 2 diabetes: a 24-week, double-blind, randomized trial. *Diabetes Care* **30** 217–223.
- Schuit FC, Derde MP & Pipeleers DG 1989 Sensitivity of rat pancreatic A and B cells to somatostatin. *Diabetologia* **32** 207–212.
- Schuit F, De Vos A, Farfari S, Moens K, Pipeleers D, Brun T & Prentki M 1997 Metabolic fate of glucose in purified islet cells. Glucose-regulated anaplerosis in beta cells. *Journal of Biological Chemistry* **272** 18572–18579.
- Sekine N, Cirulli V, Regazzi R, Brown LJ, Gine E, Tamarit-Rodriguez J, Girotti M, Marie S, MacDonald MJ, Wollheim CB *et al.* 1994 Low lactate dehydrogenase and high mitochondrial glycerol phosphate dehydrogenase in pancreatic beta-cells. Potential role in nutrient sensing. *Journal of Biological Chemistry* **269** 4895–4902.
- Shah P, Basu A, Basu R & Rizza R 1999 Impact of lack of suppression of glucagon on glucose tolerance in humans. *American Journal of Physiology* **277** E283–E290.
- Shah P, Vella A, Basu A, Basu R, Schwenk WF & Rizza RA 2000 Lack of suppression of glucagon contributes to postprandial hyperglycemia in subjects with type 2 diabetes mellitus. *Journal of Clinical Endocrinology and Metabolism* **85** 4053–4059.
- Shiota C, Rocheleau JV, Shiota M, Piston DW & Magnuson MA 2005 Impaired glucagon secretory responses in mice lacking the type 1 sulfonylurea receptor. *American Journal of Physiology. Endocrinology and Metabolism* **289** E570–E577.
- Singh V, Brendel MD, Zacharias S, Mergler S, Jahr H, Wiedenmann B, Bretzel RG, Plockinger U & Strowski MZ 2007 Characterization of somatostatin receptor subtype-specific regulation of insulin and glucagon secretion: an *in vitro* study on isolated human pancreatic islets. *Journal of Clinical Endocrinology and Metabolism* **92** 673–680.
- Slavin BG, Ong JM & Kern PA 1994 Hormonal regulation of hormone-sensitive lipase activity and mRNA levels in isolated rat adipocytes. *Journal of Lipid Research* **35** 1535–1541.
- Sloop KW, Cao JX, Siesky AM, Zhang HY, Bodenmiller DM, Cox AL, Jacobs SJ, Moyers JS, Owens RA, Showalter AD *et al.* 2004 Hepatic and glucagon-like peptide-1-mediated reversal of diabetes by glucagon receptor antisense oligonucleotide inhibitors. *Journal of Clinical Investigation* **113** 1571–1581.
- Song Z, Levin BE, McArdle JJ, Bakhos N & Routh VH 2001 Convergence of pre- and postsynaptic influences on glucosensing neurons in the ventromedial hypothalamic nucleus. *Diabetes* **50** 2673–2681.
- Sorensen H, Brand CL, Neschen S, Holst JJ, Fosgerau K, Nishimura E & Shulman GI 2006 Immunoneutralization of endogenous glucagon reduces hepatic glucose output and improves long-term glycemic control in diabetic ob/ob mice. *Diabetes* **55** 2843–2848.
- Strowski MZ, Parmar RM, Blake AD & Schaeffer JM 2000 Somatostatin inhibits insulin and glucagon secretion via two receptor subtypes: an *in vitro* study of pancreatic islets from somatostatin receptor 2 knockout mice. *Endocrinology* **141** 111–117.
- Strowski MZ, Cashen DE, Birzin ET, Yang L, Singh V, Jacks TM, Nowak KW, Rohrer SP, Patchett AA, Smith RG *et al.* 2006 Antidiabetic activity of a highly potent and selective nonpeptide somatostatin receptor subtype-2 agonist. *Endocrinology* **147** 4664–4673.
- Van Tine BA, Azizeh BY, Trivedi D, Phelps JR, Houslay MD, Johnson DG & Hruby VJ 1996 Low level cyclic adenosine 3',5'-monophosphate accumulation analysis of [des-His1, des- Phe6, Glu9] glucagon-NH2 identifies glucagon antagonists from weak partial agonists/antagonists. *Endocrinology* **137** 3316–3322.
- Tschritter O, Stumvoll M, Machicao F, Holzwarth M, Weisser M, Maerker E, Teigeler A, Haring H & Fritsche A 2002 The prevalent Glu23Lys polymorphism in the potassium inward rectifier 6.2 (KIR6.2) gene is associated with impaired glucagon suppression in response to hyperglycemia. *Diabetes* **51** 2854–2860.
- Tuduri E, Filiputti E, Carneiro EM & Quesada I 2008 Inhibition of Ca^{2+} signaling and glucagon secretion in mouse pancreatic alpha-cells by extracellular ATP and purinergic receptors. *American Journal of Physiology. Endocrinology and Metabolism* **294** E952–E960.
- Uehara S, Muroyama A, Echigo N, Morimoto R, Otsuka M, Yatsushiro S & Moriyama Y 2004 Metabotropic glutamate receptor Type 4 is involved in autoinhibitory cascade for glucagon secretion by α -cells of islet of Langerhans. *Diabetes* **53** 998–1006.
- Unger RH & Orci L 1975 The essential role of glucagon in the pathogenesis of diabetes mellitus. *Lancet* **1** 14–16.
- Vieira E, Salehi A & Gylfe E 2007 Glucose inhibits glucagon secretion by a direct effect on mouse pancreatic alpha cells. *Diabetologia* **50** 370–379.
- Vons C, Pegorier JP, Girard J, Kohl C, Ivanov MA & Franco D 1991 Regulation of fatty-acid metabolism by pancreatic hormones in cultured human hepatocytes. *Hepatology* **13** 1126–1130.
- Vozzi C, Ullrich S, Charollais A, Philippe J, Orci L & Meda P 1995 Adequate connexin-mediated coupling is required for proper insulin production. *Journal of Cell Biology* **131** 1561–1572.
- Wakelam MJO, Murphy GJ, Hruby VJ & Houslay MD 1986 Activation of two signal-transduction systems in hepatocytes by glucagon. *Nature* **323** 68–71.
- Wang F, Adrian TE, Westermark GT, Ding X, Gasslander T & Permert J 1999 Islet amyloid polypeptide tonally inhibits beta -, alpha -, and delta-cell secretion in isolated rat pancreatic islets. *American Journal of Physiology. Endocrinology and Metabolism* **276** E19–E24.
- Wendt A, Birnir B, Buschard K, Gromada J, Salehi A, Sewing S, Rorsman P & Braun M 2004 Glucose inhibition of glucagon secretion from rat α -cells is mediated by GABA released from neighboring β -cells. *Diabetes* **53** 1038–1045.
- Winzell MS, Brand CL, Wierup N, Sidelmann UG, Sundler F, Nishimura E & Ahren B 2007 Glucagon receptor antagonism improves islet function in mice with insulin resistance induced by a high-fat diet. *Diabetologia* **50** 1453–1462.
- Xu E, Kumar M, Zhang Y, Ju W, Obata T, Zhang N, Liu S, Wendt A, Deng S & Ebina Y 2006 Intra-islet insulin suppresses glucagon release via GABA-GABAA receptor system. *Cell Metabolism* **3** 47–58.
- Yamato E, Noma Y, Tahara Y, Ikegami H, Yamamoto Y, Cha T, Yoneda H, Ogihara T, Ohboshi C, Hirota M *et al.* 1990 Suppression of synthesis and release of glucagon by glucagon-like peptide-1 (7–36 amide) without affect on mRNA level in isolated rat islets. *Biochemical and Biophysical Research Communications* **167** 431–437.
- Yoon JC, Puigserver P, Chen G, Donovan J, Wu Z, Rhee J, Adelman G, Stafford J, Kahn CR, Granner DK *et al.* 2001 Control of hepatic gluconeogenesis through the transcriptional coactivator PGC-1. *Nature* **413** 131–138.
- Yoshimoto Y, Fukuyama Y, Horio Y, Inanobe A, Gotoh M & Kurachi Y 1999 Somatostatin induces hyperpolarization in pancreatic islet [alpha] cells by activating a G protein-gated K^{+} channel. *FEBS Letters* **444** 265–269.
- Young A 2005 Inhibition of glucagon secretion. *Advances in Pharmacology* **52** 151–171.
- Zaitsev SV, Efanov AM, Efanova IB, Larsson O, Ostenson CG, Gold G, Berggren PO & Efendic S 1996 Imidazoline compounds stimulate insulin release by inhibition of K(ATP) channels and interaction with the exocytotic machinery. *Diabetes* **45** 1610–1618.
- Zhao C, Wilson MC, Schuit F, Halestrap AP & Rutter GA 2001 Expression and distribution of lactate/monocarboxylate transporter isoforms in pancreatic islets and the exocrine pancreas. *Diabetes* **50** 361–366.
- Zhou H, Zhang T, Harmon JS, Bryan J & Robertson RP 2007a Zinc, not insulin, regulates the rat alpha-cell response to hypoglycemia *in vivo*. *Diabetes* **56** 1107–1112.
- Zhou H, Zhang T, Oseid E, Harmon J, Tonooka N & Robertson RP 2007b Reversal of defective glucagon responses to hypoglycemia in insulin-dependent autoimmune diabetic BB rats. *Endocrinology* **148** 2863–2869.

Received in final form 30 July 2008

Accepted 31 July 2008

Made available online as an Accepted Preprint
31 July 2008