“Pancreatic α-cells and glucagon—neglected metabolic actors”
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Although its function in the regulation of glucose homeostasis in healthy subjects is recognized, the role of glucagon secreted by the α-cells of the pancreatic islets has long been underappreciated in the etiology of type 2 diabetes (T2D) compared with its rival hormone insulin. Numerous studies of recent decades highlight its key role in the development of fasting and postprandial hyperglycemia in T2D patients. The pathophysiology of T2D is characterized not only by insulin resistance and β-cell dysfunction, but also by hyperglucagonemia in the fasting state and lack of glucagon suppression following oral glucose and exaggerated glucagon responses to mixed meal ingestion. The XIIth Servier-IGIS Symposium sought to provide an update on glucagon expression and secretion and its fundamental role in physiology and in the pathology of T2D. It also reviewed the new therapeutic approaches opened up by knowledge of glucagon and α-cells, the exciting advances made in islet transplantation, generation of functional β-cells by α-cell reprogramming, to replace conventional exogenous insulin therapy, and the encouraging use of incretin in monotherapy or in combination therapy. The present Digest summarizes the main topics debated at the meeting.
When, in 1921, Banting and Best tested their first pancreatic extracts in depancreatized dogs, they noticed that a mild, but reproducible hyperglycemia preceded the hoped-for hypoglycemic effect. The phenomenon was attributed to the presence of a glucogenic substance in the pancreatic extracts by Murlin and colleagues in 1923 who gave it the name “glucagon” or mobilizer of glucose. Roger Assan and his coworkers at the Hôtel Dieu (Paris) were the first to report extremely high circulating glucagon levels in ten patients with severe diabetes ketoacidosis and how these levels rapidly fell on treatment. The more universal character of the absolute or relative hyperglucagonemia of diabetes was established by Unger et al in 1971. Nowadays, it is admitted that diabetes is manifested as insufficient release of the hypoglycemic hormone insulin combined with impaired regulation of glucagon secretion. The abnormalities of glucagon secretion are twofold: too much glucagon is secreted during hyperglycemia and too little is released during hypoglycemia. During the XIIth symposium of the International Group on Insulin Secretion (IGIS), supported by an unrestricted educational grant from Servier, the foremost international specialists in the field of the α-cells, glucagon, and diabetes presented their most recent research findings. Below is a summary of the main points addressed.

I- Birth and death of the α-cell

1. Structure of the islets of Langerhans

The islets of Langerhans are scattered throughout the pancreatic tissue and make up less than 2% of the organ’s total mass. The islets, first observed by Paul Langerhans in 1869, contain five distinct cell subtypes, each associated with the secretion of an endocrine hormone (Figure 1). In the mouse, insulin-secreting β-cells make up an estimated 60% to 80% of the islet cells and tend to segregate to the islet core whilst the remaining cells are arranged in the so-called mantle region. The next most prominent cell type corresponds to glucagon-secreting α-cells representing 10% to 20% of the islet cells, followed by smaller numbers of the somatostatin-secreting δ-cells, pancreatic polypeptide (PP) PP-cells and ghrelin-secreting ε-cells (Lefebvre, Rorsman, Lectures). In humans, non-β-cells are often observed both at the periphery and also seemingly in clusters within the center of islets. Such a difference in islet structure between humans and rodents suggests species-specific paracrine regulation, which will be discussed in Chapter III (Kulkarni, Lecture).
2. Origin of α-cells and control of differentiation

*Pancreatic endocrine cells*

The pancreas originates from a dorsal and ventral bud of the foregut endoderm, which are later referred to as dorsal and ventral lobe, or the “tail” and “head” of the adult pancreas. In mouse, the first hormone-expressing cells are detected at E9.5, most of which elaborate glucagon; the first insulin-expressing cells are seen at E10.5 and the majority coexpress glucagon. A second wave of hormone-expressing cells is detected from E13.5 onward and includes glucagon-, insulin-, somatostatin-, ghrelin-, and PP-expressing cells. Migration of differentiated cells and formation of the islets of Langerhans take place during the tertiary transition (E16.5 to birth). The perinatal period is marked by rapid islet cell proliferation and the coalescence of endocrine cells into their final, compact islet structure (Kaestner, Lecture; Nuria et al, unpublished).

Human pancreatic endocrine cells originate from ductal cell precursors in fetal life. Limited studies on post-mortem pancreas in human pregnancy indicate a marginal increase (~1.4-fold) of β-cell mass with no change in islet or β-cell size compared with the larger expansion of islet mass in pregnant rats (2- to 5-fold). The endocrine population expands during early infancy. In adults, subsequent proliferation is thought to involve replication of existing islet cells or a combination of replication and neogenesis (Clark, Lecture; Cnop et al, unpublished). However, islet cell proliferation declines dramatically with age, both in rodents and humans.

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**Figure 1.** Anatomical characteristics of pancreatic islets (from Kawamori et al, unpublished)

(A) Immunohistochemical analysis of mouse pancreatic islets. Insulin-positive β-cells (blue) are located in the core region of the islet. Glucagon-positive α-cells (red) and somatostatin positive δ-cells (green) are located in the mantle area of the islet. A schematic image of the structure is shown on the right. (B) Schematic image of the structure of human islets. α-cells observed in the center of the islets face to intra-islet vascular vessels and are also surrounded by β-cells.
b. α-cells

α-cells are one of the four epithelial cell types of the intestine— with enterocytes, goblet cells, and Paneth cells— arising from common multipotent cells in the intestinal crypts, deriving from the endoderm. All these enteroendocrine cells differentiate as cells migrate up the crypt-villus axis turning over every 3 to 4 days; they actively self-renew and differentiate throughout the life of an animal from a large reservoir of stem cells (Lei- ter, Lecture; Li et al, unpublished). The differentiation of α-cells is governed by numerous transcription factors (Figure 2). The Pdx1 gene is activated in the progenitor cells of the foregut endoderm as early as E8.5 in the mouse. During early development, Pdx1 is expressed in the entire epithelium. In later fetal stages and in the adult pancreas, low levels of Pdx1 expression are found in the entire pancreas, including α-cells, while high expression is restricted to β-cells, a subset of δ-cells, and occasional ductal epithelial cells. In the various models of Pdx1-deficiency, an increase in α-cell number is observed. Whether this is due to loss of inhibition of α-cell development by β-cells— whose number is greatly reduced— or to transdifferentiation of β-cells into α-cells is not known.5 Pdx1+ cells differentiate into Neurogenin 3+ (Ngn3 or Neurog3) endocrine progenitor cells. Cells expressing Ngn3 at high levels are observed only during embryonic development. These cells do not co-express glucagon or insulin, but give rise to all pancreatic endocrine cells (Kaestner, "Pancreatic α-cells and glucagon-neglected metabolic actors").
Figure 3. Schematic model of endocrine subtype specification during pancreatic development (from Courtney et al, unpublished). A: Following redirection towards the pancreatic lineage (Pdx1-expressing cells), the endocrine program is initiated as outlined by the expression of Ngn3 in all endocrine progenitors. These cells have the potential to become either an α-cell or a second progenitor with β- and δ-cell potentials and able to express both Arx and Pax4. Mutual inhibitory interactions between Arx and Pax4 lead to “competition” between the two transcription factors resulting in subtype allocation. If Arx prevails, Pax4 expression is extinguished and an α-cell fate is chosen. If Pax4 predominates and Arx expression is extinguished, the resulting progenitor cell (β/δ) is poised to undergo a second round of competitive fate allocation. In this second event, a hypothetical “factor X” is envisioned to promote the δ-cell fate and to display a mutual inhibitory interaction with Pax4 similar to the interaction between Arx and Pax4 in the first round of cell fate allocation. B-D: Fate changes in the case of Pax4- (B), Arx- (C), or combined Arx-/Pax4- (D) deficiency.
Lecture). Recent evidence has shown that, in fact, Ngn3-expressing progenitor cells pass through different phases of competence where early Ngn3-expressing cells give rise exclusively to α-cells, whereas expression of Ngn3 in later pancreatic developmental stages gives rise successively to β-, PP-, and finally δ-cells.\(^6\) Following the initiation of the endocrine program, a set of transcription factors are required to direct Ngn3-positive cells towards the four mature endocrine cell fates. These factors can be subdivided into early-acting factors (such as Nkx2.2, Nkx6.1, Pax4 or Arx) that are coexpressed with Ngn3 in endocrine precursors and late factors (including Pax6, Isl1, MafA or Pdx1) that are detected in more mature cells (Collombat, Lecture).

3. β- and α-cell life: mass plasticity and cell lifespan and longevity

In animal models, β-cell mass flexibly adapts to insulin requirement (plasticity) which is thought to be regulated by an equilibrium of programmed cell death and neogenesis and/or replication of existing β-cells. Conditions associated with increased demand for insulin as insulin resistance and obesity result in larger β-cell mass in rodents (30-fold) compared with the very modest increases with BMI in humans (1.2-fold) (Clark, Lecture).

Excessive nutrient intake is reported to induce hyperplasia and/or hypertrophy of α-cells. α-cell mass is increased in diabetes. Interestingly, α-cell–specific insulin receptor knockout (αIRKO) mice\(^10\) exhibited a progressive increase in β-cell area, while α-cell area was unchanged by aging, indicating a relative decrease in α-cells in the islets. Thus insulin signaling is likely involved in the proliferation of α-cells (Kulkarni, Lecture).

The half-life of β-cells in young rodents (<1 year old) has been estimated to be 30 to 60 days; cell divisions in mouse islets decrease with age and are very low in animals at age one year, suggesting that mouse islet cells are largely post-mitotic by 12 months. In humans the “birthdate” of β-cells was shown to be in the first three decades of life and no substantial proportion of cell division occurred subsequently. Replication of human adult islet β-cells has been estimated to be 10-fold less than that in adult mouse and to be highest in children under the age of 5 years. Interestingly, using mathematical modeling of lipofuscin body accumulation—a feature of aging in post-mitotic cells—Cnop et al demonstrated that both α- and β-cell populations are largely established by the age of 20 in humans with little evidence for continuous turnover or replication after this age\(^11\) (Clark, Lecture; Cnop et al, unpublished).

II. Regulation of glucagon expression

1. Structure of the glucagon gene

The glucagon gene (Gcg) is expressed in the α-cells of the endocrine pancreas but also in the L-cells of the intestine and in specific areas of the brain.\(^12\) Signal peptide is cleaved from preproglucagon encoded by Gcg mRNA to produce proglucagon, which serves as a precursor for multiple peptides (Figure 4).\(^13\) Glucagon is produced in islet α-cells through cleavage by prohormone convertase 2 (Pcsk2), whereas glucagon-like peptides (GLP-1 and GLP-2) are produced simultaneously in intestinal L-cells and some neurons of the central nervous system through cleavage by prohormone convertase 1 (Pcsk1). Thus, pancreatic proglucagon is mostly processed to glucagon. However, GLP-1 can also be found in islets co-localized with glucagon under certain conditions such as β-cell damage. Several other peptides produced by alternative or incomplete cleavage of proglucagon, ie, glicentin and oxyntomodulin, have been described but their physiological role is not well characterized (Hayashi, Lecture; Hayashi, unpublished; Irwin, Lecture; Irwin and Prentice, unpublished).
2. Transcription factors involved in glucagon gene transcription

a. The other main transcription factors
Foxa1 and Foxa2 are present in the α-cells both during development and in adulthood. Data from mutant mice suggest that Foxa1 is involved in glucagon gene transcription while Foxa2 directs α-cell differentiation more globally. Maf B is expressed specifically in adult α-cells, although it is present in both developing α- and β-cells. Experiments on mice deficient for Maf B showed that this factor regulates important steps in the differentiation of both insulin- and glucagon-producing cells.

b. Hypothesis on the regulation of glucagon gene expression
Philippe and colleagues proposed that glucagon gene expression results from a default pathway: the absence of β-cell−specific factors such as Pdx1, Pax4, and Nkx6.1 would allow for the glucagon gene to be expressed and for the differentiation of the α-cell in the presence of other islet-specific factors. However, since mutant mice lacking Pax6, Foxa2, or Arx have no or few α-cells, this suggests that these factors are the most critical for α-cell differentiation (but not for cell proliferation, as the total number of endocrine cells is not affected by their absence) (Philippe, Lecture; Gosmain et al, unpublished).

III- Regulation of glucagon secretion

It is often assumed that peripheral glucagon concentrations do not accurately reflect the actual α-cell secretion rate, because of hepatic clearance of glucagon. However, the data are controversial. The concentrations of glucagon in plasma are low, with fasting concentrations in healthy individuals around 10 pmol/L. Being an essential part of glucose regulation, glucagon secretion is tightly linked to plasma glucose concentrations, with increases up to 40 pmol/L when glucose levels are lowered to 2-3 mmol/L and decreases to 1-2

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**FOCUS on Pax-6**

Numerous transcription factors are involved in the transcription of the glucagon gene (Figure 2). Pax-6 is expressed in all pancreatic endocrine cells, in the nose, eyes, and central nervous system, and is required for maintaining expression of genes essential for the function of the α-, β-, and δ-cells (Philippe et al, unpublished data). Pax-6 binding is critical to the basal expression of the glucagon gene and to the regulation of proglucagon processing to glucagon in α-cells since inhibition of Pax-6 expression or Pax-6 deletion in mice resulted in a strong decrease in glucagon mRNA levels. Note that Pax-6 is also critical for L-cell differentiation and Gcg gene expression in these cells, suggesting that GLP-1 production could also depend on Pax-6 (Philippe, Lecture; Gosmain et al, unpublished).

**Figure 4. Structure of preproglucagon and derived peptides (from Hayashi et al, unpublished).** Multiple peptides including glucagon, glucagon-like peptide (GLP)-1 and GLP-2 are produced from proglucagon. The sites of cleavage by prohormone convertases, Pcsk 1 and Pcsk 2, are also shown. The receptors for preproglucagon-derived peptides are also depicted.
pmol/L when glucose levels are elevated (to around 10-12 mmol/L) (Holst et al, unpublished). Emerging work reveals a complex but sophisticated regulatory mechanism for the modulation of glucagon output from the α-cells, with effects from pancreatic and endocrine hormones including insulin, somatostatin, epinephrine and incretins, Zn²⁺, nutrients, and the central and autonomic nervous pathways (Kulkarni, Rorsman, Lectures).

Figure 5. Hypothetical model for the regulation of glucagon secretion by insulin and GLP-1 (from Kawamori et al, unpublished). (A) In a high glucose state, stimulated insulin secretion by β-cells acts on the insulin receptor on the surface of α-cells and then suppresses glucagon secretion in a paracrine manner. In a low glucose state, decreased insulin secretion by β-cells is recognized by α-cells as a reduction of insulin signaling in α-cells through insulin receptors, and α-cells then increase glucagon secretion in response. (B) GLP-1 directly suppresses glucagon secretion by α-cells through slight increase of cAMP followed by inhibition of N-type Ca²⁺ channels (27) GLP-1 also potentiates insulin secretion by β-cells and then suppresses glucagon secretion through insulin effects on α-cells. Glucose stimulates insulin secretion by β-cells and suppresses glucagon from α-cells through insulin effects, while glucose can stimulate glucagon secretion by α-cells.
1. Regulation of glucagon secretion by insulin and incretins

The distribution and arrangement of different islet cell types are important for physiological regulation between the cells since the blood flows from the center of the islets towards the periphery, i.e., from β-cells to non-β-cells, suggesting that secreted insulin regulates hormone secretion by other islet cell types. This architecture is typically preserved in rodent islets, while in humans non-β-cells are often observed both at the periphery and also seemingly in clusters within the center of islet (Figure 1). This implies several possibilities; 1) rodent cellular hierarchy in the islets does not apply to human islets, or 2) human islets consist of several clover leaf–like “rosettes”, with each rosette resembling the basic islet architecture observed in rodent islets. Whether the blood flow is similar to that seen in rodents within each rosette in the human “islet” is currently unclear (Kulkarni, Lecture).

a. Role of insulin

In vivo and in vitro studies indicate that insulin suppresses glucagon secretion. Data on the αIRKO mouse provided the first direct genetic evidence for a significant role for insulin signaling in the regulation of α-cell function in both normoglycemic and hypoglycemic states in vivo. Whereas their glucagon response to hypoglycemia in the presence of fasting-induced hypoinsulinemia was impaired, the knockouts exhibited significantly enhanced glucagon response to hyperinsulinemia-induced hypoglycemia. In contrast, glucagon secretion was enhanced in control mice in the first conditions but suppressed in the second. This suggested that the αIRKO mice were unable to sense the variations in ambient insulin and therefore to adapt their glucagon secretion. Interestingly, streptozotocin (STZ)-induced hyperglycemia secondary to hypoinsulinemia induced a paradoxical similar increase in plasma glucagon levels both in control and αIRKO mice. Further, in STZ-treated mice, normalization of hyperglycemia by phloridzin treatment decreased plasma glucagon to levels comparable to those of normoglycemic untreated mice. These findings suggested that hyperglycemia itself stimulates glucagon secretion by α-cells in vivo in the absence of insulin, and that the intra-islet effect of insulin plays a central role in physiological suppression of glucagon secretion induced by high glucose. It was also demonstrated that hyperglycemia itself is able to stimulate glucagon secretion independently of insulin since it induced a large increase in glucagon secretion under supraphysiological hyperinsulinemia in both control and αIRKO mice. It is worth noting that this phenomenon might, in part, involve other modulators of glucagon secretion including glutamate, neurotransmitters, and the nervous system, while alternatively a direct effect of low glucose on glucagon secretion is also possible. A model for the regulation of glucagon secretion is proposed in Figure 5A by Kulkarni et al: in states of hyperglycemia the greater insulin secretion by β-cells activates insulin signaling in α-cells and represses glucagon secretion. On the other hand, in hypoglycemic states the consequent low levels of insulin would allow the α-cells to sense the reduction in ambient insulin leading to a lack of activation of insulin signaling proteins which in turn leads to the stimulation of glucagon secretion. This would occur in addition to direct stimulation by low glucose itself. A recent clinical study reported that this mechanism is actually feasible in humans (Kulkarni, Lecture).

b. Role of incretins

Incretins are hormones released from enteroendocrine cells in the gut that act to potentiate glucose clearance in response to the ingestion of food. This potentiation, termed the “incretin effect,” is due to the insulinotropic capacity of incretins. In fact, incretins are thought to be responsible for between 50% and 70% of the insulin release from pancreatic β-cells in response to an oral glucose load. There are two known mammalian incretin hormones: glucose-dependent insulinotropic peptide (GIP), and GLP-1.
Incretin genes

GIP and GLP-1 are each encoded by distinct genes in mammalian genomes. The Gip gene solely encodes GIP and its expression is restricted to the intestinal K-cells. As already mentioned, GLP-1 is encoded by the proglucagon (Gcg) gene. Mammalian GLP-2 is primarily involved in intestinal proliferation and function, bone breakdown, and neuroprotection. Interestingly, while glucagon activity is strongly conserved across vertebrate species, the role of GLP-1 differs between mammals and fish and the role of GLP-2 has only been defined in mammalian species. The function of the GIP hormone has only been described in a limited number of mammalian species with a few other diverse vertebrate species possessing a GIP-like gene. Both Gip and Gcg exist as single copy genes in mammalian genomes, and reside within stable genomic neighborhoods with a strong conservation of the flanking gene order. The glucagon and GLP-1 sequences are largely invariant among mammals.

Incretin functions

Functionally, these hormones are nearly identical in their ability to stimulate insulin secretion, with each being able to compensate in the event of a loss of function of the other, although each incretin has a specific receptor that is unable to be activated by the other incretin peptides. GIP is the first incretin produced following an enteral dose of glucose, and is secreted from the K-cells of the duodenum and the jejunum. GLP-1 is produced shortly thereafter by the L-type enteroendocrine cells of the ileum and colon. In addition to stimulating insulin secretion, they also promote β-cell survival by inhibition of apoptosis and stimulation of proliferation. The incretin hormones also have physiological effects on insulin target tissues, as liver, adipose tissue, and skeletal muscles, thereby increasing insulin sensitivity, glucose uptake, and metabolism. The primary difference between GIP and GLP-1 is the non-insulinotropic activity of GLP-1, but not GIP. GLP-1 acts on the stomach to inhibit gastric emptying and thus regulate nutrient uptake, as well as on the brain to increase satiety and reduce nutrient consumption (Irwin, Lecture; Irwin and Prentice, unpublished).

GIP and GLP-1 act through receptors located on the plasma membrane of target tissues. The presence of GLP-1 receptors in α-cells is a highly controversial question. Administration of the GLP-1 receptor antagonist exendin 9-39 is associated with elevations of plasma glucagon concentration, suggesting that endogenous GLP-1 inhibits glucagon secretion. The intra-islet hypothesis that inhibition of glucagon secretion is secondary to stimulation of β-cells is incompatible with the observation that GLP-1 inhibition of both basal and stimulated glucagon secretion is also observed in patients with no residual β-cells, or after a treatment with a powerful insulin receptor antagonist (Holst, unpublished). Moreover, GLP-1 treatment significantly suppressed plasma glucagon in IRKO mice to the same extent as in controls. Further, an oral glucose load suppressed plasma glucagon more than did intraperitoneal glucose, where GLP-1 secretion is not stimulated, but elevates blood glucose to the same extent. These data imply that GLP-1 can suppress glucagon secretion directly and independently of insulin (Kulkarni, Lecture). Accordingly, De Marinis et al reported that expression of GLP-1 receptors on α-cells is less than 0.2% of its expression in β-cells; thus GLP-1 can induce a small elevation in cAMP activating PKA followed by selective inhibition of N-type Ca²⁺ ion channels, leading to the suppression of glucagon exocytosis (Figure 5B). Recently, in vitro experiments in rats showed that GLP-1–induced inhibition of glucagon secretion involves somatostatin secreted from neighboring δ-cells (Holst, Lecture; Holst et al, unpublished). In contrast to GLP1, GIP receptors are expressed abundantly in α-cells and their activation stimulates electrical activity significantly, leading to an increase in Ca²⁺, which causes glucagon exocytosis to accelerate through activation of L-type Ca²⁺ ion channels (Kulkarni, Lecture).
Pancreatic α-cells and glucagon-neglected metabolic actors

Role in diabetes

Glucagon secretion exhibits characteristic abnormalities in type 2 diabetes (T2D). Very often patients have fasting hyperglucagonemia, and exaggerated responses to meal tests. Whereas in healthy controls intravenous and oral glucose brought about the same suppression of glucagon secretion, the diabetic subjects showed a paradoxical increase and lack of suppression after the oral challenge for the first 45 to 60 min, but the suppression after intravenous isoglycemic glucose infusions was nearly normal.23 The same observation was made in patients with type 1 diabetes (T1D) and no residual β-cells,29 suggesting that the difference cannot result from disturbed intra-islet interaction between β- and α-cells. Recently, Lund et al30 demonstrated that the differential consequences of oral and intravenous glucose could be the result of the combined effects of 3 hormones: GLP-1 suppresses glucagon, GLP-2 reduces the suppression, while GIP reverses the suppression and causes clear stimulation for the first 30 min. Interestingly, it was recently shown that GLP-1-induced insulin stimulation and glucagon inhibition contribute equally to the glucose-lowering effect of GLP-1 in T2D patients31 (Holst, Lecture; Holst et al, unpublished).

2. Other factors

Uncoupling protein-2 (UCP2) is expressed in pancreatic β- and α-cells,32 as well as in other tissues, and has been shown to contribute to mitochondrial proton leakage. In the cell-cell, such activity may uncouple glucose metabolism from ATP synthesis thereby impeding the mechanism that triggers insulin secretion. The development of STZ-induced hyperglycemia is significantly less severe in UCP2KO than in wild-type mice. Interestingly, UCP2-deficient α- and β-cells had chronically higher cellular reactive oxygen species (ROS) levels than the wild-type prior to STZ application. Higher ROS in UCP2-deficient β-cells was associated with improved glucose-stimulated insulin secretion. However, higher ROS in UCP2-deficient α-cells was associated with impaired glucose-regulated glucagon secretion, leading to an attenuation of STZ-induced hyperglycemia33 (Wheeler, Lecture; Hardy et al, unpublished).

Somatostatin inhibits both glucagon and insulin secretion. Its effects on glucagon are most likely mediated by somatostatin receptor subtype 2, which is highly expressed in α-cells. Somatostatin activates K+ channels, which induce membrane hyperpolarization and reduce Ca2+-dependent exocytosis.34

GABA, a major neurotransmitter that inhibits neuronal firing in the central nervous system and is produced in the β-cells, also directly inhibits glucagon secretion by hyperpolarizing the α-cell plasma membrane.35

Glutamate, which has been shown to increase in parallel with glucagon, may be another important regulator of paracrine signaling to the α-cell. Glutamate, when secreted in response to low glucose, likely modulates plasma membrane potential and acts as a glucagon-positive regulator.36

Zinc may also act as a paracrine regulator of the α-cell. In β-cells, zinc is required for normal insulin crystallization, the formation of dense core granules, and is released along with insulin. In rat α-cells, zinc acts by increasing the activity of KATP channels, which leads to the inhibition of glucagon secretion. Conversely, in mouse α-cells, the inhibitory effects of zinc on glucagon secretion involves intracellular zinc transport by calcium channels and modulation of the cellular redox state. A recent report also indicated that a sudden decrease in zinc secretion during hypoglycemia triggers glucagon secretion, suggesting that zinc, like insulin, mediates a zinc switch-off signal for glucagon during glucose deprivation in perfused islets in mice.37 However, studies in human and mouse islets showed zinc to have no effects on glucagon secretion. Mice lacking ZnT8 zinc transporter in β-cells reduce first-phase insulin secretion but maintain normal glucagon secretion (less zinc is secreted from
ZnT8βKO β-cells, leading to reduced extracellular zinc levels). Like wild-type islets, in ZnT8βKO islets the cumulative inhibitory effects of other paracrine factors and potentially direct glucose sensing prevailed in the presence of high glucose. Overall, in this study, zinc secreted by β-cells does not appear to act as an inhibitor of glucagon secretion (Wheeler, Lecture; Hardy et al, unpublished).

Figure 6. Glucose dependence of pancreatic hormone secretion by mouse (A) and human (B) pancreatic islets (from Walker et al, unpublished). Data for insulin, glucagon and somatostatin were recorded from the same islets at 0-20 mM (mouse) and 1-20 mM glucose (man). Data are mean values ± S.E.M. of 8 (A) and 7-12 (B) experiments. In (B), the individual experiments were conducted on islets from 3-4 different pancreases. Shaded area indicates range of glucose concentrations over which most of the regulation of the inhibition of glucagon secretion occurs. All values are statistically different (*P<0.05, or better) for all values measured at 3 mM glucose and above except for insulin secretion in mouse islets where the difference first becomes statistically different at 10 mM, †P<0.05 (or better) for glucagon secretion at 20 mM glucose vs that at 5 mM (mouse) or 6 mM (human).
3. Effects of glucose and role of $K_{\text{ATP}}$ channels

In human and mouse islets, glucagon secretion is inhibited at glucose concentrations <6 mM (with a nearly maximal inhibition by 3 mM), but paradoxically is “stimulated” at higher glucose concentrations: 20 mM glucose is significantly less inhibitory than 5 mM (mouse islets) and 6 mM (human islets) (Figure 6). Interestingly, the effects of glucose on glucagon secretion, at least for the responses to concentrations up to 6 mM, seem not to be mediated by paracrine effects from $\beta$- or $\delta$-cells since these glucose concentrations are not associated with any major stimulation of insulin or somatostatin secretion. Thus, and as already noted in this review, it seems likely that the $\alpha$-cell is not only under paracrine control but is also equipped with an intrinsic regulation to respond to variations of ambient glucose. The nature of this intrinsic regulation remains poorly defined but ATP-sensitive potassium channels ($K_{\text{ATP}}$-channels) of the same type as those found in $\beta$-cells seem to be involved. Human $\alpha$-cells contain $K_{\text{ATP}}$-channels and their activation leads to repolarization. Rorsman et al proposed a unifying hypothesis that integrates both paracrine and intrinsic regulation of glucagon secretion. During hypoglycemia (ie, induced by exercise), glucagon secretion is enhanced due to slight activation of the $K_{\text{ATP}}$-channels with resultant firing of large-amplitude action potentials (Figure 7A). An increase in glucose (by ingestion of a glucose-rich meal) inhibits glucagon secretion by closure of the $K_{\text{ATP}}$-channels. The resulting membrane depolarization leads to a decreased amplitude of the $\alpha$-cell action potential and reduced activation of voltage-gated P/Q-type $\text{Ca}^{2+}$-channels.

![Figure 7. Model for the regulation of glucagon secretion (from Walker et al, unpublished). The legend is detailed in the text (Chapter III.3).](image-url)
which culminates in suppression of exocytosis (Figure 7B). Following ingestion of a mixed meal (rich in glucose, amino acids, and lipids), glucagon secretion is switched off by a combination of K_{ATP}-channel closure (as in Figure 7B) and activation of paracrine inhibitory signaling (indicated by the -) due to parallel stimulation of secretion in the neighboring \(\beta\)- and \(\delta\)-cells. The latter supersedes the stimulatory effects of amino and free fatty acids (Figure 7C). In the fasted state (and starvation), however, when plasma concentrations of free fatty and amino acids are increased by mobilization of bodily depots, the low plasma glucose levels ensure minimal stimulation of exocytosis in \(\beta\)- and \(\delta\)-cells. In the absence of inhibitory paracrine signals derived from these cells, the stimulatory effect of low glucose (mediated by activation of K_{ATP}-channels) is amplified by the presence of amino and free fatty acids (Figure 7D) (Rorsman, Lecture; Walker et al, unpublished).

4. Role of the central and the autonomic nervous systems

- Glucose-sensing cells are located at several anatomical sites: the mouth, gut, hepatoportal vein area, brainstem, and hypothalamus (mainly the arcuate nucleus and paraventricular nucleus). It is now well established that glucose-sensing cells present in the central nervous system are either excited by a rise in blood glucose (glucose-excited or GE neurons) or by fall in blood glucose concentrations (glucose-inhibited or GI neurons). These neurons are thought to be responsible for activation of the sympathetic and parasympathetic branches of the autonomic nervous system, which control glucagon and insulin secretion. On \(\alpha\)-cells, the sympathetic neurotransmitter norepinephrine binds to \(\beta_2\)-adrenergic receptor, which stimulates glucagon secretion, whereas on \(\beta\)-cells it binds to \(\alpha_2\)-adrenergic receptors, which inhibits insulin secretion. Activation of the sympathoadrenal system, which induces release of epinephrine into the blood by the adrenals, combines with nervous secretion of norepinephrine directly to the islet cell level to stimulate glucagon secretion and to inhibit insulin secretion. Acetylcholine, the main neurotransmitter of the parasympathetic nervous system, stimulates secretion of both glucagon and insulin.

- Central detection of hypoglycemia also controls counterregulation as demonstrated by the secretion of glucagon and catecholamines induced by intracerebroventricular injection of 2-DG. Interestingly, glucose-induced GABA secretion in the ventromedial hypothalamus blocks the hyperinsulinemic hypoglycemia-induced activation of glucagon secretion. Besides the ventromedial hypothalamus, the glucoregulatory response to hypoglycemia is also controlled by glucose-sensitive neurons of the brainstem.

- The mechanisms of glucose sensing by GE and GI neurons are still incompletely defined. Some mechanisms involved in neuronal hypoglycemia detection and subsequent control of glucagon secretion can be mentioned:
  - In a mouse model of the glucose transporter Glut2 gene inactivation, glucose-stimulated insulin secretion is prevented and glucagon secretion in response to insulin-induced hypoglycemia or 2-DG-induced neuroglucopenia is suppressed. Transgenic complementation studies indicate that GLUT2 expression in astrocytes but not in neurons is important for the counterregulatory response to hypoglycemia.
  - Glucokinase participates in both hyper- and hypoglycemia detection. Pharmacological intracerebroventricular inhibition of glucokinase prevents normal counterregulation to hyperinsulinemic hypoglycemia. Interestingly, recurrent hypoglycemia, which induces impaired counterregulation to subsequent hypoglycemic episodes (a model of hypoglycemia-associated autonomic failure), is associated with an increase in glucokinase activity in the hypothalamus.
  - Central inhibition of K_{ATP}-channels prevents glucagon secretion in response to hyperinsulinemic hypoglycemia. In contrast, activation of this channel in the
ventromedial hypothalamus amplifies the counterregulatory hormone responses to hypoglycemia in normal and recurrently hypoglycemic rats.44

- Inhibition of the metabolic sensor AMP-activated kinase in the arcuate nucleus and ventromedial hypothalamus impairs early glucagon and catecholamine responses to hypoglycemia. AMP-activated kinase may also be involved in the counterregulatory response to hypoglycemia or to neuroglucopenia45 (Thorens, Lecture; Thorens, unpublished).

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IV- Role of glucagon in metabolism

The radioimmunoassay described by Unger and coworkers in 1959 has made it possible to establish the physiological role of glucagon as the “hormone of energy need” in circumstances like hypoglycemia, starvation, physical exercise, and adaptation to extruterine life (Lefebvre, Lecture).

1. Physiological role of glucagon

Both glucagon and insulin are pivotal in systemic energy homeostasis, and the balance between these two hormones determines the metabolic state of various organs in response to changes in energy status. During postprandial hyperglycemia, insulin secretion by β-cells is stimulated while glucagon secretion by α-cells is suppressed, leading to a lowering of blood glucose levels. In contrast, in starvation-induced hypoglycemia, glucagon secretion is promoted while insulin secretion is reduced, causing elevated blood glucose levels (Kulkarni, Lecture). Note that the response to hypoglycemia is not only the suppression of the hyperglycemic responses, but also a set of positive counterregulation mechanisms aimed at preventing a fall in blood glucose concentrations that could otherwise threaten brain function and survival of the individual (detailed in Chapter 2).

Glucagon action is mediated by a G protein-coupled receptor (GCGR/Gcgr) that is coupled to adenyl cyclase. Glucagon also mediates an increase in intracellular calcium in a phospholipase C-dependent manner and activates AMP-activated kinase and c-Jun N-terminal kinase. Binding sites for glucagon have been identified in liver, kidney, intestinal smooth muscle, brain, adipose tissue, heart, and β-cells. There is controversy regarding the presence of the Gcgr in pancreatic α-cells. Activation of the Gcgr in hepatocytes mediates the primary actions of glucagon: ketogenesis and increased hepatic glucose production—glucagon increases the net hepatic glucose output through increased expression of gluconeogenesis enzyme and glycogen degradation without altering the gluconeogenesis flux (Cherrington, Lecture). Glucagon has potent hypolipidemic actions and reduces triglycerides and very-low-density lipoprotein release as well as the deposition of triacylglycerol in the liver, in addition to reducing cholesterol levels and stimulating fatty acid oxidation (Charron, Lecture).

The mechanisms regulating the degradation and clearance of glucagon remain incompletely understood. Glucagon is catabolized by the enzyme neutral endopeptidase 24.11 in pigs, as well as in vitro by dipeptidyl peptidase-4 at pharmacological levels. Hepatic clearance of glucagon seems to be 20%-30% of the portal content, with the kidney contributing to the major portion of peripheral glucagon removal (D’Alessio, Charron, Cherrington, Lectures; D’Alessio, unpublished).

Using knockout and transgenic technology, genetically modified animal models have been developed to clarify the physiological role of glucagon. Homozygous glucagon-GFP knock-in mice (Gcggfp/gfp) lack most, if not all, peptides derived from the glucagon gene and thus provide an opportunity to analyze the metabolic impact of glucagon deficiency without the influence of GLP-1 overexpression. The mutant displays α-cell hyperplasia but normoglycemia, despite complete glucagon deficiency, probably allowed by the lower plasma insulin levels and glucagon-independent mechanisms that maintain gluconeogenesis (Hayashi, Lecture; Hayashi, unpublished). Mutant mice with glucagon receptor deficiency (Gcgr−/−) are characterized by severe α-cell hyperplasia, hyperglucagonemia, and profound elevations of GLP1. They show decreased blood glucose (with decreased glycogenolysis and gluconeogenesis), decreased capacity of the liver to oxidize lipid, with development of hepatic steatosis in some instances, but are protected from diet-induced obesity (D’Alessio, Lecture; D’Alessio, unpublished; Hayashi, Lecture; Hayashi, unpublished). Interestingly, in Gcgr−/− mice administration of a double dose of STZ led to β-cell destruction, as in wild-type mice,
but none of the foregoing clinical or laboratory manifestations of diabetes appeared, unlike in wild-type mice. Fasting glucose levels and oral and intraperitoneal glucose tolerance test results were normal with no increase in insulin level above the basal value, suggesting that insulin is not involved in the normal glucose tolerance of Gcgr⁻/⁻ mice.49 Finally, in transgenic mice that over-express Gcgr in insulin-producing cells (RiP-Gcgr),50 islets displayed a significant increase in insulin secretion in response to glucagon compared with that of controls, suggesting that the regulatory role of glucagon in insulin release may be associated with the number of glucagon-binding sites on insulin cells. Furthermore, RiP-Gcgr mice displayed enhanced glucose tolerance and a small, but significant increase in insulin cell volume compared with littermate controls. Thus glucagon action also seems to be involved in the regulation of insulin cell mass and in the potentiation of glucose-stimulated insulin secretion by increasing insulin cell competency (Charron, Lecture; Vuguin and Charron, unpublished).

2. Altered glucagon secretion and counterregulation in diabetes
While elevations in blood glucose suppress glucagon levels in nondiabetic subjects, diabetic individuals, in contrast, have a blunted or absent α-cell response to hyperglycemia and plasma glucagon remains inappropriately elevated at comparable levels of blood glucose.2 T2D patients have hepatic insulin resistance and inappropriate fasting glucagon secretion to drive excessive hepatic glucose production. The postprandial rise of glucagon is increased in T2D, providing an additional factor to impair insulin action, working against glucose homeostasis. Moreover, glucagon secretion is increased by normal α-cell stimuli, such as intravenous arginine and protein-rich meals, to a greater extent in T2D than nondiabetic individuals2 (D’Alessio, Lecture; D’Alessio, unpublished).

In health, early defenses against hypoglycemia include reduction in β-cell insulin secretion, pancreatic glucagon release, epinephrine secretion by the adrenal medulla, and sympathetic nervous system activation. Later defenses include cortisol and GH secretion as well as hepatic auto-regulation which may also help to restore euglycemia. The first of the hormone defenses lost during the course of T1D is glucagon, which occurs despite the normal number and histological appearance of α-cells in the pancreatic islets. Glucagon counterregulation (GCR) is also defective in advanced T2D. The mechanism by which hypoglycemia stimulates GCR and how it is impaired in insulin-deficient diabetes is still unclear and may include impaired blood glucose-sensing in the α-cells, autonomic dysfunction, and/or loss of an insulin “switch-off” signal from the β-cells. Using mathematical modeling to analyze and reconstruct the GCR control network, it was predicted that lack of β-cell signaling to the α-cells can be viewed as a key network deficiency which impairs the GCR by contributing to two separate network abnormalities: (i) absence of a β-cell switch-off trigger and mainly (ii) increase in intraislet auto-feedback-independent glucagon release. Importantly, in vivo defective GCR can be repaired by two different α-cell inhibitor signals, insulin and somatostatin, which upon switch-off trigger pulsatile GCR.51 Such an α-cell inhibitor-based GCR repair treatment can be added to a variety of existing insulin replacement therapies to treat diabetes and is expected to stabilize blood glucose control and improve safety by reducing the risk of hypoglycemia (Farhy, Lecture; Farhy and McCall, unpublished).

V- Therapeutic perspectives
Diabetes is a major and increasing health problem worldwide which presently affects over 150 million people. Today’s treatment includes lifestyle changes (dietary adjustments, increased physical activity) with addition of metformin as a first-line phar-
macological therapy. When this is insufficient, the choice until recently has been addition of sulfonylureas, glinides, α-glucosidase inhibitors, GLP-analogues and dipeptidyl IV inhibitors, thiazolidinediones, or insulin, or a combination thereof. These treatments offer improved glycemic control, but are also associated with significant adverse events such as hypoglycemia, weight gain, and gastrointestinal discomfort. Moreover, especially at later disease stages, it becomes increasingly difficult to maintain physiological blood glucose levels and to avoid secondary macro- and microvascular complications, such as cerebrovascular accidents, myocardial infarction, and neuropathy. Therefore, other treatment options have received much attention in recent years. Below is an overview of therapeutic targets.

1. **Insulin replacement therapy**
Insulin replacement therapy stabilizes some of the metabolic disturbances of diabetes, but is not a cure and typically cannot prevent future severe chronic complications that stem from the hyperglycemic episodes produced by inaccurate insulin doses.

2. **Pancreatic islet transplantation**
Islet transplantation is a promising approach in attempts to replace conventional exogenous insulin therapy. However, its substantial benefits conferring insulin independence and ameliorating hyperglycemia diminish over the first five years in most patients. Furthermore, there is a limited supply of high-quality donor pancreases and the use of islets from multiple donors is typically required to achieve insulin-independence. Additionally, pancreas transplantation involves major risks associated with both the surgery and the long-term post-transplant immunosuppressive treatment, so this procedure is seldom used to treat diabetes. An attractive alternative that circumvents such invasive complicated surgery is the transplant of donor allogeneic islets. Islets may be infused into the recipient portal vein by a minimally invasive technique. Furthermore, pancreatic islets have been reported to maintain their functionality and viability in culture, thereby allowing for pre-transplant ex vivo manipulation procedures and possibly eliminating the need for chronic use of powerful immunosuppressive agents. Infusion of islet suspensions via the portal vein results in their engraftment into the recipient liver, yet the rate of islet engraftment is unfortunately low as a result of several mechanisms. Numerous gene delivery studies have been performed with the purpose of protecting the allografts from immune rejection, apoptosis and inflammation. Recently, adeno-associated viruses have emerged as excellent vectors to target pancreatic β-cells and also confer long-term expression. For example, gene delivery of VEGF to pancreatic islets improved graft survival and promoted revascularization (Kieffer, Lecture; Tudurí and Kieffer, unpublished).

3. **The generation of functional β-cells**
Alternative sources of functionally competent, insulin-secreting β-cells as substitutes for donor islets to meet the clinical need for transplantation therapy are under investigation. Interestingly, recent evidence of an inherent plasticity of mature pancreatic cells has fuelled interest in in vivo (re-)generation of β-cells, through β-cell self-replication, differentiation from putative precursor cells, or by reprogramming from endogenous cell types. The use of human embryonic stem cells or of induced pluripotent stem cells may constitute an unlimited source of replacement β-cells. However, to date, despite a number of established protocols, both the efficiency of the in vitro differentiation/programming and the function of the derived β-cells remain limited.

   a. **The use of enteroendocrine cells**
Given the similar secretion patterns of GLP-1, GIP, and insulin, targeting insulin ex-
pression to either K- or L-cells of the intestine may be able to recapitulate meal-regulated physiological release of insulin, thereby eliminating the requirement for exogenous insulin. Importantly, enteroendocrine cells contain prohormone convertases, which are required to process proinsulin into mature bioactive insulin, and also secretory vesicles, suggesting that they could potentially process, store, and secrete insulin in a similar fashion to pancreatic β-cells. From these observations, ectopic expression of insulin from K-cells was performed and the experiments showed that the insulin produced was sufficient to maintain glucose homeostasis in the transgenic animals and to protect them from developing diabetes following β-cell damage. However, more investigations and improvements are needed before application in human subjects. Several studies have attempted to generate β-cells in vivo by ectopic expression of defined transcription factors in cells from the gut, given that the pancreas naturally develops from the gut. For example, adenoviral-induced overexpression of MafA (Ad-MafA) in rat intestinal cells in vivo promoted their differentiation into insulin-producing cells, resulting in increased plasma insulin levels and amelioration of hyperglycemia after STZ treatment. However, the insulin-producing intestinal cells did not secrete more insulin in response to an oral glucose load. Further investigation is clearly required to develop safe and effective vectors and approaches to make glucose-responsive insulin-producing cells (Kieffer, Lecture; Tudurí and Kieffer, unpublished).

b. Conversion of α-cells into functional β-cells
As described in Chapter I2c, forced Pax4 overexpression in glucagon-producing cells resulted in an increase of β-like cells. This indicates that a switch from α- to β-cell fate is possible. This discovery has led to the exciting possibility that Pax4 could be used for “reprogramming” of α-cells to β-cells. Recently, it was shown that following diphtheria toxin-mediated ablation of >99% of β-cells in transgenic mice, the islet remnant mainly composed of α-cells was reprogrammed to upregulate β-cell transcription markers (as Pdx1 and Nkx6.1) and cells co-expressed glucagon and insulin transiently, before turning to the mature and functional β-cell fate. Thus α-cells can be reprogrammed to become β-cells. However, it is unclear whether the newly-formed β-cells are capable of replacing original, fully functional β-cells, as many mice in both studies are diabetic and this raises the question of the source of α-cells to be reprogrammed for use in human transplantation (Kaestner, Lecture; Bramswig and Kaestner, unpublished).

4. Incretin-based therapy
In 2005, the treatment choices for T2D were expanded by the introduction of incretin-based therapy, considering the pleiotropic antidiabetic effects of GLP-1. A challenge in the development has been the short half-life of GLP-1 (1-2 minutes), due to the rapid inactivation through truncation by removal of the N-terminal dipeptide end by DPP-4. To overcome this shortcoming of native GLP-1 as a therapeutic agent, two strategies have been explored and developed in clinical practice: GLP-1 receptor agonists and DPP-4 inhibitors.

- The GLP-1 receptor agonists have high affinity for the receptors and are largely resistant to inactivation by DPP-4. They have therefore the ability to achieve long-standing GLP-1 receptor activation. There are two kinds of GLP-1 receptor agonists: exendin-4-based compounds (Exenatide (Byetta®, Amylin/Lilly) and lixisenatide (Sanofi Aventis)) and true GLP-1 analogues (liraglutide (Victoza®, Novo Nordisk)). Both drugs reduce blood glucose and lower hemoglobin A1c in diabetic subjects, with concurrent improvements in insulin secretion and reduction in plasma glucagon. Ways to prolong the half-lives of GLP-1 receptor agonists are under investigation, so as to avoid repeated administration.
- DPP-4 inhibitors inhibit the catalytic site of DPP-4 and prevent the inactivation of endogenous GLP-1. They also increase GIP levels, along with insulin secretion,
duce glucagon secretion and improve blood glucose. DPP-4 inhibitors are all orally active small molecules: sitagliptin (Januvia®, Merck) was introduced clinically in 2006, followed by vildagliptin (Galvus®, Novartis, 2008), saxagliptin (Onglyza®, BMS/AstraZeneca, 2009) and (in Japan only so far) alogliptin (Takeda) (D’Alessio, Lecture; D’Alessio, unpublished).

Incretin-based therapy can be used in several indications in patients with T2D with insufficient glycemic control, as monotherapy or in combination with metformin, sulfonylurea, thiazolidinediones, and insulin. Several recent studies have shown that patients treated with metformin and incretin-based therapy have considerably less hypoglycemia and no weight gain (DPP-4 inhibitors) or weight loss (GLP-1 receptor agonists), compared with treatment with metformin and sulfonylurea which is associated with high incidences of hypoglycemia and weight gain. However, there are still no long-term studies with incretin-based therapies that include cardiovascular hard end-point data, although data from clinical studies have shown high safety with very low risk of adverse events, and the cost of the treatment is considerably higher than that of sulfonylurea.

One potential new indication for incretin-based therapy is T1D, since GLP-1 may preserve and even possibly restore β-cell function, as well as potentially expanding the β-cell mass and also inhibiting glucagon secretion. Administration of pharmacologic amounts of GLP-1 to hyperglycemic T1D subjects with minimal β-cell function caused a 3-4 mM drop in blood glucose coincident with a 40%-50% decrease in plasma glucagon.

An important potential future development of incretin-based therapy is also the development of compounds that stimulate the release of endogenous GLP-1. The cellular mechanisms responsible for nutrient-regulated GLP-1 secretion are all potential targets, such as sugar, amino acids, fat, main macronutrients (the “preload concept,” ie, ingestion of a small amount of macronutrients prior to ingestion of the main meal may augment incretin hormone secretion during the main meal). An alternative approach is gene delivery systems to express GLP-1 in β-cells, and this has resulted in increased β-cell proliferation and protection against STZ-induced diabetes in injected mice. Finally, manipulation of proglucagon processing, like adenovirus-mediated prohormone convertase overexpression in islet cells,55 can lead to the conversion of hyperglycemic glucagon-secreting α-cells into hypoglycemic GLP-1-secreting cells, a tactic that might be useful in the context of diabetes (Kieffer, Lecture; Tuduri and Kieffer, unpublished). Such alteration of proglucagon processing was observed in vivo after islet injury and could explain, at least in part, the high circulating concentrations of GLP1 and glucagon following bariatric surgery (D’Alessio, personal communication; Madsen, Lecture; Jensen et al, unpublished).

Based on the knowledge that high glucagon contributes to hyperglycemia in T2D, attempts have been undertaken to develop glucagon receptor antagonists for the treatment. However, this approach to treat diabetes has not been successfully developed yet. Moreover, acute administration of glucagon stimulates energy expenditure and inhibits food intake. Thus an approach to treat T2D would be to stimulate the glucagon receptors provided that the hyperglycemic effect of glucagon is prevented. This may be achieved by simultaneous stimulation of GLP-1 receptors (Ahren, Lecture; Ahren, unpublished).

5. Other incretins and bioactive gastro-entero-pancreatic hormones

Other incretins and bioactive gastro-entero-pancreatic hormones can improve insulin secretion as GIP receptor agonists. However, GIP stimulates glucagon secretion, so inhibition of GIP receptors, rather than stimulation, would be a therapeutic target. Oxyntomodulin analogues are also potential therapeutic agents since oxyntomodulin is released together with GLP-1 and is an agonist for both GLP-1 and glucagon re-
ceptors. PYY\textsubscript{3-36} alone or in combination with oxyntomodulin, cholecystokinin, and ghrelin receptor antagonism could also be used (Ahren, Lecture).

VI- Conclusion

Four decades of glucagon research have firmly established its hormonal status and physiologic roles in normal glucose homeostasis and its pathogenic roles in diabetes. It now seems that the “glucagonocentric hypothesis” of diabetes has surpassed the “insulinocentric hypothesis” of diabetes established in the first half of the 19th century. What is obvious is that the morphologic, physiologic, and clinical findings spanning these last years point to the vital but underappreciated paracrine action of insulin on juxtaposed α-cells. Future studies in normal and diabetic human subjects should identify the extent to which reduction of Gcgr signaling coupled or not to insulin therapy produces a compelling therapeutic benefit without incurring a risk of adverse events (Lefebvre, Lecture).
Lectures during IGIS meeting and unpublished reviews

1. Bo Ahren (Lund) “The future of incretin- and glucagon- based treatments”
   Ahrén B, *The future of incretin-based therapy – novel avenues – novel targets*
2. Maureen Charron (New York) “Overview of the glucagon receptor”
   Vuguin P M, Charron M J, *Novel Insight Into Glucagon Receptor Action: Lessons From Knockout and Transgenic Mouse Models*
3. Alan Cherrington (Nashville) “Regulation of liver metabolism by glucagon”
4. Anne Clark (Oxford) “Lifespan of islets cells in man vs rodents”
   Cnop M et al. *Longevity of human islet α- and γ-cells*
5. Patrick Collombat (Nice) “α-cell fate determination: role of Pax genes”
   Courtney M et al. In vivo conversion of adult α-cells into β-like cells: a new research avenue in the context of Type 1 diabetes
6. David A. D’Alessio (Cincinnati) “Role of dysregulated glucagons secretion in type 2 diabetes”
   D’Alessio D, *The role of dysregulated glucagon secretion in Type 2 Diabetes*
7. Leon S.Farhy (Charlottesville) “Pancreatic network control of glucagon secretion in hypoglycaemia counterregulation”
   Farhy L S, McCall A L, *Optimizing Reduction in Basal Hyperglucagonaemia to Repair Defective Glucagon Counterregulation in Insulin Deficiency*
8. Yoshitaka Hayashi (Nagoya) “Metabolic impact of glucagon deficiency”
   Hayashi Y, *Metabolic Impact of Glucagon Deficiency*
9. Jens Holst (Copenhagen) “Regulation of glucagon secretion by incretins”
   Holst J et al. *Regulation of glucagon secretion by incretins*
10. David Irwin (Toronto) “Molecular evolution of the glucagons and incretin genes”
11. Klaus Kaestner (Philadelphia) “Gene expression profile in the developing islet”
    Bramswig N C, Kaestner K H, *Transcriptional Regulation of α-Cell Differentiation*
12. Timothy Kieffer (Vancouver) “Regulating and engineering GLP-1 and GIP production”
    Tudurí E, Kieffer T J, *Reprogramming gut and pancreas endocrine cells to treat diabetes*
13. Rohit Kulkarni (Boston) “Role of insulin signalling in the α-cell fate”
    Kawamori D et al. *Growth factor signaling in the regulation of α-cell fate*
14. Pierre Lefebvre (Liège) “Milestones in glucagon research”
    Li H J et al. *Basic helix loop helix transcription factors and enteroendocrine cell differentiation*
    Gosmain Y et al. *Glucagon gene expression in the endocrine pancreas: the role of the transcription factor Pax6 in α-cell differentiation, glucagon biosynthesis and secretion*
17. Patrik Rorsman (Oxford) “Glucose control of α cell function”
    Walker J N et al. *Regulation of glucagon secretion by glucose: paracrine, intrinsic or both?*
18. Bernard Thorens (Lausanne) “Brain glucose sensing and neural regulation of glucagon secretion”
    Thorens B, *Brain glucose sensing and neural regulation of insulin and glucagon secretion*
19. Michael Wheeler (Toronto) “Direct vs indirect effects of glucose on glucagon”
    Hardy A B et al. *Regulation of glucagon secretion by zinc: characterisation of the Znt8BKO mouse model.*
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27. DeMartis YZ et al. GLP-1 inhibits and adrenaline stimulates glucagon release by differential modulation of N- and L-type Ca2+ channel-dependent exocytosis. Cell Metab. 2010;11:543-553.
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