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Pancreatic Glucagon-Like Peptide 1: What is known?

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Abstract

Glucagon-like peptide-1 (GLP-1) is an incretin hormone encoded together with glucagon by the proglucagon gene. It has been widely accepted that GLP-1 and glucagon are derived from distinct post-translational processing of proglucagon in a tissue specific manner. GLP-1 is produced in intestinal L-cells where proglucagon is processed by prohormone convertase 1/3 (PC1/3), while glucagon is produced in pancreatic α-cells via PC2-mediated cleavage. Nonetheless, emerging evidence has now demonstrated GLP-1 is also produced in pancreatic islets, although its concentration is much lower than glucagon. Further studies have shown GLP-1 production and secretion can be up-regulated by various factors, in particular, hyperglycemia and β-cell damage. The importance of locally produced GLP-1 in pancreas for β-cell function has started to be recognized. Similar to circulating GLP-1, α-cell produced GLP-1 can promote insulin secretion, protect β-cells and enhance β-cell proliferation, thus is vital for β-cell function. This review focuses on these recent discoveries regarding GLP-1 production in pancreatic islets and its action within pancreatic tissue.

Keywords: GLP-1; Alpha cells; Islets; Diabetes; PC1/3

GLP-1: An Overview

Glucagon-like peptide-1 (GLP-1) is one of the peptide hormones encoded by the proglucagon gene, which gives rise to a number of individual peptides through posttranslational processing [1-5]. Based on its origin within the intestine and its vital role in stimulating insulin secretion, GLP-1 is considered a member of the incretin family. GLP-1 is intimately involved in the regulation of circulating blood glucose, acting to increase insulin secretion and sensitivity in a glucose-dependent manner. It is well established that GLP-1 has many effects on multiple organ systems related to nutrient ingestion and blood glucose homeostasis [6,7]. In the gastrointestinal system, GLP-1 inhibits gastrointestinal motility, reduces gastric acid secretion, and slows gastric emptying [8-11]. GLP-1 has been shown to suppress appetite in both normal and obese individuals, thereby reducing food intake [12-15]. In the pancreas, GLP-1 stimulates insulin secretion from β-cells in a blood-glucose dependent manner, while also inhibiting glucagon secretion in α-cells, thus playing an essential role in blood glucose regulation [6,7].

GLP-1 and glucagon share ~50% homology, deriving from the same precursor-proglucagon. The diversification takes place at the post-translational stage in a tissue-specific manner (Figure 1). In pancreatic α-cells, proglucagon is mainly cleaved by prohormone Convertase 2 (PC2), giving rise to glucagon, Intervening Peptide-1 (IP-1), Glicentin-Related Polypeptide (GRPP), and the Major Proglucagon Fragment (MPGF) [16-18]. In contrast, in enteroendocrine L-cells, proglucagon is processed by prohormone convertase 1/3 (PC1/3), resulting in the production of GLP-1, GLP-2, glicentin, and several other small peptides [19,20].

Nonetheless, emerging evidence has now demonstrated that GLP-1 is also produced in pancreatic α-cells, although normally at much lower levels than glucagon. This review focuses on recent discoveries on GLP-1 production in pancreatic islets and its action within pancreatic tissue. There are a number of outstanding and comprehensive reviews regarding general GLP-1 biology, physiology, and its therapeutic use, but few focusing specifically on GLP-1 production in pancreas as well as its function and regulation within the pancreas [7,21-24].

The History of GLP-1 and the Debate Regarding Its Tissue-Specific Expression

GLP-1 and its fellow proglucagon-derived peptide, GLP-2, were first identified during investigation of the gene encoding the glucagon precursor, preproglucagon [1,4,25]. Following the report that these "glucagon-like" peptides are encoded with glucagon on the proglucagon gene, and flanked in the precursor molecule by pairs of basic amino acids that are typically associated with the processing of hormones, it was suggested that the two peptides, in addition to glucagon, must therefore result from the posttranslational processing of proglucagon [1,4]. Since then, it has been discovered that posttranslational processing of proglucagon by different Prohormone Convertases (PC), namely PC1/3 and PC2, results in the production of final peptide products from differing cell types (Figure 1). Thus, the decision of which hormones are cleaved from the proglucagon precursor is ultimately a result of differential expression of the PCs in respective tissues. The production of glucagon is a result of PC2-mediated cleavage of proglucagon in islet-resident α-cells, whereas the production of GLP-1 within intestinal L-cells is the result of PC1/3 cleavage (Figure 1). Furthermore, GLP-1 exists in three different forms: the full length GLP-1 (1-37), the N-terminally truncated GLP-1 (7-37), and an amidated version of the truncated form-GLP-1 (7-36) amide. Among them, GLP-1 (7-36) amide has the highest biological activity, although some other studies have shown the non-amidated and amidated forms of the truncated GLP-1 peptide are equally effective in stimulating insulin release from perfused rat pancreas [2,26-30].

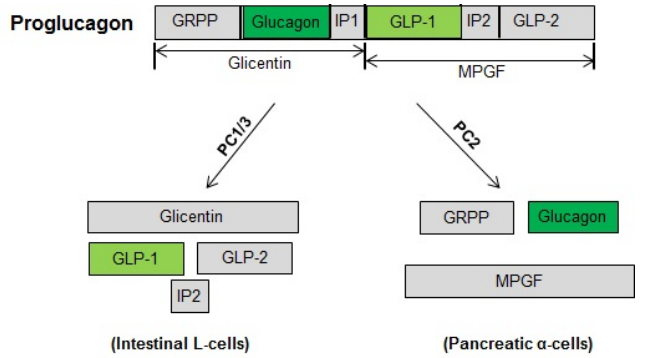

Figure 1: Diagram of proglucagon processing pathways. GRPP: Glicentin-Related Polypeptide; IP: Intervening Peptide; MPGF: Major Proglucagon Fragment.

The debate regarding tissue-specific GLP-1 expression arose as researchers attempted to specify the tissues and cell types responsible for the expression of this incretin hormone. Although the original evidence suggesting the existence of GLP-1 was a result of cDNA analysis from pancreatic extracts, genuine evidence of its expression was first reported in studies involving human gastrointestinal mucosa [3,31]. Studies utilizing immunohistochemistry and radioimmunoassay in conjunction with chromatography soon suggested that GLP-1 production was limited primarily to the intestinal tract [32-35]. GLP-1 was identified within glucagon-producing cells of the pancreas using antibodies against GLP-1, in addition to localization within cells of the small intestine. However, when pancreatic extracts were examined via HPLC, GLP-1 and GLP-2 immunoreactivity was detected within a single large peptide, instead of as two smaller, individual peptides, as would have been expected if proglucagon were processed to free GLP-1 in the pancreas [32-34]. To further investigate whether free GLP-1 was produced in the pancreas, a series of secretion studies were performed using isolated, perfused porcine pancreas and ilium. It was concluded that, while GLP-1 was secreted as an individual, cleaved peptide product from intestinal cells, it was not cleaved into an individual product in the pancreas, but rather was secreted as a single large peptide with GLP-2 and IP-2 [32-35].

On the other hand, the production of GLP-1 within the pancreas in addition to intestinal L-cells has also gained support from other studies. Immunohistochemical studies using various monoclonal and polyclonal antibodies against GLP-1 revealed immune reactivity within both pancreatic α-cells and intestinal L-cells of all species studied [36-38]. Studies utilizing HPLC performed on extracts of human pancreas, glucagonoma, and intestine, detected free GLP-1 in all three tissue types, although the GLP-1 concentrations in the pancreas were considerably lower in comparison with glucagon [32,35]. Additional evidence is also provided via liquid chromatography-purified peptides from human and porcine pancreas lysates, followed by mass spectrometry and amino acid sequencing [39]. Furthermore, other secretion studies suggested that, not only was GLP-1 produced within the pancreas, but that it was also secreted, although the biological activity of the secreted GLP-1 detected in these studies was questioned, as primarily the large molecular form of GLP-1, GLP-1 (1-37) was detected in pancreatic secretions from perfused pancreas [40,41]. Based on these early studies, it was concluded that the majority of proglucagon is cleaved to glucagon in the pancreas, while a small amount of proglucagon is cleaved to produce GLP-1 [32,35,39-41].

More recently, Habener and Stanojevic proposed that the production of GLP-1 in α-cells is a characteristic of undifferentiated α-cells, which they named pro-α-cells [42]. Their hypothesis is largely based on the facts that the GLP-1 processing enzyme PC1/3 is co-expressed with GLP-1 in the α-cells of developing endocrine and in the models of α-cell hyperplasia [42]. However, this hypothesis cannot reconcile with the detection of bioactive GLP-1, but not PC1/3, in the α-cells of normal adult islets.

Emerging Evidence Supporting Production of GLP-1 in Pancreatic Islets

With the development of antibodies with increasing specificity, and with the technical advancements, researchers have now been able to more reliably identify whether bioactive GLP-1 is produced within islets. Anti-GLP-1 antibodies that are generated specifically for the truncated and C-terminally amidated biologically active form of GLP-1 are now available from commercial sources (Figure 2 shows an example). As a result of these improvements in detection technologies, more evidence supporting the production of GLP-1 in pancreatic α-cell has emerged.

GLP-1 production in α-cell has been clearly supported using immortalized α-cell lines. Several studies have shown bioactive GLP-1 is not only expressed in α-cells, but that it is secreted from these cells into the culture media, and the secretion of GLP-1 is dependent on glucose concentration [43-45]. In addition, expression of GLP-1 can be up-regulated in response to high glucose concentration [46]. As a further support for these findings, PC1/3, the prohormone Convertase responsible for GLP-1 cleavage, has been detected in these α-cells, although its expression is considerably lower than that of PC2 [43-45].

GLP-1 has also been detected in isolated primary human and rodent islets. In general, GLP-1 can be successfully detected in the lysates of rodent or human islets regardless the ex vivo culturing condition [43,45,47-49]. Detection of GLP-1 secretion from the islets into the culture media, however, is more complicated. For instance, in one study, GLP-1 was detected in the human islet extracts cultured for 24 hours in media containing 5 mM glucose, but not in the culture supernatant, suggesting GLP-1 was either not secreted into the media, or its concentration in the medium was below the detection limits [46]. Data from other studies suggest the latter is the case [43,48-50]. Indeed, studies in which human islets were cultured for 3 days or longer have shown considerable amounts of GLP-1 in the supernatants [43,49]. More recently, using an advanced methodology named Peptide Hormone Acquisition through Smart Sampling Technique-Mass Spectrometry (PHASST-MS), which is a peptidomics platform that employs high resolution liquid chromatography-mass spectrometry to identify secreted peptide hormones, Taylor et al has detected both full-length GLP-1 (1-37) and bioactive GLP-1 (7-37) in the media of cultured human islets [51]. In addition, High glucose concentration and β-cell damage has been shown to stimulate GLP-1 secretion from cultured islets and allow it to be readily detected [45,48,49,52]. Finally, an extensive study using islets isolated from normal and Type 2 diabetic donors has definitively characterized the presence of a local GLP-1 production system within human islets [48]. In the study, Marchetti et al. [48] analyzed intact human islets as well as FACS-sorted α and β-cell fractions of the islets for GLP-1

expression. Using confocal microscopy, western blotting and mass spectrometry assays, they confirmed that GLP-1 was produced in and secreted from human islet cells-specifically the α-cells, in a glucose-dependent manner. They also found GLP-1 was significantly higher in islets from type 2 diabetic donors than that from non-diabetic donors [48].

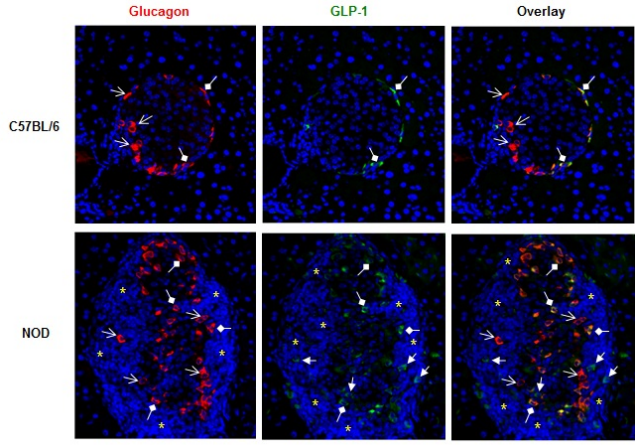

Figure 2: Glucagon and GLP-1 production in mouse pancreatic islets. Pancreatic slices from C57BL/6 or NOD mice were co-stained with anti-glucagon (Cell Signaling Technology, Danvers, MA) (red) and anti-amidated GLP-17-36 (Abcam, Cambridge, MA) (green) antibodies. Nuclei staining (blue) was included to mark all of the cells. GLP-1 was readily detected in the islets from both types of mice, although there was some difference. Most notably, in the normal C57BL/6 mouse, GLP-1 was produced in some α-cells, but mostly co-expressed with glucagon (diamond-head arrows); while in NOD mice with insulitis, cells that express only GLP-1, not glucagon, became detectable (close-head arrows). Open-head arrows mark the examples of cells that produced only glucagon. Detection of glucagon only or GLP-1 only positive cells suggests the antibodies are specific to the corresponding cleaved products. The * marks the area with infiltrating lymphocytes (insulitis). Note: the lymphocytes excused α-cells (defined by proglucagon expression) on their infiltrating path, and some of these α-cells have switched to only produce GLP-1.

Furthermore, studies have demonstrated that the forms of GLP-1 secreted from the pancreatic islets are biologically active [43.47-49,53]. The biological functionality of α-cell produced GLP-1 can be evaluated by whether it activates GLP-1 receptor (GLP-1R) and/or whether it potentiates insulin secretion from islet β-cells. Using COS-7 or BHK cells transfected with GLP-1R cDNA, which allows them to act as functional sensor of GLP-1, studies have showed that, when islet culture supernatants were added to these cells, cAMP, the second messenger of GLP-1R activation, significantly increased compared to controls, demonstrating that biologically active GLP-1 was present in the islet cell culture media [43,49]. The bioactivity of islet-cell released GLP-1 has also been confirmed by measuring insulin secretion from isolated islets that were exposed to conditioned media containing human islet culture supernatant [48]. Their data showed the islets cultured in the conditioned media had significantly more insulin release in response to glucose than controls, and this phenomenon can be blocked by GLP-1R antagonist [48]. Taken together, these studies have demonstrated that the bioactive GLP-1 is secreted from the isolated islets ex vivo.

In addition to the study of isolated islets, the production of pancreatic GLP-1 has been verified *in vivo* by a number of studies on both rodent and human pancreas. When lysates of the pancreata obtained from streptozotocin-treated rats were examined via radioimmunoassay specific for various forms of amidated GLP-1, significant amounts of biologically active GLP-1 could be readily detected [44]. Immunohistochemistry showed strong GLP-1 immunoreactivity throughout the pancreatic islets of these streptozotocin-treated rats, while only faint immunoreactivity could be detected on the periphery of islets from control animals [44]. In fact, using monoclonal antibodies that specifically recognize the biologically active form of GLP-1, quite a few studies have shown co-localization of GLP-1 (7-36) amide with glucagon within α-cells of pancreatic islets [47,54,55] (Figure 2). Furthermore, double immunogold staining and Electron Microscopy (EM) have shown that the biologically active GLP-1 co-localizes with glucagon in the same granules in pancreatic α-cells of murine models [54]. A study using Psammomys obesus, a model of Type 2 diabetes, revealed the presence of biologically active GLP-1 within α-cells, which was increased with the development and progression of diabetes [43].

The Role of PC1/3 in GLP-1 Production in A-Cells

Studies on the localization of primarily PC1/3 within intestinal cells in conjunction with previous reports regarding the L-cells as the primary site of GLP-1 production strongly pointed to PC1/3 as the prohormone convertase responsible for the processing of proglucagon to GLP-1, supported by studies utilizing cell lines expressing either PC1/3 or PC2 [26,27,56-59]. Building on these findings in conjunction with the detection of small quantities of GLP-1 in αTC1.6 cells, studies were performed to determine if this α-cell line also expressed PC1/3. Unlike the easily detectable PC2 that is responsible for the processing of glucagon, no PC1/3 could be detected using Northern blotting analysis. However, in another study, using immunohistochemical analyses with antibodies against PC1/3 and PC2, the presence of small quantities of PC1/3 was detected within these αTC1.6 cells [26]. Using prolonged exposure time in the Northern blotting assays, PC1/3 mRNA was successfully detected, although its level was considerably lower than PC2 mRNA [26]. These data suggest that PC1/3 is expressed in αTC1.6 cells at very low level, and thus may be responsible for the low level of GLP-1 production in these α-cells.

Although PC1/3 is abundant in the endocrine pancreas, it is typically associated with β-cells within islets, as PC1/3 is required in the processing of proinsulin within these cells [60]. Little, if any, PC1/3 is typically detected within α-cells in normal pancreas [43,44,54,55]. However, studies have suggested that PC1/3 expression is upregulated in α-cells during periods of β-cell stress or regeneration [44,45,54,55]. For instance, PC1/3 expression significantly increased in rat islets that were cultured at 11mM or 25 mM glucose compared to that at 5 mM glucose, and GLP-1 secretion increased in accordance [45]. In rats treated with multiple low-doses of streptozotocin, both PC1/3 and GLP-1 expression significantly increased within α-cells [44]. In the study, the researchers observed PC1/3 expression in 95% of α-cells in the islets of STZ-treated rats, while only in 9% of α-cells in normal rats, which is in agreement with the production of amidated GLP-1 in these animals. Interestingly, β-cells showed no such increase in the expression of PC1/3 in animals treated with STZ [44]. The upregulation of PC1/3 within the α-cells may thus be a means by

which to help alleviate hyperglycemia via increasing GLP-1 production in islets.

A similar conclusion was drawn by Kilimnik et al. [54] while studying PC1/3 expression in common murine models of β-cell regeneration: ob/ob, db/db, and NOD mice, as well as healthy mice at time points typically associated with islet cell expansion such as neonatal and pregnant mice. In all models of β-cell regeneration, α-cell hyperplasia was also a key feature, to varying degrees depending on the model being investigated. In addition, in all models, PC1/3 expression could be detected within at least a subset of α-cells. Particularly in neonatal, pregnant, and prediabetic NOD mouse pancreas, co-localization of GLP-1 and PC1/3 was a typical feature of α-cells. Thus, α-cell hyperplasia and the ensuing activation of PC1/3 expression within α-cells may play a vital role in supporting β-cell proliferation, and this interaction underlines the importance of the intraislet role of GLP-1 within the endocrine pancreas [54].

Likewise, we had similar observations during a study involving the development and progression of diabetes in db/db and NOD mice. Little to no PC1/3 staining was detected in the α-cells of normal mice, even in GLP-1-positive cells, likely due to levels of PC1/3 were too low to be detected via immunohistochemistry assays. However, with the onset of diabetes and as it progressed, PC1/3 expression became easily observable in α-cells [55]. Furthermore, the ratio of GLP-1-positive α-cells to glucagon-positive α-cells showed a significant and progressive increase with the development of diabetes in db/db mice, supporting up regulation of PC1/3 may be a means by which α-cells switch from the expression of primarily glucagon to GLP-1 in an attempt to ameliorate hyperglycemia [55].

The Function of A-Cell-Produced GLP-1 within Pancreatic Islets

It is well established that in pancreas, GLP-1 is essential for maintaining β-cell function. GLP-1 not only stimulates insulin secretion from the β-cells, but also promotes β-cell survival and proliferation. However, the source of GLP-1 acting on these β-cells remains to be clarified. Previous studies identifying these functions of GLP-1 are largely based on application of exogenous GLP-1, GLP-1R agonists or antagonists. Since α-cell-produced GLP-1 has historically not been recognized or appreciated, its insulinotrophic and glucagonostatic effects are attributed to GLP-1 in circulation, which is largely produced in the intestinal tract. With recent confirmation on GLP-1 production within islets, efforts start to be taken to determine the function of this locally produced incretin. Emerging evidence suggests that, similar to the circulating GLP-1, α-cell produced GLP-1 plays a vital role in maintaining β-cell function.

α-cell produced GLP-1 promotes insulin secretion

Studies utilizing isolated islets in culture have demonstrated that α-cell-produced GLP-1 can promote insulin secretion. As referenced earlier, islets cultured in conditioned media containing human islet supernatants have shown significantly stronger Glucose-Stimulated Insulin Secretion (GSIS) response than controls, which can be blocked by GLP-1R antagonists [48]. When the conditioned media derived from islets cultured in high-glucose media-and thus containing more GLP-1, was used to treat normal human islets, these islets showed stronger GSIS than controls that used condition media from low-glucose islet culture [48]. Another support for the role of α-cell produced GLP-1 on insulin secretion comes from forced PC1/3 expression in α-cells [61]. It has been shown that introduction of PC1/3 cDNA into the α-cells, but not the β-cells, in the isolated islets led to not only significant increase in GLP-1 production by transduced α-cells, but also a significant increase in insulin production and peak insulin output compared to control islets when challenged with high glucose [61]. This indicates increased GLP-1 production in α-cells improves insulin secretion from β-cells in response to high glucose.

α-cell produced GLP-1 protects β-cells

Studies with isolated rat islets have shown that locally-produced GLP-1 is vital to the maintenance of β-cell function, especially in situations of β-cell stress or damage [43,45,52]. For instance, rat islets significantly increase GLP-1 secretion when treated with the β-cell toxin STZ ex vivo, while glucagon release has not changed under the same condition [45]. Similarly *in vivo*, dramatic increases in GLP-1 production within islet α-cells has been observed in STZ-treated rats compared with untreated controls [44]. Incubation of mouse islets with cytokine cocktail containing IL-1β, IFNγ, and TNFα, which induces β-cell stress and apoptosis, resulted in robust GLP-1 secretion from the isolated islets [52]. Chronic hyperglycemia as result of diabetes has also been reported to result in increased release of GLP-1 from isolated islets as assessed with islets from normal versus diabetic animals [43]. The induction of GLP-1 production and secretion by islet α-cells as a result of SDF-1 signaling following β-cell injury has also been reported to enhance β-cell survival [52]. Indirect evidence in support of the protective effect of α-cell-produced GLP-1 on β-cells came from studies in which PC1/3 was upregulated in α-cells [61,62]. When PC1/3-transduced islets, in which GLP-1 production was significantly increased, were treated with IL-1β, an inducer of β-cell apoptosis, the islets were protected from apoptosis, while the control islets exhibited significant increase in cell death [61]. Taken together, these data have demonstrated GLP-1 production and secretion from islets increase in response to β-cell damage, suggesting it may be a protective mechanism in the attempts to overcome impaired β-cell function and hyperglycemia.

α-cell produced GLP-1 may promote β-cell replication

Studies involving mouse models of hyperglycemia have shown that GLP-1 produced by α-cells may play a vital role in the expansion or regeneration of β-cells. Up-regulation of intra-islet GLP-1 and PC1/3 in α-cells has been observed in models of β-cell expansion and regeneration, including pregnant and neonatal mice, suggesting GLP-1 produced by α-cells within the islet may play a role in β-cell proliferation [54]. This view gained support from a study in which GLP-1 production in α-cells was significantly increased by overexpression of PC1/3. The mice that were transplanted with PC1/3-transduced α-cells showed improvements in response to STZ-induced diabetes, and tended to have, on average, larger islets and a larger insulin-positive area within islets, while displaying an increased number of replicating β-cells when compared to controls [62]. In addition, the cytokine IL-6 has been shown to regulate α-cell mass expansion through stimulating GLP-1 production in α-cells [50,63]. Taken together, these data suggest α-cell produced GLP-1 may play an important role in β-cell proliferation.

Regulation of GLP-1 Production in A-Cells

The signals responsible for regulating the expression of GLP-1 versus glucagon within α-cells has been an important question from the time of the first emerging publications reporting the local

expression of GLP-1 within islets. Current studies suggest metabolic stress, PC1/3 expression, and some cytokines may influence GLP-1 expression by α-cells. We will review each of the factors below.

Glucose

Hyperglycemia and β-cell damage increase GLP-1 production and secretion by islet α-cells, suggesting glucose is a key regulator for intra-islet GLP-1 production and secretion. Indeed, upregulation of GLP-1 in α-cells has been observed in most diabetic models including STZ-induced diabetes, ob/ob mice, db/db mice, and human Type 2 diabetes patients [44,45,48,54,55]. For example, in db/db mice, a model of Type 2 diabetes, GLP-1 production in α-cells was found increased with the progression of diabetes [55]. In addition, islets isolated from hyperglycemic animals and Type 2 diabetic patients had significantly higher GLP-1 concentrations in culture supernatant than did those from control animals [43,48]. In culture, higher glucose concentration in the culture media appears to induce more GLP-1 secretion [45,48,49]. All of these studies support that glucose is a key regulator for intra-islet GLP-1 production and secretion. Interestingly, in one study, GLP-1 concentrations in the supernatants of islets cultured for 24 hours in media containing 3, 12, or 25 mM glucose did not show any significant differences with increasing glucose concentration. However, when islets were maintained for 6 days in culture, a significant glucose dose-dependent increase in GLP-1 secretion was observed, suggesting that chronic hyperglycemia is required for the upregulation of GLP-1 secretion by α-cells [43,47].

IL-6

The cytokine IL-6, produced primarily by adipose tissue, may contribute to the induction of insulin resistance. On the other hand, skeletal muscle contraction as a result of exercise is also associated with IL-6 release, which promotes efficient nutrient uptake and whole-body insulin sensitivity [64-66]. IL-6 has been reported to directly act on α-cells, which have the highest concentration of IL-6 receptors of any cell type within the endocrine pancreas [63]. It has been shown that IL-6 stimulates α-cell proliferation, inhibits α-cell apoptosis, and upregulates both proglucagon mRNA and glucagon secretion in both murine and human islets [63]. In addition, IL-6 has been reported to be vital for high-fat diet induced expansion of α-cell mass [63]. Whether IL-6 affects GLP-1 production in α-cells has also been examined. Isolated human islets were exposed to IL-6. IL-6 significantly increased both the constitutive and the acute arginine-stimulated release of GLP-1 from the islets. Furthermore, islets cultured in conditioned culture medium containing secreted GLP-1 had improved insulin release under high glucose conditions, which could be reversed via addition of the GLP-1R antagonist. To lend further credence to their findings, the researchers examined FACS-sorted α-cells from the dissociated human islets, and confirmed that the increase in GLP-1 that was associated with exposure of intact islets to IL-6 was specifically a result of increased GLP-1 production by α-cells [50]. Analysis of gene expression of isolated human α-cells revealed upregulation of both the preproglucagon gene mRNA and PC1/3 mRNA in response to IL-6 exposure. Taken together, these studies have demonstrated that α-cells are a significant source of biologically active GLP-1, and the production of GLP-1 by these cells is responsive to extra pancreatic stimuli such as circulating levels of cytokine IL-6. Based on these results, the authors propose that production of GLP-1 by α-cells may be an effective means by which GLP-1 acts in a paracrine manner within islets to enhance β-cell function, especially during times of β-cell stress [50].

SDF-1

The Stromal Cell-Derived Factor-1 (SDF-1), a chemokine involved in mediating tissue repair following cellular injury, has been shown to be able to regulate GLP-1 expression in α-cells [52]. Using both isolated immortalized α-cells and isolated, intact murine islets, Liu et al. [52] reported that β-cell signals can affect proglucagon processing in a paracrine manner. They were able to show that the injury-induced expression of SDF-1 by β-cells could act in a paracrine manner on α-cells. Treatment of the αTC.1 cell line revealed that exposure to SDF-1 stimulated the proliferation of α-cells and switched their expression from that of primarily glucagon to GLP-1 by induction of PC1/3 expression within treated α-cells. Furthermore, treatment of both human and murine islets with SDF-1 induced the production and secretion of GLP-1. SDF-1 and GLP-1 were then able to act in a concerted manner to additively enhance β-cell mass, promoting growth and survival of those cells following injury [52].

Summary Remarks

Accumulative evidence has shown that bioactive GLP-1 is produced in the α-cells of pancreatic islets. The intra-islet production of GLP-1, together with its processing enzyme PC1/3, increases in response to hyperglycemia and β-cell injuries. Similar to circulating GLP-1, α-cell produced GLP-1 can promote insulin secretion, protect β-cells and enhance β-cell proliferation, thus is vital for β-cell function. Up-regulation of GLP-1 production and secretion from α-cells appears to be a common protective mechanism that is adopted by the islets to help alleviate hyperglycemia and β-cell damage.

Despite the progress that has been made regarding α-cell-produced GLP-1, many questions remain to be answered. For example, how much does the islet-produced GLP-1 contribute to the overall GLP-1 effect *in vivo*? Since GLP-1 half-life is very short, and most GLP-1 produced from L-cells may have been degraded before it reaches pancreas. So one hypothesis is GLP-1 that casts the insulinotropic and glucagonostatic effect is produced locally in the islets, not from intestinal L-cells. Apparently, a stringent system is needed to determine the relative contribution of GLP-1 produced in α-cells vs L-cells. Another interesting question is related to increatin therapy. Since GLP-1 based incretins have been in use for clinical treatment of Type 2 diabetes, it would be interesting and important to know whether these drugs modulate endogenous GLP-1 production in pancreas. Furthermore, an unknown but important question is whether the locally produced GLP-1 modulates glucagon production and secretion from α-cell. Given that they are produced form the same precursor molecule and often in the same cell, one would expect that a feed-back mechanism exists so that the production of the two hormones is maintained at a balance.

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