Updating the Role of α-Cell Preproglucagon Products on GLP-1 Receptor–Mediated Insulin Secretion

Darleen Sandoval

Diabetes 2020;69:2238–2245 | https://doi.org/10.2337/dbi19-0027

While the field of islet biology has historically focused its attention on understanding β-cell function and the mechanisms by which these cells become dysfunctional with diabetes, there has been a scientific shift toward greater understanding of other endocrine cells of the islet and their paracrine role in regulating the β-cell. In recent years, many questions and new data have come forward regarding the paracrine role of the α-cell and specifically preproglucagon peptides in regulating insulin secretion. The role of intestinally secreted glucagon-like peptide 1 (GLP-1) in regulation of insulin secretion has been questioned, and a physiological role of pancreatic GLP-1 in regulation of insulin secretion has been proposed. In addition, in the last 2 years, a series of studies demonstrated a physiological role for glucagon, acting via the GLP-1 receptor, in paracrine regulation of insulin secretion. Altogether, this work challenges the textbook physiology of both GLP-1 and glucagon and presents a critical paradigm shift for the field. This article addresses these new findings surrounding α-cell preproglucagon products, with a particular focus on GLP-1, in the context of their roles in insulin secretion and consequently glucose metabolism.

Type 2 diabetes mellitus (T2DM) results in defects in multiple target organs. On the one hand, there is insulin resistance in many tissues including the liver and skeletal muscle. On the other hand, there is both β-cell death and dysfunction resulting in deficient nutrient-induced insulin responses. While the field of islet biology has historically focused its attention on understanding β-cell function and the mechanisms by which these cells become dysfunctional with diabetes, there has been a scientific shift toward greater understanding of other endocrine cells of the islet and their paracrine role in regulating the β-cell.

In recent years, many questions and new data have come forward regarding the role of the α-cell in insulin regulation. Data from our group have questioned the role of intestinally secreted glucagon-like peptide 1 (GLP-1) in regulation of glucose homeostasis, and data from multiple groups suggested a physiological role of pancreatic-produced GLP-1 in regulation of insulin secretion (1–4). In addition, in the last 2 years, a series of critical studies demonstrated a role for glucagon, acting via the GLP-1 receptor (GLP-1R), in paracrine regulation of insulin secretion. The purpose of this article is to discuss these new findings surrounding α-cell preproglucagon products, with a particular focus on challenging the incretin role of GLP-1, in the context of their roles in insulin secretion and consequently glucose metabolism.

Evidence That GLP-1 Is an Incretin

In the early 1960s, two studies published the same year found that glucose was lower but insulin was higher after isocaloric loads of glucose when administered orally (5) or directly into the jejunum (6) versus when administered intravenously in humans. Based on these data, the term "incretin effect" was coined to describe this phenomenon of higher insulin responses when nutrients were ingested versus infused. Together with authors of previous studies demonstrating that intestinal mucosa extracts administered to rats could lower blood glucose, these authors hypothesized that a gut-derived humoral substance contributed to the regulation of insulin secretion. Ten years later, it was demonstrated that an intravenous infusion of gastric inhibitory polypeptide (GIP) (aka glucose-dependent insulinotropic peptide) resulted in an increase in insulin immunoreactivity and improvement of glucose tolerance in humans, supporting its role as an incretin (7). However, in anesthetized rats, administration of gut extracts that had GIP removed did not completely ablate nutrient-induced

University of Michigan, Ann Arbor, MI

Corresponding author: Darleen Sandoval, darleens@umich.edu

Received 8 April 2020 and accepted 10 August 2020

© 2020 by the American Diabetes Association. Readers may use this article as long as the work is properly cited, the use is educational and not for profit, and the work is not altered. More information is available at https://www.diabetesjournals.org/content/license.

insulin responses, suggesting the presence of additional incretins (8).

More than 10 years after the discovery of GIP, another incretin was finally discovered. GLP-1, a protein coded by the same gene that makes glucagon, was found to stimulate insulin release in rat islets (9) and when infused in humans (10). In fact, the latter study found that GIP was less effective at increasing insulin levels and concluded that GLP-1 was a physiological incretin in humans. More than 30 years later, GLP-1 is effectively targeted to treat the hyperglycemia of T2DM and, due to its additional anorectic properties, is also an effective treatment of obesity.

While the pharmacological benefit of targeting GLP-1 signaling is clear, there have long been questions regarding the physiological GLP-1 system. Anatomically, the greatest concentration of enteroendocrine cells that secrete GLP-1 is located in the distal gut and has limited direct access to nutrients (11). Although much work has been done focused on direct nutrient-induced stimulation of GLP-1 from enteroendocrine cells, many neural or humoral factors (including GIP) secreted from the upper gut have been suggested to provide a feed-forward release of GLP-1 from distal enteroendocrine cells (reviewed in Sandoval and D'Alessio [12]). However, even if feed-forward regulation explains the nutrient-induced release of GLP-1, questions arise as to whether GLP-1 circulates in high enough concentrations or if the half-life is long enough for intestinally secreted GLP-1 to act as a hormone on β-cell GLP-1R. GLP-1 is rapidly degraded by a protease, dipeptidyl peptidase 4 (DPP4), resulting in only ~10% of intestinally secreted GLP-1 reaching the circulation (13). Whatever is not cleaved by DPP4 is cleared by the liver. Given these clearance challenges and based on the route of blood circulation, it seems unlikely that GLP-1 secreted by the gut could circulate to the heart and back to the pancreas in sufficient amounts to allow it to act as a hormone that regulates insulin secretion.

One possibility is that GLP-1 is not a hormone but instead is a neurotransmitter. Activation of GLP-1R on nerves that innervate the portal vein lowers fasting and postprandial glucose levels (14–17). We also know that central nervous system (CNS) administration of GLP-1 improves hepatic insulin sensitivity in healthy (16,18) and obese animals (19), increases hepatic glycogen synthesis (20), promotes peripheral glucose uptake (16,20), and increases insulin secretion in healthy and obese rodents (18–20). These data suggest that CNS GLP-1R mediate multiple aspects of glucose control. However, mice with specific CNS or nodose ganglia–specific GLP-1R ablation from birth have normal glucose tolerance on chow diet and the expected impairment on an HFD and also have comparable improvements in glucose tolerance in response to a long-acting GLP-1 agonist as compared with their genetic control mouse littermates (21). Thus, acute manipulation of these neuronal GLP-1R populations does regulate glucose homeostasis, but these receptors are not, in and of themselves, necessary for long-term glucose control. Further, it still remains unclear as to whether intestinally secreted GLP-1 could activate CNS GLP-1R populations, and, if not, what source of GLP-1 might physiologically act on these receptors.

We know that infusing exogenous GLP-1 peripherally increases insulin secretion and that administration of a GLP-1R antagonist reduces nutrient-induced insulin responses, but what evidence do we have that intestinally derived GLP-1 is physiologically involved in regulating insulin secretion? Most of that work has focused on the impact of administration of a GLP-1R antagonist, which impairs glucose tolerance and insulin secretion in both humans and rodents. However, mice null for the GLP-1R, or mice that have been administered the GLP-1R antagonist, exendin 9-39 (Ex9), have impairment in glucose tolerance whether that glucose is delivered by mouth, which increases circulating GLP-1 levels, or via an intraperitoneal (IP) injection, which does not increase circulating GLP-1 (22).

To test the role of intestinal GLP-1 secretion, two studies have looked at the necessity and sufficiency of *Gcg* expression in the gut. One recent study performed a loss-of-function experiment with Cre lox-P technology targeted to preproglucagon (*Gcg*), the gene that encodes GLP-1, comparing total gut with distal gut *Gcg* knockouts (23). Supporting a role of intestinal GLP-1 in regulating glucose homeostasis, the whole-gut *Gcg* knockout mice have impaired oral, but not IP, glucose tolerance. Consistent with reduced circulating GLP-1 levels in the whole-gut *Gcg* knockout mice, gastric emptying rate was reduced; however, insulin responses to glucose were intact. In comparison, mice with only distal gut *Gcg* removal had impaired oral and a slight impairment of IP glucose tolerance with normal circulating nutrient-induced GLP-1 and insulin secretion. Together these data suggest that the primary impact of intestinally derived GLP-1 on glucose tolerance is through regulation of gastric emptying rate.

For testing of sufficiency, in another study intestinal *Gcg* was selectively reactivated across the whole gut while the remaining tissues (i.e., pancreas and brain) remained devoid of *Gcg* (24–26). For interrogation of the specific role of GLP-1, these mice were administered glucose with and without Ex9. Ex9 had no impact on glucose tolerance (either oral or IP) in mice with whole-body deficiency of *Gcg*, indicating that Ex9 is a true GLP-1R antagonist in vivo (vs. an inverse agonist, as has been suggested previously [27]). Mice with selective intestinal *Gcg* reactivation had normal postprandial circulating levels of both total and active GLP-1 but surprisingly had no impairment of glucose tolerance in response to Ex9. In contrast, selective reactivation of pancreatic *Gcg* expression was sufficient to completely restore the response to Ex9. This was in response to both oral administration and IP glucose administration. We interpreted these results to mean that intestinal GLP-1 was dispensable for glucose tolerance and that there is a pancreatic

source of GLP-1 that regulates insulin secretion in a paracrine fashion.

Although the results from those studies (23,24) seem at odds, it is interesting that removal of *Gcg* expression from the distal (but not proximal) gut reduced fasting glucose but resulted in normal glucose-stimulated levels of both active and total GLP-1 and yet these animals still had impairments of oral but not IP glucose tolerance (23). GLP-1R knockout mice, on the other hand, have impairments in both oral and IP glucose tolerance (22), while β-cell–specific GLP-1r knockout mice have normal oral but impaired IP glucose tolerance (28). One possibility is that the different sources of GLP-1 regulate different aspects of β-cell function and/or glucose homeostasis. Regardless, together these data indicate that intestinally secreted GLP-1 is partly necessary for glucose tolerance, seemingly through its impact on gastric emptying rate, but not sufficient for the ability of Ex9 to impair glucose tolerance. These data also underscore the potential role of α-cell–derived GLP-1 in insulin secretion.

Detection of α-Cell GLP-1

Prohormone convertase 1/3 (PC1/3), the enzyme that posttranslationally processes GLP-1 (and GLP-2 and oxyntomodulin) from proglucagon, is more highly expressed in the gut and CNS compared with the pancreas (29). However, α-cell PC1/3 activity and/or expression is found in embryonic and neonatal mice, in pregnant dams, and in many models of metabolic stress including streptozotocin-induced and genetically induced diabetes, and detection increases with increasing glucose concentrations (4,30–34). Another mouse model with an α-cell knockout of PC1/3 which resulted in a ∼35% reduction of islet GLP-1 in chow-fed animals lead to impaired glucose tolerance in HFD-fed mice administered a low dose of streptozotocin (1). These data indicate that there is a pool of α-cell–derived GLP-1 that regulates glucose tolerance and increases under conditions of metabolic stress.

However, part of the hesitance about accepting that the α-cell produces physiologically active GLP-1 comes from the fact that pancreatic secretion of intact GLP-1 is low and sometimes undetectable. Generally, GLP-1 is difficult to assess in mice. In addition to degradation by DPP4, mouse GLP-1 has also be reported to undergo fast endoproteolytic metabolism (35). Regardless of these limitations, protein extraction of pancreata from wild-type control mice revealed detectable levels of both total and active GLP-1 (via radioimmunoassay for three isoforms of GLP-1 summed for total GLP-1) in one study (36) but only detectable levels of total (via radioimmunoassay for the carboxy terminus) and not active (via a radioimmunoassay for the N-terminus) GLP-1 in another study (37). Besides potential differences in the assays used, what might account for these differences is unknown. Nutrient status of the animals was not stated in either study, and it is not clear whether this could impact tissue GLP-1 levels.

GLP-1 secreted from islets has also been studied in mouse pancreas perfusion studies. Sampling during perfusion studies is done from the portal vein and under anesthesia. Under these conditions, portal vein levels of active GLP-1 in response to glucose were found to be low and often undetectable (27). For GLP-1 to act in a paracrine fashion does not require its entry into the circulation, so this result, in and of itself, does not rule out a physiological role for α-cell GLP-1. In support of this, mice that only express *Gcg* in the pancreas have total GLP-1 level in the plasma that is less than half that of animals that express *Gcg* only in the intestine (24). Further, given the role of the autonomic nervous system in regulating glucagon secretion, it is also possible that islet GLP-1 production is blunted by the anesthesia.

Based on the rationale that quantifying pancreatic GLP-1 production is complicated by the fact that the number of α-cells is quite low, a recent study isolated, and then dispersed, islets into single cells before staining for GLP-1. They found not only GLP-1–producing α-cells but also that a proportion of α-cells produce GLP-1, a proportion produces glucagon, and still another proportion produces both peptides (2). They also revealed that 70% vs. 50% of α-cells produced GLP-1 in healthy human versus mouse islets, indicating that α-cell–produced GLP-1 may be important for humans as well. These data reveal a critical piece of information regarding the heterogeneity of α-cells surrounding proglucagon peptides and support the possibility that at least a portion of α-cells produce GLP-1 and are optimally placed to regulate insulin secretion in a paracrine fashion.

Role of Glucagon Signaling in Insulin Secretion

Glucagon has traditionally been thought of as a counter-regulatory hormone, with secretion being stimulated when plasma glucose levels are low and inhibited when plasma glucose levels are high such that there is a reciprocal relationship between insulin and glucagon secretion (38). Additionally, Roger Unger has long argued the importance of postprandial suppression of glucagon in normal glucose regulation (39). Further, insulin-induced suppression of glucagon (i.e., intraislet hypothesis) has been thought to play a role in hyperglycemia, as conditions of decreased insulin secretion or α-cell insulin resistance (e.g., T2DM) leads to less postprandial suppression of glucagon (40), with hyperglucagonemia being a characteristic of T2DM (41).

Despite all this, there are also early data showing that glucagon applied to islets stimulates insulin secretion (42). The question of whether this was a physiological or pharmacological effect and whether glucagon was regulating insulin secretion via its own receptor or via the GLP-1R was debated. At the time of these initial studies, the data suggested that glucagon acted on the GLP-1R only at pharmacological concentrations (43). Competitive binding assays in purified rat islets showed that glucagon binding on β-cells could be reduced by 42% in the presence of

GLP-1 (42). However, glucagon-induced cAMP production was inhibited by both a glucagon antagonist and Ex9 (42). In perifusion studies, Ex9 suppressed high-dose glucagon stimulation of insulin secretion in cells exposed to 20 mmol/L glucose, which is a very high glucose concentration for islets but was a standard experimental design at the time, while GLP-1 binding was inhibited by high glucagon concentrations. However, at low doses glucagon action seems to be mediated by the glucagon receptor, not the GLP-1R, as Ex9 does not block its affects (42). The addition of Ex9 alone (in the absence of added glucagon or GLP-1) lowers insulin release stimulated by glucose alone (42), suggesting that β-cell GLP-1R are partially occupied by an agonist.

More recent modeling work has confirmed and extended these findings. Fluorescence resonance energy transfer assays that detect cAMP as a read-out for glucagon and GLP-1R activation revealed that glucagon is a nonconventional GLP-1R agonist that is inhibited by Ex9 (44). Although glucagon was less potent than GLP-1, it had full agonist properties on the GLP-1R. The authors postulated that glucagon is a dual agonist of the glucagon receptor and GLP-1R at the high concentrations that may exist in islets and that the net effect is that glucagon and GLP-1 regulation of the GLP-1R and downstream signaling are coordinated so that β-cell function is enhanced in the healthy state or "repaired" under conditions of metabolic stress. A caveat to this is that the concentrations of glucagon achieved in this study may still reflect pharmacological levels, as estimations of islet glucagon levels that are sufficient to stimulate insulin levels are ~1,000-fold lower (45).

Recently, three independent research groups explored the role of glucagon action on β-cell using three different genetic tools. The first report, from the Holst laboratory, used mouse pancreatic perfusions in control and in a variety of genetic mice including their α-cell–ablated mouse (27). The importance of this technique is that the islet cytoarchitecture is maintained intact. The next study used perifusion of islets isolated from a variety of genetic models (46), and the last study inhibited α-cell activity using designer receptors exclusively activated by designer drugs (DREADDs) [47]). Despite the different strategies, the findings between these studies and the prior studies were generally similar and demonstrated that glucagon stimulates insulin secretion at high, but not low, glucose levels and the effect is blunted by either genetically or pharmacologically blocking GLP-1R signaling. This general finding was further validated in human islets (46). The nutrient conditions were also important, as the presence of amino acids (potent glucagon secretagogues) magnifies the impact of glucagon action on β-cell GLP-1R. These data demonstrate an additional physiological paracrine role for glucagon in regulating insulin secretion under postprandial conditions. A proposed model illustrating the integration of the role of glucagon and GLP-1 on the GLP-1R in regulation of insulin secretion is illustrated in Fig. 1.

Postprandial Regulation of the α-Cell

If we presume that α-cell production both of glucagon and of GLP-1 is critical for regulating insulin levels, there are several open questions. One question is, what determines the peptides being produced and are they produced from the same cell or from different α-cells? Much recent work has found a high degree of heterogeneity among β-cell populations in a variety of characteristics including developmental origins and their transcriptomes (48). More research is now accumulating regarding the heterogeneity of α-cells as well. Unlike β-cells, the electrical activity of α-cells is not coupled. One reason for this lack of coordination may be the anatomy of the islet. α-Cells are dispersed throughout (or around for rodents) the islet, and this anatomical variability could generally create a greater degree of heterogeneity in function. In fact, different α-cells within the same islet exhibit different calcium activities across the physiological range of glucose levels, and that calcium activity does not correlate with glucagon secretion (49). This could certainly provide conditions whereby differential α-cells secrete differential proglucagon peptides.

Generally, we know surprisingly little about the intracellular regulation of glucagon secretion. As mentioned above, calcium channel activity is heterogenous among α-cells, leading to no consistent answer about the role of activity of this particular channel in glucagon secretion (50). Direct innervation of islets or circulatory factors such as catecholamines have been proposed to be major regulators of glucagon secretion. However, glucagon responses to glucose when studied in isolated islets are similar to what is seen in vivo, suggesting that intraislet factors rather than nervous system or blood flow influence glucagon regulation (50). Paracrine models have proposed that products from nearby β- or δ-cells, including insulin, Zn^{2+}, GABA, and/or serotonin, inhibit glucagon secretion (12). However, none of these factors alone can suppress glucagon in dispersed α-cells (50).

In addition, GLP-1, insulin, and somatostatin, peptides that all increase postprandially, all suppress glucagon. Conversely, another postprandial hormone, GIP, has been found to increase glucagon (51). There are also mixed results in terms of the impact of various nutrients on glucagon secretion, especially in humans. Ingestion of glucose alone (52,53) suppresses glucagon, but other macronutrients or their metabolites also regulate glucagon secretion. For example, free fatty acids (54) and ketones (55) have been reported to decrease glucagon, but proteins (amino acids) potently stimulate glucagon secretion (52). Free fatty acids have also been reported to suppress the ability of arginine to stimulate glucagon secretion in humans (54), suggesting that glucagon responses to a mixed meal should be restrained compared with when only arginine is administered in humans. Further emphasizing the complex interactions of nutrients on the α-cell, in isolated islets exposed to low glucose conditions, free fatty acid metabolism provides fuel to sustain glucagon

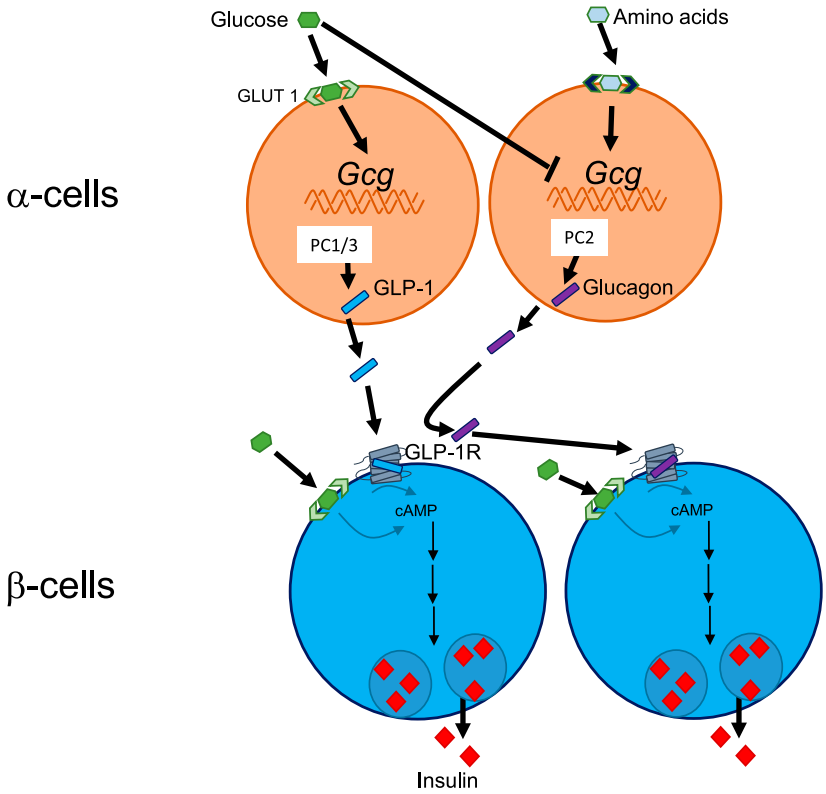

Figure 1—Working model of the role of GLP-1 and glucagon from the α-cell in regulation of insulin secretion through the GLP-1R. Some, but not all, α-cells secrete GLP-1, which displaces glucagon binding to the GLP-1R. While glucagon secretion is suppressed by glucose alone, some amino acids are potent glucagon secretagogues. Likely under mixed-meal conditions, both glucagon and GLP-1 secretion from heterogenous α-cells increase and act on the GLP-1R to increases insulin secretion in a glucose-dependent manner.

levels (56), and long-term exposure to free fatty acids potentiates glucagon release (57). Lastly, the three recent studies that found that glucagon stimulates insulin secretion through the β-cell GLP-1R all found this effect to be magnified in the presence of amino acids under ex vivo conditions (27,46,47). Using in vivo studies in mice, it has been shown that plasma and portal glucagon increases in response to a liquid mixed meal but not in response to oral glucose alone (58). However, the insulin levels appeared (this was not statistically compared) to be higher with the glucose versus the liquid meal administration despite the fact that rough calculations suggest the caloric load was higher with the liquid mixed meal. Altogether, these data highlight both the potential nutrient specificity and the complexity of the system. The varied impacts of neural, paracrine, endocrine, and/or nutrient signals on glucagon secretion may relate to the α-cell heterogeneity discussed above but also emphasize how understanding regulation of glucagon secretion is critical for understanding its role in regulating insulin secretion and glucose homeostasis.

Integration of the New "Incretin" Model

It is critical to consider how these new data should be integrated into a new "incretin" model of glucoregulation. One way to address this is by examining the phenotype of animals devoid of both glucagon and GLP-1. If GLP-1 and glucagon are both important for increasing postprandial insulin, then glucose tolerance and insulin secretion should be greatly impaired in animals that are devoid of both peptides. An initial study that used diphtheria toxin ablation of *Gcg* in enteroendocrine L cells or α-cells found that combined L- and α-cell ablation reduced the postgavage glucose peak and had minimal overall impact on oral glucose tolerance but did impair IP glucose tolerance (59). Conversely, specific α-cell ablation improved oral but had minimal impact on IP glucose tolerance (59). Using a slightly different diphtheria toxin–inducible α-cell ablation, Traub et al. (1) demonstrated that these mice have an age-induced improvement of IP and oral glucose tolerance but normally elevated insulin. Although the removal of glucagon was certainly not complete, the data suggest that reduction of circulating glucagon reduces its capability to increase hepatic glucose production and leads to an overall improvement of glucose tolerance. Thus, these models suggest that ablation of α-cells in total, and consequently glucagon levels, leads to improved and not impaired glucose homeostasis.

One complication of these models is that an increase in another glucoregulatory peptide could compensate for the loss of another critical glucoregulatory peptide. The increase in plasma GLP-1 in the glucagon receptor knockout

Table 1—Changes in oral, IP, insulin, and incretin responses across the various genetic models targeted to Gcg and GLP-1R signaling

Mouse	OGTT	IPGTT	Insulin	Incretins
Manipulation: gut and/or islet GLP-1 peptide or signaling decreased				
Whole-body GLP-1R knockout (22)	Impaired	Impaired	Reduced?	↑ glucagon
Gcg gut knockout (23)	Impaired	Normal	Normal	No GLP-1
Gcg distal gut knockout (23)	Impaired	Impaired	Normal	Normal GLP-1
Pcsk1 α-cell deletion (1)	Normal	Impaired	↓	↓ islet GLP-1, ↑ islet glucagon
GLP-1R β-cell knockout (28)	Normal	Impaired	Not studied	Not studied
Gcg pancreas RA; gut knockout (24)	Normal	Normal	Normal	50% ↓ GLP-1
Manipulation: GLP-1 normal; glucagon is decreased				
Gcg gut RA (24)	Improved	Improved	Normal	Normal
Gcg α-cell deletion 6 weeks post-DTR (1)	Improved	Normal	OGTT normal, ↑ IPGTT normal	↑ GLP-1
Gcg α-cell ablation (59)	Improved	Normal	Not studied	Not studied
Manipulation: both GLP-1 and glucagon decreased				
Gcg null (24)	Improved	Improved	Normal in vivo, ↓ in vitro	↑ GIP?
Gcg L-cell + α-cell ablation (59)	Improved	Impaired	Not studied	Not studied

DTR, diphtheria toxin receptor; IPGTT, intraperitoneal glucose tolerance test; OGTT, oral glucose tolerance test; RA, reactivated.

mouse is one such example. However, with GLP-1 and GIP, there is theoretically no need for one or the other to increase in compensation, as physiological levels of GIP are sufficient to allow a full improvement in glucose tolerance in response to a DPP4 inhibitor and vice versa (26). This concept of GIP compensating for the lack of GLP-1 with DPP4 inhibitor action may apply to physiological regulation of insulin secretion as well (60). Insulin responses to an intravenous (24) and oral glucose (D.S., unpublished observations) load are normal in animals devoid of both GLP-1 and glucagon, but islets isolated from these mice had reduced basal and stimulated insulin levels (46). In this case, as GLP-1 and glucagon are absent, the normal physiological increases in intestinal GIP may be sufficient to retain normal insulin responses. These data also highlight the critical interaction of the variety of signals that regulate insulin secretion.

How does the knowledge gained from the studies demonstrating that glucagon stimulates insulin secretion through the GLP-1R (27,47,58) inform the prior data interpretations that pancreatic and not intestinal secreted GLP-1 is necessary for glucose tolerance (24)? First, these findings suggest the possibility that the lack of response to Ex9 in the intestinally reactivated *Gcg* and the full response to Ex9 in the pancreatic reactivated *Gcg* mice are due to pancreatic glucagon, rather than GLP-1, signaling on the GLP-1R. This interpretation would mean that the entirety of Ex9 action on impairing glucose tolerance is through postprandial glucagon action on the GLP-1R. In addition, competition binding studies demonstrate that glucagon is readily displaced in the presence of GLP-1 and accordingly, even in the perfused pancreas, GLP-1 is significantly more potent than glucagon on the GLP-1R (27). It is important to note that the nutrient conditions are important in shifting of glucagon from its glucose-stimulating to its insulin-stimulating action. Specifically, ad libitum–fed mice administered a liquid mixed meal are more responsive to glucagon actions on the β-cell (58). The pancreatic *Gcg*-reactivated animals were administered glucose alone and were studied in the fasted state, conditions of low glucagon action on the β-cell. Regardless, the current body of work suggests that both peptides regulate the β-cell through GLP-1R signaling, but the process of posttranslational preproglucagon processing makes it difficult to generate genetic models that distinguish between the impacts of α-cell–derived glucagon versus GLP-1 on local GLP-1R signaling.

Interestingly, when the phenotypes of the various genetic models targeted to *Gcg* or GLP-1R signaling that have been generated are discussed in concert, some important findings fall out (Table 1). One is that the impacts on glucose tolerance in the developmental versus the adult genetic manipulations have a much stronger glucose phenotype. The other is that the absence of glucagon has a potent impact on improving oral glucose tolerance (sometimes IP glucose tolerance as well). This does not necessarily mean that glucagon or GLP-1 action on insulin secretion and glucoregulation is not important. However, if the primary role of GLP-1 and glucagon is to improve glucose homeostasis through increased insulin levels, then knockout of both peptides or their signaling should impair, not improve, glucose tolerance. Thus, instead, it is possible that given the balance of the two actions (increasing insulin or increasing glucose), glucagon's action on raising glucose is more powerful. This highlights a limitation of the currently available experimental models and why the body of literature needs to be considered as a whole rather than as individual findings.

This is a rapidly evolving model, and it is anticipated that the expansion of our knowledge on α-cell heterogeneity will go a long way in driving further evolution in our thinking of how α-cell proglucagon products regulate glucose homeostasis. It seems likely that α-cell heterogeneity contributes to some α-cells secreting GLP-1 in response to glucose and some secreting glucagon when both amino acids and glucose are present. The end result is postprandially stimulated insulin secretion, no matter the macronutrient present.

It is interesting that the function of both GLP-1 and glucagon is tightly coupled to glucose availability. GLP-1 action is blunted during hypoglycemia, and glucagon action on the liver is suppressed by hyperglycemia. Both aberrant glucagon secretion and aberrant GLP-1 secretion are seen in T2DM as well (61). For example, glucose-induced suppression of glucagon and glucagon response to hypoglycemia are both reduced with T2DM (62). Generally, the incretin response is also blunted with T2DM and is suggested to be due to reductions in sensitivity to both GLP-1 and GIP (63). Thus, in T2DM, defects in at least three peptides important for action on the β-cells are dysfunctional. Altogether, these data emphasize the importance of glucose availability and potentially the balance of these two glucoregulatory proglucagon-derived peptides within the islet in regulating insulin secretion and glucose control.

Conclusions

Whether α-cell–derived GLP-1 and/or glucagon is critical for regulating insulin secretion, what these data are telling us is that the traditional incretin model is inadequate. The reality is that there have been several open questions about the role for GLP-1 as an endocrine incretin. The traditional characterization of reciprocal roles of insulin and glucagon, in terms of glucoregulation, is also a simplified assessment of glucagon action. Instead, under postprandial conditions, proglucagon peptides from the α-cell represent redundant signals that respond to the variety of fluctuating nutrient conditions, allowing for fine-tuning of insulin responses and overall glucose control in the face of a variety of metabolic conditions, and all of these feedback signals are critical during a meal and their dysfunction is critical in the disease process.

Funding. This work is supported in part by National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, awards DK117635, DK121995, and DK107282 and by an American Diabetes Association grant (1-19-IBS-252).

Duality of Interest. No potential conflicts of interest relevant to this article were reported.

References

1. Traub S, Meier DT, Schulze F, et al. Pancreatic α cell-derived glucagon-related peptides are required for β cell adaptation and glucose homeostasis. Cell Rep 201718:3192–3203

2. de Souza AH, Tang J, Yadev AK, et al. Intra-islet GLP-1, but not CCK, is necessary for β-cell function in mouse and human islets. Sci Rep 202010:2823

3. Linnemann AK, Neuman JC, Battiola TJ, Wisinski JA, Kimple ME, Davis DB. Glucagon-like peptide-1 regulates cholecystokinin production in β-cells to protect from apoptosis. Mol Endocrinol 2015;29:978–987

4. Nie Y, Nakashima M, Brubaker PL, et al. Regulation of pancreatic PC1 and PC2 associated with increased glucagon-like peptide 1 in diabetic rats. J Clin Invest 2000;105:955–965

5. Elrick H, Stimmer L, Hlad CJ, Arai Y. Plasma insulin response to oral and intravenous glucose administration. J Clin Endocrinol Metab 1964;24:1076–1082

6. McIntyre N, Holdsworth CD, Turner DS. New interpretation of oral glucose tolerance. Lancet 1964;2:20–21

7. Dupre J, Ross SA, Watson D, Brown JC. Stimulation of insulin secretion by gastric inhibitory peptide in man. J Clin Endocrinol Metab 1973;37:826–828

8. Ebert R, Unger H, Creutzfeldt W. Preservation of incretin activity after removal of gastric inhibitory polypeptide (GIP) from rat gut extracts by immunoadsorption. Diabetologia 1983;24:449–454

9. Schmidt WE, Siegel EG, Creutzfeldt W. Glucagon-like peptide-1 but not glucagon-like peptide-2 stimulates insulin release from isolated rat pancreatic islets. Diabetologia 1985;28:704–707

10. Kreymann B, Williams G, Ghatei MA, Bloom SR, Williams G, Bloom SR. Glucagon-like peptide-1 7-36: a physiological incretin in man. Lancet 1987;2:1300–1303

11. Eissele R, Göke R, Willemer S, et al. Glucagon-like peptide-1 cells in the gastrointestinal tract and pancreas of rat, pig and man. Eur J Clin Invest 1992;22:283–291

12. Sandoval DA, D'Alessio DA. Physiology of proglucagon peptides: role of glucagon and GLP-1 in health and disease. Physiol Rev 2015;95:513–548

13. Deacon C, Johnsen AH, Holst J. Degradation of glucagon-like peptide-1 by human plasma in vitro yields an N-terminally truncated peptide that is a major endogenous metabolite in vivo. J Clin Endocrinol Metab 1995;80:952–957

14. Ionut V, Hucking K, Liberty IF, Bergman RN. Synergistic effect of portal glucose and glucagon-like peptide-1 to lower systemic glucose and stimulate counter-regulatory hormones. Diabetologia 2005;48:967–975.

15. Vahl TP, Tauchi M, Durler TS, et al. Glucagon-like peptide-1 (GLP-1) receptors expressed on nerve terminals in the portal vein mediate the effects of endogenous GLP-1 on glucose tolerance in rats. Endocrinology 2007;148:4965–4973

16. Ayala JE, Bracy DP, James FD, Julien BM, Wasserman DH, Drucker DJ. The glucagon-like peptide-1 receptor regulates endogenous glucose production and muscle glucose uptake independent of its incretin action. Endocrinology 2009;150:1155–1164.

17. Ionut V, Zheng D, Stefanovski D, Bergman RN. Exenatide can reduce glucose independent of islet hormones or gastric emptying. Am J Physiol Endocrinol Metab 2008;295:E269–E277

18. Sandoval DA, Bagnol D, Woods SC, D'Alessio DA, Seeley RJ. Arcuate glucagon-like peptide 1 receptors regulate glucose homeostasis but not food intake. Diabetes 2008;57:2046–2054

19. Burmeister MA, Ferre T, Ayala JE, King EM, Holt RM, Ayala JE. Acute activation of central GLP-1 receptors enhances hepatic insulin action and insulin secretion in high-fat-fed, insulin resistant mice. Am J Physiol Endocrinol Metab 2012;302:E334–E343

20. Knauf C, Cani PD, Perrin C, et al. Brain glucagon-like peptide-1 increases insulin secretion and muscle insulin resistance to favor hepatic glycogen storage. J Clin Invest 2005;115:3554–3563

21. Sisley S, Gutierrez-Aguilar R, Scott M, D'Alessio DA, Sandoval DA, Seeley RJ. Neuronal GLP1R mediates liraglutide's anorectic but not glucose-lowering effect. J Clin Invest 2014;124:2456–2463

22. Scrocchi LA, Brown TJ, MaClusky N, et al. Glucose intolerance but normal satiety in mice with a null mutation in the glucagon-like peptide 1 receptor gene. Nat Med 1996;2:1254–1258

23. Song Y, Koehler JA, Baggio LL, Powers AC, Sandoval DA, Drucker DJ. Gut-proglucagon-derived peptides are essential for regulating glucose homeostasis in mice. Cell Metab 2019
24. Chambers AP, Sorrell JE, Haller A, et al. The role of pancreatic preproglucagon in glucose homeostasis in mice. Cell Metab 2017;25:927–934.e3
25. Kim KS, Hutch CR, Wood L, Magrisso IJ, Seeley RJ, Sandoval DA. Glycemic effect of pancreatic preproglucagon in mouse sleeve gastrectomy. JCI Insight 2019;4:e129452
26. Hutch CR, Roelofs K, Haller A, et al. The role of GIP and pancreatic GLP-1 in the glucoregulatory effect of DPP-4 inhibition in mice. Diabetologia 2019;62:1928–1937
27. Svendsen B, Larsen O, Gabe MBN, et al. Insulin secretion depends on intra-islet glucagon signaling. Cell Rep 2018;25:1127–1134.e2
28. Smith EPP, An Z, Wagner C, et al. The role of β cell glucagon-like peptide-1 signaling in glucose regulation and response to diabetes drugs. Cell Metab 2014;19:1050–1057
29. Mojsov S, Heinrich G, Wilson IB, Ravazzola M, Orci L, Habener JF. Preproglucagon gene expression in pancreas and intestine diversifies at the level of post-translational processing. J Biol Chem 1986;261:11880–11889
30. Ellingsgaard H, Ehses JA, Hammar EB, et al. Interleukin-6 regulates pancreatic alpha-cell mass expansion. Proc Natl Acad Sci U S A 2008;105:13163–13168
31. Ellingsgaard H, Hauselmann I, Schuler B, et al. Interleukin-6 enhances insulin secretion by increasing glucagon-like peptide-1 secretion from L cells and alpha cells. Nat Med 2011;17:1481–1489
32. Kilimnik G, Kim A, Steiner DF, Friedman TC, Hara M. Intraislet production of GLP-1 by activation of prohormone convertase 1/3 in pancreatic α-cells in mouse models of β-cell regeneration. Islets 2010;2:149–155
33. Marchetti P, Lupi R, Bugliani M, et al. A local glucagon-like peptide 1 (GLP-1) system in human pancreatic islets. Diabetologia 2012;55:3262–3272
34. Davis EM, Sandoval DA. Glucagon-like peptide-1: actions and influence on pancreatic hormone function. Compr Physiol 2020;10:577–595
35. Windeløv JA, Wewer Albrechtsen NJ, Kuhre RE, et al. Why is it so difficult to measure glucagon-like peptide-1 in a mouse? Diabetologia 2017;60:2066–2075
36. Gelling RW, Du XQ, Dichmann DS, et al. Lower blood glucose, hyperglucagonemia, and pancreatic cell hyperplasia in glucagon receptor knockout mice. Proc Natl Acad Sci U S A 2003;100:1438–1443
37. Galsgaard KD, Winther-Sørensen M, Ørskov C, et al. Disruption of glucagon receptor signaling causes hyperaminoacidemia exposing a possible liver-alpha-cell axis. Am J Physiol Metab 2018;314:E93–103
38. Unger RH, Orci L. Paracrinology of islets and the paracrinopathy of diabetes. Proc Natl Acad Sci U S A 2010;107:16009–160012
39. Unger RH. The Banting Memorial Lecture 1975. Diabetes and the alpha cell. Diabetes 1976;25:136–151
40. Meier JJ, Kjems LL, Veldhuis JD, Lefèbvre P, Butler PC. Postprandial suppression of glucagon secretion depends on intact pulsatile insulin secretion: further evidence for the intraislet insulin hypothesis. Diabetes 2006;55:1051–1056
41. Knop FK, Vilsbøll T, Madsbad S, Holst JJ, Krarup T. Inappropriate suppression of glucagon during OGTT but not during isoglycaemic i.v. glucose infusion contributes to the reduced incretin effect in type 2 diabetes mellitus. Diabetologia 2007;50:797–805
42. Moens K, Flamez D, Van Schravendijk C, Ling Z, Pipeleers D, Schuit F. Dual glucagon recognition by pancreatic beta-cells via glucagon and glucagon-like peptide 1 receptors. Diabetes 1998;47:66–72
43. Thorens B. Expression cloning of the pancreatic beta cell receptor for the gluco-incretin hormone glucagon-like peptide 1. Proc Natl Acad Sci U S A 1992;89:8641–8645
44. Chepurny OG, Matsoukas M-T, Liapakis G, et al. Nonconventional glucagon and GLP-1 receptor agonist and antagonist interplay at the GLP-1 receptor revealed in high-throughput FRET assays for cAMP. J Biol Chem 2019;294:3514–3531
45. Finan B, Capozzi ME, Campbell JE. Repositioning glucagon action in the physiology and pharmacology of diabetes. Diabetes 2020;69:532–541
46. Capozzi ME, Svendsen B, Encisco SE, et al. β cell tone is defined by proglucagon peptides through cAMP signaling. JCI Insight 2019;4:e126742
47. Zhu L, Dattaroy D, Pham J, et al. Intra-islet glucagon signaling is critical for maintaining glucose homeostasis. JCI Insight 2019;5:e127994
48. Hughes JW, Ustione A, Lavagnino Z, Piston DW. Regulation of islet glucagon secretion: beyond calcium. Diabetes Obes Metab 2018;2(Suppl. 2):127–136
49. Le Marchand SJ, Piston DW. Glucose suppression of glucagon secretion: metabolic and calcium responses from alpha-cells in intact mouse pancreatic islets. J Biol Chem 2010;285:14389–14398
50. Le Marchand SJ, Piston DW. Glucose decouples intracellular Ca2+ activity from glucagon secretion in mouse pancreatic islet alpha-cells. PLoS One 2012;7:e47084
51. Opara EC, Go VLW. Influence of gastric inhibitory polypeptide (GIP) and glucose on the regulation of glucagon secretion by pancreatic alpha cells. Regul Pept 1991;32:65–73
52. Unger RH, Aguilar-Parada E, Müller WA, Eisentraut AM. Studies of pancreatic alpha cell function in normal and diabetic subjects. J Clin Invest 1970;49:837–848
53. Vieira E, Salehi A, Gylfe E. Glucose inhibits glucagon secretion by a direct effect on mouse pancreatic alpha cells. Diabetologia 2007;50:370–379
54. Gerich JE, Langlois M, Schneider V, Karam JH, Noacco C. Effects of alterations of plasma free fatty acid levels on pancreatic glucagon secretion in man. J Clin Invest 1974;53:1284–1289
55. Edwards JC, Howell SL, Taylor KW. Fatty acids as regulators of glucagon secretion. Nature 1969;224:808–809
56. Briant LJB, Dodd MS, Chibalina MV, et al. CPT1a-dependent long-chain fatty acid oxidation contributes to maintaining glucagon secretion from pancreatic islets. Cell Rep 2018;23:3300–3311
57. Collins SC, Salehi A, Eliasson L, Olofsson CS, Rorsman P. Long-term exposure of mouse pancreatic islets to oleate or palmitate results in reduced glucose-induced somatostatin and oversecretion of glucagon. Diabetologia 2008;51:1689–1693
58. Capozzi ME, Wait JB, Koech J, et al. Glucagon lowers glycemia when β-cells are active. JCI Insight 2019;5:e129954
59. Pedersen J, Ugleholdt RK, Jørgensen SM, et al. Glucose metabolism is altered after loss of L cells and α-cells but not influenced by loss of K cells. Am J Physiol Endocrinol Metab 2013;304:E60–E73
60. Flock G, Baggio LL, Longuet C, Drucker DJ. Incretin receptors for glucagon-like peptide 1 and glucose-dependent insulinotropic polypeptide are essential for the sustained metabolic actions of vildagliptin in mice. Diabetes 2007;56:3006–3013
61. Pearson MJ, Unger RH, Holland WL. Clinical trials, triumphs, and tribulations of glucagon receptor antagonists. Diabetes Care 2016;39:1075–1077
62. D'Alessio D. The role of dysregulated glucagon secretion in type 2 diabetes. Diabetes Obes Metab 2011;13(Suppl. 1):126–132
63. Højberg PV, Zander M, Vilsbøll T, et al. Near normalisation of blood glucose improves the potentiating effect of GLP-1 on glucose-induced insulin secretion in patients with type 2 diabetes. Diabetologia 2008;51:632–640