I. INTRODUCTION

Obesity and type 2 diabetes mellitus (T2DM) are major public health burdens of modern society, providing a growing medical and economic challenge. There is a pressing need for new therapies that improve glycemia and/or body weight and that have driven recent research in academic and industrial laboratories. Over the last 10 years multiple drugs have been developed for the treatment of T2DM that utilize the signaling systems of products of the preproglucagon gene (Gcg). Of these, most widely recognized are those that utilize the glucagon-like peptide 1 (GLP-1) signaling system. There is also long-standing interest in glucagon antagonists to treat T2DM, but recent developments indicate a potential role for glucagon agonists as components of multireceptor agents for obesity and diabetes. While there are many excellent reviews on GLP-1 physiology and pharmacology (53, 95, 174, 211) or glucagon physiology and pharmacology (61, 101, 142, 148, 195), few have addressed the physiology and pharmacology of both of these peptides in one review. These two closely related compounds, with distinct roles in metabolism, are now both compelling drug targets, and understanding their physiology is timely. Moreover, the considerable effort expended on study of the Proglucagon (ProG) peptides over the past several decades has revealed a more complex set of interactions and overlapping regulation for glucagon and GLP-1 that may also be important in diabetes pharmacology.

II. PREPROGLUCAGON GENE EXPRESSION AND ORGAN-SPECIFIC PROCESSING

Gcg is genetically expressed in a specific population of enteroendocrine cells (L-cells) of the intestinal mucosa, pancreatic islet α-cells, and a discrete set of neurons within the nucleus of the solitary tract (NTS; Refs. 150, 197).
on the regulation of Gcg transcription stretch back over three decades, although the process is still not completely understood. The Gcg promoter has four enhancer elements and a cAMP response element. Gcg expression is increased by elevations of cAMP or exposure to amino acids through these elements (133, 449), which are also targets for a number of homeodomain protein transcription factors (198). There is mounting evidence for cell-specific regulation of Gcg expression in the pancreas and intestine. In islet α-cells, Pax6 and MafB promote, whereas insulin inhibits, Gcg transcription through specific intermediates at distinct DNA binding regions (139, 198). In contrast, insulin increases Gcg expression in intestinal endocrine cells (60, 446). The latter effect is mediated by effectors of the canonical Wnt signaling pathway, β-catenin and transcription factor 7 like 2 (TCF7L2), a pathway that seems to be specific for control of intestinal Gcg transcription (60). Beyond nutrient and hormonal regulation, it is well established that bowel resection or injury causes a large increase in Gcg expression, although the mediators of this response are not clear. The existence of separate mechanisms to regulate transcriptional control of Gcg parallels the distinct patterns of prohormone processing in the major cell types producing ProG peptides.

In addition to glucagon and GLP-1, ProG contains the bioactive proteins glucagon like peptide-2 (GLP-2) and oxyntomodulin as well as fragments such as glicentin, glicentin-related pancreatic polypeptide (GRPP), and major proglucagon fragment (MPGF) that are of unclear functional significance (FIGURE 1). The relative amounts and forms of these ProG peptides in any one cell type depend on tissue-specific posttranslational modification by prohormone convertases (PC). In the α-cell, high PC2 expression produces predominantly glucagon (173), while in the intestinal L-cells and neurons of the NTS predominance of PC1/3 expression yields GLP-1, oxyntomodulin, and GLP-2 as the physiologically relevant products (237, 401, 416). PC2 is also found within the brain but is not colocalized with Gcg, and only trace amounts of glucagon have been detected in the CNS (237), supporting the idea that PC1/3 processing of Gcg predominates there. There is PC1/3 expression in α-cells, albeit at lower levels than PC2, and increasing evidence for islet production of GLP-1. Within the intestine, the density of Gcg expression and ProG synthesis increases from the proximal to distal gut and expression is highest in the colon.

III. PHYSIOLOGICAL IMPORTANCE OF GLUCAGON AND GLP-1

It is now generally accepted that GLP-1 has a broad role in glucose homeostasis, in great part through stimulation of nutrient-induced insulin release (227, 275) and by reducing glucagon secretion (435). In contrast, the best-recognized function of glucagon as a counterregulatory hormone that is released when glucose levels decrease below basal levels, and stimulate hepatic glucose production (88). A role for both peptides has been proposed in the pathogenesis of T2DM. On the one hand, the reduced GLP-1 levels sometimes observed in T2DM patients (414) have led to a suggestion that inappropriately low levels of GLP-1 reduce the insulin response to a given glucose load. On the other hand, postprandial GLP-1 levels are increased by ~10-fold after roux-en-Y gastric bypass and vertical sleeve gastrectomy, two highly efficacious bariatric surgeries associated with improved if not resolved diabetes and normalized body weight in obese patients (26, 298). In contrast, elevated glucagon levels are implicated in the

![FIGURE 1. Posttranslational processing of proglucagon. The proglucagon gene encodes proglucagon, a peptide that is differentially processed based on the relative activities of the prohormone convertases 1/3 and 2. In the α-cells of the pancreatic islet, prohormone convertase 2 (Psck2) predominates and glucagon, glicentin-related pancreatic polypeptide (GRPP), intervening peptide 1 (IP1), and a proglucagon fragment are the more prevalent products. In the intestinal L-cell and specific CNS neurons, prohormone convertase 1 (Psck1) action is relatively greater and proglucagon is cleaved to GLP-1, GLP-2, oxyntomodulin, glicentin, and IP2. Most recent evidence indicates that α-cells have some PC 1/3 activity, and it is likely that neurons and L-cells also have PC 2.](http://physrev.physiology.org/)

high basal glucose production seen with T2DM (72). As a result, numerous effective pharmaceutical compounds to treat T2DM are either on the market or in development that increase GLP-1 action, and/or reduce the activity of glucagon (148). However, the actions of glucagon have been less tractable, and no glucagon receptor antagonists have yet reached the clinic. Surprisingly, dual glucagon and GLP-1 agonists are in development that have balanced actions of the two peptides such that weight loss and improved glucose control have been reported in preclinical models (79). In the following sections of this review, we will discuss the physiological functions of both glucagon and GLP-1 with a goal of describing how these related signaling systems act in health and disease, and their potential as therapeutic agents.

IV. RECEPTOR DISTRIBUTION AND FUNCTION

A. GLP-1 Receptor Distribution and Signaling

The GLP-1 receptor (GLP-1r) is found within the pancreas, lung, adipose tissue, kidney, heart, vascular smooth muscle, and in a number of specific nuclei in the CNS (54, 135). Most of what is known about GLP-1r signaling comes from studies of β-cell function. The stimulation of insulin secretion by activation of the GLP-1r is primarily due to activation of a stimulatory G protein, generation of cAMP, and promotion of the activities of protein kinase A (PKA) and the exchange protein activated by cAMP 2 (EPAC2) (175). These mediators control transcription of key genes including proinsulin, glucose transporter-2 (GLUT2), subunits of the ATP-sensitive potassium channel (KATPase) and the secretory vesicle related protein α-SNAP, likely through cAMP responsive element binding protein (CREB) (449). However, the GLP-1r also activates other pathways to influence gene transcription including those mediated by extracellular signal-regulated kinase 1/2 (Erk 1/2), and phosphoinositol 3-kinase (PI3K). Increased PKA and EPAC2 activity potentiate the closure of the KATPase initiated by β-cell glucose metabolism, regulate membrane electrical activity, and promote Ca2+ influx, to amplify glucose-stimulated insulin exocytosis (175, 252). A key feature of GLP-1r signaling is the dependence on increases in glucose flux into the β-cell to be fully manifest, i.e., GLP-1 is a weak insulin secretagogue at basal concentrations of ambient glucose. The mechanism for this important phenomenon has not yet been explained, but there is evidence that PKA has variable actions on the KATPase that is dependent on the energy state of the cell (240). In the fasting state, when intracellular ADP levels are elevated, PKA hyperpolarizes the β-cell membrane by increasing KATPase conductance, but when ADP levels are reduced by increased glucose metabolism, PKA contributes to KATPase closure and depolarization. The minimal effectiveness of GLP-1 to stimulate insulin secretion under conditions of basal or low glucose is a characteristic of other GI hormones as well (68). Given the benefit of insulin secretagogues that are muted when their action could cause hypoglycemia, it is notable that the mechanism for this has not been more fully developed.

Recent findings implicate the transcription factor TCF7L2 in GLP-1 β-cell signaling, an important connection in that genome-wide association studies have noted TCF7L2 polymorphisms to be linked with the risk for T2DM. While the control of GLP-1r expression is not fully understood, there is now evidence to support a role for TCF7L2 and the Wnt signaling pathway in β-cell synthesis of the receptor. Moreover, a component of GLP-1r-mediated gene transcription is dependent on β-catenin/TCF7L2 (242). Finally, humans with specific single nucleotide polymorphisms of the TCF7L2 gene have diminished insulin responses to oral glucose and intravenous GLP-1 (310, 355, 411). The latter findings, in particular, raise the possibility that the GLP-1 system has a role in the pathogenesis of diabetes.

The role of GLP-1 in the β-cell goes beyond its role as an insulin secretagogue, and considerable attention has been given to actions related to β-cell growth and differentiation. However, to some degree, the signaling mechanisms overlap. Specifically, GLP-1r activation of the PKA pathway in the β-cell also contributes to β-cell replication and reduces apoptosis. Indeed, GLP-1r activation benefits β-cell survival in the presence of multiple apoptotic conditions including hyperglycemia, hyperlipidemia, inflammatory cytokines, and oxidative stress (97). However, the PKA pathway does not seem to be the principle means by which the GLP-1r controls β-cell growth and apoptosis. For example, in β-MIN6 cells, GLP-1 administration reduces H2O2-induced apoptosis both through cAMP and ERK1/2/PI3K signaling (179). Upstream of ERK1/2 activation are both PKA and an adaptor protein, β-arrestin, that binds to the GLP-1r and is necessary for full GLP-1r signaling (380). In β-MIN6 cells, GLP-1 induces a cAMP-dependent activation of ERK1/2 within minutes, followed by a later β-arrestin-dependent increase of ERK1/2 signaling that occurs over an hour (324). Moreover, in MIN6 cells, β-arrestin is necessary for the antiapoptotic effects of GLP-1. Activation of both β-arrestin and Akt, a prosurvival kinase, contributes to initiation of β-catenin-dependent Wnt signaling, a pathway established in cancer biology to be critical for cell proliferation and survival. Activation of β-arrestin and Akt leads to accumulation of cytosolic β-catenin and subsequent translocation to the nucleus where it forms a complex with TCF7L2 (242), a transcription factor that activates expression of Wnt target genes (433). Wnt signaling via β-catenin and TCF7L2 is necessary for the full effects of the
long-acting GLP-1 agonist Ex4, as both siRNA silencing of β-catenin and a dominant negative insertion of TCF7L2 in INS-1 cells blunted the ability of Ex4 to stimulate β-cell proliferation (242). In addition, pancreatic-specific deletion of TCF7L2 impairs GLP-1-induced insulin secretion from isolated mouse islets (73a). These data illustrate the wide-ranging signaling pathways induced by GLP-1r activation to regulate islet function. The findings from in vitro studies demonstrate that GLP-1r activation is connected to the key pathways that are essential for regulation of β-cell proliferation (242). One of the reasons that this area of GLP-1 physiology has received intense study is the potential clinical application to T2DM, a progressive disease that is thought to involve increased rates of β-cell death. However, although much has been learned about β-cell growth from this work, a connection to human disease has not been established.

Similar to GLP-1r actions in the β-cell, CNS GLP-1r signaling is dependent on nutrient availability. Williams et al. (434) were the first to demonstrate that the ability of peripherally administered Ex4 to reduce food intake was blunted in fasted compared with fed animals. This has been confirmed in other studies where fasting, glucose deprivation with 2-deoxyglucose, or diets high in fat or fructose and low in carbohydrate, also blunts the anorectic action of GLP-1r agonists (44, 163, 277, 352). Part of this response is dependent on leptin, since administration of this peptide to increase low, fasting levels to those typical of the fed state, restores the anorectic action of Ex4 (434). In addition, a recent body of work suggests that CNS fuel-sensing pathways also play a critical role in GLP-1 actions in the brain. For example, AMPK, a highly conserved fuel-sensing enzyme, is activated when cellular fuels are depleted and thus is activated by fasting, stimulates nutrient entry into the cell, and upregulates several metabolic pathways that regenerate ATP levels. Pharmacological activation of AMPK specifically within the CNS blunts the anorectic action of GLP-1r agonists in hypothalamic and/or hindbrain nuclei (44, 163, 352). Suppression of food intake by Ex4 in the hindbrain involves multiple intracellular signaling pathways including rapid suppression of AMPK and stimulation of the MAPK (163) and PI3K/Akt pathways (343). Finally, it is important to note that in rodents Ex4 has an effect to lower food intake that is 100-fold more potent than GLP-1 (21). It is not clear whether this is due to differences in rates of metabolism of GLP-1 and Ex4 in the brain, or other factors such as distinct interactions of the peptides with the GLP-1r (255). Regardless, understanding how neurons are activated by GLP-1r agonists is of physiological and clinical importance.

The distribution of the CNS GLP-1r includes neurons in the hypothalamus, the hindbrain, and amygdala, and there is evidence from studies in rodents that GLP-1 mediates distinct functions in specific populations of neurons. For example, GLP-1r located within either the paraventricular nucleus of the hypothalamus or the hindbrain suppress food intake without causing visceral illness, the term given to specific behaviors rats and mice exhibit when exposed to toxins that is analogous to nausea and malaise (219). In contrast, GLP-1r administered to the arcuate nucleus does not affect feeding but reduces hepatic glucose production (353). Lastly, GLP-1 injected specifically in the amygdala causes visceral illness but not anorexia (219). Based on these findings, suppression of food intake by GLP-1r may be mediated by neural populations distinct from those that cause visceral illness in rats, or nausea in humans. This finding has relevance to clinical medicine where beneficial effects of pharmacological GLP-1r agonists on satiety and weight loss can be limited by gastrointestinal side effects.

It is unknown how or whether the peripheral and central GLP-1r systems interact. The half-life of GLP-1 is <2 min in humans and rodents, limiting the time over which plasma GLP-1 can directly activate CNS neurons (168). An alternative route for circulating GLP-1 to stimulate the brain is through visceral afferent neurons, traveling primarily in the vagus nerve. GLP-1r mRNA is expressed in the neural cell bodies of the nodose ganglion (281, 406) that contribute the afferent tracts supplying the thoracic and abdominal viscera, and are contained in the vagus. GLP-1 increases vagal afferent neuronal activity (41, 203), and hepatic portal venous infusion of a GLP-1r antagonist, exendin-[9–39] (Ex9), impairs glucose tolerance (406). While some studies report that infusions of GLP-1 or Ex9 directly into the portal vein do not affect food intake in rats (216, 344), there has been one report that portal venous administration of GLP-1 reduces feeding (23). Furthermore, both bilateral subdiaphragmatic truncal vagotomy in rats, and afferent denervation of mice with capsaicin, significantly blunts the anorectic effects of peripherally administered GLP-1 and Ex4, respectively (1, 344, 390). Since Ex4 has a plasma half-life of 30 min, it has a greater chance to reach the CNS via the circulation. This may explain why vagotomy blunts the effect of Ex4 on food intake acutely, but has little effect on suppression of food intake over several hours (229). Interestingly, the patterns of cFos activation in the brain of Ex4-treated animals with and without vagotomy differ, supporting a model whereby vagal afferents are necessary for the full effect of Ex4 on the CNS (204).

GLP-1r are also located within the intestine, making paracrine activation of adjacent gastrointestinal nerve terminals a plausible signaling pathway. Intraperitoneal injections of Ex4 activate both submucosal and myenteric neurons of the rat duodenum (421), but it is not known whether this is due to direct or indirect activation. More recent data demonstrate that subdiaphragmatic vagotomy has a potent inhibitory effect on glucose tolerance and blunts GLP-1r-induced suppression of glucose intake while a specific ablation of the
hepatic branch of the vagus has no effect (161). These data suggest that the major source of vagal activation may be from intestinal rather than hepatic innervation. Thus, while more research is needed to understand the complexity of GLP-1r signaling in the gastrointestinal tract, a functional neuronal link between the peripheral and CNS GLP-1 systems is suggested as a means of regulating both satiety and glucose homeostasis. While understanding these pathways is in the early stages, they may become important pharmacological targets as enterally available GLP-1r agonists are under development.

B. Tissue Distribution of the Glucagon Receptor

Both the sequences of glucagon and the glucagon receptor (GCGR) are highly conserved across mammalian species (14). Like GLP-1, binding of glucagon to the GCGR activates adenylyl cyclase through G proteins of the Gs subtype with subsequent generation of cAMP and activation of PKA and other downstream mediators as the major mode of intracellular signaling (311). Evidence for a GCGR was first demonstrated using hepatic membrane preparations (314, 315, 337), and the richest source of glucagon binding is in the liver and kidney (14). Lesser binding occurs in heart, adipose tissue, the CNS, adrenal gland, and spleen. GCGR gene expression is greatest in liver, with more modest mRNA levels in the kidney, heart, spleen, ovary, and the pancreatic islets (54, 103). Consistent with the relative receptor expression, the liver and kidney play the major role in glucagon clearance, accounting for ~70% of the removal from the circulation (14, 89, 373). Much of glucagon removal is through receptor-mediated endocytosis, but there is evidence for endovascular proteolysis by dipeptidyl peptidase IV (318) and enzymatic degradation at the level of the plasma membrane (31, 117). The half-life of glucagon in circulating plasma is relatively short: 2, 5, and 7 min in rats, dogs, and humans, respectively (14, 316).

The expression of the glucagon receptor gene (Gcgr) in islet cells and hepatocytes increases in response to a rise in ambient glucose (297). Conversely, exposure to chronically elevated glucagon levels decreases expression. This latter response is likely mediated through signaling pathways initiated by cAMP in that it is mimicked by forskolin and reversed by somatostatin (2). Studies of Gcgr expression are generally consistent with binding studies and support a model whereby glucagon signaling is partially regulated by the available number of receptors. But while changes in GCGR expression could be important for modulating glucagon action, to date there are no definitive studies connecting the expression level of the GCGR with relative glucagon action. In contrast to the GCGR, pancreatic GLP-1r expression does not change with glucose or cAMP (2).

V. SECRETION OF PROGLUCAGON PEPTIDES BY α- AND L-CELLS

A. GLP-1 Secretion

Intestinal GLP-1 is secreted postprandially by enteroendocrine L-cells which increase in density along the intestine, from relatively few in the duodenum to progressively greater numbers in the jejunum, and the highest amount in the ileum and colon (16). L-cells are located within the intestinal epithelium and have apical processes that extend into the gut lumen giving these cells direct access to ingested nutrients (107), and indeed, carbohydrates (217), proteins (217), and lipids (447) all individually stimulate GLP-1 secretion. While studies with isolated enteroendocrine cells, or intestinal cell lines, suggest direct effects of nutrients to stimulate GLP-1 release, the degree to which this occurs in vivo is not clear. Neural, endocrine, and paracrine mechanisms have all been proposed to explain the response of L-cells to nutrients (286).

The molecular basis for GLP-1 secretion from L-cells is not as clearly defined as it is for some other hormones such as insulin release from islets. Nevertheless, there have been some parallels reported. For example, as in β-cells, KATP channels and the sulfonylurea receptor are expressed in L-cells (296, 331), and closure of KATP channels, regulated by increases in ATP as a consequence of glucose metabolism, leads to GLP-1 secretion (301, 331). However, the role of the KATP channel may differ between L-cell populations as the KATP blocker glibenclamide stimulated glucose-induced GLP-1 secretion in ileum, but not colon explants (130). It is important to note that in clinical trials sulfonylureas do not significantly affect GLP-1 secretion from T2DM subjects.

Current evidence suggests that a variety of G protein-coupled receptors (GPCR) play a key role in GLP-1 secretion. Gα-coupled receptors that increase cAMP and activate PKA, and its associated exchange proteins, are associated with GLP-1 release both in vitro and in vivo (38, 39, 332). One of these, the G protein-coupled bile acid receptor 1, or TGR5, is a target of bile acids that stimulates release of GLP-1 (187, 381). Although bile acids also act on a nuclear transcription factor, farnesoid X receptor, TGR5 is the primary receptor population responsible for bile acid-induced GLP-1 secretion (206). Interestingly, increasing bile acids within the intestinal lumen are well-known to increase ProG peptides. For example, infusion of bile directly into the ileum of dogs increases gut glucagon-like immunoreactivity (282) and infusion of a specific bile salt, deoxycholate, increases plasma enteroglucagon levels when infused into the colon in humans (3). Recent in vitro data using both primary enteroendocrine cells and an intestinal cell line in-
dicated that although bile acids do activate TGR5 and promote GLP-1 release, pharmacological TGR5 agonists are more potent secretagogues and also enhance L-cell responses to calcium and glucose-induced GLP-1 secretion (302) compared with naturally occurring bile acids.

Other GPCRs link lipid absorption to L-cell secretions as well. Specifically, GPR119, GPR120, and GPR40 are activated by long-chain fatty acids and their derivatives (178), and this increases GLP-1 secretion. GPR119 is a G\(\alpha\)-coupled receptor that is expressed in \(\beta\)-cells, the CNS, and is also highly colocalized to L-cells within the gastrointestinal tract. GPR119 agonists increase GLP-1 secretion (63) while GPR119 knockout mice have blunted GLP-1 responses to an oral glucose load (233). In contrast to GPR119, a G\(\alpha\)-coupled receptor, GPR120 and GPR40 are G\(\alpha\)-coupled receptors, and act primarily through PKC. Activation of both GPR40 and GPR120 is associated with GLP-1 secretion (106, 166, 183, 248); however, their role may be more pharmacological than physiological. Members of these fatty acid binding GPCR are under active investigation as drug targets for the treatment of T2DM.

Still another set of nutrient-sensing receptors linked to GLP-1 secretion are “sweet taste receptors” that are expressed both in the tongue, where they play a role in sweet taste perceptions, and also in the GI tract (191), where the function is less clearly understood. However, when intestinal sweet taste receptors are activated with a nonnutritive agonist, GLP-1 is secreted, and mice null for one such sweet taste receptors are activated with a nonnutritive drug targets for the treatment of T2DM.

The higher density of L-cells in the lower intestine suggests that GLP-1 secretion after meals comes mostly from the ileum and colon. Indeed, while nutrient infusions into ligated sections of the duodenum and ileum, respectively, both cause significant increases in portal vein concentrations of GLP-1, ileal infusion causes significantly greater excursions of GLP-1 (165). However, the upper gut may have important indirect effects on GLP-1 secretion from the lower gut. For example, both concentrations of plasma GLP-1 levels rise rapidly after a meal (71) prior to nutrient transit to the distal gut (18, 115). This disparity between the rates of nutrient passage along the gut and postprandial GLP-1 secretion suggests feed-forward neural or paracrine signals from the duodenum to the ileum that result in increased GLP-1 secretion (37, 152). The case for a neural mechanism to connect the presence of nutrients in the upper intestine to L-cells lower in the gut has been suggested by a number of experimental models. Nutrient-induced GLP-1 secretion with duodenal infusions is delayed when the gut is transected/reanastomosed below the duodenum compared with when it is simply ligated; the latter manipulation presumably maintains neural innervation (336). Bilateral subdiaphragmatic vagotomy in conjunction with transection completely abolishes nutrient-induced GLP-1 secretion, suggesting a gut-brain-gut axis in regulating GLP-1 secretion. In rodents, human enteroendocrine cells, and the isolated perfused porcine ileum model, cholinergic agonists, which bind to receptors in both the enteric and parasympathetic nervous system, act via types 1, 2, and 3 muscarinic receptors to stimulate GLP-1 secretion (12, 37, 153, 332). Conversely, norepinephrine and \(\alpha\)-adrenergic agonists inhibit (153) and \(\beta\)-adrenergic agonists stimulate (99, 312) GLP-1 secretion, suggesting that enteric, parasympathetic, and sympathetic neural innervation are all important in the regulation of GLP-1 secretion. In humans, the rise in plasma GLP-1 following ingestion of oral glucose is blunted by atropine (18, 115). While effects of atropine on GI motility cannot be discounted in this effect, there was a significant reduction of plasma GLP-1 corrected for plasma glucose. Thus, while there are many details yet to understand, the bulk of published evidence supports a neural contribution to GLP-1 secretion (312).

Recent evidence also suggests pharmacological stimulation of GLP-1 secretion. The commonly used anti-diabetes drug metformin has been shown to increase plasma GLP-1 levels in T2DM subjects (284), an effect that is thought to be mediated by direct luminal effects of the drug. In addition, GLP-1 seems to affect L-cell secretion. In studies with human subjects receiving the GLP-1r antagonist exendin-(9–39) (Ex9), plasma concentrations of GLP-1 are consistently higher when GLP-1 action is blocked (346, 348, 439), suggesting a physiological role for GLP-1 to inhibit its own secretion. While it is possible that this is due to a reduction of receptor-mediated clearance by Ex9, there are compatible findings from clinical trials with dipeptidyl-peptidase 4 (DPP-4) inhibitors that are not likely to affect this process. DPP4 inhibitors reduce the metabolic inactivation of GLP-1 and increase circulating concentrations of the intact peptide (32, 409). However, these drugs have been noted to consistently reduce the levels of total GLP-1, a measure reflective of GLP-1 secretion. It is not known whether GLP-1 interacts directly with L-cells or whether the influence is indirect.

A. Glucagon Secretion

Glucagon is the chief secretory product of \(\alpha\)-cells and has been the principle measure of \(\alpha\)-cell function. Endogenous glucagon levels are highest in the venous drainage of the pancreas and the hepatic portal vein, consistent with the liver as principle target. Hepatic clearance of glucagon has

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been reported as being 20–40% of the portal content by most (89, 192, 373), but not all (82) groups, with the kidney contributing the major remaining portion of peripheral glucagon removal (89). There is no evidence that the other cell types that express Gcg, either enteroendocrine L-cells or neurons in the hindbrain, contribute to plasma glucagon levels. Like the L-cell, a good case can be made for nutrient, paracrine, and neural inputs in the control of glucagon secretion. The major physiological stimuli for glucagon release is mixed nutrient or protein ingestion, activation of the autonomic nervous system (ANS), and hypoglycemia (101, 142). As suggested by the diversity of these regulatory factors, control of secretion at the level of the islet is complex and multilayered. Similar to secretion of insulin by β-cells, α-cell secretion of glucagon occurs with release of a stored pool of peptide initiated by specific stimuli. Moreover, the α-cell shares much of the secretory machinery associated with insulin secretion from β-cells, including membrane surface receptors, the KATP, specific ion channels, and exocytotic proteins. While there is convincing evidence to support a range of factors in the control of glucagon output, important questions remain as to how these factors interact, and under which circumstances.

Although controversial for many years, there is now good evidence that changes in plasma glucose have direct effects on glucagon secretion, mediated through changes in α-cell electrical activity, that involve KATP channels (59, 143, 251). Importantly, increased availability of human islets for research has been consistent with the results from rodent islet studies, supporting intrinsic sensing of glucose as a mechanism involved in α-cell secretion (251, 329). Initial observations that α- and β-cells had similar glucose transport, metabolism, and inhibition of the KATP channels yet opposite secretory responses was at first difficult to explain. However, recent evidence suggests that differences in resting electrical characteristics and ion channel function downstream of KATP channel closure can explain much of the reciprocal pattern of glucagon and insulin secretion at relative hypo- and hyperglycemia (251, 329, 341). This model is depicted in FIGURE 2. In addition to glucose, it has long been appreciated that amino acids are important regulators of the α-cell. Protein meals or infusions of amino acids stimulate glucagon release, and arginine is commonly used to stimulate glucagon secretion in research studies.

There are substantial differences in glucagon release from isolated α-cells compared with whole islets (118, 142, 237a). One major implication of this observation is that other islet cells have important roles in α-cell regulation. Endocrine cells in islets, which are exposed to high concentrations of local products either in the interstitium or microcirculation, have been implicated in the control of glucagon secretion. Insulin suppresses glucagon release (142), acting either through the vasculature of the islet as a hormone, or through local cell-to-cell contact. Addition of insulin to isolated islets or pancreata reduces, while removal

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**FIGURE 2.** Glucoregulation of glucagon versus insulin exocytosis. An increase in plasma glucose is utilized by both α- and β-cells to generate ATP. The increase in ATP opens potassium ATP channels and subsequently causes membrane depolarization. In β-cells, this activates calcium channels increasing intracellular calcium and stimulating the release of insulin via exocytosis. While KATP channel activation also activates calcium channels in α-cells, α-cells also express N-type sodium channels which are also activated by membrane depolarization. This process blocks, rather than excites, exocytosis.
of insulin with antibodies increases, glucagon release. Consistent with this, acute reduction of β-cell mass by 70% with streptozotocin causes hyperglucagonemia and consequent hyperglycemia in mice (177). Genetic deletion of the insulin receptor specifically from α-cells causes hyperglucagonemia and mild hyperglycemia, with increased glucagon release in response to arginine and insulin-induced hypoglycemia (208). In healthy humans, increased insulin secretion during a hyperglycemic glucose clamp suppressed the glucagon response (19), demonstrating the potency of insulin inhibition of the α-cell. However, any role of endogenous insulin on the α-cell response to low glucose concentrations is likely to be tonic, since β-cell secretion is minimal at glucose concentrations where α-cells only start to increase secretion (389). In keeping with this, there is a body of work suggesting that a rapid decline of islet insulin is necessary for appropriate glucagon secretion in response to hypoglycemia (176). The issue of α-cell regulation by insulin as glucose levels fall has not been fully resolved, but it seems that insulin does contribute measurably to the suppression of glucagon after meals (268). In an elegant study in dogs, Greenbaum et al. (140) demonstrated that insulin is necessary for the suppression of glucagon, and stimulation of somatostatin, during progressive hyperglycemia. Other compounds released from β-cells have been shown to inhibit glucagon release, including zinc (118), γ-aminobutyric acid (17), and glutamate (142), but the ultimate importance of these compounds is not clear.

The third major endocrine cell type in the islet, δ-cells, produces somatostatin which suppresses α-cells primarily through the somatostatin-2 receptor (384). δ-Cells have been proposed to have a paracrine role based on their anatomical features, as they radiate processes that extend through the islet and about other cell types. In addition, there is evidence that the major product of δ-cells, somatostatin-14, acts locally in the islet. Exogenous somatostatin is a potent inhibitor of glucagon secretion, and administration of a somatostatin-2 receptor agonist reduces fasting glucagon and glucose concentrations (384). While deletion of the prosomatostatin gene in transgenic mice does not affect basal glucagon or insulin levels in vivo or in isolated islets, these animals have significantly greater glucagon secretion in response to arginine than controls, an effect retained by isolated islets (158). Together these data suggest that effects of somatostatin are minimal under basal conditions but become important when nutrient stimuli are involved.

Several recent papers suggest that other α-cell products may in fact regulate glucagon secretion. α-Cells from islets of both primates and mice secrete glutamate and express ionotropic glutamate receptors (iGlutR; 47). In addition, glutamate stimulates release of glucagon, and the effect of glutamate seems to be important for a normal glucagon response to low glucose concentrations since inhibition of iGlutR impaired the hypoglycemic counterregulation in mice (47). α-Cells also express glucagon receptors, and when exposed to increasing concentrations of glucagon increase intracellular cAMP and membrane capacitance, a surrogate for exocytosis (250). While these experiments demonstrate glucagon receptor signaling in α-cells, it is not possible to discern whether this process stimulates or inhibits glucagon secretion. Regardless, the addition of apparent autocrine control to glucagon secretion lends another level of detail to a complex system.

The autonomic nervous system (ANS) plays a central role in the regulation of glucagon secretion, particularly as it relates to hypoglycemic counterregulation. Activation of both limbs of the ANS, parasympathetic and sympathetic, increases the release of glucagon, as does epinephrine released from the adrenal gland (389). Signaling through adrenergic receptors increases cAMP in α-cells (80b), and catecholaminergic signaling seems to be synergistic with hypoglycemia in stimulating glucagon release (341). Several of the peptides that act as postganglionic parasympathetic neurotransmitters, such as acetylcholine, vasoactive intestinal polypeptide, gastrin releasing peptide, and pituitary adenylyl cyclase activating polypeptide, can stimulate α-cell secretion (8), and genetic disruption of autonomic neurons in the islet predispose mice to hypoglycemia (342). Recent work emphasizes the role of glucose sensing neurons centered in the ventromedial hypothalamus (VMH) as mediating the CNS counterregulatory response (102, 239). Activation of KATP channels in the VMH augments (263), and closure of these channels (112) blunts, the glucagon and epinephrine responses to hypoglycemia. Efferent output from the VMH in response to hypoglycemia depends on the relative GABA/glutamate tone in these nuclei (398, 452). It seems likely that the brain response to hypoglycemia extends to other sites in the hypothalamus and hindbrain, but at present the specific areas governing islet function are not known.

Similar to insulin, glucagon release is also affected by the actions of enteric peptides (100, 142). Glucose-dependent insulinotropic polypeptide (GIP) stimulates glucagon release (80b, 100), likely through direct actions on the GIP receptor expressed on α-cells (80b). Since GIP secreted after meals is a potent stimulus for insulin secretion, which would tend to decrease α-cell secretion, there may be opposing effects of direct and indirect actions in the postprandial state. Many other peptides expressed in the gastrointestinal tract, including cholecystokinin, vasoactive intestinal polypeptide, and gastric releasing peptide (100), also stimulate glucagon release. However, it is not clear whether the mechanism is endocrine or neural since these peptides also function as neurotransmitters and are components of islet innervation (8).
Of great importance, the other major proG peptide, GLP-1, is a key regulator of glucagon secretion. Activation of the GLP-1 receptor inhibits glucagon release from isolated islets or ex vivo perfused pancreas (100). However, there is some controversy over the mechanism whereby this occurs. GLP-1 increases the secretion of hormones from both β- and δ-cells, and so could act indirectly to reduce glucagon release (100, 142); this is considered to be a major part of the inhibitory role of GLP-1 on the α-cell. In addition, there is evidence that GLP-1 affects electrical activity and secretion of α-cells even in the absence of changes in somatostatin or insulin (80b). The major question debated in this area is whether α-cells express the GLP-1 receptor. While some groups have demonstrated the presence of very low level expression of the GLP-1 receptor (80b, 333), others have been unable to document any expression at all (300, 399). Moreover, GLP-1 receptor activation generates cAMP which tends to be associated with increased glucagon release. However, a recent study supports a model in which low numbers of GLP-1 receptors on α-cells, with capacity to generate proportionately small amounts of cAMP, mediate suppressive effects through discrete inhibition of N-type calcium channels (80b). This elegant formulation provides one possible explanation as to how low and high intra-α-cellular cAMP concentrations can have opposite effects on glucagon release. This model requires confirmation but has the potential to rectify an area of controversy in glucagon regulation and provide a template for examining the role of other regulatory factors acting through GPCR.

Overall, considerable progress has been made in the past decade in the understanding of α-cell regulation. One interpretation of the totality of these data is as a complex, multilayered model controlling secretion of an important regulatory hormone. The current model of α-cell secretion seems to be more similar to than different from the physiology of β-cells, and also shares some mechanisms with L-cell secretion. A consistent theme across these cell types is dense integration of control by nutrient substrates, neural, endocrine, paracrine, and autocrine inputs to secretion. Work in recent years has shifted the debate from what is the most important signal controlling α-cell secretion, to how the myriad factors involved interact and work together. This seems a likely direction of future research for regulation of GLP-1 secretion as well. A key difference between the regulation of α- and L-cells is that it seems to be more complex in the former. Because glucagon has a key role during fasting, after meals, and hypoglycemia, whereas GLP-1 is released primarily during nutrient absorption, α-cells are subject to a greater range of controlling factors. While there appears to be some overlap in regulatory forces such as ambient glucose, somatostatin, and β-cell constituents, it seems likely that these also have specific roles as well. With evidence growing that the α-cell secretes other important compounds with key physiological effects, such as GLP-1, acetylcholine, and glutamate, the possibility for differential release of these compounds will need to be considered in future work.

VI. EFFECTS OF α-CELL PRODUCTS ON ISLET FUNCTION

In addition to regulation by systemic factors, the pancreatic islet is subject to local control through paracrine and autocrine mechanisms (FIGURE 3). The α-cell participates in this system, primarily by secreting products that regulate β-cell function. Understanding research in this area requires consideration of the differences in islet architecture that exist between rodents and humans (383), and the heterogeneity of islet size and organization within species (33, 113). It is well-established that in mice and rats the organization of the islet includes a mantle of α- and δ-cells on the periphery of the islet, surrounding a core of β-cells. Estimates of relative cell composition in rodent islets are ~75–80% β- and 20–15% α-cells, and most β-cells have contact with other β-cells (46, 49, 383). In humans, the major endocrine cell products are insulin, glucagon, and GLP-1. These hormones are secreted from the α-cell, β-cell, and δ-cell, respectively, and act in concert to regulate glucose metabolism. Glucagon and GLP-1 are key regulators of glucose homeostasis, influencing glucose production and uptake, respectively. Glucagon has a stimulating effect on glucose production, whereas GLP-1 has an inhibitory effect on glucose absorption. The interplay between these hormones is crucial for maintaining blood glucose levels within a normal range.
types are spread more heterogeneously throughout the islet, most β-cells have contact with either α- or δ-cells, and the percentage of β-cells per islet is 40–60% (33, 46, 113). Based on recent analyses of large numbers of human pancreata, the frequency of contacts between α- and β-cells appears to be much greater than previously estimated, as is the location of islet endocrine cells in close proximity to the microvasculature (33). It is notable that in humans, smaller islets have cellular architecture that resembles rodents, i.e., a mantle of α-cells surrounding a β-cell core with a higher percentage of β-cells than larger islets; these smaller islets also have greater relative insulin secretion (33, 113). These recent studies on the organization of islet anatomy, particularly in humans, have shifted the focus from interactions mediated through the microcirculation to control through direct cell-to-cell contacts.

Neural regulation of islet function is well established, although the nature and magnitude of effects are still under debate. In general, sympathetic nervous system activity is thought to be important in settings where there is enhanced demand for glucose, i.e., hypoglycemia and physical activity, and parasympathetic activity is important before and during meal ingestion (8, 389, 396). Parasympathetic cholinergic signaling mediates cephalic insulin secretion, a response most clearly apparent in rats, but also in humans (5, 394). There is limited but suggestive evidence that glucagon secretion can be mediated in this manner (105, 365, 377). Recent data suggest a difference between the density of autonomic innervation of rodent and human α- and β-cells, with rats and mice having greater density of neural fibers in islets (339). While this has led to the hypothesis that neural regulation of islet secretion is more important in rodents, and paracrine control in humans, this has not been formally tested, and not all studies have demonstrated reduced innervation of human islet cells (141).

The GCGR is expressed by islet β-cells, and it has long been appreciated that supraphysiological amounts of glucagon stimulate insulin release in vitro and in vivo (207, 212, 274). Similar to other peptide secretagogues, glucagon amplifies glucose-stimulated insulin secretion primarily through mechanisms activated by increased cAMP, and does so in both rodent and human islets (182, 274). In isolated human islets studied in culture, antagonism of the GCGR impairs insulin secretion in response to increased glucose in the media, suggesting a tonic role for islet glucagon action in maintaining glucose competence (273); similar results have been reported in isolated rat β-cells and islets (182, 207). Consistent with the actions of glucagon to potentiate insulin secretion, dispersed β-cells had greater secretory responses to elevated glucose when attached to an α-cell (440). Thus a body of evidence has been generated that supports islet glucagon in the potentiation of insulin secretion, most likely through paracrine and cell-to-cell interaction.

Mice with transgenic overexpression of the GCGR in β-cells have evidence of enhanced secretory function (129). These mice have increased insulin release in response to glucose and glucagon compared with wild-type controls, as well as greater β-cell mass and insulin content. Consequently their blood glucose levels throughout the day are modestly reduced, and they have markedly improved glucose tolerance. In contrast, mice with a global deletion of the GCGR have a more complex islet phenotype. These animals have enhanced insulin secretion to IP glucose, but this effect appears to be due to increased production and action of GLP-1, which is greatly increased in these animals that develop α-cell hyperplasia and excess production of ProG peptides (10). Mice with a global deletion of Gcg have lower plasma insulin but similar blood glucose levels as control animals (159). In response to an IP glucose load, the Gcg knockouts have increased insulin secretion and improved glucose tolerance (120). However, a potential explanation for this observation is increased circulating GIP concentrations and expression of GIP in the islets (120). One interpretation of these findings is that a loss of the actions of glucagon and GLP-1 is compensated for by another secretagogue. Although it would be useful to study the question in mice with a β-cell specific knockout of the GCGR, the results from genetic mouse models currently available support glucagon as having a role in the regulation of insulin release.

While glucagon and GLP-1 are α-cell products that promote insulin secretion, a novel peptide fragment of glucagon, glucagon[19–29] or miniglucagon, has been proposed to have an inhibitory role (24, 74). Miniglucagon is a constituent of α-cell secretory granules that is released with glucagon, but also produced from the metabolism of glucagon at target tissues like the liver. The fragment is produced by the combined activity of proteases that cleave at the Arg-Arg residues at glucagon(18,19) (117). Miniglucagon does not act through the GCGR but rather has been proposed to work through an as yet unidentified receptor that causes hyperpolarization of β-cells and attenuation of the effect of secretagogues that require voltage-gated calcium channels to stimulate insulin release (75). In the perfused rat pancreas, subpicomolar concentrations of miniglucagon inhibit insulin release (74). Although work with miniglucagon has been limited and led primarily by one laboratory, the potential for α-cells to make products that modulate insulin secretion both up and down is interesting and fits with the need for islet regulation across the fed and fasting states.

ProG processing differs fundamentally in α-cells and endocrine L-cells based on the predominant hormone proconvertase present in these cell types, and the common belief for many years was that this led to distinct, nonoverlapping production of ProG peptides. To summarize this concept, PC2 in α-cells was believed to account for almost
formulation, the endocrine pancreas produced only glucagon and the gut only GLP-1/GLP-2. However, findings from studies over the past decade have refined the view of α-cell ProG processing sufficiently that it is possible to consider an islet GLP-1 signaling system. Similarly, deletion of PC 1/3 from enteroendocrine cells markedly impairs ProG processing in the intestine, causing elevated tissue levels of ProG and glucagon, suggesting a capacity for NH₂-terminal processing by L-cells. This function has been raised as a possible explanation for the parallel increases in postprandial GLP-1 and glucagon in subjects with gastric bypass (201, 348).

While PC2 is essential for normal ProG processing in normal adult α-cells (122, 123), it is now clear that PC1/3 activity is also variably present as well (426). This has been observed in embryonic and neonatal mice, with pregnancy, and in a variety of prediabetic and diabetic states (214, 294, 397, 437). Thus GLP-1 seems to be generated from ProG in the α-cell, albeit in lower amounts than glucagon. In cultured α-cell lines or isolated islets, high media glucose concentrations increase PC 1/3 expression and cellular GLP-1 content (265, 430). Moreover, intact GLP-1[7–36]amide is secreted from isolated rat islets (262, 430), and from isolated human islets and α-cells (257), in culture. Interruption of GLP-1r signaling in isolated rodent islets or pancreata, using receptor antagonists or gene knockout, reduces basal (262) and glucose-stimulated insulin secretion (116). These findings have recently been corroborated in a mouse model with β-cell-specific deletion of the GLP-1r (378). Based on studies of β-cell lines or isolated islets with blockade or gene deletion of the GLP-1r, it has been suggested that there is constitutive activity of the GLP-1r in the absence of agonist ligands (367). However, given the mounting evidence for GLP-1 production in the islet, this proposal requires reconsideration. Finally, infusion of exendin-(9–39) to fasting humans, with unstimulated and low circulating GLP-1 levels, significantly decreases insulin secretion during glucose clamps (346, 359). These findings suggest a role for GLP-1r signaling that is independent of nutrient-induced L-cell secretion and can be taken as support for the action of local islet GLP-1. Overall, there has been an accumulation of evidence to support a role for local production of GLP-1 in the islet in the regulation of insulin secretion as a paracrine factor.

Beyond a role in normal islet function, α-cell GLP-1 seems to be involved in response to stress and illness as well. In rats treated with the β-cell toxin streptozotocin, there is an acute increase in islet PC 1/3 and ProG expression, and increased processing of the pro-peptide to GLP-1 (295). Moreover, increased GLP-1 signaling in the islet was implicated in the recovery from injury. In mice with a deletion of the glucagon receptor, α-cell hyperplasia leads to increased ProG expression, with massive elevations of plasma GLP-1 (128). These elevated levels of islet-derived circulating GLP-1 contribute to glucose lowering and delayed gastric emptying, as well as glucose-stimulated insulin release (10). Islet GLP-1 production and action is also mediated by the cytokine interleukin (IL)-6, which is released in response to exercise, obesity, and diabetes (109, 110). Expression of the IL-6 receptor is relatively high on α-cells, and IL-6 signaling increases ProG transcription and GLP-1 production in mice. There are no available data to support this in humans, but a recent study has demonstrated elevated levels of IL-6 and GLP-1, which were significantly correlated, in humans with critical illness (202). IL-6 stimulated GLP-1 release from α- and L-cells, increases insulin secretion through the actions of GLP-1 (110), and is necessary for the maintenance of glucose tolerance in response to diet-induced obesity (109). These data suggest that IL-6, released from adipose tissue and skeletal muscle, regulates insulin secretion in part through local production of GLP-1 in the islet. Finally, virally mediated transgenic overexpression of PC 1/3 in α-cells increased production of islet GLP-1, enhanced insulin secretion, and protected islets from the detrimental effects of high concentrations of cytokines (432). Islets producing more GLP-1 were also more effective in reducing hyperglycemia when transplanted into diabetic mice. Overall, the findings from these and other studies suggest that a paracrine system of islet GLP-1 signaling plays a role in several adaptations to metabolic stress. Understanding the control of the relative production of α-cell GLP-1 and glucagon would seem to hold promise for therapeutic development.

Not all factors released from α-cells and implicated in paracrine signaling are ProG-derived peptides. Recent studies have demonstrated production of acetylcholine by α-cells and regulation of insulin secretion through the β-cell muscarinic M3 receptor (340). In human islets, endocrine cells positive for glucagon also contain the vesicular acetylcholine transporter and choline acetyltransferase. These proteins are contained in vesicles distinct from those containing glucagon, suggesting separate secretory systems in the α-cell. Reduction of ambient glucose caused release of acetylcholine from a subset of islets, while increased glucose did not. Experimental interventions to increase acetylcholine increased insulin release from islets, and this could be blocked by muscarinic receptor antagonists. Likewise, reductions of acetylcholine release decreased insulin secretion from cultured islets. These studies in isolated human islets demonstrate that release of acetylcholine from α-cells, independent of autonomic nerves, provides paracrine regulation of β-cell secretion.

More recently, the incretin GIP has been demonstrated to be produced and secreted from α-cells (119). This interesting
finding builds on the coincident production of proGIP and ProG in K/L enteroendocrine cells by demonstrating coexpression in the endocrine pancreas as well. In the α-cell, proGIP is processed by proconvertase 2 into a truncated GIP$_{1-30}$ form that is distinct from the longer, GIP$_{1-42}$ produced in the gut. However, GIP$_{1-30}$ is equipotent to full-length GIP as an insulin secretagogue and like glucagon and GLP-1 seems to contribute to glucose competence as interference with its action attenuates glucose-stimulated insulin release. In keeping with this novel discovery in islets, mice with a deletion of ProG gene have increased circulating GIP levels, production and release of GIP from islets, and localization of GIP immunostaining to both α- and β-cells (120). Expression of GIP in β-cells seems to be ectopic as it is not seen in wild-type mice. These findings support the notion that a component of the incretin effect is mediated locally in the islet, by the classical incretins.

VII. REGULATION OF GLUCOSE HOMEOSTASIS BY PROGLUCAGON PEPTIDES

A. GLP-1

Over 50 years ago, it was discovered that insulin secretion in response to a glucose load was greater with oral compared with intravenous glucose administration (266). It is now understood that this response is the result of insulino-tropic intestinal peptides, predominantly GIP and GLP-1, which are secreted in response to ingested nutrients and function to stimulate the pancreas to secrete insulin. This is termed the incretin effect to describe the effect of GI factors, incretins, to stimulate internal secretions, i.e., insulin. Although GLP-1 has several effects that contribute to glucose homeostasis, its stimulation of insulin secretion is thought to be the primary mode by which it lowers blood glucose. However, it is important to note that physiologically and pharmacologically GLP-1, and GLP-1r agonists, regulate glycemia through both insulin-dependent and -independent modes of action.

GLP-1 also reduces glucagon levels and inhibits gastric emptying rate, two insulin-independent mechanisms by which GLP-1 affects glucose homeostasis. By reducing glucose entry into the gut, GLP-1-induced inhibition of gastric emptying rate reduces postprandial glucose excursions. Exogenous administration of GLP-1 certainly inhibits gastric emptying rate in healthy (285, 290, 429) and T2DM subjects (435) and inhibits antral and stimulates pyloric motility (358), all contributing to a blunting of nutrient entry into the intestine. These studies involve exogenous administration of GLP-1 which creates some difficulty in reconciling the physiological versus pharmacological effects. Several studies have infused the GLP-1 receptor antagonist exendin-(9–39) (Ex9) during test meals to assess the effects of endogenous GLP-1 on gastric emptying rate. The results to date are not conclusive as three studies did not demonstrate an effect of Ex9 on gastric emptying rate (279, 293, 349), while another reported a modest but significant effect (83). The cause for this discrepancy is not clear, but differences in caloric content of the meal, differing methodologies and experimental conditions, and possibly variability among healthy humans are possible explanations for the indefinite results of the Ex9 studies. Interestingly, GLP-1 receptor knockout mice do not have increased gastric emptying rate, per se (232), suggesting that these receptors are not necessary for regulation of gastric emptying rate. While the physiological role of GLP-1 on gastric emptying rate remains to be determined, the ability of pharmacological GLP-1 agonists to inhibit gastric emptying rate is incontrovertible, and this offers a potential mechanism for controlling postprandial glycemic excursions when used in the treatment of T2DM.

A number of studies have reported effects of GLP-1 on glucose and lipid metabolism in the liver. However, this area is much less clear than for glucagon because in contrast to the GCGR which is expressed in abundance by hepatocytes, it has been very difficult to demonstrate the GLP-1r in the liver (300, 322, 333). In addition, despite attempts by a number of investigators, an alternative receptor to mediate GLP-1 effects in the liver has not been convincingly demonstrated, nor do alternative forms of the known GLP-1r seem to account for hepatic effects of the peptide. At present, it is unclear whether differences in GLP-1r expression in the liver are due to species differences, specifically between humans and mice, or whether the small amount of detection of the GLP-1r is due to contamination of GLP-1r expressed in the vasculature of the liver rather than the parenchyma. The GLP-1r is expressed on hepatic portal vagal afferents leading to a more likely scenario that hepatic effects of GLP-1 are indirectly mediated by CNS action.

Infusion of GLP-1 in humans during experimental conditions with clamped insulin and glucagon concentrations reduces hepatic glucose production by 20–30%, a common finding by two different groups of investigators (321, 366). The mechanism explaining this observation is not clear and is subject to the same questions about direct or indirect actions of GLP-1 on the liver as have been raised for other responses. The effect of GLP-1 on hepatic glucose production is not explained by differences in free fatty acid levels or rates of lipolysis (366). There is accumulating evidence that GLP-1 effects on hepatic glucose metabolism may be neurally mediated. Central nervous system administration of GLP-1 (15) and specific administration directly into the arcuate nucleus of the hypothalamus improves hepatic insulin sensitivity (333) and increases hepatic glycogen synthesis (223). Moreover, neural GLP-1 sensors have been demonstrated in the vasculature of the hepatic portal venous system, where GLP-1 signaling has been observed to affect fasting and post-challenge glycemia (15, 186, 187, 321, 366).

In keeping with this novel discovery in islets, mice with a deletion of ProG gene have increased circulating GIP levels, production and release of GIP from islets, and localization of GIP immunostaining to both α- and β-cells (120). Expression of GIP in β-cells seems to be ectopic as it is not seen in wild-type mice. These findings support the notion that a component of the incretin effect is mediated locally in the islet, by the classical incretins.
towards lipid oxidation and away from lipogenesis. There are parallels with both peptides acting to shift the liver not been directly compared with those of glucagon, there While the effects of GLP-1 on hepatic fat metabolism have chronic GLP-1 administration to reduce body weight. 388); these effects appear to be independent of the effect of mice, administration of GLP-1 agonists reduces hepatic steation (86, 300), an effect reported in humans as well (145, 388); these effects appear to be independent of the effect of chronic GLP-1 administration to reduce body weight. While the effects of GLP-1 on hepatic fat metabolism have not been directly compared with those of glucagon, there are parallels with both peptides acting to shift the liver towards lipid oxidation and away from lipogenesis.

B. Glucagon

The cardinal endocrine action of glucagon, to increase hepatic glucose production, has been known for nearly 100 years, dating from the time when pancreatic extracts were first being tested as a treatment for diabetes (238). Subsequent work demonstrated effects of glucagon to counter hypoglycemia and led to the general principle that it has a role opposing that of insulin to maintain plasma glucose in times of stress, fasting, or exercise (238). The development of a radioimmunoassay sensitive enough to measure levels in the blood provided insights into the major regulatory influences on glucagon (404). The endocrine mechanism of glucagon action is based on the effects of exogenous glucagon to increase hepatic glucose output in animals, humans, and a number of in vitro systems, and removal of circulating glucagon with a neutralizing antibody to reduce blood glucose (35, 195).

The actions of glucagon to increase hepatic glucose production are mediated primarily through the cAMP/PKA signaling pathway (195, 419). Glycogenolysis is promoted, and glycogenesis inhibited, primarily through the activation of phosphorylase kinase and its downstream target glycogen phosphorylase (210, 443). The balance between glycogen breakdown and synthesis is primarily a function of relative insulin and glucagon effects on hepatocytes, i.e., the relative cAMP signal, as well as the level of glycogen stores (443). Genetic or pharmacological interventions that increase glycogen synthesis relative to glycogenolysis promote glucose tolerance (210, 319), and the cellular physiology controlling the flux through these pathways is being explored for drug targets. GCGR signaling also restrains glycolysis by modifying glycolytic enzymes, particularly fructose-2,6 bisphosphatase, regulating the flux between glucose-6-phosphate and fructose biphosphate, and inhibiting pyruvate kinase activity (195). Thus the immediate effects of increased GCGR signaling in the liver are reduction of glucose oxidation and rapid mobilization of stored glucose for export to the circulation.

Central to the effect of glucagon on hepatic glucose metabolism is regulation of phosphoenolpyruvate carboxykinase (Pepck) transcription. Pepck expression varies with the metabolic state, with increases during fasting and suppression by insulin (195). Glucagon increases PEPCk levels through CREB and Fox01, transcription factors downstream of PKA (436). PEPCk catalyzes the conversion of the TCA cycle product oxaloacetate into phosphoenolpyruvate which is a key step in gluconeogenesis from lactate, pyruvate, and alanine. Overexpression of Pepck can increase blood glucose (387), and deletion of Pepck decreases gluconeogenesis (42, 138). While Pepck expression has been used as a surrogate for glucagon action and/or rates of gluconeogenesis, recent findings suggest a more complex picture in which PEPCk mRNA or protein levels are only one of several factors governing flux from the tricarboxylic acid (TCA) cycle to blood glucose (42). In addition to PEPCk, glucagon increases hepatic production of several other key genes involved in glucose production including those coding for peroxisome proliferator activated receptor-γ coactivator 1 (PGC-1) and glucose-6-phosphatase (G6P). Overall, glucagon has effects on several levels of hepatic gluconeogenic gene expression that promote s sustained glucose production even after the finite glycogen supply is depleted. Gluconeogenesis is an energetically demanding process, requiring 6 mol of high-energy phosphate bonds for each mole of glucose produced, and is tightly linked to TCA cycle activity (42). A recent paper supports GCGR signaling as a means of connecting energy production with gluconeogenesis. In this report, glucagon mediated the enhanced rates of hepatic lipid oxidation characteristic of fasting, effects that were due to GCGR activation of PPARα and p38MAPK signaling to increase the expression of enzymes involved in β-oxidation (246). These findings are compatible with the long-standing view that glucagon contributes to hepatic fatty acid oxidation and ketogenesis at several metabolic steps that reduce cellular malonyl CoA and dis inhibit carnitine palmitoyl transferase 1 (121, 148). Importantly, elimination of the GCGR was associated with increased liver triglyceride content during...
fasting, indicating that the effects of glucagon on β-oxidation could extend to hepatic lipid balance as well. These findings are supported by the recent demonstration that GCGR signaling is needed for the correction of hepatic steatosis by exercise (29).

In another recent study, glucagon action, either from exogenous peptide or increases of endogenous peptide with fasting or exercise, shifted the balance of hepatic energetics as reflected in adenine nucleotide levels, increasing AMP relative to ATP (28). This effect was dependent on signaling through AMPK and required gluconeogenesis through PEPCK for the full effect on AMP/ATP. The increase in hepatocyte AMP/ATP due to GCGR signaling was proposed to be secondary to abrupt intracellular energy deficits, such as those occurring from suppression of glycolysis or initiation of gluconeogenesis, two actions of glucagon. Once these metabolic shifts occurred, activation of AMPK mediated a robust program of effects to increase lipid oxidation or initiation of gluconeogenesis, two actions of glucagon. The hallmark setting for glucagon action is during hypoglycemia counterregulation, when levels increase and hepatic glycogen output in the postabsorptive state but less than 10% after 36 h of fasting (234, 379). In most acute experiments, using pancreatic clamps as a means to control glucagon and insulin concentrations, it is the glycogenolytic effects of glucagon that are most apparent (328). Promotion of gluconeogenesis by glucagon requires the provision of glucose precursors, primarily lactate, alanine, and glycerol. Increased delivery of these compounds to the liver is not directly regulated by glucagon, but requires the gradual disinhibition of lipolysis and proteolysis due to reductions of circulating insulin as the duration of fasting lengthens. With extended periods of starvation, preservation of protein stores becomes paramount and gluconeogenesis is tightly controlled by precursor supply (48).

Glucagon-stimulated fatty acid oxidation contributes to ketogenesis in settings where delivery of free fatty acids to the liver are increased, such as extended fasting and uncontrolled type 1 diabetes (101). Recent work indicates that the transcription factor Foxa2, which controls the expression of genes involved in fatty acid oxidation and ketogenesis (441, 450), is activated both by fasting and glucagon (409a). Glucagon signaling leads to acetylation of Foxa2, which facilitates its localization in the nucleus and transcriptional activity through pathways downstream of cAMP (409a). Experimental activation of Foxa2 increased fat oxidation and ketogenesis, and improved hyperglycemia, hyperinsulinemia, and hepatic steatosis in obese and diabetic animals. Notably, insulin signaling leads to phosphorylation of Foxa2, its exit to the cytoplasm, and inactivation of transcription of its gene targets (442). This system presents yet another example of opposing regulation by insulin and glucagon to promote the metabolic shifting from the fed to fasted state and back again.

The model emerging from preclinical studies in animal models is generally consistent with physiological studies in humans. Studies using somatostatin to inhibit insulin and glucagon secretion, with selective replacement of one or both hormones, have demonstrated that after an overnight fast glucagon is necessary to support normal fasting glucose levels (36, 65); basal insulin replacement without glucagon results in hypoglycemia. On the other hand, plasma insulin and hepatic insulin action also play an important role in postabsorptive glycemic regulation (327). Given that plasma glucagon concentrations do not increase significantly until glucose concentrations drop below 4.5 mM, the effect of glucagon on fasting hepatic glucose production is not the result of stimulated secretion. Rather, the effects of glucagon to promote glycogenolysis and initiate gluconeogenesis occur in the setting of decreased insulin action. In other words, a threshold is crossed whereby the effect of insulin to inhibit intrahepatocyte generation of cAMP is surpassed by the action of glucagon to increase adenylate cyclase activity.

That glucagon-driven glycogenolysis and gluconeogenesis follow different temporal patterns is well established. During fasting, hepatic glycogen stores are steadily depleted such that glycogenolysis contributes ~50% of liver glucose output in the postabsorptive state but less than 10% after 36 h of fasting (234, 379). In most acute experiments, using pancreatic clamps as a means to control glucagon and insulin concentrations, it is the glycogenolytic effects of glucagon that are most apparent (328). Promotion of gluconeogenesis by glucagon requires the provision of glucose precursors, primarily lactate, alanine, and glycerol. Increased delivery of these compounds to the liver is not directly regulated by glucagon, but requires the gradual disinhibition of lipolysis and proteolysis due to reductions of circulating insulin as the duration of fasting lengthens. With extended periods of starvation, preservation of protein stores becomes paramount and gluconeogenesis is tightly controlled by precursor supply (48).

While the importance of hepatic glucagon action is clear in the fasting state, the role of glucagon action in prandial metabolism is less clear. Suppression of plasma glucagon levels contributes minimally to glycemia after glucose ingestion in humans (370). There is a paucity of information available about postprandial glucagon effects with mixed nutrient meals, which maintain or elevate plasma glucagon. The question of glucagon regulation of blood glucose following meals awaits the more general availability of the effective glucagon receptor antagonists that have been developed by industrial laboratories for targeted clinical research. Consistent with the model above that emphasizes a niche in the regulation of fasting metabolism, glucagon does not have important actions in the regulation of hepatic glucose uptake (419).

The hallmark setting for glucagon action is during hypoglycemic counterregulation, when levels increase and hepatic glucose increases dramatically (69). Glycogenolysis provides the most rapid source of glucose for release, but glu-
coneogenesis is also activated, and combined with a supply of glucose precursors liberated through the activity of catecholamines, also contributes meaningfully to the response (146). Thus the synergy of catecholamines and hypoglycemia to stimulate glucagon release also combine in the end-organ response to return glucose levels to normal.

Glucagon is also released during exercise and contributes to maintenance of blood glucose during this metabolic stressor (28, 422). Similar to hypoglycemia, the effects of glucagon interact with the varying amounts of catecholamines secreted in response to exercise, and to the usually low circulating levels of insulin in this setting, to ensure adequate glucose output. With protracted exercise, the effects of glucagon to promote lipid oxidation become increasingly important to preserve limited glucose and provide energy (423).

### C. Genetic Models of the Proglucagon System

Recent studies of mouse models that are null for genes encoding \( Gcg \), the GCGR, prohormone convertases, and factors involved in \( \alpha \)-cell development (\( Arx \)) and glucagon signaling (\( G_{\alpha} \)) have provided new and important information on the physiology of proglucagon peptides. Comparisons among the phenotypes of these models have been one of the important advances in the field in recent years.

Two different strains of \( Gcg \) knockout mice have been generated. One strategy involved insertion of a green fluorescent protein cDNA sequence and a diphtheria toxin cassette which resulted in \( Gcg \) deficiency in the pancreas, intestine, and presumably the CNS, although this was not specifically examined (120, 159). These mice have improved IP and oral glucose tolerance, increased glucose-stimulated insulin secretion (both in vivo and in vitro), and \( \alpha \)-cell hyperplasia (120). Interestingly, these mice have decreased levels of insulin when measured during their usual feeding (159), suggesting that the impact of \( Gcg \) expression on insulin secretion may be nutrient-dependent.

An inducible \( Gcg \) knockout mouse has recently been generated by inserting the human diphtheria toxin receptor plus a green fluorescent protein into the \( Gcg \) gene (305). This insertion does not affect function of the gene until the animals are exposed to exogenous administration of diphtheria toxin which kills cells expressing its receptor. Interestingly, with this model \( Gcg \) was knocked down only in enteroendocrine L-cells and \( \alpha \)-cells, but not CNS neurons. These mice had mild improvements of oral, but impaired IP, glucose tolerance. The ability of diphtheria toxin to reduce \( Gcg \) expression in this model was short-lived in the intestine where cell turnover is more rapid compared with the pancreas. Thus studying these animals 1 wk after administration resulted in a pancreas-specific downregulation of \( Gcg \), and these animals had normal IP glucose tolerance (305).

Together with the data on the chronic deletion of \( Gcg \), it seems that L-cell expression of \( Gcg \) is essential for IP, while glucagon is essential for oral, glucose tolerance. This is striking given the fact that plasma levels of GLP-1 have negligible increases during an IPGTT. However, the data do leave open the question of the role of CNS \( Gcg \) in regulating glucose homeostasis.

Another genetic model of reduced \( Gcg \) action is deletion of the prohormone convertases that process \( Gcg \)-derived peptides to GLP-1 (and GLP-2, and oxyntomodulin; Pcsk1) or glucagon (Pcsk2). Mice null for Pcsk1 do not produce GLP-1 or GLP-2 and are smaller (reduced length and weight) than wild-type controls, but only the heterozygote mouse had any impairment of IP glucose tolerance (453). Notably, patients with a Pcsk1 mutation are obese, hyperphagic, have reactive hypoglycemia, and have enteronecocrine dysfunction (114, 188, 215). In fact, Pcsk1 was found to be the third most prevalent genetic contributor to obesity (67). While the obesity is consistent with data supporting brain \( Gcg \) expression as crucial for regulation of energy homeostasis (22), Pcsk1 also processes proopiomelanocortin, a key neurotransmitter in the regulation of energy homeostasis.

Pcsk2 null mice that do not produce glucagon from \( Gcg \) are similar to whole body \( Gcg \) knockout animals with mild hypoglycemia, improved glucose tolerance (122), and \( \alpha \)-cell proliferation (122, 123). This similarity is striking since prohormone convertases are involved in the posttranslational processing of proinsulin and proopiomelanocortin, among other peptides, yet it is the effects of the \( Gcg \) deletion that are most pronounced.

Comparing the phenotype of the \( Gcg \) null to the respective knockout of \( Glp-1r \) and \( Gcgr \) provides important clues regarding the physiology of the ProG system. GCGR null animals are phenotypically similar to \( Gcg \) null mice, with lower fasting glucose levels, improved glucose tolerance, and \( \alpha \)-cell hyperplasia (10, 245). In contrast, GLP-1r knockouts have impaired oral and IP glucose tolerance, and no major changes in islet architecture (64, 128, 303, 363). GCGR null animals have enhanced whole body insulin sensitivity (381), whereas the GLP-1r null animal has specific impairments in hepatic but not peripheral insulin action (15).

Interestingly, secretagogue-dependent increases in insulin secretion and reduction in gastric emptying rate also play a role in mediating improvements in overall glucose tolerance in many of these models. In fact, a caveat to comparing the phenotype of the \( Gcg \) null to the GCGR null mouse is the increase in plasma and islet GLP-1 levels in the GCGR null mouse (10). To eliminate this confound, dual genetic elimination of the GCGR and GLP-1r were generated. These mice were found to have fasting glucose levels and IP glu-
cose tolerance that was comparable to wild-type controls, but maintained improved oral glucose tolerance, reduced rates of gastric emptying, and impaired glucose-stimulated insulin secretion seen in the GCGR null animals (10). Interestingly, the dual GLP-1/glucagon receptor null mice have increased sensitivity to GIP-induced insulin secretion compared with the Gcg null mice that have increased plasma levels of GIP (120).

In addition to the role of Gcg on function, there is also a role for Gcg in differentiation of islets. For example, along with the improvement in glucose homeostasis, another key feature common to Gcg null, Pcsk2, and GCGR null animals is α-cell hyperplasia (FIGURE 4). This seems to be a common feature of the interruption of glucagon signaling, since it has been described in mice treated with anti-GCGR antibody or antisense oligonucleotides as well (144). In animals that lack glucagon (Pcsk2 null mice), glucagon replacement has been shown to reverse the α-cell proliferation, indicating that loss of glucagon and/or glucagon signaling is required for α-cell proliferation (425). This is supported by the fact that mice with dual elimination of the GLP-1 and glucagon receptors still have α-cell hyperplasia (10). Further research has demonstrated that liver specific GCGR signaling (58, 245) is not only required but that there is an, as of yet unknown, circulatory factor released when hepatic GCGR signaling is absent that stimulates α-cell hyperplasia (245). This was demonstrated by the fact that α-cell hyperplasia occurred even in “normal” islets that were transplanted to the kidney capsule of whole body or liver-specific GCGR null animals (245).

In summary, Gcg, GCGR, and the GLP-1r are all required for various components of β-cell function, and hepatic GCGR signaling is required for normal α-cell growth and differentiation. Underscoring the importance of the Gcg system, each of these models has developmental adaptations that result in upregulation of alternative enteroglucagon systems and contribute to the maintenance of glucose homeostasis.

VIII. REGULATION OF ENERGY HOMEOSTASIS BY PROGLUCAGON PEPTIDES

A. GLP-1

The regulation of energy homeostasis involves the balance between caloric expenditure to caloric ingestion. When these two variables are matched calorie for calorie, body weight is stable. This process is largely thought to be regulated by the CNS in response to peripheral levels of circulating and stored nutrients, hormones, and neuronal feedback from the periphery. While the roles of GLP-1 and glucagon on glucoregulation largely oppose one another, the two peptides have similar actions to cause negative energy balance, albeit through different sides of the energy

![Figure 4](http://physrev.physiology.org/)

**FIGURE 4.** Summary of the genetics of α-cell hyperplasia. A number of mouse models have been generated to understand Gcg biology. While models that target GLP-1 action tend to be obese (Pacak1/3 null), or have mild obesity and/or glucose intolerance (GLP-1r null), models that target glucagon signaling (Gcg null, GCGR null, GLP-1r + Glcar null) all have pronounced α-cell hyperplasia. While the GCGR null also has a compensatory increase in GLP-1, the GCGR/GLP-1r double KO still has α-cell hyperplasia indicating that increases in GLP-1 signaling are not responsible for this effect. Liver-specific GCGR null animals also have α-cell hyperplasia indicating that hepatic receptors are necessary for the effect.
balance scale. However, the role of proglucagon peptides in energy homeostasis has largely focused on GLP-1 rather than glucagon. Part of this is based on the fact that the posttranslational processing of neuronal Gcg is via PC1/3 and thus their primary product occurring naturally in the CNS is GLP-1 (94, 150, 197).

Many of the hindbrain neurons expressing Gcg relay information from the viscera carried by primary afferent neurons and have rich axonal projections to the hypothalamus, hindbrain, and amygdala (150, 244), and also to vagal efferent catecholaminergic and serotonergic neurons (243). While expression of Gcg is localized to cells in the hindbrain, GLP-1 has been detected throughout the hypothalamus by immunostaining of nerve fibers (197, 393, 416), supporting its role as a ligand for GLP-1r in key areas such as the paraventricular and arcuate nuclei. In the paraventricular nucleus, GLP-1 immunoreactive terminals are found on corticotropin release hormone neurons and in oxytocinergic neurons were stained positive for GLP-1 both within the paraventricular nucleus and the supraoptic nucleus (393). Thus, while the neuronal expression of Gcg seems to be discrete, GLP-1r have widespread expression within the rodent CNS (54, 135). In contrast, it is unclear how much glucagon is produced within the brain, and the relatively less common CNS glucagon receptors have not been well characterized (54).

Despite recent advances, much of the neurophysiology of Gcg neurons remains unclear. The factors that control hindbrain production and secretion of ProG products are in an early stage of understanding. For example, ex vivo studies demonstrate that Gcg neuronal activity is stimulated by leptin (167, 180), cholecystokinin (CCK), and epinephrine (168), but not PYY or melanotan II, or most importantly, GLP-1 (168). Consistent with this latter finding, GLP-1r are not located on Gcg neurons, suggesting that peripheral GLP-1 does not directly regulate activity of these neurons. Using c-fos as a marker for in vivo neuronal activation demonstrates that experimental gastric distention (417) and noxious and nutrient stimuli (124, 335) increase Gcg neuronal activation. Most studies surrounding Gcg neuronal function are pharmacological. For example, GLP-1 administration directly into the CNS has a potent, but short-lived, inhibitory effect on food intake that is blocked by coadministration of Ex9 (392, 402). Peripherally administered GLP-1 has a limited effect on food intake in rats (292), in great part due to its rapid inactivation in the circulation (194), suggesting that the anorectic effect of GLP-1 is mediated primarily via CNS GLP-1 receptors.

CNS GLP-1 is also implicated in long-term energy balance as production is responsive to changes in feeding status and adiposity. Specifically, hindbrain Gcg gene expression and hypothalamic GLP-1 protein are and are reduced after acute and chronic restriction of food intake (137, 180), while high-fat diet and obesity increase hindbrain Gcg expression (221). In addition, blocking CNS GLP-1 either by chronically blocking GLP-1r with CNS administration of Ex9 (22, 267, 402), or by using viral-mediated RNA interference to reduce Gcg expression (22), increases adiposity; in contrast, 1 wk of CNS administration of GLP-1 in high-fat-fed mice decreases adiposity independent of food intake (299). GLP-1r KO mice also have increased adiposity, although this effect is dependent on background strain (154, 363, 364). Notably, chronic intracerebroventricular Ex9 administration to wild-type C57BL/6J mice fed a high-fat, very-low-carbohydrate diet causes weight loss (221). These results contradict most other work indicating that GLP-1 signaling is associated with negative energy balance. One explanation for this is that the effects of GLP-1r blockade are counteracted by the shift in dietary macronutrients through specific CNS nutrient-sensing (44, 162, 164, 277, 332).

The role of CNS GLP-1 in regulating long-term energy balance seems to be integrated with CNS leptin signaling. For example, the reduction of hindbrain Gcg expression and hypothalamic GLP-1 protein levels by fasting is prevented by maintaining leptin concentrations with exogenous administration (137, 180). Leptin replacement also maintains the anorectic ability of both GLP-1, and Ex4 that is typically lost with fasting (434). Consistent with these in vivo responses, leptin depolarizes Gcg neurons (167), and interestingly, deletion of the leptin receptor from Gcg neurons results in mice that are hyperphagic with rapid weight gain but increased metabolic rate and normal glucose tolerance (362).

It is notable that the interaction between leptin and GLP-1 is bidirectional. For example, the GLP-1r antagonist Ex9 delivered directly into the fourth cerebral ventricle attenuates the anorexic effects of leptin (451). The interactions of GLP-1 and leptin fit with current concepts of energy homeostasis, whereby visceral signals that have short-term actions on satiety, and signals responsive to adiposity, are linked. However, there may be differences between physiological and pharmacological regulation. For example, neuronal activation in the hindbrain in response to administration of Ex4, as reflected in c-Fos expression by NTS neurons, is reduced rather than potentiated by leptin treatment (434). Regardless, at present, it is reasonable to suggest that physiological fluctuations in leptin modulate the CNS GLP-1 system and the role of GLP-1 in long- and short-term energy balance.

While there is a wealth of data demonstrating an important role of GLP-1 to regulate food intake, the data on whether it also regulates energy expenditure is less consistent. In mice, 1 wk of centrally administered GLP-1 prevented weight loss-induced reductions in energy expenditure (299), suggesting a positive role for brain GLP-1 on energy
Interestingly, like GLP-1, activation of afferent neurons involved in food intake without changing hepatic glycogen levels (408). A study in rabbits demonstrated that glucagon suppressed food intake and consequently blood glucose levels. However, a direct, effect of GLP-1 on energy expenditure (372). Finally, long-acting GLP-1 agonists do not seem to regulate energy expenditure in patients (34, 155). Taken together, we believe these data reflect that GLP-1’s effects on reducing food intake are much more definitive than any effects on energy expenditure.

B. Glucagon

It is well established that glucagon also leads to negative energy balance, mostly based on studies with exogenous administration of peptide, or pathological states where it is secreted to high levels. Two key features about glucagon modulation of energy balance that distinguishes it from GLP-1 are 1) that glucagon both inhibits food intake and stimulates energy expenditure and 2) glucagon does not induce visceral illness at doses that cause anorexia (126). In addition, while the effects of GLP-1 to regulate energy balance have not been attributed to actions in specific brain regions, how glucagon influences the CNS is not well understood, and most studies were conducted over 20 years ago before techniques for mapping specific brain functions were developed. Regardless, it is now clear that pharmacological administration of glucagon increases resting energy expenditure and decreases food intake across multiple species (51, 77, 78, 125, 172, 237b, 261, 280, 306, 351, 360, 386, 427).

Consistent with a physiological role to regulate food intake, plasma glucagon levels increase postprandially (200, 235, 403), and administration of glucagon antibodies prior to a meal, to immunoneutralize circulating peptide, increases meal size (236, 237b). However, the mechanism for this effect of glucagon has not been specifically delineated, and most of what is known in this area comes from pharmacological studies. Like GLP-1, CNS administration of glucagon inhibits food intake (185, 228), although the precise distribution of glucagon receptors in the CNS is lacking. Early work focused on a peripheral site of action that was tied to glucagon’s ability to increase hepatic glucose production and consequently blood glucose levels. However, a study in rabbits demonstrated that glucagon suppressed food intake without changing hepatic glycogen levels (408). Interestingly, like GLP-1, activation of afferent neurons that innervate the portal vein has also been implicated in glucagon action. Infusion of glucagon into the portal vein has a dose-dependent effect to reduce food intake (427). Interestingly, complete vagotomy eliminates the ability of glucagon to suppress food intake, while specifically sparing the hepatic branch of the vagus preserves glucagon-induced anorexia (127).

A blunted anorexic effect in fasted animals (44, 352, 434). These latter points are supported by the fact that glucagon has a dose-dependent effect to reduce food intake (427). Together with the data on GLP-1 action within the portal vein, these data suggest that glucagon-induced satiety is via portal vein action while GLP-1-induced satiety is via intestinal innervation.

Another alternative to a CNS site of glucagon action is brown adipose tissue (BAT), a target organ that has come to the forefront as a mediator of energy expenditure. BAT is a highly metabolic tissue that, when stimulated, generates heat that increases local and sometimes core body temperature. In some studies, glucagon increases both core body temperature (90) and BAT mass and temperature (30, 90, 226, 444), indicative of an increase in energy expenditure. BAT is highly activated during cold exposure as a way to generate heat, and instead, cold exposure increases both plasma and BAT glucagon levels (147), suggesting a physiological role for glucagon in nonshivering thermogenesis. Some suggest that the impact of glucagon on BAT is only physiologically important during cold exposure as glucagon increases BAT temperature more potently in cold-acclimatized rats compared with rats kept at room temperature (90).

Glucagon activates BAT in vitro (199), suggesting direct regulation of this tissue. However, in vivo, propranolol, a nonspecific β-adrenergic receptor antagonist (85), blocks the effect of glucagon on BAT thermogenesis, suggesting an important indirect role mediated through the adrenergic nervous system as well. Coadministration of glucagon and epinephrine additively stimulate energy expenditure, yet the effect of glucagon is still present in adrenalectomized rats (77), dissociating glucagon action from epinephrine levels per se. One speculation is that glucagon interacts with the SNS at other tissues (e.g., the CNS). Overall these data suggest that glucagon and the sympathetic nervous system have integrated effects on regulating energy expenditure via BAT activity. Despite these interesting findings in animal models, an important role of BAT in human energy balance remains a debated issue.

Given that glucagon increases energy expenditure and reduces food intake (FIGURE 5), it is not surprising that many (30, 80a, 360) but not all (213) studies demonstrate weight loss with chronic administration. While the glucagon receptor knockout mouse has a lean phenotype (10), they also have developmental adaptations for the lack of glucagon,
one being an increase in plasma GLP-1 (10). Regardless, the ability of chronic glucagon to suppress body weight makes it an interesting target for treatment of obesity. The recent investment of pharmaceutical companies towards combined therapeutics offers an opportunity to take advantage of the weight-reducing effects of glucagon while offsetting its hyperglycemic effects by combining with other hypoglycemic agents, such as GLP-1.

IX. ROLE OF PROGLUCAGON PEPTIDES IN DIABETES AND OTHER PATHOLOGICAL STATES

A. Pathophysiology of Glucagon in Diabetes

Similar to insulin secretion, glucagon release in persons with diabetes is abnormal, and a number of defects have been identified from studies of diabetic subjects. In states of poorly controlled diabetes with severe insulin deficiency or ketoacidosis, plasma glucagon concentrations are extremely high (101). However, fasting hyperglucagonemia of a more modest degree is present in many T2DM patients even without metabolic decompensation, and a good case can be made that inappropriate fasting glucagon drives inappropriate HGP in diabetic patients (20, 326). Similar to the diabetic β-cell, the α-cell in diabetic subjects has abnormal sensitivity to glucose with less suppression during hyperglycemia from enteral or parenteral sources, and plasma glucagon levels after eating mixed nutrient meals are generally higher with T2DM (101, 405, 420). In contrast, there is evidence for sluggish or reduced glucagon responses to hypoglycemia (101, 131). It is not clear how this inappropriate response to low glucose is mediated, but the functional result suggests another form of α-cell glucose insensitivity. There is emerging evidence that α-cell dysfunction in diabetes has a genetic basis as a common polymorphism in the KIR6.2 gene, which predisposes to T2DM and is related to blunted glucose-induced suppression of glucagon (361, 400).

A number of studies in humans support a role for glucagon in postabsorptive glucose regulation, with estimates that it accounts for 40–50% of fasting hepatic glucose production (20, 84a, 241). These findings are compatible with those of rodent models demonstrating important glucagon effects to support fasting blood glucose levels (35, 128). In classic studies performed soon after the discovery that somatostatin inhibits β-cell secretion, it was demonstrated that hyperglycemia and ketogenesis were substantially improved in insulinopenic T1DM subjects when plasma glucagon was reduced (132). Moreover, in T2DM subjects, suppression of islet hormone secretion by somatostatin reduced basal hepatic glucose production significantly, and this effect was enhanced when insulin was given at basal levels (101). Together these findings exemplify the basis for concluding that glucagon action contributes to pathogenic as well as physiological control of fasting glucose control.

In subjects with T2DM, plasma glucagon levels are not normally suppressed after carbohydrate containing meals or during oral glucose tolerance testing. Thus, in diabetic subjects with persistent fasting hyperglucagonemia, postprandial hepatic glucose production remains elevated at fasting levels, rather than making the abrupt postprandial decline typical of nondiabetic subjects (15). In healthy subjects the suppression of glucagon secretion after meals is a key factor in shifting hepatic metabolism from glucose production to a glucose clearance (42). Nondiabetic subjects given somatostatin with infusions of glucagon to maintain basal levels during a glucose bolus had significantly greater glycemic excursions than when glucagon was allowed to follow the normal postprandial decline (370). A similar pattern was seen in subjects with T2DM (371). Overall a substantial body of evidence indicates that failure to suppress fasting levels of glucagon after eating contributes to hyperglycemia, an effect that is magnified in the setting of reduced plasma insulin.

B. Pathophysiology of GLP-1 in Diabetes

In a now classic paper, Nauck et al. (288) reported that subjects with T2DM had a blunted incretin effect, a find-
subjects, this is likely a function of general response to GLP-1 is impaired compared with nondiabetic for larger populations of subjects. And while the insulin deficiencies of GLP-1 have not been substantiated, at least

disease, consistent with the animal literature demonstrating that glucagon regulates energy homeostasis.

D. Proglucagon Peptides in Bariatric Surgery

The number of bariatric surgical procedures has surged in the last two decades in response to the unrelenting increase in the prevalence of obesity (40). Procedures such as gastric bypass (GB) and vertical sleeve gastrectomy (VSG) cause significant weight loss in most patients (66) and also reduce obesity-related comorbidities (62, 134, 260, 376). Of great interest has been the rapid correction of diabetic hyperglycemia by GB and VSG soon after surgery and before substantial weight loss (317, 356, 395). The magnitude and reproducibility of these findings have spurred research into the mechanism behind this effect, with GLP-1, and to a lesser extent glucagon, receiving considerable attention as potential mediators of metabolic changes after bariatric surgery.

Persons with GB have enormous increases in postprandial GLP-1 secretion compared with subjects without surgery (190, 224, 230, 307, 348). Peak plasma levels of GLP-1 are 5- to 10-fold elevated, and this response persists for years after surgery (76, 225, 231, 410). Of note, GLP-1 levels are also substantially increased after VSG despite the significant differences in surgical anatomy compared with GB (283, 307). The cause of increased L-cell secretion following surgery is unclear. The simplest explanation is that both GB and VSG cause increased flux of ingested nutrients to the small intestine (87, 338), and secretion of GLP-1 is known to be dependent on rate of glucose entrance into the gut (56, 357). However, the details of this process are still unclear, and this remains an area of active research.
The increased plasma GLP-1 in GB is associated with an enhanced incretin effect. Nondiabetic subjects with GB had a threefold greater contribution of GLP-1 to their postprandial insulin response than nonoperated controls (348). Similarly, diabetic subjects with GB also had a disproportionate reduction of insulin secretion during infusion of a GLP-1R antagonist (196, 201). While it has been proposed that GLP-1 could contribute to the increased satiety following GB, recent studies in mice with GLP-1R gene deletions have demonstrated normal weight reduction following GB or VSG (276, 438, 445).

With a greater number of patients having GB, the long-term complications of this procedure have become more defined (205). One of the more dramatic of these is a syndrome of hyperinsulinemic hypoglycemia that occurs several years after surgery (304, 368). Affected subjects have normal glucose regulation during fasting but recurrent hypoglycemia 1–2 h after eating. One hypothesis proposed for this syndrome is that it is due to enhanced GLP-1 sensitivity or action in a subset of GB patients. Indeed, in one study patients with recurrent postprandial hypoglycemia had higher plasma GLP-1 levels compared with asymptomatic GB subjects (136). This has not been the case in all such comparisons (348, 350), a variability suggesting that plasma GLP-1 is not the only factor involved in the hypoglycemic syndrome. A recent study demonstrates that blocking the GLP-1r with Ex9 corrects postprandial hypoglycemia in affected subjects while having a much smaller effect on blood glucose and insulin secretion in asymptomatic GB subjects (347). These results suggest that increased GLP-1 action contributes to the post-GB hypoglycemic syndrome and raises the possibility that a combination of elevated plasma GLP-1 and enhanced sensitivity to its actions mediate this effect.

Several groups have reported that postprandial glucagon secretion is also increased following GB (52, 348, 350, 369). It is not currently understood why plasma glucagon is two- to fourfold higher in GB patients and the observation is generally inconsistent with the improved glucose tolerance in diabetic subjects after surgery. However, it is important to note that glucose dynamics are changed considerably following GB and VSG, with elevated gastric emptying (57), accelerated absorbance of enteral carbohydrate in the early phase of meals, an early and elevated glucose peak, and a rapid decline to or below baseline glycemia (52, 338, 350). One hypothesis for why plasma glucagon levels are elevated in this setting is as a response to the need for a shorter period of hepatic suppression of glucose production. This has been suggested in a recent study of GB subjects in whom the profile of endogenous glucose production paralleled that of plasma glucagon (52). The mechanism by which the distinct profile of plasma glucagon is regulated in GB is not known, but is likely to provide new insights into the regulation of α-cell secretion.

X. ROLE OF PROGLUCAGON PEPTIDES IN THERAPEUTICS

A. Glucagon Antagonists and Agents That Reduce Glucagon Signaling

Based on the evidence supporting glucagon as a significant contributor to abnormal glucose regulation in T2DM, targeting glucagon action is reasonable therapeutic strategy that has been pursued for the past 30 years. Despite compelling findings from clinical and preclinical work, there has not yet been an approved drug that acts primarily by limiting glucagon secretion or signaling. This is due in great part to problems that may be encountered when glucagon signaling is substantially reduced, problems that have been reported in animal models and humans (9, 73). The mouse models that have been developed to study the physiology of glucagon action include animals with interruption of glucagon receptor function (128, 303) as well as α-cell secretion (151, 160). These animal lines tend to have lower fasting glucose and significantly improved glucose tolerance than control mice and provide proof of principle that long-lasting blockade of glucagon signaling could be an effective means of reducing diabetic hyperglycemia. However, despite preclinical data indicating that reduced glucagon action lowers, and resets, the level of glucose that animals defend, there are concerns raised by the development of α-cell hyperplasia and profound elevations of circulating proglucagon-related peptides in mice with GCGR or proglucagon deletion (9) and in humans with GCGR mutations (448). In addition to the expansion of endocrine cells, animals deficient in glucagon signaling also have increased total pancreatic weight reflecting expansion of the exocrine compartment. In addition to this apparent drive to cellular growth that occurs when GCGR signaling is abolished, reductions in hepatic glucagon signaling also lead to reduced lipid oxidation (151, 246). More recent work raises the possibility that absent GCGR signaling reduces the tolerance and recovery of hepatocytes to toxic injury (375). These findings provide a cautionary note to therapeutic strategies that include long-term interference with glucagon signaling, particularly in the liver.

Blockade of glucagon action in humans has been possible with several small molecule GCGR antagonists developed by pharmaceutical companies, but the information available from these clinical programs is limited. One of these compounds, BAY27-9955, was demonstrated to block the actions of exogenous glucagon in nondiabetic subjects (308), but was not further developed and no information on treatment of diabetic subjects with fasting hyperglycemia or endogenous hyperglucagonemia was published. A small molecule, MK0893, lowered fasting glucose significantly in diabetic subjects in a dose-responsive manner, but these results were only presented in preliminary form (111). Similar findings have been reported with LY2409021, with
dose-dependent reductions in fasting and postprandial glucose (209). As the pharmacological approach most analogous to genetic models of reduced GCGR signaling, antagonists have obvious potential as drugs to lower glucose and treat diabetes. However, the data from preclinical studies raise concerns that adverse effects such as α-cell hyperplasia, hepatic steatosis, and susceptibility to hepatic toxicity could occur in treated patients. Moreover, the effects of these agents on glucose counterregulation would need to be carefully assessed.

In reality, there are a number of available drugs to treat diabetes that act in part by inhibiting glucagon secretion. Both sulfonylureas and exogenous insulin inhibit glucagon secretion to a modest degree (309, 330), although the effects are variable and the specific contribution of this action to their therapeutic benefit has not been determined. In addition, two new classes of drugs, GLP-1 receptor agonists and DPP-4 inhibitors, have both been demonstrated to lower blood glucose at least partially through reductions of plasma glucagon. Exogenous administration of GLP-1 reduces plasma glucagon levels, a response reported with the early human use of this peptide (289). In a recent study islet hormone secretion was blocked with an infusion of somatostatin, and insulin and glucagon were replaced to match the levels seen after GLP-1 administration. The various combinations of insulin and glucagon levels in the healthy subjects studied supported glucagon lowering and insulin stimulation as approximately equivalent in the GLP-1 control of fasting glucose (156). Administration of pharmacological amounts of GLP-1 to hyperglycemic type 1 diabetic subjects with minimal β-cell function reduced blood glucose by ~4 mM, associated with a 40–50% decrease in plasma glucagon (291). Thus increasing plasma GLP-1 activity with either injectable analogs or by protecting endogenous peptide with DPP-4 inhibitors are approaches to reduce glucagon action.

Both exenatide and liraglutide lower blood glucose and hemoglobin A1c in T2DM subjects, improve insulin secretion, and reduce plasma glucagon (93). Exenatide has effects similar to native GLP-1 to reduce glucagon concentrations in subjects with type 1 diabetes (104), and this has been proposed to account for the lower fasting blood glucose in these subjects. With chronic administration, exenatide and liraglutide reduce plasma glucagon with concurrent increases of insulin. Although reductions of plasma glucagon are likely to contribute to the therapeutic effect, it is not possible at present to attribute the relative contribution of this action. DPP4 inhibitors are also in use for the treatment of diabetes, with a number of agents having similar efficacy now available (81). DPP4 cleaves NH2-terminal dipeptides from a number of naturally occurring peptides, but the effects of DPP4 inhibitors to improve hyperglycemia in patients with T2DM seems to be mostly due to the protection of plasma GLP-1. These drugs have similar actions as GLP-1 receptor agonists on islet hormone secretion, with their major effects being to stimulate insulin secretion, and reduce plasma glucagon. With chronic use of DPP4 inhibitors and lower blood glucose levels, changes in plasma glucagon can appear to be greater than those of insulin (6). Thus there has been a tendency to focus on the effects of these agents on α-cells. However, the relative contribution of glucagon suppression to diabetic control by these drugs has also not been proven.

Recent work suggests that metformin, the most commonly used oral medication to treat T2DM, may in fact lower blood glucose as a hepatic glucagon antagonist. It has long been appreciated that metformin reduces hepatic glucose production in humans with diabetes (70, 84). This action is independent of increased insulin secretion or insulin sensitivity and has been attributed to suppression of glycogenolysis (70) and/or gluconeogenesis (385). In cultured hepatocytes, metformin, or the related compound phenformin, inhibits generation of cAMP or activation of PKA by glucagon and reduce glucose production (272). These effects were corroborated in vivo, with mice treated with metformin resistant to the hyperglycemic effects of glucagon. These effects have not been validated in diabetic humans but raise the possibility that antagonism of glucagon has been one of the most common means of treating diabetes heretofore. It is noteworthy that metformin treatment is very well tolerated and has not been associated with hepatic toxicity in humans.

B. GLP-1 Agonists and DPP-4 Inhibitors

The effectiveness of GLP-1 to stimulate insulin secretion, and activate other processes that lower blood glucose (reduce plasma glucagon, delay gastric emptying, suppress hepatic glucose production), is central to the development of drugs that work through the GLP-1r system. Peptide agonists of the GLP-1r that are resistant to metabolism by DPP4 are now widely used to treat diabetes (7, 93). The development of incretin-based drugs has been one of the major advances in diabetes medicine in recent years. GLP-1r agonists recapitulate most of the physiological actions of native GLP-1, and also have pharmacological effects on satiety (247). Small molecule DPP4 inhibitors also stimulate insulin and reduce glucagon, actions that can be attributed to GLP-1r signaling (96), although the precise mechanism of action of these agents is not yet fully understood.

Three GLP-1 receptor agonists are currently available for the management of hyperglycemia in patients with type 2 diabetes (53, 93). Exenatide (Byetta), a synthetic replica of exendin-4, has been available clinically since 2005 and is administered twice daily. Liraglutide (Victoza), a GLP-1 analog modified to include a fatty acid side chain to facilitate albumin binding, has a longer half-life and is suitable for...
for once daily injection; this agent received approval in 2010. An extended-release form of exenatide, exenatide ER (Bydureon), is administered weekly and has been available since 2012. Because they are peptides, these agents are administered subcutaneously. The key pharmacological characteristics of the commercially available GLP-1 RAs are summarized in Table 1. Exenatide BID and exenatide ER are predominantly eliminated through glomerular filtration, while liraglutide is thought to be eliminated through a receptor-based mechanism and not through renal clearance (189, 254).

The development and study of GLP-1r agonists has provided new insights into the actions of native GLP-1. In particular, effects of GLP-1r signaling on α-cell secretion, gastric motility, and satiety have become even more apparent with pharmacological dosing in clinical studies. These actions of GLP-1r agonists are believed to have a major role in their therapeutic benefits, and may in some patients outstrip their insulinotropic actions. There remain major questions as to how the β-cell and non-β-cell actions of GLP-1 are mediated, especially since the GLP-1r is expressed minimally, if at all, by putative target tissues like the α-cell, gastric musculature, or the liver. Thus indirect mechanisms, neural or paracrine (406, 418), have been proposed for many of the responses to systemic GLP-1.

Another aspect of GLP-1 physiology that carries over to pharmacology is the effect of GLP-1 agonists, but not inhibitors of DPP-4, to reduce food intake and cause weight loss (413). In clinical trials with exenatide or liraglutide, subjects predictably lose several kilograms of body weight and frequently report a decrease in appetite (93). The neural pathways leading to this response are not currently established, and both direct actions in the CNS and activation of peripheral neural circuits have been proposed to mediate this response (23). The effects of liraglutide on body weight in diabetic patients have prompted investigation into its use as a treatment for obesity in nondiabetic humans (13). It remains an open question as to whether and how the satiety/anorectic actions of GLP-1r agonists are related to the major side effects of these drugs, nausea and vomiting. It has been noted that most patients have improvement in nausea within the first few weeks of treatment with a GLP-1r agonist, while weight loss can persist over months and years. Better understanding of the neural mechanisms of these processes would benefit the therapeutic utility of these agents.

The trend in drug development for GLP-1r agonists is manipulation of pharmacokinetics to improve convenience and compliance (259). Agents that have to be administered once every week or 2 wk have the advantage of reducing the number of injections patients need to take. Interestingly, data are emerging to support pharmacodynamic differences between constant GLP-1r agonism with long-acting agents and activation/deactivation with short-acting agents. Thus it appears that long-acting GLP-1 agonists have a greater effect to suppress glucagon while short-acting agents have a greater effect to delay gastric emptying (92). Moreover, there is evidence that a more constant exposure to GLP-1 action causes less nausea than with rapid-acting compounds (45, 92, 181, 334). These findings have potential implications for the use of different GLP-1r agonist formulations in particular patients, and it seems likely that there are important underlying mechanisms to explain these effects.

The mechanism of action of DPP-4 inhibitors is still under investigation. The simple view that doubling the amount of active incretins in the circulation is sufficient to account for the clinical effects has been questioned (72). Studies in mice suggest that GLP-1 is active in the intestine where it acts through afferent nerves to mediate actions to lower blood glucose (418). DPP-4 activity has also been described in pancreatic islets as well (91), so it is conceivable that treatment with DPP-4 inhibitors could act by protecting locally produced GLP-1. Similar to the case for GLP-1r agonists, the use of DPP-4 inhibitors to learn more about the physiology of glucose regulation is rich with possibility.

There has been some recent concern regarding possible adverse effects from GLP-1r agonists, and to a lesser extent DPP inhibitors with diseases of the exocrine pancreas (4), particularly pancreatitis. Preclinical studies have not con-

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**Table 1.** *Key pharmacological characteristics of commercially available GLP-1 receptor agonists*

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Exenatide BID (Byetta)</th>
<th>Liraglutide (Victoza)</th>
<th>Exenatide ER (Bydureon)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Description</strong></td>
<td>Synthetic exendin-4</td>
<td>Human GLP-1 modified with amino acid substitution and acyl chain addition</td>
<td>Exenatide contained in hydrolyzable polymer microspheres</td>
</tr>
<tr>
<td><strong>Administration</strong></td>
<td>Subcutaneous injection twice daily before meals</td>
<td>Subcutaneous injection once daily, any time</td>
<td>Subcutaneous injection, once weekly, any time but immediately after suspension</td>
</tr>
<tr>
<td><strong>Half-life</strong></td>
<td>2.4 h</td>
<td>13 h</td>
<td>– 2 wk</td>
</tr>
<tr>
<td><strong>Time to peak concentration</strong></td>
<td>2.1 h</td>
<td>8–12 h</td>
<td>6–7 wk</td>
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</table>

GLP-1, glucagon-like peptide 1.
C. Dual Glucagon/GLP-1 Receptor Coagonists

A novel and exciting recent approach to the treatment of diabetes has been the development of hybrid peptides that activate more than one receptor to generate an effect (345). These agents are touted as mimicking the broad range of hormonal changes associated with bariatric surgery, and the need to utilize more than one ligand-receptor system to achieve significant weight loss. Some of the first compounds developed using this strategy were glucagon/GLP-1 coagonists, peptides engineered to activate the cognate receptors of both peptides in different relative potencies (79, 80). The rationale behind this line of drug development is that both glucagon and GLP-1 bind specific and distinct receptor populations in the brain to cause satiety (23, 193), and activating these together might have synergistic results. In an initial report, two hybrid peptides, one with balanced GCGR and GLP-1R potency and a second with sevenfold greater activity at the GLP-1R, reduced body weight and fat, increased energy expenditure, and dramatically improved glucose tolerance in obese mice and rats (80). The effects on weight loss were significantly greater than that of a GLP-1R-only agonist, and this additive response supports different activation of different neural pathways for glucagon and GLP-1 to cause weight loss. Very similar findings were reported with a different GLP-1/glucagon coagonist including a careful demonstration of superiority of weight loss compared with a GLP-1R agonist, and a convincing demonstration of dependence on both receptors for full activity (313). In humans, while GLP-1 infusion alone had no effect on energy expenditure, and glucagon infusion alone raised both blood glucose levels and energy expenditure, coadministration of both peptides increased energy expenditure but did not raise glucose levels (391).

It is clear from studies of GLP-1R\textsuperscript{−/−} mice and comparisons of coagonists with different relative potencies at the two receptors that GLP-1R agonism ameliorates the hyperglycemic effects of glucagon (71, 72, 289). Based on the limited work in this area, peptides with balanced agonism for the GLP-1/GCGRs seem to have the most therapeutic promise (79, 80). While greater glucagon potency confers greater energy expenditure and suppression of food intake, there seems to be a threshold beyond which glucose control worsens despite weight loss. The potential for a GLP-1R/GCGR coagonist to lower blood glucose has also been demonstrated with oxyntomodulin (98), a proglucagon product that activates both receptors albeit less potently than the
cognate ligands. A single amino acid substitution at position 3 in oxyntomodulin abolishes agonism at the GCGR; this analog does not activate fatty acid oxidation through the GCGR, but has improved effects on glucose regulation compared with the native compound. Thus the coupling of GLP-1 activity, to mitigate any hyperglycemic effects, with glucagon allows the catabolic actions of both peptides on energy balance to give an augmented response compared with either alone.

XI. CONCLUSION

This review challenges the simple model that Gcg encodes peptides, particularly GLP-1 and glucagon, in a strictly tissue-specific fashion to regulate opposite ends of glucose fluctuations, e.g., GLP-1 as an insulin secretagogue that improves glucose homeostasis and glucagon as a counter-regulatory hormone with a hepatic centric mode of action. In fact, these peptides act at several levels to regulate glucose homeostasis but also have broader actions on energy and nutrient metabolism that are sometimes in opposition and sometimes complementary. Importantly, these two proglucagon peptides have important pharmacological actions and are likely to be a focus of investigation and drug development for the foreseeable future. Overall, a role for both glucagon and GLP-1 in the pathogenesis and treatment of T2DM is possible, and this is underscored by the fact that combined GLP-1/glucagon therapies are effective hypoglycemic and weight loss agents (FIGURE 6).

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