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Biologic actions and therapeutic potential of the proglucagon-derived peptides

Daniel J Drucker

SUMMARY

The actions of the structurally related proglucagon-derived peptides (PGDPs)—glucagon, glucagon-like peptide (GLP)-1 and GLP-2—are focused on complementary aspects of energy homeostasis. Glucagon opposes insulin action, regulates hepatic glucose production, and is a primary hormonal defense against hypoglycemia. Conversely, attenuation of glucagon action markedly improves experimental diabetes, hence glucagon antagonists may prove useful for the treatment of type 2 diabetes. GLP-1 controls blood glucose through regulation of glucose-dependent insulin secretion, inhibition of glucagon secretion and gastric emptying, and reduction of food intake. GLP-1-receptor activation also augments insulin biosynthesis, restores β-cell sensitivity to glucose, increases β-cell proliferation, and reduces apoptosis, leading to expansion of the β-cell mass. Administration of GLP-1 is highly effective in reducing blood glucose in subjects with type 2 diabetes but native GLP-1 is rapidly degraded by dipeptidyl peptidase IV. A GLP-1-receptor agonist, exendin 4, has recently been approved for the treatment of type 2 diabetes in the US. Dipeptidyl-peptidase-IV inhibitors, currently in phase III clinical trials, stabilize the postprandial levels of GLP-1 and gastric inhibitory polypeptide and lower blood glucose in diabetic patients via inhibition of glucagon secretion and enhancement of glucose-stimulated insulin secretion. GLP-2 acts proximally to control energy intake by enhancing nutrient absorption and attenuating mucosal injury and is currently in phase III clinical trials for the treatment of short bowel syndrome. Thus the modulation of proglucagon-derived peptides has therapeutic potential for the treatment of diabetes and intestinal disease.

KEYWORDS energy, glucose, insulin, islets, obesity

REVIEW CRITERIA

PubMed was searched using the search terms "incretins, GLP-1, GLP-2 and proglucagon-derived peptides".

DJ Drucker is the Director of the Banting and Best Diabetes Centre, a Staff Physician at the Toronto General Hospital, and a Professor of Medicine at the University of Toronto, Canada.

Correspondence
Toronto General Hospital, Banting and Best Diabetes Centre, 200 Elizabeth Street, MBRW4R-402, Toronto, Ontario M5G 2C4, Canada
d.drucker@utoronto.ca

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INTRODUCTION

Before the cDNAs and genes encoding proglucagon were cloned, pancreatic glucagon was the principal proglucagon-derived peptide (PGDP) with well-characterized biologic actions. However, antisera directed against glucagon detected not only circulating 29-amino-acid pancreatic glucagon, but also larger circulating gut-derived forms termed enteroglucagons. Cloning the cDNAs and genes unmasked the complexity of proglucagon and the structural relationships between glucagon, oxyntomodulin, and two glucagon-like peptides (GLP-1 and GLP-2), all of which are coencoded within a single proglucagon gene (Figure 1).[1] The PGDPs are derived from a single common proglucagon precursor expressed in islet α cells, gut enteroendocrine L cells, and in brainstem neurons. Glucagon and the GLPs serve important roles in the control of energy ingestion, gastrointestinal motility, nutrient absorption and glucose homeostasis. The PGDPs exert well-defined actions via distinct G-PROTEIN-COUPLED RECEPTORS (GPCRs), which are structurally related members of family B of the GPCR superfamily.[2] This review provides an update on recent insights into the biologic actions and therapeutic potential of glucagon, the GLPs and other PGDPs.

GLUCAGON ACTION

Studies of post-translational proglucagon processing demonstrate that proglucagon is cleaved in the α cells of the pancreas to yield 29-amino-acid glucagon and a larger, unprocessed polypeptide, designated 'major proglucagon fragment'.[3] In contrast, glucagon is not generated in the gut, where processing within enteroendocrine L cells in the gut produces glicentin and oxyntomodulin (Figure 1). Prohormone convertase 2 (PC2) is essential for liberation of pancreatic glucagon, and PC2 knockout mice demonstrate fasting hypoglycemia and improved GLUCOSE TOLERANCE[4] consistent with greatly reduced circulating levels of bioactive glucagon.

Glucagon levels are low in the postprandial state and increase with fasting or the development of hypoglycemia (Figure 2). The dominant actions of pancreatic glucagon converge on regulation of hepatic glucose production. Glucagon opposes the actions of insulin at the hepatocyte, and activation of hepatic glucagon-receptor signaling regulates a transcriptional network involving the transcription factor cAMP response element binding protein (CREB), its coactivator peroxisome proliferator-activated receptor-gamma coactivator 1 (PGC1), and a genetic program regulating GLUCONEOGENESIS. Type 2 diabetes is associated with failure of meal-associated glucagon suppression; inappropriately elevated levels of circulating glucagon, together with insulin deficiency or INSULIN RESISTANCE, contribute to the metabolic derangements characteristic of diabetes.[5]

Conversely, glucagon secretion is stimulated by hypoglycemia, and repeated hypoglycemic episodes lead to the development of impaired counter-regulation characterized by deficient or absent glucagon secretion in response to hypoglycemia.[6] The pathophysiology of dysregulated glucagon secretion in diabetes remains unclear, but probably involves defective suppression of glucagon secretion by insulin or other β-cell products.[6] Insulin resistance in α cells or reduced levels of circulating insulin lead to dysinhibition of glucagon secretion following meal ingestion, whereas intraislet hyperinsulinemia and the lack of a normal physiologic decline in levels of intraislet insulin may contribute to defective glucagon secretion in patients with type 1 diabetes.[7,8] The potent and rapid actions of glucagon on hepatic glucose production have led to the use of injectable glucagon for the treatment of severe symptomatic hypoglycemia in insulin-treated subjects. Less commonly, glucagon may be administered to facilitate gastrointestinal motility during radiologic imaging studies, or as an emergency treatment for refractory hypotension, anaphylaxis, or bradyarrhythmias.

Glucagon action is mediated by a specific GPCR coupled to the activation of adenylate cyclase. Targeted disruption of the glucagon receptor (Gcgr) in mice results in hypoglycemia, circulating hyperglucagonemia, increased pancreatic mass, and islet α-cell hyperplasia.[9] The central importance of hepatic Gcgr signaling for control of blood glucose has led to exploration of the therapeutic potential of Gcgr antagonists for the treatment of type 2 diabetes.

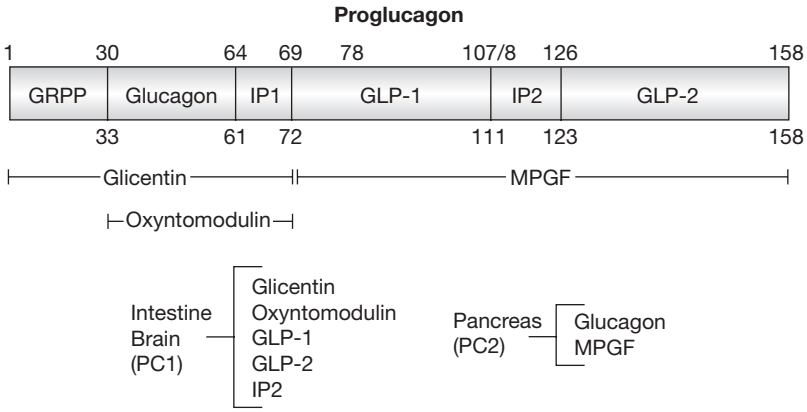

Figure 1 Structure of proglucagon and the proglucagon-derived peptides. The numbers refer to the amino acid position within proglucagon, starting at the N-terminal amino acid. Differences between the numerals in the top and bottom rows reflect processing and removal of spacer amino acids between the peptides. GLP, glucagon-like peptide; GRPP, glicentin-related pancreatic polypeptide; IP, intervening peptide; MPGF, major proglucagon fragment. PC, prohormone convertase.

Genetic reduction of hepatic Gcgr expression using ANTISENSE TECHNOLOGIES results in amelioration of hyperglycemia, improved glucose tolerance, reduced levels of free fatty acids via reduction of lipolysis, and increased levels of circulating glucagon.[10,11] Furthermore, reduction in hepatic Gcgr expression is also associated with induction of pancreatic GLP-1 production and increased circulating levels of GLP-1, probably contributing to the improved glucose tolerance and enhanced insulin secretion in leptin-resistant and insulin-resistant diabetic db/db mice.

Experimental reduction in glucagon activity has also been achieved using immuno-neutralizing antisera or small-molecule Gcgr antagonists. Gcgr antagonists lower blood glucose in normal and diabetic rodents in short-term studies, whereas immunoneutralization of endogenous glucagon normalizes blood glucose in rats with streptozotocin-induced diabetes.[12] Furthermore, administration of a small-molecule Gcgr antagonist to human subjects attenuates glucagon-stimulated hepatic glucose production in short-term studies of human subjects without diabetes.[13] Hence, there continues to be interest in strategies primarily directed at attenuation of glucagon action; however, the risks of hypoglycemia and α-cell hyperplasia need to be carefully considered in the context of sustained reduction of glucagon action for the treatment of diabetes. Remarkably, both pramlintide and

GLOSSARY

G-PROTEIN-COUPLED RECEPTORS
Heptahelical transmembrane-domain membrane-spanning receptors that transduce the actions of peptide hormones

GLUCOSE TOLERANCE
The ability to assimilate and clear glucose efficiently from the systemic circulation

GLUCONEOGENESIS
The *de novo* generation of glucose from substrate precursors that occurs predominantly in the liver, and to a lesser extent, in the kidney

INSULIN RESISTANCE
A metabolic state characterized by an impairment in the ability to clear glucose at a given plasma insulin concentration

ANTISENSE TECHNOLOGIES
Nucleic-acid sequences complementary to transcribed RNA sequences resulting in translational attenuation or mRNA degradation

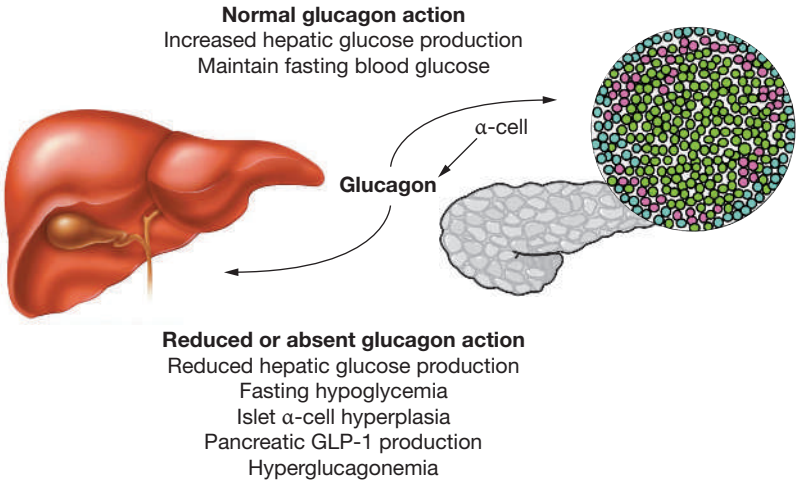

Figure 2 Representation of normal glucagon action on the liver and pancreas. The β cells in the core of the islets are shown in turquoise, whereas the non-β cells (α and δ cells) are green and pink. GLP, glucagon-like peptide.

exenatide (exendin 4), the two newest drugs approved for the treatment of type 2 diabetes, exert their actions partly through inhibition of glucagon secretion.[14] Furthermore, clinical studies using dipeptidyl-peptidase-IV (DPP4) inhibitors (see below) indicate that suppression of plasma glucagon is an important feature associated with the mechanism of action of these drugs in diabetic human subjects.[15] Hence, these observations lend further credence to the important role of inappropriately elevated levels of plasma glucagon in the pathophysiology of the metabolic derangements of type 2 diabetes.

INTESTINAL PROGLUCAGON-DERIVED PEPTIDES

Post-translational processing of proglucagon in enteroendocrine L cells leads to the liberation of glicentin, oxyntomodulin, two intervening peptides (IP-1 and IP-2), and two GLPs (GLP-1 and GLP-2). This intestinal processing of proglucagon requires PC1, and genetic elimination of PC1 action results in the impaired generation of both GLP-1 and GLP-2.[16] As mentioned above, in rare instances, PC1 expression may be induced in the islet α-cell during experimentally induced diabetes, resulting in enhanced generation of pancreatic GLP-1.

Glicentin and oxyntomodulin

Glicentin is a 69-amino-acid peptide that contains the sequence of pancreatic glucagon together with amino-terminal and carboxy-terminal extensions (Figure 1). Administration of exogenous glicentin stimulates growth of the small bowel in rodents;[17] however, a separate receptor for glicentin has not yet been identified. The actions of glicentin on gut motility overlap with those of GLP-1 and are inhibited by the GLP-1 receptor (GLP-1R) antagonist exendin (9–39), a truncated peptide derived from amino acids 9 to 39 of the sequence of the GLP-1R agonist exendin (see below).[18]

Oxyntomodulin is a 37-amino-acid peptide that contains the sequence of 29-amino-acid glucagon and 8 additional amino acids at the carboxyl terminus. Oxyntomodulin exerts stimulatory effects on gastric acid secretion and inhibits food intake in both rodent and human studies.[19,20] Furthermore, repeated self-administration of oxyntomodulin three times daily before meals for 4 weeks reduced appetite and produced ~2.3 kg of weight loss in overweight or obese human subjects.[21] The anorectic actions of oxyntomodulin require an intact GLP-1R signaling system;[22] the existence of a putative separate oxyntomodulin receptor has not yet been clearly demonstrated.

Glucagon-like peptide 1

Two equipotent forms of GLP-1 are generated in gut endocrine cells; a glycine-extended form GLP-1 (7–37) and the amidated peptide, GLP-1 (7–36) amide. Plasma levels of GLP-1 are low in the fasting state and rise rapidly following meal ingestion. The majority of GLP-1 is produced in enteroendocrine L cells located in the distal gut, predominantly in the ileum and colon. Hence, the rapid initial increase in circulating GLP-1 within minutes of meal ingestion occurs before digested nutrients reach the distal bowel, invoking the production of both neural and hormonal signals arising from the proximal gut as indirect mediators of GLP-1 secretion.[23] GLP-1 is cleared rapidly from the circulation and exhibits a very short half-life of several minutes.[24] The principal determinants of the levels of active plasma GLP-1 include enzymatic inactivation by DPP4 and neutral endopeptidase, and renal clearance.

The predominant actions of exogenously administered GLP-1 regulate blood glucose via inhibition of appetite, glucagon secretion and gastric emptying, and stimulation of insulin secretion (Figure 3). The actions of GLP-1 on the islet β and α cells are glucose-dependent; hence GLP-1 no longer stimulates insulin or inhibits glucagon secretion once plasma glucose

returns to normal.[25] Furthermore, unlike the actions of insulin secretagogues exerting their actions through the sulfonylurea receptor and ATP-sensitive potassium channels, GLP-1R agonists also enhance insulin biosynthesis through induction of insulin gene transcription leading to increased levels of insulin mRNA transcripts and replenishment of β-cell insulin content.[26] Although treatment with GLP-1R agonists improves insulin sensitivity in diabetic rodents and human subjects, whether this is a direct or indirect action of GLP-1 remains uncertain.

GLP-1 also exerts proliferative and cytoprotective actions on rodent and human islet β cells through engagement of signal-transduction pathways linked to mitogenesis and cell survival.[27] GLP-1R activation leads to increased levels of cAMP, enhanced phosphorylation of protein kinase B (also known as Akt) and pancreas duodenal homebox-1 (Pdx1), and increased expression and activity of insulin receptor substrate-2 (IRS2), key components of pathways important for β-cell cytoprotection. Treatment of normoglycemic or diabetic rodents with GLP-1R agonists leads to expansion of the β-cell mass, and an increased number of islets.[28–30] Conversely, GLP-1R activation reduces APOPTOSIS in isolated β cells in response to cytokines, and in experimental models of β-cell injury.[31,32] The proliferative and cytoprotective actions of GLP-1 have been demonstrated in studies using *in vitro* human islet cells cultured in the presence of high levels of glucose and free fatty acids.[33,34] Hence, there is considerable interest in determining whether long-term therapy with GLP-1R agonists in human subjects will prevent the deterioration of β-cell function that is commonly observed after several years of type 2 diabetes.

GLP-1 also exerts potent effects on reduction of appetite, probably through direct and indirect effects on hypothalamic satiety centers and inhibition of gastric emptying.[35,36] The reduction in gastric emptying and CNS activation observed following GLP-1R-agonist administration produces the dose-limiting side effects of nausea and vomiting and is a key determinant of the maximum tolerated dose of GLP-1R agonist in human subjects. Inhibition of gastric emptying following GLP-1 administration markedly attenuates meal-related GLYCEMIC EXCURSION, and is frequently associated with reduced rather than increased levels of plasma insulin.[37] The vagus nerve is an important component of the GLP-1R-activated signaling pathway regulating gastric emptying and satiety. Experimental afferent vagotomy eliminates the inhibitory effects of GLP-1 on gastric emptying.[38] Furthermore, the recombinant GLP-1–albumin protein, albugon, increases the expression of proto-oncogene protein c-fos, a marker of neuronal activation in the hypothalamus, and inhibits both food intake and gastric emptying without directly accessing the central nervous system.[39] Moreover, the anorectic actions of GLP-1 are abolished following subdiaphragmatic total truncal vagotomy or transection of the brainstem–hypothalamic pathway in rodents,[40] further illustrating the importance of ascending vagal pathways for transmission of the GLP-1R-dependent anorectic signal.

GLP-1Rs are also expressed in the heart, and administration of GLP-1 improves cardiovascular function in the setting of experimental cardiac injury.[41] The actions of GLP-1 on the heart may be direct, through generation of cAMP in cardiomyocytes, and/or indirect, by improvement of the metabolic environment through control of blood glucose, insulin, and free fatty acids.[42] Remarkably, a small pilot study of GLP-1 administration in 10 human subjects for 72 h following acute myocardial infarction and angioplasty demonstrated significant improvements in left-ventricular function and reduced wall-motion abnormalities.[43]

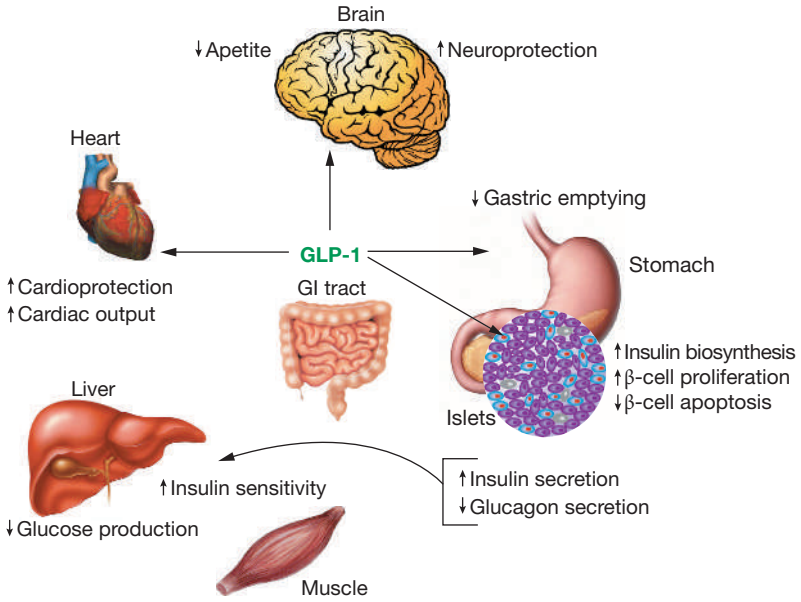

Figure 3 Actions of glucagon-like peptide 1 on multiple target tissues. GI, gastrointestinal; GLP-1, glucagon-like peptide 1.

GLOSSARY

APOPTOSIS
The process of programmed cell death attributable to activation of a family of enzymes designated caspases

GLYCEMIC EXCURSION
The pattern of, and quantitative change in, blood glucose after a glucose-rich stimulus, such as a meal

The physiologic actions of GLP-1 have been delineated using GLP-1R antagonists, immunoneutralizing antisera and GLP-1R knockout mice. Exendin (9–39) is a lizard-venom-derived GLP-1R antagonist that binds to the GLP-1R and exhibits minimal cross-reactivity for related receptors in family B of the GPCR superfamily (see below).[22] Studies employing acute administration of exendin (9–39) demonstrate an essential role for endogenous GLP-1 in the control of glucose-dependent insulin secretion, glucagon secretion, and gastric emptying. Similarly, repeated administration of exendin (9–39) increases food intake and promotes weight gain in rodent studies. Conversely, GLP-1R knockout mice exhibit fasting hyperglycemia, reduced glucose-stimulated insulin secretion,[44] reduced numbers of large islets, and enhanced susceptibility to islet or neuronal injury. Hence, basal levels of GLP-1 are essential for metabolic control of glucose homeostasis and regulation of islet growth and survival.

Therapy with glucagon-like peptide 1 receptor agonists for the treatment of diabetes

Intravenous or subcutaneous administration of GLP-1 rapidly lowers blood glucose in the majority of subjects with type 2 diabetes.[45,46] Remarkably, the effects of GLP-1 on inhibition of gastric emptying and glucagon secretion also lower blood glucose in subjects with type 1 diabetes.[45,47] GLP-1 administration has been studied in patients with type 2 diabetes of diverse etiologies, and is effective in lowering blood glucose in diabetic subjects irrespective of etiology.[48] Continuous administration of GLP-1 by subcutaneous infusion for 6 weeks in obese subjects with type 2 diabetes produced significant improvements in fasting and postprandial glycemia, reduced levels of free fatty acids and decreased levels of fructosamine and glycosylated hemoglobin A (HbA_1c), markers of intermediate and long term glycemic control, respectively.[49] Remarkably, subcutaneous GLP-1 therapy was well tolerated and also improved insulin sensitivity, in association with a mean weight loss of 1.6 kg.[49] GLP-1 administration also maintained a stable level of glucose control, and had a stimulatory effect on insulin secretion and enhanced insulin sensitivity, in elderly lean subjects with type 2 diabetes in a 12-week continuous-infusion study.[50] Hence, intermittent or continuous administration of native GLP-1 is an effective (albeit impractical) treatment for type 2 diabetes. As GLP-1 exhibits a very short circulating half-life,[24] development of GLP-1-based therapeutic approaches has focused on degradation-resistant, long-acting GLP-1R agonists with a longer duration of action *in vivo*.

Exendin 4 is a naturally occurring GLP-1R agonist originally isolated from the venom of the *Heloderma suspectum* lizard.[51] Exendin 4 binds to and activates the mammalian GLP-1R, yet is encoded by a distinct gene from lizard GLP-1.[52] Exendin 4 mimics all of the actions of GLP-1, but is several orders of magnitude more potent as a glucose-lowering agent *in vivo*. Exendin 4 (also known as exenatide in human studies) lowers blood glucose in both normal and diabetic human subjects, with nausea as the principal dose-limiting side effect following acute administration.

The efficacy of exendin 4 for the treatment of type 2 diabetes was examined in a series of three randomized, double-blind, phase III clinical trials. Subjects with type 2 diabetes that was not optimally controlled by metformin alone, sulfonylurea alone, or metformin plus sulfonylurea, were randomized to receive twice-daily injections of either saline (placebo) or exendin 4, either 5 μg or 10 μg twice-daily for 30 weeks. Exendin 4 treatment produced significant reductions in HbA_1c in all three treatment cohorts, with 40–50% of patients achieving an HbA_1c of 7% or less.[53–55] The principal treatment-associated side effect was nausea, which tended to decrease over time, and an increased rate of mild to moderate hypoglycemia was observed in patients receiving concomitant sulfonylurea therapy. There were no major problems with severe hypoglycemia, consistent with the observations that the counter-regulatory glucagon response to hypoglycemia is preserved in patients treated with exendin 4.[56] Remarkably, exendin 4 therapy was associated with prevention of weight gain or with modest weight loss over the 30-week treatment period.[53–55] Furthermore, open-label extension studies in patients continuing on exendin 4 demonstrate a significantly greater mean weight loss of over 10 lb (4.6 kg) at 82 weeks. Exendin 4 was approved by the FDA for the treatment of type 2 diabetes in the US in April, 2005.

Liraglutide is a human DPP4-resistant acylated GLP-1 analog which binds noncovalently to human albumin, thereby exhibiting an extended pharmacokinetic profile following a single

injection in human subjects.[57] Liraglutide therapy produces all of the expected actions of GLP-1R agonists, including restoration of β-cell sensitivity to glucose, improvement in first-phase insulin secretion, inhibition of glucagon secretion, and enhancement of insulin sensitivity. Daily administration of 0.75 mg liraglutide for 12 weeks lowered fasting glucose, improved the proinsulin:insulin ratio and decreased HbA_1c by 0.7% without patient weight gain.[58] More-recent studies have employed considerably higher doses of liraglutide.

CJC-1131 is a human GLP-1 analog formulated with a reactive chemical linker at the carboxyl terminus, enabling the drug to form a covalent bond with albumin following subcutaneous administration. CJC-1131 exhibits a reduced affinity for the GLP-1R relative to native GLP-1 but, in rodents, mimics the full spectrum of GLP-1 actions *in vivo*.[29] Similarly, albugon is a recombinant GLP-1–albumin protein that reduces food intake, inhibits gastric emptying, and potentiates glucose-dependent insulin secretion in preclinical studies.[59] Although CJC-1131 and albugon are predicted to exhibit prolonged pharmacokinetic profiles, there is only limited human clinical data with CJC-1131 and no available human data with albugon; hence the potential role and utility of albumin-based GLP-1 therapies in the treatment of type 2 diabetes require further investigation.

Dipeptidyl peptidase IV

DPP4, also known as CD26, is a widely expressed peptidase that is found in two principal molecular forms: a circulating soluble protein with catalytic activity, and a slightly larger membrane-spanning form that is also capable of transducing intracellular signals independent of its retained catalytic activity. DPP4 cleaves peptide substrates possessing a position-2 N-terminal alanine or proline. Although dozens of chemokines and regulatory peptides are theoretical pharmacologic substrates for DPP4, relatively few peptides have been demonstrated to be physiologic substrates for DPP4 cleavage *in vivo*.

DPP4 regulates the activity of multiple peptides capable of regulating glucose metabolism, including glucagon, glucose-dependent insulinotropic polypeptide (GIP), gastrin-releasing peptide, pituitary adenylate cyclase activating polypeptide, and GLP-1.[60] GIP is a circulating 42-amino-acid peptide produced in a nutrient-dependent manner in the proximal small bowel and, like GLP-1, is also a potent stimulator of glucose-dependent insulin secretion.[61] Both GLP-1 and GIP are cleaved and inactivated by DPP4, and inhibition of DPP4 activity results in reduced blood glucose, together with increased circulating levels of both these INCRETIN HORMONES.

Although the plasma levels of GIP and GLP-1 are only modestly elevated after DPP4 inhibition, the available evidence suggests that they represent the two principal peptides essential for transducing the glucoregulatory actions of the DPP4 inhibitors. Mice with mutations in the genes encoding both the GIP and GLP-1 receptors are completely resistant to the glucose-lowering actions of multiple chemically distinct DPP4 inhibitors.[62]

Remarkably, DPP4 is essential for glucose homeostasis and incretin degradation because the DPP4 knockout mouse exhibits reduced blood glucose and increased plasma levels of both GLP-1 and GIP following glucose challenge.[63] Similarly, rats with a naturally occurring inactivating mutation in the *DPP4* gene exhibit improved glucose tolerance in association with increased plasma levels of GLP-1.

Treatment of diabetic rodents with DPP4 inhibitors results in improved glucose tolerance, enhanced glucose-stimulated insulin secretion, and preservation or expansion of islet mass. Similarly, DPP4 inhibitors lowered blood glucose in diabetic human subjects over a 4-week treatment period,[15,64] principally via inhibition of plasma glucagon and potentiation of glucose-dependent insulin secretion. More recent information demonstrates that addition of the DPP4 inhibitor vildagliptin to metformin leads to improved β-cell function over a 12-week study period;[65] in a small open-label extension study of the same patients, vildagliptin led to a significant sustained reduction in HbA_1c sustained over 52 weeks.[66] Although mice with genetic inactivation of the gene encoding DPP-4 are resistant to diet-induced obesity,[67] rodents or human subjects treated with DPP4 inhibitors do not exhibit perturbations in foods intake or weight loss.

DPP4 exerts a large number of actions in the immune system,[68] raising theoretical concerns about the long-term safety of DPP4 inhibition. Nevertheless, it remains unclear whether highly specific DPP4 inhibitors that target the catalytic site of the enzyme without disrupting the signaling capacity of the membrane-anchored

GLOSSARY

INCRETIN HORMONES
Gut-derived peptides that potentiate nutrient-stimulated insulin secretion

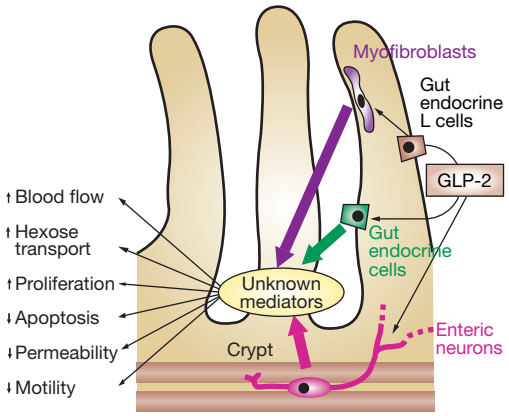

Figure 4 The actions of glucagon-like peptide 2 in the gastrointestinal mucosa. GLP-2, glucagon-like peptide 2.

protein will produce any meaningful alterations in immune function in subjects with type 2 diabetes.[68] Similarly, although a large number of peptides may be potential substrates for DPP4, whether chronic DPP4 inhibition will produce unexpected safety issues as a result of changes in cleavage of one or more regulatory peptides or chemokines cannot be predicted based on currently available information. Nevertheless, the available data from ongoing clinical trials suggest that DPP4 inhibitors appear to be safe for the treatment of type 2 diabetes.

Glucagon-like peptide 2

GLP-2 is a 33-amino-acid peptide structurally related to glucagon and GLP-1 that is located carboxy terminal to the GLP-1 sequence within proglucagon. GLP-2 is secreted along with GLP-1 from the enteroendocrine L cell in a nutrient-dependent manner. GLP-2 is also a substrate for DPP4, and DPP4-resistant GLP-2 analogs exhibit reduced degradation and enhanced potency *in vivo*.[69] The actions of GLP-2 are mediated by a separate GLP-2 receptor (GLP-2R), a member of the glucagon/GLP-1R superfamily.[70] The GLP-2R is predominantly expressed in the intestine, but not in small-bowel enterocytes or colonocytes; hence many of the actions of GLP-2 are likely to be indirect, through as-yet-unidentified mediators. Intestinal injury is associated with increased circulating levels of the gut-derived PGDPs,[71] and a link between PGDPs and bowel growth was established following a report of a patient with a glucagonoma presenting with marked small-bowel hyperplasia. Subsequent studies demonstrated that exogenous administration of GLP-2 increases small-bowel growth via stimulation of crypt-cell proliferation and inhibition of apoptosis.[17]

The principal actions of GLP-2 on the gut include inhibition of gastrointestinal motility and gastric acid secretion, stimulation of nutrient absorption, and reduction of intestinal epithelial permeability (Figure 4). Although pharmacologic levels of GLP-2 inhibit food intake in rodents, GLP-2 does not produce satiety or inhibition of gastric emptying in normal human subjects.[72] The physiologic actions of GLP-2 on mucosal growth have been examined in mice using a partial GLP-2R antagonist; this agent, GLP-2 (3–33), markedly prevented the adaptive mucosal regrowth normally observed in response to refeeding, through its effects on both cell proliferation and apoptosis.[73] Hence endogenous GLP-2 is essential for changes in mucosal growth in response to nutrient availability. Moreover, GLP-2 markedly reduces the severity of intestinal injury in both the small and large bowel. The protective and regenerative actions of GLP-2 have been detected following small-bowel resection, enteritis induced by nonsteroidal anti-inflammatory agents, chemotherapy, or vascular ischemia. Similarly, GLP-2 attenuates mucosal injury and improves clinical outcomes in the large bowel of rodents with experimental injury.

A principal feature of GLP-2 action is the suppression of apoptosis, detectable in the intestinal mucosal epithelium in *in vivo* models of experimental injury,[74,75] or in cells expressing a GLP-2R. Moreover, GLP-2 also inhibits bone resorption and promotes calcium absorption in human studies.[76,77]

The proabsorptive, cytoprotective and regenerative properties of GLP-2 have prompted assessment of whether exogenous GLP-2 administration may be useful for enhancing energy absorption in human subjects with short-bowel syndrome. A pilot study of twice-daily GLP-2 administration for 35 days in human subjects with short-bowel syndrome demonstrated significant improvements in energy absorption and lean body mass, together with increases in mucosal thickness detected in small-bowel biopsies.[78] A degradation-resistant GLP-2 analog, teduglutide, is currently being evaluated in phase III clinical trials for the treatment of short-bowel syndrome.[79]

CONCLUSIONS

The biologic actions of glucagon, GLP-1 and GLP-2 are focused on the intake, absorption, retention and disposal of energy. Hence there is considerable interest in ascertaining whether the actions of these peptides may be therapeutically useful for the treatment of human diseases. Glucagon-receptor antagonism significantly ameliorates the severity of experimental diabetes; however, data on the use of glucagon-receptor antagonists in human subjects are extremely limited. Furthermore, the safety of chronic glucagon-receptor blockade merits careful scrutiny. The first GLP-1R agonist, exendin 4, has been approved for the treatment of type 2 diabetes; however, long-term efficacy and safety data in human diabetic subjects are not yet available. There is great interest in ascertaining whether therapy with exendin 4 will continue to be associated with sustained weight loss in treated subjects.

Furthermore, the actions of GLP-1R agonists to stimulate β-cell proliferation and reduce apoptosis raise the possibility that these agents may provide durable benefits for prevention of deterioration in β-cell function and, ideally, for restoration of defective β-cell function in diabetic subjects; whether this concept will be validated in long-term human studies remains to be proven. Several recent reports have also linked increased circulating levels of GLP-1 with the development of nesidioblastosis and hypoglycemia in a small number of patients following gastric bypass, a finding that requires additional investigation.[80,81] Potentiation of incretin action by inhibition of DPP4 activity appears promising as an alternative therapeutic approach; again, however, the long-term safety and efficacy of DPP4 inhibitors in human subjects with diabetes have not yet been demonstrated.

Similarly, although GLP-2 enhances nutrient absorption and facilitates mucosal regeneration in preclinical studies, there is only limited human clinical data on the safety and effectiveness of GLP-2 therapy in patients with intestinal disorders. Taken together, the increasing understanding of the biologic importance of GLPs for the control of energy homeostasis, along with the development of pharmaceutical strategies for enhancing the action of both GLP-1 and GLP-2, suggests that clinicians may increasingly utilize clinical strategies based on PGDP action for the control of diabetes and intestinal disorders.

KEY POINTS

- The structurally related proglucagon-derived peptides are produced in the pancreas, gut and brain, and regulate complementary aspects of energy homeostasis
- The glucagon-like peptide 1 receptor agonist exenatide has recently been approved for treatment of type 2 diabetes
- Exenatide (exendin 4) and the amylin agonist pramlintide (another drug used to treat type 2 diabetes) also inhibit glucagon secretion
- Dipeptidyl peptidase IV cleaves incretin hormones such as glucagon-like peptide 1, and drugs that inhibit this enzyme are in phase III trials for treatment of type 2 diabetes
- Glucagon-like peptide 2 is currently in phase III clinical trials for treatment of short-bowel syndrome
- Thus various strategies that modulate proglucagon-derived peptides show therapeutic potential in both diabetes and intestinal disease

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The author declared competing interests; go to the online article for details.