

Minireview: The Glucagon-Like Peptides

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ABSTRACT

The glucagon-like peptides GLP-1 and GLP-2 are produced in enteroendocrine L cells of the small and large intestine and secreted in a nutrient-dependent manner. GLP-1 regulates nutrient assimilation via inhibition of gastric emptying and food intake. GLP-1 controls blood glucose following nutrient absorption via stimulation of glucose-dependent insulin secretion, insulin biosynthesis, islet proliferation, and neogenesis and inhibition of glucagon secretion. Experiments using GLP-1 antagonists and GLP-1 receptor^{-/-} mice indicate that the glucoregulatory actions of GLP-1 are essential for glucose homeostasis. In the central nervous system, GLP-1 regulates hypothalamic-pituitary function and GLP-1-activated circuits mediate the

CNS response to aversive stimulation. GLP-2 maintains the integrity of the intestinal mucosal epithelium via effects on gastric motility and nutrient absorption, crypt cell proliferation and apoptosis, and intestinal permeability. Both GLP-1 and GLP-2 are rapidly inactivated in the circulation as a consequence of amino-terminal cleavage by the enzyme dipeptidyl peptidase IV (DP IV). The actions of these peptides on nutrient absorption and energy homeostasis and the efficacy of GLP-1 and GLP-2 in animal models of diabetes and intestinal diseases, respectively, suggest that analogs of these peptides may be clinically useful for the treatment of human disease. (*Endocrinology* 142: 521–527, 2001)

THE MAMMALIAN proglucagon gene encodes two glucagon-like peptides (GLPs), glucagon-like peptide-1 (GLP-1) and glucagon-like peptide-2 (GLP-2), that exhibit approximately 50% amino acid identity to pancreatic glucagon. The biological actions of glucagon, GLP-1, and GLP-2 converge, at multiple levels, on the regulation of nutrient assimilation and energy homeostasis. As GLP-1 and GLP-2 exert multiple beneficial effects in experimental models of diabetes and intestinal dysfunction, respectively, analogs of these peptides are currently being evaluated in clinical trials for the treatment of human disease. The aim of this review is to explore recent advances in our understanding of the biology of these peptides. The reader is referred to several recent reviews for a more comprehensive overview of the established GLP literature (1–3).

Synthesis, Secretion, and Degradation of GLP-1 and GLP-2

Processing of proglucagon in the islet A cell gives rise primarily to 29 amino acid glucagon and the unprocessed major proglucagon fragment (MPGF). A larger number of proglucagon-derived peptides (PGDPs) are liberated in enteroendocrine cells of the small and large bowel (Fig. 1). The generation of an intestinal profile of PGDPs is contingent on the expression of the prohormone convertase enzyme PC1/3 in the L cell (4). The biological actions and physiological importance of glicentin and oxyntomodulin, cosecreted with

the glucagon-like peptides (GLPs) from gut endocrine cells (Fig. 1), remain uncertain.

The intestinal PGDPs are synthesized and secreted in a nutrient-dependent manner in both rodents and humans. Nutrients, fatty acids, and dietary fiber up-regulate proglucagon mRNA transcripts and PGDP secretion in the gastrointestinal tract (5). Although the majority of gut endocrine L cells are located in the distal ileum and colon, the circulating levels of GLP-1 and GLP-2 rise within minutes of food ingestion. Hence, nutrients, primarily fat and carbohydrates, likely stimulate endocrine (possibly GIP (glucose-dependent inhibitory polypeptide) and gastrin-releasing peptide) and neural mediators that activate GLP secretion from the distal intestine (6, 7). Indeed, pharmacological or surgical vagotomy significantly attenuates meal-stimulated increases in GLP secretion (8). Somatostatin-28 exerts a tonic inhibitory effect on GLP secretion, and immunoneutralization of somatostatin increases intestinal GLP release from the perfused porcine ileum (9). The potential inhibitory role of insulin in the regulation of intestinal GLP synthesis and secretion remains unclear, although treatment of diabetic rats with insulin decreases the levels of circulating intestinal PGDPs (10).

Following an initial nutrient-stimulated rise in circulating levels of GLP-1 and GLP-2, the levels of the bioactive forms of these peptides fall rapidly, largely due to renal clearance and the N-terminal degradation of both peptides by dipeptidyl peptidase IV (11–16). This widely expressed enzyme cleaves GLP-1 and GLP-2 at the position 2 alanine, resulting in the generation of inactive GLP-1^{9–36amide}, GLP-1^{9–37}, and GLP-2^{3–33}, respectively. The expression of DP IV in the gut and vascular endothelium is consistent with findings that the majority of immunoreactive GLP-1 entering the portal venous circulation has already been inactivated by N-terminal cleavage, accounting for its short $t_{1/2}$ of several minutes (17). Although the $t_{1/2}$ of GLP-2 is several times greater than that of GLP-1 (18), the biological importance of DP IV for GLP-2

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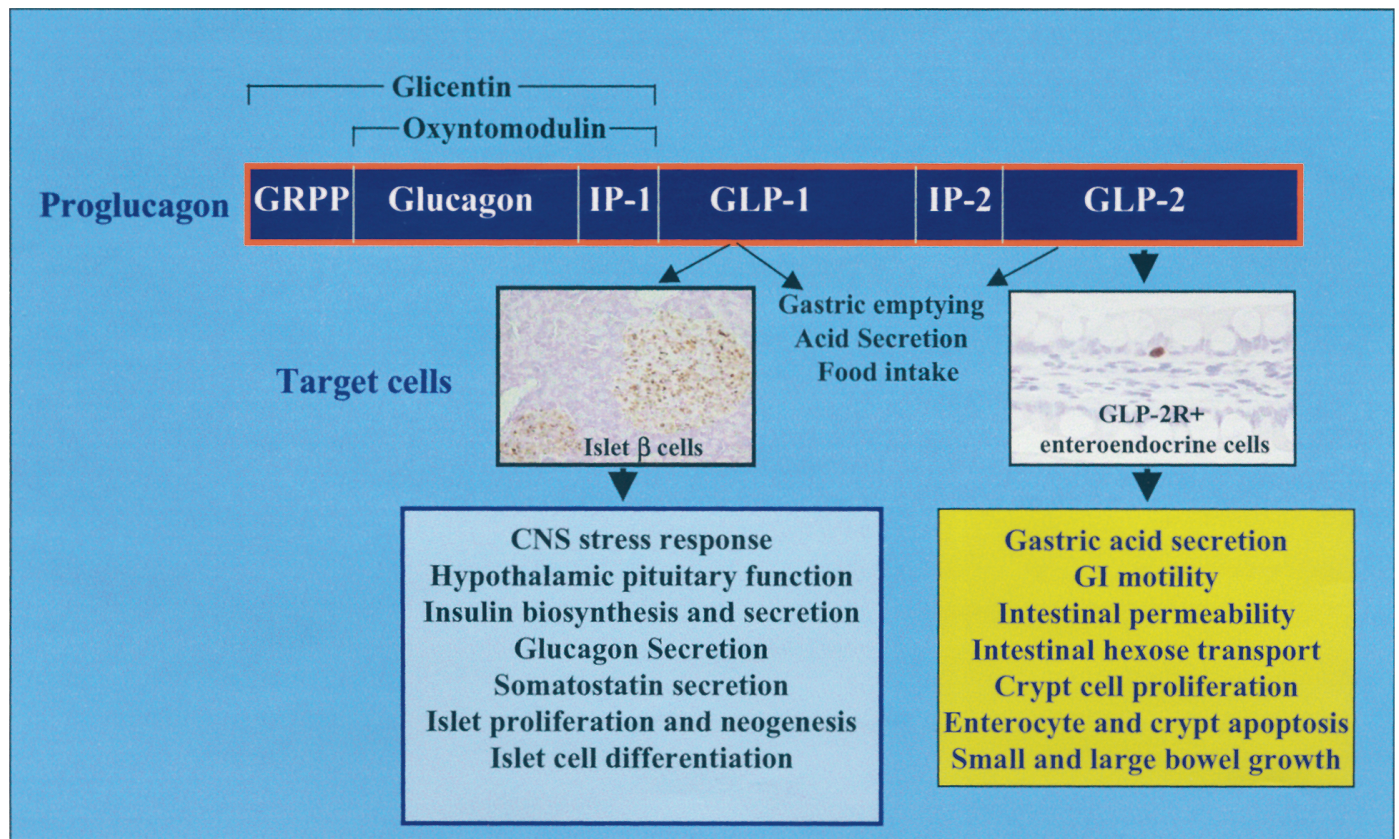


FIG. 1. Structure of proglucagon and biological actions of GLP-1 and GLP-2. The principal target cell types for GLP-1 (islet β cells stained with insulin antiserum) and GLP-2 (intestinal endocrine cells stained with GLP-2 receptor antiserum) are shown below the peptide sequences. The biological actions of the peptides are summarized below. GRPP, Glicentin-related pancreatic polypeptide; IP, intervening peptide; GLP-2R, glucagon-like peptide-2 receptor.

inactivation is illustrated by studies in wild-type and DP IV mutant rats demonstrating that larger doses of exogenous GLP-2 are required to achieve comparable intestinotrophic effects in the presence of active DP IV enzyme (15).

The rapid DP IV-mediated inactivation of GLP-1 suggests that DP IV inhibition may represent a useful strategy for prolonging GLP-1 action leading to sustained lowering of blood glucose *in vivo*. DP IV inhibitors stimulate insulin secretion and improve glucose tolerance in diabetic rodents (19). Furthermore, mice with genetic disruption of the DP IV gene exhibit increased levels of bioactive GLP-1 and GIP and enhanced glucose clearance following oral glucose challenge (20). GLPs are also cleared by the kidney (13) and by non-DP IV-dependent mechanisms, including the enzyme neutral endopeptidase 24.11 (GLP-1) (21).

The importance of a colon in continuity with the small bowel for maintaining normal to enhanced levels of GLP-1 and GLP-2 has been demonstrated in human subjects with ileostomies and colonic resections (22, 23). Little is known about the levels of circulating GLP-2 in the setting of intestinal disease. Patients with mild to moderate intestinal inflammation exhibit increased levels of bioactive GLP-2 (24), due in part to a decrease in levels of circulating DP IV. In contrast, patients with major small bowel resection or patients with inflammatory bowel disease exhibit reduced levels of circulating GLP-2 (24, 25).

Physiological Actions and Therapeutic Potential of GLP-1

GLP-1 action in the CNS

The increasing interest in GLP-1 action stems from its ability to lower blood glucose through activation of several diverse but complementary physiological systems. GLP-1 regulates nutrient intake via effects on gastric emptying (26, 27) and short-term regulation of feeding behavior (28, 29). Whether intestinal-derived GLP-1 stimulates CNS GLP-1R+ (GLP-1 receptor) neuronal circuits remains a subject of active investigation. Intracerebroventricular administration of GLP-1 or the GLP-1 antagonist exendin (9–39) inhibits or stimulates food intake in rodent studies, respectively. However, ICV GLP-1 produces only a transient reduction in food ingestion (29) and disruption of GLP-1 receptor signaling results in lean mice with normal food intake, even after several months of high fat feeding (30, 31). Furthermore, mice with combined disruption of leptin and GLP-1 action do not eat more or gain additional weight compared with ob/ob mice with leptin deficiency alone (32). Finally, transgenic mice with sustained elevations in circulating exendin-4, a unique lizard peptide that exhibits ~ 50% amino acid identity to mammalian GLP-1 and functions as a potent GLP-1 agonist, eat normally and do not exhibit growth disturbances despite months of continuous exendin-4 expression (33).

Taken together, these observations suggest that GLP-1 is not essential for physiological control of nutrient intake and body weight regulation *in vivo*.

An alternative explanation for the anorexic effects of CNS GLP-1 derives from studies demonstrating that GLP-1R+ neurons are activated as part of the stress response, such as following lithium chloride or lipopolysaccharide administration. ICV administration of GLP-1 produces conditioned taste aversion (34) and GLP-1 and lithium chloride induce similar patterns of neuronal *c-fos* activation (35, 36), demonstrating that CNS GLP-1R signaling is activated in response to aversive stimuli. Conversely, GLP-1 antagonists attenuate stress-induced increases in colonic motility (37) and abrogate the activation of CNS neurons in response to lithium chloride or lipopolysaccharide (38, 39). The finding of an exaggerated corticosterone response to restraint stress in GLP-1R^{-/-} mice provides further evidence linking GLP-1R signaling with the central response to stress-associated stimulation (40). The available evidence suggests that GLP-1 induced anorexia and taste aversion are mediated by different CNS pathways (41).

GLP-1 may also regulate the hypothalamic pituitary axis (HPA) via effects on LH, TSH, CRH, oxytocin, and vasopressin secretion (42–44). These GLP-1 actions do not appear to be essential for HPA function, as GLP-1R^{-/-} mice cycle normally, are fertile, and exhibit normal basal levels of plasma osmolarity, corticosterone, thyroid hormones, estradiol, and testosterone (40). Conversely, transgenic mice with sustained elevations in circulating exendin-4 are fertile and do not exhibit significant disturbances in eating or drinking behavior (33).

GLP-1 and β cell function

GLP-1 increases levels of β cell cAMP and insulin gene transcription and stimulates glucose-dependent insulin release (45); however, unlike other depolarizing agents such as the sulfonylureas, β cell GLP-1R signaling is glucose dependent (46). Despite the presumed importance of protein kinase A for β cell GLP-1 signaling, GLP-1-stimulated activation of insulin gene transcription and cytosolic calcium influx is PKA-independent (47, 48). GLP-1 also increases pdx-1 gene expression and binding activity, likely via a PI-3-kinase-dependent pathway (49). Elimination of GLP-1 receptor signaling in β cells is associated with reduced intracellular cAMP, and defective glucose-stimulated calcium influx (50).

Studies using GLP-1 antagonists reveal an essential role for GLP-1 as an incretin mediating postprandial nutrient disposal. Elimination of GLP-1 action using GLP-1 immunoneutralizing antisera or the antagonist exendin (9–39) increased glycemic excursion and reduced insulin secretion following oral nutrient ingestion in baboons, rats, and humans (51–54). Surprisingly, GLP-1 action is also essential for control of fasting glycemia and glucose clearance following nonenteral glucose challenge (30, 55). These latter observations are likely attributable to the importance of GLP-1 for basal β cell function and for inhibition of glucagon secretion. Whether the inhibitory effect of GLP-1 on glucagon secretion is direct, or indirect, perhaps mediated via insulin and/or somatostatin, remains unclear. In contrast to the role of

GLP-1 for glucose homeostasis following both enteral and nonenteral glucose challenge, the role of GIP appears more restricted as GIP regulates glucose absorption and glycemic excursion only following enteral glucose challenge (55).

GLP-1 and regulation of islet growth and differentiation

Exogenous GLP-1 stimulates islet cell proliferation in Umea +/? mice (56) and increases islet cell proliferation in INS-1 cells via a PI3-kinase-dependent pathway (49). Incubation of pancreatic exocrine AR4J cells with exendin-4 or GLP-1 induced differentiation to an endocrine islet phenotype, with expression of GLUT-2 and glucokinase associated with acquisition of islet cell-like properties including glucagon and insulin immunopositivity and glucose-dependent insulin secretion (57). Administration of GLP-1 or exendin-4 for 10 days to neonatal diabetic rats following partial pancreatectomy stimulated expansion of β cell mass via induction of islet proliferation and islet neogenesis (58). Similarly, GLP-1 and exendin-4 enhanced ductal pdx-1 expression, stimulated insulin secretion, lowered blood glucose and increased islet size and β cell neogenesis in +/+ and diabetic db/db mice (59). These findings using both cell and animal models strongly suggest that activation of ductal and islet GLP-1R signaling leads to increased β cell mass. Nevertheless, GLP-1R signaling is not invariably sufficient or necessary for induction of islet neogenesis and β cell hyperplasia as evident from studies of metallothionein promoter-exendin-4 and ob:ob:GLP-1R^{-/-} mice (32, 33).

Extrapancreatic actions of GLP-1

The glucagon-like peptide 1 receptor (GLP-1R) is expressed in the pancreatic islets, the gastrointestinal tract, kidney, heart, lungs, CNS, and possibly in adipose tissue (60–62). Although several studies have suggested that GLP-1 may enhance glucose clearance in an insulin-independent manner, more recent data suggests that the majority of GLP-1 actions on glucose clearance are mediated by changes in the insulin to glucagon ratio (63). The putative importance and physiological significance of GLP-1 actions in muscle and adipose tissue remain unclear. GLP-1 administered iv or by ICV injection increases heart rate and blood pressure in rats (64). These effects can be blocked by iv or ICV administration of the antagonist exendin (9–39) and bilateral vagotomy blocked the cardiovascular effects of ICV, but not peripherally administered GLP-1 (65). Clinically significant effects of GLP-1 on heart rate and blood pressure in human studies have not yet been reported.

GLP-1 and the treatment of diabetes

Studies in diabetic rodents demonstrate that GLP-1 and exendin-4 reduce blood glucose and hemoglobin A1c, increase insulin secretion and insulin mRNA, and promote weight loss and reduced adipose tissue mass (59, 66–68). The ideal mode and frequency of GLP-1 agonist administration for the treatment of diabetes remains under investigation. Although once daily administration of exendin-4 lowers glucose and HbA1c, twice daily exendin-4 administration was required to reduce food intake and decrease visceral fat dep-

osition and body weight in Zucker rats (67). The glucose-lowering actions of GLP-1 in studies of diabetic patients are secondary to inhibition of gastric emptying and glucagon secretion, and stimulation of insulin secretion. GLP-1 also lowers appetite in short-term studies of patients with type 2 diabetes (69, 70); however the long-term effects of GLP-1 or exendin-4 on body weight in diabetic subjects have not yet been reported. The finding that short-term GLP-1 infusion normalized fasting plasma glucose in patients with type 2 diabetes following secondary sulfonylurea failure suggests that activation of GLP-1R signaling may reverse diabetes-associated defects in the failing β cell (71, 72). Although results from long-term clinical studies using GLP-1 to treat human diabetes are not yet available, preliminary evidence suggests that GLP-1 maintains its glucose-lowering effects after several weeks in human subjects with type 2 diabetes (73).

The rapid inactivation and short $t_{1/2}$ of GLP-1 has stimulated interest in longer-acting GLP-1 analogs that exhibit a more prolonged duration of action *in vivo*. Sustained GLP-1 action may be achieved by the use of selective amino acid substitutions that confer DP IV-resistance or related molecules such as lizard exendin-4, fatty acid derivation of the molecule, and optimization of GLP-1 formulations to achieve enhanced levels of the bioactive peptide. Alternatively, inhibition of GLP-1 degradation through use of DP IV inhibitors may also represent a viable strategy for lowering blood glucose (74). A potential advantage of DP IV inhibitors is the concomitant potentiation of the activity of GIP and PACAP (11), GLP-1-related peptides that also exhibit insulinotropic activity *in vivo*. The long-term safety and optimal mode of DP IV inhibitor administration requires further investigation in light of findings that DP IV, also known as CD26 modulates cleavage of numerous chemokines with possible implications for regulation of immune and inflammatory responses (75). Complementary strategies for enhancing the effectiveness of GLP-1 treatment regimens including the development of enteral or mucosal GLP-1 delivery systems (76, 77). The multiple glucose-lowering actions of GLP-1, taken together with its effects on suppression of food intake and stimulation of islet neogenesis, provides a powerful rationale for evaluating the clinical effectiveness of agents that enhance GLP-1R signaling for the treatment of diabetes.

Biological Actions and Therapeutic Potential of GLP-2

Intestinal trophic properties of GLP-2

The finding that islet proglucagon complementary DNAs from several nonmammalian species did not contain a GLP-2 sequence led to suggestions that the lack of evolutionary conservation of the GLP-2 sequence may be consistent with a nonessential role for GLP-2 in physiological systems. Subsequent studies demonstrated that GLP-2 is indeed conserved in both vertebrate and nonvertebrate genomes, and intestinal RNA transcripts encoding GLP-2 are generated as a result of tissue-specific RNA splicing in fish, chicken, and lizards (78, 79). The biological role of GLP-2 as an intestinal trophic peptide was deduced in experiments demonstrating that administration of exogenous GLP-2 to mice stimu-

lated intestinal crypt cell proliferation leading to enhanced growth of the intestinal mucosal epithelium (80). These findings explain the correlation between human glucagon-producing tumors and small bowel hyperplasia (81, 82) and are consistent with multiple observations linking injury of the intestinal epithelium to enhanced production and secretion of the PGDPs in rodent and human studies (2). The growth promoting effects of GLP-2 appear restricted to the gastrointestinal tract, as no evidence for cell proliferation was detected in extraintestinal tissues in mice after 3 months of daily GLP-2 administration (83).

GLP-2 rapidly stimulates intestinal hexose transport and inhibits both meal-stimulated gastric acid secretion and gastric motility, actions that appear independent of the trophic effects of the peptide (84–86). GLP-2 also enhances barrier function in the murine intestinal epithelium via effects on both transcellular and paracellular pathways, with significant changes in tissue conductance noted within 4 h of GLP-2 administration (87). The importance of enteral nutrition for intestinal mucosal epithelial growth may be explained in part by the stimulatory effects of nutrients on GLP-2 secretion (14, 16), as GLP-2 infusion prevented parenteral nutrition-associated gut mucosal hypoplasia in rats (88).

Therapeutic potential of GLP-2

The intestinotrophic and antiapoptotic properties of GLP-2 in the small and large bowel of normal rodents (83, 89, 90) suggests that exogenous GLP-2 administration may prevent or ameliorate the effects of intestinal injury. Administration of GLP-2 to rats following major small bowel resection enhanced endogenous intestinal adaptation, with significant increases in mucosal weight, villus height, sucrase activity, and D-xylose absorption detected in GLP-2-treated rats (91). Similarly, sc administration of GLP-2 for 35 days produced increases in bowel histology, energy retention, weight gain, and lean body mass in human subjects with short bowel syndrome (92).

GLP-2 significantly ameliorated the extent of inflammation-associated injury in the murine small bowel following indomethacin administration, as evidenced by significant reductions in mortality, disease activity scores, intestinal cytokine expression, and bacterial infection in GLP-2-treated mice (93). The reparative effects of GLP-2 are not confined to the small bowel, as mice with dextran sulfate-induced colitis exhibit reduced weight loss and attenuation of intestinal injury following GLP-2 administration (94). The finding that GLP-2 enhanced mucosal mass and reduced mortality in rats with vascular intestinal ischemia (95), taken together with data demonstrating the functional integrity of the GLP-2-GLP-2R axis in the neonatal rat (96), suggests that GLP-2 may be useful for preventing ischemic intestinal injury in the neonatal gut *in vivo*.

The GLP-2 receptor

The actions of GLP-2 are transduced by a recently cloned GLP-2 receptor (GLP-2R), a new member of the glucagon/GLP-1 receptor superfamily (97). The GLP-2R was cloned from stomach, small bowel, and hypothalamus complementary DNA libraries, is highly specific for GLP-2, and does not

recognize supraphysiological concentrations (10 nM) of glucagon, GLP-1, exendin-4, and GIP (97). The GLP-2R gene was localized to human chromosome 17p13.3 and GLP-2R expression is highly tissue specific, with RNA transcripts detected in the stomach, small and large bowel, and central nervous system (97, 98). GLP-2R expression has been localized to distinct subpopulations of gut endocrine cells in the stomach, small bowel, and colon (98). These findings suggest a model for GLP-2 action whereby the biological effects of GLP-2 are mediated by GLP-2-stimulated factors liberated from gut endocrine cells in different regions of the gastrointestinal epithelium (Fig. 1).

As gut endocrine cell lines expressing the endogenous GLP-2 receptor have not yet been reported, studies of GLP-2 receptor signaling have been carried out in transfected heterologous cell types (97, 99, 100). Activation of GLP-2R signaling in fibroblasts transfected with the GLP-2 receptor increases cAMP and AP-1-dependent pathways but has no effect on intracellular calcium (99). Consistent with the putative indirect effects of GLP-2 on intestinal growth, 10 nM GLP-2 had no direct effect on stimulation of fibroblast cell proliferation *in vitro* (99). The finding that GLP-2 significantly reduces apoptosis in the intestinal crypt compartment following gut injury (93) has prompted studies of GLP-2 receptor signaling and apoptotic pathways. Remarkably, BHK-GLP-2R cells exhibit decreased apoptotic cell death and reduced activation of caspase-3 following GLP-2 treatment *in vitro* (100). The effects of GLP-2 on apoptosis were independent of protein kinase A and associated with reduced activation of caspase-8 and caspase-9-like activities, decreased cleavage of polyADP ribose polymerase, and diminished cytochrome c release (100). These findings, taken together with data from animal studies, suggest that GLP-2 may also act directly on gut endocrine cells to reduce cellular injury, which in turn permits the enteroendocrine cell to liberate additional factors that protect the adjacent crypt compartment from apoptotic stimuli *in vivo*.

GLP-1 and GLP-2: Future Research Directions

The pleiotropic effects of GLP-1 in normal and diabetic subjects, taken together with the series of exciting studies linking GLP-1R signaling to islet neogenesis and proliferation, have fostered considerable interest in evaluating the efficacy of GLP-1 in the clinic as a treatment for human diabetes. Whether chronic GLP-1 administration will lead to sustained glucose lowering over months to years remains to be determined. Similarly, the long-term effects of GLP-1 on food intake and body weight in diabetic human subjects require further investigation. Optimization of GLP-1 delivery systems and development of safe, well-tolerated GLP-1 formulations that exhibit prolonged bioactivity *in vivo* remain major challenges for implementation of successful GLP-1 therapeutics programs. Much less is known about the biological actions of GLP-2. The recent finding that GLP-2 may act as a central satiety factor will stimulate additional studies of the role of GLP-2 in the central nervous system. The enteroendocrine localization of GLP-2R expression implies the existence of as yet unidentified mediators of GLP-2 action in the gut. The efficacy and safety of GLP-2 in human

subjects with intestinal disease requires future evaluation in controlled clinical trials. Taken together, recent studies have revealed that the glucagon-like peptides exert an increasing number of physiological actions on regulation of nutrient absorption and assimilation via actions on the gut, pancreas and central nervous system. As diabetes, obesity, and intestinal diseases are characterized by multiple defects in energy absorption and nutrient homeostasis, the potential therapeutic efficacy of GLP-1 and GLP-2 in the treatment of these disorders merits ongoing clinical investigation.

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