The Extrapancreatic Effects of Glucagon-Like Peptide-1 and Related Peptides
Rania Abu-Hamdah, Atoosa Rabiee, Graydon S. Meneilly, Richard P. Shannon, Dana K. Andersen and Dariush Elahi
The Extrapancreatic Effects of Glucagon-Like Peptide-1 and Related Peptides

Rania Abu-Hamdah, Atoosa Rabiee, Graydon S. Meneilly, Richard P. Shannon, Dana K. Andersen, and Dariush Elahi
Johns Hopkins University School of Medicine, Departments of Surgery (R.A.-H., A.R., D.K.A., D.E.) and Medicine (A.R., D.E.), Johns Hopkins Bayview Medical Center, Baltimore, Maryland 21224-2780; University of British Columbia (G.S.M.), Department of Medicine, Vancouver, British Columbia, Canada V5Z 1M9; and University of Pennsylvania School of Medicine (R.P.S.), Department of Medicine, Philadelphia, Pennsylvania 19104-2646

Context: Glucagon-like peptide-1 (GLP-1) 7-36 amide, an insulinotropic hormone released from the intestinal L cells in response to nutrient ingestion, has been extensively reviewed with respect to β-cell function. However GLP-1 receptors are abundant in many other tissues. Thus, the function of GLP-1 is not limited to the islet cells, and it has regulatory actions on many other organs.

Evidence Acquisition: A review of published, peer-reviewed medical literature (1987 to September 2008) on the extrapancreatic actions of GLP-1 was performed.

Evidence Synthesis: The extrapancreatic actions of GLP-1 include inhibition of gastric emptying and gastric acid secretion, thereby fulfilling the definition of GLP-1 as an enterogastrone. Other important extrapancreatic actions of GLP-1 include a regulatory role in hepatic glucose production, the inhibition of pancreatic exocrine secretion, cardioprotective and cardiotropic effects, the regulation of appetite and satiety, and stimulation of afferent sensory nerves. The primary metabolite of GLP-1, GLP-1 (9-36) amide, or GLP-1m, is the truncated product of degradation by dipeptidyl peptidase-4. GLP-1m has insulinomimetic effects on hepatic glucose production and cardiac function. Exendin-4 present in the salivary gland of the reptile, Gila monster (Heloderma suspectum), is a high-affinity agonist for the mammalian GLP-1 receptor. It is resistant to degradation by dipeptidyl peptidase-4, and therefore has a prolonged half-life.

Conclusion: GLP-1 and its metabolite have important extrapancreatic effects particularly with regard to the cardiovascular system and insulinomimetic effects with respect to glucose homeostasis. These effects may be particularly important in the obese state. GLP-1, GLP-1m, and exendin-4 therefore have potential therapeutic roles because of their diffuse extrapancreatic actions. (J Clin Endocrinol Metab 94: 1843–1852, 2009)

Glucagon-like peptide-1 (7-36) amide (GLP-1) is a 29-amino acid hormone secreted from the L cells of the small intestine by site-selective cleavage of the preproglucagon gene product (1). After the delineation of the DNA sequence of preproglucagon by Bell et al. (2) in hamster and man, the presence of two related peptides, GLP-1 and glucagon-like peptide-2, was proposed. Lopez et al. (3) isolated the same sequences in bovine preproglucagon cDNA. Habener’s group then isolated the same sequence in the rat (4) and showed that GLP-1 was a potent insulinotropic agent (5) that qualified for the designation of an incretin (6, 7). GLP-1 is a mammalian brain-gut axis hormone that is also an endocrine paracrine hormone, an autonomic nervous system neurotransmitter (1, 8), and a natriuretic hormone (9).

GLP-1 is rapidly degraded to GLP-1 (9-36) amide, also referred to as GLP-1m, by the action of dipeptidyl peptidase-4 (DPP-4) (10, 11), which results in a circulating half-life for GLP-1 of 2 min (12) and is also cleared from the circulation by renal excretion (1). Peptides with alanine, proline, and hydroxyproline found in the N-terminal domain are cleaved by DPP-4, and GLP-1 is cleaved at the His 7, Aln 8 position leading to the

Abbreviations: CNS, Central nervous system; DPP-4, dipeptidyl peptidase-4; GLP-1, glucagon-like peptide-1; GLP-1R, GLP-1 receptor; GLUT, glucose transporter; IR, insulin receptor; PYY, peptide YY; T2DM, type 2 diabetes mellitus.
formation of the GLP-1m (13). Exendin-4, a GLP-1 receptor (GLP-1R) agonist, is present in the saliva of Gila monster (Heloderma suspectum). It shares 53% of its amino acid sequence with the N-terminal region of mammalian GLP-1 (14). Exendin-4 has an extra nine amino acid residues at its C-terminus. A major difference between the two agents is that the second amino acid of exendin-4 is glycine, which is resistant to DPP-4 cleavage. It has a circulating half-life of 30 min in man (15, 16).

GLP-1 and exendin-4 have been shown to be potent in- sulinotropic in both normal and diabetic subjects, and their roles as mediators of insulin release have therefore received much attention (12). Previous studies showed that exenatide (synthetic exendin-4) treatment resulted in lowering fasting and postprandial plasma glucose concentrations in patients with type 2 diabetes mellitus (T2DM) (17). GLP-1 is present and secreted from the L cells of several mammals including pig and rat (1, 18), and the peptide sequence of GLP-1 is identical in mouse, rat, and human (19, 20).

GLP-1 and exendin-4 have also been shown to have trophic effects on the β-cell (21). One mechanism responsible for the expansion of β-cell mass is inhibition of apoptosis shown for both GLP-1 and exendin-4 by Farilla et al. (22). The effect of GLP-1 on apoptosis appears to be mediated by the GLP-1R, and the expression of the GLP-1R in a nonpancreatic cell line renders these cells sensitive to the inhibition of programmed cell death by GLP-1 (23). It has also been shown that human islets treated with GLP-1 have a down-regulation of caspase 3 at the levels of mRNA of the active protein and an up-regulation of the antiapoptotic protein Bel-2 (22). A second mechanism responsible for the expansion of β-cell mass is enhanced cell proliferation. Mice treated with GLP-1 or exendin-4 show increased cell prolifera- tion by bromodeoxyuridine labeling or by tritiated thymi- dine incorporation within the islets. The insulin-expressing cells are stimulated to proliferate by administration of GLP-1 in vivo (23). GLP-1 increases levels of β-cell cAMP and insulin gene transcription and stimulates glucose-dependent insulin release (24); however, unlike other depolarizing agents (such as the sulfonylureas), β-cell GLP-1R signaling is glucose dependent (24). GLP-1 also increases the gene expression and binding activity of transcription factor pancreatic and duodenal homeobox gene 1 most likely by a phosphatidylinositol-3-kinase-dependent pathway (25). Elimination of GLP-1R signaling in β-cells is associ- ated with reduced intracellular cAMP and defective glucose-stimulated calcium influx (25).

The GLP-1R has been localized to the stomach, duodenum, exocrine pancreas, brainstem, thalamus, hypothalamus, hippocampus, heart, lung, and kidney, as well as the pancreatic islets (26). Furthermore, GLP-1 binding sites have been found in muscle cells, adipocytes, and the liver (27–30). The findings of GLP-1R outside of the islets provides strong evidence that GLP-1 has many extrasilet effects and corroborates other studies (31–34) that show physiological effects of GLP-1 on a variety of extrapancreatic functions. This review summarizes the function of GLP-1 on different organs including the stomach, heart, liver, and the central nervous system (CNS).

**GLP-1 Actions on Gastrointestinal Function**

The inhibitory function of GLP-1 on gastric emptying and gastric acid secretion confirms the role of GLP-1 as an important entero-gastrone, a hormone that inhibits proximal events of the gastrointestinal tract in a negative feedback manner (26). Nauck et al. (35) showed that iv administration of GLP-1 (7-36) amide and GLP-1 (7-37) has similar, profoundly inhibitory effects on the gastric emptying of a liquid mixed test meal in healthy, normoglycemic volunteers (Fig. 1) and that the effect of GLP-1 on gastric emptying is dose dependent and highly significant with physiological concentrations of approximately 25 pmol/liter. In eight healthy male volunteers, Schirra et al. (36) investigated the effect of different doses of GLP-1(7-36) amide (0.125 nmol/kg, 0.25 nmol/kg, or placebo) administered sc 5 min before the mixed meal. They quantified the pattern of gastric emptying of a mixed meal (300 kcal) as well as pancreatic secretion, antroduodenal motility, and the glycemic response and the release of insulin, C-peptide, and glucagon. The lag period or the time to reach maximal velocity of gastric emptying was dose-dependently prolonged in response to the sc infusion of GLP-1. Maximal emptying velocity, total emptying rate, and the exponential emptying rate were not changed, however (36). This study also showed that the sc infusion of GLP-1 resulted in a dose-dependent inhibition of duodenal and antral motility; that both doses of GLP-1 resulted in coordinated antroduodenal contractions; that GLP-1 initially reduced, then transiently stimulated the secretion of pancreatic enzymes; that both doses of GLP-1 resulted in a delayed postprandial insulin peak and enhanced total insulin release; and that the postprandial response of pancreatic polypeptide and glucagon was diminished. In another study, Schirra et al. (37) showed antro-pyloro-duodenal motility in humans and the actions of endogenously released GLP-1 on endocrine pancreas secretion. In their study of nine healthy volunteers, they used GLP-1R antagonist exendin (9-39) to test whether GLP-1 acts as an incretin and/or as an entero-gastrone in humans. They showed that the endogenously released GLP-1 significantly enhanced postprandial insulin secre-
tion and suppressed the secretion of glucagon (37). During the fasting and postprandial state, antro-duodenal motility was inhibited by GLP-1, which qualifies GLP-1 as an entero gastrone. They also showed that the stimulation of pyloric motility that is induced by intestinal glucose was mediated by GLP-1. The presence of food in the gut causes the L cells of the intestine to release GLP-1 into the circulation, which not only stimulates the pancreas to produce insulin but also slows gastric emptying and may lead to a decrease in appetite (38–43).

The mechanisms by which GLP-1 inhibits gastric emptying appear to be complex and to involve communication with the central and peripheral nervous systems (44, 45). Gastric distension increases the expression of c-Fos in brainstem neurons that produce GLP-1 (46). In addition, administration of GLP-1 centrally resulted in reduction of food intake (47), which is accompanied with increased expression of the c-Fos in the brainstem of the rat (48, 49). The denervation of vagal afferent fibers abolishes the effects of GLP-1 on gastric emptying in the rat (50). The stimulation to the CNS is most likely responsible for the reduction in food intake, inhibition of gastric emptying, as well as inhibitory action on gastric motor function (47, 50). These actions are most likely mediated by increased action potential and calcium influx in neurons of the nodose ganglion (51).

Although small peptides such as GLP-1 and exendin-4 are capable of rapidly crossing the blood-brain barrier and directly accessing the CNS, higher molecular weight GLP-1R agonists, such as albumin-bound GLP-1, that do not cross the blood-brain barrier are still capable of inhibiting gastric emptying and food intake (52). These findings indicate the importance of ascending vagal afferents for GLP-1R-dependent control of gastrointestinal motility. Interestingly, studies by Meier et al. (53) showed that antagonizing the delaying effects of GLP-1 on gastric emptying by using a prokinetic agent such as erythromycin resulted in an augmentation of the insulin secretory response after meal ingestion. GLP-1Rs are also directly expressed in the stomach on gastric parietal cells, where GLP-1 may directly regulate gastric acid secretion (54). However, the effects of GLP-1 on gastric acid secretion were found to be absent in vagotomized human subjects (55). Hence, considerable evidence supports the importance of vagal innervation for GLP-1 regulation of gastric secretion and motility.

It should be noted that the effect of delayed gastric emptying has been generally demonstrated with physiological or supra-physiological exogenously administered GLP-1 (56, 57). Therefore, it remains unclear whether endogenously released GLP-1 has a significant effect on gastric emptying. Studies in healthy baboons have shown that with intragastric infusion of glucose and D-xylose (a marker for rate of emptying of glucose from stomach), plasma levels of D-xylose were similar when the effects of GLP-1 were blocked with exendin (9-36) amide or with a specific monoclonal antibody to GLP-1 (44, 58). Those findings suggested that gastric emptying is not increased when the effects of GLP-1 are blocked, at least in the baboon. The use of a DPP-4 inhibitor, which increases plasma levels of GLP-1, might be expected to delay gastric emptying, but Vella et al. (59) failed to observe any changes in the gastric emptying of a solid meal in patients with T2DM who were treated with such an inhibitor.

Most recently, an iv-oral hyperglycemic clamp study in humans was reported during which 75 g glucose containing D-xylose was ingested. During the entire clamp, plasma glucose levels were held at a steady level despite the ingestion of glucose. Two studies were conducted, with blockade of GLP-1R in one. The rate of appearance of ingested D-xylose was not different between the two studies, indicating that endogenously released GLP-1 has at best only a modest effect on gastric emptying (60).

In some endocrine systems, negative feedback mechanisms regulate secretion of the hormone. The classic example is reproductive hormone regulation by the hypothalamus. Exogenous infusion of hormone may also exert negative feedback regulation of the endogenously released hormone. An example of this is the documented suppression of C-peptide levels when insulin is infused (61). In this context, we are not aware of any data that demonstrate regulation of endogenously released GLP-1 when it is infused exogenously.

**GLP-1 and the Cardiovascular System**

Early studies demonstrated the presence of transcripts for GLP-1Rs in the heart (62), but only recently has the cellular distribution of the receptors been localized. Ban et al. (63) identified GLP-1Rs via immunohistochemistry in cardiomyocytes and coronary and vascular endothelial cells as well as smooth muscle in mice.

Previous studies have shown that exogenous administration of GLP-1 exhibits both inotropic and chronotropic activity. The extent to which GLP-1 (7-36) exerts cardio vascular effects via increased inotropic and chronotropic actions appears to depend on the integrity of the autonomic nervous system. For example, Barragan et al. (64) showed that iv administration of GLP-1 dose-dependently increases arterial blood pressure and heart rate in anesthetized rats. Ahren (26) showed that GLP-1 increased both systolic and diastolic pressures under anesthesia and that those effects were not prevented by reserpine, propranolol, or phentolamine, suggesting a direct action of GLP-1. Yamamoto et al. (65) showed similar effects after peripheral and central administration of GLP-1 in anesthetized rats, observing dose-dependent increases in blood pressure and heart rate. Barragan et al. (66) showed that administration of the GLP-1R agonist, exendin-4, also increases blood pressure and heart rate in anesthetized rats. Notably, the pressor and chronotropic responses seen in rodents were not evident in normal conscious, chronically instrumented dogs over a dose range of 1–20 pmol · kg⁻¹ · min⁻¹ (67). Nikolaidis et al. (67) showed that myocardial function and cardiac output were improved after administration of GLP-1 in conscious, chronically instrumented canine models of cardiac injury or heart failure. GLP-1 increased cardiac output and reduced left ventricular end diastolic pressure in association with reduced systemic vascular resistance, and it improved myocardial insulin sensitivity and myocardial glucose uptake in dogs with rapid pacing-induced dilated cardiomyopathy (67). A clinical study showed that GLP-1 improves left ventricular ejection fraction and functional status in patients with congestive heart failure, without affecting heart rate or blood pressure, suggesting a mechanism other than direct inotropic or chronotropic effects (68).
GLP-1 has been shown to reduce infarct size in the isolated perfused rat heart subjected to complete coronary artery occlusion (69, 70). When the cAMP inhibitor Rp-cAMP was present, the infarct-sparing actions of GLP-1 were abolished, implicating a cAMP-dependent mechanism. In these studies, GLP-1 was also associated with increased Akt expression, although these investigators did not measure myocardial glucose uptake. In contrast, Zhao et al. (71) demonstrated that GLP-1 (7-36) in normal rat hearts altered resting contractility and heart rate through a non-Akt-dependent mechanism. Specifically, these investigators showed that GLP-1, in contrast to insulin, had no effect on Akt phosphorylation or activation and did not result in increased glucose transporter (GLUT)-4 translocation, despite increased phosphorylation or activation and did not result in increased myocardial glucose uptake. Rather, GLP-1 (7-36) increased p38 MAPK activation, nitric oxide expression, and GLUT-1 translocation. Under circumstances of low flow ischemia, GLP-1 (7-36) mitigated myocardial stunning. Thus, the cellular signaling effects of GLP-1 (7-36) vary depending on the experimental circumstances (partial vs. complete coronary artery occlusion).

Remarkably, GLP-1 also exerts beneficial effects on cardiac function in human subjects after myocardial infarction and angioplasty. In one study, a 72-h infusion of GLP-1 in patients with acute myocardial infarction and ejection fractions less than 40% resulted in significantly improved left ventricular ejection fraction and improved regional and global wall motion scores, and it was associated with earlier hospital discharge (34) (Fig. 2). In a randomized study of the effects of GLP-1 on patients undergoing coronary artery bypass grafting, Sokos et al. (72) showed that the need for inotropic support and exogenous insulin was significantly reduced in patients who received GLP-1. Whether the beneficial effects of GLP-1 on the injured heart are primarily directed via activation of cardiac GLP-1R signaling or indirectly via GLP-1R-dependent improvement in levels of glucose and insulin requires further investigation. The findings of a direct, cardioprotective effect in the isolated perfused rat and mouse heart argue strongly for the former (63, 71). However, it remains to be determined whether the cardioprotective effects are attributable to the increase in myocardial glucose uptake and glycolytic ATP or activation of distinct but related cellular pathways implicated in ischemic pre- or postconditioning.

The agonists of GLP-1R have been shown to have vascular and cardiac actions in humans as well as in rodents; these actions include the effects on cardiac output, blood pressure, contractility (65, 66, 68, 73), and cardioprotection (34, 67, 70, 74). Previous studies showed that GLP-1 is believed to exert its action through heptahelical G protein-coupled receptor (GLP-1R), which is functionally associated with adenylate cyclase through the stimulatory Gs (75, 76). Whether these mechanisms are operative and account for the putative beneficial effects of GLP-1 agonists remains to be determined conclusively. These studies will have important implications on the ultimate role of these agonists because chronic cAMP generation may be deleterious in clinical cardiovascular conditions. Moreover, the dose of the GLP-1 agonists that elicits a beneficial cardiovascular effect tends to be greater than the native peptide, raising important considerations of ligand-receptor interaction.

The demonstration that GLP-1 (9-36) amide, the principal metabolite of GLP-1, improves myocardial glucose uptake and ventricular contractility in dogs with pacing-induced dilated cardiomyopathy suggests that some of the cardiovascular effects of native GLP-1 may be mediated by a mechanism independent of the known GLP-1R (77). Ban et al. showed that GLP-1 (9-36) had favorable effects on posts ischemic contractile dysfunction in mice when administered after but not before occlusion. These investigators have suggested a two pathway schema for cardiovascular actions of GLP-1. The first depends on the GLP-1R action for inotropic, glucose uptake, ischemic preconditioning, and mild vasodilatory actions. The second pathway depends on the rapid degradation of GLP-1 to GLP-1m. Their data are compatible with the notion that although GLP-1m is not an inotrope, it has a small, significant cardioprotective effect in the setting of ischemic reperfusion injury. This is due to an increase of glucose uptake and vasodilation through a nitric oxide/cGMP-dependent pathway (63, 78, 79). These findings have important implications for the role of DPP-4 inhibition in the clinical utility of incretin biology.

Although the majority of experimental studies have used acute exposure to GLP-1 in assessing cardiovascular effects, a recent study by Poornima et al. (79) has examined the effects of 3 months of continuous infusion of GLP-1. These investigators demonstrated that chronic infusions of GLP-1 improved survival and preserved cardiac function in a rodent model of diabetes and hypertension that develops dilated cardiomyopathy and dies prematurely. These studies indicate that the salutary effects of
GLP-1 on cardiovascular performance are sustained after chronic exposure. Thus, the emerging cardiovascular profile of GLP-1 together with its effective antilipemic actions portend significant clinical benefits in the treatment of T2DM where therapies that reduce macrovascular outcomes have been elusive.

**GLP-1 Actions on the Liver**

The hepatoportal region may be an important site of action of GLP-1 because there is a rapid degradation of GLP-1 in the plasma after its secretion into the mesenteric venous bed. During the postprandial phase, the concentration of GLP-1 increases in the mesenteric-portal venous system.

The effect of GLP-1 on hepatic glucose production has been reviewed by D’Alessio et al. (80). In vitro studies supporting GLP-1 effects on liver cells are most convincing from the laboratory of Valverde et al. (27) who showed that GLP-1 promotes glycogen accumulation in cultured rat liver cells. They observed that GLP-1 increases the activity of glycogen synthase-A, decreases the activity of glycogen phosphorylase-A, and promotes the incorporation of labeled glucose into glycogen in isolated rat hepatocytes. They also showed that these effects of GLP-1 are concentration dependent and increase with increasing levels of glucose. These effects of GLP-1 can also be reproduced with exendin and blocked by GLP-1R antagonist exendin (9-39) amide. The increased glycogen accumulation by GLP-1 or insulin is significantly reduced when glucagon is added to the media, which is also accompanied by a significant reduction in cAMP (27, 81, 82).

An in vivo dog study by Dardevet et al. (32) suggested the presence of GLP-1 sensors or receptors in the hepatoportal region. They showed that the insulin-independent effect of GLP-1 on hepatic glucose uptake is consistent with the presence of specific GLP-1Rs that could activate kinases and/or factors involved in glycogen synthesis and glucose uptake.

In a study to evaluate the beneficial therapeutic effects of exendin on hepatic steatosis in ob/ob mice (16), it was shown that GLP-1 and exendin-4 both have the potential for a direct lipid-lowering effect on hepatocytes, making either peptide a potential candidate for the treatment of non-alcoholic fatty liver disease. The presence of GLP-1Rs in isolated rat hepatocytes was shown in this study by an immunoblot analysis. However, in contrast to the study of Valverde et al. (27), GLP-1 and exendin-4 resulted in a marked increase in cAMP production; when the hepatocytes were pretreated with the GLP-1R antagonist exendin (9-39), the activity of cAMP was significantly reduced to below basal levels.

In addition to its role in hepatic glucose uptake and glycogen formation, GLP-1 may also mediate the regulation of hepatic glucose output by insulin. The hepatic insulin receptor (IR) and the hepatocyte membrane-bound GLUT2 have been shown to be internalized into the endosomal compartment after feeding or insulin administration (83), and the internalization of hepatocyte GLUT2 appears to mediate the suppression of hepatic glucose output by insulin (84). The endocytosis of the hepatic IR and GLUT2 appears coupled, suggesting an IR-GLUT2 complex on the hepatocyte plasma membrane (85). In states of apocrine diabetes, such as chronic pancreatitis, the internalization of the IR-GLUT2 complex is impaired, and hepatic glucose production becomes unresponsive to suppression by insulin (86). Treatment with GLP-1 in rats with chronic pancreatitis was found to reverse this impairment (87), suggesting a role for GLP-1 in the regulation of IR and GLUT2 endocytosis, and therefore hepatic glucose production.

Our group showed that an infusion of GLP-1 in obese volunteers resulted in an increase in glucose uptake that was not the result of increased endogenous insulin secretion (88). These results were similar to what Dardevet et al. (32) found, i.e. that pharmacological doses of GLP-1 resulted in increased glucose utilization, independent of changes in insulin. Such human studies and others in pigs and dogs suggest an important extrapancreatic effect of the principal metabolite of GLP-1, GLP-1 (9-36) amide, or GLP-1m. GLP-1m was previously found to lack insulinotropic activity and has therefore been considered to be biologically inactive (89). It has been shown that infusion of GLP-1 (7-36) amide results in high levels of GLP-1m because of its cleavage by DPP-4 in plasma (11, 90). Indeed, we and others have shown that when steady-state levels were achieved during infusion of full-length peptide, approximately 80% of the circulating plasma levels of peptide were in the form of GLP-1m (88, 90). Therefore we hypothesized that the insulinomimetic action of GLP-1 might be due to GLP-1m formation, and we undertook glucose clamp studies in lean and obese subjects with the aim of elucidating the effects of GLP-1m. Glucose turnover was measured during two 2-h euglycemic clamp studies in which saline or GLP-1m was infused from 0 to 60 min. Half of the volunteers underwent a third clamp in which the known GLP-1R antagonist was included.

In lean subjects, no glucose infusion was necessary to sustain euglycemia during saline or GLP-1m infusion. However, in obese subjects glucose infusion was necessary during GLP-1m infusion because of a marked (>50%) inhibition of hepatic glucose production. Plasma insulin levels remained constant in lean subjects but rose significantly in obese subjects after termination of the peptide infusion. During GLP-1R blockade, infusion of glucose was immediately required, on starting GLP-1m infusions, in all subjects because of a more dramatic reduction in hepatic glucose production and a delayed and modest insulinotropic response (33). Thus, GLP-1m inhibits hepatic glucose production and is a weak insulinotropic agent (Fig. 3). These properties are especially apparent and pronounced in obese subjects and only become apparent in lean subjects during GLP-1R blockade. These previously unrecognized antidiabetogenic actions of GLP-1m, which is always generated when GLP-1 (7-36) is administered or secreted, suggest a role for GLP-1m as a therapeutic agent in controlling blood glucose (33).

The observation noted above that GLP-1m improved myocardial glucose uptake and ventricular contractility in dogs with dilated cardiomyopathy equally as GLP-1 (7-36) suggests that a putative GLP-1m receptor may be present in cardiac tissue (77). Increased functional recovery and cardiomyocyte viability after ischemic reperfusion injury in isolated hearts from wild-type mice were also observed in mice lacking a functional GLP-1R.
The presence of GLP-1 and the GLP-1R in the CNS indicate that the brain, particularly in the paraventricular nucleus (48, 95, 96), solitary tract (94), and its receptors are widespread throughout the brain. GLP-1 is synthesized in the caudal part of the nucleus of the solitary tract (94), and its receptors are involved in a variety of functions, including suppression of hepatic glucose production (33) strongly suggests that GLP-1 also acts centrally in addition to its actions on the peripheral system. It has been shown previously that injection of GLP-1 intracisternally causes a delay of liquid gastric emptying (50). Nakade et al. (97) showed that the peripheral sympathetic nervous system and the central corticotropin-releasing factor receptors are involved in the central GLP-1-mediated delay of solid gastric emptying in rats.

Nishizawa et al. (98) showed that administration of GLP-1 into the portal vein increases the firing frequency in the vagal afferents in rats. This suggests that the release of GLP-1 from the gut is rapidly signaled to the brain through this afferent pathway (98). This may represent the functional basis for neurally mediated inhibition of gastric emptying, gastric acid secretion, and exocrine pancreatic secretion by GLP-1, effects that have been shown to require intact sensory and efferent parasympathetic nerves (26).

Intracerebroventricular administration of GLP-1R antagonists inhibits food intake in rodents (96, 99), and GLP-1Rs have been localized to hypothalamic nuclei, which are important for the regulation of satiety. Repeated intracerebroventricular administration of GLP-1 in rats produces weight loss (Fig. 4), whereas intracerebroventricular administration of the GLP-1R antagonist exendin (9-39) for 3 d produced weight gain, and exendin (9-39) administered together with the central orexigenic agent neuropeptide Y resulted in an increased food intake and weight gain compared with that observed with neuropeptide Y alone (100). It should be noted that the L cells corelease GLP-1 and peptide YY (PYY), and immunohistological studies have shown that these peptides are colocalized and coreleased from these cells. PYY (3-36), the major circulating form of PYY, has been shown to be a potent orexigenic agent in rats and man (101, 102). Evidence therefore supports the corelease of GLP-1 and PYY as having important roles as mediators of satiety. Repeated intracerebroventricular administration of GLP-1 in rats produces weight loss (Fig. 4), whereas intracerebroventricular administration of the GLP-1R antagonist exendin (9-39) for 3 d produced weight gain, and exendin (9-39) administered together with the central orexigenic agent neuropeptide Y resulted in an increased food intake and weight gain compared with that observed with neuropeptide Y alone (100).

Despite these reports of an insulin-independent effect of GLP-1 (or GLP-1m) on the liver, and despite other in vitto studies that demonstrate effects that are attributable to a presumed hepatic GLP-1R, it is acknowledged that there is controversy with respect to GLP-1 (or GLP-1m) effects in the liver, muscle, and adipose tissue (31), and the presence and/or species differentiation of an identifiable receptor in these tissues (91, 92). GLP-1m has been administered in humans by other investigators (89, 93) and in general did not show any effect. However, the design of these studies did not allow for evaluation of effects of GLP-1m because it was infused along with GLP-1, or with DPP-4 inhibitors, and tracers were not used to determine site-specific glucose kinetics. Our observation that GLP-1m infusion results in the suppression of hepatic glucose production (33) strongly suggests a role of GLP-1m on hepatic glucose production. In the absence of measurements of hepatic glucose dynamics, these effects would appear to result in enhanced insulin sensitivity after GLP-1 infusion.

**GLP-1 and the CNS**

GLP-1 is synthesized in the caudal part of the nucleus of the solitary tract (94), and its receptors are widespread throughout the brain, particularly in the paraventricular nucleus (48, 95, 96). The presence of GLP-1 and the GLP-1R in the CNS indicate that

![FIG. 3. The effect of GLP-1m on hepatic glucose production in man. Rates of appearance of glucose (Ra, top panel) in 12 lean (left) and 12 obese (right) volunteers who received GLP-1 (9-36) amide (GLP-1m) or saline from 0 to 60 min. Rates of appearance of glucose (bottom panel) in seven lean (left) and six obese (right) volunteers who received GLP-1m from 0 to 60 min. Exendin (9-39) amide was infused from 60 to 60 min (mean ± se). * Significant difference between the two studies at indicated times (33).](image-url)

![FIG. 4. The effect of intracerebroventricular (icv) GLP-1 on body weight in the rat. Body weight after daily icv injection of GLP-1 or saline. The solid circles represent animals given 3 nmol GLP-1, and open circles represent control animals that received saline (100).](image-url)
GLP-1 and PYY are secreted not only from L cells in the small intestine (from duodenum to ileum, with the greatest concentration in the ileum) but also from mammalian taste cells. Egan and colleagues (106) have shown that human duodenal L cells and taste cells of the tongue express the sweet taste receptor G protein gustducin, which is probably involved in the regulation of GLP-1 release. These investigators have shown that in many L cells, GLP-1, gustducin, and PYY are colocalized. They also have shown that GLP-1 is produced in two subsets of mammalian taste cells (type 2 and type 3) and that GLP-1Rs are present on adjacent intragammmal afferent nerve fibers (107). It is possible that GLP-1 (and PYY) activate the CNS events resulting in an anorexigenic effect, before stimulating islet hormones (108). Chronic peripheral administrations of GLP-1R agonists (Exendin, Liraglutide) have been consistently associated with reductions in food intake and weight loss in rats and humans (109–112). However, in a study of continuous sc administration of GLP-1 for 6 wk at a rate of 4.8 pmol·kg⁻¹·min⁻¹, only 1.9 kg of weight loss was documented (113). Furthermore, in a 12-wk continuous sc administration study of a lower dose of GLP-1 (1.5 pmol·kg⁻¹·min⁻¹), there was no weight loss (114). Therefore, it is possible that, in humans, reduction of appetite by GLP-1 is manifested only acutely and does not persist long term. Alternatively, to demonstrate weight loss with exogenous GLP-1 administration, much larger doses are required.

Cabou et al. (115) showed that brain GLP-1R signaling simultaneously controls heart rate, femoral arterial blood flow, and glucose utilization in an awake free-moving mouse, and that brain GLP-1 signaling regulates reactive nitric oxide and reactive oxygen species that are likely important for the coordinated regulation of metabolic and cardiovascular function. An increase in vagus nerve activity was associated with brain to periphery signaling, implying that the action of GLP-1R signaling for control of nitric oxide and reactive oxygen species is also glucose dependent (115). Previous studies showed that GLP-1 was able to relax the femoral artery tone in a dose-response manner in rats (116) and that it is associated with vasodilatation induced by acetylcholine (117). On the other hand, when GLP-1 or its analogs is infused systemically in humans, it does not induce hyperor hypotension as has been seen in animals (68, 118).

Conclusions

Our understanding of the extrapancreatic effects of the incretin hormones has expanded exponentially over the past two decades, and it is clear that GLP-1, exendin-4, and GLP-1m all have actions beyond the pancreatic islets (Fig. 5). The roles of these peptides on peripheral organs such as the gastrointestinal tract, CNS, and heart appear well established. The roles of GLP-1 and GLP-1m in the liver remain to be clarified and await consensus on the localization of receptors for GLP-1 and GLP-1m on the hepatocyte. Although many of the extrapancreatic effects of GLP-1 appear to be insulinomimetic, it is possible that some mechanisms of action by GLP-1 and/or GLP-1m are independent of insulin-regulated pathways. That GLP-1 has a broad range of effects in nutrient metabolism and energy balance is now clear.

FIG. 5. GLP-1 actions on peripheral tissues. GLP-1 acts directly on the endocrine pancreas, heart, stomach, and brain, whereas actions on liver and muscle are direct and/or indirect (45).

It is also clear that an exciting spectrum of possible therapeutic applications is rapidly emerging.

Acknowledgments

The authors acknowledge with thanks the assistance of Ms. Melissa Scudder in the preparation of the manuscript. We gratefully acknowledge the support of the Alan McGavin Medicine Endowment of the University of British Columbia.

Address all correspondence and requests for reprints to: Dariush Elahi, Ph.D., Departments of Surgery and Medicine, Johns Hopkins Bayview Medical Center, 4940 Eastern Avenue, Baltimore, Maryland 21224-2780. E-mail: delahi1@jhmi.edu.

This work has been supported in part by National Institutes of Health Grants AG 00599 and AG 623175.

Disclosure Summary: The authors have nothing to disclose.

References


77. Yamamoto H, Lee CE, Marcus JN, Williams TD, Overton JM, Lopez ME, Barragán JM, Rodríguez RE, Blázquez E
76. Ban K, Noyan-Ashraf MH, Hoefer J, Bolz SS, Drucker DJ, Husain M
66. Elahi D, Nagulesparan M, Hershcopf RJ, Muller DC, Tobin JD, Blix PM, Salehi M, Vahl TP, D’Alessio DA
65. Bose AK, Mocanu MM, Carr RD, Yellon DM
61. Zhao T, Parikh P, Bhashyam S, Bolukoglu H, Poornima I, Shen YT, Shannon RP
58. Zhao T, Parikh P, Bhashyam S, Bolukoglu H, Poornima I, Shen YT, Shannon RP
59. Bose AK, Mocanu MM, Carr RD, Yellon DM
52. Zhou T, Zhao T, Parikh P, Bhashyam S, Bolukoglu H, Poornima I, Shen YT, Shannon RP
49. Zhou T, Zhao T, Parikh P, Bhashyam S, Bolukoglu H, Poornima I, Shen YT, Shannon RP
47. Zhou T, Zhao T, Parikh P, Bhashyam S, Bolukoglu H, Poornima I, Shen YT, Shannon RP
44. Zhou T, Zhao T, Parikh P, Bhashyam S, Bolukoglu H, Poornima I, Shen YT, Shannon RP
43. Zhou T, Zhao T, Parikh P, Bhashyam S, Bolukoglu H, Poornima I, Shen YT, Shannon RP
42. Zhou T, Zhao T, Parikh P, Bhashyam S, Bolukoglu H, Poornima I, Shen YT, Shannon RP
41. Zhou T, Zhao T, Parikh P, Bhashyam S, Bolukoglu H, Poornima I, Shen YT, Shannon RP
35. Zhou T, Zhao T, Parikh P, Bhashyam S, Bolukoglu H, Poornima I, Shen YT, Shannon RP
34. Zhou T, Zhao T, Parikh P, Bhashyam S, Bolukoglu H, Poornima I, Shen YT, Shannon RP


108. Egan JM, Margolskee RF 2008 Taste cells of the gut and gastrointestinal chemosensation. Mol Interv 8:78–81


