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Glucagon-like peptide-1 and glucagon-like peptide-2

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The glucagon-like peptides (glucagon-like peptide-1 (GLP-1) and glucagon-like peptide-2 (GLP-2)) are released from enteroendocrine cells in response to nutrient ingestion. GLP-1 enhances glucose-stimulated insulin secretion and inhibits glucagon secretion, gastric emptying and feeding. GLP-1 also has proliferative, neogenic and antiapoptotic effects on pancreatic β -cells. More recent studies illustrate a potential protective role for GLP-1 in the cardiovascular and central nervous systems. GLP-2 is an intestinal trophic peptide that stimulates cell proliferation and inhibits apoptosis in the intestinal crypt compartment. GLP-2 also regulates intestinal glucose transport, food intake and gastric acid secretion and emptying, and improves intestinal barrier function. Thus, GLP-1 and GLP-2 exhibit a diverse array of metabolic, proliferative and cytoprotective actions with important clinical implications for the treatment of diabetes and gastrointestinal disease, respectively. This review will highlight our current understanding of the biology of GLP-1 and GLP-2, with an emphasis on both well-characterized and more novel therapeutic applications of these peptides.

Key words: diabetes; obesity; food intake; intestinal disease; cell proliferation; apoptosis; insulin secretion.

Glucagon-like peptide-1 (GLP-1) is an incretin hormone that regulates blood glucose level through its combined actions on the stimulation of glucose-dependent insulin secretion and the inhibition of glucagon secretion, gastric emptying and food intake.¹ GLP-1 also increases β -cell mass via a stimulation of β -cell proliferation and neogenesis, and an inhibition of β -cell apoptosis.² The observation that the pharmacological

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administration of GLP-1 and related analogues can reduce elevated fasting and postprandial blood glucose levels in diabetic human subjects has generated intense interest in the development of GLP-1R agonist-based therapies for the treatment of diabetes mellitus.

GLP-2 is a 33 amino acid peptide that regulates energy homeostasis via acute and chronic effects on gut motility, and nutrient ingestion and absorption. In rodents, exogenous GLP-2 administration promotes the growth and survival of epithelial cells within the small and large bowel mucosa via an inhibition of apoptotic cell death and a stimulation of cellular proliferation.¹ GLP-2 also enhances barrier function and increases the resistance to and recovery from a variety of experimental models of gut injury.¹ The reported actions of GLP-2 have fostered the initiation of clinical studies to assess the ability of GLP-2 to improve nutrient absorption and epithelial integrity and restore functional bowel mass in human patients with intestinal disease.

The actions of GLP-1 and GLP-2 are mediated via unique G protein-coupled receptors. The GLP-2 receptor (GLP-2R) is expressed in a highly tissue-specific manner, predominantly in the gastrointestinal tract and brain.³ In contrast, the GLP-1 receptor (GLP-1R) has a more widespread distribution and is expressed in a number of tissues, including the pancreas, intestine, stomach, central nervous system (CNS), heart, pituitary, lung and kidney.⁴⁻⁶

Despite the potential therapeutic benefits of GLP-1 and GLP-2, the durations of their action are limited owing to a rapid inactivation of these peptides by the ubiquitous protease dipeptidyl peptidase-IV (DPP-IV). Consequently, the inhibition of DPP-IV activity or the development of DPP-IV-resistant glucagon-like peptide analogues offers additional therapeutic options for treating human disease.

SYNTHESIS, SECRETION AND METABOLISM OF GLP-1 AND GLP-2

GLP-1 and GLP-2 are co-encoded within the proglucagon gene, which, in mammals, gives rise to a single mRNA transcript that is expressed in the α -cells of the endocrine pancreas, in the enteroendocrine L-cells of the intestine and in the hypothalamus and brainstem in the CNS.^{7,8} The proglucagon mRNA is translated into a single 160 amino acid precursor protein that undergoes tissue-specific post-translational processing to produce several biologically active proglucagon-derived peptides (PGDPs), including glucagon in the pancreatic α -cells and glicentin, oxyntomodulin, GLP-1 and GLP-2 in the intestine and brain (Figure 1). Glucagon is the major counter-regulatory hormone to insulin and is essential for maintaining blood glucose levels in the physiological range during the post-absorptive state. Oxyntomodulin has inhibitory effects on gastrointestinal secretion and motility, and stimulatory effects on pancreatic enzyme secretion and intestinal glucose uptake.⁹ More recently, it has been demonstrated that oxyntomodulin can reduce food intake in both rodents and humans.^{10,11} In contrast, the physiological actions of glicentin are poorly defined. GLP-1 and GLP-2 exhibit trophic effects in the pancreas and intestine, respectively, and play important roles in the regulation of nutrient assimilation and energy homeostasis (see below).

The PGDP sequences within the proglucagon precursor are flanked by pairs of basic amino acids, and the post-translational processing of proglucagon is carried out by prohormone convertases, which are endoproteolytic enzymes that cleave C-terminal to paired basic amino acid residues.¹² Although the prohormone convertase enzymes responsible for the production of GLP-1 and GLP-2 in the CNS have not been

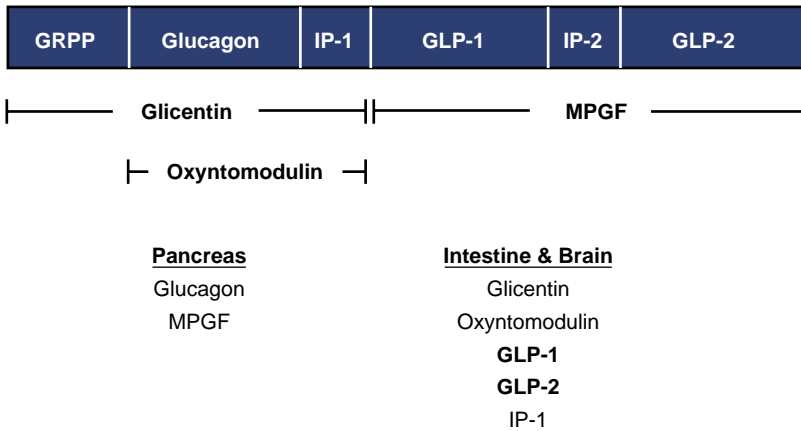


Figure 1. Mammalian proglucagon structure and tissue-specific post-translational processing of the proglucagon-derived peptides. GRPP, glicentin-related polypeptide; IP-1 and IP-2, intervening peptide-1 and -2; MPGF, major proglucagon fragment; GLP-1 and GLP-2, glucagon-like peptide-1 and -2.

definitively established, prohormone convertase 1/3 has been localized to intestinal L-cells and is both necessary and sufficient for the post-translational processing of proglucagon to GLP-1 and GLP-2.¹³ In contrast, prohormone convertase 2 is expressed in the islet α -cell and is essential for the generation of 29 amino acid pancreatic glucagon.¹⁴

GLP-1 and GLP-2 are secreted in a 1:1 ratio from intestinal L-cells¹⁵, the majority of which are located in the distal ileum and colon.¹⁶ The major stimulus for GLP-1 and GLP-2 secretion is the ingestion of nutrients, including glucose, fatty acids and dietary fibre.¹⁷ Fasting plasma levels of the biologically active forms of GLP-1 and GLP-2 in healthy humans are 5–10 and 15–20 pM, respectively, and increase 2–5-fold following food ingestion, the absolute peak level being dependent on the size and nutrient composition of the meal.^{18,19} When nutrients are ingested, the release of GLP-1 and GLP-2 into the circulation occurs in a bi-phasic manner, consisting of a rapid (within 10–15 minutes) early phase followed by a more prolonged (30–60 minutes) second phase.²⁰ The distal location of most L-cells that produce GLP-1 and GLP-2 makes it unlikely that the rapid nutrient-stimulated increase in plasma levels of these peptides is due to a direct effect of nutrients on the L-cell. Indeed, studies in rodents and humans clearly indicate that the vagus nerve, the neurotransmitter gastrin-releasing peptide and the hormone glucose-dependent insulinotropic peptide all contribute to the rapid release of GLP-1 and GLP-2 from distal L-cells in response to nutritional stimuli.¹⁷ In contrast, the second phase of peptide secretion probably results from a direct stimulation of the L-cell by digested nutrients.²¹ Thus, nutrient-induced stimulatory signals are transmitted to intestinal L-cells indirectly, via neural and endocrine effectors, and also by direct interaction with these cells, to mediate the first and second phase, respectively, of GLP-1 and GLP-2 secretion.

The half-life of circulating biologically active GLP-1 is less than 2 minutes²², whereas GLP-2 is more stable, with a half-life of approximately 5–7 minutes.^{23,24} The relatively short circulating half-lives of the bioactive forms of these peptides can be attributed to renal clearance and enzymatic inactivation. DPP-IV, a serine protease that cleaves dipeptides from the amino terminus of oligopeptides or proteins that have a proline or

alanine residue in the penultimate position²⁵, is a critical determinant of GLP-1/GLP-2 degradation. DPP-IV cleaves GLP-1 and GLP-2 at the alanine residue in position 2, yielding the inactive peptides GLP-1 (9–37/36NH₂) and GLP-2 (3–33). DPP-IV expression is fairly widespread and is found on the surface of circulating white blood cells and in cells constituting the vascular endothelium of the small intestine, adjacent to the sites of GLP-1 and GLP-2 secretion.^{25,26} Thus, the majority of GLP-1 and GLP-2 entering the portal circulation has already been inactivated by DPP-IV prior to entry into the systemic circulation. The kidney provides the major route of clearance for both GLP-1 and GLP-2²⁷, and patients with uraemia or chronic renal insufficiency have elevated levels of circulating GLP-1 relative to healthy control individuals.^{28,29}

GLP-1 AND GLUCOSE HOMEOSTASIS

GLP-1 elicits multiple actions in the pancreas and in extra-pancreatic tissues that lead to the reduction of blood glucose (Figure 2). The first physiological action to be described for GLP-1 was the augmentation of glucose-stimulated insulin secretion.^{30–32} GLP-1 binds to its specific receptor on the pancreatic β -cell and stimulates insulin secretion through mechanisms that involve an inhibition of ATP-sensitive K⁺ channels (K_{ATP}) and subsequent β -cell depolarization, elevations in intracellular Ca²⁺ level, an inhibition of voltage-dependent K⁺ channels and direct effects on the β -cell exocytotic machinery.^{33,34} The intracellular signalling events that modulate GLP-1-regulated insulin release include activation of the cAMP/protein kinase A (PKA), cAMP/guanine-nucleotide exchange factor and phosphatidylinositol-3 kinase (PI-3K)/protein kinase C (PKC) ζ pathways.^{34–36} Unlike other insulin secretagogues, GLP-1 also promotes insulin gene transcription, mRNA stability and biosynthesis, and thus has the capacity to replenish depleted β -cell insulin stores.^{37,38}

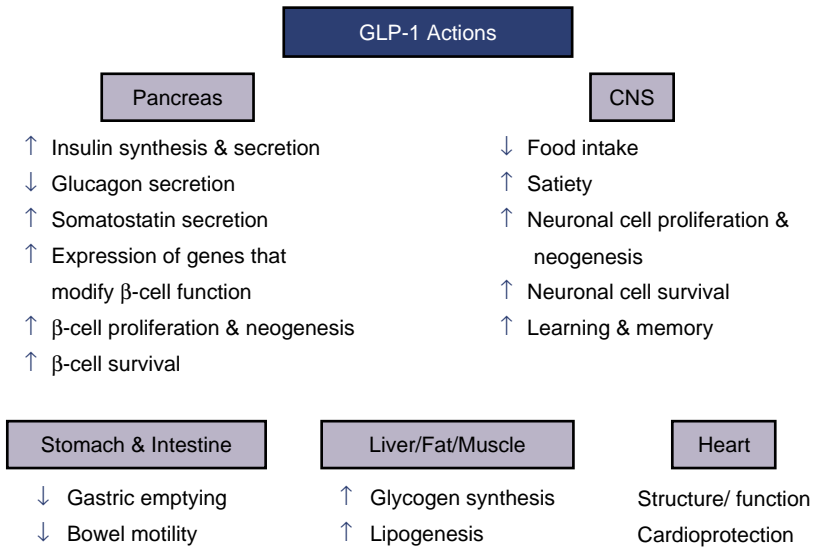


Figure 2. Pancreatic and extra-pancreatic glucagon-like peptide-1 receptor agonist-dependent actions. CNS, central nervous system.

In addition to its ability to stimulate glucose-dependent insulin secretion by direct interaction with the pancreatic β -cell GLP-1R, GLP-1 may also stimulate insulin secretion indirectly via neural mechanisms. It has been estimated that more than half of the GLP-1 secreted from intestinal L-cells is inactivated by DPP-IV, while the majority of the remaining intact peptide is inactivated as it passes through the liver.^{26,39} It is thus likely that only small amounts of bioactive GLP-1 actually reach the pancreas intact. Studies using ganglionic blockers in rats or chemical denervation experiments in mice following capsaicin treatment suggest that endogenously released GLP-1 can stimulate insulin secretion in part by a sensory neural reflex that probably initiates in the hepatoportal system.^{40,41}

GLP-1 can also increase the steady-state levels of mRNA transcripts for key components of the molecular machinery involved in β -cell exocytosis, such as the sulphonylurea receptor and the inwardly rectifying potassium channel (Kir 6.2), molecular subunits of the β -cell K_{ATP} channel. Similarly, GLP-1 also regulates β -cell K_{ATP} channel function via the prevention of glucose-dependent inhibition of K_{ATP} channel activity.⁴²

Studies in both rodents and humans reveal that GLP-1 can improve the ability of the β -cell to sense and respond to glucose, thereby conferring glucose sensitivity to previously resistant β -cells.^{43,44} A potential mechanism whereby GLP-1 could restore β -cell glucose responsiveness is suggested by studies demonstrating that GLP-1 can upregulate the expression of glucose transporters and glucokinases, components of the β -cell glucose sensor.^{45,46}

GLP-1 also lowers blood glucose levels by inhibiting the secretion of glucagon.⁴⁷ The inhibitory effects of GLP-1 on glucagon secretion may occur through direct interaction with GLP-1 receptors on pancreatic α -cells⁴⁸ or indirectly through GLP-1-mediated stimulation of insulin and/or somatostatin secretion.⁴⁹

Both animal and human studies demonstrate that GLP-1 delays gastric emptying and intestinal motility, thereby slowing the transit of nutrients from the stomach to the small intestine and attenuating meal-associated elevations in plasma glucose levels.^{50,51} The inhibitory effects of GLP-1 on the gut are likely to involve both CNS- and intestinal-derived GLP-1.⁵² The mechanism whereby peripheral GLP-1 inhibits gastrointestinal motility appears to involve either direct interaction with CNS centres that regulate visceral motility or an indirect mechanism via vagal afferent pathways.⁵²

GLP-1 may also regulate glucose disposal through peripheral actions on liver, skeletal muscle and adipose tissue. GLP-1 has been shown to increase glucose incorporation into glycogen in isolated rat hepatocytes and skeletal muscle⁵³, and enhance insulin-stimulated glucose metabolism in 3T3 L1 adipocyte cultures and isolated rat adipocytes.^{54,55} However, subsequent studies have failed to support a direct extra-pancreatic role for GLP-1 in liver, muscle or adipose tissue.^{56,57} Whether GLP-1 has direct effects on glucose disposal independent of changes in the levels of islet hormones in humans remains unclear. A number of studies in healthy and diabetic humans suggest that GLP-1 can increase glucose disappearance, independent of insulin and glucagon^{58–60}; in contrast, other studies indicate that GLP-1 has no direct influence on glucose disposition.^{61–65} Recent evidence suggests that the effects of GLP-1 may involve a suppression of endogenous glucose production rather than an increase in peripheral glucose disposal.⁶⁶ The mechanism whereby GLP-1 could mediate these extra-pancreatic effects in humans, independent of changes in the insulin:glucagon ratio, remains uncertain. There is no consistent evidence from rodent or human studies that GLP-1 receptors are present in liver, fat or muscle tissues.^{67–71} Since GLP-1R binding,

signal transduction pathways and the effects of GLP-IR agonists and antagonists in these non-pancreatic cell types differ from those observed with the known pancreatic GLP-IR, it seems likely that GLP-I may mediate its effects in extra-pancreatic tissues via a 'second' or related GLP-I receptor.⁷²⁻⁷⁶

The importance of GLP-I as a physiological regulator of glucose homeostasis is illustrated by studies in which GLP-I action has been reduced or eliminated. Inhibition of GLP-I activity with exendin (9-39), a specific GLP-IR antagonist, leads to impaired glucose tolerance and diminished levels of glucose-stimulated insulin in rodent, baboon and human studies.⁷⁷⁻⁷⁹ Similarly, mice with a targeted genetic disruption of the GLP-I receptor (GLP-IR^{-/-}) are characterized by mild fasting hyperglycaemia, glucose intolerance in the face of an oral or intraperitoneal glucose challenge, and decreased circulating levels of insulin following glycaemic stimulation.⁸⁰ Moreover, the relative importance of basal GLP-I levels for glucoregulation is demonstrated in human studies in which the administration of exendin (9-39) produces significant elevations in the levels of fasting glucose and glucagon, suggesting that GLP-I may have a tonic inhibitory effect on the α -cell.⁸¹

More recent experiments have demonstrated that GLP-I has proliferative and neogenic effects on pancreatic β -cells. Studies with the INS-1 β -cell line demonstrate that GLP-I can activate the expression of immediate early genes, whose products are transcription factors that play a role in genetic programmes regulating islet cell proliferation and differentiation.^{82,83} The treatment of islet hormone-negative AR42J pancreatic exocrine cells with GLP-I promotes their conversion into cells that produce and secrete insulin in a glucose-dependent manner.⁸⁴ Moreover, the treatment of rat and human pancreatic ductal cell lines with GLP-I causes them to differentiate into cells exhibiting endocrine properties.^{85,86} GLP-I has also been shown to accelerate both the differentiation and maturation of human and porcine fetal islet cells.^{87,88} In both normal and diabetic rodents, short-term treatment with GLP-IR agonists leads to improved glucose tolerance, enhanced β -cell proliferation and neogenesis, leading to increased β -cell mass.⁸⁹⁻⁹¹ In db/db mice and Goto-Kakizaki rats, rodent models of type 2 diabetes, GLP-IR agonist administration is associated with increased β -cell mass and a delayed onset of diabetes.^{92,93} Moreover, GLP-IR agonist administration during the prediabetic neonatal period prevented the development of adult-onset diabetes in rats following experimentally induced intrauterine growth retardation.⁹⁴ A key feature revealed by these animal studies is that the beneficial effects of GLP-IR agonists on glucose homeostasis may be sustained for prolonged periods following the suspension of GLP-IR agonist treatment.

In addition to stimulating cell proliferation and differentiation, GLP-I has also been shown to have cytoprotective effects. GLP-I reduces apoptosis in rodent islets and islet cell lines exposed to cytotoxic agents^{95,96} and preserves morphology, improves glucose-stimulated insulin secretion and inhibits apoptosis in freshly isolated human islets.⁹⁷ Similarly, GLP-I reduces the lipotoxic effects of fatty acids in both human islets and rodent cell lines.⁹⁸ GLP-IR agonist administration was associated with proliferative and anti-apoptotic effects in both the endocrine and exocrine compartments of the pancreas in Zucker diabetic rats⁹⁹ and significantly reduced the number of apoptotic β -cells in db/db mice as well as following streptozotocin administration to wildtype mice.^{92,96}

A direct role for the GLP-IR in the proliferative, neogenic and anti-apoptotic actions of GLP-I is supported by studies examining GLP-I-dependent effects in the presence of the GLP-IR antagonist exendin (9-39) in normal cells and rodents, or in separate studies of GLP-IR^{-/-} mice. Exendin (9-39) blocks the GLP-IR agonist-mediated

differentiation of human pancreatic ductal cells⁸⁶ and inhibits the anti-apoptotic effects of GLP-1 in the MIN6 β -cell line.⁹⁵ GLP-1R^{-/-} mice have fewer large β -cell clusters, and GLP-1R^{-/-} islets exhibit an altered α -cell topography¹⁰⁰, display an impaired ability to regenerate their β -cell mass following partial pancreatectomy¹⁰¹ and manifest an increased susceptibility to streptozotocin-induced apoptosis.⁹⁶

The signal-transduction pathways downstream of GLP-1R activation coupled to stimulation of cell proliferation have been examined in islets and islet cell lines and appear to include trans-activation of the epidermal growth factor receptor, which leads to increased PI-3K activity and a subsequent activation of PKC ζ .¹⁰² The precise mechanisms involved in GLP-1-dependent β -cell differentiation/neogenesis are poorly defined but may involve the activation of PKC and mitogen-activated protein kinase.⁸⁴ Notably, a common observation following the GLP-1R agonist treatment of β -cells is an increase in levels of PDX-1, a transcription factor essential for pancreatic development and β -cell function.^{84-87,103,104} The molecular mechanisms implicated in GLP-1-dependent anti-apoptotic pathways appear to be diverse and involve multiple signalling pathways. Studies in rodents and experiments with isolated islets or β -cell lines demonstrate that the anti-apoptotic effects of GLP-1 are associated with reduced levels of the pro-apoptotic markers active caspase-3 and poly-ADP-ribose polymerase cleavage, and an upregulation of anti-apoptotic factors including Bcl-2, Bcl-xL and inhibitor of apoptosis protein-2.^{92,95,98,99,105} The cumulative experimental evidence indicates that the intracellular signalling pathways that mediate GLP-1-dependent cytoprotective effects include:

1. cAMP/PKA;
2. PI-3K-dependent activation of the prosurvival kinase Akt/protein kinase B;
3. activation of nuclear factor-kappa B activity;
4. cAMP/PKA-mediated phosphorylation and activation of cAMP response element binding protein (CREB), subsequent CREB-dependent activation of insulin receptor substrate-2 and induction of Akt/protein kinase B.^{92,98,106,107}

As β -cell mass is reduced and β -cell apoptosis is increased in autopsy studies of pancreata from patients with type 2 diabetes¹⁰⁸, the ability of GLP-1 to promote β -cell proliferation and neogenesis, and inhibit β -cell apoptosis, raises the possibility that GLP-1 therapy has the potential to preserve or even restore functional β -cell mass in diabetic individuals.

GLP-1 AND FEEDING BEHAVIOR

Numerous studies in rodents have demonstrated that the central (intracerebroventricular) or peripheral administration of GLP-1R agonists leads to the inhibition of food intake and reductions in body weight.¹⁰⁹⁻¹¹¹ Moreover, GLP-1 also inhibits food intake and promotes satiety in normal, obese and diabetic humans¹¹²⁻¹¹⁴, suggesting that GLP-1 could play an important role in controlling appetite and body weight. However, GLP-1R^{-/-} mice exhibit normal feeding behaviour and body weight, indicating that GLP-1R signalling may not be essential for the regulation of satiety and maintenance of body weight.⁸⁰

There is considerable interest in how GLP-1 mediates its effects on ingestive behaviour, and experimental evidence indicates that GLP-1 probably modifies food

intake via a number of different mechanisms. It has been proposed that the inhibitory effects of GLP-I on food intake can be mediated indirectly, by its ability to slow gastric emptying, thereby promoting gastric distension and a sensation of satiety. In addition, GLP-I receptors are expressed in the hypothalamus and nucleus of the solitary tract (NTS), CNS regions that are thought to be important for regulating appetite and satiety.^{5,115} Thus, GLP-I could also modify feeding by direct interaction with these CNS centres. Alternatively, it has been suggested that the inhibitory effect on food intake could reflect a secondary physiological response to the GLP-I-dependent activation of aversive signalling pathways that produce visceral illness.^{116–118}

Binding sites or receptors for GLP-I have been detected in numerous CNS regions, including the hypothalamus, NTS, subfornical organ (SFO) and area postrema (AP).^{5,115,119,120} The SFO and AP represent blood–brain barrier-free CNS locations and have also been shown to play a role in the regulation of feeding.¹²¹ Although GLP-I is produced in CNS neurons located in the NTS, studies in rats have demonstrated that peripherally administered GLP-I can also gain access to the CNS through interaction with GLP-I receptors in the SFO and AP.¹²² However, the relative importance of peripheral versus central GLP-I for appetite regulation remains to be elucidated.

GLP-I AND THE CARDIOVASCULAR SYSTEM

Studies using anaesthetized rats or conscious calves and central or intravenous GLP-I administration are associated with increases in heart rate and systolic, diastolic and mean arterial blood pressure.^{123,124} Consistent with these effects, GLP-I receptors are expressed in the heart and in the NTS and AP, CNS regions known to regulate cardiovascular function.^{125–127} The stimulatory effects of GLP-I on the rat cardiovascular system are independent of catecholamine action, are blocked by administration of the GLP-IR antagonist exendin (9–39) and appear to be mediated by both direct and indirect GLP-IR-dependent effects on the nervous system and heart.¹²⁴ More recent studies using telemetric monitoring to measure heart rate and blood pressure in freely moving rats demonstrated that GLP-IR-dependent increases in heart rate and blood pressure are associated with the activation of (i) neuronal activity in several autonomic control regions of the rat CNS, (ii) GLP-I-sensitive hypothalamic and medullary catecholamine neurons that innervate sympathetic preganglionic neurons, and (iii) neuronal activity in the adrenal medulla, suggesting that central GLP-I regulates cardiovascular function by activating the sympathetic nervous system.¹²⁸ An essential role for GLP-IR signalling in the maintenance of normal cardiac structure and function is demonstrated in studies using GLP-IR^{-/-} mice. GLP-IR^{-/-} hearts are characterized by increased septal and posterolateral myocardial wall thickness, and GLP-IR^{-/-} mice display abnormal cardiac haemodynamic responses to external stress.¹²⁹

In contrast to its stimulatory effects on the cardiovascular system in animals, limited studies in humans indicate that GLP-IR agonist administration has no significant effects on heart rate or blood pressure.^{130–132} Moreover, GLP-I may also have cardioprotective effects under certain conditions. A 14 day infusion of GLP-I prevents the development of hypertension, improves endothelial function and reduces renal and cardiac damage in Dahl salt-sensitive rats maintained on a high-salt diet.¹³³ The antihypertensive effect of GLP-I in these hypertension-prone rats is attributed

to a GLP-1-dependent increase in salt and water excretion¹³³, actions recently demonstrated in human subjects.¹³⁴ Additionally, a 72 hour infusion of GLP-1 in patients with acute myocardial infarction and severe left ventricular systolic dysfunction following successful reperfusion with primary angioplasty led to improved regional and global left ventricular function and was associated with reduced in-hospital mortality rate and length of hospitalization.¹³⁵

GLP-1 AND NEUROPROTECTION

Comparable to actions observed in studies of pancreatic β -cells, GLP-1 also exerts proliferative, neogenic and anti-apoptotic effects on neuronal cells. GLP-1R agonist treatment of PC12 cells stimulates neurite outgrowth, enhances nerve growth factor-induced differentiation and improves cell survival after nerve growth factor withdrawal.¹³⁶ GLP-1R agonists prevent glutamate-induced apoptosis in cultured rat hippocampal neurons and restore cholinergic marker activity in the basal forebrain of ibotenic acid-treated rats, a rodent model of neurodegeneration.¹³⁷ Furthermore, GLP-1R-activated pathways seem to be important for learning and memory. GLP-1R agonist administration is associated with enhanced learning in rats, an effect that can be blocked by the co-administration of exendin (9–39).¹³⁸ In contrast, GLP-1R^{-/-} mice exhibit deficits in learning that can be overcome by hippocampal *Glp1r* gene transfer.¹³⁸ GLP-1R^{-/-} mice are also more susceptible to kainate-induced seizures and hippocampal neuronal degeneration, whereas GLP-1R agonist treatment prevents kainate-induced apoptosis in wildtype animals.¹³⁸ These observations have led to the suggestion that GLP-1R agonists may potentially be useful for the treatment of neurological disorders, including the neuropathy that results as a secondary complication of diabetes.¹³⁹ In contrast, endogenous GLP-1 has also been implicated in the pathogenesis of β -amyloid protein-induced neurotoxicity as a continuous co-infusion of a GLP-1R antagonist with β -amyloid protein prevented memory impairment and hippocampal apoptosis in rats.¹⁴⁰

GLP-1 AND THE TREATMENT OF TYPE 2 DIABETES

Numerous studies have demonstrated that GLP-1 can enhance glucose-stimulated insulin secretion and lower fasting and postprandial blood glucose levels in individuals with type 2 diabetes. The administration of GLP-1 by continuous subcutaneous infusion for 6 weeks increased insulin secretion, reduced fasting and postprandial glucose levels, lowered haemoglobin A_{1c} (HbA_{1c}) values, and decreased food intake and body weight in patients with type 2 diabetes¹⁴¹, indicating that GLP-1 retains its effectiveness, even with continuous long-term treatment. As obesity can be a contributing factor to the pathogenesis of diabetes, the ability of GLP-1 to reduce food intake and promote satiety and weight loss provides an additional means for improving glycaemia in these individuals. GLP-1 also lowers fasting and postprandial glucose and reduces the meal-associated insulin requirement in human patients with type 1 diabetes, probably via its inhibitory effects on glucagon secretion and/or gastric emptying.^{142–144} Moreover, the insulinotropic and glucagonostatic effects of GLP-1 are glucose dependent^{145–147}; thus under conditions of normoglycaemia, GLP-1 has no effect on the levels of plasma insulin or glucagon.

The ability of GLP-I to lower blood glucose levels and promote weight loss, combined with its potential to preserve or restore functional β -cell mass, has generated considerable interest in the use of GLP-I as a therapeutic agent for the treatment of diabetes. Moreover, the observation that GLP-I secretion is deficient in type 2 diabetics suggests that the restoration of normal GLP-I concentrations in these patients may be beneficial.^{19,148} However, because the plasma half-life of native GLP-I is very short, owing to its rapid inactivation by DPP-IV, continuous infusion or multiple injections of GLP-I are required to attain adequate glycaemic control. Consequently, alternative therapeutic strategies have been devised focused on the generation of GLP-I analogues that are resistant to DPP-IV and the development of compounds that inhibit DPP-IV activity.

DPP-IV-RESISTANT GLP-IR AGONISTS AND DPP-IV INHIBITION AS ALTERNATIVE THERAPEUTIC STRATEGIES

A number of structurally unique GLP-IR agonists have been developed with prolonged activity such that once- or twice-daily injections of these molecules are potentially as efficacious as continuous GLP-I infusion for the treatment of experimental or clinical diabetes. Exendin-4 is a naturally occurring, DPP-IV-resistant GLP-IR agonist originally isolated from the venom of the *Heloderma suspectum* lizard¹⁴⁹, and exenatide is a synthetic version of exendin-4. A single subcutaneous injection of exenatide in patients with poorly controlled type 2 diabetes significantly reduced fasting and postprandial glucose concentrations, in association with increased levels of plasma insulin and decreased plasma glucagon.¹⁵⁰ Four weeks of twice-daily exendin-4/exenatide treatment significantly reduced postprandial glucose and HbA_{1c} levels¹⁵¹, and, when used in combination with metformin and/or sulphonylurea treatment, also lowered postprandial plasma glucose and HbA_{1c} in diabetic patients with poor glycaemic control.¹⁵² The results of recently completed phase 3 clinical trials demonstrated that the exenatide treatment of subjects previously treated with metformin and/or sulphonylureas, significantly reduced HbA_{1c} levels and prevented weight gain in patients with type 2 diabetes.

Liraglutide, is a fatty-acyl-derivatized, DPP-IV-resistant human GLP-I analogue. Addition of the fatty acid group enables non-covalent binding to serum albumin, thereby prolonging the circulating half-life and reducing renal clearance. In humans, the half-life of liraglutide is approximately 12 hours, suggesting that a once-daily dosing regimen may be all that is required therapeutically. A single subcutaneous injection of liraglutide at bedtime reduces fasting glucose, increases prandial insulin and reduces glucagon secretion, gastric emptying and postprandial glucose excursions in type 2 diabetics.¹⁵² Once-daily injection of liraglutide for 1 week was associated with improved 24 hour glycaemia and islet α - and β -cell function, as well as reduced 24 hour plasma glucagon and endogenous glucose release in diabetic patients.¹⁵³ In more long-term studies, 12 weeks of once-daily liraglutide not only reduced HbA_{1c}, fasting glucose and proinsulin:insulin levels, but also decreased body weight in patients with type 2 diabetes.¹⁵⁴

Additional efforts to prolong GLP-I action in vivo include the development of GLP-IR receptor agonists that form covalent bonds with serum albumin¹⁵⁵ or sustained release formulations of exenatide; however, studies with these molecules

have thus far been limited to preclinical experiments in animals or very preliminary (phase 1/2) human clinical studies.

An alternative approach for enhancing the action of GLP-1 for the treatment of diabetes involves the prevention of GLP-1 degradation by inhibiting the activity of the enzyme DPP-IV. The physiological importance of DPP-IV for glucose regulation is exemplified by an analysis of mice with a targeted disruption of the DPP-IV gene that exhibit enhanced glucose clearance, increased insulin secretion, resistance to diet-induced obesity and augmented levels of GLP-1 following glucose challenge.^{156,157} Several orally active agents that inhibit DPP-IV activity are currently under evaluation. Numerous studies using both normal and diabetic animals clearly demonstrate that the acute pharmacological inhibition of DPP-IV activity increases both endogenous and exogenous plasma levels of intact, biologically active GLP-1 and leads to enhanced insulin secretion and improved glucose tolerance.^{158–162} In longer-term studies, 1 week of twice-daily oral treatment with the DPP-IV inhibitor P32/98 improved glucose tolerance, increased pancreatic insulin content and stimulated islet neogenesis and β -cell survival in streptozotocin-induced diabetic rats¹⁶³, whereas 12 weeks of a similar treatment regimen was associated with sustained improvements in glucose tolerance, β -cell function and peripheral insulin sensitivity in VDF Zucker rats, a rodent model of type 2 diabetes.^{164,165}

In comparison, published human studies with DPP-IV inhibitors are more limited. In healthy males, a single-dose escalation study with P32/98 led to increased circulating levels of intact, bioactive GLP-1 and improved oral glucose tolerance.¹⁶⁶ In a 4 week study, the DPP-IV inhibitor NVP DPP728, administered two or three times a day, reduced fasting and postprandial blood glucose levels and decreased HbA_{1c} values in patients with relatively mild type 2 diabetes.¹⁶⁷ Similarly, once-daily administration of the DPP-IV inhibitor LAF237 improved glycaemic excursions, reduced fasting glucose and decreased meal-stimulated levels of plasma glucagon in a 4 week study.¹⁶⁸ Despite the promising therapeutic potential of DPP-IV inhibition for the treatment of type 2 diabetes, DPP-IV has been implicated in the co-stimulation and activation of T cells.¹⁶⁹ Furthermore, in addition to GLP-1 and GLP-2, DPP-IV has a wide variety of substrates encompassing neuropeptides, peptide hormones and chemokines.²⁵ Thus, the relative safety of long-term use of DPP-IV inhibitors in humans clearly necessitates ongoing investigation.

GLP-2

Injury to the gastrointestinal tract is associated with a diverse number of changes designed to optimize nutrient absorption and preserve energy retention (intestinal adaptation). Multiple lines of evidence implicate one or more PGDPs in the hormonal response to gut injury. Experimental intestinal injury is associated with increased levels of circulating PGDPs, and an index patient with a glucagon-producing tumour presented with a massively enlarged small bowel, thereby indirectly implicating one or more PGDPs as the agent responsible for bowel growth. This patient exhibited intestinal villous hyperplasia together with reduced gut motility, both features completely resolving following resection of the enteroglucagon-producing tumour.¹⁷⁰

Subsequent studies of the trophic properties of the PGDPs have demonstrated that both glicentin and GLP-2 increase small bowel mass in mice.¹⁷¹ Nevertheless,

GLP-2 was significantly more potent, relative to glicentin, consistent with earlier studies that failed to show a significant diminution of endogenous intestinal adaptation following the immunoneutralization of glicentin in bowel-resected rats.¹⁷²

The sequence of GLP-2 is conserved within all mammalian proglucagon genes characterized to date, although not as well as that of GLP-1, which exhibits 100% amino acid identity in most mammalian proglucagon genes.¹⁷³ Following the demonstration that a twice-daily administration of GLP-2 significantly increased bowel growth and nutrient absorption in mice^{171,174}, the intestinotrophic activity of GLP-2 was examined in rats. Surprisingly, although significant increases in mucosal villous height were observed in GLP-2-treated rats, small bowel mass was not significantly increased following subcutaneous GLP-2 administration to normal rats¹⁷⁵, although a continuous infusion of GLP-2 did increase mucosal DNA content and both galactose and glycine absorption in rats.¹⁷⁶ Subsequent studies revealed that GLP-2, which contains an N-terminal alanine at position 2, was rapidly degraded by the ubiquitously expressed protease DPP-IV¹⁷⁵, the same enzyme previously shown to degrade GLP-1 and glucose-dependent insulintropic polypeptide.¹⁷⁷

Consistent with the importance of DPP-IV for the control of GLP-2 activity, the native GLP-2 molecule was significantly more intestinotrophic following administration to rats harbouring an inactivating mutation in the DPP-IV gene.¹⁷⁵ Furthermore, GLP-2 molecules containing amino acid substitutions that conferred resistance to DPP-IV-mediated degradation exhibited a significantly longer circulating half-life in vivo and considerably greater intestinotrophic activity in wildtype rats.¹⁷⁵ Although the repeated administration of a DPP-IV inhibitor to rats and mice did not enhance the intestinotrophic properties of endogenous GLP-2, the co-administration of exogenous GLP-2 together with a DPP-IV inhibitor augmented the intestinotrophic response beyond that seen with GLP-2 alone.¹⁷⁸

Studies demonstrating the trophic effects of GLP-2 on the small bowel of normal rodents^{171,174,179,180} were followed by experiments examining the effects of GLP-2 in rodent models of gut injury (Table 1). The intravenous infusion of GLP-2 prevented the development of small bowel mucosal villous hypoplasia in rats maintained on parenteral nutrition.^{181–183} Similarly, the co-infusion of GLP-2 together with parenteral nutrition resulted in increased mucosal mass, protein and DNA content in the small but not the large bowel of tumor-bearing rats; however, no significant effects of GLP-2 were observed on tumor growth after an 8 day treatment period.¹⁸⁴ The increased thickness of the small bowel mucosa observed following

Table 1. Effects of glucagon-like peptide-2 in rodent models of gut injury.

Disease	Experimental model	Species
Colitis	Dextran sulphate	Mice
Short bowel syndrome	MSBR	Rats
Small bowel enteritis	Non-steroidal anti-inflammatory agents, genetic	Mice, rats
Intestinal mucositis	Chemotherapy	Mice, rats
Allergic enteritis	Immune sensitivity	Mice
Ischaemic enteritis	Superior mesenteric artery occlusion	Rats

the development of experimental rodent diabetes correlates with increased circulating levels of GLP-2¹⁸⁵ and is partially attenuated by the administration of GLP-2 immunoneutralizing antisera.¹⁸⁶

Whether GLP-2 plays a role in development, growth or maturation of the fetal or neonatal gut remains unclear. Mice with a dominant negative *Pax6* transcription factor mutation fail to develop GLP-2-containing enteroendocrine cells yet exhibit comparatively normal growth of the neonatal small and large bowel.¹⁸⁷ Although GLP-2 and the GLP-2R are expressed during late gestation in premature fetal pigs and rats^{188,189}, immunoreactive GLP-2 was not detected in the plasma of E98 fetal pigs, and exogenous GLP-2 administration did not stimulate intestinal growth in premature fetal pigs.¹⁸⁸ In contrast, the administration of GLP-2 to neonatal pigs or rats produced significant increases in intestinal weight and mucosal thickness. Hence, the GLP-2:GLP-2R axis appears coupled to control of intestinal growth during the neonatal, but not the fetal period.

Multiple models of small bowel injury exhibit some degree of improvement following GLP-2 administration. Indomethacin-induced murine enteritis is associated with septicaemia, mucosal ulceration and a high rate of mortality. The administration of h[Gly2]-GLP-2 prior to, concomitant with or following indomethacin administration markedly reduced mortality in mice, in association with significantly fewer intestinal ulcerations.¹⁹⁰ The protective effects of h[Gly2]-GLP-2 were associated with both an enhanced repair of gut mucosa via the induction of crypt cell proliferation, and a suppression of apoptosis in the crypt compartment of the gut epithelium.¹⁹⁰ GLP-2 administration similarly reduced the gross and histological parameters of mucosal inflammation and cytokine (interferon- γ and interleukin-2) expression in Fischer 344 rats prone to the development of spontaneous enteritis.¹⁹¹

The trophic and cytoprotective activities of GLP-2 have been examined following intestinal resection or experimental burn injury. Circulating levels of GLP-2 are increased following bowel resection^{192,193}, and rats subjected to a major small bowel resection exhibited a significant increase in histological parameters of mucosal growth and sucrase activity in the jejunum following exogenous treatment with a GLP-2 analogue.¹⁹⁴ Similarly, the administration of GLP-2 to burned rats for 5 days increased protein content in the small bowel and reduced immunosuppression, as measured by normalization of the lymphocyte proliferative response to mitogenic stimulation.¹⁹⁵ Significant benefits of GLP-2 on preservation of intestinal epithelium and reduction in mortality have also been noted following experimental intestinal ischaemia induced by superior mesenteric artery occlusion. A 3 day treatment of rats with a GLP-2 analogue increased mucosal DNA and protein content, and reduced mortality from 50 to 25%.^{196,197}

GLP-2 administration has also produced beneficial effects on the gastrointestinal epithelium of rodents treated with chemotherapeutic agents. Administration of h[Gly2]-GLP-2 to 5-fluorouracil-treated rats augmented intestinal recovery and reduced histological parameters of mucosal injury.¹⁹⁸ Similarly, h[Gly2]-GLP-2 significantly improved survival, reduced bacteraemia, attenuated epithelial injury and inhibited crypt apoptosis in the murine gut epithelium after the administration of irinotecan hydrochloride or 5-fluorouracil.¹⁹⁹ Furthermore, the protective effects of h[Gly2]-GLP-2 on survival and weight loss were preserved following the cyclical administration of chemotherapy and h[Gly2]-GLP-2 over a 3 week period in tumour-bearing mice.¹⁹⁹

Although the predominant intestinotrophic effects of GLP-2 are detected in the small bowel, a much weaker but detectable effect of GLP-2 has been observed in the colon of GLP-2-treated animals. The induction of colitis via administration of enteral dextran sulphate produces considerable weight loss and disruption of mucosal epithelial integrity in the colon of mice. Mice receiving twice-daily injections of h[Gly2]-GLP-2 and oral dextran sulphate for 10 days exhibited a marked reduction of weight loss, together with reduced intestinal cytokine expression and a significant improvement in histological parameters of mucosal epithelial integrity.²⁰⁰

The actions of GLP-2 are mediated by a specific GLP-2R²⁰¹, a seven-transmembrane domain G protein-coupled receptor related in sequence to members of the glucagon-secretin G protein-coupled receptor family.²⁰² The rodent and human GLP-2 receptors are coupled to dose-dependent increases in cAMP accumulation in heterologous cell lines transfected with the cognate receptors.^{201,203} Only modest effects on cell proliferation are observed following direct exposure of cells to GLP-2 in vitro.^{203,204}

The GLP-2 receptor has been localized by immunocytochemistry to human enteroendocrine cells²⁰⁵ and by in situ hybridization to murine enteric neurons.²⁰⁶ These findings imply a model for GLP-2 action that invokes the liberation of signals, either from enteroendocrine cells or neurons, which mediate the pleiotropic actions of GLP-2 on the gut epithelium. Given the importance of the anti-apoptotic actions of GLP-2 in experimental models of intestinal injury^{190,199,207}, understanding how GLP-2R activation leads to cell survival may have therapeutic relevance. GLP-2R stimulation robustly inhibits apoptotic pathways in cells expressing the transfected GLP-2R in vitro.^{199,208,209}

SUMMARY

The ability of GLP-1R agonists to regulate blood glucose levels and food intake in humans, combined with their potential to promote β -cell growth and survival, has provoked a considerable amount of interest in examining the potential therapeutic benefits of protracted GLP-1R agonist activity in diabetic individuals. Furthermore, GLP-1R agonists such as exenatide demonstrate considerable efficacy in 6–12 month clinical trials in human subjects with type 2 diabetes. Whether long-term GLP-1R stimulation will also exhibit trophic effects on human β -cells, or produce sustained enhancement of insulin secretion, remains to be determined. Moreover, although continuous GLP-1 infusion has not been associated with tachyphylaxis in diabetic patients¹⁴¹, the ability of longer-term GLP-1R agonist administration to sustain efficacy, after months or years of treatment, is still an open question. Likewise, the chronic effects of GLP-1R stimulation on food intake and body weight are not known. Similarly, although DPP-IV inhibitors appear to represent promising agents for the enhancement of incretin action in diabetic subjects, there is little information about long-term use of these drugs in diabetic humans. Preliminary studies of exogenous GLP-2 administration in human subjects with short-bowel syndrome demonstrated significant improvements in nutrient absorption, fluid retention, body weight and lean body mass.²¹⁰ Whether sustained beneficial effects on nutrient absorption, energy retention and bone mineral density will continue to be observed in larger numbers of patients treated for more extended time periods remains to be determined.

Practice points

- GLP-1 and GLP-2 are secreted in response to nutrient ingestion and may be used therapeutically for the treatment of diabetes and intestinal disease, respectively
- GLP-1 receptor agonists are administered via subcutaneous injection and lower glucose while preventing weight gain in diabetic subjects
- GLP-1R agonists also improve insulin secretion, prevent β -cell death and stimulate the formation of new β -cells, raising the possibility that therapy with these agents might produce clinically meaningful and sustained improvements in β cell function
- augmentation of incretin (GLP-1) action for the treatment of type 2 diabetes may also be achieved by preventing GLP-1 degradation using inhibitors of the enzyme DPP-IV
- GLP-2 enhances nutrient absorption and promotes the restoration of intestinal function, thereby exhibiting therapeutic potential for the treatment of patients with intestinal failure secondary to short-bowel syndrome

Research agenda

- the precise role of GLP-1R agonists in the cardiovascular and central nervous systems, particularly in human subjects, remains unclear
- similarly, although DPP-IV inhibitors have the advantage of being administered orally, their ability to modify the activities of other regulatory peptides and potential effects on immune function, raise theoretical safety concerns with respect to the long-term use of DPP-IV inhibitors in humans
- the observations that GLP-2 reduces gut permeability and enhances barrier function in both normal animals and in the setting of experimental intestinal injury raises the possibility that pharmacological GLP-2 administration might be useful for repairing intestinal barrier function in a number of human gastrointestinal disorders; however, the mechanisms underlying GLP-2 action, and the downstream mediators liberated in response to GLP-2 stimulation that promote gut growth, have not been identified
- furthermore, the physiological importance of endogenously secreted GLP-2 for nutrient absorption, intestinal integrity and mucosal growth remains uncertain

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REFERENCES

1. Drucker DJ. Biological actions and therapeutic potential of the glucagon-like peptides. *Gastroenterology* 2002; **122**: 531–544.
2. Brubaker PL & Drucker DJ. Minireview: glucagon-like peptides regulate cell proliferation and apoptosis in the pancreas, gut, and central nervous system. *Endocrinology* 2004; **145**: 2653–2659.
3. Estall JL & Drucker DJ. Dual regulation of cell proliferation and survival via activation of glucagon-like peptide-2 receptor signaling. *J Nutr* 2003; **133**: 3708–3711.
4. Wei Y & Mojsov S. Distribution of GLP-1 and PACAP receptors in human tissues. *Acta Physiol Scand* 1996; **157**: 355–357.
5. Merchenthaler I, Lane M & Shughrue P. Distribution of pre-pro-glucagon and glucagon-like peptide-1 receptor messenger RNAs in the rat central nervous system. *J Comp Neurol* 1999; **403**: 261–280.
6. Satoh F, Beak SA, Small CJ et al. Characterization of human and rat glucagon-like peptide-1 receptors in the neurointermediate lobe: lack of coupling to either stimulation or inhibition of adenylyl cyclase. *Endocrinology* 2000; **141**: 1301–1309.
7. Drucker DJ & Asa S. Glucagon gene expression in vertebrate brain. *J Biol Chem* 1988; **263**: 13475–13478.
8. Novak U, Wilks A, Buell G et al. Identical mRNA for proglucagon in pancreas and gut. *Eur J Biochem* 1987; **164**: 553–558.
9. Schjoldager B, Mortensen PE, Myhre J et al. Oxyntomodulin from distal gut. Role in regulation of gastric and pancreatic functions. *Dig Dis Sci* 1989; **34**: 1411–1419.
10. Cohen MA, Ellis SM, Le Roux CW et al. Oxyntomodulin suppresses appetite and reduces food intake in humans. *J Clin Endocrinol Metab* 2003; **88**: 4696–4701.
11. Dakin CL, Gunn I, Small CJ et al. Oxyntomodulin inhibits food intake in the rat. *Endocrinology* 2001; **142**: 4244–4250.
12. Seidah NG & Chretien M. Proprotein and prohormone convertases: a family of subtilases generating diverse bioactive polypeptides. *Brain Res* 1999; **848**: 45–62.
13. Dhanvantari S, Seidah NG & Brubaker PL. Role of prohormone convertases in the tissue-specific processing of proglucagon. *Mol Endocrinol* 1996; **10**: 342–355.
14. Furuta M, Yano H, Zhou A et al. Defective prohormone processing and altered pancreatic islet morphology in mice lacking active SPC2. *Proc Natl Acad Sci USA* 1999; **94**: 6646–6651.
15. Orskov C, Holst JJ, Knuhtsen S et al. Glucagon-like peptides GLP-1 and GLP-2, predicted products of the glucagon gene, are secreted separately from pig small intestine but not pancreas. *Endocrinology* 1986; **119**: 1467–1475.
16. Sjolund K, Sanden G, Hakanson R et al. Endocrine cells in human intestine: an immunocytochemical study. *Gastroenterology* 1983; **85**: 1120–1130.
17. Brubaker PL & Anini Y. Direct and indirect mechanisms regulating secretion of glucagon-like peptide-1 and glucagon-like peptide-2. *Can J Physiol Pharmacol* 2003; **81**: 1005–1012.
18. Xiao Q, Boushey RP, Drucker DJ et al. Secretion of the intestinotropic hormone glucagon-like peptide 2 is differentially regulated by nutrients in humans. *Gastroenterology* 1999; **117**: 99–105.
19. Vilsboll T, Krarup T, Deacon CF et al. Reduced postprandial concentrations of intact biologically active glucagon-like peptide 1 in type 2 diabetic patients. *Diabetes* 2001; **50**: 609–613.
20. Hermann C, Goke R, Richter G et al. Glucagon-like peptide-1 and glucose-dependent insulin-releasing polypeptide plasma levels in response to nutrients. *Digestion* 1995; **56**: 117–126.
21. Roberge JN & Brubaker PL. Secretion of proglucagon-derived peptides in response to intestinal luminal nutrients. *Endocrinology* 1991; **128**: 3169–3174.
22. Kieffer TJ, McIntosh CHS & Pederson RA. Degradation of glucose-dependent insulinotropic polypeptide and truncated glucagon-like peptide I in vitro and in vivo by dipeptidyl peptidase IV. *Endocrinology* 1995; **136**: 3585–3596.
23. Tavares W, Drucker DJ & Brubaker PL. Enzymatic and renal-dependent catabolism of the intestinotropic hormone glucagon-like peptide-2 in the rat. *Am J Physiol* 1999; **278**: E134–E139.
24. Hartmann B, Harr MB, Jeppesen PB et al. In vivo and in vitro degradation of glucagon-like peptide-2 in humans. *J Clin Endocrinol Metab* 2000; **85**: 2884–2888.
25. Mentlein R. Dipeptidyl-peptidase IV (CD26)—role in the inactivation of regulatory peptides. *Regul Pept* 1999; **85**: 9–24.

26. Hansen L, Deacon CF, Orskov C et al. Glucagon-like peptide-1-(7–36)amide is transformed to glucagon-like peptide-1-(9–36)amide by dipeptidyl peptidase IV in the capillaries supplying the L cells of the porcine intestine. *Endocrinology* 1999; **140**: 5356–5363.
27. Ruiz-Grande C, Pintado J, Alarcon C et al. Renal catabolism of human glucagon-like peptides I and 2. *Can J Physiol Pharmacol* 1990; **68**: 1568–1573.
28. Orskov C, Andreasen J & Holst JJ. All products of proglucagon are elevated in plasma from uremic patients. *J Clin Endocrinol Metab* 1992; **74**: 379–384.
29. Meier JJ, Nauck MA, Kranz D et al. Secretion, degradation, and elimination of glucagon-like peptide I and gastric inhibitory polypeptide in patients with chronic renal insufficiency and healthy control subjects. *Diabetes* 2004; **53**: 654–662.
- *30. Kreymann B, Williams G, Ghatei MA et al. Glucagon-like peptide-1 7–36: a physiological incretin in man. *Lancet* 1987; **2**: 1300–1304.
- *31. Mojsov S, Weir GC & Habener JF. Insulinotropin: glucagon-like peptide I (7–37) co-encoded in the glucagon gene is a potent stimulator of insulin release in the perfused rat pancreas. *J Clin Invest* 1987; **79**: 616–619.
- *32. Holst JJ, Orskov C, Nielsen OV et al. Truncated glucagon-like peptide I, an insulin-releasing hormone from the distal gut. *FEBS Lett* 1987; **211**: 169–174.
33. Gromada J, Bokvist K, Ding WG et al. Glucagon-like peptide I (7–36) amide stimulates exocytosis in human pancreatic beta-cells by both proximal and distal regulatory steps in stimulus-secretion coupling. *Diabetes* 1998; **47**: 57–65.
34. MacDonald PE, Wang X, Xia F et al. Antagonism of rat beta-cell voltage-dependent K⁺ currents by exendin 4 requires dual activation of the cAMP/protein kinase A and phosphatidylinositol 3-kinase signaling pathways. *J Biol Chem* 2003; **278**: 52446–52453.
35. Gromada J, Holst JJ & Rorsman P. Cellular regulation of islet hormone secretion by the incretin hormone glucagon-like peptide I. *Pflugers Arch* 1998; **435**: 583–594.
36. Kang G, Joseph JW, Chepurny OG et al. Epac-selective cAMP analog 8-pCPT-2'-O-Me-cAMP as a stimulus for Ca²⁺-induced Ca²⁺ release and exocytosis in pancreatic beta-cells. *J Biol Chem* 2003; **278**: 8279–8285.
- *37. Drucker DJ, Philippe J, Mojsov S et al. Glucagon-like peptide I stimulates insulin gene expression and increases cyclic AMP levels in a rat islet cell line. *Proc Natl Acad Sci USA* 1987; **84**: 3434–3438.
38. Fehmman H-C & Habener JF. Insulinotropic hormone glucagon-like peptide-1(7–37) stimulation of proinsulin gene expression and proinsulin biosynthesis in insulinoma β TC-1 cells. *Endocrinology* 1992; **130**: 159–166.
39. Deacon CF, Pridal L, Klarskov L et al. Glucagon-like peptide I undergoes differential tissue-specific metabolism in the anesthetized pig. *Am J Physiol* 1996; **271**: E458–E464.
40. Balkan B & Li X. Portal GLP-1 administration in rats augments the insulin response to glucose via neuronal mechanisms. *Am J Physiol Regul Integr Comp Physiol* 2000; **279**: R1449–R1454.
41. Ahren B. Sensory nerves contribute to insulin secretion by glucagon-like peptide-1 in mice. *Am J Physiol Regul Integr Comp Physiol* 2004; **286**: R269–R272.
42. Moritz W, Leech CA, Ferrer J et al. Regulated expression of adenosine triphosphate-sensitive potassium channel subunits in pancreatic beta-cells. *Endocrinology* 2001; **142**: 129–138.
43. Holz GG, Kuhlreiber WM & Habener JF. Pancreatic beta-cells are rendered glucose-competent by the insulinotropic hormone glucagon-like peptide-1(7–37). *Nature* 1993; **361**: 362–365.
44. Byrne MM, Gliem K, Wank U et al. Glucagon-like peptide-1 improves the ability of the beta-cell to sense and respond to glucose in subjects with impaired glucose tolerance. *Diabetes* 1998; **47**: 1259–1265.
45. Wang Y, Perfetti R, Greig NH et al. Glucagon-like peptide-1 can reverse the age-related decline in glucose tolerance in rats. *J Clin Invest* 1997; **99**: 2883–2889.
46. Wang Y, Egan JM, Raygada M et al. Glucagon-like peptide-1 affects gene transcription and messenger ribonucleic acid stability of components of the insulin secretory system in RIN 1046-38 cells. *Endocrinology* 1995; **136**: 4910–4917.
47. Matsuyama T, Komatsu R, Namba M et al. Glucagon-like peptide-1 (7–36) amide: a potent glucagonostatic and insulinotropic hormone. *Diabetes Res Clin Pract* 1988; **5**: 281–284.
48. Heller RS, Kieffer TJ & Habener JF. Insulinotropic glucagon-like peptide I receptor expression in glucagon-producing alpha-cells of the rat endocrine pancreas. *Diabetes* 1997; **46**: 785–791.

49. Samols E, Bonner-Weir S & Weir GC. Intra-islet insulin–glucagon–somatostatin relationships. *Clin Endocrinol Metab* 1986; **15**: 33–58.
50. Wettergren A, Schjoldager B, Mortensen PE et al. Effect of GLP-I on gastric motility and gastric and pancreatic secretion in man. *Digestion* 1993; **54**: 384–385.
51. Tolessa T, Gutniak M, Holst JJ et al. Glucagon-like peptide-I retards gastric emptying and small bowel transit in the rat: effect mediated through central or enteric nervous mechanisms. *Dig Dis Sci* 1998; **43**: 2284–2290.
52. Imeryuz N, Yegen BC, Bozkurt A et al. Glucagon-like peptide-I inhibits gastric emptying via vagal afferent-mediated central mechanisms. *Am J Physiol* 1997; **273**: G920–G927.
53. Morales M, Lopez-Delgado MI, Alcantara A et al. Preserved GLP-I effects on glycogen synthase activity and glucose metabolism in isolated hepatocytes and skeletal muscle from diabetic rats. *Diabetes* 1997; **46**: 1264–1269.
54. Egan JM, Montrose-Rafizadeh C, Wang Y et al. Glucagon-like peptide-I(7–36) amide (GLP-I) enhances insulin-stimulated glucose metabolism in 3T3-L1 adipocytes: one of several potential extrapancreatic sites of GLP-I action. *Endocrinology* 1994; **135**: 2070–2075.
55. Miki H, Namba M, Nishimura T et al. Glucagon-like peptide-I(7–36)amide enhances insulin-stimulated glucose uptake and decreases intracellular cAMP content in isolated rat adipocytes. *Biochim Biophys Acta* 1996; **1312**: 132–136.
56. Furnsinn C, Ebner K & Waldhausl W. Failure of GLP-I (7–36) amide to affect glycogenesis in rat skeletal muscle. *Diabetologia* 1995; **38**: 864–867.
57. Nakagawa Y, Kawai K, Suzuki H et al. Glucagon-like peptide-I(7–36)amide and glycogen synthesis in the liver. *Diabetologia* 1996; **39**: 1241–1242.
58. D’Alessio DA, Kahn SE, Leusner CR et al. Glucagon-like peptide I enhances glucose tolerance both by stimulation of insulin release and by increasing insulin-independent glucose disposal. *J Clin Invest* 1994; **93**: 2263–2266.
59. Meneilly GS, McIntosh CH, Pederson RA et al. Effect of glucagon-like peptide I on non-insulin-mediated glucose uptake in the elderly patient with diabetes. *Diabetes Care* 2001; **24**: 1951–1956.
60. Egan JM, Meneilly GS, Habener JF et al. Glucagon-like peptide-I augments insulin-mediated glucose uptake in the obese state. *J Clin Endocrinol Metab* 2002; **87**: 3768–3773.
61. Orskov L, Holst JJ, Moller J et al. GLP-I does not acutely affect insulin sensitivity in healthy man. *Diabetologia* 1996; **39**: 1227–1232.
62. Toft-Nielsen M, Madsbad S & Holst JJ. The effect of glucagon-like peptide I (GLP-I) on glucose elimination in healthy subjects depends on the pancreatic glucoregulatory hormones. *Diabetes* 1996; **45**: 552–556.
63. Larsson H, Holst JJ & Ahren B. Glucagon-like peptide-I reduces hepatic glucose production indirectly through insulin and glucagon in humans. *Acta Physiol Scand* 1997; **160**: 413–422.
64. Ryan AS, Egan JM, Habener JF et al. Insulinotropic hormone glucagon-like peptide-I-(7–37) appears not to augment insulin-mediated glucose uptake in young men during euglycemia. *J Clin Endocrinol Metab* 1998; **83**: 2399–2404.
65. Vella A, Shah P, Reed AS et al. Lack of effect of exendin-4 and glucagon-like peptide-I-(7,36)-amide on insulin action in non-diabetic humans. *Diabetologia* 2002; **45**: 1410–1415.
66. Prigeon RL, Quidusi S, Paty B et al. Suppression of glucose production by GLP-I independent of islet hormones: a novel extrapancreatic effect. *Am J Physiol Endocrinol Metab* 2003; **285**: E701–E707.
67. Bullock BP, Heller RS & Habener JF. Tissue distribution of messenger ribonucleic acid encoding the rat glucagon-like peptide-I receptor. *Endocrinology* 1996; **137**: 2968–2978.
68. Campos RV, Lee YC & Drucker DJ. Divergent tissue-specific and developmental expression of receptors for glucagon and glucagon-like peptide-I in the mouse. *Endocrinology* 1994; **134**: 2156–2164.
69. Blackmore PF, Mojsov S, Exton JH et al. Absence of insulinotropic glucagon-like peptide-I(7–37) receptors on isolated rat liver hepatocytes. *FEBS Lett* 1991; **283**: 7–10.
70. Valverde I, Merida E, Delgado E et al. Presence and characterization of glucagon-like peptide-I(7–36) amide receptors in solubilized membranes of rat adipose tissue. *Endocrinology* 1993; **132**: 75–79.
71. Wheeler MB, Lu M, Dillon JS et al. Functional expression of the rat glucagon-like peptide-I receptor, evidence for coupling to both adenyl cyclase and phospholipase-C. *Endocrinology* 1993; **133**: 57–62.
72. Montrose-Rafizadeh C, Yang H, Wang Y et al. Novel signal transduction and peptide specificity of glucagon-like peptide receptor in 3T3-L1 adipocytes. *J Cell Physiol* 1997; **172**: 275–283.

73. Yang H, Egan JM, Wang Y et al. GLP-1 action in L6 myotubes is via a receptor different from the pancreatic GLP-1 receptor. *Am J Physiol* 1998; **275**: C675–C683.
74. Villanueva-Penacarrillo ML, Delgado E, Trapote MA et al. Glucagon-like peptide-1 binding to rat hepatic membranes. *J Endocrinol* 1995; **146**: 183–189.
75. Idris I, Patiag D, Gray S et al. Exendin-4 increases insulin sensitivity via a PI-3-kinase-dependent mechanism: contrasting effects of GLP-1. *Biochem Pharmacol* 2002; **63**: 993–996.
76. Luque MA, Gonzalez N, Marquez L et al. Glucagon-like peptide-1 (GLP-1) and glucose metabolism in human myocytes. *J Endocrinol* 2002; **173**: 465–473.
77. D'alesio DA, Vogel R, Prigeon R et al. Elimination of the action of glucagon-like peptide 1 causes an impairment of glucose tolerance after nutrient ingestion by healthy baboons. *J Clin Invest* 1996; **97**: 133–138.
78. Edwards CM, Todd JF, Mahmoudi M et al. Glucagon-like peptide 1 has a physiological role in the control of postprandial glucose in humans: studies with the antagonist exendin 9–39. *Diabetes* 1999; **48**: 86–93.
79. Wang Z, Wang RM, Owji AA et al. Glucagon-like peptide-1 is a physiological incretin in rat. *J Clin Invest* 1995; **95**: 417–421.
- *80. Scrocchi LA, Brown TJ, MaClusky N et al. Glucose intolerance but normal satiety in mice with a null mutation in the glucagon-like peptide 1 receptor gene. *Nat Med* 1996; **2**: 1254–1258.
81. Schirra J, Sturm K, Leicht P et al. Exendin(9–39)amide is an antagonist of glucagon-like peptide-1(7–36)amide in humans. *J Clin Invest* 1998; **101**: 1421–1430.
82. Susini S, Roche E, Prentki M et al. Glucose and glucoincretin peptides synergize to induce c-fos, c-jun, junB, zif-268, and nur-77 gene expression in pancreatic beta(INS-1) cells. *Faseb J* 1998; **12**: 1173–1182.
83. Buteau J, Roduit R, Susini S et al. Glucagon-like peptide-1 promotes DNA synthesis, activates phosphatidylinositol 3-kinase and increases transcription factor pancreatic and duodenal homeobox gene 1 (PDX-1) DNA binding activity in beta (INS-1)-cells. *Diabetologia* 1999; **42**: 856–864.
84. Zhou J, Wang X, Pineyro MA et al. Glucagon-like peptide 1 and exendin-4 convert pancreatic AR42J cells into glucagon- and insulin-producing cells. *Diabetes* 1999; **48**: 2358–2366.
85. Hui H, Wright C & Perfetti R. Glucagon-like peptide 1 induces differentiation of islet duodenal homeobox-1-positive pancreatic ductal cells into insulin-secreting cells. *Diabetes* 2001; **50**: 785–796.
86. Zhou J, Pineyro MA, Wang X et al. Exendin-4 differentiation of a human pancreatic duct cell line into endocrine cells: involvement of PDX-1 and HNF3beta transcription factors. *J Cell Physiol* 2002; **192**: 304–314.
87. Movassat J, Beattie GM, Lopez AD et al. Exendin 4 up-regulates expression of PDX 1 and hastens differentiation and maturation of human fetal pancreatic cells. *J Clin Endocrinol Metab* 2002; **87**: 4775–4781.
88. Hardikar AA, Wang XY, Williams LJ et al. Functional maturation of fetal porcine beta-cells by glucagon-like peptide 1 and cholecystokinin. *Endocrinology* 2002; **143**: 3505–3514.
89. Edvell A & Lindstrom P. Initiation of increased pancreatic islet growth in young normoglycemic mice (Umea-1?). *Endocrinology* 1999; **140**: 778–783.
90. Xu G, Stoffers DA, Habener JF et al. Exendin-4 stimulates both beta-cell replication and neogenesis, resulting in increased beta-cell mass and improved glucose tolerance in diabetic rats. *Diabetes* 1999; **48**: 2270–2276.
91. Perfetti R, Zhou J, Doyle ME et al. Glucagon-like peptide-1 induces cell proliferation and pancreatic-duodenum homeobox-1 expression and increases endocrine cell mass in the pancreas of old, glucose-intolerant rats. *Endocrinology* 2000; **141**: 4600–4605.
92. Wang Q & Brubaker PL. Glucagon-like peptide-1 treatment delays the onset of diabetes in 8 week-old db/db mice. *Diabetologia* 2002; **45**: 1263–1273.
93. Tourrel C, Bailbe D, Lacorne M et al. Persistent improvement of type 2 diabetes in the Goto-Kakizaki rat model by expansion of the beta-cell mass during the prediabetic period with glucagon-like peptide-1 or exendin-4. *Diabetes* 2002; **51**: 1443–1452.
94. Stoffers DA, Desai BM, DeLeon DD et al. Neonatal exendin-4 prevents the development of diabetes in the intrauterine growth retarded rat. *Diabetes* 2003; **52**: 734–740.
95. Hui H, Nourparvar A, Zhao X et al. Glucagon-like peptide-1 inhibits apoptosis of insulin-secreting cells via a cyclic 5'-adenosine monophosphate-dependent protein kinase A- and a phosphatidylinositol 3-kinase-dependent pathway. *Endocrinology* 2003; **144**: 1444–1455.

- *96. Li Y, Hansotia T, Yusta B et al. Glucagon-like peptide-1 receptor signaling modulates beta cell apoptosis. *J Biol Chem* 2003; **278**: 471–478.
97. Farilla L, Bulotta A, Hirshberg B et al. GLP-1 inhibits cell apoptosis and improves glucose responsiveness of freshly isolated human islets. *Endocrinology* 2003; **144**: 5149–5158.
98. Buteau J, El-Assaad W, Rhodes CJ et al. Glucagon-like peptide-1 prevents beta cell glucolipototoxicity. *Diabetologia* 2004; **47**: 806–815.
99. Farilla L, Hui H, Bertolotto C et al. Glucagon-like peptide-1 promotes islet cell growth and inhibits apoptosis in Zucker diabetic rats. *Endocrinology* 2002; **143**: 4397–4408.
100. Ling Z, Wu D, Zambre Y et al. Glucagon-like peptide 1 receptor signaling influences topography of islet cells in mice. *Virchows Arch* 2001; **438**: 382–387.
101. De Leon DD, Deng S, Madani R et al. Role of endogenous glucagon-like peptide-1 in islet regeneration after partial pancreatectomy. *Diabetes* 2003; **52**: 365–371.
102. Buteau J, Foisy S, Joly E et al. Glucagon-like peptide 1 induces pancreatic beta-cell proliferation via transactivation of the epidermal growth factor receptor. *Diabetes* 2003; **52**: 124–132.
103. Stoffers DA, Kieffer TJ, Hussain MA et al. Insulinotropic glucagon-like peptide 1 agonists stimulate expression of homeodomain protein IDX-1 and increase islet size in mouse pancreas. *Diabetes* 2000; **49**: 741–748.
104. Perfetti R, Zhou J, Doyle ME & Egan JM. Glucagon-like peptide-1 induces cell proliferation and pancreatic-duodenum homeobox-1 expression and increases endocrine cell mass in the pancreas of old, glucose-intolerant rats. *Endocrinology* 2000; **141**: 4600–4605.
105. Li Y, Hansotia T, Yusta B et al. Glucagon-like peptide-1 receptor signaling modulates B cell apoptosis. *J Biol Chem* 2003; **278**: 471–478.
106. Kwon G, Pappan KL, Marshall CA et al. cAMP dose-dependently prevents palmitate-induced apoptosis by both protein kinase A- and cAMP-guanine nucleotide exchange factor-dependent pathways in beta-cells. *J Biol Chem* 2004; **279**: 8938–8945.
107. Jhala US, Canettieri G, Sreaton RA et al. cAMP promotes pancreatic beta-cell survival via CREB-mediated induction of IRS2. *Genes Dev* 2003; **17**: 1575–1580.
108. Butler AE, Janson J, Bonner-Weir S et al. Beta-cell deficit and increased beta-cell apoptosis in humans with type 2 diabetes. *Diabetes* 2003; **52**: 102–110.
- *109. Turton MD, O'Shea D, Gunn I et al. A role for glucagon-like peptide-1 in the central regulation of feeding. *Nature* 1996; **379**: 69–72.
110. Meeran K, O'Shea D, Edwards CM et al. Repeated intracerebroventricular administration of glucagon-like peptide-1-(7–36) amide or exendin-(9–39) alters body weight in the rat. *Endocrinology* 1999; **140**: 244–250.
111. Rodriguez de Fonseca F, Navarro M & Alvarez E. Peripheral versus central effects of glucagon-like peptide-1 receptor agonists on satiety and body weight loss in Zucker obese rats. *Metabolism* 2000; **49**: 709–717.
112. Flint A, Raben A, Astrup A et al. Glucagon-like peptide 1 promotes satiety and suppresses energy intake in humans. *J Clin Invest* 1998; **101**: 515–520.
113. Naslund E, Barkeling B, King N et al. Energy intake and appetite are suppressed by glucagon-like peptide-1 (GLP-1) in obese men. *Int J Obes Relat Metab Disord* 1999; **23**: 304–311.
114. Gutzwiller JP, Drewe J, Goke B et al. Glucagon-like peptide-1 promotes satiety and reduces food intake in patients with diabetes mellitus type 2. *Am J Physiol* 1999; **276**: R1541–R1544.
115. Larsen PJ, Tang-Christensen M & Jessop DS. Central administration of glucagon-like peptide-1 activates hypothalamic neuroendocrine neurons in the rat. *Endocrinology* 1997; **138**: 4445–4455.
116. Rinaman L. Interoceptive stress activates glucagon-like peptide-1 neurons that project to the hypothalamus. *Am J Physiol* 1999; **277**: R582–R590.
117. Rinaman L. A functional role for central glucagon-like peptide-1 receptors in lithium chloride-induced anorexia. *Am J Physiol* 1999; **277**: R1537–R1540.
118. Seeley RJ, Blake K, Rushing PA et al. The role of CNS glucagon-like peptide-1 (7–36) amide receptors in mediating the visceral illness effects of lithium chloride. *J Neurosci* 2000; **20**: 1616–1621.
119. Goke R, Larsen PJ, Mikkelsen JD et al. Distribution of GLP-1 binding sites in the rat brain: evidence that exendin-4 is a ligand of brain GLP-1 binding sites. *Eur J Neurosci* 1995; **7**: 2294–2300.
120. Calvo JC, Yusta B, Mora F et al. Structural characterization by affinity cross-linking of glucagon-like peptide-1(7–36)amide receptor in rat brain. *J Neurochem* 1995; **64**: 299–306.

121. Meier JJ, Gallwitz B, Schmidt WE et al. Glucagon-like peptide 1 as a regulator of food intake and body weight: therapeutic perspectives. *Eur J Pharmacol* 2002; **440**: 269–279.
122. Orskov C, Poulsen SS, Moller M et al. Glucagon-like peptide 1 receptors in the subfornical organ and the area postrema are accessible to circulating glucagon-like peptide 1. *Diabetes* 1996; **45**: 832–835.
123. Edwards CM, Edwards AV & Bloom SR. Cardiovascular and pancreatic endocrine responses to glucagon-like peptide-1 (7–36) amide in the conscious calf. *Exp Physiol* 1997; **82**: 709–716.
124. Barragan JM, Eng J, Rodriguez R et al. Neural contribution to the effect of glucagon-like peptide-1-(7–36) amide on arterial blood pressure in rats. *Am J Physiol* 1999; **277**: E784–E791.
125. Cechetto DF. Central representation of visceral function. *Fed Proc* 1987; **46**: 17–23.
126. Ferguson AV. The area postrema: a cardiovascular control centre at the blood-brain interface? *Can J Physiol Pharmacol* 1991; **69**: 1026–1034.
127. Wei Y & Mojsov S. Tissue-specific expression of the human receptor for glucagon-like peptide 1: brain, heart and pancreatic forms have the same deduced amino acid sequences. *FEBS Lett* 1995; **358**: 219–224.
128. Yamamoto H, Lee CE, Marcus JN et al. Glucagon-like peptide-1 receptor stimulation increases blood pressure and heart rate and activates autonomic regulatory neurons. *J Clin Invest* 2002; **110**: 43–52.
129. Gros R, You X, Baggio LL et al. Cardiac function in mice lacking the glucagon-like peptide-1 receptor. *Endocrinology* 2003; **144**: 2242–2252.
130. Toft-Nielsen MB, Madsbad S & Holst JJ. Continuous subcutaneous infusion of glucagon-like peptide 1 lowers plasma glucose and reduces appetite in type 2 diabetic patients. *Diabetes Care* 1999; **22**: 1137–1143.
131. Edwards CM, Stanley SA, Davis R et al. Exendin-4 reduces fasting and postprandial glucose and decreases energy intake in healthy volunteers. *Am J Physiol Endocrinol Metab* 2001; **281**: E155–E161.
132. Fineman MS, Bicsak TA, Shen LZ et al. Effect on glycemic control of exenatide (synthetic exendin-4) additive to existing metformin and/or sulfonylurea treatment in patients with type 2 diabetes. *Diabetes Care* 2003; **26**: 2370–2377.
133. Yu M, Moreno C & Hoagland KM. Antihypertensive effect of glucagon-like peptide 1 in Dahl salt-sensitive rats. *J Hypertens* 2003; **21**: 1125–1135.
134. Gutzwiller JP, Tschopp S, Bock A et al. Glucagon-like peptide 1 induces natriuresis in healthy subjects and in insulin-resistant obese men. *J Clin Endocrinol Metab* 2004; **89**: 3055–3061.
135. Nikolaidis LA, Mankad S, Sokos GG et al. Effects of glucagon-like peptide-1 in patients with acute myocardial infarction and left ventricular dysfunction after successful reperfusion. *Circulation* 2004; **109**: 962–965.
136. Perry T, Lahiri DK, Chen D et al. A novel neurotrophic property of glucagon-like peptide 1: a promoter of nerve growth factor-mediated differentiation in PC12 cells. *J Pharmacol Exp Ther* 2002; **300**: 958–966.
137. Perry T, Haughey NJ, Mattson MP et al. Protection and reversal of excitotoxic neuronal damage by glucagon-like peptide-1 and exendin-4. *J Pharmacol Exp Ther* 2002; **302**: 881–888.
138. During MJ, Cao L, Zuzga DS et al. Glucagon-like peptide-1 receptor is involved in learning and neuroprotection. *Nat Med* 2003; **9**: 1173–1179.
139. Perry T & Greig NH. The glucagon-like peptides: a double-edged therapeutic sword? *Trends Pharmacol Sci* 2003; **24**: 377–383.
140. Oka J, Suzuki E & Kondo Y. Endogenous GLP-1 is involved in beta-amyloid protein-induced memory impairment and hippocampal neuronal death in rats. *Brain Res* 2000; **878**: 194–198.
- *141. Zander M, Madsbad S, Madsen JL et al. Effect of 6-week course of glucagon-like peptide 1 on glycaemic control, insulin sensitivity, and beta-cell function in type 2 diabetes: a parallel-group study. *Lancet* 2002; **359**: 824–830.
142. Creutzfeldt WO, Kleine N, Willms B et al. Glucagonostatic actions and reduction of fasting hyperglycemia by exogenous glucagon-like peptide 1(7–36) amide in type I diabetic patients. *Diabetes Care* 1996; **19**: 580–586.
143. Dupre J, Behme MT, Hramiak IM et al. Glucagon-like peptide 1 reduces postprandial glycemic excursions in IDDM. *Diabetes* 1995; **44**: 626–630.
144. Dupre J, Behme MT, Hramiak IM et al. Subcutaneous glucagon-like peptide 1 combined with insulin normalizes postcibal glycemic excursions in IDDM. *Diabetes Care* 1997; **20**: 381–384.
145. Weir GC, Mojsov S, Hendrick GK et al. Glucagonlike peptide 1 (7–37) actions on endocrine pancreas. *Diabetes* 1989; **38**: 338–342.

146. Goke R, Wagner B, Fehmann HC et al. Glucose-dependency of the insulin stimulatory effect of glucagon-like peptide-1 (7–36) amide on the rat pancreas. *Res Exp Med* 1993; **193**: 97–103.
147. Nauck MA, Heimesaat MM, Behle K et al. Effects of glucagon-like peptide I on counterregulatory hormone responses, cognitive functions, and insulin secretion during hyperinsulinemic, stepped hypoglycemic clamp experiments in healthy volunteers. *J Clin Endocrinol Metab* 2002; **87**: 1239–1246.
148. Vaag AA, Holst JJ, Volund A et al. Gut incretin hormones in identical twins discordant for non-insulin-dependent diabetes mellitus (NIDDM)—evidence for decreased glucagon-like peptide I secretion during oral glucose ingestion in NIDDM twins. *Eur J Endocrinol* 1996; **135**: 425–432.
149. Eng J, Kleinman WA, Singh L et al. Isolation and characterization of exendin 4, an exendin 3 analogue from *Heloderma suspectum* venom. *J Biol Chem* 1992; **267**: 7402–7405.
150. Kolterman OG, Buse JB, Fineman MS et al. Synthetic exendin-4 (exenatide) significantly reduces postprandial and fasting plasma glucose in subjects with type 2 diabetes. *J Clin Endocrinol Metab* 2003; **88**: 3082–3089.
151. Egan JM, Meneilly GS & Elahi D. Effects of 1-mo bolus subcutaneous administration of exendin-4 in type 2 diabetes. *Am J Physiol Endocrinol Metab* 2003; **284**: E1072–E1079.
152. Juhl CB, Hollingdal M, Sturis J et al. Bedtime administration of NN2211, a long-acting GLP-1 derivative, substantially reduces fasting and postprandial glycemia in type 2 diabetes. *Diabetes* 2002; **51**: 424–429.
153. Degen KB, Juhl CB, Sturis J et al. One week's treatment with the long-acting glucagon-like peptide I derivative liraglutide (NN2211) markedly improves 24-h glycemia and alpha- and beta-cell function and reduces endogenous glucose release in patients with type 2 diabetes. *Diabetes* 2004; **53**: 1187–1194.
154. Madsbad S, Schmitz O, Ransam J et al. Improved glycemic control with no weight increase in patients with type 2 diabetes after once-daily treatment with the long-acting glucagon-like peptide I analog Liraglutide (NN2211): a 12-week, double-blind, randomized, controlled trial. *Diabetes Care* 2004; **27**: 1335–1342.
155. Kim JG, Baggio LL, Bridon DP et al. Development and characterization of a glucagon-like peptide I-albumin conjugate: the ability to activate the glucagon-like peptide I receptor in vivo. *Diabetes* 2003; **52**: 751–759.
- *156. Marguet D, Baggio L & Kobayashi T. Enhanced insulin secretion and improved glucose tolerance in mice lacking CD26. *Proc Natl Acad Sci USA* 2000; **97**: 6874–6879.
157. Conarello SL, Li Z, Ronan J et al. Mice lacking dipeptidyl peptidase IV are protected against obesity and insulin resistance. *Proc Natl Acad Sci USA* 2003; **100**: 6825–6830.
158. Ahren B, Holst JJ, Martensson H et al. Improved glucose tolerance and insulin secretion by inhibition of dipeptidyl peptidase IV in mice. *Eur J Pharmacol* 2000; **404**: 239–245.
159. Pederson RA, White HA, Schlenzig D et al. Improved glucose tolerance in Zucker fatty rats by oral administration of the dipeptidyl peptidase IV inhibitor isoleucine thiazolidide. *Diabetes* 1998; **47**: 1253–1258.
160. Deacon CF, Hughes TE & Holst JJ. Dipeptidyl peptidase IV inhibition potentiates the insulinotropic effect of glucagon-like peptide I in the anesthetized pig. *Diabetes* 1998; **47**: 764–769.
161. Balkan B, Kwasnik L, Miserendino R et al. Inhibition of dipeptidyl peptidase IV with NVP-DPP728 increases plasma GLP-1 (7–36 amide) concentrations and improves oral glucose tolerance in obese Zucker rats. *Diabetologia* 1999; **42**: 1324–1331.
162. Pauly RP, Demuth HU, Rosche F et al. Improved glucose tolerance in rats treated with the dipeptidyl peptidase IV (CD26) inhibitor Ile-thiazolidide. *Metabolism* 1999; **48**: 385–389.
163. Pospisilik JA, Martin J, Doty T et al. Dipeptidyl peptidase IV inhibitor treatment stimulates beta-cell survival and islet neogenesis in streptozotocin-induced diabetic rats. *Diabetes* 2003; **52**: 741–750.
164. Pospisilik JA, Stafford SG, Demuth HU et al. Long-term treatment with the dipeptidyl peptidase IV inhibitor P32/98 causes sustained improvements in glucose tolerance, insulin sensitivity, hyperinsulinemia, and beta-cell glucose responsiveness in VDF (fa/fa) Zucker rats. *Diabetes* 2002; **51**: 943–950.
165. Pospisilik JA, Stafford SG, Demuth HU et al. Long-term treatment with dipeptidyl peptidase IV inhibitor improves hepatic and peripheral insulin sensitivity in the VDF Zucker rat: a euglycemic-hyperinsulinemic clamp study. *Diabetes* 2002; **51**: 2677–2683.
166. Hoffman T, Glund K, McIntosh CHS et al. DPPIV inhibition as treatment of type II diabetes. In Mizutani S et al (ed.) *Cell-surface Aminopeptidases: Basic and Clinical Aspects*. Amsterdam: Elsevier, 2001, pp. 381–387.

167. Ahren B, Simonsson E, Larsson H et al. Inhibition of dipeptidyl peptidase IV improves metabolic control over a 4-week study period in type 2 diabetes. *Diabetes Care* 2002; **25**: 869–875.
- *168. Ahren B, Landin-Olsson M & Jansson PA. Inhibition of dipeptidyl peptidase-4 reduces glycemia, sustains insulin levels, and reduces glucagon levels in type 2 diabetes. *J Clin Endocrinol Metab* 2004; **89**: 2078–2084.
169. Fleischer B. CD26: a surface protease involved in T-cell activation. *Immunol Today* 1994; **15**: 180–184.
170. Gleeson MH, Bloom SR, Polak JM et al. Endocrine tumour in kidney affecting small bowel structure, motility, and absorptive function. *Gut* 1971; **12**: 773–782.
- *171. Drucker DJ, Ehrlich P & Asa SL. Induction of intestinal epithelial proliferation by glucagon-like peptide 2. *Proc Natl Acad Sci USA* 1996; **93**: 7911–7916.
172. Gregor M, Menge H, Stossel R et al. Effect of monoclonal antibodies to enteroglucagon on ileal adaptation after proximal small bowel resection. *Exp Clin Endocrinol* 1993; **101**: 297–302.
173. Irwin DM. Molecular evolution of proglucagon. *Regul Pept* 2001; **98**: 1–12.
174. Drucker DJ, Deforest L & Brubaker PL. Intestinal response to growth factors administered alone or in combination with h[Gly2]-glucagon-like peptide 2. *Am J Physiol* 1997; **273**: G1252–G1262.
- *175. Drucker DJ, Shi Q & Crivici A. Regulation of the biological activity of glucagon-like peptide 2 in vivo by dipeptidyl peptidase IV. *Nat Biotechnol* 1997; **15**: 673–677.
176. Kato Y, Yu D & Schwartz MZ. Glucagonlike peptide-2 enhances small intestinal absorptive function and mucosal mass in vivo. *J Pediatr Surg* 1999; **34**: 18–20.
177. Kieffer TJ, McIntosh CH & Pederson RA. Degradation of glucose-dependent insulinotropic polypeptide and truncated glucagon-like peptide 1 in vitro and in vivo by dipeptidyl peptidase IV. *Endocrinology* 1995; **136**: 3585–3596.
178. Hartmann B, Thulesen J, Kissow H et al. Dipeptidyl peptidase IV inhibition enhances the intestinotrophic effect of glucagon-like peptide-2 in rats and mice. *Endocrinology* 2000; **141**: 4013–4020.
179. Tsai CH, Hill M, Asa SL et al. Intestinal growth-promoting properties of glucagon-like peptide-2 in mice. *Am J Physiol* 1997; **273**: E77–E84.
180. Tsai CH, Hill M & Drucker DJ. Biological determinants of intestinotrophic properties of GLP-2 in vivo. *Am J Physiol* 1997; **272**: G662–G668.
181. Chance WT, Foley-Nelson T, Thomas I et al. Prevention of parenteral nutrition-induced gut hypoplasia by coinfusion of glucagon-like peptide-2. *Am J Physiol* 1997; **273**: G559–G563.
182. Kitchen PA, Fitzgerald AJ, Goodlad RA et al. Glucagon-like peptide-2 increases sucrase-isomaltase but not caudal-related homeobox protein-2 gene expression. *Am J Physiol Gastrointest Liver Physiol* 2000; **278**: G425–G428.
183. Ghatei MA, Goodlad RA, Taheri S et al. Proglucagon-derived peptides in intestinal epithelial proliferation: glucagon-like peptide-2 is a major mediator of intestinal epithelial proliferation in rats. *Dig Dis Sci* 2001; **46**: 1255–1263.
184. Chance WT, Sheriff S, Foley-Nelson T et al. Maintaining gut integrity during parenteral nutrition of tumor-bearing rats: effects of glucagon-like peptide 2. *Nutr Cancer* 2000; **37**: 215–222.
185. Fischer KD, Dhanvantari S, Drucker DJ et al. Intestinal growth is associated with elevated levels of glucagon-like peptide-2 in diabetic rats. *Am J Physiol* 1997; **273**: E815–E820.
186. Hartmann B, Thulesen J, Hare KJ et al. Immunoneutralization of endogenous glucagon-like peptide-2 reduces adaptive intestinal growth in diabetic rats. *Regul Pept* 2002; **105**: 173–179.
187. Hill ME, Asa SL & Drucker DJ. Essential requirement for Pax6 in control of enteroendocrine proglucagon gene transcription. *Mol Endocrinol* 1999; **13**: 1474–1486.
188. Petersen YM, Burrin DG & Sangild PT. GLP-2 has differential effects on small intestine growth and function in fetal and neonatal pigs. *Am J Physiol Regul Integr Comp Physiol* 2001; **281**: R1986–R1993.
189. Lovshin J, Yusta B, Iliopoulos I et al. Ontogeny of the glucagon-like peptide-2 receptor axis in the developing rat intestine. *Endocrinology* 2000; **141**: 4194–4201.
- *190. Boushey RP, Yusta B & Drucker DJ. Glucagon-like peptide 2 decreases mortality and reduces the severity of indomethacin-induced murine enteritis. *Am J Physiol* 1999; **277**: E937–E947.
191. Alavi K, Schwartz MZ, Palazzo JP et al. Treatment of inflammatory bowel disease in a rodent model with the intestinal growth factor glucagon-like peptide-2. *J Pediatr Surg* 2000; **35**: 847–851.
192. Ljungmann K, Hartmann B, Kissmeyer-Nielsen P et al. Time-dependent intestinal adaptation and GLP-2 alterations after small bowel resection in rats. *Am J Physiol Gastrointest Liver Physiol* 2001; **281**: G779–G785.

193. Dahly EM, Gillingham MB, Guo Z et al. Role of luminal nutrients and endogenous GLP-2 in intestinal adaptation to mid-small bowel resection. *Am J Physiol Gastrointest Liver Physiol* 2003; **284**: G670–G682.
194. Scott RB, Kirk D, MacNaughton VK et al. GLP-2 augments the adaptive response to massive intestinal resection in rat. *Am J Physiol* 1998; **275**: G911–G921.
195. Chance WT, Sheriff S, McCarter F et al. Glucagon-like peptide-2 stimulates gut mucosal growth and immune response in burned rats. *J Burn Care Rehabil* 2001; **22**: 136–143.
196. Prasad R, Alavi K & Schwartz MZ. Glucagonlike peptide-2 analogue enhances intestinal mucosal mass after ischemia and reperfusion. *J Pediatr Surg* 2000; **35**: 357–359.
197. Prasad R, Alavi K & Schwartz MZ. GLP-2alpha accelerates recovery of mucosal absorptive function after intestinal ischemia/reperfusion. *J Pediatr Surg* 2001; **36**: 570–572.
198. Tavakkolizadeh A, Shen R, Abraham P et al. Glucagonlike peptide 2 (glp-2) promotes intestinal recovery following chemotherapy-induced enteritis. *Curr Surg* 2000; **57**: 502.
- *199. Boushey RP, Yusta B & Drucker DJ. Glucagon-like peptide (GLP)-2 reduces chemotherapy-associated mortality and enhances cell survival in cells expressing a transfected GLP-2 receptor. *Cancer Res* 2001; **61**: 687–693.
200. Drucker DJ, Yusta B, Boushey RP et al. Human [Gly2]GLP-2 reduces the severity of colonic injury in a murine model of experimental colitis. *Am J Physiol* 1999; **276**: G79–G91.
- *201. Munroe DG, Gupta AK, Kooshesh F et al. Prototypic G protein-coupled receptor for the intestinotrophic factor glucagon-like peptide 2. *Proc Natl Acad Sci USA* 1999; **96**: 1569–1573.
202. Mayo KE, Miller LJ, Bataille D et al. International union of pharmacology. XXXV. The glucagon receptor family. *Pharmacol Rev* 2003; **55**: 167–194.
203. Yusta B, Somwar R, Wang F et al. Identification of glucagon-like peptide-2 (GLP-2)-activated signaling pathways in baby hamster kidney fibroblasts expressing the rat GLP-2 receptor. *J Biol Chem* 1999; **274**: 30459–30467.
204. Velazquez E, Ruiz-Albusac JM & Blazquez E. Glucagon-like peptide-2 stimulates the proliferation of cultured rat astrocytes. *Eur J Biochem* 2003; **270**: 3001–3009.
205. Yusta B, Huang L, Munroe D et al. Enteroendocrine localization of GLP-2 receptor expression in humans and rodents. *Gastroenterology* 2000; **119**: 744–755.
206. Bjerknes M & Cheng H. Modulation of specific intestinal epithelial progenitors by enteric neurons. *Proc Natl Acad Sci USA* 2001; **98**: 12497–12502.
207. Burrin DG, Stoll B, Jiang R et al. GLP-2 stimulates intestinal growth in premature TPN-fed pigs by suppressing proteolysis and apoptosis. *Am J Physiol Gastrointest Liver Physiol* 2000; **279**: G1249–G1256.
208. Yusta B, Boushey RP & Drucker DJ. The glucagon-like peptide-2 receptor mediates direct inhibition of cellular apoptosis via a cAMP-dependent protein kinase-independent pathway. *J Biol Chem* 2000; **275**: 35345–35352.
209. Yusta B, Estall J & Drucker DJ. GLP-2 receptor activation engages Bad and glycogen synthase kinase 3 in a protein kinase A-dependent manner and prevents apoptosis following inhibition of phosphatidylinositol 3-kinase. *J Biol Chem* 2002; **277**: 24896–24906.
- *210. Jeppesen PB, Hartmann B, Thulesen J et al. Glucagon-like peptide 2 improves nutrient absorption and nutritional status in short-bowel patients with no colon. *Gastroenterology* 2001; **120**: 806–815.