CHAPTER 6

Gastrointestinal Peptide Hormones Regulating Energy and Glucose Homeostasis

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Gastrointestinal hormones synthesized in functionally distinct populations of enteroendocrine cells and neurons play diverse roles in regulation of energy intake, nutrient absorption, and nutrient disposal. Most gut hormones are secreted at low basal levels in the fasting state, and plasma levels of most gut peptides increase rapidly but transiently after nutrient ingestion. The effects of gut hormones are increasingly complex and include regulation of food intake, exocrine secretion, gut motility, mucosal growth, nutrient absorption, and pancreatic endocrine function. Gut hormones also communicate with regulatory centers in the central nervous system (CNS) via afferent ascending nerves. This chapter focuses on the biology of the proglucagon-derived peptides (PGDPs) and gut hormones with related actions on control of insulin secretion and energy balance.

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PROGLUCAGON GENE STRUCTURE AND THE PROGLUCAGON-DERIVED PEPTIDES

Before the molecular biology era, the best characterized member of the proglucagon peptide family was 29-amino-acid glucagon produced in pancreatic α cells. Antisera directed against pancreatic glucagon were shown to cross-react with immunoreactive peptides in gut extracts that were also detected in the circulation (1). These peptides, originally known collectively as "enteroglucagons," were subsequently shown to consist of 2 principal forms: a larger protein of 69 amino acids designated glicentin, and a smaller 37-amino-acid peptide named oxyntomodulin (2). After cloning of complementary DNA (cDNA) and genes encoding mammalian

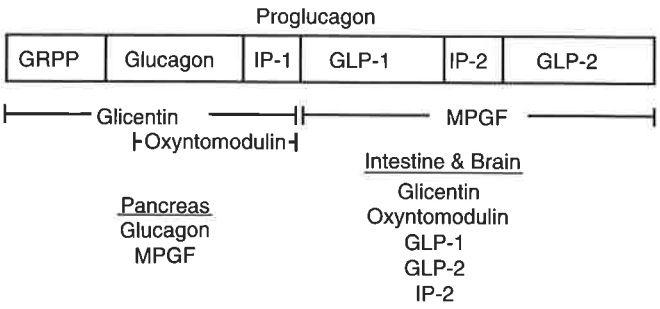

FIG. 6-1. Structural organization of proglucagon gene and the proglucagon-derived peptides (PGDPs). Proglucagon undergoes tissue-specific posttranslational processing in the pancreas, intestine, and brain to liberate the indicated PGDPs. GRPP, glicentin-related pancreatic polypeptide; GLP-1 and GLP-2, glucagon-like peptide-1 and -2; IP-1 and IP-2, intervening peptides 1 and 2; MPGF, major proglucagon fragment.

preproglucagon, the structural relation among various PGDPs was clearly elucidated (Fig. 6-1). Two PGDPs related in sequence to glucagon, designated glucagon-like peptide-1 (GLP-1) and -2 (GLP-2) were found to be coencoded together with glucagon in a single proglucagon precursor (3–5). The human proglucagon gene is located on the long arm of chromosome 2 (6) and consists of six exons, with the sequences of glucagon and the GLPs encoded within separate exons (see Fig. 6-1). To date, no variants of the human proglucagon gene have been linked to heritability of specific human diseases.

The mammalian genome encodes a single proglucagon gene that is transcribed to yield identical proglucagon messenger RNA (mRNA) transcripts predominantly in three cell types: pancreatic α cells, gut enteroendocrine L cells, and neurons in the caudal brainstem. The proglucagon mRNA transcript is structurally identical in all three tissues (7–9); hence, diversity in the generation of tissue-specific profiles of PGDPs is accomplished through tissue- and cell-specific expression of the prohormone convertases, proteases that differentially process proglucagon to yield either 29-amino-acid glucagon in the islet α cell or glicentin, oxyntomodulin, and both GLP-1 and GLP-2 in the gut L cell (see Figs. 6-1 and 6-2). Experimental studies have provided evidence supporting an essential role for PC2 in the cleavage of proglucagon to yield glucagon in pancreatic α cells, together with a larger, incompletely processed, secreted polypeptide designated major proglucagon fragment (10–13). The importance of PC2 for the generation of mature proglucagon is exemplified by the phenotype of mice with a targeted disruption of the *PC2* gene. These mice exhibit mild hypoglycemia and markedly increased levels of incompletely processed proglucagon, together with deficiency of mature 29-amino-acid glucagon (14).

In contrast with the importance of PC2 for generation of glucagon in islet α cells, PC1/3 appears to be required for processing of proglucagon in intestinal L cells (11,15). PC1 knockout mice exhibit multiple defects in prohormone processing including failure to generate significant amounts of GLP-1 and GLP-2 (16). Although levels of mature GLP-1 are reduced in human subjects with an inactivating mutation of PC1, small amounts of bioactive GLP-1 can be generated in the absence of a functional PC1 enzyme (17). Intriguingly, PC1/3 has been localized to α cells in the embryonic pancreas, raising the possibility that GLP-1 may be liberated during development of the endocrine pancreas (18). PC1/3 expression, together with increased production of bioactive GLP-1, has also been localized to α cells in the setting of experimental diabetes; however, the biological implications of this finding remain uncertain (18,19).

Regulation of Proglucagon Gene Expression

In the pancreas, proglucagon gene expression is stimulated by fasting and hypoglycemia, but is inhibited by insulin (20, 21), whereas in the intestine, proglucagon gene expression is up-regulated by nutrients (22). Gastrin-releasing peptide (GRP) and glucose-dependent insulinotropic polypeptide (GIP) (23) have been shown to increase levels of intestinal proglucagon mRNA transcripts in rodents. Fasting reduces and refeeding stimulates intestinal proglucagon gene expression (24), and a high-fiber diet (25) and short-chain fatty acids are potent inducers of proglucagon mRNA transcripts in enteroendocrine cells (26,27). Intestinal resection is associated with increased levels of proglucagon mRNA transcripts in the remnant intestine (28,29); however, the signals and mechanisms underlying this up-regulation remain unknown. Agents that activate the cAMP and protein kinase A (PKA) signaling pathways also increase proglucagon gene expression in the pancreas and intestine (8,30,31). Protein hydrolysates directly stimulate intestinal proglucagon gene expression through induction of gene transcription in part via DNA-promoter elements that mediate the response to cyclic 3′,5′-adenosine monophosphate (cAMP) (32).

Intestinal proglucagon gene expression has been studied using primary cultures of intestinal cells, enteroendocrine cell lines, and transgenic mice. A primary determinant of intestinal proglucagon gene expression in studies of nontransformed intestinal cells appears to be the level of intracellular cAMP (8). Although agents such as phorbol esters, cholinergic agonists, calcium ionophores, and both GIP and GRP stimulate PGDP secretion in these cultures (33), only activators of cAMP generation enhanced proglucagon biosynthesis. Similarly, agents acting through the cAMP-dependent pathway appear to be the principal factors regulating proglucagon gene expression in mouse enteroendocrine glucagon SV40T antigen (GLUTag) (31,33) and secretin tumor cell line (STC-1) cells (34). Although GRP increases proglucagon gene transcription in STC-1 cells via the cAMP-response element (CRE) (23), GRP has not yet been shown to stimulate proglucagon gene expression in primary cell cultures or in the rodent intestine *in vivo*.

Much less is known about the factors regulating the human proglucagon gene, largely because of the paucity of suitable models for studying human proglucagon gene expression.

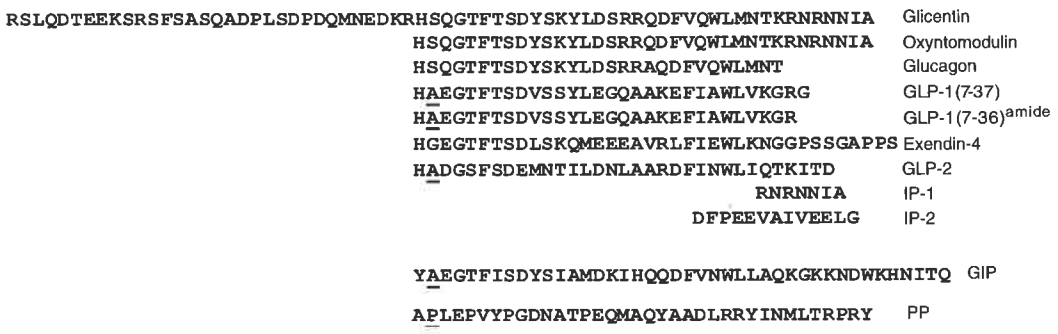

FIG. 6-2. Amino acid sequences of the proglucagon-derived peptides (PGDPs), exendin-4, glucose-dependent insulinotropic polypeptide (GIP), and pancreatic polypeptide (PP). The lizard-derived exendin-4 shares 53% amino acid sequence identity with mammalian glucagon-like peptide-1 (GLP-1). Peptides that contain an alanine or proline at position 2 (indicated by underlining) are substrates for cleavage by the enzyme dipeptidyl peptidase-IV. IP-1 and IP-2, intervening peptides 1 and 2.

Transgenic mice expressing a human proglucagon gene promoter growth hormone transgene in the gut provide a potential model for studies of the human proglucagon gene promoter (35). Although nutrients up-regulated expression of the endogenous murine proglucagon gene, feeding had no effect on the human growth hormone (hGH) transgene, suggesting that important control elements for nutrient-regulated control of human proglucagon promoter activity do not reside within the 1.6 kb of transgene human proglucagon gene promoter sequences (36). The control of human proglucagon gene expression has also been examined in NCI-H716 cells, a cell line derived from a human adenocarcinoma exhibiting features of endocrine differentiation. Multiple factors, including palmitic acid, oleic acid, meat hydrolysate, carbachol, and GRP stimulate PGDP secretion from NCI-H716 cells. In contrast with results of studies in rodent cells, cAMP enhanced GLP-1 secretion but failed to increase levels of proglucagon mRNA transcripts in NCI-H716 cells (37). Similarly, insulin, phorbol myristate acetate, or forskolin, all known regulators of rodent proglucagon gene expression, had no effect on proglucagon gene expression in NCI-H716 cells, and transfection studies using either the human or rodent proglucagon gene promoters demonstrate that NCI-H716 cells do not support transcriptional activation of the isolated proglucagon gene promoter sequences (38). Hence, the available data suggest that NCI-H716 cells may express the human proglucagon in a constitutive, nonregulated manner.

Cis-acting sequences within the rat proglucagon gene promoter have been identified as important control regions for islet cell–specific gene expression. Transcription factors important for islet cell–specific proglucagon gene expression include Brn4, Pax-6, Cdx-2/3, Isl-1, and members of the hepatocyte nuclear factor 3 (HNF-3 or Foxa) family (39–46). Pax6 and Cdx-2/3, in association with a coactivator protein, p300, interact synergistically to regulate proglucagon gene expression in islet cells (47). Pax-2 binds elements within the proglucagon gene promoter (48), but whether Pax-2 plays a role in the control of proglucagon gene expression remains uncertain (49). Similarly, although Brn4 is a potent activator of islet proglucagon gene expression, targeted disruption of the *Brn4* gene in mice does not produce abnormalities in α-cell development or changes in the levels of pancreatic glucagon mRNA transcripts (50). The proglucagon gene 5′-flanking sequences contain a CRE that confers cAMP responsivity to proglucagon gene transcription in pancreas and intestine (8,30,31,51).

In contrast with our understanding of proglucagon expression in pancreatic islets, less is known about the factors that specify proglucagon gene expression in the intestine, partly because of the limitations of models for analysis of enteroendocrine gene transcription. Cell transfection studies using immortalized STC-1 or GLUTag cell lines indicate that DNA sequences between −1,252 and −2,292 in the rat proglucagon promoter are essential for specifying intestinal proglucagon expression (52). Several transcription factors thought to be important for control of islet proglucagon gene transcription may not be essential for enteroendocrine gene transcription. Genetic inactivation of the murine Foxa1 (*HNF-3α*) gene results in mice with mild hypoglycemia and inappropriately low levels of pancreatic proglucagon mRNA transcripts; however, levels of intestinal proglucagon mRNA transcripts are normal in Foxa1 mutant mice (46,53). Similarly, although members of the Foxa3 (*HNF-3β*) family have been implicated in the control of proglucagon gene transcription in transfection studies, levels of pancreatic and intestinal proglucagon mRNA transcripts are normal in Foxa3$^{-/-}$ mice (54).

The *Pax-6* gene is important for cell lineage development in the gut and pancreas, as well as for control of pancreatic and intestinal proglucagon gene expression. Global inactivation of the murine *Pax-6* gene results in major defects in formation of islet cell lineages (55), whereas mice homozygous for a dominant negative form of *Pax-6* (SEY^{Neu}) have significantly reduced levels of proglucagon mRNA transcripts in the pancreas (56) and both the small and large bowel, indicating that this transcription factor is essential for proglucagon gene expression in both islet and enteroendocrine cells (57). Conversely, increased expression of *Pax-6* in primary intestinal cell cultures or in the rodent intestinal epithelium is associated with up-regulation of the levels of proglucagon gene expression (58).

Control of Proglucagon-Derived Peptide Secretion

Glucagon, the principal secretory product from islet α cells, is secreted in response to hypoglycemia; however, the mechanism for sensing hypoglycemia and stimulating α-cell secretion likely involves the central and peripheral nervous system. In contrast, insulin and other factors secreted from the β cell, such as γ-aminobutyric acid (GABA) and zinc, appear to be the predominant factors inhibiting islet α-cell secretion. Glicentin, oxyntomodulin, GLP-1, and GLP-2 (Fig. 6-2) are cosecreted from enteroendocrine L cells, which are predominantly localized to the distal ileum and colon (59,60). GLP-1–immunopositive cells may also be located in more proximal regions of the small bowel, in cells that produce both GLP-1 and GIP (61). Multiple forms of GLP-1 are released *in vivo,* including GLP-1(1-37) and GLP-1(1-36)NH_2, which appear to be biologically inactive (62–64), and GLP-1(7-37) and GLP-1(7-36)NH_2, which are biologically active. A major proportion of GLP-1 is amidated at the C-terminal glycine residue [GLP-1(1-36)NH_2 and GLP-1(7-36)NH_2], likely via the activity of peptidylglycine α-amidating monooxygenase (65). C-terminal amidation may enhance the survival of GLP-1 in plasma (66). Both GLP-1(7-37) and GLP-1(7-36)NH_2 appear equipotent (66–68); however, most circulating GLP-1 in humans is GLP-1(7-36)NH_2 (69). In pigs and rats, approximately half of the GLP-1 is glycine extended, whereas in dogs, the glycine-extended forms of GLP-1 predominate.

GLP-1 secretion from intestinal endocrine cells is stimulated by neural signals, endocrine factors, and direct nutrient contact with gut L cells (70). Intestinal PGDP secretion is regulated by several intracellular signals, including PKA, PKC, and calcium (33,71,72). A combination of studies using fetal rat intestinal cultures, the perfused rat ileum, and experiments performed in humans, rodents, and dogs have demonstrated that GLP-1 secretion is regulated directly by nutrients such as fatty acids, butyrate, peptones, or amino acids such as glutamine. Nevertheless, the rapid increase in plasma levels of the PGDPs within minutes of nutrient ingestion invokes a role for both neural and endocrine factors in control of L-cell secretion (70,73–75). Several neurotransmitters, including muscarinic agonists (76,77), regulate L-cell secretion both *in vitro* and *in vivo.* In humans, basal plasma levels of intact GLP-1 typically are within the 5- to 10-pM range in the fasting state, and increase to approximately 50 pM after meal ingestion (69,78). A small but detectable defect in meal-stimulated GLP-1 secretion has been observed in some subjects with obesity and type 2 diabetes (78).

Mixed meals appear to be the most potent stimulus for GLP-1 secretion; however, individual nutrients including glucose, fatty acids, and dietary fiber also stimulate PGDP secretion (74,79–81). As most GLP-1–producing L cells, and hence stored GLP-1, is localized to the distal portion of the small intestine, a role for neural, endocrine mediators, or both in the rapid, nutrient-stimulated increase in plasma GLP-1 seems likely (70,82). Candidate mediators for indirect stimulators of GLP-1 include the more proximally located duodenal hormone GIP and the neurotransmitter acetylcholine (77,82–84). Nevertheless, species-specific differences have been identified in studies of GLP-1 secretion because GIP does not stimulate GLP-1 secretion in humans (85,86), whereas the neuropeptide GRP stimulates GLP-1 secretion in both humans and rodents (87). Further evidence for the importance of GRP derives from studies of mice with inactivation of the GRP receptor that exhibit a reduced plasma GLP-1 response to gastric glucose (88). The neuropeptide calcitonin gene–related peptide may also regulate GLP-1 release (89). The vagus nerve plays a major role in controlling GLP-1 release from the distal L cells in response to ingested nutrients (90). Intestinal GLP-1 secretion appears to be inhibited by insulin and somatostatin-28 (33,91), as well as by the neuropeptide galanin (92).

PROGLUCAGON-DERIVED PEPTIDE METABOLISM AND CLEARANCE

Little is known about the metabolism of glucagon, glicentin, and oxyntomodulin; however, the kidney plays a major role in the catabolism and clearance of all three peptides (93,94). Glucagon action is terminated via extracellular and intracellular degradation pathways. Glucagon-degrading activity within hepatic endosomes has been attributed to cathepsins B and D (95), and glucagon and GLP-1 are substrates for the widely expressed membrane-bound neutral ectopeptidase (NEP) 24.11 (96). The clearance and degradation of GLP-1 has received considerable attention because of the therapeutic potential of the peptide. The half-life of circulating native GLP-1 is less than 2 minutes (97,98), principally because of the protease activity of dipeptidyl peptidase-IV (DPP-IV), an aminopeptidase that specifically cleaves dipeptides from the amino terminus of proteins containing an alanine or proline at position 2.

Oxyntomodulin and GLP-2 are also substrates for DPP-IV (99,100). DPP-IV is widely expressed in a large number of cell types in many tissues including the vascular endothelium of the small intestine, directly adjacent to the sites of GLP-1 release (97,101,102). Furthermore, in addition to a cell-associated membrane-bound form, a soluble DPP-IV molecule is also found in the circulation. DPP-IV catalyzes the cleavage of GLP-1 at the position 2 Ala residue to yield GLP-1(9-37) or GLP-1 (9-36)NH_2. More than 50% of GLP-1 is metabolized to its N-terminal truncated form by DPP-IV within 2 minutes of peptide administration (103). Complementary evidence for the importance of DPP-IV in GLP-1 metabolism is derived from studies of rodents with mutations or inactivation of the *DPP-IV* gene. *DPP-IV*–deficient rats exhibit a prolonged half-life of GLP-1 (104,105), and mice with a targeted inactivation of DPP-IV exhibit increased levels of plasma GLP-1 (106). Moreover, rats and mice with mutant or inactivated *DPP-IV* genes exhibit improved glucose tolerance and increased levels of circulating intact GLP-1. DPP-IV is also critical for GLP-1 inactivation in humans, because intravenous or subcutaneous GLP-1 is rapidly degraded (within 30 minutes) to GLP-1(9-36)NH_2 after administration of GLP-1 to healthy or diabetic human subjects, and this N-terminally shortened peptide accounts

for more than 75% of the immunodetectable circulating GLP-1 (98).

Although GLP-1 (9-36)NH_2 functions as a weak competitive pharmacologic antagonist of the GLP-1 receptor at the β cell and in the gastrointestinal system (107), experiments in anesthetized pigs treated with DPP-IV inhibitors revealed that GLP-1 (9-36)NH_2 could paradoxically elicit modest insulin-independent antihyperglycemic effects (108). In contrast, infusion of GLP-1 (9-36)NH_2 in healthy human subjects had no effects on glucose tolerance, insulin secretion or sensitivity, or GLP-1 action (109). Thus, the biological importance of N-terminally cleaved GLP-1 (9-36)NH_2 remains uncertain. NEP 24.11 also exhibits endoproteolytic activity on GLP-1 and may also contribute to the metabolism of GLP-1 (96,110).

Because GLP-2 is cosecreted with GLP-1, factors identified as important for GLP-1 secretion are similarly important for GLP-2 secretion, predominantly nutrients (111–113). GLP-2 also contains an alanine at position 2 and is degraded by DPP-IV (100). However, the half-life of exogenously administered GLP-2 is comparatively longer than that measured for GLP-1, at ~7 minutes (114). Although limited information is available about perturbations in levels of GLP-2 in human disease, patients with extensive resection of the small and large bowel exhibit significant reductions in circulating levels of GLP-2 after meal stimulation, whereas preservation of the colon appears important for maintaining levels of GLP-2 in adult human subjects (115,116). In contrast, the colon may be less important for preservation of plasma levels of GLP-2 in infants with nutrient malabsorption consequent to intestinal surgery (117). Patients with inflammatory bowel disease often exhibit increased circulating levels of GLP-2, in association with reductions in plasma levels of DPP-IV activity (118).

The primary route of clearance for GLP-1 and GLP-2 appears to be through the kidney via mechanisms that include glomerular filtration and tubular catabolism (119–122). Patients with uremia have increased levels of circulating, immunoreactive GLP-1 (123), and bilateral nephrectomy or ureteral ligation in rats is associated with increases in the circulating half-life of GLP-1 (119). A role for tissues other than the kidney, such as the liver and lung, in GLP-1 clearance has not been clearly established (119).

GLUCAGON RECEPTOR FAMILY

Separate receptors have been identified for glucagon, GLP-1, GLP-2, and GIP (124); however, the mechanisms underlying actions of PGDPs such as glicentin and oxyntomodulin remain poorly understood.

GLUCAGON RECEPTOR

The glucagon receptor (Gcgr) is a member of the seven-transmembrane–spanning G protein–coupled receptor superfamily, and it responds to glucagon with increases in both intracellular cAMP and intracellular calcium (125). Oxyntomodulin is capable of binding to and activating both the glucagon and GLP-1 receptors; however, the anorectic actions of oxyntomodulin appear to require a functional GLP-1 receptor (126).

The human *Gcgr* gene is localized to chromosome 17q25, and several reports have described an association between a Gly40Ser Gcgr mutation and an increased incidence of type 2 diabetes; however, this finding has not been confirmed in different populations with type 2 diabetes (127,128). Furthermore, cells expressing a transfected *Gcgr* containing the Gly40Ser mutation exhibit decreased affinity for glucagon *in vitro,* and human subjects with the Gly40Ser mutation exhibit a paradoxically decreased glycemic response to glucagon infusion *in vivo* (128,129). Hence, the biological significance of the Gly40Ser substitution remains uncertain.

Gcgr mRNA transcripts have been detected in liver, islet β cells, brain, adipocytes, heart, and kidney. Rat Gcgr mRNA transcripts have also been detected in tissues such as spleen, thymus, adrenal gland, intestine, ovary, and testis where glucagon action remains poorly defined (130). Glucagon binding sites have been identified in multiple brain regions, and Gcgr transcripts have been detected in cortex, cerebellum, hypothalamus, and brainstem; however, the biological actions of glucagon in specific CNS regions remain unclear (131).

Loss of glucagon action has been studied using antisense oligonucleotides directed against the Gcgr in diabetic mice (132,133), or in $Gcgr^{-/-}$ mice. Remarkably, overlapping phenotypes were seen in these experiments, including increased circulating levels of glucagon, mild fasting hypoglycemia together with improved glucose tolerance in $Gcgr^{-/-}$ mice, decreased body weight, and increased pancreatic mass associated with considerable hyperplasia of islet α cells (134,135). Furthermore, reduction of Gcgr expression in db/db mice was associated with lower levels of blood glucose, triglycerides, and free fatty acids (132,133). The improvement in glycemic control after loss of Gcgr function in these rodent studies appears to be attributable in part to increased circulating levels of bioactive GLP-1 derived from the pancreas. Gcgr expression is regulated in a tissue-specific manner with glucose and cAMP, important for Gcgr expression in hepatocytes (136,137), whereas glucose increased but cAMP and the glucocorticoid dexamethasone decreased levels of Gcgr mRNA transcripts in rat islets (138).

GLUCAGON-LIKE PEPTIDE-1 RECEPTOR

The glucagon-like peptide-1 receptor (GLP-1R) was cloned from a rat pancreatic islet cDNA library (139). The human *GLP-1R* gene has been mapped to chromosome 6, band p21.1 (140); however, human *GLP-1R* mutations or polymorphisms have not been linked to the development of type 2 diabetes. The GLP-1R is expressed in lung; kidney; stomach; heart; intestine; α, β, and δ cells of the pancreatic islets; and multiple regions of the CNS (131,141–143).

The GLP-1R couples to multiple G proteins, including $G\alpha_s$, $G\alpha_{q/11}$, and $G\alpha_{i1,2}$, leading to activation of several intracellular

signaling pathways (144), increased adenylate cyclase and phospholipase C, and activation of PKA and PKC, respectively (139,145,146). GLP-1R activation also increases intracellular calcium (147–149), phosphatidylinositol-3 kinase (PI_3K), and mitogen-activated protein kinase (MAPK) signaling pathways (144,150). Little is known about the factors that regulate levels of GLP-1R mRNA. GLP-1R expression is down-regulated in response to GLP-1, activation of PKC, high glucose, or dexamethasone, whereas treatment of diabetic rodents with DPP-IV inhibitors is associated with up-regulation of pancreatic GLP-1R expression (151).

The GLP-1R undergoes homologous and heterologous desensitization and internalization in islet cell lines, in association with receptor phosphorylation (152–154). However, desensitization of the GLP-1R has not been observed after long-term administration of GLP-1R agonists *in vivo*. Exendin (9-39), an N-terminally truncated peptide derived from the lizard GLP-1R agonist exendin-4, functions as a relatively specific GLP-1 receptor antagonist (155,156) and is commonly used to examine the consequences of transient loss of GLP-1R action.

GLUCAGON-LIKE PEPTIDE-2 RECEPTOR

The GLP-2 receptor was cloned from hypothalamic and intestinal cDNA libraries and is also coupled to cAMP generation when expressed in heterologous cell lines (157). GLP-2R expression is highly restricted, predominantly to the stomach, small and large bowels, and the CNS (158–160). Immunocytochemistry localized the GLP-2R to human enteroendocrine cells (158) and specific regions of the murine and rat CNS (159,160), whereas *in situ* hybridization has localized the receptor to neurons in the CNS and the murine enteric nervous system (161,162). The GLP-2R is highly specific for GLP-2 and is not activated by physiologically relevant concentrations of related members of the glucagon peptide superfamily (157,163).

GLP-2 dose-dependently activates adenylyl cyclase, cAMP formation, and PKA in cells expressing a heterologous rat or human GLP-2R, as well as in primary cell cultures from the CNS and the intestinal mucosa (157,160,164,165). Furthermore, GLP-2R activation activates c-fos in cells transfected with the GLP-2R (164) and in the murine gastrointestinal tract (161). GLP-2 also activates constitutive nitric oxide synthase (NOS) activity and endothelial NOS protein abundance in the gut, which appears to regulate GLP-2–induced intestinal blood flow and glucose uptake (166).

Activation of the GLP-2 receptor is also associated with enhanced cell survival. GLP-2R activation inhibits cycloheximide-induced apoptosis in a PKA-independent manner (167); in contrast, PKA regulates the antiapoptotic properties of GLP-2R signaling after inhibition of PI_3K (168) in transfected fibroblasts or exposure to glutamate in hippocampal neurons (160). Although GLP-2 administration promotes rapid growth of the intestinal epithelium after administration *in vivo* (169), whether GLP-2R activation can directly stimulate cellular proliferation requires further study. Incubation of colonic intestinal cells or rat astrocytes with GLP-2 results in increased cell proliferation (170–172); however, baby hamster kidney (BHK) fibroblasts transfected with the GLP-2 receptor did not exhibit a significant mitogenic effect in response to GLP-2 (164). Furthermore, GLP-2 treatment paradoxically inhibits cell proliferation in epithelial cells derived from the small intestine, yet stimulates cell proliferation in cell lines derived from the colon in a GLP-2R–independent manner (173). Hence, the molecular basis for GLP-2R activation promoting mitogenesis appears complex, likely indirect, and highly cell-type or tissue specific.

GLUCOSE-DEPENDENT INSULINOTROPIC POLYPEPTIDE RECEPTOR

Genetic mapping and linkage studies have localized the human glucose-dependent insulinotropic polypeptide receptor (*GIPR*) gene to chromosome 19 band q13.3 (174). The GIPR is a member of the seven-transmembrane domain, heterotrimeric, G protein–coupled Gcgr superfamily (124, 175), and it is expressed in the pancreas, stomach, small intestine, adipose tissue, adrenal cortex, lung, pituitary, heart, testis, vascular endothelium, bone, and brain (175,176).

Activation of GIPR signaling leads to generation of cAMP and increases in intracellular calcium in pancreatic islet cells, adipocytes, osteoblasts, endothelial cells, and heterologous cell lines transfected with GIPR (176–179). The GIPR also signals via PI_3K, MAPK, and phospholipase A_2 pathways (180–182).

The GIPR undergoes rapid and reversible homologous desensitization (73) *in vitro*. Studies using the βTC3 islet cell line implicate a role for regulator of G-protein signaling 2 (RGS-2), G-protein receptor kinase 2 (GRK-2), and β-arrestin-1 in GIP-induced receptor desensitization (183). GIP action is diminished in human subjects with type 2 diabetes (86), and defective GIP action in Zucker diabetic fatty rats has been correlated with reduced levels of GIPR mRNA transcripts in pancreatic islets (184).

The absence of GIP action has been studied in mice with genetic disruption of the GIPR (185). GIPR knockout mice exhibit a mild defect in glucose tolerance (185), but they are resistant to the development of obesity and insulin resistance after high-fat feeding (186). Despite the roles of GIP and GLP-1 as the two dominant incretins regulating insulin secretion, combined disruption of both the GLP-1 and GIP receptors produces only modest impairment of glucose-dependent insulin secretion (187,188).

BIOLOGICAL ACTIONS OF GLUCAGON

Glucagon exerts a number of important physiologic actions in tissues such as the liver, the endocrine pancreas, the vascular bed, and the gastrointestinal tract. Glucagon regulates hepatic glucose production via activation of glycogenolysis

and gluconeogenesis and by inhibition of glycolysis. Glucagon modifies the activity of enzymes important for glucose production and modulates expression of genes encoding enzymes in the glycolytic or gluconeogenic pathways (189). Glucagon also regulates fatty acid metabolism via reduction of malonyl coenzyme A (CoA) and stimulation of fatty acid oxidation. The cAMP-dependent transcription factor CREB is a downstream mediator for glucagon action. CREB, together with activation of the nuclear receptor coactivator peroxisome proliferator-activated receptor (PPAR) gamma coactivator-1 (PGC-1) and suppression of PPARγ activity, ultimately results in activation of hepatic gluconeogenesis (190).

Glucagon increases cAMP and stimulates lipolysis in adipocytes, thereby providing free fatty acids as substrate for fat-burning tissues. Glucagon also inhibits insulin-stimulated glucose transport in adipocytes (191). In the peripheral vascular system, glucagon acts as a vasodilator via effects on local vascular tone, and glucagon increases cardiac output and heart rate, possibly via direct effects on the heart. Pharmacologic doses of glucagon increase renal blood flow, glomerular filtration rate (GFR), and urinary electrolyte excretion; however, lower concentrations of glucagon do not affect renal blood flow, GFR, or solute excretion (192). Although the kidney exhibits significant gluconeogenic capacity, the available evidence does not support a role for endogenous glucagon in the control of renal glucose output (193).

The β cell expresses receptors for glucagon coupled to cAMP and stimulation of insulin secretion. The threshold for glucagon-stimulated cAMP accumulation in isolated β cells is ~1 nM glucagon, which is greater than the concentrations required for cAMP stimulation by GLP-1 or GIP (194). The physiologic importance of intraislet or circulating glucagon for β-cell physiology remains unclear, given the high concentrations of glucagon required to stimulate the β cell *in vitro.*

GLUCAGON ADMINISTRATION IN HUMAN SUBJECTS

The most common use of glucagon therapeutically is in the acute management of severe hypoglycemia. Diabetic patients with hypoglycemia generally respond quickly, with a rapid increase in blood glucose, after glucagon administration (195,196). Glucagon is also used to inhibit gastrointestinal motility during radiologic or endoscopic investigations, and glucagon administration has been shown to benefit selected patients with refractory bronchospasm or symptomatic bradycardia (197,198).

BIOLOGICAL ACTIONS OF GLICENTIN

Glicentin is a 69-amino-acid PGDP that contains the sequence of 29-amino-acid glucagon flanked by peptide extensions at both the amino and carboxy termini (see Fig. 6-2). Although cosecreted with GLP-1 and GLP-2 from gut L cells, the specific biological actions of glicentin remain elusive. Glicentin has been shown to activate signal transduction pathways leading to stimulation of intracellular calcium and reduction of cAMP formation in smooth muscle cells derived from rabbit antrum (199). Furthermore, administration of glicentin to rodents induces small-bowel growth in some (169,200) but not all studies (201). To date, a distinct receptor responsible for transducing the biological actions of glicentin has not yet been identified.

OXYNTOMODULIN

Oxyntomodulin contains the sequence of 29-amino-acid pancreatic glucagon together with an 8-amino-acid carboxy-terminal extension (see Fig. 6-1). The original biological action described for oxyntomodulin was stimulation of acid secretion from the oxyntic glands in the stomach (202). Additional actions ascribed to oxyntomodulin include stimulation of intestinal glucose uptake (203), regulation of insulin secretion (204), reduction of gastric emptying (205), and inhibition of meal-stimulated gastric acid secretion (205–207). More recent studies have demonstrated an inhibitory effect of oxyntomodulin on food intake after both intracerebroventricular and peripheral administration in rats (208,209) and after intracerebroventricular administration in mice (126). Oxyntomodulin infusion also produces satiety and inhibits food intake after short-term intravenous infusion in human subjects (210). Although oxyntomodulin is a weak agonist at both the GLP-1 and glucagon receptors (211–214), the anorectic actions of oxyntomodulin are blocked by the GLP-1R antagonist exendin (9-39) (208) and are eliminated in the absence of a functional GLP-1R (126). Given the overlapping actions of oxyntomodulin with glucagon and GLP-1 and the lack of evidence for a distinct oxyntomodulin receptor, the available evidence suggests that many of the pharmacologic actions of oxyntomodulin represent heterologous activation of related PGDP receptor systems.

BIOLOGICAL ACTIONS OF GLUCAGON-LIKE PEPTIDE-1

GLP-1 exerts multiple physiological actions leading to control of energy intake and nutrient assimilation (Table 6-1) (215). The original physiologic role described for GLP-1 was that of an incretin hormone. GLP-1 is secreted after nutrient ingestion and stimulates insulin secretion in a glucose-dependent manner (85,216,217). GLP-1 also increases insulin gene transcription, mRNA stability, and biosynthesis by mechanisms that involve both cAMP/PKA-dependent and -independent pathways, as well as increases in the levels of intracellular Ca^{2+} (145,218,219). GLP-1 increases expression of the sulfonylurea receptor (SUR1) and inwardly rectifying potassium channel (Kir 6.2) in β cells, and it prevents glucose-dependent inhibition of K_{ATP}-channel activity (220).

TABLE 6-1. *Summary of glucagon-like peptide-1 action*

Pancreas
Stimulates glucose-dependent insulin secretion
Increases insulin gene transcription, messenger RNA stability, and biosynthesis
Inhibits glucagon secretion
Stimulates somatostatin secretion
Enhances β-cell responsivity and glucose competence
Induces β-cell neogenesis and proliferation
Inhibits β-cell apoptosis
Gastrointestinal tract
Inhibits gastric emptying
Inhibits gastric acid secretion
Cardiovascular system
Increases heart rate and blood pressure in rodents
Improves myocardial contractility
Reduces cardiomyocyte apoptosis
Central nervous system
Inhibits food and water intake
Stimulates luteinizing hormone–releasing hormone secretion
Increases thyroid-stimulating hormone, luteinizing hormone, adrenocorticotropic hormone, and corticosterone/cortisol secretion *in vivo*
Thyroid, lung, and kidney
Stimulates calcitonin secretion from C cells in the thyroid gland
Enhances mucous secretion, pulmonary muscle relaxation, and surfactant secretion in lung
Promotes diuresis and natriuresis in kidney

The physiologic importance of GLP-1 has been demonstrated using GLP-1R antagonists, immunoneutralizing antisera, and GLP-1R knockout mice. Elimination of GLP-1 activity with GLP-1 immunoneutralizing antisera or the GLP-1R antagonist exendin (9-39) results in impaired glucose tolerance and diminished glucose-stimulated insulin levels in animals and humans (221–224). Similarly, mice with a targeted inactivation of the *GLP-1R* gene (GLP-1R$^{-/-}$) are glucose intolerant and exhibit defective glucose-stimulated insulin secretion (225). GLP-1 confers glucose sensitivity to β cells, thereby improving the ability of the endocrine pancreas to sense and respond to glucose (226,227); however, GLP-1R signaling is not required for preservation of β-cell glucose sensitivity in the mouse (228). The demonstration that GLP-1 up-regulates the expression of components of the β-cell glucose sensing system (i.e., glucose transporters and glucokinase) may provide a partial mechanism for the effects of GLP-1 on β-cell glucose responsivity (229,230).

GLP-1 also inhibits glucagon and stimulates somatostatin secretion (217). The increase in somatostatin secretion appears to be direct, via GLP-1Rs on somatostatin-secreting pancreatic δ cells (231), whereas the inhibitory effect of GLP-1 on glucagon secretion may be indirect, perhaps through stimulation of insulin and somatostatin, both of which inhibit glucagon secretion. However, GLP-1 may also inhibit glucagon secretion directly, via interaction with GLP-1Rs on α cells (142). Basal GLP-1 signaling in the fasting state is important for glucoregulation because administration of the antagonist exendin (9-39) to humans increases fasting glucose and glucagon, suggesting that even the low basal levels of GLP-1 exert a tonic inhibitory effect on glucagon-secreting α cells (156). The insulinotropic and glucagonostatic effects of GLP-1 are glucose-dependent (232,233). Thus, when blood glucose levels decrease, GLP-1 no longer stimulates insulin secretion or inhibits glucagon secretion, thereby reducing the possibility of hypoglycemia.

GLP-1 administration also leads to a number of changes in β-cell differentiation and function, including induction of proliferation and neogenesis of pancreatic β cells, reduced apoptosis, and increased differentiation of exocrine-like cells toward a more differentiated β-cell phenotype (234). GLP-1 activates the expression of immediate early genes which encode for transcription factors that regulate islet cell proliferation and differentiation. Furthermore, the differentiation of pancreatic exocrine cells toward a differentiated endocrine and β-cell–like phenotype is associated with induction of genes required for glucose-sensing and insulin gene expression (235–237). The molecular mechanisms mediating the GLP-1R–dependent activation of the endocrine differentiation pathway are poorly understood but may involve synergy with transforming growth factor-β and coordinate changes in Smad transcription factor activity (238).

GLP-1R agonists also stimulate β-cell neogenesis and proliferation, and increase β-cell mass in both normal and diabetic rodents (235,239–242). Conversely, elimination of GLP-1R signaling in the mouse is associated with reduced numbers of large β-cell clusters and alterations in islet α-cell topography (243), and GLP-1R$^{-/-}$ mice exhibit a reduced adaptive response and greater hyperglycemia after partial pancreatectomy (244). The intracellular signal transduction pathways whereby GLP-1 mediates its proliferative effects have not been clearly defined, but they likely involve the induction of PI_3K, epidermal growth factor receptor transactivation, and activation of p38 MAPK and PKCξ (245,246).

GLP-1 may also regulate β-cell mass via increasing the resistance to apoptosis (246–250). GLP-1 inhibits apoptosis in β-cell lines and in nontransformed rodent β cells. Similarly, treatment of diabetic mice or rats with GLP-1R agonists reduces β-cell apoptosis (247,251,252). Furthermore, GLP-1R signaling appears to be essential for β-cell survival because GLP-1R$^{-/-}$ mice exhibit more severe hyperglycemia and enhanced sensitivity to the cytotoxic effects of streptozotocin on β cells *in vivo* (247). Importantly, the antiapoptotic actions of GLP-1 have been demonstrated in experiments using human islets (249,250). Incubation of freshly isolated human islets with GLP-1 for 72 hours reduced the expression of proapoptotic genes, improved cell viability, and enhanced glucose-stimulated insulin secretion (249). Complementary studies demonstrated that GLP-1 prevented apoptosis induced by high glucose or palmitate in human islets *in vitro* through mechanisms involving PKB and Akt (250).

In the gastrointestinal tract, GLP-1 inhibits pentagastrin- and meal-induced gastric acid secretion and gastric emptying (253,254). The reduction of gastric emptying attenuates meal-associated excursions in blood glucose, appears

dependent on the vagus nerve, and likely involves both central and peripheral GLP-1 actions (255,256). Vagal afferent denervation or GLP-1R antagonism with exendin (9-39) eliminates the effects of central and peripheral GLP-1 on gastric emptying. Furthermore, a recombinant GLP-1-albumin protein (Albugon) that does not appear to cross the blood–brain barrier retains the ability to inhibit gastric emptying (256), which is consistent with the importance of ascending GLP-1R–dependent pathways for control of gut motility.

GLP-1 increases systolic, diastolic, and mean arterial blood pressure and heart rate in rats (257) and increases heart rate in calves (258). The effects of GLP-1 on the cardiovascular system are mediated through central and peripheral mechanisms (257,259,260). In experimental models of cardiovascular dysfunction such as pacing-induced ventricular failure or coronary occlusion and reperfusion in dogs, GLP-1 administration significantly improved ventricular function (261,262). GLP-1 may also exert a direct effect on cardiovascular parameters through interaction with GLP-1Rs in the heart (263). GLP-1 improved myocardial glucose uptake and cardiac contractility in dogs with pacing-induced heart failure (262). Furthermore, GLP-1 improved regional wall motion recovery after transient coronary artery occlusion and a 24-hour reperfusion period in dogs (261). Moreover, a 72-hour infusion of GLP-1 in patients after acute myocardial infarction and angioplasty resulted in markedly improved ventricular function (264). Conversely, GLP-1R signaling appears essential for normal cardiac structure and function because GLP-1R$^{-/-}$ mice exhibit ventricular hypertrophy and an impaired cardiovascular response to external stress (265).

Intracerebroventricular administration of GLP-1 inhibits short-term food and water intake in rodents (225,266,267), and peripheral administration promotes satiety and suppresses energy intake in healthy, diabetic, and obese humans (268–270). Pharmacologic administration of GLP-1 may modify feeding behavior through direct interaction with satiety centers, or through mechanisms involving induction of interoceptive stress and visceral illness (271,272). The recombinant GLP-1-albumin protein Albugon rapidly inhibits food intake and activates neuronal c-fos expression without directly penetrating the CNS (256). Administration of either GLP-1 or noxious substances such as lithium chloride results in a similar pattern of neuronal c-fos activation in rats and elicits comparable aversive responses consistent with induction of visceral illness (272–275). Thus, the observation that excess GLP-1 induces nausea and reduction of food intake in human subjects may reflect activation of central aversive signaling pathways.

Consistent with the cytoprotective actions of GLP-1 on β cells, activation of GLP-1R signaling promotes neuronal survival in diverse modes of cytotoxic injury (276,277). Furthermore, GLP-1R$^{-/-}$ mice exhibit a learning deficit that is improved after localized restoration of CNS GLP-1R signaling and display enhanced sensitivity to neuronal injury and increased seizure activity after kainate administration (278). These findings have engendered interest in the potential use of GLP-1R agonists as neuroprotective agents (279).

GLP-1 also modulates components of the hypothalamic-pituitary axis. GLP-1 stimulates cAMP formation and thyroid-stimulating hormone (TSH) release from cultured mouse pituitary thyrotrophs and isolated rat anterior pituitary cells (280) and enhances luteinizing hormone-releasing hormone secretion from rodent hypothalamic neuronal cell lines. Furthermore central GLP-1 administration stimulates TSH, luteinizing hormone, corticosterone, and vasopressin secretion in rats (281,282); however, GLP-1R$^{-/-}$ mice do not exhibit a major impairment of hypothalamic-pituitary function (283). Nevertheless, short-term infusions of GLP-1 transiently increase plasma levels of adrenocorticotropic hormone and cortisol in healthy human subjects (284). Notably, experimental evidence in rodents clearly indicates that systemic administration of GLP-1 or exendin-4 results in rapid activation of brainstem proglucagon neurons, indicating that peripheral GLP-1 is capable of activating the CNS circuits that, in turn, produce GLP-1 in the brain (285).

GLUCAGON-LIKE PEPTIDE-1 RECEPTOR AGONISTS AND THE TREATMENT OF TYPE 2 DIABETES

GLP-1 reduces blood glucose levels through stimulatory effects on insulin secretion, reduction of glucagon secretion and gastric emptying, and indirectly through promotion of satiety leading to weight loss and improved insulin sensitivity. Short-term administration of native GLP-1 rapidly decreases plasma glucose in patients with type 2 diabetes (286–288). Furthermore, postprandial administration of native GLP-1 significantly attenuated meal-related glycemic excursion in diabetic patients, and a 3-week trial of preprandial subcutaneous GLP-1 injections improved postprandial glycemic control and reduced plasma glucagon in subjects with type 2 diabetes (289). The efficacy of native GLP-1 for the treatment of type 2 diabetes was illustrated in studies of continuous subcutaneous GLP-1 infusion for 6 weeks. Subjects treated with GLP-1 exhibited improved β-cell function, decreased fasting and postprandial glucose levels, a significant reduction in HbA1c, in association with modest weight loss and improved insulin sensitivity (290). Hence, there are considerable data from human clinical studies that administration of native GLP-1 exhibits therapeutic utility in the treatment of diabetic patients (291,292).

Nevertheless, the therapeutic use of native GLP-1 would require multiple daily subcutaneous injections or continuous administration via subcutaneous infusion, because of the short circulating half-life of the native peptide *in vivo* (98,103,293,294). Accordingly, considerable effort has been devoted to development and characterization of GLP-1R agonists that are resistant to DPP-IV–mediated degradation, and that exhibit more prolonged durations of action *in vivo.* Exendin-4 is a naturally occurring GLP-1–related peptide isolated from the venom of the Heloderma suspectum lizard (295). Exendin-4 exhibits 53% identity to mammalian GLP-1, is a potent agonist at the GLP-1 receptor (155), and is

encoded by a separate gene distinct from lizard GLP-1 (296). Analysis of exendin-4 action in preclinical studies demonstrates that exendin-4 is a long-acting GLP-1R agonist that exhibits a full spectrum of GLP-1R–dependent actions in normal and diabetic rodents (297–299).

Exendin-4, subsequently renamed Exenatide for clinical use, also has been shown to potently reduce blood glucose in human subjects with type 2 diabetes. Acute intravenous infusion of exendin-4 to healthy volunteers reduced fasting and postprandial glucose in association with inhibition of gastric emptying and reduced food intake (300). Repeated subcutaneous administration of exendin-4 for 4 weeks improved blood glucose and significantly decreased HbA1c (301). The clinical efficacy of Exenatide administration was examined in a series of phase 2 and 3 trials in patients with type 2 diabetes. Addition of Exenatide twice daily to treatment regimens previously encompassing metformin, a sulfonylurea, or both agents produced a significant reduction in HbA1c with prevention of weight gain in a 4-week study (302,303). Exenatide has completed phase 3 clinical trials as add-on therapy in diabetic patients with inadequate glycemic control who were treated previously with metformin or a sulfonylurea agent, or both, and is the first GLP-1R agonist approved for the treatment of type 2 diabetes.

Additional GLP-1R–based agonists in clinical development include Liraglutide, a human DPP-IV–resistant analogue that exhibits noncovalent binding to albumin and a prolonged pharmacokinetic profile after once daily administration (304,305). Liraglutide has completed phase 2 clinical trials and significantly reduced HbA1c with no associated weight gain in a 12-week monotherapy study (306). CJC-1131 is a human GLP-1 analogue that forms a covalent bond to human serum albumin and exhibits GLP-1R–dependent actions in preclinical studies (242). CJC-1131 also has been shown to effectively reduce glycemia in 12-week studies of subjects with type 2 diabetes. Taken together, the available evidence strongly suggests that one or more injectable GLP-1R agonists may be used for the treatment of type 2 diabetes.

ENHANCING INCRETIN ACTION VIA INHIBITION OF DIPEPTIDYL PEPTIDASE-IV

The rapid degradation of native GLP-1 has fostered efforts directed at preventing GLP-1 degradation for the treatment of diabetes. DPP-IV, also known as CD26, is a widely expressed cell surface–associated peptidase that also circulates as a soluble form in the plasma. DPP-IV cleaves peptides at the position 2 alanine and appears to be essential for normal glucose homeostasis, because mice with genetic inactivation of CD26 exhibit increased levels of GLP-1 and reduced glycemic excursion after glucose challenge (106). Because DPP-IV is the dominant enzyme regulating GLP-1 degradation (98,103,106), considerable efforts have focused on the development of DPP-IV inhibitors for the treatment of type 2 diabetes. DPP-IV inhibitors reduce blood glucose, stimulate insulin secretion, and increase the levels of intact GLP-1 and GIP in preclinical models of diabetes (307–309). Moreover, administration of DPP-IV inhibitors to human subjects with type 2 diabetes results in significant reduction of glycemia, together with decreased levels of circulating glucagon and an improvement in the insulin/glucose ratio (310,311). Several DPP-IV inhibitors currently are in late-stage clinical development for the treatment of type 2 diabetes (291,292,312).

BIOLOGICAL ACTIONS OF GLUCAGON-LIKE PEPTIDE-2

GLP-2 is a 33-amino-acid peptide cosecreted with GLP-1 from gut endocrine cells in a nutrient-dependent manner (112,113). The principal biological consequence of exogenous GLP-2 administration is the expansion of the mucosal epithelium in the small bowel (169), caused by enhanced crypt cell proliferation and a reduction in enterocyte apoptosis (313,314). The intestinotrophic actions of GLP-2, which are summarized in Figure 6-3, have been demonstrated in mice (169,315), rats (100,316,317), pigs (318,319), and human subjects (320). GLP-2 also acutely enhances hexose transport (321,322), reduces epithelial permeability (323), enhances gut barrier function (324), and reduces gastric motility and acid secretion (325,326) after exogenous administration *in vivo* (see Fig. 6-3). Whether GLP-2 action is essential for the growth and survival of the developing or adult mucosal epithelium remains uncertain. Although GLP-2(3-33) modestly attenuates exogenous GLP-2 action, it exhibits properties of both a partial antagonist and a weak agonist (327); hence, optimal tools for analysis of loss of GLP-2 action have not yet been identified.

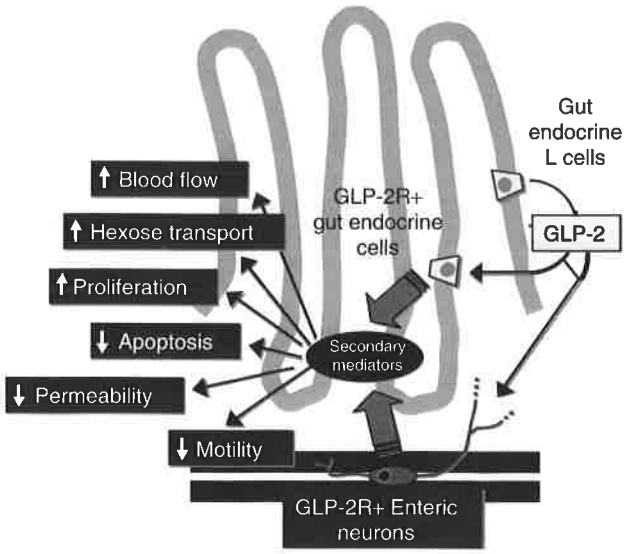

FIG. 6-3. The actions of glucagon-like peptide-2 (GLP-2) in the gastrointestinal epithelium. GLP-2 secreted from gut endocrine cells acts via gut endocrine cells, myofibroblasts, or enteric neurons to promote diverse actions in the gut epithelium. GLP-2R, glucagon-like peptide-2 receptor.

The trophic and cytoprotective actions of GLP-2 also have been examined in the setting of experimental intestinal injury. GLP-2 facilitates mucosal adaptation in rats after major small-bowel resection (317), and intravenous GLP-2 prevents mucosal hypoplasia in rats fed parenterally (328). The extent of mucosal damage is markedly attenuated by exogenous GLP-2 in mice with dextran-sulphate–induced colitis (329) or after indomethacin-induced enteritis (330). Similarly, GLP-2 administration improved intestinal disease activity scores and survival in mice with chemotherapy-induced enteritis (331), in rats with autoimmune enteritis (332), and in rats after occlusion of the superior mesenteric artery (333). Hence, pharmacologic GLP-2 administration promotes intestinal healing and prevents mucosal injury in a diverse number of experimental models of gut injury (234,334).

GLUCAGON-LIKE PEPTIDE-2 ADMINISTRATION TO HUMAN SUBJECTS

Because exogenous GLP-2 administration significantly increases the surface area and absorptive capacity of the small-bowel epithelium, GLP-2 or structurally related GLP-2 analogues may exhibit therapeutic potential for the treatment of human subjects with short bowel syndrome (335). Administration of native GLP-2, 400 μg subcutaneously twice daily for 35 days, to human subjects without a terminal ileum and colon significantly improved energy absorption and body weight, and increased lean body mass. GLP-2–treated subjects exhibited reduced enteral fluid loss and reduced gastric emptying but no change in small-bowel transit time (320). The potential therapeutic efficacy of a more potent DPP-IV–resistant GLP-2 analogue currently is being examined in separate clinical trials of patients with Crohn's disease or short bowel syndrome.

GLP-2 also appears to regulate bone mass through direct effects on bone resorption. Administration of GLP-2 twice daily to human subjects with short bowel syndrome reduced markers of bone turnover in a 5-week study (336). Because food ingestion is known to regulate gut hormone release and bone resorption, the relation between gut peptides and bone resorption was studied after single-dose administration of various peptides in healthy postmenopausal women. Although administration of GIP and GLP-1 had no significant effect on bone turnover, GLP-2 reduced circulating levels of the C-terminal telopeptide region of type I collagen and decreased urinary excretion of dihydropyrimidine dehydrogenase (DPD)/creatinine, both markers of bone resorption (337).

GLUCOSE-DEPENDENT INSULINOTROPIC POLYPEPTIDE

Synthesis and Secretion

GIP, a 42-amino-acid peptide and the first incretin hormone to be identified (338,339), is synthesized in and released from intestinal K cells in response to nutrient ingestion. GIP was originally identified on the basis of its ability to inhibit gastric acid secretion in dogs, and subsequently was shown to potentiate glucose-stimulated insulin secretion (338,339). The sequence of GIP is highly conserved across species, with more than 90% identity at the amino acid level for human, rat, mouse, porcine, and bovine GIP. The peptides encoded within the N- and C-terminal segments of pro-GIP have no known biological function.

GIP is expressed predominantly in the stomach and in the K cells of the proximal small intestine. GIP mRNA also has been detected in the rat submandibular gland (73). There is little information about the factors that regulate GIP gene expression; however, nutrients up-regulate levels of GIP mRNA in the rat duodenum and submandibular salivary gland, whereas fasting significantly reduces levels of GIP mRNA (340,341).

GIP secretion reflects the rate of nutrient absorption, rather than the simple presence of nutrients in the small intestine. Fat is a potent stimulus for GIP secretion in humans, whereas in rodents and pigs, carbohydrates are more effective secretagogues (342). GIP contains an alanine at position 2 and is a substrate for enzymatic inactivation by DPP-IV (293). The half-life of biologically active intact GIP(1-42) is estimated to be less than 2 minutes in rats (103, 343) and approximately 7 and 5 minutes in healthy subjects and patients with type 2 diabetes, respectively (343). After intravenous infusion of GIP in humans, intact bioactive GIP accounted for approximately 40% of the total detectable amount of immunoreactive GIP, whereas only about 20% of total immunoreactive GLP-1 remained intact in the same studies (343). GIP is cleared through the kidney, and levels of GIP are increased in patients with uremia or chronic renal failure.

Biological Actions of Glucose-Dependent Insulinotropic Polypeptide

The increasingly diverse actions of GIP on various tissues are summarized in Table 6-2. The dominant actions of GIP on the islet β cell are the enhancement of glucose-dependent insulin secretion via increase of intracellular cAMP, inhibition of adenosine triphosphate–sensitive K^+ channels, increase in intracellular Ca^{2+}, and engagement of the β-cell secretory machinery (344). GIP also enhances insulin gene transcription and up-regulates the expression of glucose transporters and glucokinase, which are components of cellular glucose sensors (345). GIP functions as a β-cell growth factor for islet β cells *in vitro* via activation of cAMP/PKA, MAPK, and PI_3K-dependent pathways (346,347). Whether GIP is also important for growth and survival of islet β cells *in vivo* remains uncertain.

GIP also regulates adipocyte lipid metabolism (342) including stimulation of glucose uptake and increases the sensitivity of insulin-stimulated glucose transport. GIP enhances fatty acid production and insulin-stimulated incorporation of fatty acids into triglyceride, augments lipoprotein lipase

TABLE 6-2. *Summary of glucose-dependent insulinotropic polypeptide action*

Pancreas
Enhances glucose-stimulated insulin secretion
Stimulates insulin gene expression
Promotes β-cell proliferation and reduces β-cell apoptosis
Adipose tissue
Stimulates insulin-dependent glucose uptake
Increases insulin receptor affinity
Enhances fatty acid synthesis and incorporation into triglyceride
Augments lipoprotein lipase synthesis and activity
Reduces glucagon-stimulated lipolysis
Bone
Stimulates alkaline phosphatase activity and collagen type I messenger RNA
Increases bone mineral density in rodents
Other tissues
Up-regulates intestinal hexose transport
Stimulates glucocorticoid secretion in rodents
Modulates vascular bed-type–dependent endothelial tone

synthesis and activity, and reduces glucagon-stimulated lipolysis in adipose tissue. GIP may also have lipolytic effects in adipocytes (348). The signaling mechanism(s) activated by GIP in adipocytes have not been fully elucidated (342). Intriguingly, GIPR null mice are resistant to diet-induced obesity and exhibit relative reductions in adipose tissue mass after high fat feeding, together with a reduction in expression of the key enzyme Acyl CoA:diacylglycerol transferase 1 (Dgat1) in adipose tissue (186).

GIP may also regulate bone formation in osteoblast cells including increases in alkaline phosphatase activity and levels of collagen type I mRNA (178). GIP treatment increases bone mineral density in the ovariectomized rat (349), although acute administration of GIP to human subjects was not associated with changes in markers of bone turnover (337). GIP stimulates glucocorticoid secretion in rats via a cAMP/PKA-dependent signaling pathway (350). Although GIP does not appear to regulate cortisol secretion in healthy human subjects (351), aberrant expression of the GIPR in adrenocortical adenomas is associated with the pathogenesis of meal-induced Cushing syndrome (351–353).

Glucose-Dependent Insulinotropic Polypeptide Administration in Human Subjects

Because GIP stimulates glucose-dependent insulin secretion, several studies have examined the therapeutic potential of GIP for the treatment of type 2 diabetes. Remarkably, although GIP is a potent insulinotropic agent in healthy humans, GIP action is significantly diminished in human subjects with type 2 diabetes (86,354). Unlike GLP-1, GIP does not significantly inhibit glucagon secretion or gastric emptying in humans (355,356). Although GIP analogs engineered for resistance to DPP-IV action exhibit enhanced insulinotropic properties in preclinical studies (357), the potential of these analogs to reduce blood glucose in human subjects with type 2 diabetes has not been carefully examined. Hence, the available evidence suggests that GIP is unlikely to be a therapeutic candidate for the treatment of diabetic human subjects (358).

Pancreatic Polypeptide

Pancreatic polypeptide (PP) is a structurally related member of the peptide YY/neuropeptide Y (PYY/NPY) family. PP is a 36-amino-acid peptide predominantly expressed in the endocrine pancreas; however, rare PP-immunopositive enteroendocrine cells have been described in some but not all species (359). PP appears to preferentially recognize the NPY 4 receptor (360). PP secretion is stimulated by food ingestion and exercise, and vagal tone is an important determinant regulating PP secretion in rodents and human subjects.

Studies in rodents have also demonstrated an anorectic role for either centrally or peripherally administered PP (361). Intriguingly, PP not only reduces food intake but also increases energy expenditure after intraperitoneal administration in genetically obese mice (362). Similarly, transgenic mice with PP overexpression in pancreatic islets exhibit reduced milk intake during the neonatal period, with decreased food intake together with reduced body weight a feature of older PP transgenic mice (362). Although the available evidence clearly implicates PP as a regulator of food intake, whether PP is essential for body weight homeostasis has not yet been determined.

The biological actions of PP in human subjects remain somewhat obscure; however, PP, like PYY(3-36), exerts anorectic actions *in vivo.* Administration of PP reduces food intake in healthy human subjects (363) and in patients with Prader–Willi syndrome (364).

Orexins

The orexin peptide families, also referred to as the hypocretins, are produced predominantly in the CNS. Original studies of orexin biology linked orexin action to the control of feeding behavior (365), whereas more recent data have implicated a role for orexins in the regulation of arousal and sleep physiology (366,367). Orexin-like immunoreactivity and orexin receptor mRNA transcripts have also been localized to the enteric nervous system in both the submucosal and myenteric ganglia (368). Intriguingly, fasting activates orexin plus neurons in the gut, and circulating levels of plasma orexin-A are increased after fasting in both rodents and humans (369). Intriguingly, orexin is also secreted from islet α and β cells in a glucose-dependent manner, and administration of orexin-A increases plasma levels of glucagon and glucose in fasted rats (370). Both orexin and the OX2 receptor have been localized to enteroendocrine cells; however, the precise function of the orexin system in gut endocrine cells remains poorly understood.

In summary, enteroendocrine peptides exert increasingly complex actions on the control of gut motility, epithelial integrity, cytoprotection, satiety, and pancreatic endocrine function. The actions of many of these peptides, as revealed through studies using antagonists and genetic loss of function mutants, are essential for control of glucose and energy homeostasis. Moreover, GLP-1 and GLP-2 agonists are being evaluated in clinical trials for the treatment of diabetes and short bowel syndrome. Hence, understanding the pleiotropic actions of these peptides has relevance for understanding the biology of gut hormone action and the potential treatment of specific human diseases.

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