

MINIREVIEW

Glucagon-Like Peptides: Regulators of Cell Proliferation, Differentiation, and Apoptosis

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Peptide hormones are secreted from endocrine cells and neurons and exert their actions through activation of G protein-coupled receptors to regulate a diverse number of physiological systems including control of energy homeostasis, gastrointestinal motility, neuroendocrine circuits, and hormone secretion. The glucagon-like peptides, GLP-1 and GLP-2 are prototype peptide hormones released from gut endocrine cells in response to nutrient ingestion that regulate not only energy absorption and disposal, but also cell proliferation and survival. GLP-1 expands islet mass by stimulating pancreatic β -cell proliferation and induction of islet neogenesis. GLP-1 also promotes cell differentiation, from exocrine cells or immature islet progenitors, toward a more differentiated β -cell phenotype. GLP-2 stimulates cell proliferation in the gastrointestinal mucosa, leading to expansion

of the normal mucosal epithelium, or attenuation of intestinal injury in experimental models of intestinal disease. Both GLP-1 and GLP-2 exert antiapoptotic actions *in vivo*, resulting in preservation of β -cell mass and gut epithelium, respectively. Furthermore, GLP-1 and GLP-2 promote direct resistance to apoptosis in cells expressing GLP-1 or GLP-2 receptors. Moreover, an increasing number of structurally related peptide hormones and neuropeptides exert cytoprotective effects through G protein-coupled receptor activation in diverse cell types. Hence, peptide hormones, as exemplified by GLP-1 and GLP-2, may prove to be useful adjunctive tools for enhancement of cell differentiation, tissue regeneration, and cytoprotection for the treatment of human disease. (*Molecular Endocrinology* 17: 161–171, 2003)

THE PROGLUCAGON GENE (see Fig. 1) encodes the sequences of glucagon and several structurally related glucagon-like peptides, collectively referred to as the proglucagon-derived peptides (PGDPs) (1, 2). Pancreatic glucagon is generated in islet A cells via the action of prohormone convertase 2 and regulates hepatic glucose flux through control of glycogenolysis and gluconeogenesis. In contrast, prohormone convertase 1/3 expression in gut endocrine cells results in the liberation of two larger peptides that both contain the sequence of glucagon, oxyntomodulin, and glicentin (Fig. 1), two intervening peptides, and two glucagon-like peptides, GLP-1 and GLP-2 (3, 4). In the brain, posttranslational processing liberates a profile of PGDPs that overlaps the pattern seen in the pancreas and gut (5, 6).

Abbreviations: GLP-1 and -2, Glucagon-like peptide 1 and 2; GLP-1R and -2R, GLP-1 and -2 receptors; GLUT-2, glucose transporter 2; GPCR, G protein-coupled receptor; GSK-3, glycogen synthase kinase 3; ICC, islet-like cell cluster; NIP, nestin-positive islet-derived progenitor; PACAP, pituitary adenylate cyclase-activating peptide; PGDP, proglucagon-derived peptide; PI-3K, phosphatidylinositol-3-kinase; PKA, protein kinase A; PKC, protein kinase C; PDX-1, pancreatic duodenal homeobox-1; STZ, streptozotocin; VIP, vasoactive intestinal peptide.

Although the metabolic actions of glucagon have been clearly delineated over several decades, the structural identity of the glucagon-like peptides remained unclear until the molecular cloning of the mammalian cDNAs and genes was reported in the early 1980s (1, 2, 7). The peptide sequence of mammalian GLP-1 is identical in mice, rats, and humans. The GLP-2 amino acid sequence is also highly conserved, with only one (rat) or two (mouse) amino acid differences compared with the human sequence (8). Although initial studies of GLP-1 action using GLP-1(1–37) or GLP-1(1–36)amide concluded that these peptides were devoid of metabolic activities, subsequent experiments using N-terminal truncated peptides beginning at the position 7 His residue revealed that both GLP-1(7–36)amide and GLP-1(7–37) were potent insulinotropic peptides both *in vitro* (9), and in rodents, pigs, and human subjects *in vivo* (10–12). Similarly, the actions of GLP-2 have been delineated and are directed toward regulation of the function and proliferation of the gut epithelial mucosa (13).

GLP-1 not only stimulates glucose-dependent insulin secretion, but also increases somatostatin (14) and inhibits glucagon secretion (15), gastric emptying (16), and gastric acid secretion (17), and reduces food in-

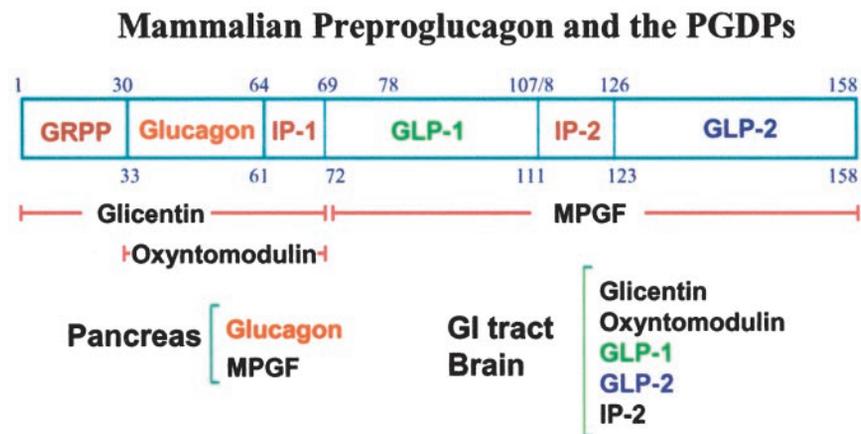


Fig. 1. Structure of Mammalian Proglucagon and Tissue-Specific Processing of Proglucagon into the PGDPs

The numbers above and below the proglucagon sequence refer to specific amino acid residues within proglucagon. IP, Intervening peptide.

take after both intracerebroventricular (18) or peripheral administration (19). Similarly, GLP-2 promotes nutrient absorption and inhibits both gastric acid secretion and gut motility when administered peripherally. Taken together, these actions of GLP-1 and GLP-2 provide a scientific rationale for assessing the effectiveness of these peptides, or structurally related agonists, in the treatment of type 2 diabetes and intestinal insufficiency, respectively (20, 21).

EFFECTS OF GLP-1 ON CELLULAR DIFFERENTIATION AND PROLIFERATION

β -Cell Differentiation and Exocrine Cell Lines

The actions of GLP-1 are mediated by the glucagon-like peptide-1 receptor (GLP-1R), a 463-amino-acid member of the G protein-coupled receptor (GPCR) superfamily (22). GLP-1R is expressed on islet β -cells (9, 22), and GLP-1R activation leads to activation of cAMP and both protein kinase A-dependent and -independent actions (23–25). In addition to enhancement of proinsulin biosynthesis and insulin secretion in islet cells, GLP-1 agonists induce features of cellular differentiation in exocrine cell lines. Exposure of rat AR42J cells to GLP-1 for 24–48 h leads to an initial increase in levels of cAMP and cellular proliferation, followed by cessation of proliferation and expression of the islet hormones, insulin, glucagon, and somatostatin, in up to 50% of cells (26). Furthermore, GLP-1 treatment also induced expression of mRNAs for glucose transporter-2 (GLUT-2) and glucokinase, in association with the capacity to release insulin in a glucose-dependent manner (26). A similar set of experiments was carried out using two pancreatic ductal cell lines, rat ARIP and human PANC-1 cells (27). GLP-1 or exendin-4 (a potent lizard GLP-1R agonist) treatment for 72 h of ARIP cells, which express

endogenous pancreatic duodenal homeobox-1 (PDX-1), promoted cell aggregation, with some heterogeneity in the number of cells responding (27). GLP-1 treatment also induced insulin expression in a majority of ARIP cells. Native PANC-1 cells that do not express PDX-1 did not differentiate into insulin-producing cells in response to GLP-1; however, transfection with PDX-1 was sufficient for establishing GLP-1-dependent differentiation into insulin-producing cells (27). Furthermore, PANC-1 cells transfected with PDX-1 exhibited increased expression of the endogenous GLP-1R.

The importance of PDX-1 and hepatocyte nuclear factor-3 β for GLP-1R-dependent cell differentiation has also been examined in Capan-1 cells, derived from a human pancreatic ductal carcinoma, which express the GLP-1R. A subset, approximately 10% of Capan-1 cells, normally contain insulin and/or glucagon immunopositivity, and after several days of exposure to 0.1 nM exendin-4, approximately 40% of the cells exhibited immunopositivity for insulin and/or glucagon (28). Treatment of Capan-1 cells with exendin-4 increased MAPK activity but not cell proliferation and was associated with increased cAMP accumulation and enhanced expression of glucokinase, GLUT-2, BETA2/NeuroD, hepatocyte nuclear factor-3 β (Foxa2), and PDX-1. Exendin-4 promoted the nuclear localization of PDX-1, which was abolished after coadministration of the protein kinase A (PKA) inhibitor H-89 (28).

GLP-1 agonists also enhanced islet cell differentiation in the β -cell line β lox5, an immortalized cell line derived from human islet β -cells infected with a retrovirus encoding SV40 T antigen. Treatment of β lox5 cells expressing transfected PDX-1 with the GLP-1 agonist exendin-4 resulted in induction of insulin gene expression; however, exendin-4 alone, in the absence of PDX-1 expression, had no effect on cell differentiation despite inducing expression of the cAMP response element binding protein (29). Although ex-

endin-4 treatment of cells *in vitro* before transplantation under the kidney capsule was required for induction of C-peptide secretion from engrafted cells, continuous treatment with exendin-4 *in vivo* did not result in C-peptide secretion, suggesting that sustained administration of exendin-4 was not invariably associated with enhanced β -cell function or survival (29).

β -Cell Differentiation and Fetal or Adult Islet Precursors

Several studies have used fetal islet cell precursors to examine whether exposure to GLP-1R agonists is associated with enhanced differentiation of previously immature islet precursors. Incubation of fetal porcine islet-like cell clusters (ICCs) with 100 nM GLP-1 for periods ranging from hours to several days resulted in enhanced glucose-stimulated insulin secretion, with increased formation of β -cells from undifferentiated cells, in association with increased numbers of PDX-1+ cells (30). Furthermore, transplantation of GLP-1-treated ICCs into severe combined immunodeficient mice resulted in increased numbers of β -cells that appeared functionally mature as assessed by subsequent studies of glucose-induced insulin release *in vivo* (30). Exendin-4 induced PDX-1 expression but did not increase the number of insulin-immunopositive cells in human ICCs prepared from 16- to 24-wk gestation human fetal pancreata (31). After transplantation of the human ICCs under the kidney capsule of athymic rats, a 10-d treatment with exendin-4 was initiated commencing 48 h after transplantation. Eight weeks after ICC transplantation, the human ICCs from exendin-4-treated rats exhibited glucose-stimulated insulin secretion, whereas rats implanted with ICCs in the absence of exendin-4 administration did not exhibit increases in human C peptide after glucose challenge (31). Hence, these results suggest that administration of GLP-1 agonists promotes differentiation of functional β -cells both *in vitro* and *in vivo*.

GLP-1Rs have also been detected, using both immunocytochemistry and RT-PCR, on a subpopulation of nestin-immunopositive cells, designated nestin-positive islet-derived progenitor (NIP) cells, within human pancreatic islets and ducts (32, 33). GLP-1 increased intracellular calcium in nestin-positive cells at basal (5.6 mM) but not high (20 mM) glucose, and these actions were blocked by the GLP-1R antagonist exendin(9–39). Expansion of NIP cultures for 7–12 d in the presence of GLP-1 promoted changes in cell morphology and the appearance of insulin-immunopositive cells in 5–30% of NIP cultures, effects that were also blocked by the GLP-1R antagonist exendin(9–39). Furthermore, approximately 30% of NIP clones treated with exendin-4 exhibited increased insulin secretion. Transfection of rat PDX-1 into long-term NIP cultures enhanced GLP-1 responsiveness as assessed by insulin promoter activity. Intriguingly, NIP clones express the proglucagon gene when they approach confluence and secrete GLP-1 into the culture medium,

raising the possibility that under some conditions, GLP-1 may act in an autocrine or paracrine manner, to regulate islet cell differentiation (33).

β -Cell Proliferation

The signal transduction mechanisms activated by the GLP-1R coupled to islet cell proliferation have been studied in immortalized mouse β TC-9 and rat INS-1 cells (34). Glucose and GLP-1 synergistically increased the expression of immediate early genes, including *c-fos*, *c-jun*, JunB, *zif-268*, and *nur-77* in islet INS-1 cells, and these effects were markedly attenuated by the L-type Ca^{2+} channel blocker nifedipine (34). GLP-1 also increased thymidine incorporation, phosphatidylinositol 3-kinase (PI-3K) activity, and PDX-1 DNA binding in a dose-dependent manner, and these actions were blocked by the PI-3K inhibitors wortmannin and LY294002 (35). GLP-1 together with glucose increased levels of PDX-1, GLUT-2, glucokinase, and insulin mRNAs in INS-1 cells and GLP-1R activation increased the nuclear translocation of PDX-1 and enhanced PDX-1 binding to insulin promoter elements in RIN1046–38 islet cells (36). Furthermore the proliferative effects of GLP-1 were not confined to INS-1 cells but were also demonstrated in primary rat islet cell cultures (35).

Analysis of specific signal transduction pathways activated by GLP-1 using INS-1 cells, MIN6 cells, and normal rat β -cells demonstrated increased ERK 1/2, p38 MAPK, and protein kinase B activities in association with nuclear translocation of the atypical protein kinase C (PKC) ζ isoform in both INS-1 cells and in normal rat β -cells (37, 38). Functional evidence implicating a role for PKC ζ in GLP-1-stimulated islet cell proliferation derives from observations that a dominant negative PKC ζ protein attenuated, whereas expression of a constitutively active PKC ζ mutant stimulated, islet cell proliferation (37).

The importance of GLP-1 for stimulation of islet cell proliferation was originally demonstrated in lean 20-d-old normoglycemic mice (Umea +/-) after 2 d of GLP-1 administration (39). Similarly, once daily administration of exendin-4 stimulated islet neogenesis and β -cell proliferation in normal rats whereas daily administration of exendin-4 for 10 d after partial pancreatectomy decreased blood glucose and stimulated pancreatic regeneration in rats via enhancement of islet neogenesis and β -cell proliferation (40). Remarkably, glucose tolerance remained significantly improved even several weeks after cessation of exendin-4 treatment (40). GLP-1 agonists also stimulated β -cell proliferation, expansion of β -cell mass, and islet neogenesis in both young and old Wistar rats, in association with increased islet PDX-1 expression and islet insulin content (41). Treatment of neonatal Goto-Kakizaki rats with GLP-1 or exendin-4 from d 2–6 resulted in stimulation of β -cell neogenesis and proliferation as measured by 5-bromo-2'-deoxyuridine labeling, with persistent expansion of β -cell mass detected at 2 months

of age despite the transient short-term exposure to GLP-1R agonists within the first week of life (42). Short-term 5-d neonatal exposure to GLP-1 or exendin-4 also stimulated islet neogenesis in Wistar rats treated with a single dose of streptozotocin (STZ) at birth (43). Similarly, administration of exendin-4 to db/db mice lowered blood glucose, enhanced islet neogenesis, up-regulated PDX-1 expression, and increased β -cell mass (44). Comparable results were obtained after treatment of db/db mice with the long acting GLP-1 analog NN221 (45). Treatment of db/db mice twice daily with sc NN221 200 μ g/kg for 2 wk significantly increased the β -cell proliferation rate and β -cell mass (45). Enhanced GLP-1R signaling appears to promote increased PDX-1 transcription *in vivo*, as treatment of transgenic mice containing a PDX-1 promoter-LacZ transgene with exendin-4 for 14 d resulted in increased pancreatic LacZ expression, predominantly localized to epithelial cells surrounding large and small pancreatic ducts (44).

The physiological importance of GLP-1R activity for islet proliferation has been examined in GLP-1R knockout (GLP-1R $^{-/-}$) mice. Although islet area is normal in the absence of GLP-1R signaling, GLP-1R $^{-/-}$ mice exhibit decreased numbers of large islets and abnormal islet topography, with significantly increased numbers of centrally located α -cells (46). To examine whether GLP-1R $^{-/-}$ β -cells exhibit a reduced capacity for proliferation in response to a metabolic stress, β -cell mass was determined in insulin-resistant ob/ob:GLP-1R $^{-/-}$ double-mutant mice (47). These experiments revealed marked up-regulation of insulin gene expression and significantly increased islet numbers and islet area despite the absence of a functional GLP-1R in leptin-deficient GLP-1R $^{-/-}$ mice (47). In contrast, GLP-1R $^{-/-}$ mice exhibit more severe hyperglycemia and impaired β -cell regeneration after partial pancreatectomy (48). Hence, the importance of endogenous GLP-1R signaling for the adaptive islet proliferative response to metabolic stress and pancreatic injury appears context specific.

GLP-2 AND STIMULATION OF INTESTINAL EPITHELIAL PROLIFERATION

Although the GLP-2 sequence is highly conserved in mammalian proglucagon genes, initial characterization of anglerfish pancreatic islet cDNAs revealed the presence of sequences encoding GLP-1 but not GLP-2, suggesting that the biological activity of GLP-2 may be unimportant in certain species (7). Later studies demonstrated that alternative RNA splicing gives rise to GLP-2-containing mRNA transcripts in fish, chicken, and lizards (8, 49), and daily GLP-2 administration was subsequently shown to promote crypt cell proliferation leading to expansion of the intestinal mucosal epithelium in mice (50).

The histological consequences of repeated GLP-2 administration are most evident in the small intestinal

epithelium, which exhibits elongated villi due predominantly to enhanced crypt cell proliferation and decreased enterocyte apoptosis (51, 52). GLP-2-treated murine enterocytes appear longer and exhibit increased numbers of microvilli (53). The proliferative effects of GLP-2 have been demonstrated in the small bowel of mice, rats, pigs, and humans after exogenous peptide administration (50, 54–56). Furthermore, GLP-2 is also weakly mitogenic for cells in the stomach and colon (57, 58).

The proliferative effects of exogenous GLP-2 contribute to intestinal epithelial regeneration in the setting of small-bowel enteritis (59–63) and colitis (64) and after major small-bowel resection (56, 65, 66). Increased circulating levels of GLP-2 are associated with development of small-bowel hyperplasia in experimental rodent diabetes (67), and immunoneutralization of GLP-2 reduced small-bowel epithelial proliferation in diabetic rats (68). The presence of enteral nutrients is not required for the trophic effects of GLP-2, as exogenous GLP-2 enhances the mass of the small-bowel epithelial mucosa in normal or tumor-bearing rats maintained on parenteral nutrition (69, 70).

THE GLP-2 RECEPTOR (GLP-2R) AND CELL PROLIFERATION

The molecular cloning of the cDNA encoding the GLP-2R has enabled delineation of mechanisms coupling GLP-2R activation to intestinal cell proliferation. The GLP-2R is structurally related to the glucagon and GLP-1 receptors and is coupled to cAMP generation in cells expressing a transfected human or rat GLP-2R (71, 72). Exogenous GLP-2 increased AP-1-dependent transcriptional activity and immediate early gene expression and weakly stimulated cell proliferation in BHK fibroblasts expressing a stably transfected rat GLP-2R (71, 72). The GLP-2R is expressed in a highly tissue-specific manner predominantly in the gastrointestinal tract (71, 73). A combination of immunocytochemistry and *in situ* hybridization experiments has localized GLP-2R expression to human enteroendocrine cells and murine enteric neurons (73, 74). In the murine gut, GLP-2 stimulates division of columnar, and not mucous progenitor cells, in association with activation of nuclear *c-fos* expression in enteric ganglia, followed by subsequent *fos* activation in crypt cells that do not directly express the GLP-2R (74). Hence, the GLP-2-dependent stimulation of intestinal epithelial proliferation *in vivo* appears indirect (Fig. 2) and is regulated by as yet unidentified downstream mediators of GLP-2 action (13, 75).

GLP RECEPTOR SIGNALING AND APOPTOSIS

The demonstration that administration of GLP-1 or GLP-2 leads to expansion of islet or intestinal epithelial

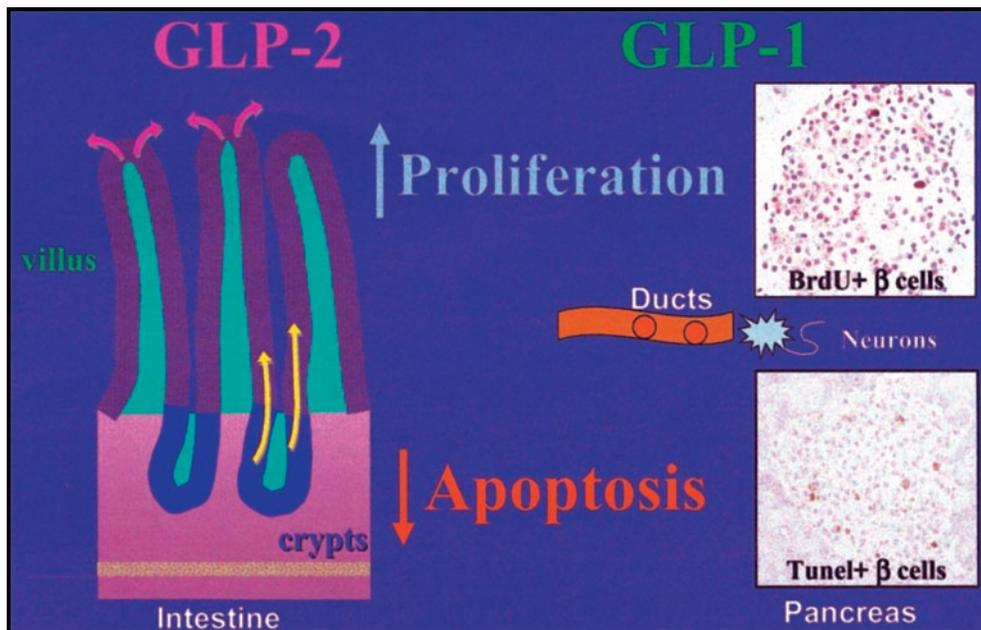


Fig. 2. GLP-2 Actions in the Gastrointestinal Mucosal Epithelium

The actions of GLP-2 are predominantly indirect and mediated by activation of GLP-2Rs in enteroendocrine cells or enteric neurons.

mass, respectively, has fostered studies directed at determining whether these peptides exert their effects via stimulation of cell proliferation alone or via both enhanced proliferation and decreased apoptosis (Fig. 2). Although the number of detectable apoptotic islet and intestinal epithelial cells is normally low in uninjured normal tissue, induction of experimental islet or intestinal injury leads to increased numbers of apoptotic cells in the endocrine pancreas and gut epithelium. Treatment of rat islets with the GLP-1 analog NN2211 reduced cytokine-induced apoptosis *in vitro* (76), and GLP-1 increased cell survival and reduced caspase activation in BHK fibroblasts expressing a transfected GLP-1R (77). Similarly, treatment of mice with exendin-4 reduced β -cell apoptosis induced by STZ, whereas GLP-1R $^{-/-}$ mice exhibit increased susceptibility to STZ-induced β -cell apoptosis (77). Furthermore, exendin-4 directly reduced the extent of apoptotic cell death in purified rat β -cells exposed to a combination of cytotoxic cytokines, consistent with a direct action for β -cell GLP-1R signaling in promoting resistance to cellular apoptosis (77).

The antiapoptotic properties of GLP-1 agonists have been demonstrated in Zucker diabetic rats and db/db mice. A 2-d continuous infusion of recombinant GLP-1 was associated with a marked increase in islet size and β -cell mass, formation of new islet-like clusters, and extraslet insulin-positive cells (78). GLP-1-treated rats exhibited increased numbers of Ki-67-positive cells in both the endocrine and exocrine pancreas, with aggregates of mitotic cells detected in association with small and medium-sized islets. GLP-1-treated rats also exhibited reduced numbers of ap-

optotic cells in the exocrine parenchyma. Remarkably, the percentage of apoptotic β -cells in this study was found to be greater than 20% and was significantly reduced in rats treated with GLP-1 (78). Treatment of normoglycemic db/db mice with daily exendin-4 for 2 wk prevented the progression to hyperglycemia, in association with increased β -cell mass, enhanced numbers of 5-bromo-2'-deoxyuridine+ islet cells, and reduced numbers of TUNEL+ apoptotic β -cells (79). Exendin-4-treated rats also exhibited increased levels of pancreatic Akt1 and p44 MAPK and reduced expression of activated caspase-3 (79).

The antiapoptotic actions of GLP-1 agonists have been demonstrated in cultured fetal rat hippocampal neurons that exhibit GLP-1-dependent increases in cAMP formation. Both GLP-1 and exendin-4 significantly reduced the extent of glutamate-induced cell death in short-term cultures of hippocampal neurons (80). Furthermore, both GLP-1 and exendin-4 reduced depletion of choline acetyltransferase immunoreactivity, a marker for cholinergic neurons in the basal forebrain, after administration of ibotenic acid (80). Hence, the demonstration that GLP-1R activation reduces cell death in transfected fibroblasts, islet β -cells, and neurons suggests that direct coupling to antiapoptotic signaling pathways may represent a generalized feature of GLP-1R action in diverse cell types (Fig. 3).

Considerable experimental evidence from animal studies *in vivo*, and experiments with transfected cells *in vitro* links activation of GLP-2R signaling to attenuation of apoptotic pathways. The nonsteroidal antiinflammatory agent indomethacin induces crypt compartment apoptosis in the murine small- and

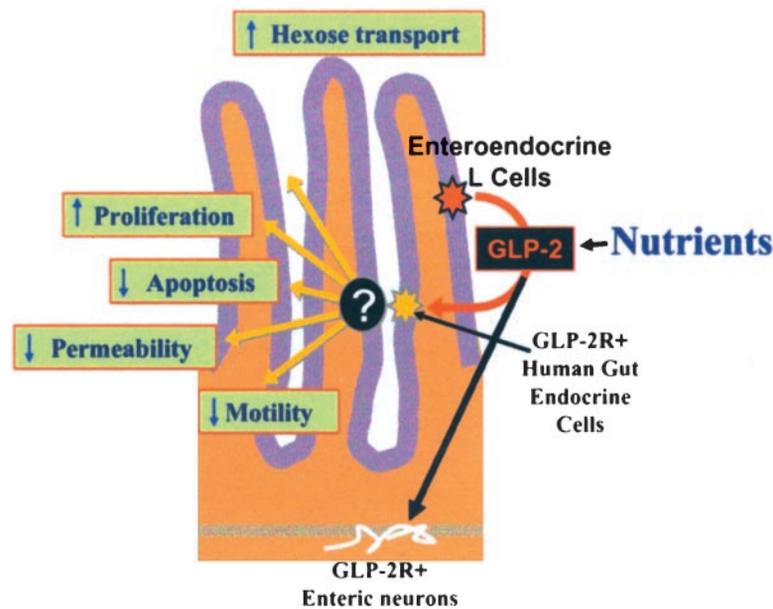


Fig. 3. Proliferative and Antiapoptotic Effects of GLP-1 and GLP-2 in the Pancreas and Intestine, Respectively

GLP-1 stimulates cell proliferation in pancreatic ductal cells and islets, and exerts antiapoptotic actions on islet β -cells and neurons. GLP-2 stimulates intestinal crypt cell proliferation and inhibits apoptosis in the crypt and enterocyte compartments of the gut epithelium.

large-bowel epithelium, and pretreatment of mice with a GLP-2 analog markedly reduced mortality, decreased the extent of mucosal injury, and suppressed the appearance of apoptotic cells in the gut epithelium (59). Furthermore, administration of the cytotoxic chemotherapeutic agents irinotecan or 5'-fluorouracil produces significant epithelial damage and apoptosis in a position-dependent manner along the crypt-to-villus axis that was markedly attenuated by pretreatment with a potent GLP-2 agonist (81). Exogenous GLP-2 infusion also reduced epithelial apoptosis in the small bowel of premature parenterally fed piglets (55).

Heterologous cells that express a transfected GLP-2R exhibit enhanced survival after external injury in the presence of GLP-2, which reflects decreased cellular apoptosis. BHK-GLP-2R cells exposed to cycloheximide exhibit reduced viability, morphological features of apoptosis and DNA laddering, and reduced viability; these parameters are markedly attenuated after incubation with GLP-2 agonists (82). Furthermore, GLP-2 reduced activation of caspase-3, caspase-8, and caspase-9, decreased poly(ADP-ribose) polymerase cleavage, and reduced mitochondrial cytochrome *c* release in BHK-GLP-2R cells *in vitro* (82). The antiapoptotic effects of GLP-2 in the setting of cycloheximide-induced injury were cAMP dependent yet protein kinase A independent. Similarly, GLP-2 increased cell survival after cycloheximide in the presence of the phosphatidylinositol 3-kinase, and MAPK inhibitors LY294002 and PD98054, respectively (82).

The direct antiapoptotic effects of GLP-2 in BHK cells do not require activation of the survival kinase

Akt, p90Rsk, or p70 S6 kinase, as GLP-2 reduced caspase activation and cytochrome *c* release after LY294002 in the absence of Akt, p90Rsk, or p70 S6 phosphorylation. GLP-2R activation in BHK-GLP-2R cells is coupled to inhibition of glycogen synthase kinase 3 (GSK-3) through phosphorylation of Ser21 in GSK-3 α and Ser9 in GSK-3 β in a PI-3K-independent, PKA-dependent manner. GLP-2 also reduced the magnitude of LY294002-induced mitochondrial localization of the proapoptotic Bcl family Bad and Bax proteins and stimulated Bad phosphorylation at Ser155 in a PI-3K-independent, PKA-dependent manner (83). Although the antiapoptotic properties of GLP-2 have not yet been directly demonstrated in gut endocrine cells or enteric neurons, the structurally related pituitary adenylate cyclase activating peptide (PACAP) promotes neuronal survival via cAMP-dependent mechanisms in sympathetic neurons (84).

GPCRs, PEPTIDE HORMONES, AND ENGAGEMENT OF APOPTOTIC PATHWAYS

Several peptide hormones structurally related to the glucagon-secretin superfamily exert either pro- or antiapoptotic actions in diverse cell types (Table 1). Glucose-dependent insulinotropic peptide exerts both proliferative and antiapoptotic actions in the immortalized INS-1 islet cell line (85), and PACAP promotes neuronal survival in cerebellar neurons (86). Vasoactive intestinal peptide (VIP) reduces apoptosis in ovarian follicles (87), and both VIP and PACAP reduced

Table 1. Representative Peptide Hormone GPCRs Linked to Pro- and Antiapoptotic Signaling Pathways

| Receptor | Cell/Tissue | Action |
|--------------|-------------------------------|---|
| CCK | Pancreas | Increased acinar apoptosis (106, 107) |
| CRH | Hypothalamic neurons | Reduced amyloid β -associated apoptosis (108) |
| Gastrin | Pancreatic cells, tumors | Reduced (AR42J) (93) or increased (109) apoptosis |
| GIP | Islet cells | Reduced β -cell apoptosis (85) |
| GLP-1 | Islet cells, neurons | Reduced β -cell and hippocampal neuronal apoptosis (77, 80) |
| GLP-2 | Gut epithelium | Decreased crypt and villous apoptosis (59, 81) |
| Neurotensin | Cancer cells | Reduced apoptosis (110) |
| PTH/PTHrP | Bone, diverse cell types | Suppression or activation of apoptosis (100, 111, 112) |
| PACAP | Neuroendocrine cells, T cells | Reduction of apoptosis (113, 114) |
| Somatostatin | Cancers, neuroendocrine cells | Induction of apoptosis (115, 116) |
| VIP | Neurons, lymphocytes | Neuroprotection, lymphocyte survival (88, 117) |

CCK, Cholecystokinin.

thymocyte apoptosis induced by glucocorticoid withdrawal (88) and inhibited Fas ligand expression and NF- κ B activation in T lymphocytes in a cAMP-dependent manner (89). Similarly, antiapoptotic actions have been detected after activation of CRH (90), FSH (91), adrenomedullin (92), gastrin (93), TSH (94), and substance P/neurokinin-1 (95) receptors, whereas receptors for angiotensinogen 1 (96), opioids (97), calcitonin gene-related peptide (98), natriuretic peptide(s) (99), PTH (100), and somatostatin (101) have been linked to enhanced apoptosis in diverse cell types. Hence, control of cell survival via peptide hormone-activated GPCR signaling (Table 1) may be an increasingly recognized mode of action of specific regulatory peptides in diverse cell types.

UNANSWERED QUESTIONS AND FUTURE RESEARCH DIRECTIONS

The available evidence clearly indicates that activation of GLP receptors (Figs. 2 and 3), and more broadly, related classes of GPCRs, may be important determinants of cell survival, particularly in the setting of tissue injury. For example, adrenergic receptor signaling modifies cardiomyocyte survival in a ligand- and receptor-specific manner (102, 103), and vasoactive peptides including endothelin, angiotensin II, VIP, atrial natriuretic peptide, and adrenomedullin exert both trophic and either pro- or antiapoptotic actions through specific subclasses of GPCRs (104). Considerable evidence implicates progressive β -cell failure as an inevitable feature accompanying the progression of type 2 diabetes in affected human subjects (105). Accordingly, strategies directed at enhancing islet neogenesis and β -cell proliferation, and/or preservation of existing β -cell mass via reduced susceptibility to apoptosis may prove to be useful in preventing loss of β -cell function in the diabetes clinic. Whether chronic therapy with GLP-1 agonists will prove useful for enhancement or preservation of β -cell mass in patients with type 2 diabetes will require long-term clinical

studies and/or improvements in our currently limited ability to assess β -cell mass in human subjects. Similarly, it remains unclear whether GLP-1 agonists may enhance cell preservation and reduce β -cell apoptosis in the setting of islet transplantation. Furthermore, the signal transduction pathways activated by the GLP-1R in human ducts or β -cells that transduce proliferative or antiapoptotic signals have not yet been determined. In contrast to the direct actions of GLP-1 on islet cell survival, the trophic and antiapoptotic actions of GLP-2 leading to expansion of the intestinal epithelial mucosa are largely indirect (Fig. 3), and the identity of the specific downstream mediators that convey GLP-2-activated mitogenic and cytoprotective signals to the stomach and small and large intestinal epithelium remain unknown. Furthermore, the emerging clinical use of long acting GLP analogs, with inherent proliferative and antiapoptotic actions, suggests that ongoing surveillance of tissues such as the pancreatic ductal (GLP-1) or colonic (GLP-2) epithelium appears prudent. Given the emerging interest in the therapeutic use of GLP analogs for the treatment of diabetes and intestinal disease, a more detailed understanding of the cellular pathways coupling GPCR signaling to control of cell proliferation and survival seems warranted.

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REFERENCES

- Bell GI, Santerre RF, Mullenbach GT 1983 Hamster proglucagon contains the sequence of glucagon and two related peptides. *Nature* 302:716–718
- Heinrich G, Gros P, Lund PK, Bentley RC, Habener JF 1984 Pre-proglucagon messenger ribonucleic acid: nucleotide and encoded amino acid sequences of the rat pancreatic complementary deoxyribonucleic acid. *Endocrinology* 115:2176–2181
- Mojsov S, Heinrich G, Wilson IB, Ravazzola M, Orci L, Habener JF 1986 Preproglucagon gene expression in pancreas and intestine diversifies at the level of post-translational processing. *J Biol Chem* 261:11880–11889
- Orskov C, Holst JJ, Poulsen SS, Kirkegaard P 1987 Pancreatic and intestinal processing of proglucagon in man. *Diabetologia* 30:874–881
- Larsen PJ, Tang-Christensen M, Holst JJ, Orskov C 1997 Distribution of glucagon-like peptide-1 and other proglucagon-derived peptides in the rat hypothalamus and brainstem. *Neuroscience* 77:257–270
- Lui EY, Asa SL, Drucker DJ, Lee YC, Brubaker PL 1990 Glucagon and related peptides in fetal rat hypothalamus *in vivo* and *in vitro*. *Endocrinology* 126:110–117
- Lund PK, Goodman RH, Dee PC, Habener JF 1982 Pancreatic proglucagon cDNA contains two glucagon-related coding sequences arranged in tandem. *Proc Natl Acad Sci USA* 79:345–349
- Irwin DM 2001 Molecular evolution of proglucagon. *Regul Pept* 98:1–12
- Drucker DJ, Philippe J, Mojsov S, Chick WL, Habener JF 1987 Glucagon-like peptide I stimulates insulin gene expression and increases cyclic AMP levels in a rat islet cell line. *Proc Natl Acad Sci USA* 84:3434–3438
- Kreymann B, Ghatei MA, Williams G, Bloom SR 1987 Glucagon-like peptide-1 7–36: a physiological incretin in man. *Lancet* 2:1300–1304
- Orskov C, Holst JJ, Nielsen OV 1988 Effect of truncated glucagon-like peptide-1 [proglucagon-(78–107) amide] on endocrine secretion from pig pancreas, antrum, and nonantral stomach. *Endocrinology* 123:2009–2013
- Mojsov S, Weir GC, Habener JF 1987 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* 79:616–619
- Drucker DJ 2001 Glucagon-like peptide 2. *J Clin Endocrinol Metab* 86:1759–1764
- D'Alessio DA, Fujimoto WY, Ensink JW 1989 Effects of glucagonlike peptide I(7–36) on release of insulin, glucagon, and somatostatin by rat pancreatic islet cell monolayer cultures. *Diabetes* 38:1534–1538
- Komatsu R, Matsuyama T, Namba M, Watanabe N, Itoh H, Kono N, Tarui S 1989 Glucagonostatic and insulinotropic action of glucagonlike peptide I-(7–36)-amide. *Diabetes* 38:902–905
- Willms B, Werner J, Holst JJ, Orskov C, Creutzfeldt W, Nauck MA 1996 Gastric emptying, glucose responses, and insulin secretion after a liquid test meal: effects of exogenous glucagon-like peptide-1 (GLP-1)-(7–36)amide in type 2 (non-insulin dependent) diabetic patients. *J Clin Endocrinol Metab* 81:327–332
- Schjoldager BTG, Mortensen PE, Christiansen J, Orskov C, Holst JJ 1989 GLP-1 (glucagon-like peptide 1) and truncated GLP-1, fragments of human proglucagon, inhibit gastric acid secretion in humans. *Dig Dis Sci* 34:703–708
- Turton MD, O'Shea D, Gunn I, Beak SA, Edwards CMB, Meeran K, Choi SJ, Taylor GM, Heath MM, Lambert PD, Wilding JPH, Smith DM, Ghatei MA, Herbert J, Bloom SR 1996 A role for glucagon-like peptide-1 in the central regulation of feeding. *Nature* 379:69–72
- Flint A, Raben A, Astrup A, Holst JJ 1998 Glucagon-like peptide 1 promotes satiety and suppresses energy intake in humans. *J Clin Invest* 101:515–520
- Drucker DJ 2001 Development of glucagon-like peptide-1-based pharmaceuticals as therapeutic agents for the treatment of diabetes. *Curr Pharm Des* 7:1399–412
- Drucker DJ 2001 Minireview: the glucagon-like peptides. *Endocrinology* 142:521–527
- Thorens B 1992 Expression cloning of the pancreatic β cell receptor for the gluco-incretin hormone glucagon-like peptide 1. *Proc Natl Acad Sci USA* 89:8641–8645
- Skoglund G, Hussain MA, Holz GG 2000 Glucagon-like peptide 1 stimulates insulin gene promoter activity by protein kinase A-independent activation of the rat insulin I gene cAMP response element. *Diabetes* 49:1156–1164
- Kashima Y, Miki T, Shibasaki T, Ozaki N, Miyazaki M, Yano H, Seino S 2001 Critical role of cAMP-GEFII/Rim2 complex in incretin-potentiated insulin secretion. *J Biol Chem* 276:46046–46053
- Kang G, Chepurny OG, Holz GG 2001 cAMP-regulated guanine nucleotide exchange factor II (Epac2) mediates Ca^{2+} -induced Ca^{2+} release in INS-1 pancreatic β -cells. *J Physiol* 536:375–385
- Zhou J, Wang X, Pineyro MA, Egan JM 1999 Glucagon-like peptide 1 and exendin-4 convert pancreatic AR42J cells into glucagon- and insulin-producing cells. *Diabetes* 48:2358–2366
- Hui H, Wright C, Perfetti R 2001 Glucagon-like peptide 1 induces differentiation of islet duodenal homeobox-1-positive pancreatic ductal cells into insulin-secreting cells. *Diabetes* 50:785–796
- Zhou J, Pineyro MA, Wang X, Doyle ME, Egan JM 2002 Exendin-4 differentiation of a human pancreatic duct cell line into endocrine cells: involvement of PDX-1 and HNF3 β transcription factors. *J Cell Physiol* 192:304–314
- de la Tour D, Halvorsen T, Demeterco C, Tyrberg B, Itkin-Ansari P, Loy M, Yoo SJ, Hao E, Bossie S, Levine F 2001 β -Cell differentiation from a human pancreatic cell line *in vitro* and *in vivo*. *Mol Endocrinol* 15:476–483
- Hardikar AA, Wang XY, Williams LJ, Kwok J, Wong R, Yao M, Tuch BE 2002 Functional maturation of fetal porcine β -cells by glucagon-like peptide 1 and cholecystokinin. *Endocrinology* 143:3505–3514
- Movassat J, Beattie GM, Lopez AD, Hayek A 2002 Exendin 4 up-regulates expression of PDX 1 and hastens differentiation and maturation of human fetal pancreatic cells. *J Clin Endocrinol Metab* 87:4775–4781
- Zulewski H, Abraham EJ, Gerlach MJ, Daniel PB, Moritz W, Muller B, Vallejo M, Thomas MK, Habener JF 2001 Multipotential nestin-positive stem cells isolated from adult pancreatic islets differentiate *ex vivo* into pancreatic endocrine, exocrine, and hepatic phenotypes. *Diabetes* 50:521–533
- Abraham EJ, Leech CA, Lin JC, Zulewski H, Habener JF 2002 Insulinotropic hormone glucagon-like peptide-1 differentiation of human pancreatic islet-derived progenitor cells into insulin-producing cells. *Endocrinology* 143:3152–3161
- Susini S, Roche E, Prentki M, Schlegel W 1998 Glucose and glucocorticoid peptides synergize to induce c-fos, c-jun, junB, zif-268, and nur-77 gene expression in pancreatic β (INS-1) cells. *FASEB J* 12:1173–1182
- Buteau J, Roduit R, Susini S, Prentki M 1999 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 β (INS-1)-cells. *Diabetologia* 42:856–864

36. Wang X, Cahill CM, Pineyro MA, Zhou J, Doyle ME, Egan JM 1999 Glucagon-like peptide-1 regulates the β cell transcription factor, PDX-1, in insulinoma cells. *Endocrinology* 140:4904-4907
37. Buteau J, Foisy S, Rhodes CJ, Carpenter L, Biden TJ, Prentki M 2001 Protein kinase C ζ activation mediates glucagon-like peptide-1-induced pancreatic β -cell proliferation. *Diabetes* 50:2237-2243
38. Gomez E, Pritchard C, Herbert TP 2002 cAMP dependent protein kinase and Ca²⁺ influx through L-type voltage gated calcium channels mediate Raf-independent activation of extracellular regulated kinase in response to glucagon like peptide-1 in pancreatic β -cells. *J Biol Chem* 277:48146-48151 (first published 2 October 2002; 10.1074/jbc.M209165200)
39. Edvell A, Lindstrom P 1999 Initiation of increased pancreatic islet growth in young normoglycemic mice (Umeå +/?). *Endocrinology* 140:778-783
40. Xu G, Stoffers DA, Habener JF, Bonner-Weir S 1999 Exendin-4 stimulates both β -cell replication and neogenesis, resulting in increased β -cell mass and improved glucose tolerance in diabetic rats. *Diabetes* 48:2270-2276
41. Perfetti R, Zhou J, Doyle ME, Egan JM 2000 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* 141:4600-4605
42. Tourrel C, Bailbe D, Lacorne M, Meile MJ, Kergoat M, Portha B 2002 Persistent improvement of type 2 diabetes in the Goto-Kakizaki rat model by expansion of the β -cell mass during the prediabetic period with glucagon-like peptide-1 or exendin-4. *Diabetes* 51:1443-1452
43. Tourrel C, Bailbe D, Meile M-J, Kergoat M, Portha B 2001 Glucagon-like peptide-1 and exendin-4 stimulate β -cell neogenesis in streptozotocin-treated newborn rats resulting in persistently improved glucose homeostasis at adult age. *Diabetes* 50:1562-1570
44. Stoffers DA, Kieffer TJ, Hussain MA, Drucker DJ, Egan JM, Bonner-Weir S, Habener JF 2000 Insulinotropic glucagon-like peptide-1 agonists stimulate expression of homeodomain protein IDX-1 and increase β -cell mass in mouse pancreas. *Diabetes* 49:741-748
45. Rolin B, Larsen MO, Gotfredsen CF, Deacon CF, Carr RD, Wilken M, Knudsen LB 2002 The long-acting GLP-1 derivative NN2211 ameliorates glycemia and increases β -cell mass in diabetic mice. *Am J Physiol* 283:E745-E752
46. Ling Z, Wu D, Zambre Y, Flamez D, Drucker DJ, Pipeleers DG, Schuit FC 2001 Glucagon-like peptide 1 receptor signaling influences topography of islet cells in mice. *Virchows Arch* 438:382-387
47. Scrocchi LA, Hill ME, Saleh J, Perkins B, Drucker DJ 2000 Elimination of GLP-1R signaling does not modify weight gain and islet adaptation in mice with combined disruption of leptin and GLP-1 action. *Diabetes* 49:1552-1560
48. De Leon DD, Deng S, Madani R, Ahima RS, Drucker DJ, Stoffers DA, Role of endogenous glucagon-like peptide-1 in islet regeneration following partial pancreatectomy. *Diabetes*, in press
49. Chen YE, Drucker DJ 1997 Tissue-specific expression of unique mRNAs that encode proglucagon-derived peptides or exendin 4 in the lizard. *J Biol Chem* 272:4108-4115
50. Drucker DJ, Ehrlich P, Asa SL, Brubaker PL 1996 Induction of intestinal epithelial proliferation by glucagon-like peptide 2. *Proc Natl Acad Sci USA* 93:7911-7916
51. Tsai C-H, Hill M, Drucker DJ 1997 Biological determinants of intestinotrophic properties of GLP-2 *in vivo*. *Am J Physiol* 272:G662-G668
52. Tsai C-H, Hill M, Asa SL, Brubaker PL, Drucker DJ 1997 Intestinal growth-promoting properties of glucagon-like peptide 2 in mice. *Am J Physiol* 273:E77-E84
53. Benjamin MA, McKay DM, Yang PC, Cameron H, Perdue MH 2000 Glucagon-like peptide-2 enhances intestinal epithelial barrier function of both transcellular and paracellular pathways in the mouse. *Gut* 47:112-119
54. Drucker DJ, Shi Q, Crivici A, Sumner-Smith M, Tavares W, Hill M, DeForest L, Cooper S, Brubaker PL 1997 Regulation of the biological activity of glucagon-like peptide 2 *in vivo* by dipeptidyl peptidase IV. *Nat Biotechnol* 15:673-677
55. Burrin DG, Stoll B, Jiang R, Petersen Y, Elnif J, Budington RK, Schmidt M, Holst JJ, Hartmann B, Sangild PT 2000 GLP-2 stimulates intestinal growth in premature TPN-fed pigs by suppressing proteolysis and apoptosis. *Am J Physiol* 279:G1249-G1256
56. Jeppesen PB, Hartmann B, Thulesen J, Graff J, Lohmann J, Hansen BS, Tofteng F, Poulsen SS, Madsen JL, Holst JJ, Mortensen PB 2001 Glucagon-like peptide 2 improves nutrient absorption and nutritional status in short-bowel patients with no colon. *Gastroenterology* 120:806-815
57. Ghatei MA, Goodlad RA, Taheri S, Mandir N, Brynes AE, Jordinson M, Bloom SR 2001 Proglucagon-derived peptides in intestinal epithelial proliferation: glucagon-like peptide-2 is a major mediator of intestinal epithelial proliferation in rats. *Dig Dis Sci* 46:1255-1263
58. Drucker DJ, DeForest L, Brubaker PL 1997 Intestinal response to growth factors administered alone or in combination with human [Gly²]glucagon-like peptide 2. *Am J Physiol* 273:G1252-G1262
59. Boushey RP, Yusta B, Drucker DJ 1999 Glucagon-like peptide 2 decreases mortality and reduces the severity of indomethacin-induced murine enteritis. *Am J Physiol* 277:E937-E947
60. Alavi K, Schwartz MZ, Palazzo JP, Prasad R 2000 Treatment of inflammatory bowel disease in a rodent model with the intestinal growth factor glucagon-like peptide-2. *J Pediatr Surg* 35:847-851
61. Kato Y, Yu D, Schwartz MZ 1999 Glucagon-like peptide-2 enhances small intestinal absorptive function and mucosal mass *in vivo*. *J Pediatr Surg* 34:18-20
62. Prasad R, Alavi K, Schwartz MZ 2000 Glucagon-like peptide-2 analogue enhances intestinal mucosal mass after ischemia and reperfusion. *J Pediatr Surg* 35:357-359
63. Prasad R, Alavi K, Schwartz MZ 2001 GLP-2 α accelerates recovery of mucosal absorptive function after intestinal ischemia/reperfusion. *J Pediatr Surg* 36:570-572
64. Drucker DJ, Yusta B, Boushey RP, DeForest L, Brubaker PL 1999 Human [Gly²]-GLP-2 reduces the severity of colonic injury in a murine model of experimental colitis. *Am J Physiol* 276:G79-G91
65. Scott RB, Kirk D, MacNaughton WK, Meddings JB 1998 GLP-2 augments the adaptive response to massive intestinal resection in rat. *Am J Physiol* 275:G911-G921
66. Sigalet DL, Martin GR 2000 Hormonal therapy for short bowel syndrome. *J Pediatr Surg* 35:360-363; discussion 364
67. Fischer KD, Dhanvantari S, Drucker DJ, Brubaker PL 1997 Intestinal growth is associated with elevated levels of glucagon-like peptide-2 in diabetic rats. *Am J Physiol* 273:E815-E820
68. Hartmann B, Thulesen J, Hare KJ, Kissow H, Orskov C, Poulsen SS, Holst JJ 2002 Immunoneutralization of endogenous glucagon-like peptide-2 reduces adaptive intestinal growth in diabetic rats. *Regul Pept* 105:173-179
69. Chance WT, Foley-Nelson T, Thomas I, Balasubramanian A 1997 Prevention of parenteral nutrition-induced gut hypoplasia by coinfusion of glucagon-like peptide-2. *Am J Physiol* 273:G559-G563

70. Chance WT, Sheriff S, Foley-Nelson T, Thomas I, Balasubramaniam A 2000 Maintaining gut integrity during parenteral nutrition of tumor-bearing rats: effects of glucagon-like peptide 2. *Nutr Cancer* 37:215-222
71. Munroe DG, Gupta AK, Kooshesh F, Vyas TB, Rizkalla G, Wang H, Demchyshyn L, Yang ZJ, Kamboj RK, Chen H, McCallum K, Sumner-Smith M, Drucker DJ, Crivici A 1999 Prototypic G protein-coupled receptor for the intestinotrophic factor glucagon-like peptide 2. *Proc Natl Acad Sci USA* 96:1569-1573
72. Yusta B, Somwar R, Wang F, Munroe D, Grinstein S, Klip A, Drucker DJ 1999 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* 274:30459-30467
73. Yusta B, Huang L, Munroe D, Wolff G, Fantaska R, Sharma S, Demchyshyn L, Asa SL, Drucker DJ 2000 Enteroendocrine localization of GLP-2 receptor expression. *Gastroenterology* 119:744-755
74. Bjerknes M, Cheng H 2001 Modulation of specific intestinal epithelial progenitors by enteric neurons. *Proc Natl Acad Sci USA* 98:12497-12502
75. Drucker DJ 2002 Gut adaptation and the glucagon-like peptides. *Gut* 50:428-435
76. Bregenholt S, Moldrup A, Knudsen LB, Petersen JS, The GLP-1 derivative NN2211 inhibits cytokine-induced apoptosis in primary rat β cells. *Proc 61st Annual Meeting of the American Diabetes Association, Philadelphia, 2001, A31 (Abstract 125-OR)*
77. Li Y, Hansotia T, Yusta B, Ris F, Halban PA, Drucker DJ (29 October 2002) Glucagon-like peptide-1 receptor signaling modulates β cell apoptosis. *J Biol Chem* 10.1074/jbc.M209423200
78. Farilla L, Hui H, Bertolotto C, Kang E, Bulotta A, Di Mario U, Perfetti R 2002 Glucagon-like peptide-1 promotes islet cell growth and inhibits apoptosis in Zucker diabetic rats. *Endocrinology* 143:4397-4408
79. Wang Q, Brubaker PL 2002 Glucagon-like peptide-1 treatment delays the onset of diabetes in 8 week-old db/db mice. *Diabetologia* 45:1263-1273
80. Perry T, Haughey NJ, Mattson MP, Egan JM, Greig NH 2002 Protection and reversal of excitotoxic neuronal damage by glucagon-like peptide-1 and exendin-4. *J Pharmacol Exp Ther* 302:881-888
81. Boushey RP, Yusta B, Drucker DJ 2001 Glucagon-like peptide (GLP)-2 reduces chemotherapy-associated mortality and enhances cell survival in cells expressing a transfected GLP-2 receptor. *Cancer Res* 61:687-693
82. Yusta B, Boushey RP, Drucker DJ 2000 The glucagon-like peptide-2 receptor mediates direct inhibition of cellular apoptosis via a cAMP-dependent protein kinase-independent pathway. *J Biol Chem* 275:35345-35352
83. Yusta B, Estall J, Drucker DJ 2002 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* 277:24896-24906
84. Chang JY, Korolev VV 1997 Cyclic AMP and sympathetic neuronal programmed cell death. *Neurochem Int* 31:161-167
85. Trumper A, Trumper K, Horsch D 2002 Mechanisms of mitogenic and anti-apoptotic signaling by glucose-dependent insulinotropic polypeptide in β (INS-1)-cells. *J Endocrinol* 174:233-246
86. Villalba M, Bockaert J, Journot L 1997 Pituitary adenylate cyclase-activating polypeptide (PACAP-38) protects cerebellar granule neurons from apoptosis by activating the mitogen-activated protein kinase (MAP kinase) pathway. *J Neurosci* 17:83-90
87. Flaws JA, DeSanti A, Tilly KI, Javid RO, Kugu K, Johnson AL, Hirshfield AN, Tilly JL 1995 Vasoactive intestinal peptide-mediated suppression of apoptosis in the ovary: potential mechanisms of action and evidence of a conserved antiapoptotic role through evolution. *Endocrinology* 136:4351-4359
88. Delgado M, Garrido E, Martinez C, Leceta J, Gomariz RP 1996 Vasoactive intestinal peptide and pituitary adenylate cyclase-activating polypeptides (PACAP27) and PACAP38) protect CD4+CD8+ thymocytes from glucocorticoid-induced apoptosis. *Blood* 87:5152-5161
89. Delgado M, Ganea D 2001 Vasoactive intestinal peptide and pituitary adenylate cyclase-activating polypeptide inhibit expression of Fas ligand in activated T lymphocytes by regulating c-Myc, NF- κ B, NF-AT, and early growth factors 2/3. *J Immunol* 166:1028-1040
90. Lezoualc'h F, Engert S, Berning B, Behl C 2000 Corticotropin-releasing hormone-mediated neuroprotection against oxidative stress is associated with the increased release of non-amyloidogenic amyloid β precursor protein and with the suppression of nuclear factor- κ B. *Mol Endocrinol* 14:147-159
91. Chun SY, Eisenhauer KM, Minami S, Billig H, Perlas E, Hsueh AJ 1996 Hormonal regulation of apoptosis in early antral follicles: follicle-stimulating hormone as a major survival factor. *Endocrinology* 137:1447-1456
92. Oehler MK, Norbury C, Hague S, Rees MC, Bicknell R 2001 Adrenomedullin inhibits hypoxic cell death by up-regulation of Bcl-2 in endometrial cancer cells: a possible promotion mechanism for tumour growth. *Oncogene* 20:2937-2945
93. Todisco A, Ramamoorthy S, Witham T, Pausawasdi N, Srinivasan S, Dickinson CJ, Askari FK, Krametter D 2001 Molecular mechanisms for the antiapoptotic action of gastrin. *Am J Physiol* 280:G298-G307
94. Sato H, Abe Y, Noguchi M, Kurokawa K, Sakai H 1999 Inhibitory effect of thyrotropic hormone on apoptosis induced by actinomycin D in a functioning rat thyroid cell line. *Endocr J* 46:309-315
95. DeFea KA, Vaughn ZD, O'Bryan EM, Nishijima D, Dery O, Bunnett NW 2000 The proliferative and antiapoptotic effects of substance P are facilitated by formation of a β -arrestin-dependent scaffolding complex. *Proc Natl Acad Sci USA* 97:11086-11091
96. Leri A, Claudio PP, Li Q, Wang X, Reiss K, Wang S, Malhotra A, Kajstura J, Anversa P 1998 Stretch-mediated release of angiotensin II induces myocyte apoptosis by activating p53 that enhances the local renin-angiotensin system and decreases the Bcl-2-to-Bax protein ratio in the cell. *J Clin Invest* 101:1326-1342
97. Goswami R, Dawson SA, Dawson G 1998 Cyclic AMP protects against staurosporine and wortmannin-induced apoptosis and opioid-enhanced apoptosis in both embryonic and immortalized (F-11 κ 7) neurons. *J Neurochem* 70:1376-1382
98. Sakuta H, Inaba K, Muramatsu S 1996 Calcitonin gene-related peptide enhances apoptosis of thymocytes. *J Neuroimmunol* 67:103-109
99. Suenobu N, Shichiri M, Iwashina M, Marumo F, Hirata Y 1999 Natriuretic peptides and nitric oxide induce endothelial apoptosis via a cGMP-dependent mechanism. *Arterioscler Thromb Vasc Biol* 19:140-146
100. Turner PR, Mefford S, Christakos S, Nissenson RA 2000 Apoptosis mediated by activation of the G protein-coupled receptor for parathyroid hormone (PTH)/PTH-related protein (PTHrP). *Mol Endocrinol* 14:241-254
101. Sharma K, Srikant CB 1998 G protein coupled receptor signaled apoptosis is associated with activation of a cation insensitive acidic endonuclease and intracellular acidification. *Biochem Biophys Res Commun* 242:134-140
102. Adams JW, Brown JH 2001 G-proteins in growth and apoptosis: lessons from the heart. *Oncogene* 20:1626-1634
103. Singh K, Communal C, Sawyer DB, Colucci WS 2000 Adrenergic regulation of myocardial apoptosis. *Cardiovasc Res* 45:713-719

104. Filippatos GS, Gangopadhyay N, Lalude O, Parameswaran N, Said SI, Spielman W, Uhal BD 2001 Regulation of apoptosis by vasoactive peptides. *Am J Physiol* 281: L749–L761
105. Turner RC, Cull CA, Frighi V, Holman RR 1999 Glycemic control with diet, sulfonylurea, metformin, or insulin in patients with type 2 diabetes mellitus: progressive requirement for multiple therapies (UKPDS 49). UK Prospective Diabetes Study (UKPDS) Group. *JAMA* 281: 2005–2012
106. Gukovskaya AS, Gukovsky I, Jung Y, Mouria M, Pandolf SJ 2002 Cholecystokinin induces caspase activation and mitochondrial dysfunction in pancreatic acinar cells. Roles in cell injury processes of pancreatitis. *J Biol Chem* 277:22595–22604
107. Trulsson LM, Svanvik J, Permert J, Gasslander T 2001 Cholecystokinin octapeptide induces both proliferation and apoptosis in the rat pancreas. *Regul Pept* 98:41–48
108. Pedersen WA, McCullers D, Culmsee C, Haughey NJ, Herman JP, Mattson MP 2001 Corticotropin-releasing hormone protects neurons against insults relevant to the pathogenesis of Alzheimer's disease. *Neurobiol Dis* 8:492–503
109. Kanno N, Glaser S, Chowdhury U, Phinizy JL, Baiocchi L, Francis H, LeSage G, Alpini G 2001 Gastrin inhibits cholangiocarcinoma growth through increased apoptosis by activation of Ca^{2+} -dependent protein kinase C- α . *J Hepatol* 34:284–291
110. Somai S, Gompel A, Rostene W, Forgez P 2002 Neutrotensin counteracts apoptosis in breast cancer cells. *Biochem Biophys Res Commun* 295:482–488
111. Amling M, Neff L, Tanaka S, Inoue D, Kuida K, Weir E, Philbrick WM, Broadus AE, Baron R 1997 Bcl-2 lies downstream of parathyroid hormone-related peptide in a signaling pathway that regulates chondrocyte maturation during skeletal development. *J Cell Biol* 136: 205–213
112. Chen HL, Demiralp B, Schneider A, Koh AJ, Silve C, Wang CY, McCauley LK 2002 Parathyroid hormone and parathyroid hormone-related protein exert both pro- and anti-apoptotic effects in mesenchymal cells. *J Biol Chem* 277:19374–19381
113. Cavallaro S, Copani A, D'Agata V, Musco S, Petralia S, Ventura C, Stivala F, Travali S, Canonico PL 1996 Pituitary adenylate cyclase activating polypeptide prevents apoptosis in cultured cerebellar granule neurons. *Mol Pharmacol* 50:60–66
114. Campard PK, Crochemore C, Rene F, Monnier D, Koch B, Loeffler JP 1997 PACAP type I receptor activation promotes cerebellar neuron survival through the cAMP/PKA signaling pathway. *DNA Cell Biol* 16:323–333
115. Szende B, Zalatnai A, Schally AV 1989 Programmed cell death (apoptosis) in pancreatic cancers of hamsters after treatment with analogs of both luteinizing hormone-releasing hormone and somatostatin. *Proc Natl Acad Sci USA* 86:1643–1647
116. Sharma K, Patel YC, Srikant CB 1996 Subtype-selective induction of wild-type p53 and apoptosis, but not cell cycle arrest, by human somatostatin receptor 3. *Mol Endocrinol* 10:1688–1696
117. Said SI, Dickman KG 2000 Pathways of inflammation and cell death in the lung: modulation by vasoactive intestinal peptide. *Regul Pept* 93:21–29

