The Glucagon-like Peptide-2 Receptor Mediates Direct Inhibition of Cellular Apoptosis via a cAMP-dependent Protein Kinase-independent Pathway*

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Glucagon and the glucagon-like peptides regulate metabolic functions via signaling through a glucagon receptor subfamily of G protein-coupled receptors. Activation of glucagon-like peptide-2 receptor (GLP-2R) signaling maintains the integrity of the intestinal epithelial mucosa via regulation of crypt cell proliferation. Because GLP-2 decreases mortality and reduces intestinal apoptosis in rodents after experimental injury, we examined whether GLP-2R signaling directly modifies the cellular response to external injury. We show here that activation of GLP-2R signaling inhibits cycloheximide-induced apoptosis in baby hamster kidney fibroblasts expressing a transfected GLP-2 receptor. GLP-2 reduced DNA fragmentation and improved cell survival, in association with reduced activation of caspase-3 and decreased poly(ADP-ribose) polymerase cleavage and reduced caspase-8 and caspase-9-like activities. Both GLP-2 and forskolin reduced mitochondrial cytochrome c release and decreased the cycloheximide-induced cleavage of caspase-3 in the presence or absence of the PKA inhibitor H-89. Similarly, GLP-2 increased cell survival following cycloheximide in the presence of the kinase inhibitors PD98054 and LY294002. These findings provide evidence that signaling through G protein-coupled receptors of the glucagon superfamily is directly linked to regulation of apoptosis and suggest the existence of a cAMP-dependent protein kinase-, phosphatidylinositol 3-kinase-, and mitogen-activated protein kinase-independent pathway coupling GLP-2R signaling to caspase inhibition and cell survival.

Glucagon and the glucagon-like peptides are co-encoded within a common precursor, proglucagon, that is expressed in a tissue-specific manner, giving rise to glucagon in the pancreatic A cells and glucagon-like peptides-1 and 2 in the endocrine cells of the gastrointestinal tract (1, 2). Proglucagon-derived peptides play important roles in regulation of metabolic function following nutrient assimilation (2). Glucagon regulates hepatic glucose production and maintains plasma glucose in a narrowly defined physiological range by opposing the actions of insulin at the hepatocyte. Glucagon-like peptide-1 (GLP-1) is secreted from the gut following nutrient ingestion and controls glycemia via stimulatory and inhibitory effects on insulin and glucagon, respectively (2, 3). In contrast, glucagon-like peptide-2 (GLP-2) exerts its principal actions on nutrient homeostasis proximal to nutrient absorption via regulation of the integrity of the mucosal epithelium (4, 5).

In addition to metabolic effects regulating fuel homeostasis, glucagon-like peptides also exert specific actions on cell proliferation and tissue regeneration. Glucagon potentiates proliferation of rat hepatocytes (6) and stimulates hepatic DNA synthesis following partial hepatectomy in vivo (7). GLP-1 increases islet proliferation in mice (8) and enhances islet regeneration and lowers blood glucose in rats following partial pancreatectomy (9). GLP-1 also appears to stimulate islet neogenesis via induction of pdx-1 expression in normal and diabetic rodents (10).

GLP-2 stimulates intestinal crypt cell proliferation in normal rats and mice leading to villus hyperplasia and expansion of the mucosal epithelium (4, 11, 12). The beneficial effects of GLP-2 in experimental models of intestinal injury have largely been attributed to enhancement of mucosal regeneration via GLP-2-dependent stimulation of crypt proliferation (13–16). Although GLP-2 inhibits apoptosis in the crypt compartment following administration of the nonsteroidal anti-inflammatory agent indomethacin (14), the mechanisms coupling GLP-2 signal to anti-apoptotic effects in a direct or indirect manner remain unknown. Because intestinal cell lines expressing the endogenous GLP-2 receptor have not yet been identified, we have now examined the effects of GLP-2 receptor signaling on cell death in heterologous cells expressing the transfected rat GLP-2 receptor.

EXPERIMENTAL PROCEDURES

Materials—Tissue culture medium, serum, and other supplements, including G418, were from Life Technologies, Inc. Cycloheximide, forskolin, protease inhibitor mixture (P-2714), and 4′,6-diamidino-2-phenylindole were purchased from Sigma. Recombinant human [Gly2]-GLP-2 was a kind gift from NPS Allelix Inc. (Mississauga, Canada). The caspase inhibitors Z-VAD-fmk and Z-YVAD-fmk and the kinase inhibitors H89 and LY294002 were obtained from Calbiochem (San Diego, CA). Ac-IETD-pNA and Ac-LEHD-pNA were from BIOSOURCE International (Camarillo, CA). PD98059 was obtained from New England

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1 The abbreviations used are: GLP, glucagon-like peptide; GLP-2R, GLP-2 receptor; X-gal, 5-bromo-4-chloro-3-indolyl β-D-galactopyranoside; BHK, baby hamster kidney; PBS, phosphate-buffered saline; PARP, poly(ADP-ribose) polymerase; CHAPS, 3-(3-cholamidopropyl) dimethylammonio)-1-propanesulfonic acid; PKA, cAMP-dependent protein kinase; PI, phosphatidylinositol; GPCR, G protein-coupled receptor; Erk, extracellular signal-regulated kinase; NGF, nerve growth factor; CHX, cycloheximide; pNA, p-nitroanilide.
GLP-2 Receptor and Apoptosis

Biolas (Beverly, MA). X-gal was purchased from BioShop Canada Inc. (Burlington, Canada). All electrophoresis and immunoblotting reagents were purchased from Bio-Rad. The pCRE/β-galactosidase reporter plasmid (17) and the expression vector MrT(AB) (18) were gifts from R. D. Cone (Portland, OR) and G. S. McKnight (Seattle, WA), respectively.

Cycloheximide was dissolved in anhydrous ethyl alcohol, stock solutions were stored at −70 °C or used immediately. Cycloheximide was dissolved in anhydrous ethyl alcohol, stock solutions were stored at −70 °C or used immediately.

Biotinylated secondary antibodies were conjugated to horseradish peroxidase and an enhanced chemiluminescence commercial kit (Amersham Pharmacia Biotech). Primary antibodies reactive to the 40 kD band in the CellTiter 96 aqueous assay (Promega, Madison, WI).

The extent of PKA inhibition achieved by the transfected MtR(AB) dominant negative expression vector and carrier DNA for a total of 20 μg. After glycerol shock, cells were cultured in serum-depleted medium for 15–17 h followed by cycloheximide in the presence or absence of the indicated drugs, and then fixed in 2% paraformaldehyde. Transfected, β-galactosidase-expressing cells were identified by histochemistry with X-gal (2 mg/ml in PBS containing 5 mM potassium ferricyanide, 5 mM potassium ferrocyanide, and 100 mM MgCl₂). The percentage of β-galactosidase-positive cells remaining in the plate that showed evidence of apoptosis was determined by counting >200 cells from at least 15–20 different fields. Transfected cells were scored as apoptotic when appearing rounded-up with a shrunken morphology, as opposed to the flat appearance of viable, healthy cells (see Fig. 5c, upper right panel).

CAMP-dependent Protein Kinase Activity Measurement—The extent of PKA inhibition achieved by the transfected MrT(AB) dominant negative expression plasmid was assessed indirectly by determining its ability to inhibit both h[γ2]-GFP-2 and forskolin-induced transcriptional activation of a CRE/β-galactosidase reporter plasmid, as described previously (19). Cells were transfected with 3 μg of CRE reporter plasmid and h[γ2]-GFP-2 or forskolin-induced CAMP-dependent protein kinase activity was measured in vitro using a fluorescent-labeled kemptide (PepTag nonradioactive CAMP-dependent protein kinase assay; Promega). Phosphorylation of the kemptide alters the net charge of the peptide, allowing the phosphorylated form to be separated from the nonphosphorylated form on an agarose gel at pH 6. PKA activity was determined as the kemptide phosphorylation that could be inhibited by 10 μM protein kinase inhibitor-(5–22)-amide peptide (Upstate Biotechnology, Lake Placid, NY).

Mitochondria and Cytosol Isolation—Cells were resuspended in ice-cold mitochondrial isolation buffer (10 mM Hepes-KOH, pH 7.4, 200 mM mannitol, 70 mM sucrose, 1 mM EGTA, and protease inhibitor mixture (1:100 dilution)) and lysed at 4 °C with a glass Dounce homogenizer. After pelleting nuclei and unbroken cells at 770 g for 10 min, the supernatant was centrifuged for 15 min at 10,000 x g to collect the heavy membrane fraction enriched for mitochondria. The newly obtained supernatant containing the cytosol was further centrifuged for 15 min at 20,800 x g to remove the light membrane fraction and then stored at −70 °C. The mitochondria-enriched pellet was washed once in mitochondrial isolation buffer and then resuspended in the same buffer and stored at −70 °C until use. Protein concentration in both cytosolic and mitochondrial fractions was determined before preparing the samples for immunoblot analysis as described above.

Statistical Analysis—For assessment of statistical significance, data were analyzed using analysis of variance, and group comparisons were done using the Bonferroni multiple comparison post-test. RESULTS

The glucagon-like peptide-2 receptor is expressed in a highly tissue-specific pattern primarily in the intestine and brain (20). Despite ongoing attempts by our laboratory and others, intestinal cell lines that express the endogenous GLP-2 receptor...
have not yet been identified. Accordingly we have studied GLP-2R signaling and its putative coupling to anti-apoptotic pathways in BHK fibroblasts stably transfected with the rat GLP-2 receptor (19). Incubation of wild type BHK cells or BHK-GLP-2R cells with cycloheximide alone at concentrations (5–80 μM) that inhibit protein synthesis by greater than 95% produced a visibly detectable decrease in cell viability. In contrast, incubation of cells with cycloheximide and h[Gly2]-GLP-2 or forskolin (or 8-bromo-cyclic AMP) resulted in significantly improved cell survival (Fig. 1, A and B). Furthermore, 1,9-dideoxyforskolin, a forskolin analog that does not stimulate adenylate cyclase, had no effect on the cycloheximide-induced decrease in cell viability (data not shown). Cycloheximide-induced cell death was reduced by the pancaspase inhibitor Z-VAD-fmk but not by Z-YVAD-fmk, an inhibitor of caspase-1-like activity (Fig. 1C). The extent of cycloheximide-induced cell death was markedly attenuated in the presence of ZnCl₂, a known inhibitor of caspase-3-mediated apoptosis (21). Morphologically, BHK-GLP-2R cells exhibited characteristic nuclear features consistent with apoptosis including chromatin condensation and margination as well as nuclear fragmentation following treatment with cycloheximide (Fig. 1D). DNA fragmentation was induced by cycloheximide and reduced by treatment of cells with h[Gly2]-GLP-2 (Fig. 1E). Taken together, these findings are consistent with the cycloheximide-dependent activation of apoptosis in BHK-GLP-2R cells in vitro.

Consistent with the importance of caspase activation for cycloheximide-induced cellular injury, the levels of the p17 active subunit of caspase-3 were increased in a time-dependent manner following treatment with cycloheximide and reduced following treatment of cells with either h[Gly2]-GLP-2 or forskolin (Fig. 2A). Similarly, the cycloheximide-induced cleavage of PARP was clearly attenuated following treatment with either h[Gly2]-GLP-2 or forskolin (Fig. 2A). In contrast, the protective effects of h[Gly2]-GLP-2 but not forskolin on both caspase-3 and PARP cleavage were absent in control BHK-pcDNA3 cells (Fig. 2B), consistent with the importance of the GLP-2R for transduction of the GLP-2-mediated signal to downstream apoptosis pathways.

Because caspase-3 processing and PARP cleavage are distal events that follow activation of upstream signaling molecules, we assessed the effects of GLP-2 on the activation of more proximal caspase enzymes following cycloheximide treatment of BHK cells. Analysis of initiator caspase activity using synthetic tetrapeptide substrates that are preferentially cleaved by caspase-8 (IETD-pNA) or caspase-9-like (LEHD-pNA) proteases showed that both h[Gly2]-GLP-2 and forskolin significantly inhibited the induction of IETD-pNA and LEHD-pNA cleaving activity following cycloheximide treatment of BHK-GLP-2R cells (Fig. 3). In contrast, only forskolin, but not h[Gly2]-GLP-2 inhibited activation of caspase-8- and caspase-9-like activity in control BHK-pcDNA3 cells (Fig. 3), consistent with the importance of the GLP-2R for transduction of the anti-apoptotic effects of h[Gly2]-GLP-2 in vitro. The specificity of caspase induction was further illustrated by findings that caspase-1-like activity, as assessed using YVAD-pNA as a substrate, was not induced following treatment of BHK cells with similar concentrations of cycloheximide (data not shown).

The finding that GLP-2R signaling was coupled to inhibition of cycloheximide-induced caspase-9-like activity prompted us to assess mitochondrial cytochrome c release, a known upstream effector of caspase-9 activation (22). Cycloheximide treatment of BHK-GLP-2R cells was associated with a marked time-dependent induction in the levels of cytosolic cytochrome c (Fig. 4). Both h[Gly2]-GLP-2 and forskolin markedly attenuated the cycloheximide induction of cytosolic cytochrome c release from BHK-GLP-2R cells (Fig. 4). Because both GLP-2 and forskolin are known to exert their effects via activation of adenylate cyclase and downstream PKA-dependent signaling pathways (19, 20), we assessed the importance of PKA signaling for cellular apoptosis in the presence or absence of H-89, a pharmacological inhibitor of protein kinase A. H-89 alone reduced cell viability in BHK-GLP-2R cells, consistent with the importance of basal PKA signaling for cell survival in vitro (Fig. 5A). However, both h[Gly2]-GLP-2 and forskolin significantly increased cell survival after cycloheximide in the presence of H-89 (Fig. 5A), demonstrating that stimulation of PKA-dependent signaling pathways is not required for the anti-apoptotic actions of these agents (Fig. 5A). To verify that H-89 inhibited PKA activity in BHK-GLP-2R cells, we assessed basal and stimulated PKA activity following incubation of cells with h[Gly2]-GLP-2 or forskolin. PKA activity was induced following treatment of BHK-GLP-2R cells with h[Gly2]-GLP-2 or forskolin, and PKA activation by these agents was effectively blocked by H-89 in the same experiments (Fig. 5A).

These observations clearly suggest that h[Gly2]-GLP-2 inhibits cycloheximide-induced apoptosis in BHK-GLP-2R cells in a PKA-independent manner. Consistent with these findings, both h[Gly2]-GLP-2 and forskolin attenuated the cycloheximide-induced cleavage of procaspase-3 to the active caspase-3 p17 subunit in H-89-treated BHK-GLP-2R cells (Fig. 5B). To provide complementary evidence for the PKA-independent actions of GLP-2 on apoptotic pathways, PKA activity was inhibited by transfection of Mtr(AB), a plasmid encoding the dominant negative regulatory subunit of protein kinase A (18). Analysis of transfected cells following cycloheximide treatment demonstrated that both h[Gly2]-GLP-2 and forskolin produced a significant reduction in the number of apoptotic cells despite transfection of Mtr(AB) (Fig. 5C). In contrast, the activity of a PKA-dependent reporter gene, CRE-β-galactosidase, was markedly stimulated by h[Gly2]-GLP-2 or forskolin, and the stimulatory activity was significantly reduced in Mtr(AB)-transfected cells (Fig. 5C).

Because activation of both the glucagon and GLP-1 receptors has been associated with induction of mitogen-activated protein kinase activity (23, 24), we assessed the effects of PD98059, a known inhibitor of Erk1/2 activity, on cycloheximide-induced apoptosis. Incubation of BHK-GLP-2R cells with PD98059 alone had no effect on the number of viable cells in the presence or absence of cycloheximide, demonstrating that basal Erk 1/2 activity is not required for cell survival in BHK-GLP-2R cells (Fig. 6A). Furthermore, both h[Gly2]-GLP-2 and forskolin significantly increased cell viability following cycloheximide in the presence of PD98059. In contrast, the activation of phosphorylated Erk1/2 by lysophosphatidic acid or fetal calf serum was markedly attenuated in the presence of PD98059 (Fig. 6A). These findings demonstrate that the effects of GLP-2R signaling on cell survival following cycloheximide treatment are independent of Erk1/2 activation in BHK-GLP-2R cells.

Because GLP-1 activates phosphatidylinositol 3-kinase (PI 3-kinase) in INS-1 cells (25) and PI 3-kinase is known to exert anti-apoptotic effects via induction of Akt (26), we examined whether the anti-apoptotic effects of GLP-2 might be mediated via a PI 3-kinase-dependent pathway. Treatment of BHK cells with LY294002, an inhibitor of phosphatidylinositol 3-kinase signaling, significantly reduced BHK cell survival, and co-incubation of cells with both LY294002 and cycloheximide produced a further diminution in survival beyond that seen with either agent alone (Fig. 6B). In contrast, cell survival was significantly increased in cycloheximide-treated cells incubated with either h[Gly2]-GLP-2 or forskolin in the presence of...
FIG. 1. GLP-2 and forskolin protect BHK-GLP-2R cells from cycloheximide-induced apoptosis. A, phase contrast micrographs showing the appearance of untreated control cultures and of cultures exposed for 8 h to 80 μM CHX alone or in combination with 20 nM h(Gly2)-GLP-2 or 20 μM forskolin. Magnification, ×100. B, for assessment of cell viability, cells were cultured as described under “Experimental Procedures” and treated with 80 μM CHX in the presence or absence of 20 nM h(Gly2)-GLP-2 or 20 μM forskolin. Cell viability at different time points was quantified using a tetrazolium salt bioreduction assay and expressed as percentage of the values obtained from analysis of vehicle alone-treated control cultures. Data shown are the means ± S.D. from four to six independent experiments, each one performed in quadruplicate. ***, p < 0.001, CHX plus either h(Gly2)-GLP-2 or forskolin versus CHX alone. C, effect of caspase inhibitors on cell viability. BHK-GLP-2R cells were pretreated with the indicated caspase inhibitors for 45 min prior to CHX 80 μM or vehicle alone for 8 h. Cell viability was then determined as described for B. Data are the means ± S.D. (n = 4). D, changes in nuclear morphology and chromatin condensation following apoptosis induction by CHX. Cells were exposed to CHX 80 μM (panels b–d) or vehicle alone (panel a) for 8 h. Cell nuclei were visualized using fluorescence microscopy after 4',6-diamidino-2-phenylindole staining. Magnification × 1000. E, agarose gel electrophoresis of DNA extracted from cells exposed to CHX 80 μM for 0–10 h in the absence or presence of h(Gly2)-GLP-2. The densitometric profile of the gel along the horizontal axis, as indicated by the arrow, is shown in the lower panel to provide semiquantitative information on the extent of chromatin fragmentation illustrated in the upper panel. Results are representative of two independent experiments.
LY294002 at concentrations that completely block Akt activation by either lysophosphatidic acid or fetal calf serum (Fig. 6B). These results imply that although basal PI 3-kinase activity is required for BHK-GLP-2R cell survival, the effects of h[Gly2]-GLP-2 on cycloheximide-induced cell death are mediated through a phosphatidylinositol 3-kinase-independent pathway.

**DISCUSSION**

Although signaling through G-protein-coupled receptors (GPCRs) of the glucagon/GLP-1/GLP-2 receptor superfamily has not previously been reported to directly modify apoptotic pathways, recent experiments provide increasing evidence linking GPCR activation and signal transduction pathways modulating cell death. Activation of the somatostatin receptor modulates pH-dependent apoptosis in heterologous cell types (27, 28) and signaling through the PTH/PTHrP (parathyroid hormone/parathyroid hormone-related protein) receptor activates apoptotic pathways in cells of the chondrocyte and osteoblast lineages (29). Activation of the pituitary adenylate cyclase-activating peptide receptor prevented apoptosis in cerebellar neurons, and the effects of pituitary adenylate cyclase-activating peptide signaling on apoptosis were reversed following transfection of cells with a dominant negative inhibitor of protein kinase A (30), consistent with the importance of the PKA pathway for antiapoptotic action in this cell type. In contrast, our data clearly show that the antiapoptotic effects of GLP-2 are PKA-independent. Similarly, stimulation of thymocyte apoptosis following activation of β-adrenergic receptor signaling is mediated by a PKA-independent, G,α-dependent pathway (31). These findings illustrate the diversity of signal-
FIG. 5. GLP-2 and forskolin protect BHK-GLP-2R cells from cycloheximide-induced apoptosis in a PKA-independent manner. A, left panel, cultures were pretreated with H89 or vehicle alone for 1 h prior to CHX in the presence or absence of h[Gly2]-GLP-2 or forskolin. After 8 h, cell viability was determined as described for Fig. 1B and expressed as a percentage of the values for vehicle alone-treated control cells not exposed to CHX. Data are the means ± S.D. from three independent experiments, each one performed in quadruplicate. ** and ***. p < 0.01 and p < 0.001, respectively, CHX plus either h[Gly2]-GLP-2 or forskolin versus the corresponding CHX alone-treated cultures. Right panel, BHK-GLP-2R cells were pretreated with H89 or vehicle alone for 1 h and then exposed to vehicle alone or either h[Gly2]-GLP-2 or forskolin for 20 and 90 min. PKA activity in cell extracts was determined using a fluorescent kemptide assay as detailed under “Experimental Procedures.” Results are representative of two independent experiments performed in duplicate. B, Western blot analysis of cell extracts from control or H-89-treated BHK-GLP-2R cells treated as described for Fig. 5A. Extracts were analyzed by immunoblotting for caspase-3 activation or actin. C, cells were transiently transfected with RSV-β-galactosidase either alone (control, upper left panel) or in combination with a dominant negative PKA mutant expression plasmid (MtR(AB), lower left panel). After 16 h, cells were treated with CHX 80 μM in the presence or absence of 20 nM h[Gly2]-GLP-2 or 20 μM forskolin. After 4 and 8 h of CHX treatment, cultures were fixed, stained for β-galactosidase expression, and transfected flat (healthy, arrows in upper right panel) and round (apoptotic, open arrowheads in upper right panel) blue cells were counted. The data shown represent the percentage of apoptotic cells. A representative micrograph (400× magnification) of cells transfected with the β-galactosidase reporter plasmid alone and treated with CHX 80 μM for 8 h is shown in the upper right panel after histochemistry with X-gal. Arrows point to healthy β-galactosidase positive cells. Open arrowheads point to apoptotic β-galactosidase positive cells. Lower right panel, h[Gly2]-GLP-2 or forskolin-induced transcriptional activation of a pCRE/β-galactosidase reporter plasmid in the absence (control) or presence of the cotransfected MtR(AB) expression plasmid. Cells were treated for 8 h with h[Gly2]-GLP-2 or forskolin and then assayed for β-galactosidase activity. Reporter gene activity is expressed as fold induction versus vehicle-treated cells following normalization for protein content. Data are the means ± S.D. from two independent experiments each one performed in triplicate.
GLP-2 Receptor and Apoptosis

In previous studies of GLP-2 action in BHK-GLP-2R cells in the absence of cycloheximide-induced cellular injury, we did not detect GLP-2-dependent activation of intracellular calcium influx, and GLP-2 stimulation failed to activate Erk1/2, p70 S6 kinase or Akt1 kinase activity (19). The findings on the lack of Erk induction in BHK-GLP-2R cells differ from studies of NGF action in PC 12 cells, where activation of Erk activity prevents apoptosis induced by NGF withdrawal (39). Similarly, the PI 3-kinase pathway is required for the antiapoptotic effects of NGF in PC 12 cells because PI 3-kinase inhibitors such as LY294002 also inhibit the effects of NGF on cell survival (26). In contrast, activation of GLP-2R signaling reduced cell death and decreased caspase activation in the presence of H-89 or MiR(AB), LY294002, or PD98059. Hence, although elevated levels of cAMP and/or PKA may negatively regulate apoptosis in some cell types (40), the available evidence from our studies points to the existence of a GLP-2-dependent, PKA-independent anti-apoptotic pathway coupled to inhibition of caspase-3 cleavage and prevention of cell death. Similarly, the lack of effect of the inhibitors LY294002 and PD98059 on GLP-2-mediated enhancement of cell survival strongly suggest that the anti-apoptotic effects of GLP-2R are mediated via a PI 3-kinase- and mitogen-activated protein kinase-independent pathway.

The cellular targets utilized by cycloheximide for induction of cell death appear cell type-specific, remain incompletely understood, and may involve both mitochondrial and death receptor-associated pathways. Although activation of Fas leading to caspase-8 cleavage and caspase-3 activation may occur independent of the mitochondrial pathway, recent evidence suggests that caspase-8 activation may also lead to mitochondrial cytochrome c release via a cycloheximide-sensitive pathway in

are shown. Anti-Erk1/2 and anti-Akt polyclonal antibodies were used to monitor loading and transfer conditions.
specific cell types (41). Although Fas ligand was not expressed in cycloheximide-sensitive Jurkat cells prior to or following induction of apoptosis, a dominant negative Fas-associated death domain protein completely inhibited cycloheximide-induced apoptosis, strongly implicating the importance of the Fas-associated death domain adapter protein in cycloheximide-induced cell death (42). The observation that GLP-2 inhibits the cycloheximide induction of both cytochrome c release and IETD-pNA and LEHD-pNA cleaving activity is consistent with previous studies of cycloheximide action (41) and suggests that GLP-2R signaling may interact with multiple signaling pathways upstream of caspase-3 cleavage.

GLP-2 ameliorates experimental intestinal injury in rats following nutritional deprivation, intestinal resection, and vascular ischemia (15, 16, 43) and in mice following chemically induced injury to the large and small bowel (13, 14). Although the beneficial effects of GLP-2 on intestinal mucosa were originally attributed to stimulation of crypt cell proliferation (4, 12, 28), GLP-2 action in the intestine 

![Image](https://via.placeholder.com/150)

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