Frontiers in glucagon-like peptide-2: multiple actions, multiple mediators

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Dubé PE, Brubaker PL. Frontiers in glucagon-like peptide-2: multiple actions, multiple mediators. Am J Physiol Endocrinol Metab 293: E460–E465, 2007; doi:10.1152/ajpendo.00149.2007.—Glucagon-like peptide-2 (GLP-2) is a pleiotropic hormone that affects multiple facets of intestinal physiology, including growth, barrier function, digestion, absorption, motility, and blood flow. The mechanisms through which GLP-2 produces these actions are complex, involving unique signaling mechanisms and multiple indirect mediators. As clinical trials have begun for the use of GLP-2 in a variety of intestinal disorders, the elucidation of such mechanisms is vital. The GLP-2 receptor (GLP-2R) is a G protein-coupled receptor, signaling through multiple G proteins to affect the cAMP and mitogen-activated protein kinase pathways, leading to both proliferative and antiapoptotic cellular responses. The GLP-2R also demonstrates unique mechanisms for receptor trafficking. Expression of the GLP-2R in discrete sets of intestinal cells, including endocrine cells, subepithelial myofibroblasts, and enteric neurons, has led to the hypothesis that GLP-2 acts indirectly through multiple mediators to produce its biological effects. Indeed, several studies have now provided important mechanistic data illustrating several of the indirect pathways of GLP-2 action. Thus, insulin-like growth factor I has been demonstrated to be required for GLP-2-induced crypt cell proliferation, likely involving activation of β-catenin signaling. Furthermore, vasoactive intestinal polypeptide modulates the actions of GLP-2 in models of intestinal inflammation, while keratinocyte growth factor signaling is required for GLP-2-induced colonic mucosal growth and mucin expression. Finally, enteric neural GLP-2R signaling affects intestinal blood flow through a nitric oxide-dependent mechanism. Determining how GLP-2 produces its full range of biological effects, which mediators are involved, and how these mediators interact is a continuing area of active research.

growth; intestine; receptor; signaling

THE INTESTINAL EPITHELIUM is a highly dynamic tissue that serves an important role both as a barrier to the external environment and as the ultimate site for nutrient digestion and absorption. These critical functions are maintained through constant tissue renewal, driven by a high rate of crypt cell proliferation that is carefully balanced against apoptosis and exfoliation, thus maintaining the size and integrity of the epithelium. This process is highly regulated; the tropic status, or growth, of the epithelium remains relatively constant in the healthy adult but is altered by developmental stage, enteral nutrition, injury, and disease. The mechanisms regulating these processes are not wholly understood; however, nutrients, humoral factors, intrinsic factors, and pathogenic/commensal organisms are clearly involved in determining crypt cell proliferation rates and the tropic status of the epithelium. The role of humoral factors is particularly interesting, as the intestine both produces and is responsive to a wide variety of regulatory peptide hormones that function through endocrine and paracrine pathways to affect intestinal growth. Of particular interest is the hormone glucagon-like peptide-2 (GLP-2) and its multifaceted role in the regulation of intestinal growth and function in physiological and pathophysiological states.

GLP-2 Is a Multifaceted Intestinal Growth Factor

The observations that GLP-2 is a potent intestinal mitogen in rodents and possesses therapeutic potential in humans, have led to considerable interest in the mechanisms through which this hormone regulates intestinal epithelial growth (13). GLP-2 is a 33-amino acid peptide and, along with its cognate hormone GLP-1, is liberated by prohormone convertase-1/3-mediated cleavage of proglucagon in the intestinal endocrine L cell. GLP-2 is secreted in response to nutrient intake and is subsequently inactivated by dipeptidyl peptidase IV cleavage and cleared by the kidney, conferring a relatively short biological half-life of ~7 min. Consequently, the use of native GLP-2 has been superceded by a degradation-resistant analog, (Gly²)GLP-2 (Teguduglutide), in animal research models and human clinical trials. Such studies have revealed that GLP-2 is a pleiotropic hormone, affecting multiple facets of intestinal physiology. Foremost among these is the ability of GLP-2 to increase small and large intestinal weight through stimulation of epithelial cell proliferation and inhibition of apoptosis, leading to enlarged crypts and villi and, hence, an enhanced absorptive surface area. In fact, a physiological role for GLP-2 appears to be the restoration of epithelial growth following periods of fasting (38). GLP-2 also increases the capacity for carbohydrate, amino acid, and lipid absorption and increases the activity of epithelial brush-border digestive enzymes and nutrient transporters. Epithelial barrier capacity is enhanced by GLP-2 through decreases in transcellular and paracellular permeability, as well as accelerated wound closure following injury. In addition to its effects on the epithelium, GLP-2 also stimulates intestinal blood flow and inhibits gastrointestinal motility. Excitingly, GLP-2 has recently been shown to produce anti-inflammatory effects, independent of its proliferative actions (39). However, the growth-promoting actions of GLP-2 appear to be context-specific and are dependent on the developmental state and health of the intestine. For instance, in contrast to the mainly proliferative actions observed in the normal mouse, the effects of GLP-2 are largely antiapoptotic in the neonatal pig on total parenteral nutrition (5) and are anti-inflammatory in rat models of intestinal disease (39). Nonetheless, numerous animal studies have shown that GLP-2 is beneficial in settings of intestinal dysfunction, including total parenteral nutrition (TPN)-induced atrophy, short bowel syndrome (SBS), inflammatory bowel disease (IBD), neonatal intestinal dysfunction, and gut injury caused by a variety of...
observed in cells transfected with the GLP-2R, which demon-

Nevertheless, this characteristic dose-response is not
higher levels of GLP-2, reminiscent of an “inverted U-shaped”
pathway involving both PKA-dependent and -inde-

The mechanistic explanations for such a phe-
involve inhibition of glycogen synthase kinase-3
and Bad. Furthermore, in some cells, the GLP-2R can also
couple to alternate G proteins (G<sub>q</sub> and G<sub>11o</sub>) and can activate
mitogen-activated protein kinase (MAPK) pathways (14, 24).
A distinct feature of this receptor is the mechanisms by which it
undergoes desensitization. Like many GPCRs, signaling through the
GLP-2R is subject to both homologous and heterologous
desensitization; however, in contrast to most known GPCRs,
GLP-2R desensitization and internalization are independent of β-arrestin and clathrin-mediated endocytosis (14, 15).

A major obstacle in the study of the GLP-2R has been the
lack of cell lines that demonstrate endogenous GLP-2R signal-
ing. It is therefore notable that the majority of studies address-
ing the mechanisms of GLP-2R signaling have utilized cell lines transfected with the GLP-2R, including 293-EBNA (29),
COS (17, 29), BHK (14, 15, 25, 38, 44, 46), fibroblasts, DLD-1
colon cancer cells (14, 15), and HeLa cervical cancer cells (14, 24).
Only a few cell lines have been identified that endogenously
express the GLP-2R, including HeLa cells (24), CCD-18Co in-
testinal myofibroblasts (36) (and PED and PLB; unpublished
data), and FHC fetal colon cells (36). Furthermore, in the case
of HeLa (24) or CCD-18Co cells (and PED and PLB; unpublished
data), the endogenous GLP-2R shows limited activity,
with severely blunted cAMP accumulation or slight activa-
tion of MAPK, respectively. This paucity of robust cell models is
relevant, as there may be significant differences between the
signaling activities of the transfected and endogenous GLP-2R.

For instance, the endogenous GLP-2R from hypothalamus (21),
pituitary (21), intestinal mucosa (41), intestinal muscularis (38),
and fetal intestine (10) produces a reproducible GLP-2 dose-
response curve, in which peak cAMP accumulation occurs at
moderate concentrations (10<sup>-10</sup> to 10<sup>-9</sup> M) but is reduced with
higher levels of GLP-2, reminiscent of an “inverted U-shaped”
dose-response. The mechanistic explanations for such a phe-
nomenon are unknown but may potentially result from the
unique nature of GLP-2 desensitization and trafficking (14,
15) or from dose-dependent coupling to alternate G proteins
(24). Nevertheless, this characteristic dose-response is not
observed in cells transfected with the GLP-2R, which demon-
strate a maximum “plateau” of cAMP accumulation and only a
slight reduction at concentrations of 10<sup>-6</sup> M or greater, sug-
gesting that these cells may not appropriately reflect the true
nature of GLP-2R signaling in situ. Therefore, one major issue
in the field is to determine which signaling mechanisms are
relevant to the endogenous GLP-2R, especially as they relate to
the intestinal growth effects of GLP-2, and to determine the
intracellular pathways underlying the biological actions of
GLP-2 with the use of relevant cell types.

While the signaling mechanisms of the GLP-2R remain
unclear, its localization is equally curious and raises important
questions regarding the mechanisms underlying GLP-2-in-
duced intestinal growth. GLP-2R expression is restricted to the
gastrointestinal tract and central nervous system, with limited
expression in lung, cervix, and vagal afferents (24, 25, 29, 30,
45). However, the exact cellular localization of the GLP-2R,
particularly in the intestine, has been a source of some contro-
versy, with variable reports demonstrating expression in di-
verse endocrine cells, enteric neurons, and/or subepithelial
myofibroblasts (SEMFs) (see Table 1). Some authors have
suggested that these differences may be a result of method-
ological problems or species-specific expression; however, as
each cell type has now been confirmed in several species by
using multiple approaches, neither of these reasons seems
likely, and it is possible that the GLP-2R is expressed in
each of these diverse cellular localizations. While an expla-
nation for the discrepancies between the studies remains
elusive, it is nonetheless evident that neither crypt epithelial
cells nor enterocytes express the GLP-2R. This has led to
the hypothesis that GLP-2 requires an indirect signal, per-
haps functioning through a paracrine mechanism, to induce
its effects on intestinal growth (45).

Multiple Actions, Multiple Mediators

Although there is a wealth of information about the ultimate
effects of GLP-2 on intestinal physiology and on the signaling
mechanisms initiated by the GLP-2R, there are relatively few
studies addressing how these two events are functionally con-
nected. Unraveling the mechanisms behind this has been a
difficult task, which is understandable given the multiple in-
terrelated actions of GLP-2 and the complex nature of the
GLP-2R. It is clear that the diverse effects of GLP-2 require an
indirect mechanism, likely involving not one but multiple
indirect signals, interacting in a complementary fashion to
effect different intestinal responses (i.e., proliferation, apopto-
sis, digestion, absorption, barrier function, blood flow, motility,
anti-inflammation). Several studies have now provided impor-
tant mechanistic data illustrating several of the indirect path-
ways of GLP-2 action (Fig. 1).

| Table 1. Reported cellular localization of the GLP-2R in the intestine |
|------------------|------------------|------------------|------------------|------------------|
| Cell Type        | Species          | Method(s) Used   | References       |
| Enteroendocrine  | Human            | IHC, ISH         | (17, 45)         |
| cells            | Pig              | IHC, ISH, LCM/RT-PCR | (17)           |
|                  | Rat              | IHC, WB          | (30)             |
| Eнтерic neurons | Human            | IHC, ISH         | (17)             |
|                  | Pig              | IHC, ISH, LCM/RT-PCR | (17)           |
|                  | Rat              | ISH              | (30, 32)         |
|                  | Mouse            | ISH, RT-PCR      | (2)              |
| Subepithelial    | Human, rat, mouse| IHC, ISH         | (32, 35)         |
| myofibroblasts   | marmoset         |                  |                  |

IHC, immunohistochemistry; ISH, in situ hybridization; LCM, laser-capture microdissection; RT-PCR, reverse transcriptase-polymerase chain reaction; WB, Western blot.
The insulin-like growth factors (IGFs) have recently been implicated in the intestinal growth effects of GLP-2 (10). IGF-I and IGF-II are widely expressed growth factors that regulate whole body, organ, and tissue growth in development and throughout adult life. Unlike liver-derived IGF-I, which circulates in response to growth hormone (GH), intestine-derived IGF-I is thought to act in a paracrine fashion to regulate local tropic responses through the epithelial IGF-I receptor (IGF-IR) (26). The intestine is a rich source of IGFs, produced in both endocrine cells (25), intestinal subepithelial myofibroblasts (SEMFs; 34), and enteric neurons (3). The activation of IGF-IR and IGF-I signaling through bone morphogenic protein (BMP) and phosphatase and tensin homolog (PTEN) downregulates β-catenin signaling in the intestinal crypt cell (19). One role of IGF-I, such as keratinocyte growth factor (KGF) in the colon, is to regulate IGF-I in the intestine. GLP-2 increases intestinal growth, specifically by inducing small intestinal crypt cell proliferation. This conclusion is supported primarily by studies of IGF-I knockout mice, which demonstrate marked impairments in small and large intestinal growth in response to GLP-2, compared with wild-type littermates, despite normal responses to other intestinal growth factors (10). Indeed, the effect of GLP-2 on crypt cell proliferation is completely lost in the absence of IGF-I. In normal mice, GLP-2 increases the rate of proliferation in the upper half of the intestinal crypts, thus expanding the number of cells responsible for populating the epithelium. This occurs in concert with a potential effect on the epithelial stem cells, as GLP-2 also increases the number of cells expressing musashi-1, a putative stem cell marker (10). The regulation of crypt cell proliferation involves several key mediators, most notably the canonical wingless (Wnt)/β-catenin signaling system. The activation of β-catenin transcriptional signaling by Wnt proteins, R-Spondin1, or IGF-I occurs through prevention of constitutive β-catenin degradation, thereby allowing its translocation to the nucleus (8, 33). IGF-I appears to initiate β-catenin signaling through a phosphatidylinositol-3 kinase (PI3K)-dependent pathway, involving Ras activation and GSK-3β inhibition (8). Conversely, inhibition of PI3K signaling through bone morphogenic protein (BMP) and phosphatase and tensin homolog (PTEN) downregulates β-catenin signaling in the crypt cell (19). One role of β-catenin in the intestine is to drive the transcription of genes required for proliferation, such as c-myc (28) while also inhibiting genes involved in terminal cell differentiation (33). This maintains cells within the active cell cycle, thereby increasing the overall numbers of proliferating cells. We have recently demonstrated that GLP-2 is a novel activator of β-catenin signaling in the intestinal crypt, through a mechanism requiring IGF-I signaling through the IGF-IR (11). This provides a mechanistic basis for IGF-I as a mediator for GLP-2-induced proliferation and serves to link the GLP-2-IGF-I signaling system to other regulators of intestinal crypt cell fate, through common effects on β-catenin (Fig. 2).

Although intestinally derived IGF-I is the likely mediator of GLP-2-induced proliferation in a paracrine fashion, it is unclear whether GLP-2 might also depend upon nonintestinal, endocrine IGF-I. This is an important issue as the GLP-2R is expressed in both hypothalamus and pituitary (25), such that GLP-2 has the potential to affect liver-derived IGF-I through
In addition to IGF-I, keratinocyte growth factor (KGF) has also been implicated in GLP-2-induced colonic growth. KGF is a member of the fibroblast growth factor family, expressed in SEMFs throughout the gastrointestinal tract, and is a tropic factor for epithelial cells (34). KGF colocalizes with the GLP-2R in SEMFs, and immunoneutralization of KGF in mice prevents the effect of GLP-2 on colonic weight and mucosal area without affecting colonic crypt depth or small intestinal growth (32). This is in contrast to the more marked effect of IGF-I ablation on both small and large intestinal growth responses to GLP-2. Moreover, unlike IGF-I, KGF treatment produces differential effects to those of GLP-2, affecting mainly colonic growth and the differentiation of goblet cells (2, 22, 42). Indeed, Ørskov et al. (32) found that the GLP-2-induced increase in mucin expression was blocked by KGF antibodies, suggesting that the specific role of KGF may be to promote colonic goblet cell differentiation in response to GLP-2. Nonetheless, it is possible that immunoneutralization may be insufficient to block small intestinal KGF, and therefore additional studies, utilizing a knockout model, would be helpful to determine whether this effect of KGF is truly restricted to the colon. Furthermore, a more detailed study of the role for KGF in crypt cell proliferative responses would help to determine whether the KGF effect extends beyond that on the goblet cells and whether KGF may interact with the proliferative actions of IGF-I.

These studies have only begun to uncover the mechanisms through which GLP-2 induces growth in the normal intestine. Although proliferative responses in the small intestinal crypt appear to require IGF-I, it is unknown how GLP-2 alters apoptosis, permeability, or nutrient digestion or absorption in the villus epithelium. Cheeseman (6) reported that GLP-2-mediated epithelial glucose uptake, through the sodium-dependent glucose transporter, occurred through a PI3K-dependent mechanism. Furthermore, the activation of Akt in the intestinal mucosa has been implicated in the antiapoptotic actions of GLP-2 (3, 9). The PI3K-Akt pathway is a common signaling effector for multiple growth factors and cytokines, including IGF-I. However, although IGF-I activates Akt signaling in the intestinal mucosa, it appears that, unlike the response observed with β-catenin in the crypt, IGF-I signaling is not strictly required for the activation of Akt by GLP-2 (11). This suggests that, in addition to the requirement of IGF-I for proliferation, other factors exist that mediate the diverse biological actions of GLP-2. The SEMFs, for example, express and secrete a wide range of different growth factors and cytokines in addition to the IGFs and KGF that may participate in some of the effects of GLP-2 (34).

Several studies have uncovered an exciting role for GLP-2R signaling in submucosal enteric neurons. Functioning through a nitric oxide (NO)-dependent mechanism, GLP-2 acutely and dose-dependently increases intestinal blood flow (17, 18). This is associated with the activation of endothelial nitric oxide synthase (eNOS), which may be a direct consequence of GLP-2R signaling, as PKA-mediated phosphorylation at Ser1177 is sufficient to increase eNOS activity (43). However, as Ser1177 is also a phosphorylation site for Akt, this activation may be indirect, through another paracrine factor. This is potentially relevant, as Akt is a downstream kinase in IGF-IR signaling and is activated by GLP-2 treatment in the intestinal mucosa in an acute and sustained manner (3, 9). Very recently, Sigalet et al. (39) have reported that GLP-2 reduced intestinal damage and the levels of inflammatory cytokines in a rat model of IBD through a mechanism requiring vasoactive intestinal...
polypeptide (VIP)-expressing submucosal enteric neurons. One of the curious findings of this report was that, in a counterintuitive manner, GLP-2 administration reduced the rate of epithelial proliferation in this setting; given that inflammation itself induces crypt cell proliferation (27), it would seem that this effect of GLP-2 may be an indirect result of a VIP-dependent anti-inflammatory action (39). It is thus possible that NO or VIP signaling may affect GLP-2-induced intestinal growth through several indirect mechanisms. For instance, NO-induced alterations in local circulation may function as a permissive mechanism for intestinal growth; indeed, a reduction in blood flow is associated with intestinal atrophy (31). However, a direct effect of NO or VIP on epithelial growth cannot be ruled out (12, 37). Finally, although a direct growth effect of neuronal GLP-2R signaling is unknown, Bjerknes and Cheng (2) have shown that GLP-2-induced c-fos expression in the intestinal crypt is dependent on a tetrodotoxin-sensitive mechanism; a neural mechanism in the control of crypt epithelial function therefore cannot be ruled out.

Finally, the spectrum of GLP-2 actions in the intestine suggests that GLP-2-based therapy may be promising in multiple clinical settings, including TPN, SBS, neonatal intestinal dysfunction, IBD, and intestinal injury. One potential benefit is that GLP-2 therapy may be associated with fewer extragastrointestinal sequelae compared with other growth factors, given the relatively intestine-specific effects of GLP-2 in animal models. However, in light of the indirect mechanisms of GLP-2 action (Fig. 1), the mediators involved deserve consideration when the benefits and potential side effects of GLP-2 administration are being determined. For instance, it has been proposed that GLP-2 may engage alternate mediators in a dose-dependent fashion (i.e., proliferative vs. anti-inflammatory responses) (39), suggesting differential clinical benefits depending on the dose of GLP-2, as well as on the condition being treated. Furthermore, given the proliferative effects of GLP-2, one potential detriment to prolonged GLP-2 therapy may be an increased risk for tumorigenesis (7, 16). Indeed, at least one study has shown that GLP-2 administration in mice can accelerate the growth of chemically induced intestinal tumors (40). This may be especially important in IBD patients, who already possess an increased risk of gastrointestinal cancer due to chronic inflammation. Therefore, careful dose regulation and monitoring for gastrointestinal dysplasia should be considered in any clinical use of GLP-2.

Summary

It has become clear that GLP-2 functions through multiple interrelated pathways that defy simple definition. Each new discovery in this field raises important new questions, both for the actions of GLP-2 and for the integrated physiology of the intestine. Future studies will have to consider the full range of GLP-2 effects, as well as all the sites of GLP-2R expression, to elucidate the multiple mechanisms of GLP-2 action. In particular, the function of the GLP-2R in intestinal endocrine cells is unknown, although it is tempting to hypothesize a role in the regulation of intestinal peptide hormone and/or serotonin secretion. Furthermore, the recent description of GLP-2R expression in vagal afferents (30), as well as its widespread expression in the central nervous system, hypothalamus, and pituitary (25), suggest that the intestinal functions of GLP-2 may be functionally linked to currently unidentified whole body actions. Indeed, recent studies have identified bone as a target of GLP-2 action, although how these effects are mediated is completely unknown (20). A major goal of future research should therefore be not only to discover each mediator but also to determine how each interacts and contributes functionally to the diverse actions of GLP-2.

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