Intestinal hormones and growth factors: Effects on the small intestine

Laurie Drozdowski, Alan BR Thomson

Laurie Drozdowski, Alan BR Thomson, Division of Gastroenterology, University of Alberta, Edmonton AB T6G 2X8, Canada

Correspondence to: Alan BR Thomson, Division of Gastroenterology, University of Alberta, Zeidler Leducor Centre, 130 University Campus, Edmonton AB T6G 2X8, Canada. alan.thomson@ualberta.ca

Telephone: +1-780-4926490 Fax: +1-780-4927964

Received: March 28, 2007 Revised: December 10, 2007

Accepted: December 17, 2007

Published online: January 28, 2009

Abstract

There are various hormones and growth factors which may modify the intestinal absorption of nutrients, and which might thereby be useful in a therapeutic setting, such as in persons with short bowel syndrome. In part I, we focus first on insulin-like growth factors, epidermal and transferring growth factors, thyroid hormones and glucocorticosteroids. Part II will detail the effects of glucagon-like peptide (GLP)-2 on intestinal absorption and adaptation, and the potential for an additive effect of GLP2 plus steroids.

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Key words: Epidermal growth factor; Glucocorticosteroids; Insulin-like growth factor- I / II; Intestinal growth; Transforming growth factor- α -2; Hepatocyte growth factor; Keratinocyte growth factor

Peer reviewer: Martin Anlauf, MD, Department of Pathology, University of Kiel, Michaelisstrasse 11, 24105 Kiel, Germany

Drozdowski L, Thomson ABR. Intestinal hormones and growth factors: Effects on the small intestine. *World J Gastroenterol* 2009; 15(4): 385-406 Available from: URL: <http://www.wjg-net.com/1007-9327/15/385.asp> DOI: <http://dx.doi.org/10.3748/wjg.15.385>

INTRODUCTION

In this review, the hormones and growth factors to be reviewed which alter nutrient absorption include insulin-like growth factor I (IGF- I) and II, EGF, TGF- α , HGF, Erythropoietin and KGF. In Table 1 is

outlined the cell types which express those peptides, as well as the cell types in the intestine and elsewhere which express the corresponding receptors. Part I there are various hormones and growth factors which may modify the intestinal absorption of nutrients, and which might thereby be useful in a therapeutic setting, such as in persons with short bowel syndrome. Part II presented here will detail the effects of glucagon-like peptide (GLP)-2 on intestinal absorption and adaptation, and the potential for an additive effect of GLP2 plus steroids.

INSULIN-LIKE GROWTH FACTORS (IGF- I , IGF- II)

The insulin-like growth factor family consists of insulin, IGF- I, and IGF- II. The liver is the major site of synthesis for IGF- I, but it is also synthesized in other tissues including the gastrointestinal tract^[1,2]. IGF- I and IGF- II are found in human milk^[3], and these peptides are members of a complex mixture of growth factors that the neonate is exposed to during the suckling period.

Intestinal development may involve IGF- I and IGF- II^[4-6]. IGF- I receptor (IGF-1R) is detectable in the fetus and the neonate of animals^[7,8]. In humans, IGF- I, IGF- II and IGF-1R are also present in fetal intestine^[9-11].

Intestinal cells proliferate *in vitro* when exposed to IGF- I^[12-14]. Transgenic mice that over express IGF- I demonstrate increased small intestinal growth^[15], particularly in the muscle layers of the distal small intestine and large intestine^[16]. IGF- I receptor gene knockout animals have fetal growth retardation, but distinctive effects on the gastrointestinal system have not been found^[17]. This may indicate the presence of redundant systems in the gastrointestinal tract that compensate for the lack of IGF- I.

In vivo studies show variable responses depending on the dose of IGF- I that is used. Pharmacological doses of IGF- I, given orally to colostrum-deprived 5 d old piglets, increase electrolyte and nutrient absorption^[18]. Neonatal pigs fed formula supplemented with IGF- I from birth to 4 d of age had increased mucosal growth^[19]. Parenterally fed 1 d old piglets given enteral IGF- I had increased brush border maltase (BBM) disaccharidase levels^[20]. Houle *et al*^[21] administered IGF- I orally to neonatal rats, and demonstrated increases in mucosal growth and BBM enzymes. Similarly, Burrin *et al*^[19] showed increases in intestinal weight, protein, and DNA in neonatal pigs fed

Table 1 Hormones and peptides which affect intestinal nutrient absorption

Hormones/ Peptides	Cell types which express the specific peptide	Cell types in the intestine and elsewhere which express corresponding receptors
IGF I & II	Granulosa cells ^[158] Prostate stromal cells ^[159] Neonatal bone marrow-mesenchymal stem cells ^[160] Systemic sclerosis lung fibroblasts ^[161] Melanoma Cells (SK-MEL2) ^[162]	Non-Islet cell tumors ^[163] Hepatoma cells ^[164] Intestinal smooth muscle ^[165] Astrocytoma cells ^[166] Camel intestinal lamina propria ^[167] Camel epithelia of the crypt cells ^[167] Camel villi ^[167]
EGF	Rat submandibular glands cells ^[168] HaCaT cells ^[169] Porcine cumulus cells ^[170] Esophageal epithelial cell ^[171]	Keratinocytes ^[169] Urothelial cells ^[172] Fibromyoblasts ^[173] Smooth muscle cells ^[173] Fibroblasts ^[173] Colonic epithelial cells ^[174] Murine pancreatic beta cells ^[175]
TGF- α	Hypothalamic astrocytes ^[176] Granulosa cells ^[177] Ovarian theca cells ^[177] Endometrial stromal cells ^[177] Eosinophils ^[178] Colon cancer cells ^[179]	Pancreatic cancer cells ^[180] Osteoblasts ^[181] Murine conjunctival goblet cells ^[182] Fundic mucous cells ^[183] Murine intestinal epithelial cells ^[184]
HGF	Lung microvascular endothelial cells ^[185] Umbilical vein endothelial cells ^[185] Neutrophils ^[186] Monocytes ^[187] Adipocytes ^[188]	Cardiomyocytes ^[189] Liver progenitor cells ^[190] Glioblastoma cells ^[191] Myeloma cells ^[192] Tumor plasma cells ^[192] Neuroblasts ^[193] Hepatocellular carcinoma cells ^[194] Epithelial tubular cells ^[195] Antroduodenal G cells ^[196] Rectal enterochromaffin cells ^[196] Pancreatic A and B cells ^[196] Intestinal epithelial cells ^[196]
Erythropoietin	Amniotic epithelial cells ^[197] Schwann cells ^[198] Interstitial fibroblasts ^[199] Metanephric adenoma cells ^[200] Mesenchymal stromal cells ^[201]	Erythroid cells ^[202] Endothelial cells ^[203] Neuronal cells ^[203] Cardiac myocytes ^[203] Vascular smooth muscles ^[203] Murine fetal enterocytes ^[204] Intestinal epithelial cells ^[205]
KGF	Mucosal and dermal fibroblasts ^[206] Gastric fibroblasts ^[207] Endometrial stromal cells ^[208]	Alveolar epithelial type II cells ^[209] Breast cancer cells ^[210] Cholesteatoma cells ^[211] Enterochromaffin cells ^[212]

Note: All of these are in humans until stated otherwise.

orogastrically. Alexander and Carey^[18] examined the effect of oral IGF- I on neonatal piglet intestine, and found increases in D-glucose uptake in everted jejunal sleeves, independent of the intestinal mass or surface area. Lane *et al*^[22] used RT-PCR and laser scanning confocal microscopy to show that intragastric IGF- I or IGF- II increased the expression of the glucose transporters SGLT1 and GLUT2 in suckling rat intestine.

The mechanisms responsible for increased jejunal transport rates observed in tissues treated with orally administered IGF- I may include the increases in Na⁺K⁺-ATPase seen with IGF- I treatment. The PI3-kinase pathway may also be important, as preincubation with a PI3-kinase inhibitor abolishes the effects of IGF- I on ion and nutrient transport^[23].

Although pharmacological doses of IGF- I have clear effects on the neonatal intestine, studies using physiological doses show only limited effects. For example, recent studies with both neonatal pigs^[21,24] and calves^[25] have shown that the oral administration of physiological concentrations of IGF- I in formula results in measurable increases in crypt cell proliferation, but there are no demonstrable increases in intestinal mucosal mass or length.

EPIDERMAL GROWTH FACTOR AND TRANSFORMING AND TRANSFORMING GROWTH FACTOR- α (TGF- α)

EGF and TGF- α are two ligands of the EGF receptor

that have been postulated to play a role in intestinal ontogeny. Both peptides are present in human breast milk, with TGF- α at a 5-10-fold lower concentration than EGF^[26]. Transforming growth factor β (TGF β) is a family of growth factors, with the three isoforms (TGF β 1, TGF β 2, TGF β 3) having nearly identical biological activities. TGF β 1 and TGF β 2 are present in human milk^[27], and mRNA and protein corresponding to the three TGF β isoforms are found in the intestinal mucosa^[28-30]. Small intestinal development, however, is not severely impaired by the targeted disruption of the TGF β gene^[31].

EGF and TGF- α have been isolated in human 15-20 wk gestation fetal intestine, with TGF- α levels as much as 10 times higher than EGF^[32]. EGF receptor expression is detectable throughout the GI tract in the human fetus and neonate. EGF receptor is developmentally regulated with expression being low during the human suckling period, and in rodents, unlike humans, EGF receptor expression is delayed until weaning^[33].

Oral EGF administration augments gut growth and functional development in neonatal rats and pigs^[34-36]. Gene targeting studies have demonstrated that disrupting the expression of the EGF receptor in mice resulted in impaired intestinal development, and death associated with a necrotizing enterocolitis-like disorder^[37].

The postnatal development of intestinal transport and the physical composition of the BBM were examined in New Zealand White rabbits receiving EGF (40 mg/kg per day) either intraperitoneally or orogastrically from day 3 to day 17 of life. Intestinal water, Na⁺ and glucose absorption expressed per cm of intestine were significantly increased in animals receiving EGF by either route. Increased absorption induced by orogastric EGF appeared to be secondary to mucosal hyperplasia. The BBM isolated from EGF-treated animals was significantly more fluid than that of controls. These results suggest that EGF modulates the development of transport function during the postnatal period, both by stimulating mucosal growth and by inducing specific transport processes^[38].

Experimental necrotizing enterocolitis (NEC) was induced by exposure of newborn animals to asphyxia and cold stress. The newborn rats were artificially fed either with growth factor-free rat milk substitute or the same formula supplemented with 500 ng/mL of EGF^[39]. EGF supplementation of formula reduced the incidence and severity of NEC, as assessed by gross and histological scoring of the ileum. This finding suggests a potential therapeutic approach for the prevention and treatment of NEC.

HEPATOCYTE GROWTH FACTOR, ERYTHROPOIETIN, KERATINOCYTE GROWTH FACTOR AND THYROID HORMONES

Erythropoietin (Epo) is present in breast milk^[40], and

receptors for Epo are present on intestinal cells^[41]. Modest changes in small intestinal length and surface area are observed following subcutaneous Epo injections in artificially fed rat pups. Hepatocyte growth factor (HGF) is also present in breast milk, and both HGF and its receptor are present in fetal intestinal tissue^[42,43]. Keratinocyte growth factor (KGF) promotes proliferation and up-regulates SI expression in fetal human small intestine explant culture^[44]. It remains to be seen if KGF plays a physiological role in postnatal intestinal development.

The effect of thyroid hormones on the ontogeny of BBM enzymes has been examined. An *in vivo* study of lactase phlorizin hydrolase (LPH) catalytic activity, synthesis, and degradation was performed in propylthiouracil-induced hypothyroid rat pups, hypothyroid pups injected with thyroxine, and normally weaned rats. T4 regulates LPH ontogeny by posttranslational mechanisms that include altered processing and increased degradation of the BBM lactase enzyme^[45]. Monteiro *et al.*^[46] examined the role of T4 on the precocious enhancement of GLUT5 in weanling rats. Rat pups were made hypothyroid by giving the dam 0.01% propylthiouracil in the drinking water from day 18 of gestation. The hypothyroid pups and age-matched euthyroid control pups were then fed high-fructose solutions by gavage, twice a day starting at 17 d of age for 3 d. Although serum T4 levels were five times lower in the hypothyroid pups, the mRNA level for the BBM fructose transporter (GLUT5) increased in euthyroid and hypothyroid pups fed high fructose. This result paralleled the increase in fructose uptake. This suggests that during weaning, dietary fructose can precociously enhance intestinal fructose uptake and GLUT5 mRNA expression, independent of developmental increases in serum T4 levels.

GLUCOCORTICOSTEROIDS

Biochemistry

Steroids can be divided into two categories: glucocorticosteroids (GC), if potency is based on liver glycogen deposition, and mineralocorticoids, if potency is based on sodium retention. The hormones that are secreted in significant amounts are cortisol, corticosterone, and aldosterone. In humans, the major naturally occurring steroid is cortisol, while in rodents the main steroid is corticosterone.

Steroids are synthesized from cholesterol in the inner mitochondrial membrane of the adrenal glands, through a series of reactions including hydroxylations. The release of cortisol from the adrenal glands is regulated by the hypothalamic-pituitary-adrenal axis, and involves the secretion of corticotropin-releasing hormone from the hypothalamus, and the subsequent secretion of adrenocorticotropin hormone (ACTH) from the pituitary gland. The rate-limiting step in steroid synthesis is the transport of cholesterol to the inner mitochondrial membrane by sterol carrier protein 2 (SCP2), steroidogenesis activator protein polypeptide (SAP), peripheral benzodiazepine receptor (PBR), and

steroidogenic acute regulatory protein (StAR)^[47]. Because corticosteroids are not stored in adrenal tissues, the rate of secretion equals the rate of biosynthesis^[48-51].

GC are classified as lipids because they are more soluble in organic solvents than aqueous solvents. Their solubility is affected by the presence of hydroxyl or carbonyl groups^[52]. GC enter cells by diffusion across the plasma membrane. About 90% of circulating cortisol is reversibly bound to a plasma protein, the corticosteroid binding globulin (CBG)^[48,51,52]. CBG is a high affinity, low capacity plasma protein, while albumin has a low affinity and high capacity to bind steroids^[48,51-53]. The bioavailability of GC in specific tissues is determined by the presence of tissue specific metabolizing enzymes [11- β -hydroxysteroid dehydrogenase (11 β -HSD)], CBG levels, the presence of efflux proteins (such as multi-drug resistance protein 1, MDR1), and the expression of the glucocorticosteroid receptors (GR). Other levels of regulation include variations in the receptor protein (isoforms, polymorphisms), alternative receptor dimerization, co-chaperones, levels of hsp, and posttranslational modifications^[54].

Approximately 70% of corticosteroid metabolism occurs in the liver, and involves the cytochrome P450 system^[55]. Intestinal metabolism is also important, as the intestinal sites of cortisol and cortisone metabolism in humans are saturated before the hepatic sites^[56]. The majority of corticosteroids are excreted in the urine, although small amounts are detectable in fecal, biliary and pulmonary CO₂ excretions^[48,49,57].

Glucocorticosteroid receptors (GR) are 94 kDa proteins found in the cytoplasm of cells from many tissues. When ligand binding occurs, an inhibitory protein is released, the receptor dimerizes, hyperphosphorylation occurs^[58-60], and a DNA binding site is exposed (Figure 1). This conformational change, which may unmask nuclear localization signals, allows the GR to translocate to the nucleus. The GR may affect gene transcription and subsequent protein synthesis by interacting with specific nuclear binding sites (referred to as glucocorticosteroid response elements, GRE). This causes either stimulation or, less frequently, inhibition of transcription. Also, the binding of the GR homodimer to the GRE may induce rearrangement of the chromatin, allowing other transcription factors to bind to previously inaccessible DNA^[61].

The GR may also interact with other transcription factors, such as AP-1, in which case transcription is commonly inhibited^[62,63]. There are several other transcription factors that have been linked to the GR, including the p65 subunit of NF κ B^[64,65].

The unliganded GR may be associated with heat shock proteins (hsp), including hsp56, hsp70 and hsp90. There may be constant cycles of dissociation/reassociation of these components, as well as constant bi-directional shuttling of GR between the cytosol and the nucleus^[66,67]. Furthermore, the action of hsp70 and hsp90 may be further regulated, either positively or negatively, by co-chaperones^[68-71]. These proteins may

Figure 1 Simplified model of GR-mediated transcriptional modulation. (from Bamberger et al 1996).

also be involved in recruiting transport proteins to the receptor complex, regulating hormone binding to the receptor, and modulating the regulatory function of GR by disassembling transcription complexes^[70-73].

Several other factors may influence GR-mediated transcriptional activation. For example, it has been suggested that competition between transcription factors for limited coactivator molecules may lead to gene repression^[74,75]. Alternatively, cofactor effects may be restricted to designated compartments, and dynamic cofactor modules may hit promoters in a cyclic way during transcription^[72-73,76-77]. In addition to acetylation, methylation and phosphorylation of histones may influence transcriptional control^[78-80], as also could phosphorylation of the GR^[81].

Some of the effects of GC occur too rapidly to be explained at the genomic level of transcription. Distinct GR forms or a cytosolic subset of GR may interact with signal transduction pathways, which are usually associated with membrane receptor signalling events^[82]. Extensive crosstalk between steroid- and growth factor-stimulated signalling pathways occurs, and may impact the functioning of steroid receptors and genes that are regulated by steroids^[83].

A number of synthetic GC have been developed with increased potency when compared to the naturally occurring substances. The increased potency, however, comes with an increased potential for adverse effects. The high potency of the synthetic steroid is attributed to its long half life and high affinity for the GR. DEX is commonly used in the research setting due to its unique properties. The synthetic DEX differs from the naturally occurring cortisol due to the addition of a double bond between carbon 1 and 2, fluorination of the 9 position, and the addition of a methyl group. Therefore, while most GC in the plasma is bound to the CBG, DEX circulates freely, with blood concentrations unaffected by changes in CBG levels. This is particularly important in studies of development, as CBGs are known to be developmentally regulated^[84]. The placental enzyme 11- β hydroxysteroid dehydrogenase type-2 (11 β -HSD₂) protects the fetus by converting maternal cortisol into inactive cortisone, due to its dehydrogenase

activity^[85]. However, the unique structure of DEX allows it to cross the placenta by escaping deactivation by 11 β -HSD₂.

Intestinal maturation

The effect of GC on prenatal intestinal maturation was investigated by Gartner *et al.*^[86], using mice lacking a functional glucocorticoid receptor (GR^{-/-}). Although a temporal association exists between the prenatal GC surge and corresponding intestinal development, the results of this study suggest that steroids are not mandatory for prenatal intestinal maturation. Histological examination of 18 d old fetal mice failed to demonstrate any effects of the GR^{-/-} phenotype on intestinal morphology, or on measures of functional development (including enterocyte, Goblet and enteroendocrine cell numbers). LPH activity (as measured by X-gel staining) and the number of proliferating cells (as assessed by KI-67 staining) were also unaffected by the absence of GR. Thus, the rodent intestine appears to pass through a period of unresponsiveness to GC during fetal life, prior to becoming responsive in the postnatal period.

The role of GC in postnatal development has been well characterized. Indeed, adrenalectomy during the suckling period retards the functional maturation of the small intestine, while exogenous administration of GC may cause precocious maturation^[87-89]. The mechanisms by which GC may influence maturation are not completely understood. There may be direct as well as indirect effects of GC. For example, Schaeffer *et al.*^[90] injected hydrocortisone (50 g/g, *sc*) into 11 d old rats; they showed that the precocious induction of the BBM enzyme sucrase isomaltase (SI) paralleled decreases in TGF- β and the cytokine IL-1 α , and increases in TNF- α . This finding suggests that cytokine levels may mediate the effects of GC on postnatal intestinal maturation.

The development of GC responsiveness was characterized in more detail by Solomon *et al.*^[89] using trehalase as a marker of intestinal maturation, they showed three phases of responsiveness to GC in mice: (1) a prenatal phase during which time Dexamethasone (DEX) did not induce maturation; (2) an early postnatal phase (first 2 postnatal weeks) of moderate responsiveness; and (3) a subsequent phase of increasing responsiveness in the third postnatal week. Postnatal increases in responsiveness were not paralleled by increases in GR abundance, but were associated with elevated circulating T4 concentrations. This observation fits with previous work demonstrating a synergistic effect of GC and T4 on BBM enzyme expression^[91-93] also previously demonstrated three post-natal phases of responsiveness to GC. Based on the effects on SI mRNA and activity in rats, they identified (1) an early phase (day 10) when activation of the gene occurs; (2) a late phase (day 16) when changes in cell kinetics are observed; and (3) a loss of responsiveness (day 18) to GC. Although the exact timing of the various phases is not consistent between studies, it is clear that steroids

have maximal effects in the early postnatal period.

Although some studies have shown that GC are not important in fetal development^[86,89], not all researchers would agree with the suggestion that the fetus is unresponsiveness to GC. For example, Buchmiller and colleagues^[94] showed that maternal DEX administration produced increases in small intestinal length and a trend towards an increase in LPH and maltase activity in fetal rabbits. The everted sleeve technique was used to demonstrate increases in glucose and proline transport in fetal rabbits exposed to DEX. When the fetal rabbits were subdivided into categories based on uterine position, runt fetuses exposed to DEX exhibited high nutrient uptake rates, surpassing the rates of the favored fetuses. This suggests that the runt intestine may be more responsive to steroid treatment, an observation with interesting implications regarding the potential clinical treatment of low birth weight or growth retarded infants.

The effects of GC on fetal intestine were also examined in a rabbit gastroschisis model. Gastroschisis is a herniation (displacement) of the intestines through a defect on one side of the umbilical cord. Intra-amniotic DEX (0.2 μ g/g per day) infusion enhanced intestinal disaccharidase activity and glucose uptake in fetuses with experimentally induced herniation of the small intestine into the amniotic cavity^[95]. While this study looks at a specific congenital dysfunction, it clearly demonstrates that the fetal intestine is capable of responding to GC.

What is the effect of GC on the intestine of human neonates? Nanthakumar *et al.*^[96] used human intestinal xenografts to characterize the response of the developing intestine to GC. Responsiveness was determined by lactase activity and cytokine induction after a proinflammatory stimulus. Immature transplants (20 wk) responded to GC, but that this effect was lost in the mature (30 wk) transplants. This suggests that in humans there may also be a brief period of responsiveness *in utero* to GC.

Costalos *et al.*^[97] showed that in humans, maternal DEX administration increased fetal and neonatal plasma gastrin concentrations, while motilin was increased only in neonates, and vasointestinal peptide concentrations were unchanged. The authors speculate that the effects of GC on the gastrointestinal tract may be at least partially mediated by their actions on other GI hormones.

Clearly, there is contradictory evidence regarding the ability of the fetus to respond to steroids. The discrepancies may be due to the use of different indicators (trehalase, SI, nutrient uptake, morphology) of GC responsiveness. Maternal DEX administration may have a direct effect on the fetus, as DEX is known to cross the placenta^[98]. However, it is also possible that the maternal administration of DEX has an indirect effect on the fetus, through the modulation of other factors such as cytokines, hormones or growth factors.

There are many other adverse effects of the therapeutic administration of GC, such as glaucoma,

poor wound healing, muscle atrophy, hypertension, hyperglycemia/diabetes, withdrawal syndrome, increased risk of infection, GI ulcers/perforation/bleed, thin skin, poorly developed muscle, thin extremities, fat collection in abdomen and upper back ("buffalo hump"), salt and water retention "moon face", osteoporosis, avascular necrosis, and CNS effects such as increased appetite, insomnia, euphoria and frank psychoses.

GC may also be used to accelerate lung maturation and surfactant production in premature infants, although DEX is not recommended as it is associated with impaired growth and neurodevelopmental delay. GC administration reduces the incidence of NEC in infants: a prospective experiment looking at prenatal or postnatal steroid use demonstrated a reduction in NEC in neonates^[99]. Animal models of DEX administration demonstrate increased mucosal maturation, with increased enterocyte and goblet cells, coupled with a thinning of the muscularis^[100]. Unfortunately, between early postnatal administrations of DEX is associated with the development of focal small bowel perforation^[100,101]. The mechanism associated with the differential effect of GC on intestinal mucosa and muscularis is not fully understood. Although *in vitro* work on intestinal smooth muscle cells suggests that DEX alters the expression of collagen and other basement membrane elements^[102,103], *in vivo* remodelling of the extracellular matrix by DEX has not been shown.

The tissue specific effects of GC may be the result of a redistribution of growth factors. DEX administration in newborn mice alters IGF- I immunolocalization, with increased protein detected in the mucosa, and reduced levels in the muscularis^[104]. *In situ* hybridization analyses for IGF- I transcripts showed no differences in localization between the groups. In addition to its' effects on IGF- I immunolocalization, DEX (i.p. 1 µg/g, once daily) altered IGF- I binding protein composition in the mucosa in newborn mice. This alteration may be responsible for drawing IGF- I from the mesenchyme to the mucosa, and subsequently influencing intestinal maturation^[105].

Further support for the involvement of the IGF- I system in NEC comes from the work of Burrin *et al*^[106], who showed that in addition to decreases in circulating IGF- I and alterations in IGF- I binding proteins, neonatal pigs treated with DEX (subcutaneous, 1 mg/kg, for 7 d) had increased IGF- I receptor mRNA abundance. This increase was only observed in the stomach and ileum, with no effect seen in the jejunum. This corresponds well with the authors' observation that the effects of DEX are most pronounced in the ileum. The catabolic effects of DEX were also characterized in this study: small intestinal growth, particularly in the ileum, was inhibited due to increases in protein degradation, without significantly affecting protein synthesis.

GC have well documented effects on the ontogeny of BBM hydrolases. Adrenalectomy delays enzyme maturation during the third post-natal week, while exogenous GC administration may induce the precocious

appearance of SI activity^[107]. In adult animals, however, disaccharidase activities in the small intestine are unaffected by adrenalectomy or by GC administration^[108]. The administration of DEX in the neonatal period increases the intestinal uptake of sugars (Drowdowski *et al*, unpublished observations, 2005).

There may be a synergetic interaction between GC and T4 on BBM enzyme maturation. This was thought to involve the ability of T4 to increase corticosteroid-binding globulin, which subsequently reduces the clearance of hydrocortisone^[109]. However, work by McDonald and Henning^[110] demonstrated synergism between DEX and T4. This indicates that an additional mechanism must be involved since DEX does not bind to CBG. In this study, animals received daily injections of subcutaneous T4 (130 pmol/g body weight) and a non-saturating dose of DEX (0.01 µg/g body weight) from post-natal day 5 to 12. The hormones synergistically increased jejunal SI as well as ileal and duodenal alkaline phosphatase, and decreased ileal β-galactosidase activity, but did not affect jejunal or ileal LPH activity. When administered alone, T4 did not affect intestinal maturation, and DEX only partially stimulated maturation, when compared to the combination of hormones. The authors concluded that enzymes that rise post-natally responded to treatment with the combination of the T2 and DEX, while enzymes that decline post-natally show a mixed response.

In suckling rodents, GC regulate the expression of BBM enzymes such as SI-isomaltase and trehalase^[107,111,112]. However, following GC administration, it takes 12-24 h to observe increases in the transcription of these genes. This delayed time-course suggests the involvement of secondary response genes^[113]. Oesterreicher and Henning^[114] identified a region of the trehalase promoter with potential binding sites for several transcription factors. Electromobility shift assays were performed using oligonucleotides from this region, as well as nuclear extracts from jejunum of 8 d old control or DEX-treated (1 g/g body wt) mice. They found that DEX stimulated expression of GATA-4 and GATA-6 proteins. These transcription factors are recognized as being important regulators of intestinal gene expression, and may interact with other transcription factors including those from the Sp family, Cdx, HNF-1 and HNF-4^[115-120]. Indeed, GATA factors may allow cooperating transcription factors to bind to the DNA by altering the chromatin structure^[119]. Although this study failed to prove that the induction of GATA factors leads to transcriptional activation of the BBM hydrolases, it does increase our understanding of possible mediators of GC effects.

Other molecular signals that may be responsible for the ontogenic changes in intestinal gene expression include a group of transcription factors called CCAAT/enhancer binding proteins (C/EBPs). In an attempt to determine regulatory mechanisms involved in the expression of the C/EBP α, β and δ isoforms, Boudreau *et al*^[120] examined their expression in response to GC in the rat intestinal epithelial crypt-derived cell line

IEC-6, using Northern blot, transcription run-on assays, indirect immunofluorescence, Western blot, and electrophoretic mobility shift assays. Whereas C/EBP α expression was not regulated by GC, C/EBP β and δ mRNA and protein levels were rapidly induced. Moreover, C/EBP β - and δ -containing DNA binding complexes were increased by GC as determined by supershift assays, in contrast to C/EBP α containing complexes. Immunofluorescence studies showed cytoplasmic and nuclear localization for C/EBP α . This is in contrast to a restricted nuclear localization for both C/EBP β and C/EBP δ . Differential regulation by GC as well as the different localization of three C/EBP isoforms suggest a role for this class of transcription factors in the control of gene expression in intestinal epithelial cells.

Mechanisms of action

The mechanisms by which GC exert their effects may involve several other factors. Boudreau *et al.*^[121] showed that DEX increases c-fos and c-jun, and increased AP-1 DNA-binding capacity in IEC-6 cells. Ras transformation repressed the growth-inhibitory properties of DEX, and inhibited the induction of c-fos protein and mRNA. This suggests that Ras negatively modulates the response of intestinal epithelial cells to GC. Thiesen *et al.*^[122] have shown that early response genes may be involved in the effect of GC on the enhancement of intestinal absorption of nutrients that occurs after intestinal resection. This further illustrates the cross-talk that occurs between GC and intracellular signaling pathways.

GC may also exert their effects by influencing cellular proliferation, differentiation and apoptosis. Foligne *et al.*^[123] investigated the effects of 10-d bilateral adrenalectomy on morphometry, proliferation and apoptosis in the small intestine of 3 mo old Sprague-Dawley rats. Adrenalectomy led to partial atrophy and disorganization of the epithelium, with an increased number of goblet and Paneth cells. A reduction of crypt cell proliferation was paralleled by a marked increase in apoptosis in the villus.

Several other studies suggest that steroids may increase apoptosis. *In vitro* studies using rat jejunal epithelial cells (IEC-6) cells show that the locally acting budesonide increases apoptosis, while *in vivo* studies show that steroids increase apoptosis in intraepithelial lymphocytes^[124,125].

There are conflicting reports on the effect of GC on intestinal proliferation. Low to medium doses (10^{-6} - 10^{-11}) increase proliferation in IEC cells, while high doses (10^{-5}) inhibit proliferation^[126]. Other studies suggest that the effect of steroids on proliferation depends on the developmental stage of the animal^[127]. Similarly, the location of the cells along the crypt-villous axis is an important factor as DEX reduced proliferation in the upper but not the lower crypt of fetal mouse duodenal explants^[128]. This points to an important role of the adrenal glands and GC in the trophic status of the adult small intestinal mucosa. These results also highlight that the atrophy associated with GC is associated with

a reduction in proliferation together with an increase in apoptosis. It is possible that some of the negative effects of GC on the intestine could be reduced or prevented by the administration of a trophic agent.

Early exposure to GC has been associated with lasting effects on cardiovascular, endocrine and metabolic systems. For example, prenatal glucocorticoid exposure results in reduced birth weight^[129-134], increased blood pressure^[135] increased expression of the glucocorticoid receptor in visceral fat^[136], impaired coping in adverse situations^[137], increases in corticosterone levels^[135,137] hyperglycemia/hyperinsulinemia^[131,138,139], increased susceptibility of the inner ear to acoustic noise trauma^[140] and increases in PEPCK (rate-limiting enzyme in gluconeogenesis) mRNA and activity^[141]. Steroids may also influence the development of the retina, although studies have shown both a positive effect on experimentally-induced retinopathy^[142,143] and an increased risk of retinopathy of prematurity^[144]. Furthermore, neurodevelopmental problems including an increased risk of cerebral palsy^[145] have been documented following postnatal steroid administration.

It is not known if early exposure to GC results in lasting effects on intestinal function. However, because steroids are known to influence intestinal transport in adult animals^[146], and because steroids influence the ontogeny of the intestine^[110,147], it seems plausible that they may also play a role in the programming of intestinal function.

Effects on adult intestine

Does the adult small intestine remain responsive to steroids? Foligne *et al.*^[123] attempted to answer this question: three month old Sprague Dawley rats underwent bilateral adrenalectomies, and were sacrificed 10 d after surgery. Adrenalectomy modified maturation and differentiation, particularly in the proximal small intestine. A partial atrophy and disorganization of villous architecture was noted, coupled with decreases in crypt cell proliferation and increases in apoptotic cells in the upper villous region. LPH and SI were increased by adrenalectomy, while aminopeptidase N and intestinal alkaline phosphatase activities were reduced. These results indicate that the adrenal glands and GC play an important role in the trophic status of the small intestine in adult rats.

GC are used to treat various disorders including rheumatoid arthritis, inflammatory bowel disease, systemic lupus erythematosus, allergies and asthma due to their anti-inflammatory effects^[148]. Both NF κ B and AP-1 are crucial for the induction of many genes involved in inflammation. GC may interfere with the transcriptional activity of these and other factors. Alternatively, GC may also interact with a negative GRE (nGRE) and thereby inhibit transcription. Although the anti-inflammatory effects of GC may be due to negative modulation of pro-inflammatory factors (transrepression), the side effects may be the result of transactivation^[149]. This has created interest in developing "dissociating steroids", in which

the desirable transrepression effect is separated from undesirable transactivation effect.

Glucocorticoid resistance may occur as GC reduce GR expression^[150]. The importance of GC is clear from the clinical symptoms associated with a deficiency or excess of the hormones. Cortisol deficiency (Addison's disease) is characterized by postural hypotension, weight loss and hypoglycaemia GC excess (Cushing's syndrome) is characterized by hypertension, central obesity and glucose intolerance.

Quaroni *et al*^[151] found that exposing IEC cells to GC cause growth arrest, the formation of tight junctions, the appearance of tall slender microvilli, reorganization of the ER and Golgi, and decreased cdk6 as well as p^{27kip1} protein. These results are consistent with the activation of multiple genes important in the functioning of absorptive villous cells, but likely not involved in the induction of cell differentiation.

The role of the mesenchyme in mediating the effects of GR was studied by Simo *et al*^[152] GC treatment of mesenchyme-derived cell populations resulted in an accumulation of laminin at the cell surface, accompanied by enhanced expression of BBM enzymes. This effect was abolished by anti-laminin, suggesting that GC may lead to accelerate laminin organization at the epithelial-mesenchymal interface, leading to epithelial cell differentiation.

Glucocorticosteroids and sugar transport

GC alter sugar transport in a number of other tissues. GLUT4 expression in adipose tissue is diminished in response to DEX, while GLUT4 in muscle is increased^[153]. In adipocytes, DEX increased GLUT4 levels at the plasma membrane in the basal state, while GLUT4 translocation in response to insulin was inhibited.

Oral glucocorticosteroids increase intestinal sugar transport. For example, using an *in vivo* recirculation-perfusion technique in rats, Batt and Peters^[154] showed that 7 d of prednisolone increased intestinal galactose absorption per enterocyte, without influencing the intestinal mucosa or cell kinetics. Similarly, short-term pharmacological doses of prednisolone increased digestive/absorptive function, while paradoxically decreasing the epithelial cell population in rats^[155]. Long-term (28 d) administration of prednisolone or betamethasone in rats increased the activity of BBM proteins and galactose absorption, but induced atrophy of the mucosa and inhibition of cell turnover^[156].

When the proximal jejunum of humans was perfused with glucose (28 mmol), intraluminal (100 mg/L) hydrocortisone increased sodium, water and glucose absorption^[157] when compared to controls. Thiesen *et al*^[146] assessed the influence of the glucocorticosteroids budesonide and prednisone on the *in vitro* uptake of sugars in weaning male rats. The steroids had no effect on the uptake of D-glucose by SGLT1. In contrast, the uptake of D-fructose by GLUT-5 was increased with both budesonide and prednisone. The increases in the uptake

of fructose were not due to variations in the weight of the intestinal mucosa, food intake, or in GLUT-5 protein abundance or mRNA expression. This enhanced uptake of fructose was likely regulated by posttranslational processes, such as enhancement of the intrinsic activity of the transporters. There were no steroid-associated changes in mRNA expression of c-myc, c-jun, c-fos, proglucagon, or selected cytokines. However, the abundance of ileal ornithine decarboxylase mRNA was increased with Prednisone.

Thiesen *et al*^[122] further characterized the effect of steroids using a model of intestinal resection. Adult male Sprague Dawley rats underwent transection or resection of 50% of the middle portion of the small intestine. Prednisone had no effect on the *in vitro* uptake of glucose or fructose in resected animals. In contrast, in resected rats budesonide increased by over 120% the value of the jejunal maximal transport rate (Vmax) for the uptake of glucose, and increased by over 150% ileal uptake of fructose. Changes in SGLT1, GLUT5, GLUT2, and Na⁺K⁺-ATPase protein abundance and mRNA expression did not explain the enhancing effect of budesonide. The steroids reduced c-jun, ODC and proglucagon expression. These data suggest that the influence of GC on sugar uptake in resected animals may be achieved by post-translational processes involving signalling with c-jun, ODC, and proglucagon, or perhaps also other as yet unknown signals.

GLUCAGON-LIKE PEPTIDE-2 (GLP2)

Biochemistry

Proglucagon is a 160 amino acid peptide encoded by the glucagon gene, and is present in intestinal L cells and α cells of the islets of Langerhans^[213,214]. Proglucagon undergoes post-translational processing in the pancreas liberating glucagon as the main product. In the intestine, several peptide products (collectively referred to as "enteroglucagon") are produced including glucagon-like peptide-1 (GLP1) and glucagon-like peptide-2 (GLP2) (Figure 2).

Both GLP1 and GLP2 are secreted from the L cells of the distal small intestine and colon in response to enteral nutrients^[215]. Both fatty acids^[216] and glucose^[217] stimulate secretion from L cells, but protein meals do not increase GLP1 or GLP2 secretion^[218,219]. However, amino acid mixtures have been shown to stimulate GLP1 release in humans^[220], and meat hydrolysates do stimulate GLP1 secretion from rat intestinal L cells *in vitro*^[221].

The secretion of these peptides is biphasic, with an early peak within 30 min of a meal, followed by a later peak at 60-120 min^[219]. It is thought that neuroendocrine pathways may be responsible for the early secretion, as luminal nutrients are not likely to reach the distal L cells within 30 min of ingestion^[216,222].

The peptides are degraded in the plasma by dipeptidyl peptidase IV (DPPIV)^[223], with a half-life for GLP2 of 7

Figure 2 Post-translation processing of proglucagon in the pancreas and the intestinal L-cells. The numbers represent the amino acid at which enzymatic cleavage occurs.

min^[224]. DPPIV-resistant analogs (ALX-600, Teduglutide) with greater potency have been developed for clinical use. These will be discussed in more detail in later sections.

The receptor is a G-protein coupled receptor with 7 transmembrane domains, and is encoded by a single gene localized to chromosome 17p13.3. GLP2R expression is highest in the proximal small intestine, and decreases distally along the longitudinal axis^[225]. GLP2R has been localized to intestinal enteroendocrine cells in humans using immunohistochemistry^[226], to enteric neurons in mice using reverse transcription polymerase chain reaction (RT-PCR) and immunohistochemistry^[227], and to rat enterocytes using ¹²⁵I-GLP2^[228]. Orskov *et al.*^[229] found GLP2 receptors mainly on subepithelial myofibroblasts in rat, mouse, and human small and large intestine by immunohistochemistry and *in situ* hybridization. By double labelling they found that these GLP2 receptor immunoreactive cells also produce smooth muscle actin and keratinocyte growth factor (KGF). KGF antibody abolished the growth promoting effect of GLP2 in the large intestine, but not in the small intestine. This suggests that GLP2 in the gut may act by activating receptors on the subepithelial myofibroblasts, thereby causing the release of growth factors, which in turn stimulate intestinal growth. Therefore, at this time it remains unclear whether the intestinotropic effects of GLP2 are due to direct effects on enterocytes, or are mediated by secondary factors.

Physiological effects

While GLP1 has potent insulinotropic effects^[230], GLP2 has been found to be an important intestinotrophic factor. The relationship between enteroglucagon and small bowel growth was first documented by Gleeson *et al.*^[231]: a patient with an enteroglucagon-producing tumor exhibited small bowel hyperplasia, and injection of the tumor extract into mice resulted in intestinal growth^[232]. Drucker *et al.*^[233] demonstrated that GLP2 was the specific agent responsible for this effect. GLP2 appeared to exert a “tissue specific” effect on the gut, as

no changes were found in other tissues including spleen, heart, brain and liver.

GLP2 given subcutaneously adult to mice (6-43 g, twice daily, for 10 d) increases small bowel weight and crypt-villous height^[234,235]. Intestinal proliferation was increased, and apoptosis was reduced. The effect of GLP2 was not due to changes in food consumption. The effects of GLP2 were sustained, as increases in growth were still evident after three months of administration. The rapid turnover of the epithelium was thought to contribute to the lack of desensitization that was observed with prolonged GLP2 treatment.

In addition to its morphological effects, GLP2 (when administered intravenously or subcutaneously) also increases the activity and expression of BBM enzymes including SI, LPH, maltase-glucoamylase and aminopeptidase N^[236-238]. Although the effects on gastric physiology in humans have been conflicting^[239,240], inhibition of gastric emptying in pigs has been observed with GLP2 treatment^[241]. Reductions in meal-stimulated gastric acid secretion have also been observed with GLP2 administration in humans^[242]. GLP2 enhances barrier function in murine intestinal epithelium^[243], making it a potential therapeutic for disorders such as inflammatory bowel disease and necrotizing pancreatitis, both of which are characterized by increases in intestinal permeability^[243,244].

While the mechanism by which GLP2 stimulates the adaptive response is unknown, its effects on an immediate early gene, PC4/TIS7, have been observed. In postconfluent, quiescent IEC 18 cells in culture, a stable derivative of GLP2, r(gly2)GLP2, increased PC4/TIS7 expression. r(gly2)GLP2 administered intraperitoneally to mice similarly induced increased PC4/TIS7 mRNA compared with vehicle control^[245].

A number of studies aimed at establishing the effect of GLP2 on intestinal transport. Cheeseman and Tsang^[246] showed that a two hour vascular perfusion of GLP2 (4 h, 400 and 800 pmol/L) in rats increased D-glucose V_{max} by about 65% in the basolateral membrane, and by three-fold in the BBM, with a concomitant increase in SGLT1 protein^[247]. An anti-GLP antibody abolished the GLP2-induced increase in transport^[246].

Not all studies have demonstrated an effect of GLP2 on nutrient transport. For example, Brubaker *et al.*^[237] treated mice subcutaneously with GLP2 (2.5 µg) for a 10 d period, and failed to reveal increases in the absorption of glucose or maltose. Curiously, an increased capacity for nutrient digestion was found to be due to increases in BBM enzyme activities, resulting in an apparent uncoupling of digestive capacity from subsequent sugar absorption. It is unclear if there is this discrepancy in the results of these studies on the effect of GLP2 or nutrient uptake.

The rapid trafficking the glucose and fructose transportation contributes to the sugar uptake GLUT2 into the BBM following a glucose infusion of meal^[248-250]. GLUT2 protein levels in the BBM increased two-fold when luminal perfusions were increased from

0 mmol/L to 100 mmol/L glucose. One hour vascular infusion of GLP2 doubled the rate of fructose uptake into BBM following luminal fructose perfusion^[251]. Fructose absorption in this study was determined by the appearance of fructose in the vascular bed. Western blotting of biotinylated surface-exposed protein showed a doubling of GLUT2 expression in the BBM following GLP2 infusion. Thus, GLP2 may promote the insertion of GLUT2 into the BBM, thereby providing a low affinity/ high capacity route in addition to SGLT1 and GLUT5 by which fructose or glucose may be absorbed into the enterocyte.

Ramsanahie *et al*^[252] examined the effect of chronically administered GLP2 on diurnal SGLT1 expression. Rats were treated with [Gly2]GLP2 (twice daily; 1 mg/g body weight) or vehicle (control) for 10 d. GLP2 administration did not alter the diurnal increase in mRNA levels of SGLT1, GLUT2, or GLUT5. However, SGLT1 protein was increased three-fold by GLP2, and *in situ* hybridization showed that SGLT1 mRNA was distributed along the entire length of the villi. This is in contrast to what was seen in control animals, where SGLT1 was restricted to the mid and upper villus, with less SGLT1 mRNA localized to the villous tip. Also, in contrast to the diffuse staining seen in control animals, immunofluorescence microscopy showed that SGLT1 protein in GLP2 treated animals was preferentially localized to the BBM, with little or no staining in the cytoplasm. This may represent another mechanism by which GLP2 increases intestinal glucose uptake.

The effect of GLP2 on *in vivo* nutrient absorption may also be attributed to nitric oxide-dependent increases in intestinal blood flow. Infusing GLP2 (500 pmol/kg per hour) for 4 h in TPN-fed piglets led to increased portal-drained visceral (PDV) blood flow rate, intestinal blood volume, and PDV glucose uptake. GLP2 also increased intestinal constitutive nitric oxide synthase (NOS) activity and endothelial NOS protein abundance^[253]. Thus, in TPN-fed neonatal pigs, GLP2 acutely stimulates intestinal blood flow and glucose utilization, and that this response is nitric oxide-dependent.

Walsh *et al*^[254] studied GLP2 signalling in isolated rat intestinal mucosal cells expressed mRNA transcripts for the GLP2R, as well as for chromogranin A and β -tubulin III, markers for enteroendocrine and neural cells, respectively. cAMP production in response to [Gly2]GLP2, a degradation-resistant analog of GLP2, was maximal at 10^{-11} mol/L with reduced cAMP accumulation observed at higher doses. The cAMP response was abolished by pretreatment with 10^{-6} mol/L GLP2, indicating receptor desensitization. GLP2 treatment of isolated mucosal cells increased ^3H -thymidine incorporation, and this was prevented by inhibition of the protein kinase A pathway. In contrast, GLP2 did not affect p44/p42 MAPK phosphorylation or the levels of cytosolic calcium in the mucosal cell preparation. These results provide evidence that activation of the endogenous rat mucosal GLP2 receptor is linked to activation of a cAMP/protein

kinase A-dependent, growth-promoting pathway *in vitro*.

Estall *et al*^[255] examined the mechanisms regulating signaling, internalization, and trafficking of the GLP2R to identify determinants of receptor activation and desensitization. Heterologous cells expressing the transfected rat or human GLP2R exhibited a rapid, dose-dependent, and prolonged desensitization of the GLP2-stimulated cAMP response and a sustained GLP2-induced decrease in levels of cell surface receptor. Surprisingly, inhibitors of clathrin-dependent endocytosis failed to significantly decrease GLP2R internalization, whereas cholesterol sequestration inhibited ligand-induced receptor internalization and potentiated homologous desensitization. The hGLP2R localized to both Triton X-100-soluble and -insoluble (lipid raft) cellular fractions and colocalized transiently with the lipid raft marker caveolin-1. Although GLP2R endocytosis was dependent on lipid raft integrity, the receptor transiently associated with green fluorescent protein tagged-early endosome antigen 1-positive vesicles and inhibitors of endosomal acidification attenuated the reappearance of the GLP2R on the cell surface. This data demonstrates that GLP2R desensitization and raft-dependent trafficking represent distinct and independent cellular mechanisms and provide new evidence implicating the importance of a clathrin- and dynamin-independent, lipid raft-dependent pathway for homologous G protein-coupled receptor internalization.

Intestinal resection

Plasma GLP2 levels rise following intestinal resection in rats^[256-258]. When rats were subjected to a 70% midjejunioileal resection or ileal transection, and were maintained with TPN or oral feeding. Resection-induced adaptive growth in TPN- and orally-fed rats was associated with a significant positive correlation between increases in plasma bioactive GLP2 and proglucagon mRNA abundance in the colon of TPN-fed rats and in the ileum of orally fed rats^[259]. While these increases were transient in the TPN-fed group, luminal nutrients produced a sustained increase detected at 3 and 7 d post-resection. These data support a significant role for endogenous GLP2 in the adaptive response to mid-small bowel resection in both TPN and orally fed rats.

GLP2 administration in rats increases the adaptive response to massive intestinal resection^[260]. Sprague-Dawley rats were divided into two groups, with a 75% mid-jejunum-ileum resection and a sham operated group. Animals were given 0.1 $\mu\text{g/g}$ GLP2 analog (protease resistant human GLP2) or placebo given subcutaneously twice daily for 21 d. Administration of the GLP2 analog was associated with an increase of the mucosal mass in the proximal jejunum and terminal ileum.

Martin *et al*^[261] investigated the effects of GLP2 in a total parenteral nutrition (TPN)-supported model of experimental short bowel syndrome. Juvenile Sprague-Dawley rats underwent a 90% small intestinal resection, and were randomized to three groups: enteral diet and intravenous saline infusion, TPN only, or TPN +

10 µg/kg per hour GLP2. TPN plus GLP2 treatment resulted in increased bowel and body weight, villous height, intestinal mucosal surface area, crypt cell proliferation, and reduced intestinal permeability, as compared with the TPN alone animals. GLP2 increased serum GLP2 levels and intestinal SGLT-1 protein abundance as compared with either TPN or enteral groups. This demonstrates that GLP2 is capable of stimulating intestinal adaptation in the absence of enteral feeding. Because a number of hormones and growth factors have been shown to influence intestinal function, Washizawa *et al.*^[262] compared the effects of GLP2, growth hormone (GH) and keratinocyte growth factor (KGF) on markers of gut adaptation following massive small bowel resection (MSBR). KGF increased goblet cell numbers and TTF3, a cytoprotective trefoil peptide, in both the small bowel and the colon. While both GH and KGF increased colonic mucosal growth, GLP2 exerted superior trophic effects on jejunal growth. GLP2 also increased the glutathione/glutathione disulfide ratio, resulting in improved mucosal glutathione redox status throughout the bowel. Because of the differential effects of GLP2, GH and KGF on gut adaptation following MSBR, the authors conclude that a combination of these agents may be most beneficial.

Human studies with GLP2 have also been performed: a non-placebo controlled study was conducted in 8 patients with short bowel syndrome (SBS) with an end-enterostomy type of anastomosis (6 had Crohn's disease and 4 were not receiving HPN)^[263]. Treatment with GLP2 (400 µg subcutaneously twice a day for 35 d) increased intestinal absorption of energy, body weight, and lean body mass. Crypt depth and villous height were also increased in 5 and 6 patients, respectively.

The results of more recent studies of GLP2 in SBS have been reviewed^[264] and concluded that "Currently, hormonal therapy in short-bowel patients should be considered experimental and it is only recommended in research studies. The optimal duration and concentration requirements for GLP2 to induce beneficial effects on intestinal secretion, motility, morphology and most importantly absorption, are not known. Optimal dosage and administration of this new treatment to short-bowel patients may eventually result in long-term improvements in nutritional status and independence of parenteral nutrition in a larger fraction of short-bowel patients".

Intestinal development

The topic of the Development of the Infant Intestine: Implications for Nutrition Support and a consideration of trophic factors essential to intestinal development, have recently been considered^[265]. A role for GLP2 in the ontogeny of the intestine has been proposed. Lee *et al.*^[266] established that proglucagon mRNA was detectable in the rat fetus, and that immunoreactivity increased in the early neonatal period. Prohormone convertases, which are required for the liberation of GLP2 from proglucagon, are also detected in the fetal rat. Lovshin *et al.*^[267] detected GLP2R mRNA during fetal

and neonatal development in the rat, with levels being higher in the fetal and neonatal gut as compared to adult rats. High levels of GLP2 (1-33) were also detected in the circulation of 13 d old neonatal rats, and GLP2 immunoreactivity was found in the fetal rat intestine. In order to prove that fetal cells were capable of secreting GLP2, fetal rat intestinal cell cultures were studied, and were found to secrete correctly processed GLP2 (1-33). The administration of a degradation resistant GLP2 analog [h (Gly2)-GLP2] to 1 d old rat pups for a period of 10 d resulted in increases in both small bowel weight and length. Thus, the GLP2/GLP2R axis is functional in early life, and that the developing intestine is capable of synthesizing, secreting and responding to GLP2.

A subsequent study done by Petersen *et al.*^[236] on premature (92% gestation) TPN fed piglets demonstrated that in addition to increases in maltase mRNA and activity, increases in SI and aminopeptidase N activities were observed. Thus, it appears that some of the effects that GLP2 exerts on intestinal function may be related to gestational age at birth, as the premature intestine was more responsive to exogenous GLP2 than the term neonatal intestine. The authors also noted that GLP2 infused into pig fetuses *in vivo* passed into the maternal circulation^[268]. This suggests that GLP2 may pass through the placenta, and conversely, may expose the fetus to maternal GLP2. This raises the possibility that GLP2 given to pregnant animals may alter the form and function of the offspring.

Signalling events

Even before the GLP2 receptor was cloned, the role of the PI3K pathway in mediating the effects of GLP2 on intestinal sugar transport was studied by Cheeseman *et al.*^[247]: *in vivo* infusions of GLP2 produced an acceleration of sodium-dependent glucose uptake into BBM vesicles, with similar increase in SGLT-1 abundance. The effect of GLP2 could be inhibited by luminal brefeldin A, which blocks protein trafficking from the Golgi to the plasma membrane, or by the PI3K inhibitor, wortmannin. These results indicate that GLP2 is able to induce trafficking of SGLT-1 from an intracellular pool into the BBM and that PI3K may be involved in the intracellular signaling pathway in this response.

Because the GLP2R is not expressed on any intestinal cell lines, *in vitro* studies on GLP2 receptor signalling have been carried out in transfected heterologous cell types. Work done on transfected baby hamster kidney fibroblasts showed that GLP2 stimulated AP-1 dependent pathways increased cAMP, but did not change intracellular calcium levels^[269]. There is decreased apoptosis and reduced caspase-3 activation following GLP2 treatment *in vitro*^[270]. PKA, PI3K and the ERK pathways were not found to be essential for GLP2 inhibition of apoptosis, which was associated with reductions in cytochrome c release and cleavage of poly ADP-ribose polymerase (PARP).

GLP2-treated cells (10 µm, 3 d) demonstrated a greater than 10-fold increase in proliferation in Caco2

cells^[271]. This response was inhibited by PI3K and mitogen activated/extracellular signal-regulated kinase (MEK) inhibitors. A significantly greater abundance of the phosphorylated forms of both ERK-1 and ERK-2 was present in cells following GLP2. This suggests that the increase in Caco-2 proliferation in response to GLP2 may be due, at least in part, to the involvement of both the PI 3-kinase and the MAPK pathways.

The limitations of this work, however, centers around the fact that both the authors of this study as well as Yusta *et al*^[270] were unable to show, using Western blotting or RT-PCR, that Caco2 cells express endogenous GLP2R. This suggests that the proliferative effect of GLP2 on Caco2 cells may be mediated by other receptors, such as the EGFR, which mediates GLP1 induced proliferation in pancreatic β cells^[272]. Furthermore, the observation that GLP2 is able to induce proliferation in a tumor cell lines, raises a concern regarding the role of GLP2 in promoting tumor growth. Indeed, recent work by Thulesen *et al*^[273] showed that GLP2 promotes the growth of mucosal neoplasms in female C57bl mice whose colonic tumours were experimentally induced by administering a methylating carcinogen.

Kochler *et al*^[274] identified several expressed sequence tags from human cervical carcinoma cDNA libraries that correspond to GLP2R nucleotide sequences. GLP2R mRNA transcripts were detected by RT-PCR in HeLa cervical carcinoma cells and Ca Ski cervical carcinoma cells. GLP2 increased cAMP accumulation and activated ERK1/2 in HeLa cells transiently expressing the cloned human HeLa cell GLP2R cDNA. However, the GLP2R-induced activation of ERK1/2 was not mediated through G α s, adenylyl cyclase, or transactivation of the epidermal growth factor receptor, but was pertussis toxin sensitive, inhibited by dominant negative Ras, and dependent on betagamma-subunits. GLP2 also induced a significant increase in bromodeoxyuridine incorporation that was blocked by dominant negative Ras. Furthermore, GLP2 inhibited HeLa cell apoptosis induced by LY294002 in a protein kinase A-dependent, but ERK-independent, manner. These findings demonstrate that the HeLa cell GLP2R differentially signals through both G(α)/cAMP- and G(β)/G(γ)-dependent pathways, illustrating for the first time that the GLP2R is capable of coupling to multiple heterotrimeric G proteins defining distinct GLP2R-dependent biological actions.

Finally, the effects of GLP2 may be due to transactivation of other cell surface receptors. For example, GLP1 increases PI3K activity and enhances β -cell proliferation via transactivation of the EGFR^[272].

Yusta *et al*^[275] demonstrated that GLP2, in a cycloheximide-insensitive manner, enhanced survival in baby hamster kidney cells stably transfected with the rat GLP2R, reduced mitochondrial cytochrome c efflux, and attenuated the caspase-dependent cleavage of Akt, poly (ADP-ribose) polymerase, and β -catenin following inhibition of phosphatidylinositol 3-kinase

(PI3K) by LY294002. The prosurvival effects of GLP2 on LY294002-induced cell death were independent of Akt, p90 (Rsk), or p70 S6 kinase activation; were mimicked by forskolin; and were abrogated by inhibition of protein kinase A (PKA) activity. GLP2 inhibited activation of glycogen synthase kinase-3 (GSK-3) through phosphorylation at Ser (21) in GSK-3 α and at Ser (9) in GSK-3 β in a PI3K-independent, PKA-dependent manner. GLP2 reduced LY294002-induced mitochondrial association of endogenous Bad and Bax and stimulated phosphorylation of a transfected Bad fusion protein at Ser (155) in a PI3K-independent, but H89-sensitive manner, a modification known to suppress Bad pro-apoptotic activity. These results suggest that GLP2R signaling enhances cell survival independently of PI3K/Akt by inhibiting the activity of a subset of pro-apoptotic downstream targets of Akt in a PKA-dependent manner.

Rocha *et al*^[276] assessed the proliferative actions of GLP2 on the human Caco-2 cell line. GLP2 stimulated proliferation was inhibited in a dose-dependent fashion by both pertussis and cholera toxin (specific G protein inhibitors). This suggests that a G-protein-linked signaling pathway is involved with GLP2 bioactivity in Caco-2 cells. GLP2 stimulated proliferation was also augmented by 2',5'-dideoxyadenosine, which increases adenylyl cyclase. Proliferation rates were inversely proportional to changes in intracellular cAMP concentration. These findings suggest that a G-protein linked signaling pathway is involved with GLP2 bioactivity in the intestinal epithelial cell line Caco-2.

Clinical

Burrin *et al*^[277] studied 38 TPN-fed neonatal piglets infused intravenously with either saline or GLP2 at three rates (2.5, 5.0, and 10.0 nmol/kg per day for 7 d). GLP2 infusion dose-dependently increased small intestinal weight, DNA and protein content, and villus height; however, stomach protein synthesis was decreased by GLP2. Intestinal crypt and villus apoptosis decreased and crypt cell number increased linearly with GLP2 infusion rates, whereas cell proliferation and protein synthesis were stimulated only at the high GLP2 dose. The intestinal activities of caspase-3 and -6 and active caspase-3 abundance decreased, yet procaspase-3 abundance increased markedly with increasing infusion rate and plasma concentration of GLP2. The GLP2-dose-dependent suppression of intestinal apoptosis and caspase-3 activity was associated with increased protein kinase B and glycogen-synthase kinase-3 phosphorylation, yet the expression phosphatidylinositol 3-kinase was unaffected by GLP2. Intestinal endothelial nitric oxide synthase mRNA and protein expression was increased, but only at the high GLP2 dose. These authors concluded that the stimulation of intestinal epithelial survival is concentration-dependent at physiological GLP2 concentrations. However, induction of cell proliferation and protein synthesis is a pharmacological response. Moreover, they showed that GLP2 stimulates intestinal cell survival and proliferation

in association with induction of protein kinase B and glycogen-synthase kinase-3 phosphorylation and Bcl-2 expression.

Thus, GLP2 increases intestinal growth in premature, TPN-fed pigs by decreasing proteolysis and apoptosis, and enteral nutrition was not required for these effects to occur. The actions of GLP2 are transduced by the GLP2 receptor (GLP2R), which was characterized and cloned by Munroe *et al.*^[225]. GLP2R was detected in several rat tissues including the stomach, small bowel, colon, and in small quantities in other tissues such as brain and lung^[225]. This raises the possibility that GLP2 may have effects beyond the small intestine. Indeed, Haderslev *et al.*^[278] found that GLP2 administration significantly increased spinal bone mineral density in short-bowel patients with no colon, due to beneficial effects of GLP2 on bone resorption^[279]. Centrally administered GLP2 increases satiety in rodents^[280,281], while peripherally administered GLP2 does not influence gastric emptying, food intake or satiety in humans^[238,282].

Although the effects of early exposure to hormones such as GLP2 are unknown, the presence of circulating GLP2 and the detection of the GLP2R in fetal and neonatal rats^[267] suggests a role for GLP2 in regulating intestinal development. Drozdowski *et al.* (unpublished observations, 2006) has shown that GLP2 modifies intestinal morphology when given to suckling rats, and enhances sugar uptake into the intestine of rats whose mothers were given GLP2 during pregnancy and lactation. Therefore, it is possible that exposing young animals to GLP2, either directly or *via* their pregnant and lactating dams, may also have lasting effects on intestinal function.

GLP2 and glucocorticosteroids

The possibility of a growth factor or hormone acting additively or synergistically with a steroid hormone has been demonstrated in various tissues. For example, EGF potentiates the proliferative effects of progesterone and estrogen in the mammary gland^[283]. In a review, Lange^[83] discusses the cross-talk that occurs between steroid hormone receptors and intracellular signalling pathways. There is clearly evidence of non-genomic and extra-nuclear functions of steroid receptors, including the initiation of signal transduction pathways. Indeed, this type of cross-talk may explain how genes are coordinately regulated by mitogenic stimuli in hormone responsive tissues. For example, Migliaccio *et al.*^[284] reported MAPK activation by estradiol, and interactions between the progesterone receptor, the estrogen receptor and p60-Src kinase.

A synergistic effect between DEX and GLP2 may occur in the intestine, as GC have a permissive effect on several hormones including catecholamines, thyroid hormones, growth hormone and ACTH^[285]. GC mediates a permissive action, mostly for hormones, which act on G-protein coupled receptors, and increases adenylate cyclase^[286,287]. This occurs because DEX alters adenylate cyclase, enhancing the effects of cAMP generating agonists. Therefore, an interaction between DEX and GLP2 is like-

ly, as the GLP2 receptor is a G-protein coupled receptor.

Furthermore, cAMP and PKA, which are activated by GPCRs like GLP2R, may increase the steroid sensitivity of a target cell by increasing the DNA binding ability of the GR for its response elements. In a study by Rangarajan *et al.*^[288] using embryonal carcinoma cells lacking cAMP response element binding protein (CREB), activation of PKA increased hormone-dependent trans-activation of the GR. The effect of PKA was related to the DNA binding domain of the GR, as deletion of the amino-terminal or the ligand-binding domain did not alter PKA's effect. However, the absence of a consensus PKA phosphorylation site within the GR DNA binding domain led the authors to suggest that the GR is not a direct substrate for phosphorylation by PKA. Instead, they propose a multi-step process involving other cellular kinases and phosphatases that may interact with the GR.

As discussed previously, there are several pieces of evidence that suggest that GLP2 may exert its effect *via* the PI3K pathway. Steroid receptors may interact with this pathway as Simoncini *et al.*^[82] showed that the estrogen receptor binds to p85 subunit of PI3K.

Although there are no reports of the effect of DEX on PI3K in the intestine, an association has been observed in other tissues. For example, Saad *et al.*^[289] showed that DEX induced a 69% increase in the level of PI 3-kinase in adipocytes as determined by immunoblotting. Conversely, Buren *et al.*^[290] demonstrated that DEX decreases PI3K, PKB, insulin-stimulated PKB phosphorylation and glucose transport in isolated rat adipocytes, without changes in GLUT4. These results suggest that glucocorticoids, independently of the surrounding glucose and insulin concentration, impair glucose transport capacity in fat cells. Finally, Krasil'nikov *et al.*^[291] showed that prolonged exposure to DEX increased PI3K in Rous sarcoma virus-transformed hamster fibroblasts. Certainly in other tissues, such as adipose and muscle, PI3K is involved in regulating insulin-stimulated glucose uptake^[292]. In the intestine, EGF stimulated increases in intestinal glucose transport in rabbits is abolished by the PI3K inhibitor LY294002^[293]. Similarly, IGF- I associated increases in jejunal glucose uptake and Na⁺K⁺-ATPase activity are abolished by wortmannin, another PI3K inhibitor^[23]. So clearly this pathway plays an important role in transducing signals from hormones and growth factors to the proteins involved in sugar transport. Indeed, preliminary studies in the young rat show that GLP2 and DEX may stimulate the intestinal uptake of glucose, and this is essential with increased abundance of Akt and mTOR, part of the PI3K pathway.

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