DIGESTIVE PHYSIOLOGY OF THE PIG SYMPOSIUM: Gut chemosensing and the regulation of nutrient absorption and energy supply

O. J. Mace and F. Marshall
Heptares Therapeutics, BioPark, Broadwater Road, Welwyn Garden City, AL7 3AX, United Kingdom

ABSTRACT: The field of intestinal physiology has been transformed by the discovery that nutrient-sensitive chemosensors are strategically positioned within the gastrointestinal tract to regulate nutrient absorption and gut hormone secretion. It is well established that the composition of the diet modulates the absorptive capacity of the intestine and the secretion of gut peptides including glucoinsulinotropic polypeptide, glucagon-like peptide-1, and peptide YY. Through these mechanisms chemosensors, including nutrient transporters and G protein coupled receptors, are able to regulate cellular energy uptake, expenditure, and homeostasis in intestine. Over the past few decades, the molecular identities for some of the chemosensors that respond to the arrival of dietary nutrients have been revealed. This review summarizes the current understanding of intestinal chemosensors in nutrition and their ability to regulate cellular energy homeostasis through the expression of nutrient transporters and the secretion of gut peptides. In both humans and animals, this raises exciting possibilities to beneficially manipulate whole body metabolic processes. In humans, malnutrition or metabolic diseases, such as obesity and type 2 diabetes, may result from impaired intestinal chemosensing. Therefore, intestinal chemosensors associated with the neuroendocrine control of metabolism are considered targets for nutritional and pharmacological intervention, with the potential to manipulate the nutritional status of actively growing or elderly individuals and/or restore energy homeostasis during metabolic disease. In the animal world, targeting intestinal chemosensors to increase energy supply in animals is likely to affect feed consumption, gut development, health, and growth efficiency. For instance, stimulating nutrient absorption at an early age may reduce incidence of intestinal disorders postweaning and this could improve feed efficiency, impacting the economic and environmental sustainability of animal-protein production.

Key words: apical glucose transporter 2, chemosensing, enteroendocrine, intestine, H⁺-coupled peptide transporter 1


INTRODUCTION

There is immense interest in the gut chemosensory system driven by virtue of the fact that it can control whole body metabolic processes. In the human world, there are nutritional and pharmacological applications of gut chemosensing interventions to promote health during disease and regulate nutrient absorption and disposal to control metabolic disturbances and BW. In the animal world, improving feed consumption, gut development, and health is also important for growth efficiency. Increasing the amount of animal protein produced per unit of feed consumed is a major issue impacting the economic and environmental sustainability of animal-protein production. Finding the control switch to these metabolic processes is, therefore, of significant value.

Different animals have different digestive systems, which have adapted to the type of food they consume and their energy needs. Monogastric organisms, including humans and pigs, have 1 stomach. In contrast, a polygastric digestive system is found in ruminants,
such as cattle and sheep. Their differing digestive systems mean that cattle and sheep can use different types of feed. Humans and pigs must eat food that can be digested more easily but cattle and sheep can eat hay and pasture that is more difficult to digest. Therefore, intestinal nutrient chemosensing varies significantly in different species. Humans ingest a highly processed diet and the small intestine is the primary site for nutrient absorption. In other animals, for example in ruminants, the diet is far less processed and requires extensive digestion to liberate free nutrients and both the small and large intestine contribute to energy uptake. Thus, nutrient exposure along the length of the intestine differs significantly between species. However, in all species the intestine senses the constituents of the intestinal chyme to transduce nutritional information to the rest of the body. In particular, the gut plays a key role in metabolism. After the consumption of food, intestinal epithelial cells are primed to selectively absorb or eliminate substances from the diet. The intestinal epithelium is lined by a single layer of mucosal epithelial cells. Among these are absorptive epithelial cells and enteroendocrine cells (EEC). The primary purpose of absorptive epithelial cells, which account for approximately 90% of the epithelial mucosa, is to selectively take up nutrients from the intestinal lumen across the brush border membrane (BBM). This is achieved by modulating the expression of key nutrient transport proteins at the BBM to upregulate nutrient absorption (Kellett and Brot-Laroche, 2005; Kellett, 2011; Chaudhry et al., 2012; Gorboulev et al., 2012). Enteroendocrine cells are sparsely distributed throughout the intestinal mucosa and secrete gut peptides including glucoinsulinotropic polypeptide (GIP), glucagon-like peptide (GLP)-1 and -2, and peptide YY (PYY) across the basolateral membrane (BLM). These gut peptides are involved in the regulation of energy homeostasis (Murphy et al., 2006). For example, in response to nutrient intake, the insulinoitropic hormones, GIP and GLP-1, are secreted to buffer plasma glucose excursions after a meal by stimulating insulin secretion from the pancreas. Nutrient chemosensing continues past the small intestine into the large intestine. There are large differences in the activation of EEC in the small intestine, where digestion occurs to liberate nutrients, and the large intestine, where microbes generate fermentation products that depend on the dietary intake of the animal. In ruminants for example, microbial fermentation products are key sources of energy for the gut and peripheral tissues.

Nutrients are detected in the lumen of the intestine by chemosensors, strategically positioned in both absorptive epithelial cells and EEC where they transduce nutritional information to the body. Much has been learned from the study of lingual nutrient detection due to the identification of the G protein, α-gustducin, which couples to nutrient-sensitive G protein coupled receptors (GPCR) localized to the taste buds on the tongue and in the intestine (Sutherland et al., 2007). This key observation paved the way to the identification of further nutrient chemosensors common to both the tongue and intestinal epithelium. They include the carbohydrate-stimulated sweet taste receptor T1R2+T1R3, the umami taste receptor T1R1+T1R3, the AA-sensitive calcium sensing receptor (CasR), and lipid receptors GPR40, GPR119, and GPR120. There are a number of excellent reviews on chemosensing by GPCR in the intestine (Miguel-Aliaga, 2012; Reimann et al., 2012).

Key elements of the signal transduction cascade identified in taste buds are also evident in intestinal EEC (Sbarbati et al., 2010). For example, the facilitative glucose transporter (GLUT2) is found in both lingual taste cells and intestinal EEC, inviting the possibility that GLUT2 may regulate peptide secretion in the intestine (Merigo et al., 2011). This was functionally demonstrated in 2012 when GIP, GLP-1, and PYY secretion mediated by a diverse set of secretagogues, including agonists for GPR119 and T1R2+T1R3, was abolished by pharmacological inhibition of GLUT2 (Mace et al., 2012). In the intestine, chemosensors transduce information regarding the nutritional content of the lumen to regulate nutrient transporter expression and gut peptide secretion, ultimately to control energy intake, expenditure, and homeostasis.

Nutrient absorption is at the forefront of energy supply to the body. Gut development and function is vital for the health and performance of growing individuals. Gut function also declines with age, so improving gut function is critical for preventing malnutrition in an elderly population. Given that intestinal chemosensing can regulate nutrient uptake and gut peptide secretion to control energy supply and whole body metabolism, there is a strong relationship between nutrient transporter activity and the secretion of enteroendocrine-derived hormones. For example, inhibition of the Na⁺-dependent glucose/galactose co-transporter (SGLT1) or GLUT2 in rat small intestine diminishes GIP, GLP-1, and PYY secretion (Mace et al., 2012). Furthermore, GLUT2 knockout mice exhibit impaired GLP-1 secretion (Cani et al., 2007). Disturbances to the expression of nutrient transporters in absorptive epithelial cells and the secretion of gut hormones from EEC is evident during metabolic diseases, such as obesity and type 2 diabetes (Helliwell et al., 2000; Freeland et al., 2010). Understanding how nutrients influence transporter expression and gut peptide secretion is important, especially in view of the alarming rise in dietary-induced metabolic disease. Nutrient fluxes across the intestinal epithelium that extend beyond the intestine to central and peripheral sites are equally important when considering intestinal function and whole body homeostasis. However, a detailed description of
intestinal nutrient flux is beyond the scope of this review. The aim of this review is to summarize the current evidence describing the regulation of nutrient absorption and energy supply by gut chemosensing.

**INTESTINAL NUTRIENT ABSORPTION**

**Dietary Sugar Absorption**

Sugars are transported across the BBM of absorptive epithelial cells as glucose, galactose, and fructose and exit across the BLM by the facilitative transporter GLUT2 to enter the systemic circulation. The Na⁺-dependent glucose/galactose co-transporter and the facilitative fructose transporter (GLUT5) reside permanently at the BBM to accomplish this when the concentration of sugars in the lumen is low between meals. This situation changes rapidly after the ingestion of food when the concentrations of glucose in the lumen can approach several hundred millimolar (Pappenheimer, 1993). In rodent intestine in vivo, pharmacological knockout of SGLT1 using phlorizin shows that SGLT1 exhibits the Michaelis constant ($K_m$) of 20 mM and a maximum rate achieved by the system ($V_{max}$) of 40 μmol-min⁻¹-g dry weight⁻¹, despite glucose absorption increasing linearly in vivo to >100 mM (Kellett and Helliwell, 2000). Therefore, after a meal, the concentration of glucose in the lumen exceeds the capacity of SGLT1. Indeed, phlorizin only partially inhibits glucose absorption. Therefore, a growing body of evidence now describes the mobilization of GLUT2 to the BBM in response to increased luminal glucose concentrations to upregulate the absorption of glucose explaining the apparent diffusive-like kinetics of intestinal glucose absorption (Kellett et al., 2008; Gorboulev et al., 2012; Fig. 1). The expression of GLUT2 at the BBM in intestine has been described by a number of independent laboratories around the world challenging the current dogma of glucose absorption. The expression of GLUT2 at the BBM in intestine has been described in response to glucose in enterocytic cell models (Zheng and Sarr, 2012), rodents (Scow et al., 2011), in rodent models of diabetes (Helliwell et al., 2000), and in obese patients (Ait-Omar et al., 2011). At present, there are 2 favored views on the...
regulation of intestinal glucose absorption. The first is that glucose absorption involves only SGLT1 and that there is no expression of GLUT2 at the BBM (Shirazi-Beechey et al., 2011). The second is that GLUT2 is rapidly and transiently mobilized to the BBM from intracellular storage vesicles in response to increasing luminal glucose concentrations to increase the absorptive capacity of the intestine to match the dietary load (Kellett et al., 2008). The translocation of GLUT2 to the BBM occurs within minutes, is transient, and is retrieved on a similar time scale in rodents. The expression of GLUT2 at the BBM has been reported in insects, rodents, sheep, pigs, and humans, indicating that it may be an evolutionarily conserved mechanism, as reviewed by Kellett et al. (2008).

**Dietary AA and Peptide Absorption**

The final products of protein digestion include a mixture of peptides and AA. Transporters at the BBM coupled to either Na⁺ or H⁺ transport AA whereas di- and tri-peptides are transported across the BBM by the H⁺-coupled peptide transporter (PepT1). For example, in the intestine, uptake of glutamate and aspartate by the excitatory AA carrier 1 (EAAC-1) and peptides by PepT1 across the BBM are active processes coupled to the co-transport of Na⁺ and H⁺, respectively (Fan et al., 2004; Meredith, 2009). Inside the absorptive epithelial cell, peptidases contribute to the generation of AA by proteolysis of peptides. The PepT1 couples substrate transport to the electrochemical H⁺ gradient across the BBM maintained by the Na⁺-H⁺ exchanger (NHE3) which itself is energized by the sodium-potassium ATPase, that catalyses the hydrolysis of ATP to ADP, at the BLM. The EAAC-1 couples AA transport to the Na⁺ electrochemical gradient maintained by the Na⁺-K⁺-ATPase. Di-peptides and AA exit the BLM through a number of carriers. To date, the molecular identity of the BLM peptide transporter (BPT) is unknown.

Although very little is known regarding the regulation of EAAC-1 expression, it has been reported to exhibit mobilization to the membrane in C6 glioma cells to increase glutamate transport (Fournier et al., 2004). In contrast, it is well established that the expression and mobilization of PepT1 is tightly controlled by dietary, hormonal, and protein kinase regulation (Adibi, 2003). The peptide transporter 1, PepT1, was the first nutrient transporter in intestine reported to be regulated through BBM mobilization (Leibach and Ganapathy, 1996). This regulation has been demonstrated in vitro using Caco-2 cells and in rodent small intestine that endogenously express PepT1 (Walker et al., 1998; D’Souza et al., 2003; Mace et al., 2009). Expression of PepT1 at the BBM is negatively regulated by glucose. Increasing glucose concentrations in vitro diminished PepT1 expression and activity (D’Souza et al., 2003). In addition, increased concentrations of glucose in the lumen of the intestine downregulate PepT1 expression and dipeptide transport in rodent small intestine in vivo (Mace et al., 2009). This was shown to be controlled, at least in part, by SGLT1 because repolarization of the BBM with phlorizin upregulated the expression of PepT1. The expression of PepT1 and GLUT2 at the BBM appears to be reciprocally regulated (Mace et al., 2009). In contrast to the increased expression of BBM GLUT2 after streptozotocin (STZ)-induced diabetes in rat, PepT1 expression is completely abolished revealing another link between glucose and peptide transport (Gangopadhyay et al., 2002; Bikhazi et al., 2004).

**Dietary Fat Absorption**

In brief, dietary fats enter the small intestine as lipid droplets, which are emulsified by bile acids and pancreatic secretions. Triacylglycerides are hydrolyzed by luminal lipases to fatty acids and monoglycerides for absorption across the intestinal mucosa. Fatty acids and monoglycerides associate with bile acids to form micelles. Therefore, fatty acids are present within the lumen of the intestine as micelles of 4 to 8 nm spheres of mixed lipids and bile acids rather than free fatty acids. Distinct from carbohydrate, peptide, and AA absorption, micelles enter the lymphatic circulation as chylomicrons before entering the systemic circulation.

**Signaling Pathways Regulating Nutrient Transporter Expression**

The regulation of nutrient transporter expression has been investigated in a number of in vitro and in vivo experimental models and this has elucidated key signaling components involved in the mobility of nutrient transporters to and from the BBM (Fig. 2). These include depolarization of the BBM as a consequence of electrogenic nutrient absorption or intracellular metabolism, extracellular Ca²⁺ influx through voltage-gated Ca²⁺ channels (VGCC), and activation of protein kinase C (PKC). For GLUT2, expression at the BBM can be stimulated by glucose and artificial sweeteners (Mace et al., 2007a) and by pharmacological activation of VGCC (Morgan et al., 2007) and activation of PKC (Helliwell et al., 2003). The retrieval of GLUT2 from the BBM after the clearance of glucose can be experimentally achieved by repolarization of the BBM (pharmacologically performed using phlorizin to inhibit SGLT1 activity or replacing glucose with mannitol; Mace et al., 2007a), omission of extracellular Ca²⁺ or pharmacological inhibition of VGCC (Morgan et al., 2007), or inhibition of vesicular dynamics (Mace et al., 2007b). It is important to note that SGLT1...
activity is maintained in all of those studies in which GLUT2 expression at the BBM is observed to sustain a signaling cascade involving the influx of extracellular Ca\(^{2+}\), which can occur after glucose absorption by Na\(^+-\)dependent glucose/galactose co-transporter (SGLT1) or fructose absorption by glucose transporter (GLUT) 5. After the metabolism of fructose the ATP/ADP ratio increases, resulting in the inhibition of the ATP-regulated K\(^+\) channel Kir6.2 as well as sulphonylurea receptor 1 (SUR1) and depolarization of the brush border membrane (BBM). The subsequent opening of voltage-gated Ca\(^{2+}\) channels (VGCC) results in an increase in the intracellular Ca\(^{2+}\) concentration ([Ca\(^{2+}\)]\(I\)), which primes PKC. Agonism of taste receptors T1R1+T1R3 or T1R2+T1R3 activates phospholipase C \(\beta\) (PLC\(\beta\)) to catalyse the production of diacylglycerol (DAG) from phosphatidylinositol 4,5-bisphosphate (PtdIns 4,5-P\(_2\)) needed to fully activate PKC. Activation of PKC is associated with mobilization of GLUT2 and excitatory amino acid carrier 1 (EAAC-1) from cytosolic storage vesicles to the BBM and retrieval of peptide transporter (PepT) 1 from the BBM. Systemically derived hormones, glucagon-like peptide 2 (GLP) 2 stimulates GLUT2 and SGLT1 expression at the BBM through GLP-2 receptors whereas insulin promotes the retrieval of GLUT2 and expression of PepT1 through the insulin receptor (IR). Peptides may exit the cell via the basolateral membrane (BLM) peptide transporter (BPT). See online version for figure in color.
open VGCC has been shown to inhibit PepT1 activity (Sawada et al., 1999; Fig. 2).

Given the similarity in key signaling components governing multiple nutrient transporter expression at the BBM, it is clear that there is the very real potential for crosstalk between nutrient signaling pathways. In addition, paracrine networks also regulate nutrient transporter expression. Infusion of the EEC-derived hormone, GLP-2, was shown to stimulate both SGLT1 and GLUT2 expression at the BBM (Cheeseman, 1997; Au et al., 2002; Fig. 2). Increases in SGLT1 and GLUT2 expression at the BBM are also blocked pharmacologically by brefeldin A or wortmannin, indicating that phosphoinositide-3 kinase (PI3K) is involved in the intracellular signaling pathway in this response (Cheeseman, 1997; Helliwell et al., 2000). Another key metabolic hormone known to regulate the PI3K pathway and nutrient transporter expression is insulin. It is widely established that insulin can redistribute glucose transporters between cytosol and membrane through PKC and PI3K signaling pathways (Verhey et al., 1993; Yang et al., 1996; Fig. 2). Tobin et al. (2008) first demonstrated that GLUT2 expressed in the BBM was retrieved in response to insulin, addressing the earlier observation that GLUT2 is permanently expressed at the BBM of STZ-treated rodents (Corpe et al., 1996; Fig. 2). In addition, insulin stimulates mobilization of PepT1 to the BBM (Bikhazi et al., 2004). It is widely reported that application of insulin to PepT1-expressing cell lines increases PepT1 membrane density and activity (Bikhazi et al., 2004; Der-Boghossian et al., 2010). This line of evidence strongly implies that PI3K is involved in the signaling pathway modulating the expression of at least 2 key nutrient transporters at the BBM.

**INTESTINAL GUT PEPTIDE SECRETION**

The profile of intestinal hormones secreted in response to a mixed meal varies depending on the composition of the diet and the metabolic status of the individual. The reader is referred to Gribble (2012) for a comprehensive review on gut peptide secretion in response to diet. The effect of diet on the secretion of the insulinotropic peptides, GIP and GLP-1, and on GLP-2 will be discussed here briefly. After the arrival of diet, GIP concentrations in the plasma increase from approximately 10 pM to approximately 50 pM within 20 min (Cho and Kieffer, 2010). Dietary fats and carbohydrates are potent stimulators of GIP secretion (Elliott et al., 1993). Plasma GLP-1 increases from approximately 5 pM up to approximately 50 pM within 15 min after a meal (Holst, 2007). The dietary stimulation of GLP-1 has been extensively investigated and fats and carbohydrates appear to be potent stimulators (Lu et al., 2007; Nauck et al., 2011). Nutrients are also potent stimulators of GLP-2 secretion (Burrin et al., 2001).

Chronic administration of the probiotic fiber, oligofructose, to obese mice showed that the concentrations of GLP-2 and GLP-1 increased (Cani et al., 2009). In addition to the intestinotropic action of GLP-2 (Burrin and Stoll, 2002), GLP-2 release in response to glucose increases the expression of glucose transporters at the BBM (Cheeseman, 1997; Au et al., 2002).

**INTESTINAL NUTRIENT CHEMOSENSING**

**Dietary Sugar Sensing**

It is clear that SGLT1 activity and depolarization of the BBM upregulates GLUT2 and downregulates PepT1 expression at the BBM (Mace et al., 2009). This is a direct demonstration that SGLT1 acts as a chemosensor to control both glucose and peptide uptake in intestine (Mace et al., 2009). Using primary cultures of murine intestinal epithelial cells, SGLT1 has also been identified as a glucose sensor responsible for stimulating the secretion of GLP-1 from enteroendocrine L-cells (Reimann et al., 2008). Identical signaling pathways that regulate nutrient transporter expression in absorptive epithelial cells appear to stimulate GLP-1 secretion from EEC. Both GLP-2 and GLP-1 are co-localized, and their secretion by nutrient reveals a paracrine mechanism by which EEC activity can also regulate nutrient uptake in neighboring absorptive epithelial cells.

In 2007, a second family of chemosensor proteins was identified to control glucose absorption in the intestine (Mace et al., 2007a). The taste receptor family consisting of 3 monomers, T1R1, T1R2, and T1R3, was localized to both absorptive epithelial cells and EEC (Mace et al., 2007a). The monomers form heterodimers and the T1R2+T1R3 heterodimer functions as a sweet taste receptor, activated by both artificial sweeteners at low millimolar concentrations and by high concentrations of glucose (up to 500 mM; Li et al., 2002). In vitro, changes to sugar transporter expression mediated by sweet taste receptor activity have also been reported in Caco-2 cells (Le Gall et al., 2007), supporting the hypothesis that sweet taste receptors control sugar transporter expression. The localization of sweet taste receptors in the intestine goes some way to dispel the myth that glucose in the lumen fails to achieve this concentration. Such high concentrations contrast with the 24 mM value measured along the entire length of the intestine (Ferraris and Diamond, 1997). Their estimation grossly underestimated the concentration of glucose in the small intestine where glucose is concentrated at the BBM during carbohydrate digestion. Experimental observations indicate that the concentration of glucose in the small intestine is greater than 20 mM after a meal. Functional consequences in
vivo, including the mobilization of GLUT2 and PepT1, are observed when the concentration of luminal glucose exceeds 20 mM (Kellett and Helliwell, 2000; Mace et al., 2007b). Furthermore, data from Pappenheimer (1993) demonstrated that the rate of glucose production at the BBM from the digestion and hydrolysis of maltose generated a local concentration of glucose of approximately 300 mM. The expression of GLUT2 at the BBM induced by glucose (i.e., 30 to 100 mM) observed in 2000 (Kellett and Helliwell, 2000) subsequently correlated with activation of the sweet taste receptor dimer, T1R2+T1R3, by glucose at 30 to 100 mM (Li et al., 2002). The artificial sweeteners saccharin, acesulfame K, and sucralose have been used as agonists of the sweet taste receptor to reveal a functional T1R2+T1R3 receptor in intestine (Mace et al., 2007a). Activation of T1R2+T1R3 by artificial sweeteners upregulated glucose absorption by mobilizing GLUT2 to the BBM and their ability to stimulate BBM GLUT2 expression correlated to agonist potency (Mace et al., 2007a). Upregulation of BBM GLUT2 expression by artificial sweetener was inhibited by pretreatment with the phospholipase C (PLC) inhibitor, U-73122, consistent with the βγ subunit of the sweet taste receptor G protein coupling to PLC. Using an integrated in vivo whole organ approach, however, it is difficult to dissect from which epithelial cell type a sweet taste receptor signal may originate. Both absorptive and enterendocrine cells express the taste receptor subunits, T1R1, T1R2, and T1R3 (Mace et al., 2007a), so either site could be involved. This has been resolved to some extent by the elegant studies of Zheng et al. (2012), who have studied the regulation of glucose transport using intestinal cell models. In response to exposure to high concentrations of glucose, GLUT2 is mobilized from intracellular storage vesicles to the BBM membrane of 2 intestinal epithelial cell models, human Caco-2 and rat intestinal epithelial (RIE-1) cells (Zheng et al., 2012). Treatment of Caco-2 and RIE-1 cells with sweet taste receptor agonists stimulated glucose uptake by 20 to 30% at concentrations of glucose >25 mM. The increase in glucose transport mediated by sweet taste receptor activation was PKC dependent and mediated by an increase in GLUT2 (Zheng and Sarr, 2013). Therefore, artificial sweeteners appear to demonstrate biological activity at the level of the absorptive epithelial cell.

A subsequent report also confirmed that sweet taste receptor activation increases the absorptive capacity of the intestine for glucose by showing that subchronic treatment of mice with artificial sweetener increased the expression of SGLT1 (Margolskee et al., 2007). These authors have demonstrated that chronic activation of the sweet taste receptor modulates glucose transporter expression in weaning piglets and equine small intestine (Moran et al., 2010; Daly et al., 2012). In those studies, glucose absorption was assessed in vitro and data from in vivo models are still lacking. Furthermore, in vivo studies in humans and rodents do not recapitulate the in vitro findings (Brown and Rother, 2012).

Two contrasting views on the regulation of gut peptide secretion by sweet taste receptors have been proposed. Margolskee and coworkers have showed that the secretion of enterendocrine-derived hormones was, at least in part, regulated by the intestinal sweet taste receptor using mice lacking the cognate G protein, α-gustducin (Kokrashvili et al., 2009). Human studies have confirmed that the sweet taste receptor antagonist, lactisole, diminishes gut peptide secretion, adding credence to the ability of sweet taste receptors to modulate enterendocrine hormone secretion (Gerspach et al., 2011; Steinert et al., 2011). In contrast to these studies, the Gribble laboratory has not been able to detect sweet taste receptor expression using a fluorescently labeled murine L-cell model and does not detect the secretion of GLP-1 in the presence of the artificial sweetener, sucralose (Reimann et al., 2008). Clearly there are many different types of intestinal EEC and many different subsets within each class of EEC. At present, the in vivo rodent preclinical data on the function of taste receptors in intestine have been confirmed in some human studies (Brown and Rother, 2012).

In response to glucose, SGLT1 and sweet taste receptors have been shown to function as chemosensors to modulate nutrient transporter expression and stimulate gut peptide secretion. However, the nonmetabolizable SGLT1 substrate, α-methylglucopyranoside, generates a significantly decreased level of gut peptide secretion from EEC indicating that intracellular glucose metabolism is also required to generate maximum induction of gut peptide secretion (Parker et al., 2009). In support of this view, GLP-1 secretion in response to glucose in a GLUT2 knockout mouse is significantly less than its wild-type counterpart (Cani et al., 2007). The importance of electrogenic and facilitative glucose transporters to induce gut peptide secretion was recently clarified using rodent small intestine, in which both SGLT1 and GLUT2 were shown to contribute toward generation of gut peptide secretion in response to glucose (Mace et al., 2012). The same study also revealed that GLUT2 was essential to gut peptide secretion generated by a diverse range of secretagogues, including artificial sweeteners (T1R2+T1R3 agonists), agonists of the bile acid receptor, TGR5, and agonists of short chain fatty acid receptors, GPR41 and GPR43 (Mace et al., 2012).

**Dietary AA and Peptide Sensing**

It is well established that AA and peptide sensing in the intestine uses both GPCR and transporters. Electrogenic
nutrient transport across the BBM causes membrane depolarization and initiates downstream signaling cascades. Therefore, AA uptake coupled to Na\(^+\) co-transport and peptide uptake coupled to H\(^+\) co-transport means that these transporters depolarize the BBM during substrate transport and can initiate similar signaling cascades to SGLT1. Amino acids transported across the BBM by electrogenic transport, for example glutamine, which is transported by the Na\(^+\) dependent transporter, SLC38A2, have been reported to stimulate GLP-1 secretion from primary cultures of intestinal epithelial cells (Tolhurst et al., 2011). The release of GLP-2 by AA and peptides is likely to stimulate glucose transporter expression, thereby providing a further link between AA and glucose absorption. The H\(^+\) coupled peptide transporter, PepT1, has also been reported to stimulate GLP-1 secretion from the EEC model, STC-1, in response to peptides in vitro (Matsumura et al., 2005). Using an in vivo rat model, gut peptide secretion in response to the PepT1 substrate, glycylsarcosine, increased as the availability of H\(^+\) increased. This strongly indicates PepT1 to be an intestinal peptide chemosensor (Mace et al., 2012). However, it has been widely reported that peptides are weaker stimulants of EEC activity (Lu et al., 2007; Wu et al., 2010; Nauck et al., 2011) and that protein hydrolysis to AA is required for potent gut peptide secretion. Reimann et al. (2004) have described GLP-1 secretion from primary cultures of intestinal epithelial cells in response to a variety of AA, coupled to electrogenic transport and GPCR activation.

A functional T1R1+T1R3 taste receptor was revealed using an integrated in vivo rodent approach (Mace et al., 2009). The T1R1+T1R3 heterodimer detects and responds to glutamate and other nonessential AA (Li et al., 2002). Activation of intestinal T1R1+T1R3 by glutamate in rat intestine stimulated the expression of its own transporter, EAAC-1 (Mace et al., 2009). Activation of T1R1+T1R3 was also found to simultaneously increase glucose absorption via GLUT2 and inhibit di-peptide transport by downregulating PepT1 expression at the BBM (Mace et al., 2009). It is well established that GPCR undergo internalization as part of their signaling cascade and this was also evident for the taste receptors T1R1, T1R2, and T1R3, whose expression at the BBM was influenced by nutrient (Mace et al., 2007a, 2009). This line of evidence provides a direct demonstration that nutrients not only modulate their own uptake but also moderate the uptake of other nutrients through convergent signaling pathways. There is also 1 report to describe the stimulation of GLP-2 secretion after activation of T1R1+T1R3, providing yet a further link between AA sensing and glucose transporter activity or expression (Wang et al., 2011).

In addition to extracellular Ca\(^{2+}\), CasR senses aromatic AA in the intestine and activation induces gut peptide secretion (Mace et al., 2012). It is located throughout the gastrointestinal tract and AA act allosterically to improve the sensitivity of the receptor to extracellular Ca\(^{2+}\) (Geibel and Hebert, 2009). The GPCR, GPR93, is reported to act like a taste receptor to detect dietary protein hydrolysate (Choi et al., 2007a,b) and GPRC6A is sensitive to AA and divalent cations such as Ca\(^{2+}\) and Mg\(^{2+}\) (Haid et al., 2011, 2012) but their physiological relevance has yet to be uncovered.

**Dietary Fat Sensing**

Similar to sugar, AA, and peptide sensing, a number of GPCR and non-GPCR mechanisms have been identified to respond to lipids of different carbon chain length and saturation. The BBM expressed fatty acid translocase (FAT) Cluster of Differentiation 36 (CD36), responsible for long-chain fatty acid uptake, is also expressed on the tongue where it detects dietary lipids (Chen et al., 2001; Migrenne et al., 2011). Also involved in the production of the intracellular lipid messenger, oleoylethanolamide (OEA; Guijarro et al., 2010), an agonist of the lipid sensor, GPR119, is CD36 that is expressed in EEC and stimulates gut peptide secretion to modulate glucose homeostasis and decrease BW (Jones et al., 2009). More recently, GPR119 has been associated with dietary lipid handling and lipid absorption (Hansen et al., 2012). Other lipid sensors, including GPR40 and GPR120, detect medium- to long-chain fatty acids (Hirasawa et al., 2005, 2011) and stimulate gut peptide secretion, including GLP-1. In the large intestine, GPR41 and GPR43 detect short-chain fatty acids, generated by microbial fermentation of nondigestible dietary fiber. When activated, GPR41 and GPR43 localized to the large intestine stimulated the secretion of GLP-1 (Friedlander et al., 2011). Here, the release of GLP-1 identifies the presence of fatty acids in the lumen of the large intestine. In humans, we are only beginning to reveal the contribution that the large intestine makes toward energy homeostasis. In other animals including ruminants, microbial fermentation products provide a key energy source.

Because fatty acids are presented to fatty acid receptors as micelles, it would be of interest to determine whether micelles could stimulate the secretion of gut peptides from EEC. Stimulation of lipid chemosensors on EEC containing GLP-1 and GLP-2 shows that in addition to AA and proteins, dietary lipids may also regulate glucose absorption. The common theme throughout is that nutrient chemosensors regulate the absorption not only of their own ligands but also of other nutrients.
Apical, Basolateral, or Intracellular Chemosensing

The exact location of chemosensor activation is difficult to determine from in vitro and in vivo studies where nutrient agonists may be exposed to both BBM and BLM. However, there are some experimental observations that may, in part, address these questions. The use of agonists or inhibitors that do not traverse the intestinal epithelium have been used to show that signals arising from taste receptor activation, SGLT1 transport, or GLUT2 transport originate from the BBM (Mace et al., 2012). A subset of EEC only contact the basolateral surface, indicating that activation of their chemosensor population originates from agonists in the systemic circulation. Finally, some lipid agonists, such as OEA, are produced intracellularly. This lipid amide agonist of GPR119 reveals a potential cytosolic binding site. In addition, GPCR including GPR80 and GPR91, activated by citric acid cycle intermediates, may be activated through an intracellular binding site.

INTESTINAL ENERGY SUPPLY

A Model for Nutrient Transporter Regulation by Gut Chemosensors

In the intestine, SGLT1 and taste receptors stimulate the absorption of glucose through increasing the expression of GLUT2 at the BBM (Mace et al., 2007a). Sodium and glucose co-transport via SGLT1 and activation of T1R2+T1R3 after the increase in luminal glucose concentration after a meal stimulates glucose absorption by upregulating the expression of glucose transporters at the BBM (Mace et al., 2007a; Margolskee et al., 2007). The working model for the mobilization of nutrient transporters at the BBM is described in Fig. 2. Using depolarization of the BBM generated by SGLT1 to open VGCC, the increase in intracellular Ca^{2+} facilitates vesicular translocation of inactive but competent (phosphorylated) PKC from the cytosol to the BBM by increasing the affinity of PKC for phosphatidylserine in the membrane. Competent PKC is not fully activated (Newton, 1997). For PKC to become fully activated, it requires diacylglycerol (DAG). This is generated after the activation of T1R2+T1R3, which uses PLC to hydrolyze phosphatidylinositol 4,5-bisphosphate (PtdIns 4,5-P_{2}) to inositol triphosphate (IP_{3}) and DAG to complete the activation of PKC by removing the tethered pseudosubstrate region from the active site (Newton, 1997). Active PKC stimulates GLUT2 expression at the BBM. The same kinase mediates the insertion of EAAC-1 and the retrieval of PepT1 from the membrane, explaining the regulation of glutamate and peptide transport by glucose (Mace et al., 2009). Sweet taste receptor activation, also coupled to PLC and PKC, stimulates the insertion of EAAC-1 and GLUT2 (if SGLT1 is active) into the BBM and the retrieval of PepT1 in a parallel manner (Mace et al., 2009). In the absence of SGLT1 activity, GLUT2 is not expressed at the BBM because the PKC-priming Ca^{2+} signal is absent. Hence taste receptors can discriminate between glucose and glutamate to specifically promote the expression of GLUT2 and EAAC-1 at the BBM independently.

Signals arising from chemosensor activation may also originate from EEC. Paracrine regulation of glucose transporter expression in absorptive epithelial cells by release of GLP-2 from neighboring EEC has been reported (Cheeseman, 1997; Au et al., 2002). Receptors for GLP-2 have been reported in both intestinal cell lines (Sams et al., 2006) and enteric neurons (Shirazi-Beechey et al., 2011), indicating both paracrine and neuroendocrine mechanisms of action. However, there are no reports that chemosensing can occur through the enteric nervous system. It is well established that GLP-2 has a trophic action on the intestine (Yusta et al., 2012), so it is not surprising that glucose transporters are upregulated to provide additional energy supply. The ability of a number of dietary nutrients to stimulate the secretion of gut peptides from EEC and the relationship between GLP-2 and nutrient absorption makes it tempting to speculate that nutrient absorption can be modulated by agents that target the enteroendocrine gut peptide axis. This was recently revealed using an integrated rodent intestinal model in which the application of EEC hormone secretagogues co-stimulated gut peptide absorption and glucose absorption (Mace et al., 2012). Agents reported to stimulate GLP-1 and presumably the co-release of gut peptide absorption and glucose absorption (Mace et al., 2012). Agents reported to stimulate GLP-1 and presumably the co-release of GLP-2 include therapeutic agonists of multiple GPCR reported to be expressed in EEC, including GPR40, GPR119, and GPR120 (Engelstoft et al., 2008). The BBM of EEC is, therefore, a major site for the detection of nutrient arrival, coupling gut peptide secretion to the regulation of nutrient uptake and nutrient disposal into the systemic circulation.

The Control of Energy Supply by Gut Chemosensors

Ultimately, intestinal nutrient uptake is at the forefront of energy supply. The consequences for the stimulation of nutrient uptake for actively growing or elderly individuals is clear as are the consequences of overnutrition given the global rise in metabolic disease. At the molecular level, chemosensing in the intestine controls nutrient uptake through changes to nutrient transporter expression or activity. Consequently, changes to nutrient uptake by intestinal chemosensors regulate energy supply.

The intestine is a metabolically active and highly secretory organ. The epithelial mucosa completely renews every 3 to 4 d and, together with the secretion of large quantities of gut peptides, requires extensive protein synthesis. It also requires substantial energy expenditure during active nutrient uptake after meal ingestion. In
addition to meeting its own energy demands, the intestine must also deliver energy-rich molecules to the systemic circulation for use by the rest of the body. Glucose and glutamate are primary sources of energy for mucosal metabolism. The absorption of glutamate from the lumen is so efficient that it does not appear in the circulation (Gardner, 1975). Additionally, during the postprandial phase, glutamine is extracted from the systemic circulation and converted to glutamate. By using glutamate as an energy source, the intestine is able to deliver energy-rich carbon skeletons in the form of alanine or lactate to the systemic circulation (Fig. 3). The absorption of glucose and glutamate/aspartate are mutually beneficial in terms of energy supply to the intestinal mucosa and the body. Given that energy is required to power electrogenic nutrient uptake, there is a considerable energy-saving advantage to upregulating facilitative glucose transport through GLUT2 at the BBM (Walker et al., 2005). As Na+ dependent nutrient transporters operate to maximum capacity, the adenosine 5 monophosphate (AMP) to ATP ratio rises, causing activation of AMP-kinase to stimulate GLUT2 (Walker et al., 2005) and inhibit PepT1 (Pieri et al., 2010) expression at the BBM. In the fed situation, BBM-associated peptidases ensures significant AA production so there is relatively little requirement for peptide transport via PepT1. Consequently, energy-consuming peptide transport through PepT1 is downregulated.

After nutrient ingestion, the secretion of GIP and GLP-1 by nutrients stimulates the release of insulin from the pancreas and provides a negative feedback loop to internalize GLUT2 and translocate PepT1 to the BBM via the PI3K pathway (Thamotharan et al., 1999; Tobin et al., 2008). Therefore, gut chemosensors are also involved in the attenuation of glucose absorption by insulin to limit postprandial glucose excursions (Jang et al., 2007). As absorption switches off after a meal, the intestine adopts a recovery mode. The recovery of peptides from the continual desquamation of epithelial cells is performed by PepT1, which is more energy efficient than Na+ dependent AA uptake. The absorption of di- and tri-peptides using H+ is energetically more efficient than the equivalent AA coupled to Na+. Therefore, PepT1 could be considered a scavenger protein whose expression is upregulated during periods when food is absent. Increased PepT1 function is clearly observed after use of pharmacological substrates, which show significantly greater oral bioavailability after a brief fast. Furthermore, because plasma glucose concentrations exceed the luminal glucose concentration during fasting, GLUT2 is retrieved from the BBM to prevent backflux of glucose from the blood to the lumen and SGLT1 is permanently positioned at the BBM to recover glucose from the lumen.

**SUMMARY AND CONCLUSIONS**

The expression of nutrient transporters at the BBM is dynamic and controlled by the availability of transporter substrate. The available in vitro and in vivo rodent data

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**Figure 3.** Basic flow of metabolites involved in intestinal mucosal metabolism. The main pathway of energy metabolism in the intestinal mucosa are shown, through which pyruvate obtained from the glycolysis of the glucose transport 2 (GLUT2) substrates glucose or fructose is derived to participate in transamination reactions facilitated by excitatory AA carrier 1 (EAAC-1) transporter substrates, glutamate and aspartate. Coordination of the expression of GLUT2 and EAAC-1 allows the replenishment of tricarboxylic acid (TCA) metabolic intermediates, 2-oxoglutarate and oxaloacetate, when the intestinal mucosa is under intense energy demands. BBM = brush border membrane; G-3-P = glycerol-3-phosphate. See online version for figure in color.
captured from many laboratories shows that dietary nutrients alter the absorptive capacity of the intestine after activation of gut chemosensors, including electrogenic and facilitative nutrient transporters and GPCR, through nutrient transporter expression and activity. In the intestine, nutrient uptake at the BBM controls energy supply for mucosal metabolism and for the rest of the body. Glucose and glutamate are primary sources of energy for the intestine and their uptake is coordinated by taste receptors. Stimulating uptake is likely to prove beneficial for augmenting gut development and function. In the animal world, increasing feed consumption should improve growth. Furthermore, stimulating nutrient absorption at an early age may reduce incidence of intestinal disorders postweaning and, as the animal matures, improve feed efficiency. These are major issues concerning the economic and environmental sustainability of animal-protein production.

It is clear that modulating nutrient supply and the enteroneuroendocrine axis can significantly alter whole body metabolism in mammals. This is clearly seen by the remission of type 2 diabetes and BW loss after Roux-en-Y surgery (Anderwald et al., 2012). Certainly, the therapeutic manipulation of the neuroendocrine control of metabolism is an active area for research and there are clinical candidates under development that modulate metabolic homeostasis and potentially BW through modulation of gut chemosensors, nutrient uptake, and gut peptide secretion (Lind, 2012; Mudaliar and Henry, 2012). What is unclear is whether modulation of taste receptors can be used to manipulate nutrient absorption and BW of other mammals and humans, as has been observed in rodents. Currently, the jury is still out on whether activation of taste receptors can regulate nutrient transporter expression or gut peptide secretion in humans. The clinical data reported so far appear inconsistent (Brown and Rother, 2012).

LITERATURE CITED


