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Sweet taste receptor expression in ruminant intestine and its activation by artificial sweeteners to regulate glucose absorption

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ABSTRACT

Absorption of glucose from the lumen of the intestine into enterocytes is accomplished by sodium-glucose co-transporter 1 (SGLT1). In the majority of mammalian species, expression (this includes activity) of SGLT1 is upregulated in response to increased dietary monosaccharides. This regulatory pathway is initiated by sensing of luminal sugar by the gut-expressed sweet taste receptor. The objectives of our studies were to determine (1) if the ruminant intestine expresses the sweet taste receptor, which consists of two subunits [taste 1 receptor 2 (T1R2) and 3 (T1R3)], and other key signaling molecules required for SGLT1 upregulation in nonruminant intestines, and (2) whether T1R2-T1R3 sensing of artificial sweeteners induces release of glucagon-like peptide-2 (GLP-2) and enhances SGLT1 expression. We found that the small intestine of sheep and cattle express T1R2, T1R3, G-protein gustducin, and GLP-2 in enteroendocrine L-cells. Maintaining 110-d-old ruminating calves for 60 d on a diet containing a starter concentrate and the artificial sweetener Sucram (consisting of saccharin and neohesperidin dihydrochalcone; Pancosma SA, Geneva, Switzerland) enhances (1) Na^+-dependent D-glucose uptake by over 3-fold, (2) villus height and crypt depth by 1.4- and 1.2-fold, and (3) maltase- and alkaline phosphatase-specific activity by 1.5-fold compared to calves maintained on the same diet without Sucram. No statistically significant differences were observed for rates of intestinal glucose uptake, villus height, crypt depth, or enzyme activities between 50-d-old milk-fed calves and calves maintained on the same diet containing Sucram. When adult cows were kept on a diet containing 80:20 ryegrass hay-to-concentrate supplemented with Sucram, more than a 7-fold increase in SGLT1 protein abundance was noted. Collectively, the data indicate that inclusion of this artificial sweetener enhances SGLT1 expression and mucosal growth in ruminant animals. Exposure of ruminant sheep intestinal segments to saccharin or neohesperidin dihydrochalcone evokes secretion of GLP-2, the gut hormone known to enhance intestinal glucose absorption and mucosal growth. Artificial sweeteners, such as Sucram, at small concentrations are potent activators of T1R2-T1R3 (600-fold > glucose). This, combined with oral bioavailability of T1R2-T1R3 and the understanding that artificial sweetener-induced receptor activation evokes GLP-2 release (thus leading to increased SGLT1 expression and mucosal growth), make this receptor a suitable target for dietary manipulation.

Key words: ruminant, intestine, glucose absorption, taste 1 receptor 2, taste 1 receptor 3

INTRODUCTION

Ruminants have evolved a digestive system that enables microbial fermentation of plant structural carbohydrates in the rumen to produce nutrients such as proteins and short-chain FA to fulfil their energy demands. Their digestive system is not designed for efficient utilization of nonstructural carbohydrates, such as starch. However, current feeding practices for ruminants promote the consumption of diets containing high levels of starch to provide the bulk of dietary energy (Walker and Harmon, 1995). Most dietary starch is fermented in the rumen, but, depending on the grain type and the degree of processing, up to 60% of starch intake may be available in the small intestine for enzymatic hydrolysis by the host (Theurer, 1986; Nocek and Tamminga, 1991; Firkins et al., 2001).

Generally, starch is hydrolyzed in the small intestine of mammalian species by pancreatic α-amylase and brush border membrane disaccharidases to glucose (Shirazi-Beechey, 1995). In ruminants, small intestinal digestion of starch to glucose is energetically more efficient than microbial fermentation to short-chain FA

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in the rumen (Owens et al., 1986; Harmon, 2009). It has been proposed that at least 75% of starch escaping ruminal fermentation must be hydrolyzed and absorbed in the small intestine in order to provide a positive energy contribution to the host, thus avoiding energetically inefficient digestion in the large intestine (Harmon, 2009).

It has been suggested that major factors limiting small intestinal starch utilization by ruminants may include limited activity of pancreatic α-amylase or intestinal glucose absorption. Thus, a better understanding of potential limitations to starch digestion and glucose absorption in the intestines of ruminants, and methods to overcome such limitations, is essential for rational formulation of feed in order to maintain animal health and enhance growth, performance, and productivity.

Absorption of glucose in the ruminant small intestine, similar to other mammalian species, is accomplished by the apically located sodium-glucose co-transporter 1 (**SGLT1**; Shirazi-Beechey, 1995). With rumen development, expression and activity of this protein declines dramatically and becomes negligible in sheep and cow intestines (Wolffram et al., 1986; Shirazi-Beechey et al., 1989, 1991; Lescale-Matys et al., 1993; Wood et al., 2000; Dyer et al., 2003). However, intestinal infusion of ruminant sheep, through duodenal cannulae, with D-glucose and D-galactose (metabolizable substrates of SGLT1), 3-*O*-methyl α, D-glucopyranoside and methyl-α-D-glucosopyranoside (nonmetabolizable substrates of SGLT1), D-fructose (a monosaccharide not transported by SGLT1), and di(glucose-6-yl)poly(ethylene glycol)$_{600}$ (a membrane impermeable D-glucose analogue), enhanced expression of SGLT1 by over 50 fold (Shirazi-Beechey et al., 1991; Lescale-Matys et al., 1993; Wood et al., 2000; Dyer et al., 2003). This indicated that the ruminant intestine maintains the ability to upregulate its capacity to absorb increased luminal glucose, that metabolism of glucose by enterocytes is not responsible for enhanced SGLT1 expression, and that a glucose sensor expressed on the luminal membrane of intestinal epithelial cells initiates pathways controlling glucose-induced SGLT1 expression (Dyer et al., 2003).

We subsequently showed, for the first time, that the lingual epithelium sweet taste receptor, consisting of 2 subunits, taste 1 receptor 2 (**T1R2**) and 3 (**T1R3**), is expressed in mouse intestinal enteroendocrine cells and proposed that it acts as the intestinal glucose sensor (Dyer et al., 2005). The sensor (receptor) functions in association with the G-protein gustducin_(McLaughlin et al., 1992).

Convincing evidence for the involvement of gut-expressed T1R2-T1R3 heteromer and gustducin in intestinal sweet transduction and SGLT1 upregulation was provided by our studies using mice in which the genes for either the α-subunit of gustducin (*GNAT3*) or the T1R3 sweet receptor subunit (*Tas1R3*) were deleted. The elimination of sweet transduction in mice in vivo prevented the dietary monosaccharide-induced upregulation of SGLT1 expression observed in wild-type mice (Margolskee et al., 2007). Expression of SGLT1 in both types of knockout mice was identical to that of wild-type animals on a low-carbohydrate diet, implying that there is a constitutive pathway independent of luminal glucose sensing by T1R3 and α-gustducin, which maintains basal expression of SGLT1 and an inducible pathway dependent on T1R2-T1R3 (Margolskee et al., 2007). Furthermore, when included in the diet of the wild-type mice (Margolskee et al., 2007) or infused directly into the intestine (Stearns et al., 2010), artificial sweeteners also enhanced the expression of SGLT1. In contrast, the knockout mice showed no increase in SGLT1 expression in response to supplementation with sweeteners, indicating that T1R2-T1R3 and α-gustducin are required for sensing the presence of sugars and sweeteners in the intestinal lumen (Margolskee et al., 2007).

It is well established that enteroendocrine L-cells, in response to luminal monosaccharides or artificial sweeteners, secrete glucagon-like peptides-1 and -2 (**GLP-1** and **GLP-2**; Rehfeld, 2004; Jang et al., 2007; Sato et al., 2013). Insulin secretion is enhanced by GLP-1, whereas GLP-2 increases small intestinal mucosal growth (Drucker et al., 1996). This stimulation of intestinal growth is evidenced by increased crypt depth and villus height (Hartmann et al., 2000; Ramsanahie et al., 2004) and mediated by greater crypt cell proliferation (Drucker et al., 1996), reduced cell apoptosis, and a subsequent increase in the number of villus-differentiated cells (Tsai et al., 1997). It has also been shown that GLP-2 increases SGLT1 expression and intestinal glucose absorption in the rat and pig small intestine (Cheeseman, 1997; Ramsanahie et al., 2003; Cottrell et al., 2006). Our experimental evidence suggests that sensing of monosaccharides and artificial sweeteners by T1R2-T1R3, residing in murine enteroendocrine L-cells, evokes the release of GLP-2, and that this gut hormone enhances SGLT1 expression via a neuro-paracrine pathway in absorptive enterocytes (Shirazi-Beechey et al., 2011).

The major objectives of this study were to assess if (1) cow and sheep intestines express T1R2, T1R3, and other signaling elements required for SGLT1 upregulation, (2) artificial sweeteners included in the diet enhance SGLT1 expression, and (3) glucose and artificial sweeteners evoke GLP-2 release from ruminant intestinal tissue. The AA sequences of cow T1R2 and T1R3 were deduced from the bovine genome. However, due to lack of information on ovine T1R2-T1R3 AA