Expression of sweet taste receptors of the T1R family in the intestinal tract and enteroendocrine cells


Epithelial Function and Development Group, Department of Veterinary Preclinical Sciences, University of Liverpool, Liverpool L69 7QJ, U.K.

Abstract

The composition of the intestinal luminal content varies considerably with diet. It is important therefore that the intestinal epithelium senses and responds to these significant changes and regulates its functions accordingly. Although it is becoming evident that the gut epithelium senses and responds to luminal nutrients, little is known about the nature of the nutrient sensing molecule and the downstream cellular events. A prototype example is the modulation in the capacity of the gut to absorb monosaccharides via the intestinal luminal membrane Na\(^+\)/glucose cotransporter, SGLT1. The experimental evidence suggests that luminal sugar is sensed by a glucose sensor residing on the luminal membrane of the gut epithelium and linked to a G-protein-coupled receptor, cAMP/PKA (protein kinase A) pathway, resulting ultimately in modulation of intestinal monosaccharide absorption. Here we report the expression, at mRNA and protein levels, of members of the T1R sweet taste receptors, and the \(\alpha\)-subunit of the G-protein gustducin, in the small intestine and the enteroendocrine cell line, STC-1. In the small intestine, there is a highly coordinated expression of sweet taste receptors and gustducin, a G-protein implicated in intracellular taste signal transduction, throughout the gut. The potential involvement of these receptors in sugar sensing in the intestine will facilitate our understanding of intestinal nutrient sensing, with implications for better nutrition and health maintenance.

Introduction

The cells lining the inner surface of the intestinal epithelium are exposed to a luminal environment that varies dramatically with the diet. It is generally believed that the epithelium can sense chemical components of the luminal contents, and this chemosensory information appears to be important for the regulation of various aspects of intestinal function such as secretion, motility and nutrient absorption [1,2]. Although it is becoming established that the adaptation in the absorptive capacity is achieved through the modulation of expression/activity of specialized nutrient transporters resident in the enterocyte’s plasma membrane [3], a major challenge that remains is to gain an insight into the identity of the receptors that sense the changes in the luminal contents, i.e. the intestinal nutrient sensors.

This article addresses, specifically, the initial molecular recognition events involved in sugar sensing by the intestinal epithelium. We report, for the first time, that members of the T1R sweet taste receptors, and the \(\alpha\)-subunit of the G-protein gustducin, in the small intestine and the enteroendocrine cell line, STC-1. In vivo and in vitro studies using an intestinal cell line, STC-1, indicated that the increase in SGLT1 expression in response to media glucose was accompanied by an increase in intracellular cAMP. Additionally, STC-1 cells transfected with SGLT1 promoter constructs containing a glucose response
element have demonstrated that (i) the enhancement in SGLT1 promoter activity, induced by media glucose, was also accompanied by a significant increase in the level of intracellular cAMP; (ii) a membrane-permeant protein kinase A (PKA) agonist, 8-bromo-cAMP, mimicked the glucose-induced activation of the SGLT1 promoter and (iii) the glucose-induced SGLT1 promoter activity was inhibited, in a dose-dependent manner, by the PKA antagonist H-89 (N-[2-([p-bromocinnamyl]amino)ethyl]-5-isouquinolinesulphonamide). Furthermore, addition of pertussis toxin, a G-protein (Gαi)-specific inhibitor, to STC-1 cells grown in low-glucose conditions enhanced SGLT1 abundance to the same extent as observed with high glucose or 8-bromo-cAMP. Probing protein components of the intestinal brush-border membrane with a broad specificity Gα subunit antibody confirmed the presence of G-proteins associated with the luminal membrane of the intestinal epithelium [9,10]. Collectively, these data suggest that intestinal glucose sensor operates via a cAMP/PKA signalling pathway, likely linked to a GPCR, resulting in modulation of the expression of SGLT1 and the capacity of the gut to absorb monosaccharides.

Sugar sensing in the tongue

The only knowledge of sugar sensing in the mammalian gastrointestinal tract is from taste transduction mechanisms. Taste cells in the taste buds of the tongue and palate epithelia have mechanisms that can distinguish chemical compounds, such as sugars, having potential nutritional value. It has been shown recently that transduction of sweet-tasting compounds involves activation of a family of GPCRs on the apical surface of taste receptor cells. To date three members of the T1R family of sweet taste receptors have been identified and characterized. Analysis of the distribution of T1R receptors on the tongue using in situ hybridization indicates that the three T1R genes appear to be expressed in three combinations: cells expressing T1R1 and T1R3, cells expressing T1R2 and T1R3 and cells expressing only T1R3 [11,12]. Behavioural assays and knock-out mouse models have indicated that the T1R2/T1R3 receptor combination functions as a broad specificity sweet sensor for sugars and artificial sweeteners, whilst T1R1 and T1R3 work together to recognize the ‘umami’ taste of glutamate, L-amino acids and sweet proteins [13,14]. These receptor combinations are also found in 25–30% of cells in combination with gustducin, a taste-specific transducin-like heterotrimeric G-protein. The gustducin α-subunit (Gαgust) was also recently shown to be expressed in mouse proximal intestine, and the murine intestinal enteroendocrine cell line, STC-1, along with members of the bitter taste receptor, T2R, family [15].

Figure 1 | Expression of SGLT1, taste sensors T1R2 and T1R3 and α-gustducin along the length of the mouse small intestine

(A) Real-time PCR with dual-labelled Taqman probes was used to assess their expression at mRNA level along the longitudinal axis of the intestine of C57BL/6 mice. (B) Western blot analysis of luminal membranes (30 µg protein) probed with antibodies to SGLT1, T1R2, T1R3 and α-gustducin. (C) Densitometric analysis of the Western blot data shown in (B).
Expression of T1R sweet taste receptors and α-gustducin in the murine small intestinal mucosa

In view of our data suggesting the involvement of a potential G-protein-linked signalling pathway in glucose-induced SGLT1 expression we investigated the expression of T1R receptors in the mouse small intestine. To determine if T1R members are expressed in the small intestine, along with α-gustducin, reverse transcription–PCR was performed on mRNA isolated from mucosal scrapings of the proximal small intestine of CD-1 mice using specific primers based on the mouse, rat and human sequences. PCR products of the predicted size were cloned and sequenced. Sequence analysis confirmed that all were 100% homologous to the reported mouse sequences cloned from taste buds on the tongue, indicating that taste receptors are expressed in the proximal small intestine.

Having demonstrated the presence of the (sweet) taste receptors in proximal intestine we investigated the level of their expression along the longitudinal and the radial axes of the small intestine. To assess, at the mRNA level, the expression of the T1R family members and Ga\textsubscript{gust} throughout the small intestine, and along the crypt–villus axis, the technique of real-time PCR was employed with primers and Taqman probes designed specifically to detect mouse T1R1, T1R2, T1R3 and Ga\textsubscript{gust}. Protein expression was assessed by Western blot analysis using commercial antibodies to T1R2 and Ga\textsubscript{gust} (Santa Cruz) and T1R3 (Abcam).

Results indicated that all members of the T1R family and α-gustducin are expressed along the length of the small intestine (Figure 1). Expression levels were low and equivalent to those detected in the tongue using the same technique (data not shown). This suggests that either very low levels of the receptors are expressed in the cells, or the receptors may be expressed only in a subpopulation of

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**Figure 2** | Expression of T1R1, T1R2, T1R3 and α-gustducin along the crypt–villus axis of mouse small intestine

Cells were isolated from the upper villus (UV), mid to lower villus (LV) and crypt (C) regions of mouse small intestine. (A) Western blot analysis of SGLT1, villin and β-actin expression in purified luminal membranes prepared from isolated cell populations. (B) Densitometric analysis of Western blot data from (A). (C) Real-time PCR with dual-labelled Taqman probes was used to assess the expression of taste sensors, T1R1, T1R2, T1R3, and α-gustducin in RNA isolated from crypt–villus cell populations.
cells rather than in all intestinal cells along the crypt–villus axis.

Western blot analysis indicated that the antibodies to T1R2 and T1R3 each identified a single protein in the purified intestinal epithelial cell luminal membranes that was present in the proximal, mid and distal small intestinal fractions, with slightly higher levels in the mid small intestine. The $\alpha$gust mRNA expression determined by real-time PCR (Figure 2C).

Having demonstrated the presence of T1R sweet taste receptors in the mouse small intestine it is tempting to propose that they may function as the luminal sugar sensors of the gut. Work is currently underway in our laboratory to investigate this area.

The identification of the sugar receptor(s) in the intestine will provide opportunities for: (i) a better understanding of molecular and cellular events involved in nutrient sensing in the small intestine and (ii) the development of therapeutic compounds and nutritional aids to modulate the function of the receptor in the gut. This will assist in combating diseases such as obesity, diarrhoea and diabetes, and in providing nutritional supplements for infants, the elderly and athletes.

References


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