Title: Evolution of complex, discreet nutrient sensing pathways.

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Abstract

Purpose of review: The current review summarizes and discusses current research on differences elicited between sugars and non-nutritive sweeteners via sugar sensing pathways.

Recent findings: Sugars, sweeteners and sweetening agents are all perceived as sweet tasting due to their ability to bind to the type 1 taste receptor (T1R) family of sweet taste receptors in the oral cavity. The ability of a wide variety of chemical ligands to activate the sweet taste receptor highlights the importance of sweet-tasting foods during human evolution. The sweet taste receptor has been located in the gut, and differences between oral and gut sugar-sensing pathways are discussed.

Summary: Differences in the sweetness transduction cascade, and neuronal signalling may result in incretin hormone release upon activation of the sweet taste receptor from some sweeteners, but not others.

Keywords: sugar, sweeteners, nutrient sensing, T1R2/T1R3

Introduction

The use of sweeteners and sweetness enhancers is increasing, partly in response to WHO and governmental initiatives set-out to reduce sugar consumption. Sweeteners are primarily described by whether they are broken down to provide energy or not. The term nutritive sweeteners (NS) encompass all sweetening agents that provide energy (as kcal/g), whereas non-nutritive sweeteners (NNS) are undigested and therefore provide negligible energy
content. The NS category can be further broken down into sugars (glucose, fructose, sucrose, dextrose) including honey and maple syrup, or polyols – sugar alcohols such as sorbitol, mannitol and xylitol – also known as bulk sweeteners. All NS are metabolised by the body, however, polyols provide lower energy compared to sugars (1-2 kcal/g and 4 kcal/g, respectively) (1). The majority of NNS are artificially synthesized (hence, artificial sweetener), however, some are derived from natural sources; steviol glycosides is a natural extract from *Stevia rebaudiana* (2). All of these compounds stimulate oral sweet taste perception through activation of the sweet taste receptor; a heterodimer of type 1 taste receptor 2 (T1R2)/type 1 taste receptor 3 (T1R3) (3). The T1R2/T1R3 sweet taste receptor is expressed not only in the oral cavity but also in several gut cells, indicating a role in gut nutrient sensing (3). In this review, recent investigations into the physiological response to activation of the T1R2/T1R3 receptor in the oral cavity and gut in response to both sugars and sweeteners are summarised and discussed.

**The evolved importance of sugar and sweetness**

Human metabolism uses glucose as the primary energy currency; glucose is derived from dietary intake upon the digestion and subsequent absorption of complex and simple carbohydrates. Plant-derived foods are the richest source of mono- and disaccharides, and progressive hydrolysis of complex carbohydrates such as starch, ultimately provides large amounts of glucose. Fruits, nuts and berries contain high amounts of simple mono- and disaccharides such as glucose, fructose, maltose and sucrose. Digestion of starchy roots and tubers, i.e. potatoes, yams, cassava, along with cereals and grains such as wheat, barley, rice and millet yield large quantities of glucose. Natural sugars and sweeteners exist in the form of honey, maple syrup, carob, and sucrose (table sugar) has been extracted from sugar cane (*Saccharum officinarum*) and sugar beets (*Beta vulgaris*) since the 18th Century (2). These nutritive sweeteners (NS) have been used in food preparation for many years, however, more recently, the use of polyols and non-nutritive sweeteners (NNS) has increased (1).
Humans have consumed sugars throughout evolution, and it is believed that carbohydrates were an increasingly important component of hominin diets, since the advent of cooking (4). It has been suggested that cooked carbohydrates and sugars were integral to our evolution and survival to support increased energy demands of human’s relatively increased cranial capacity (4, 5). Given the high energy demands of the human brain (20% of total energy intake), a mechanism to quickly identify sweet and nutrient-dense foods would have provided a survival advantage. This has led to the evolution of complex and discreet nutrient sensing pathways both in the oral cavity and the gut.

Despite the necessity for sugar and carbohydrate intake during evolution, evidence suggests chronic high sugar consumption in the modern dietary and lifestyle landscape, may contribute towards detrimental energy homeostasis (6, 7). Over the past 20 years the UK has experienced a dramatic rise in the incidence of type-2 diabetes mellitus (T2DM) and a near parallel increase in obesity rates – itself a principal risk factor for T2DM development. Despite the multifactorial risk factors for both T2DM and obesity including hereditary genetic and lifestyle components, diet and lifestyle management is pivotal to reducing the incidence of type 2 diabetes mellitus (8).

Changes in sugar intake guidelines

The Scientific Advisory Committee on Nutrition’s review on the role of dietary carbohydrates in health (7), and the 2015 changes to the WHO dietary guidelines (9), both proposed a reduction in sugar consumption. There has been increased focus of healthcare professionals and organizations to encourage the public to reduce sugar consumption. Current recommendations are that sugar should contribute up to 10% of daily caloric intake; 30g adults and 19g children (9). Potential health benefits of a reduction in sugar intake, include the correlated reduction in total energy intake, which may help prevent obesity, and reduce risk of dental caries (7, 10). This has led to increased use of NNS, which provide sweetness
in the absence of calories, such as polyols, aspartame, sucralose and Ace-K in the food and beverage industry, largely due to their associated health benefits.

**Oral sweet taste perception, and sugar sensing**

Humans perceive a diverse range of chemical compounds as sweet tasting, including sugars (glucose, fructose, sucrose, maltose), polyols (sorbitol, erythritol), artificial sweeteners (saccharin, aspartame, cyclamate), sweet amino acids (d-tryptophan, d-phenylalanine, d-serine), and sweet proteins (monellin, brazzein, thaumatin). NNS approved for use in the EU include aspartame, sucralose, saccharin, acesulfame-k (Ace-K), cyclamate, neohesperidin and steviol glycosides(2). All of these ligands stimulate oral sweet taste perception through activation of the sweet taste receptor.

The human sweet taste receptor is a transmembrane heterodimer of type-1 taste receptor 2 and type-1 taste receptor 3 (T1R2 and T1R3, respectively), known as T1R2/T1R3. Expression of **TAS1R2** and **TAS1R3** genes encoding T1R2 and T1R3 respectively, have been reported in the circumvallate papillae and foliate papillae taste receptor cells in the mouth (11). Both T1R2 and T1R3 belong to Class C of G protein-coupled receptors (GPCRs), and feature the distinct N-terminal Venus flytrap domain (VFT), connected by a short cysteine-rich linker region to the seven transmembrane domain, typical of Class C GPCRs (12).

The T1R2 and T1R3 VFT domains carry different binding sites, with different affinities for sugars and sweeteners. Glucose, sucrose and sucralose can bind to the VFT domain of both T1R2 and T1R3. T1R2 shows broader specificity for natural sugars and NNS, including aspartame, whereas cyclamate can activate T13R through binding to the seven transmembrane domain, rather than the VFT domain. Furthermore, sweet tasting proteins (e.g. thaumatin and monellin), achieve activation through simultaneous binding of regions on T1R2 and T1R3 (13).
Ligand-binding of T1R2/T1R3 on the apical surface of taste cells activates the release of G-protein α-gustducin. However, there is evidence that different pathways can then be triggered by natural sugars versus sweeteners. Stimulation of the GCPRs by natural sugars leads to adenylate cyclase activation that depolarizes the taste cell, leading to increased cAMP concentration, this can then either directly or indirectly close basolateral potassium channels, ultimately resulting in neurotransmitter release (13).

Alternatively, binding from sweeteners leads to phospholipase C β2 activation – rather than adenylate cyclase – that produces intracellular release of Ca2+ which in-turn activates the transient receptor potential cation channel M5 (TRPM5), resulting in ATP release. Ultimately, binding of sweet taste receptors results in sweet taste perception through neurotransmitter release either from direct changes in depolarisation or by second messenger-mediated changes in intracellular Ca2+ concentration. Currently, it is believed that both pathways are triggered from the same taste cell (13). The pathways result in stimulation of sensory neurons that send signals to brain centers involved in taste perception and the reward centres. It has been suggested that there are separate, yet integrated, neuronal circuits involved in taste perception and nutritional value yet it remains largely unknown if oral and gut T1R2/T1R3 use the same neural circuitries (14). Currently, the evidence for nutritional evaluation of sweetness appears to be a result of gastric glucose availability (14).

Oral perception of sweetness is described by the intensity and duration of sweetness experienced, this has been associated to differences in binding patterns to the multiple binding sites of T1R2/T1R3 (15). Often, polyols and NNS are used in varying combinations in order to achieve a combined sweetness profile similar to sucrose, whilst providing a healthy, low-energy burden. Many NNS are considered high-intensity sweeteners as they are many times sweeter than sucrose, i.e. saccharin 200-700x, sucralose is 600x sweeter, and aspartame and Ace-K are both ~200x. Therefore they are often used at much lower concentrations than sucrose to provide equivalent sweetness to food and beverages (1).
Sugars, NS and NNS are all perceived as sweet tasting due to their ability to bind to the T1R family of sweet taste receptors in the oral cavity. This triggers the sweetness transduction cascade, in the case of glucose and other glucose-containing sugars, this elicits a cephalic response, however, evidence suggests this response is absent after NNS consumption (16). The cephalic response leads to GLP-1 release which prompts sugar transporters to localise, stimulates release of insulin, thus promoting glucose clearance from the blood.

**Gut sugar-sensing and signalling**

Nearly a decade has passed since the discovery of TAS1R2 and TAS1R3 expression in intestinal enterocytes and enteroendocrine cells. There is evidence for the co-localisation of T1R2/T1R3 and α-gustducin, in human intestinal epithelium, and that T1R2/T1R3 becomes activated in the lumen in the presence of sweet nutrients, much like in the oral cavity (17)**.

A secondary mechanism of luminal glucose-sensing has been proposed in the hormone producing enteroendocrine cells of the intestine. Under conditions of luminal glucose concentrations >30mM, sodium-dependent glucose co-transporter 1 (SGLT1) increases co-transportation of glucose and Na+ into the cell, increasing the cells’ electrogenericity. This generates action potentials and triggers cell membrane depolarization and Ca”2 entry into the cell (18). This signalling cascade results in the secretion of numerous gut hormones, including the incretin hormones; glucagon-like peptide 1 (GLP-1) and glucose-dependent insulinotropic peptide (GIP) (18).

These incretin hormones are vital in reducing blood glucose concentration, through their insulino tropic activity. Once secreted they travel and act upon their respective receptors on pancreatic beta-cells to stimulate insulin secretion and synthesis (19). GLP-1 is also involved in appetite regulation by interacting with its receptors located on the brain and stomach, decreasing food intake and increasing satiety, and decreasing gastric emptying, respectively.
Evidence supporting T1R2/T1R3 and α-gustducin’s direct involvement in GIP secretion, however is lacking (17)**.

**Differences in non-nutritive sweetener sensing**

NNS are thought to interact and activate the sweet taste receptors in the gut as they do in the mouth. Sucralose has been shown to be an agonist for T1R2/T1R3 receptors, resulting in incretin secretion \textit{in vitro}, with both mouse and human enteroendocrine cell lines (21). However, the concentration of sucralose used (50mM) is typically much higher than normal luminal concentrations (0.04-0.1mM), therefore extrapolation into a relevant physiological response is limited.

Using perfused rat small intestines, Saltiel et al. (17)** demonstrated that intra-luminal doses of sucralose, stevioside and Ace-K did not elicit GIP secretion, and only Ace-k stimulated GLP-1 release. Interestingly, intra-vascular dosing of sucralose and stevioside induced both GLP-1 and GIP secretion, while Ace-K did not stimulate any incretin release. These results suggest gut sweet taste receptors may be basolaterally located, in which case, sucralose and stevioside are unlikely to be capable of eliciting a response \textit{in vivo} since they do not cross the brush-border membrane. However, Ace-k’s ability to induce GLP-1 secretion indicate that there may be some apically located receptors. It is possible that GLP-1 secretion may only be stimulated through some specific ligand-binding to T1R2/T1R3 - with certain NNS being able to interact with the sweet taste receptors and other unable to, though further research is needed to test this hypothesis.

The traditional understanding is that NNS can be used to help lower or control blood glucose levels. \textit{In vitro} and \textit{Ex vivo} rodent studies provided evidence that NNS may increase glucose uptake in intestinal epithelium cells by activating T1R2/T1R3 and α-gustducin which may signal another transporter – the GLUT2 transporter – to translocate to the apical membrane and up-regulate glucose transport (22). Similarly, pre-incubation of Caco-2 cells with Ace-K
was reported to promote glucose uptake only in glucose concentrations >25mM, suggesting saturation of SGLT1 increased glucose uptake in the presence of Ace-K, via GLUT2 translocation (22). However, a similar study, pre-incubated cells with Ace-K and sucralose, but did not observe significant differences in glucose uptake in concentrations of glucose ranging from 2.5mM to 75mM (23). However, a recent report suggested that certain NNS may promote glucose transport via incretin secretions leading to increased blood glucose concentrations (24)*.

Since 1996, there are almost 20 publications of randomised controlled human trials (RCT) investigating the effect of NNS, on postprandial glycaemia and insulinaemia. A recent systematic review of observational studies involving / comparing NNS reported no clear consensus on the effects of NNS on glucose metabolism(24) *. Clinical trials in humans investigating the effects of NNS, including aspartame, sucralose and Ace-K, on gut hormone secretion have provided conflicting results. With some studies suggesting aspartame decreases GLP-1 secretion, and others suggesting ace-k and sucralose increase secretion (25). A recent trial conducted a 12-week, double-blind parallel study provides concurrent evidence indicating sucrалose does not affect glycaemic control (26) **. The absence of caloric burden, in combination with un-altered glucose metabolism makes most NNS and polyol sweeteners suitable for individuals with metabolic disorders such as T2DM.

**Conclusion**

The evolved redundancy of the sweet taste receptor to become activated by such a wide variety of chemical ligands, highlights the importance of sweet-tasting foods. Within an evolutionary context, a mechanism to quickly identify sweet and by association, nutrient-dense foods would have provided a survival advantage. However, in the modern dietary landscape, where not all sweet tasting molecules provide caloric energy, it appears the brain - via reward centres - and body - via glucose homeostasis - is able to access and respond appropriately to caloric vs non-caloric sweeteners.
Recent research has focused on laying the foundation of understanding nutrient-sensing pathways in the gut via the T1R family of sweet taste receptors. Animal studies, and human *in vitro* research suggests NNS can activate the gut sweet taste receptors, and under some conditions elicit incretin hormone release. However, the preponderance of evidence from human studies suggest NNS have no effect on glucose metabolism. This highlights the importance of understanding differences in the physical binding of the T1R2/T1R3 proteins and their subsequent variance in the transduction cascade, and neuronal signalling in understanding the differences in physiological response between sugars, NS and NNS.

**Key points:**

- The sweet taste receptor, T1R2/T1R3, is found in the oral cavity and throughout the intestine and binds both nutritive sweeteners (i.e. glucose, fructose, sucrose) and non-nutritive sweeteners (i.e. aspartame, acesulfame-k, sucralose).
- The evidence on whether non-nutritive sweeteners have an impact on incretin hormone secretion and glycemia in humans is unclear, with the majority of studies reporting no significant effects.
- The negligible caloric content in non-nutritive sweeteners may be the reason for the lack of physiological effect as the T1R2/T1R3 receptors may have evolved in humans to distinctly recognize sweet tasting molecules that provide energy.

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References:

Papers of particular interest, published within the annual period of review, have been highlighted as:
* of special interest
** of outstanding interest


A comprehensive study investigating the effects of sucralose, stevioside and Ace-K on GLP-1 and GIP secretion in perfused rat intestine. First study to test whether taste receptors are basolaterally or apically located.


Using physiologically relevant doses, this study found no increase in glucose uptake in enterocyte, when incubated in sucralose and Ace-k; contrary to previous older studies


This paper meticulously reviewed human clinical and observational studies from the last 20 years, investigating non-nutritive sweeteners that are approved for use and their effects on glycemia and the incretin hormones.


This double-blind randomized control study is one of few to investigate the effects of sucralose on glucose homeostasis over a long time period. The results found no significant differences in blood glucose and insulin levels between the control and experimental groups—indicating chronic sucralose consumption may not have any detrimental consequences in a healthy population.