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A bitter pill for type 2 diabetes? The activation of bitter taste receptor TAS2R38 can stimulate GLP-1 release from enteroendocrine L-cells

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ABSTRACT

The bitter taste receptor TAS2R38 is a G protein coupled receptor (GPCR) that has been found in many extra-oral locations like the gastrointestinal (GI) system, respiratory system, and brain, though its function at these locations is only beginning to be understood. To probe the receptor's potential metabolic role, immunohistochemistry of human ileum tissues was performed, which showed that the receptor was co-localized with glucagon-like peptide 1 (GLP-1) in L-cells. In a previous study, we had modeled the structure of this receptor for its many taste-variant haplotypes (Tan et al. 2011), including the taster haplotype PAV. The structure of this haplotype was then used in a virtual ligand screening pipeline using a collection of ~2.5 million purchasable molecules from the ZINC database. Three compounds (Z7, Z3, Z1) were purchased from the top hits and tested along with PTU (known TAS2R38 agonist) in *in vitro* and *in vivo* assays. The dose-response study of the effect of PTU and Z7 on GLP-1 release using wild-type and TAS2R38 knockout HuTu-80 cells showed that the receptor TAS2R38 plays a major role in GLP-1 release due to these molecules. *In vivo* studies of PTU and the three compounds showed that they each increase GLP-1 release. PTU was also chemical linked to cellulose to slow its absorption and when tested *in vivo*, it showed an enhanced and prolonged GLP-1 release. These results suggest that the GI lumen location of TAS2R38 on the L-cell makes it a relatively safe drug target as systemic absorption is not needed for a TAS2R38 agonist drug to effect GLP-1 release.

1. Introduction

The surface of GI tract epithelium is endowed with molecular sensing machinery that detects dietary constituents and gut microbial metabolites [1–4]. Many types of enteroendocrine cells have been identified and are classified for the most part by their specific contents of endocrine transmitters. Key examples include enteroendocrine I-cells containing cholecystokinin (CCK); and L-cells containing glucagon-like peptide-1 (GLP-1) and peptide tyrosine tyrosine (PYY). The release of these agents into the blood results from interaction of sensors on the cells' lumenal surface to nutrient or environmental factors in the contents of the intestine. Each has specific and necessary functions on gastrointestinal tract responses including local and systemic metabolism.

GLP-1 is derived from the transcription product of the pro-

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glucagon gene. The biologically active forms of GLP-1 are: GLP-1-(7-37) and GLP-1-(7-36)NH_2 [5]. Once in the circulation, GLP-1 has a half-life of under 2 min due to rapid degradation by the enzyme dipeptidyl peptidase-4 (DPP4) [6,7]. It is a potent anti-hyperglycemic hormone, inducing glucose-dependent stimulation of insulin secretion while suppressing glucagon secretion. Such glucose-dependent action is particularly attractive because when the plasma glucose concentration is in the normal fasting range, GLP-1 no longer stimulates insulin to cause hypoglycemia. GLP-1 restores the glucose sensitivity of pancreatic β-cells, using a mechanism involving the increased expression of GLUT2 and glucokinase. GLP-1 also inhibits pancreatic β-cell apoptosis, stimulates the proliferation and differentiation of insulin-secreting β-cells, and inhibits gastric secretion and motility [5,8,9]. This delays gastric emptying which promotes satiety and weight loss. In fact, GLP-1 analogs as well as inhibitors of endogenous GLP-1 degradation have been developed that demonstrate efficacy for treatment of type-II diabetes mellitus, which is the type associated with obesity [5,9]. Not only have the analogs been demonstrated to significantly improve insulin secretion and glucose control, they have been found to decrease gastric emptying and increase satiety resulting in weight loss-benefits [10]. L-cells also release two circulating forms of PYY: PYY1-36 and PYY3-36 [11,12]. The latter form is considered the predominant one in both fasted and fed states and is produced by the cleavage of the N-terminal Tyr-Pro residues from PYY1-36 by peptidase enzyme DPP4. PYY inhibits food intake via PYY-2 receptors expressed in neurons of the arcuate nucleus of the hypothalamus [13]. Other actions of PYY include slowing of gastric emptying and slowing small intestine motility.

Numerous studies have used animal and human models to probe the fundamental mechanistic roles of nutrient sensing receptors in the gut [14–25]. These studies have identified taste receptors (sweet, umami, and bitter) as well as fatty acid receptors (activated by a broad range of chain lengths in the fatty acids) in various enteroendocrine cells. Sweet and umami taste receptors are most likely sensing/tasting [15] energy nutrients and amino acids in the food, whereas the bitter taste receptors are potentially sensing/tasting any harmful and toxic constituents in the food. Once these food components are sensed, several metabolic pathways are activated. In the case of bitter sensing components, pathways that slow down gastric emptying and food absorption are likely activated. Many of these associations are not very well characterized especially in the context of human physiology and are being slowly uncovered. Several studies have shown that release of GLP-1 or other hormones like PYY, CCK, and ghrelin can be effected by activating one of these sensors [20,26–33]. These associations are critical to understand the detailed role of these gustatory gut sensors in food digestion.

Physiologic roles for the peptide hormones released from the enteroendocrine cells lining the lumen of the GI tract have been known for some time, but the mechanisms underlying the "sensing" and secretion of the hormones by intestinal contents were not known. Findings are now emerging that taste receptors, previously thought to be restricted to the tongue epithelium, are also present in the stomach, small intestine and colon. Several enteroendocrine cell types express TAS2R-family bitter taste receptors and T1R2/3 sweet taste receptors. In fact, the enteroendocrine cells secrete GLP-1, peptide YY (PYY) or CCK in response to bitter ligands like phenylthiocarbamide (PTC) and denatonium, which activate bitter taste receptors TAS2R38 and TAS2R47 respectively [20,25,32–35].

To understand the nutrient mediated signaling of L-cells in the context of peptide hormone release, we focused on a bitter taste receptor TAS2R38 to investigate its mechanistic role in the release of the GLP-1 hormone from L-cells. This understanding will advance novel therapeutic avenues for targeting type 2 diabetes, specifically using bitter (but safe) components of food that can target bitter taste receptors like TAS2R38. In the long term, these advances are also expected to show that some bitter constituents of foods are functional and therapeutically beneficial.

In the current study, we first investigated if the bitter taste receptor TAS2R38 is co-localized with GLP-1 in the L-cells using human GI tissue samples from the Cedars-Sinai Biobank. Then we tested if using TAS2R38 ligands can cause GLP-1 release from L-cells. Both PTU and PTC are agonists for this receptor, and we used PTU as a reference ligand for all studies. We performed an *in silico* ligand screening using our previously published structure for the taster haplotype PAV of this receptor [36] to identify novel agonists for this receptor that can be used to probe its signaling and also serve as lead compounds as potential therapeutics. This identified three novel compounds which were tested along with PTU in *in vitro* and *in vivo* assays measuring GLP-1 release. The *in vivo* studies also involved the use of PTU conjugated with cellulose to see if reducing PTU absorption, thereby prolonging its presence in the lumen, will cause prolonged GLP-1 release or not. This is important because the target receptor TAS2R38 is located in the gut and any potential therapeutic targeting of that receptor should be gut-restricted to minimize potential side-effects coming from systemic exposure of the drug.

2. Materials and methods

The human ileum tissues from Cedars-Sinai Biobank using IRB protocol 34332 were analyzed by using immunohistochemistry (IHC) methods for co-localization of TAS2R38 with GLP-1 to see if the receptor was present in the L-cells. In a parallel study, the previously determined structure of the taster haplotype PAV of TAS2R38 [36] was used in a structure-based virtual ligand screening of purchasable compounds from the ZINC database [37]. Three of the top hit molecules were purchased and tested in *in vitro* assays utilizing HuTu-80 cells and *in vivo* assays for their potential of GLP-1 release. One of the molecules was also tested with TAS2R38 knockout cells. The methods and materials used for these studies are described below.

2.1. Immunohistochemistry

The experiments were designed to determine if the TAS2R38 receptor is expressed on the human enteroendocrine L-cells. To identify if it is co-localized with GLP-1 on the native L-cells, we performed IHC using previously validated GPCR and GLP-1 antibodies by double immune-staining on human GI tissues. The antibodies used were: TAS2R38 [rabbit polyclonal (H: ab65509, Abcam)] and GLP-1 [goat polyclonal (sc-26637, Santa Cruz Biotechnology)]. The numbers of cells staining GLP-1 or TAS2R38, or both were counted visually.

2.2. Virtual ligand screening

We used the PTU-bound PAV conformation predicted previously [36] in a virtual ligand screen (VLS) study using the DOCK Blaster server [38] which has access to several compound libraries including one with ~2 million commercially available compounds from the ZINC database [39]. For each ligand molecule, this server docks multiple ligand conformations corresponding to its internal torsional degrees of freedom into a putative binding site provided by the user and ranks the molecules by a scoring function. We took the top 500 hits from this server and prioritized them using a more accurate scoring function based on an all-atom Dreiding force field [40] to select top 200 diverse small molecules corresponding to