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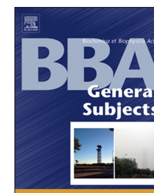

Reengineering the ligand sensitivity of the broadly tuned human bitter taste receptor TAS2R14

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ABSTRACT

Background: In humans, bitterness perception is mediated by ~25 bitter taste receptors present in the oral cavity. Among these receptors three, TAS2R10, TAS2R14 and TAS2R46, exhibit extraordinary wide agonist profiles and hence contribute disproportionally high to the perception of bitterness. Perhaps the most broadly tuned receptor is the TAS2R14, which may represent, because of its prominent expression in extraoral tissues, a receptor of particular importance for the physiological actions of bitter compounds beyond taste.
Methods: To investigate how the architecture and composition of the TAS2R14 binding pocket enables specific interactions with a complex array of chemically diverse bitter agonists, we carried out homology modeling and ligand docking experiments, subjected the receptor to point-mutagenesis of binding site residues and performed functional calcium mobilization assays.
Results: In total, 40 point-mutated receptor constructs were generated to investigate the contribution of 19 positions presumably located in the receptor's binding pocket to activation by 7 different TAS2R14 agonists. All investigated positions exhibited moderate to pronounced agonist selectivity.
Conclusions: Since numerous modifications of the TAS2R14 binding pocket resulted in improved responses to individual agonists, we conclude that this bitter taste receptor might represent a suitable template for the engineering of the agonist profile of a chemoreceptive receptor.
General significance: The detailed structure-function analysis of the highly promiscuous and widely expressed TAS2R14 suggests that this receptor must be considered as potentially frequent target for known and novel drugs including undesired off-effects.

1. Introduction

The human bitter taste provides an alarm system that prevents the involuntary ingestion of potentially toxic food items, although the levels of bitterness and toxicity are not necessarily correlated [1, 2]. The bitter responsive cells are located in the oral cavity, where they occur intermingled with sensory cells devoted to the detection of the other four basic taste qualities in units of ~100 cells, called taste buds [3]. Each bitter taste receptor cell expresses a subset of the ~25 putatively functional human bitter taste receptors (TAS2Rs) [4] that are distantly related to the class A G protein-coupled receptor (GPCR) family [5]. Until now, functional heterologous expression of TAS2Rs and screening of bitter compound libraries have resulted in the identification of agonists for 21 of the receptors [6, 7]. The functional characterization of TAS2Rs revealed that they can be grouped based on their agonist spectra into broadly, narrowly, and intermediately tuned receptors [8]. The three broadly tuned receptors are TAS2R10 [9], TAS2R14 [10], and TAS2R46 [11]. Each one of them recognizes about one-third of the bitter substances tested so far and their combined response pattern facilitates detection of about one-half of the bitter substances [6]. Thus, a considerably strong contribution to the overall bitter tasting ability of humans can be assumed. Whereas TAS2R46 exhibits a bias towards the detection of sesquiterpene lactones and related compounds, with few exceptions of structurally different natural and synthetic chemicals

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[11], TAS2R10 and TAS2R14 show fewer preferences for recognizable common structures in their agonist panels.

The ability of these broadly tuned receptors to interact with many chemically unrelated compounds has raised interest in the architecture and composition of their ligand binding pockets. Consequently, structure-function analyses have been performed and revealed that the orthosteric binding site of TAS2Rs coincides with that of class A GPCRs. Indeed, it was proved that, despite of the numerous diverse agonists, TAS2R46 possesses a unique ligand binding pocket accommodating all the agonists with overlapping, but different contact points between receptor residues and agonists [12]. Experiments performed with TAS2R10 revealed that the receptor is tailored to recognize numerous agonists at the expense of potentially higher affinities for individual agonists [13]. Although additional experimental studies have addressed the structure-function relationships of a variety of human TAS2Rs [14–21], so far, no such experiments have been done in case of TAS2R14, perhaps the most broadly tuned receptor among the human TAS2Rs [6]. However, the syntheses of agonist derivatives carrying bulky side-chains indicated that the binding pocket of this receptor is rather spacious tolerating agonist structures considerably exceeding the size of the unmodified agonists [22]. The TAS2R14 receptor is very promiscuous and its agonists seem devoid of a common chemical feature [10]. In silico pharmacophore modeling approaches combined with functional experiments failed to reveal a core structure present in all agonists, rather the existence of multiple different chemical scaffolds for TAS2R14 agonists was determined [23]. Intriguingly, numerous medicinal drugs were identified among the discovered agonists, indicating that the receptor may represent an important target for side-effects of drugs, especially since TAS2R14 is strongly expressed in heart myocytes and bitter stimulation of rodent hearts result in negative inotropy [24, 25]. Moreover, expression of TAS2R14 was detected in sinonasal cilia and flavone stimulation leads to increases ciliary beating and mucociliary clearance in primary cells suggesting an important role of this receptor in innate immunity [26].

The present work investigates the architecture of the TAS2R14 binding pocket by a combination of functional heterologous expression in mammalian cells, site directed mutagenesis, and in silico ligand docking to the homology model of this receptor. The data shed light on the binding modes of multiple structurally diverse bitter agonists within the ligand binding pocket of the most promiscuous human TAS2R.

2. Materials and methods

2.1. Bitter compounds

Aristolochic acid, flufenamic acid, genistein, picrotoxinin, (−)-α-thujone were purchased from Sigma-Aldrich at the highest available quality. Parthenolide was available from a previous study [11]. Substances were either dissolved directly in C1-buffer or first in DMSO followed by dilution in C1 buffer not exceeding a final DMSO-concentration of 1% (v/v) in the functional calcium assay.

2.2. TAS2R14 constructs

The full coding cDNA sequence of wild type TAS2R14 (corresponding to accession number NM_023922) was cloned into the eukaryotic expression vector pcDNA5FRT (Invitrogen) as described previously [23]. The vector was modified to add in frame a sequence coding for the first 45 amino acids of the rat sst3 receptor at the 5′-end and at the 3′-end a sequence coding for a herpes simplex virus glycoprotein D epitope (HSV-tag) was added [9]. Subsequent site-directed mutagenesis experiments were performed by PCR-mediated recombination [27] as detailed before [13, 28]. A complete list of the used mutagenesis primers is found in online resource 1, supplemental table 1S.

2.3. Calcium mobilization assay

HEK 293 T cells stably expressing the chimeric G protein Gα16gust44 were cultivated in DMEM supplemented with 10% fetal bovine serum, 1% penicillin/streptomycin-mixture, 1% L-glutamine at 37 °C, 5%-CO_2 and saturated air humidity. Prior to transfection, cells were plated into flat bottom, black-walled 96-well plates coated with 10 µg/mL poly-D-lysine. For transient transfection with TAS2R14 constructs Lipofectamine2000 was used exactly as published before [23]. About 24 h after transfection, cells were loaded with the calcium-sensitive dye Fluo-4 AM in the presence of 2.5 mM probenecid for 1 h at room temperature followed by 3 washing steps with buffer C1 (130 mM NaCl, 5 mM KCl, 10 mM glucose, 10 mM Hepes; pH 7.4). The plates were then transferred into an automated fluorescence plate reader (FLIPRtetra) for measurement of receptor-dependent cellular calcium responses. Each experiment was performed at least twice independently using triplicate wells per applied substance concentration.

2.4. Data analyses

Data points corresponding to individual substance concentration steps and receptor constructs were averaged, corrected for responses of identically treated mock-transfected cells and normalized for background fluorescence. Threshold concentrations were determined as the lowest substance concentrations significantly stimulating cellular calcium responses. Dose-response curves were calculated using the function $f(x) = \min + (\max - \min)/(1 + (x/EC_{50})^{nH})$ and plotted with SigmaPlot software as before [23]. Statistical comparison of results obtained for wild type and point-mutated TAS2R14 constructs was done by ANOVA followed by post-hoc tests (Bonferroni correction, Dunnet's multiple comparison test).

2.5. Immunocytochemical staining of HEK 293T cells

HEK 293T-Gα16gust44 cells were plated on poly-D-lysine coated glass cover slips and grown overnight in culture medium before transient transfection using Lipofectamine2000 as detailed above (cf. calcium mobilization assay). About 24 h after transfection, cells were subjected to immunocytochemical staining procedure as published before [13]. Briefly, the culture medium was removed and the glass cover slips were washed once with 37 °C warm PBS-buffer (140 mM NaCl, 10 mM Na_2HPO_4, 1.76 mM KH_2PO_4, 2.7 mM KCl; pH 7.4). Next, the cells were placed on ice for 30 min to block endocytosis before concanavalin A in icecold PBS was applied (1:2000) and left on the cells for 1 h. Then, extensive washes with PBS were carried out and icecold methanol/acetone mixture (1:1, v/v) was used to fix the cells for 2 min. After several washing steps with PBS buffer performed at room temperature (RT) unspecific binding sites were blocked with 5% normal goat serum in PBS for 45 min. The incubation with anti-HSV antibody (1:15000) was performed overnight at 4 °C in 5% normal goat serum in PBS. The next day, cells were washed with PBS and incubated with anti-mouse Alexa488 antiserum (1:2000) and streptavidin Alexa633 (1:1000) in 5% normal goat serum in PBS for 1 h at RT. Several washes with PBS were followed by a final rinse with ddH_2O before the glass cover slips were mounted to slides with Dako mounting medium. Cellular fluorescence was monitored by confocal laser scanning microscopy using a Leica SP2 with laser emission wavelengths 488 nm and 633 nm. For each receptor construct 4–6 randomly selected areas were evaluated using the ImageJ software to calculate efficiency of expression (expression (in%) = number of receptor expressing cells × 100/total cell number).

2.6. In silico modeling and docking

Aristolochic acid, flufenamic acid, genistein, picrotoxinin, (−)-α-thujone and parthenolide were prepared with LigPrep (version 3.6,

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