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Plasticity of the ligand binding pocket in the bitter taste receptor T2R7

Kun Liu, Appalaraju Jaggupilli, Dhanaraj Premnath, Prashen Chelikani*

From the Manitoba Chemosensory Biology Research Group and Department of Oral Biology, University of Manitoba, Children's Hospital Research Institute of Manitoba (CHRIM), Winnipeg, MB R3E 0W4. Canada

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ABSTRACT

Bitter taste receptors (T2Rs) are a group of 25 G protein-coupled receptors (GPCRs) in humans. The cognate agonists and the mechanism of ligand binding to the majority of the T2Rs remain unknown. Here we report the first structure-function analysis of T2R7 and study the ability of this receptor to bind to different agonists by site-directed mutagenesis. Screening of ligands for T2R7 in calcium based assays lead to the identification of novel compounds that activate this receptor. Quinine, diphenidol, dextromethorphan and diphenhydramine showed substantial activation of T2R7. Interestingly, these bitter compounds showed different pharmacological characteristics. To investigate the structural features in T2R7 that might contribute to the observed differences in agonist specificities, molecular model guided ligand docking and site-directed mutagenesis was pursued. Amino acids D65, D86, W89, N167, T169, W170, S181, T255 and E271 in the ligand-binding pocket were replaced and the mutants characterized pharmacologically. Our results suggest D86, S181 and W170 present on the extracellular side of transmembrane 3 (TM3), TM5 and in extracellular loop 2 (ECL2) are essential for agonist binding in T2R7. Mutations of these amino acids lead to loss-of-function. We also identified gain-of-function residues that are agonist specific. These results suggest that agonists bind at an extracellular site rather than deep within the TM core involving residues present in both ECL2 and TM helices in T2R7. Similar to majority of the Class A GPCRs, ECL2 in T2R7 plays a significant role in agonist binding and activation.

1. Introduction

Bitter taste, which is sensed by cell surface G protein-coupled receptors (GPCRs), has evolved to help mammals avoid consumption of harmful substances. In humans, taste receptor cells express 25 bitter taste receptors, termed as T2Rs. Each T2R contain 291 to 334 amino acids, share 23% to 86% sequence identity and respond to a wide range of compounds [1–3]. Similar to all GPCRs, the T2R structure consists of a short extracellular N-terminus, seven transmembrane helices (TMs), three extracellular loops (ECLs), three intracellular loops (ICLs) and a short intracellular C-terminus. Hundreds of compounds with different chemistries bind to T2Rs [4,5]. The cognate agonists for these T2Rs are yet to be elucidated [5], and a single T2R can bind to agonists with different chemistries [4]. The structural features on the T2Rs that contribute to agonist binding and activation, including the role of ECL loops remains to be investigated [6,7].

Over the past decade, many studies reported that in addition to the gustatory system T2Rs were also expressed in various extraoral human tissues, as suggested by nCounter sequencing [8], and recently reviewed [9,10]. In addition to human tissues, T2Rs were recently reported in exosomes isolated from saliva [11]. Intriguingly, the activation of T2Rs expressed in extraoral tissues leads to various physiological and pathophysiological cellular responses. For example, T2R agonists were suggested as novel targets for treating asthma [12]. It was reported that T2R agonists induce autophagy and show antimitogenic effects in airway smooth muscle [13]. In view of the emerging role of T2Rs in extraoral tissues, it is of significant biomedical importance to characterize T2Rs and identify efficient T2R ligands. A recent exome sequencing of a family susceptible to gastric and rectal cancer identified 12 novel non-synonymous single nucleotide polymorphic (nsSNP) variants in 12 different genes including TAS2R7 [14]. SNPs in TAS2R7 were also suggested to be involved in susceptibility to glioma [15].

In this report, we pursue the first structure–function analysis on T2R7. We screen a number of compounds that are potential T2R7 agonists. These compounds include reported T2R7 agonists [1,16], common bitter compounds, common prescribed antibiotics and quorum sensing molecules. Our results suggest that T2R7 is activated by novel ligands including dextromethorphan, diphenhydramine, thiamine, tobramycin and erythromycin. To understand the structure of the ligand binding pocket in T2R7, molecular model guided ligand docking and

* Corresponding author at: D319, Manitoba Chemosensory Biology Research Group, Department of Oral Biology, 780 Bannatyne Avenue, University of Manitoba, Winnipeg, MB R3E 0W4, Canada.
E-mail address: Prashen.Chelikani@umanitoba.ca (P. Chelikani).

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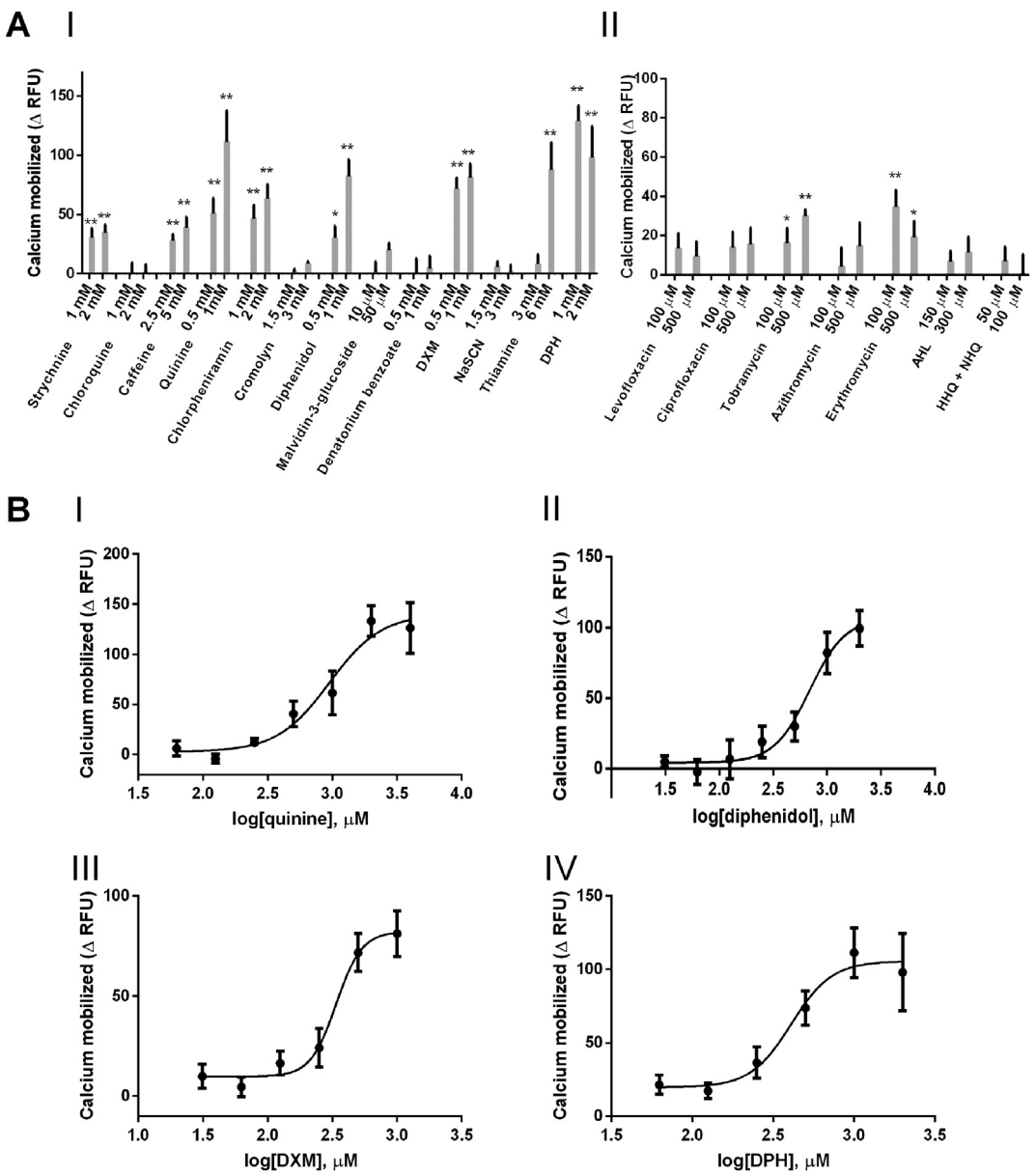

Fig. 1. T2R7 ligand screening. A. Calcium responses of T2R7 to potential agonists. T2R7 stably expressed in the HEK293T cell line was treated with different compounds. The results after subtracting the baseline responses of control HEK293T (untransfected) cells to different compounds were shown. The calcium mobilized (ΔRFUs or Relative Fluorescence Units) was detected using the calcium sensitive dye Fluo 4NW (Invitrogen), and fluorescence measured using Flex Station III microplate reader as described before [18–20]. The results were from a minimum of n = 3 experiments with each data point in triplicate. Statistical significance of response differences in control cells and T2R7 stable cells was determined as described in methods (*p < .05, **p < .01). [I] Calcium responses of T2R7 to previously reported agonists. Of the eight compounds, T2R7 showed significant responses to both concentrations of strychnine, caffeine, quinine, chlorpheniramin and diphenidol compared to control (untransfected) HEK29T cells. No response was observed with 1 mM or 2 mM chloroquine, 1.5 mM or 3 mM cromolyn, and 10 μM or 50 μM Malvidin-3-glucoside (*p < .05, **p < .01). [II] Calcium responses of T2R7 to new (unreported) bitter compounds. Among the five bitter compounds, T2R7 showed significant responses to dextromethorphan and diphenhydramine hydrochloride at both concentrations, and thiamine hydrochloride at 6 mM compared to control cells (*p < .05, **p < .01). [III] Calcium responses of T2R7 to antibiotics and quorum sensing molecules. The antibiotics were tested at two concentrations, 100 μM and 500 μM. The acyl homoserine lactones (AHLs) mixture contains C4-AHL, 3-oxo-C8-AHL and 3-oxo-C12-AHL at 50 μM and 100 μM each. Mixture of the other quorum sensing molecules contains HHQ (2-heptyl-e-hydroxy-4-quinolone) and NHQ (2-nanoyl-3-hydroxy-4-quinolone) at 25 μM and 50 μM each. The results showed that T2R7 responded to tobramycin and erythromycin at both concentrations compared with control cells (*p < .05, **p < .01). B. Concentration-dependent calcium response of T2R7 stable cells treated with quinine, diphenidol, dextromethorphan (DXM) and diphenhydramine (DPH). T2R7 expressing HEK293T stable cells were treated with different concentrations of [I] quinine ranging from 0.0625 to 4 mM, [II] diphenidol from 0.03125 to 2 mM, [III] DXM from 0.0625 to 4 mM, and [IV] DPH concentrations from 0.0625 to 4 mM. Relative fluorescence unit (ΔRFU) was calculated by subtracting the baseline response of control HEK293T cells. EC_{50} values were determined by nonlinear regression analysis using PRISM software v 6.01. The EC_{50} values for quinine, diphenidol, DXM and DPH are 983 ± 257 μM, 675 ± 186 μM, 518 ± 19 μM and 634 ± 81 μM, respectively.

site-directed mutagenesis was pursued. We identify amino acids that are essential for T2R7 agonist binding and few amino acids that might determine the specificity of ligand binding. Our results suggest that the T2R7 agonist binding pocket is not deeply buried and present on the extracellular surface. This suggests the involvement of residues in the upper part of the TM bundle are required for accommodating the ligands along with residues from ECL2 playing a significant role in agonist binding and activation.

2. Materials and methods

2.1. Materials

Human Embryonic Kidney (HEK293T) cell line was from ATCC