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SHORT COMMUNICATION

L-Theanine elicits umami taste via the T1R1 + T1R3 umami taste receptor

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Abstract L-Theanine is a unique amino acid present in green tea. It elicits umami taste and has a considerable effect on tea taste and quality. We investigated L-theanine activity on the T1R1 + T1R3 umami taste receptor. L-Theanine activated T1R1 + T1R3-expressing cells and showed a synergistic response with inosine 5′-monophosphate. The site-directed mutagenesis analysis revealed that L-theanine binds to L-amino acid binding site in the Venus flytrap domain of T1R1. This study shows that L-theanine elicits an umami taste via T1R1 + T1R3.

Keywords L-Theanine · Umami · Synergy · T1R1 + T1R3

Introduction

Taste is classified into five basic categories: salty, sweet, bitter, sour and umami. Umami taste is stimulated by compounds containing amino acids, such as L-glutamate (Glu) and L-aspartate, and 5′-ribonucleotides such as inosine 5′-monophosphate (IMP) and guanosine 5′-monophosphate. These compounds are present as mono- or disodium salts in meats, vegetables and dairy products (Yamaguchi and Ninomiya 2000). When amino acids and 5′-ribonucleotides are both present in food, umami taste intensity is synergistically enhanced and the umami taste threshold is dramatically lowered (Yamaguchi and Ninomiya 2000).

Previous studies have identified three putative G-protein-coupled receptors for umami compounds: taste-mGluR1 (Toyono et al. 2003), taste-mGluR4 (Chaudhari et al. 1996, 2000) and T1R1 + T1R3 heterodimer (Li et al. 2002; Nelson et al. 2002). Among these receptors, T1R1 + T1R3, which belongs to the class C G-protein-coupled receptor family, detects various amino acids, including Glu (Li et al. 2002; Nelson et al. 2002). T1R1 + T1R3 is thought to be the umami receptor because it shows a synergistic response, a hallmark of an umami taste, between Glu and 5′-ribonucleotides (Li et al. 2002; Nelson et al. 2002).

L-Theanine (Fig. 1), 5-*N*-ethylglutamine, is a unique amino acid present in green tea (Sakato 1949). Recently, numerous studies have reported beneficial effects of L-theanine: relaxation effects and improved learning ability (Cooper et al. 2005; Gomez-Ramirez et al. 2007; Nobre et al. 2008); cancer prevention (Friedman et al. 2007; Liu et al. 2009); and taste-improving effect (Narukawa et al. 2010). Our previous study revealed that L-theanine has umami taste and shows umami synergy with 5′-ribonucleotide in both humans and mice (Narukawa et al. 2008, 2011). Since the molecular structure of L-theanine is similar to that of Glu and L-theanine elicits umami synergy with 5′-ribonucleotide, L-theanine may activate T1R1 + T1R3. However, there have been no studies to determine whether L-theanine activates this receptor. To

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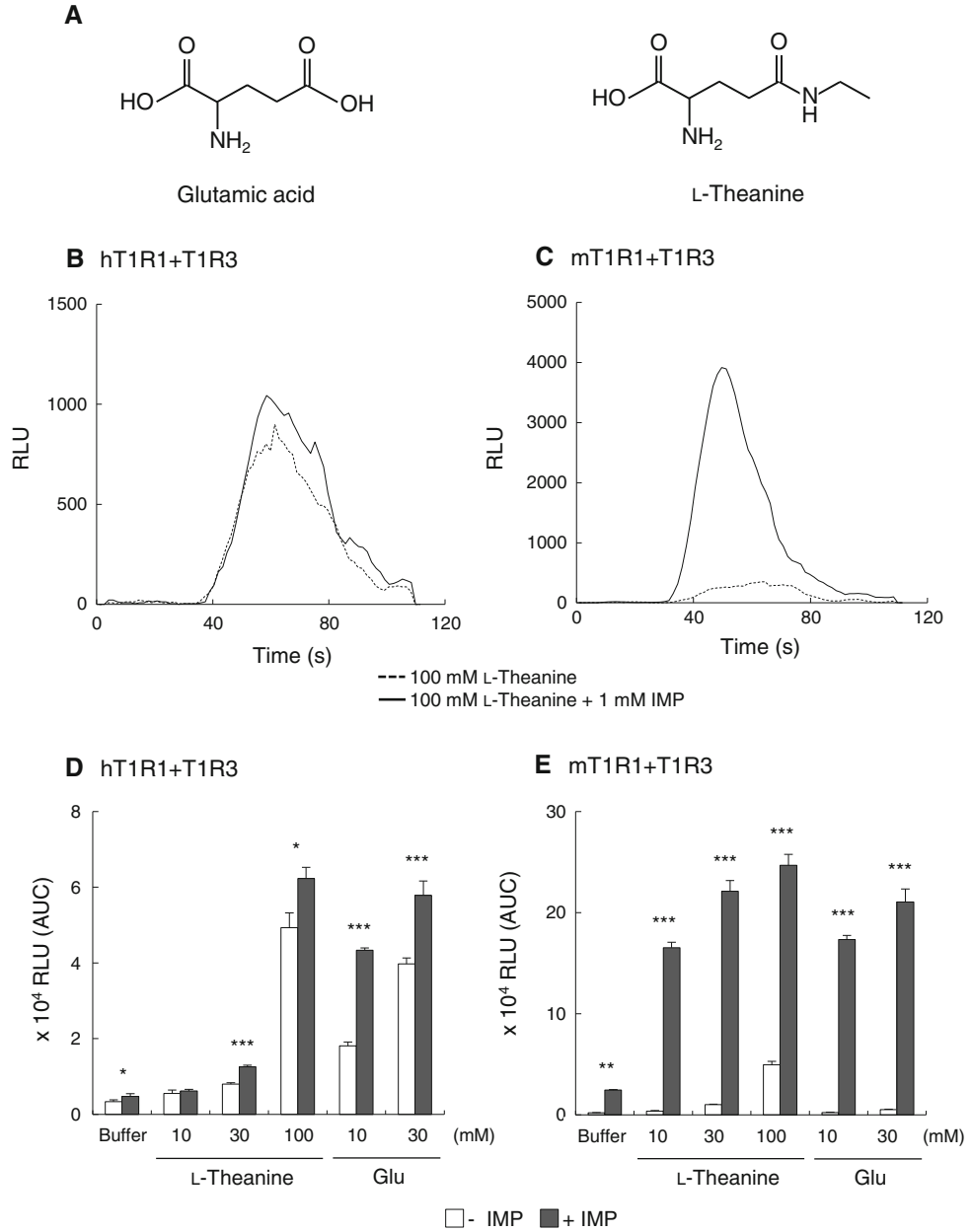

Fig. 1 Response of hT1R1 + T1R3- and mT1R1 + T1R3-expressing HEK293T cells to L-theanine. **a** Chemical structure of glutamic acid and L-theanine (5-*N*-ethylglutamine). **b**, **c** Representative line traces obtained from the Flexstation™ III assay showing luminescence changes after treatment with 100 mM L-theanine in the absence or presence of 1 mM IMP (*dash* and *solid line*, respectively) for **b** hT1R1 + T1R3- and **c** mT1R1 + T1R3-expressing cells. **d, e** Dose–response relationships to L-theanine and L-glutamate (Glu) in the absence or presence of 1 mM IMP (*white* and *black columns*, respectively) in **d** hT1R1 + T1R3- and **e** mT1R1 + T1R3-expressing cells. Values represent the mean ± SEM of the area under the curve (AUC). $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$ ($n = 4$; *t* test)

investigate whether the taste of L-theanine is detected via T1R1 + T1R3, we examined L-theanine activity using human T1R1 + T1R3 (hT1R1 + T1R3)- and mouse T1R1 + T1R3 (mT1R1 + T1R3)-expressing human embryonic kidney 293T (HEK293T) cells. We also examined the cellular response to L-theanine in the presence of IMP. Furthermore, T1R1 mutants were used to identify the L-theanine binding site.

Materials and methods

Materials

Glu, L-alanine (Ala) and IMP were purchased from Sigma (St. Louis, MO, USA). L-Theanine was from Taiyo chemical (Mie, Japan). All other reagents were of analytical grade and were from standard suppliers. The buffer for

a luminescence assay comprised 10 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), 130 mM NaCl, 10 mM glucose, 5 mM KCl, 2 mM $CaCl_2$ and 1.2 mM $MgCl_2$ and was supplemented with 0.1 % bovine serum albumin (Sigma Aldrich, St. Louis, MO, USA); the pH was adjusted to 7.4 using NaOH. The ligands were diluted to the desired concentrations in the assay buffer, and the pH values of these solutions range from 7.2 to 7.4.

Constructs for h- and mT1R1 + T1R3 umami receptors and their point mutants

hT1R1 (NCBI refseq number NM_138697.3), hT1R3 (NM_152228.1), mouse T1R1 (mT1R1) (NM_031867.2) and mT1R3 (NM_031872.2) were subcloned into the pEAK10 expression vector (Edge Biosystems, Gaithersburg, MD, USA). The Kozak consensus sequence was introduced upstream of the start codon for efficient translation. To identify key residues for L-theanine binding, we constructed four single-point mutants for hT1R1 and mT1R1: hT1R1-S172A and E301A and mT1R1-S173A and E302A.

Measurement of cellular response to the amino acid

A luminescence assay was performed as described previously (Toda et al. 2011, 2013). Briefly, HEK293T cells were transiently transfected with expression vectors for T1R1, T1R3 and $rG15_i2$ with mitochondrial apoclytin II (Inouye 2008) and maintained at 37 °C under 5 % CO_2 in DMEM supplemented with GlutaMAX (GIBCO) and 10 % FBS (GIBCO) to minimize glutamate-induced desensitization. Assays were performed after 48 h of transfection. The cellular response in each well was measured using a FlexStation™ III (Molecular Devices, Sunnyvale, CA, USA) and expressed as relative light units (RLU), which was calculated based on the area under the curve (AUC). An aliquot of 100 µl of assay buffer supplemented with 2× ligands was added at 20 s, and scanning was continued for an additional 90 s. The dose–response curves were generated using Clampfit ver. 9.2.0.09 (Molecular Devices) by fitting the data to the Hill equation. The final concentrations of test ligands were 2–100 mM L-theanine, 10 and 30 mM Glu, and mixtures of these compounds with 1 mM IMP and 30 mM Ala. Statistical analyses were performed using KyPlot version 3 (Keyence, Osaka, Japan).

Homology modeling

Homology models of hT1R1 were constructed in the open form of metabotropic glutamate receptor 1 (PDB ID; 1EWT) (Kunishima et al. 2000). Sequence alignment and energy minimization were calculated using MOE software (Chemical Computing Group Inc., Montreal, Quebec, Canada). Visualization was performed using Discovery Studio software (Accelrys, San Diego, CA, USA).

Results

Measurement of the cellular response of hT1R1 + T1R3- and mT1R1 + T1R3-expressing HEK293T cells to L-theanine application showed that the cells expressing hT1R1 + T1R3 responded strongly to Glu, which is a representative umami taste compound, whereas the mouse receptor responded weakly to Glu in the absence of IMP, as reported previously (Toda et al. 2013) (Fig. 1b–e). Both hT1R1 + T1R3- and mT1R1 + T1R3-expressing cells also responded to L-theanine in a concentration-dependent manner (10–100 mM). When both L-theanine and IMP (1 mM) were applied to T1R1 + T1R3-expressing cells, the response to L-theanine showed a large, concentration-dependent increase, particularly for the mouse receptor (Fig. 1d, e). The response to the mixture of L-theanine and IMP was much higher than that to L-theanine or IMP alone. These results suggest that L-theanine activates T1R1 + T1R3 and acts synergistically with IMP.

Next, we attempted to identify the L-theanine binding site on the umami receptor using point-mutation analysis. Both the N-terminal Venus flytrap domain (VFTD) and transmembrane domain (TMD) of T1R1 are known to be ligand binding sites (Zhang et al. 2008; Toda et al. 2013). Because L-amino acid binding site exists in T1R1 VFTD, we generated four point mutants, S172 and E301 in hT1R1 (corresponding to S173 and E302 in mT1R1), affecting L-amino acid recognition in h- and mT1R1 VFTD (Fig. 2a). Since the responses to L-theanine were decreased in the hT1R1-S172A and E301A mutants similar to the decrease for Glu, these residues were hypothesized to be involved in L-theanine recognition (Fig. 2b, d). Also, mutations at S173 and E302 in mT1R1 affected the receptor response to L-theanine, although the effect of S173 mutation was much greater than that of E302 mutation (Fig. 2c, e). These results strongly indicate that L-theanine is recognized by the L-amino acid binding site in T1R1 VFTD.

Discussion

We previously reported that L-theanine has a complicated taste, including umami, and that a synergistic effect of L-theanine and IMP can be observed in humans and mice (Narukawa et al. 2008, 2011). However, its receptive mechanism in the oral cavity was not well understood. Using a heterologous experimental system, we found that

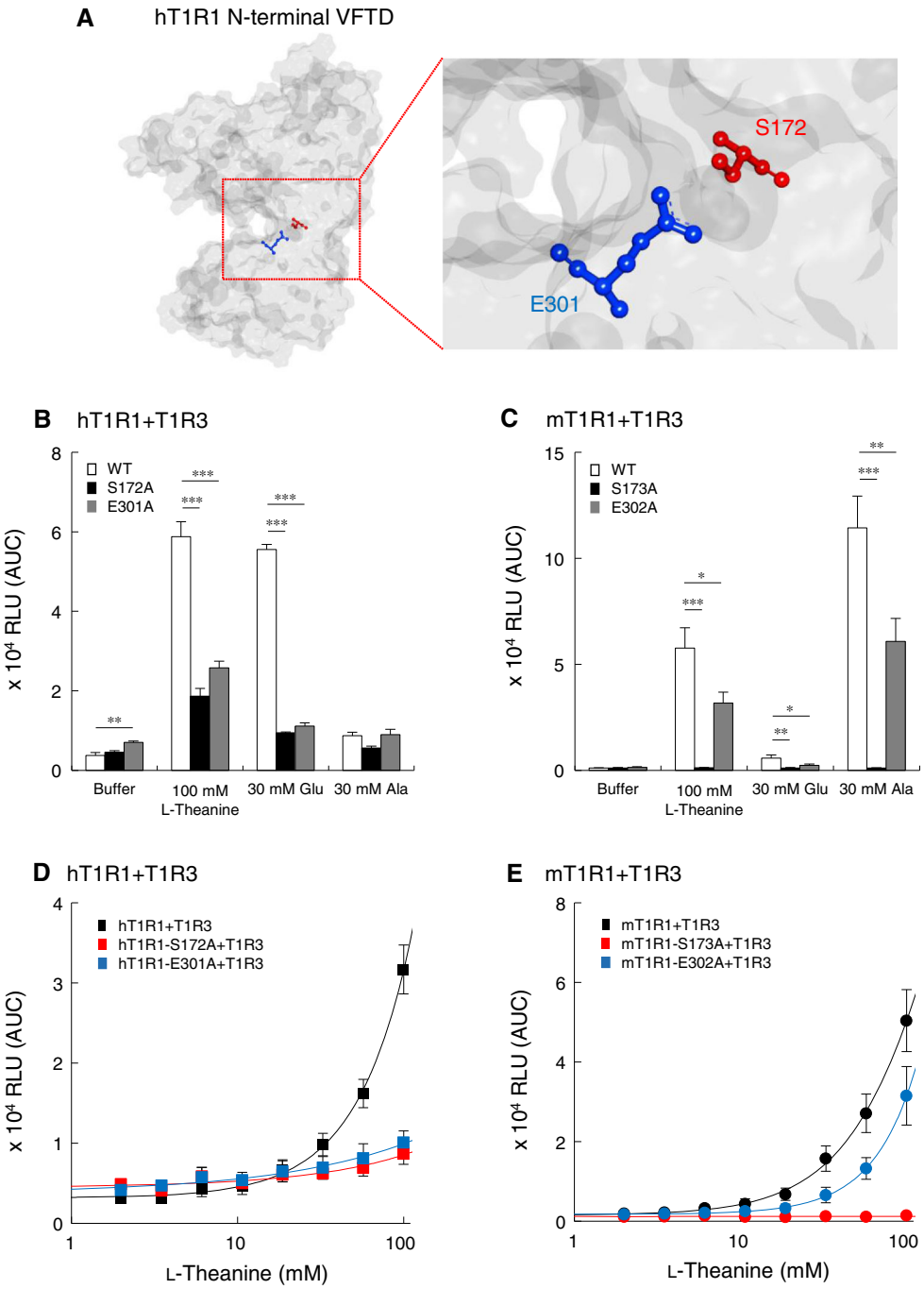

Fig. 2 Response profiles for point mutants of residues involved in amino acid recognition. **a** Mapping of key residues in a molecular model of the Venus flytrap domain (VFTD) of hT1R1. The key residues for broad L-amino acid recognition are shown in S172 (*red*) and E301 (*blue*), respectively. **b, c** HEK293T cells were transfected with **b** hT1R1-WT, S172A and E301A + T1R3 or **c** mT1R1-WT, S173A and E302A + T1R3 together with rG15i2 and stimulated with 100 mM L-theanine, 30 mM L-glutamate (Glu) and 30 mM L-alanine (Ala). $*p < 0.05$; $**p < 0.01$; $***p < 0.001$ ($n = 4$–6; Dunnett's test). **d, e** The dose–response curves to L-theanine in HEK293T cells expressing hT1R1-WT, S172A and E301A + T1R3 **d** or mT1R1-WT, S173A and E302A + T1R3 **e** together with rG15i2. Values represent the mean ± SEM of the area under the curve (AUC)

L-theanine activated the umami taste receptor T1R1 + T1R3 (Figs. 1, 2). Furthermore, the response of T1R1 + T1R3 was drastically increased by adding IMP to L-theanine. This strongly suggests that the umami taste response to L-theanine, which was observed in both humans and mice, occurred via T1R1 + T1R3.

In the previous study, the taste threshold of L-theanine was approximately 25 mM, with taste intensity increasing in a concentration-dependent manner in humans (Narukawa et al. 2008). The current study revealed that L-theanine activates hT1R1 + T1R3 in a concentration-dependent manner, and the threshold concentration of L-theanine for activating hT1R1 + T1R3 is approximately 30 mM (Figs. 1d, 2d). In previous studies, the synergistic effects of L-theanine and IMP were strongly observed in mice rather than in humans in vivo (Narukawa et al. 2008, 2011).

Consistent with the result in vivo, the L-theanine response of mT1R1 + T1R3 was strongly enhanced by the addition of IMP compared to the response of hT1R1 + T1R3 (Fig. 1b–e). These observations indicate that L-theanine is recognized by T1R1 + T1R3 in oral cavity.

Class C GPCRs have multi-ligand binding sites (Conigrave and Hampson 2010). Previous studies revealed that multiple ligand binding sites exist in both VFTD and TMD of the T1R1 subunit (Zhang et al. 2008; Toda et al. 2013). L-amino acids are recognized by the hinge region of VFTD of T1R1, whereas S807, a synthetic ligand, acts on the TMD of T1R1. The amino acid residues S172 and E301 in hT1R1 (corresponding to S173 and E302 respectively in mT1R1) play a crucial role in the response to many kinds of L-amino acids (Zhang et al. 2008; Toda et al. 2013). According to the results for L-amino acids such as Glu and Ala, the receptor response to L-theanine was drastically decreased in hT1R1-S172A and mT1R1-S173A mutants. Although the effect of mT1R1-E302 mutation in L-theanine activity was weaker than that of hT1R1-E301 mutation, L-theanine activity was decreased even in these mutants (Fig. 2b–e). In addition, the response to S807 was observed in those hT1R1 mutants-expressing cells (data not shown). These results strongly suggest that L-theanine binds to the hinge region of VFTD of T1R1, which is known as the binding site for L-amino acids.

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Conflict of interest The authors declare that they have no conflict of interest.

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