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Umami–bitter interactions: The suppression of bitterness by umami peptides via human bitter taste receptor

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ABSTRACT

Taste–taste interactions often showed in human psychophysical studies. Considering that each tastant in foodstuffs individually stimulates its responsible gustatory systems to elicit relevant taste modalities, taste–taste interaction should be performed in taste receptor cell-based assay. While umami substances have been proposed to suppress the bitterness of various chemicals in human sensory evaluation, the bitter–umami interaction has not been explored in bitter taste receptors, TAS2Rs. We investigated umami–bitter taste interactions by presenting umami peptides with bitter substance (salicin) on Ca^{2+}-flux signaling assay using hTAS2R16-expressing cells. Five representative umami peptides (Glu-Asp, Glu-Glu, Glu-Ser, Asp-Glu-Ser, and Glu-Gly-Ser) derived from soybean markedly attenuated the salicin-induced intracellular calcium influx in a time-dependent manner, respectively, while Gly-Gly, a tasteless peptide did not. The efficacies of Glu-Glu suppressing salicin-induced activation of hTAS2R16 were higher than that of probenecid, a specific antagonist of hTAS2R16. According to Ca^{2+}-flux signaling assay using the mixtures of salicin and umami peptides, all five umami peptides suppressed salicin-induced intracellular calcium influx in a noncompetitive manner. These results may provide evidence that umami peptides suppress bitter taste via bitter taste receptor(s). This is the first report which defines the interaction between bitter and umami taste in taste receptor level.

1. Introduction

Five basic tastes can be detected by the human gustatory system. A substantial amount of taste compounds in foodstuffs stimulate different transduction pathways, such as gustatory receptors, G-protein coupled receptors (GPCRs) and ion channels, and elicit taste qualities [1]. Although each tastant has a specific taste, multiple taste stimuli, tastant concentration, and other factors can enhance or suppress the taste of foodstuffs. For example, the bitterness of caffeine inhibits the sweetness of sucrose. At low concentrations, the salt–sour interaction and sour–bitter interaction enhance each taste, but saltiness suppresses bitterness [2–4].

Umami also interacts with other tastes. One feature of umami is the suppression of bitterness [5–9]. Suppressing bitter taste is important, since bitterness has a negative hedonic impact on food intake. MSG at moderate/high concentrations, MSG plus adenosine monophosphate, or sodium salts of 5′-ribonucleotides inhibit bitter tastes [5]. Monopotassium glutamate (MPG) plus inosine 5′-monophosphate (IMP) suppressed the response of C57BL/6J mice to quinine hydrochloride, according to the single-unit activity recorded in neurons [7]. Not only amino acids but also umami peptides attenuate the bitterness. Umami-active acidic oligopeptides containing glutamyl peptides, including glutamic acid (Glu)-aspartic acid (Asp), Glu-Glu, Glu-serine (Ser), and Glu-Glu-Glu, suppress the bitter flavors of protein hydrolysates [8,9].

Most of the taste interactions including umami–bitter interactions have been demonstrated on human sensory evaluation and in the chorda tympani (CT) and parabrachial nucleus (PbN) [10–15]. A cell-based assay using bitter taste receptors, TAS2Rs is regarded as an effective assay for specifically investigating umami–bitter interactions, but thus far it has not been frequently used. Twenty-five human TAS2Rs (hTAS2Rs) have been identified, all in the same transduction pathway involved in the perception of bitter taste in taste receptor cells [16]. Bitter substances bind to TAS2Rs, which then activate G-protein and phospholipase C (PLC). Phosphatidylinositol-4,5-bisphosphate is hydrolyzed subsequently, producing diacylglycerol (DAG) and inositol triphosphate (IP_3), second messengers of G-protein-coupled receptor (GPCR) signaling. Finally, intracellular Ca^{2+} level is enhanced by releasing

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Ca^{2+} from internal calcium stores, such as the endoplasmic reticulum (ER). Therefore, the enhanced intracellular Ca^{2+} level by tastants was estimated to evaluate bitterness of tastants.

In this study, we investigated whether the umami peptides suppressed bitter taste by inhibiting binding of bitter ligand to bitter taste receptors. Five soybean-derived peptides, Glu-Asp, Glu-Glu, Glu-Ser, Asp-Glu-Ser, and Glu-Glycine (Gly)-Ser [8] for umami peptides and salicin for bitter substance were used to monitor the bitterness-suppressing effects. Of 25 hTAS2Rs, hTAS2R16 was employed because hTAS2R16 was relatively expressed well and broadly activated by diverse ligands compared to most of hTAS2Rs [18]. The suppressing efficacies of five peptides were compared with that of a commercially available antagonist of hTAS2R16, probenecid. Furthermore, the types of inhibition of glutamyl peptides were defined from nonlinear regression of concentration–response curves.

2. Materials and methods

2.1. Materials

Salicin, probenecid, and Gly-Gly were purchased from Sigma–Aldrich (St. Louis, MO, USA). Three dipeptides (Glu-Asp, Glu-Glu, and Glu-Ser) and two tripeptides (Asp-Glu-Ser and Glu-Gly-Ser) were synthesized from Lugen Sci (Seoul, Republic of Korea).

2.2. Cell culture and transfection

Plasmids for hTAS2R16 and G16αgust44 were constructed as described previously [17]. hTAS2R16and G16αgust44 were cloned into a CMV promoter-based vector and expressed constitutively. hTAS2R16 was cotransfected with the G16αgust44 expression plasmid (4:1) into HEK293T cells using Lipofectamine 2000 (Invitrogen). At 18–24 h after transfection, the cells were used for measuring cellular responses. All cells were cultured at 37 °C in DMEM supplemented with 10% FBS. Mock-transfected cells were used as a control.

2.3. Bitter taste-suppressing effect in a cell-based assay

For calcium imaging, mock- or hTAS2R16-transfected cells were seeded onto 96-well black-wall imaging plates (BD Falcon Labware, Franklin Lakes, NJ, USA) for 18–26 h prior to use. After 18–26 h, cells were washed with assay buffer (130 mM NaCl, 10 mM glucose, 5 mM KCl, 2 mM $CaCl_2$, 1.2 mM $MgCl_2$, and 10 mM HEPES, pH 7.4) and loaded with 5 μM Fura-2 AM (Invitrogen) in assay buffer for 30 min at 37 °C. The cells were rinsed with assay buffer, incubated in assay buffer for 15 min, and then treated with 1 mM five peptides in the presence or absence of 1 mM salicin. The peptides were Glu-Asp, Glu-Glu, Glu-Ser, Asp-Glu-Ser, and Glu-Gly-Ser—known umami-taste, glutamyl peptides derived from soybean. Gly-Gly, non-bitter peptide, was used as a negative control. The bitterness-suppressing effects of peptides on hTAS2R16-expressing cells were compared with those of cells treated with only salicin. The fluorescence intensity of Fura-2 was measured using an Andor Luca CCD camera (Andor Technology, Belfast, UK) and an inverted fluorescence microscope (IX-71; Olympus, Tokyo, Japan) and images were analyzed using MetaMorph software (Molecular Devices, Sunnyvale, CA, USA). Normalized fluorescence was calculated = $ax + b$. The initial and maximum fluorescent ratio (340/380 nm) induced by salicin were considered as 0 and 100, respectively. Then, the values of a and b were obtained by substituting (initial fluorescent ratio, 0) and (maximum fluorescent ratio, 100) instead of (x, y). All data was obtained from 100 to 200 hTAS2R16-expressing cells.

For quantitative analysis, dose–response curves were estimated by monitoring the changes on intracellular Ca^{2+} level by salicin and/or five glutamyl peptides using FlexStation III microplate reader (Molecular Devices). Calcium-4 (FLIPR® Calcium 4 Assay Kit, Molecular Devices, Sunnyvale, USA) in assay buffer was loaded to the cells for 30 min at 37 °C and then, for 15 min at 27 °C. Each chemical was added to each well and the fluorescence intensity (λ_{EX} = 485 nm, λ_{EM} = 525 nm) indicating intracellular Ca^{2+} level was monitored for 120 s. The tastants including salicin (1×10^{-3} – 10 mM) in the presence or absence of Glu-Asp (1, 2, 3 mM)/Glu-Glu (1, 2, 3 mM)/Glu-Ser (1, 2, 4, 6 mM)/Asp-Glu-Ser (1, 1.5, 2, 3 mM)/Glu-Glu-Ser (1, 2, 3 mM) were treated to hTAS2R16-expressing cells. Y axis was performed as $\Delta F/F_0$, where ΔF was changes in Calcium-4 fluorescence intensity at each time and F_0 was the initial fluorescence intensity. Because umami peptides suppressed hTAS2R16 in narrow concentration range, the peptide-induced changes in the EC_{50} values and maximum of $\Delta F/F_0$ for salicin were investigated for suppressing bitterness instead of IC_{50}. The effective concentration (EC_{50}) was calculated by follow equation.

$$Y = C_0 + \frac{(C_s - C_0)}{1 + (EC_{50}/[X])^H}$$

where C_0 is the amplitude of the response in the absence of the salicin, C_s is the amplitude of the response at saturating salicin concentrations, $[X]$ is the salicin concentration, and H is the Hill coefficient. All experiments were at least triplicated.

3. Results and discussion

3.1. Glutamyl peptides inhibited salicin-induced Ca^{2+} response

All glutamyl peptides itself did not change intracellular fluorescence intensities, but partially inhibited the increase in intracellular [Ca^{2+}] caused by salicin (Fig. 1A–E). Glu-Asp, Glu-Glu, Glu-Ser, Asp-Glu-Ser, and Glu-Gly-Ser (1 mM) blocked 57.2%, 70.3%, 55.9%, 61.0%, and 43.7% of salicin-induced increase of Ca^{2+} influx on hTAS2R16-expressing cells, respectively. This may be the specific characteristic of umami peptides because tasteless peptide, Gly-Gly did not change salicin-induced Ca^{2+} influx on hTAS2R16-expressing cells (Fig. 1F). In addition, the blocking capacities of Glu-Glu and Asp-Glu-Ser at 1 mM on salicin-induced Ca^{2+} influx were higher than that of probenecid. Glu-Asp and Glu-Ser at 1 mM reduced salicin-induced Ca^{2+} influx which was almost identical with probenecid while Glu-Gly-Ser at 1 mM less decreased salicin-induced Ca^{2+} influx than probenecid. That is, of five glutamyl peptides, Glu-Glu and Asp-Glu-Ser were better antagonists against hTAS2R16 than probenecid at 1 mM.

The effects of glutamyl peptides on bitterness were not investigated previously in hTAS2R16-expressing cells, but in human sensory evaluation. Asp and Glu were reported to be effective at reducing the bitterness of solutions containing low concentrations of bitter amino acids [19]. According to Arai et al., Glu-Asp and Glu-Glu suppressed bitterness very effectively in psychophysical studies [8]. Glu-Ser and Glu-Gly-Ser also suppressed bitterness effectively, but less so than Glu-Asp and Glu-Glu. Glu-Gln-Glu, Glu-Glu-Glu, and Ser-Glu-Glu are also very effective, and Glu-Thr, Glu-Gly-Glu, Glu-Glu-Glu-Glu, and Glu-Glu-Glu-Glu-Glu are effective. Asp-Glu-Ser is also found in acidic fraction of chicken protein which had umami taste [17]. Maehashi et al. insisted that 1% synthesized Asp-Glu-Ser showed strong sourness, weak saltiness, and no umami in psychophysical studies, however, these characteristics may be generated from pH condition, pH 6.0. Glu-enriched plastein hydrolysates, especially acidic oligopeptides,

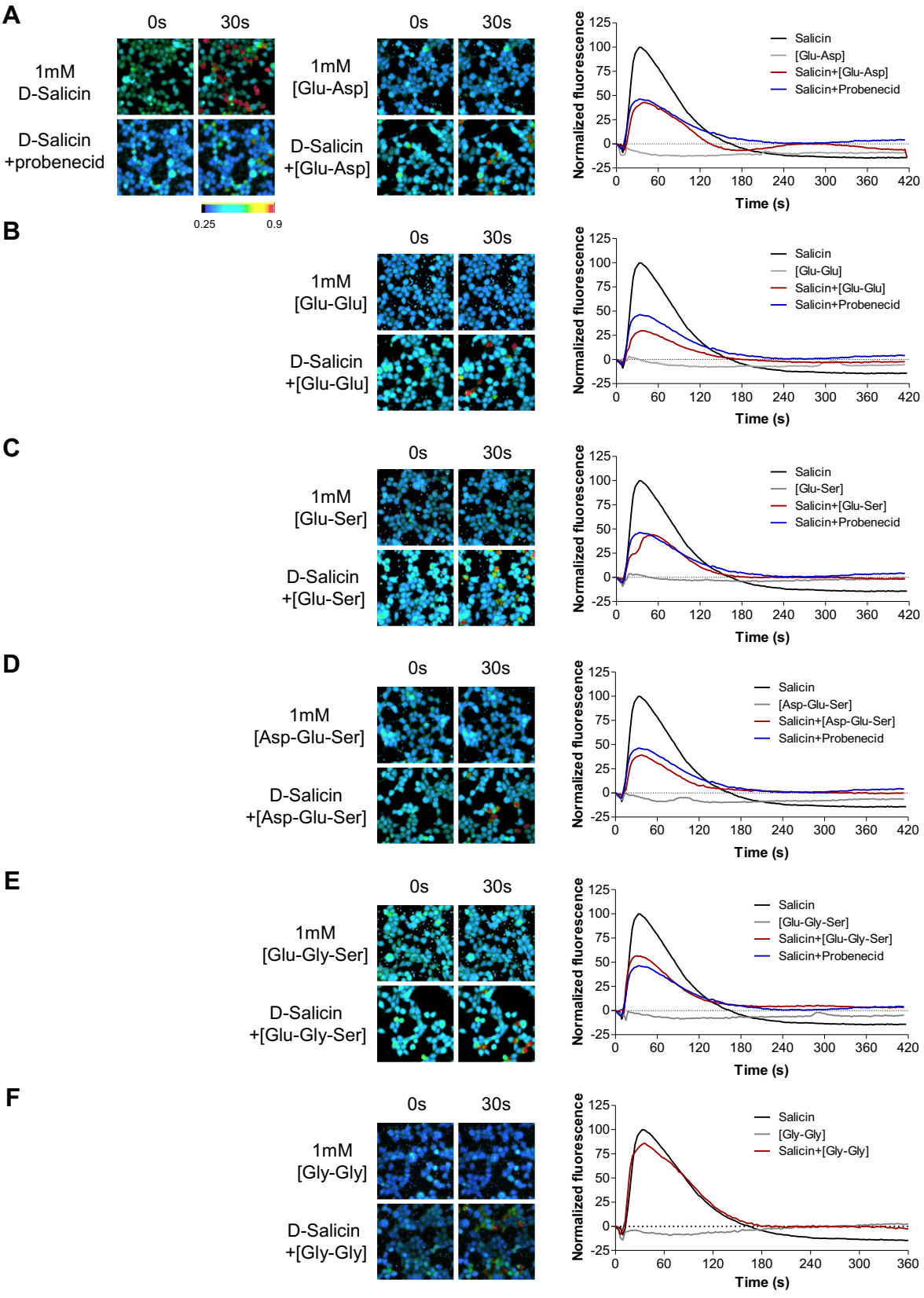

Fig. 1. Suppression of salicin-induced Ca^{2+} influx in hTAS2R16-expressing cells by five glutamyl peptides. According to calcium imaging, Glu-Asp (A), Glu-Glu (B), Glu-Ser (C), Asp-Glu-Ser (D), and Glu-Gly-Ser (E) at 1 mM partially blocked 1 mM salicin activity on hTAS2R16. Gly-Gly (F), tasteless peptide, had no effect on hTAS2R16-expressing cells.

are associated with suppressing the bitter taste of bitter substances [6,20]. Glu-Glu is considered one of the strongest bitterness-suppressing peptides in psychophysical studies [6] and this result was consistent with our result using hTAS2R16-expressing cells. On the other hand, the relation between the composition of amino acids in glutamyl peptides and inhibitory efficacy of bitterness has

to be performed in the further study to explain umami–bitter interaction.

3.2. Five glutamyl peptides were noncompetitive inhibitors on hTAS2R16-expressing cells

Then, the effects of five glutamyl peptides on salicin-induced intracellular Ca^{2+} level on hTAS2R16-expressing cells were estimated in the concentration–response curves (Fig. 2) and the types of inhibition of glutamyl peptides were estimated. Salicin (0.001–10 mM) activated hTAS2R16 and elevated intracellular Ca^{2+} level in a dose-dependent manner same as that shown in the result by Bufe et al. [22] and Maehashi et al. [23]. EC_{50} value of salicin was 0.417 mM in the absence of glutamyl peptides. The addition of glutamyl peptides in hTAS2R16-expressing cells in the presence of salicin induced the sigmoidal curves of salicin shifting to the right. The shifted EC_{50} values for salicin were 0.4472, 0.5721, and 1.595 mM by 1, 2, and 3 mM Glu-Asp, respectively; 0.5291 and 2.695 mM by 1 and 2 mM Glu-Glu, respectively; 0.4680, 0.4682, and 0.5434 mM by 1, 2, and 4 mM Glu-Ser, respectively; 0.5433 and 0.8721 mM by 1 and 1.5 mM Asp-Glu-Ser, respectively; 0.5948 mM by 1 mM Glu-Gly-Ser. Salicin-induced responses to hTAS2R16-expressing cells were highly inhibited by 3 mM Glu-Glu, 6 mM Glu-Ser, 2/3 mM Asp-Glu-Ser, and 2/3 mM Glu-Gly-Ser, so EC_{50} value of salicin was not estimated in the range of 0.001–10 mM. In addition, IC_{50} value for each glutamyl peptide was not capable to calculate because glutamyl peptides affected salicin-induced responses in the narrow concentration range. Moreover, the maximum of $\Delta F/F_0$ on sigmoid curves also decreased by glutamyl peptides in a dose-dependent manner. This was a similar inhibition pattern reported by Greene et al. [21]. A known hTAS2R16 antagonist, probenecid changed the maximum signal and EC_{50} values of salicin in dose–response curves and defined as a non-competitive allosteric inhibitor. Therefore, all five glutamyl peptides were also considered as noncompetitive allosteric inhibitors against salicin-induced Ca^{2+} influxes on hTAS2R16-expressing cells according to nonlinear regression of concentration–response curves from these experiments.

Although peptides show complex taste characteristics, consistent patterns in taste characteristics of peptides have been observed [24]. Some L-glutamyl di- and tri-peptides have an MSG-like taste and dipeptides containing Thr and Ser may be regarded important for the umami taste [6]. Furthermore, many dipeptides, including Arg-Pro, Asp-Asp, Arg-Asp, pGlu-Gln, pGlu-Gly, Val-Pro, Asp-Glu, Gly-Phe, Ala-Pro, Glu-Glu, and Thr-Glu, are also positively correlated with umami taste [25]. Glu-enriched oligopeptides and Asp-containing dipeptides also evoked an umami taste. The highly acidic (hydrophilic) L-glutamyl oligopeptides, which contain an N-terminal Glu residue and a hydrophilic C-terminal L-Glu residue in dipeptides, possessed an MSG-like flavor and an umami taste [9].

As shown in our results, the taste–taste interaction between umami and bitter taste was proved in a cellular level, unlike previous studies which were performed using psychophysical studies. Five umami peptides directly bound to one of bitter taste receptors, hTAS2R16 in the different binding region against orthosteric ligand site and interrupted the activity of hTAS2R16 agonists, salicin on hTAS2R16. Although taste–taste interaction was observed in taste receptor cells in this study, more taste interactions have to be considered in different aspects [1,26]. One is that cell-to-cell communication in taste buds has to be concerned. Type II receptor cells can detect sweet, umami, and bitter taste, while type III presynaptic cells can detect sour. After stimulation of sweet, umami, or bitter substances to each taste receptor cells, ATP is released from type II receptor cells via pannexin channels and stimulates gustatory-related nerve fibers as well as adjacent presynaptic cells. Stimulated presynaptic cells can secret neurotransmitters such as 5-HT. Released 5-HT inhibits the activation of type II receptor cells, but stimulates nerve fibers connected to type III receptor cells. The other being considered is cognitive interaction. Although single compound in the mixture stimulates each taste receptor cell, the afferent signal reaching the brain is decoded and mixture suppression occurs that the intensities of each compound in the mixture are perceived not the same as those in the single solution.

In this study we showed that umami peptides derived from soybean reduced the response to bitter taste in a cell-based assay using hTAS2R16-expressing cells. Five glutamyl peptides (Glu-Asp, Glu-Glu, Glu-Ser, Asp-Glu-Ser, and Glu-Gly-Ser) partially

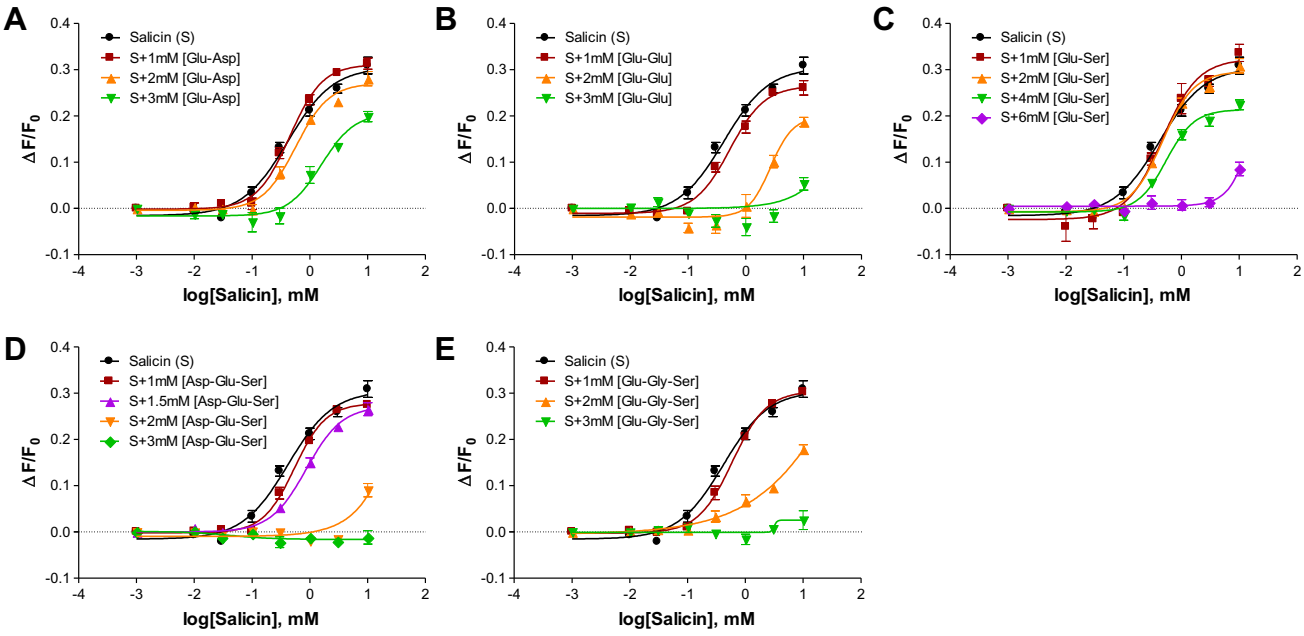

Fig. 2. Dose-dependent changes in intracellular Ca^{2+} level induced by salicin in the absence and presence of five glutamyl peptides in hTAS2R16-expressing cells. Glu-Asp (A), Glu-Glu (B), Glu-Ser (C), Asp-Glu-Ser (D), and Glu-Gly-Ser (E) shifted the salicin dose–response curve to the right with changing maximum of $\Delta F/F_0$.

suppressed the human bitter receptor hTAS2R16. Glu-Glu at 1 mM was the most effective inhibitor among five glutamyl peptides. Salicin activity on hTAS2R16-expressing cells was more suppressed by Glu-Glu than by probenecid, known hTAS2R16 antagonist. Based on glutamyl peptide-induced changes in EC_{50} values and maximum of $\Delta F/F_0$ for salicin in concentration–response curves, five glutamyl peptides noncompetitively inhibited the salicin-induced intracellular Ca^{2+} response. Taste–taste interactions are important for food product development and modification and are so complicated that many aspects have to be considered. In order to understand better about umami–bitter interaction, further research about cell-to-cell interaction and cognitive interaction is needed. However, we investigated umami–bitter taste interaction in the cellular level and these successful results will bring development about comprehension of taste–taste interaction. Additionally, our results suggest that one of the negative hedonic impacts on food intake, bitter taste can be reduced by taste mixtures, especially umami peptides. This is the first report which defines the interaction between bitter and umami taste in taste receptor level.

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