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Note

Human Bitter Taste Receptors hTAS2R8 and hTAS2R39 with Differential Functions to Recognize Bitter Peptides

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The strong bitter peptide, Phe-Phe-Pro-Arg, activated cultured cells expressing either of the known human bitter taste receptors, hTAS2R8 and hTAS2R39. The partial structure of Pro-Arg activated hTAS2R39, but did not activate hTAS2R8. These receptors may not indiscriminately recognize bitter peptides, but have a differential function in their recognition.

Key words: bitterness; taste receptor; bitter peptide

Many peptides derived from hydrolysates of casein or seed storage proteins are well known to elicit strong bitterness.[1–3] More than 200 kinds of bitter peptides have been found to date, but they vary greatly in their amino acid sequences.[4] Numerous studies have been conducted to elucidate the structure-bitterness correlation of bitter peptides, indicating the involvement of their hydrophobicity, sequence, and length.[5–8]

Bitter substances are recognized in humans by human bitter taste receptors (hTAS2Rs) which belong to a family of G-protein coupled receptors comprising 25 members and are expressed in a subset of oral taste receptor cells.[9] Several papers have reported that hTAS2R1 was activated by some bitter peptides and a casein hydrolysate.[10,11] It is likely that other hTAS2Rs which undergo activation by some other bitter peptides could exist if we consider the vast structural diversity of these peptides. This study attempts to clarify the existence of another hTAS2R that responds to bitter peptides.

We first used the highly water-soluble, strongly bitter tetrapeptide, L-Phe-L-Phe-L-Pro-L-Arg (FFPR), with a recognition threshold of almost 0.1 mM in a human sensory test.[7] This peptide was purchased from Watanabe Chemical Industries (Hiroshima, Japan) as H-Phe-Phe-Pro-Arg-OH·2HCl with 99% purity. A cell-based bitterness assay was conducted with human embryonic kidney 293T (HEK293T) cells that were transiently expressing either hTAS2R8 or hTAS2R39, which was N-terminally tagged with a 45-amino acid sequence derived from rat somatostatin receptor type 3, together with a chimeric G-protein (hGα16gust44). The response was examined by a calcium imaging analysis as previously described.[12,13] The transfected cells were washed with the assay buffer (130 mM NaCl, 10 mM glucose, 5 mM KCl, 2 mM $CaCl_2$, 1.2 mM $MgCl_2$, and 10 mM HEPES at pH 7.4) and then loaded with 5 μM fura-2 AM (Invitrogen), a fluorescent calcium indicator, for 30 min at 27 °C. The cells were washed again with the assay buffer and incubated in 100 μL of the same buffer for 10 min at 27 °C, prior to being stimulated by adding 100 μL of a 2× ligand solution. The change in the intracellular calcium ion concentration was measured for randomly selected DsRed2-positive cells that were regarded as transfected cells and presented as the ratio of the fluorescence intensity values at two wavelengths (F_{340}/F_{380}). The cells were considered to be responsive when the increase in F_{340}/F_{380} was greater than 0.1 within 30 s after applying the ligand.

The HEK293T cells expressing either hTAS2R8 or hTAS2R39 clearly responded to 10 mM FFPR in an aqueous solution at pH 7.4 (Fig. 1A and B), while similar cells expressing hTAS2R1, which has been reported as a receptor of bitter peptides,[10,11] did not respond to FFPR. We also used the cells expressing one of 22 hTAS2Rs and found that each did not show any response to FFPR (data not shown). The response of the hTAS2R8- or hTAS2R39-expressing cells to FFPR was dose-dependent with an EC_{50} value of 1.1 ± 0.8 mM in hTAS2R8 and 8.4 ± 1.8 mM in hTAS2R39 (Fig. 1C).

We also examined whether or not these receptors could also recognize the partial structures of FFPR. We used the following amino acids and dipeptides for the examination: L-phenylalanine, L-proline, L-arginine, L-Phe-L-Phe (FF), L-Phe-L-Pro (FP), and L-Pro-L-Arg (PR). These amino acids and dipeptides are known to elicit a slight or moderate degree of bitterness in a human sensory test.[4] The amino acids were purchased from Kanto Chemical Co. (Tokyo, Japan) and the dipeptides from Bachem (Bubendorf, Switzerland). We also examined the tripeptide, L-Phe-L-Pro-L-Arg (FPR), which was purchased from Bachem, but FPR could not be used as a ligand under our experimental conditions because it responded to hGα16gust44-expressing HEK293T cells which did not express hTAS2Rs (data not shown). We applied each of these ligands at 10 mM, except for FF that was used at 1 mM because of its low

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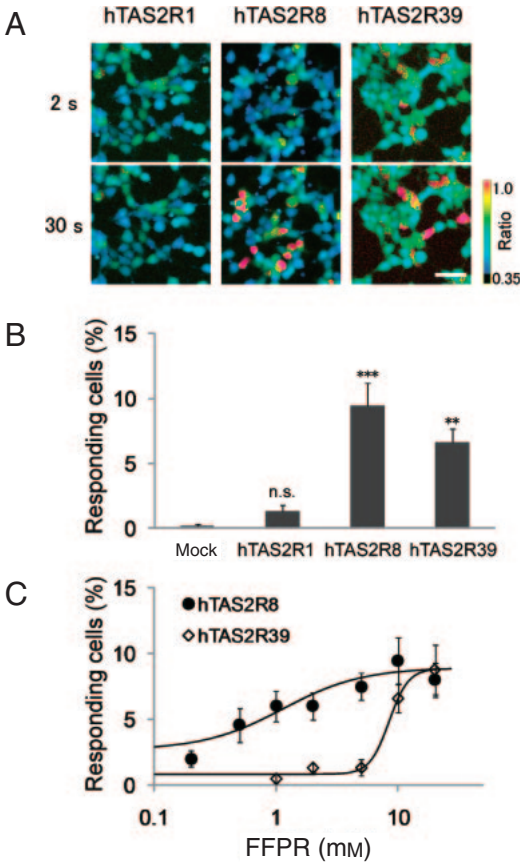

Fig. 1. Phe-Phe-Pro-Arg (FFPR)-Activated HEK293T Cells Expressing hTAS2R8 or hTAS2R39 Together with hGα16gust44.

A and B show responses of the HEK293T cells expressing either hTAS2R1, hTAS2R8, or hTAS2R39 together with hGα16gust44 to 10 mM FFPR. A, Representative ratiometric images of fura-2-loaded HEK293T cells. The top and bottom columns respectively show the representative cell images obtained 2 s and 30 s after ligand application. The color scale indicates the F_{340}/F_{380} fluorescence ratio as the pseudocolor. Scale bar, 50 µm. B, The response is regarded as positive when the increment in the F_{340}/F_{380} fluorescence ratio was larger than 0.1 within 30 s after ligand application. Each bar indicates the mean ± SEM from at least 6 independent measurements. The statistical significance of differences between the control (mock-transfected cells) and bitter taste receptor-expressing cells was tested by one-way ANOVA and subsequent Dunnett's test. ***, $p < 0.001$; **, $p < 0.01$; n.s., not significant *vs.* mock-transfected cells. C, Dose-response relationship between FFPR and HEK293T cells expressing either hTAS2R8 (circles) or hTAS2R39 (squares) together with hGα16gust44. Each point indicates the mean and SEM values from at least 3 independent measurements. Plots of the concentration *vs.* amplitude were fitted by using Hill's equations.

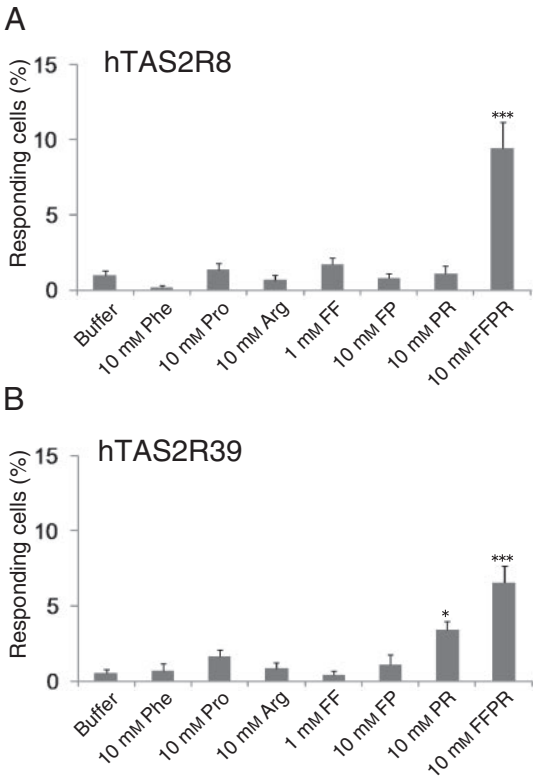

Fig. 2. Responses of HEK293T Cells Expressing Either hTAS2R8 (A) or hTAS2R39 (B) Together with hGα16gust44 to Individual Amino Acids and Di-Peptides of FFPR.

Each bar indicates the mean and SEM values from two independent measurements. The statistical significance of the differences between the responses to the buffer and ligand solutions was determined by one-way ANOVA and subsequent Dunnett's test; a p-value <0.05 is considered to be indicative of statistically significant difference. ***, $p < 0.001$; *, $p < 0.05$ *vs.* buffer.

solubility. Figure 2 shows the clear response of cells expressing hTAS2R39 for PR as well as for FFPR, whereas the cells expressing hTAS2R8 showed no response to 10 mM PR. None of the amino acids or the other dipeptides activated either of the receptors used. The result suggests that these bitter taste receptors did not indiscriminately recognize bitter peptides, although recognizing FFPR itself (Fig. 1).

Previous studies have reported hTAS2R1 to be a receptor for several bitter peptides, but no report was found on the activation of other hTAS2Rs by bitter peptides. We found for the first time in this study that the two human bitter taste receptors, hTAS2R8 and hTAS2R39, were activated by the bitter tetrapeptide, FFPR. Since the bitterness threshold of FFPR obtained in our *in vitro* experiment with hTAS2R8 was found to closely resemble the value obtained in a human sensory test,[7] hTAS2R8 could be used to measure the bitterness of some bitter peptides, including FFPR. The results of our study also indicate that not all bitter peptides could activate hTAS2R1 (Fig. 1), clearly indicating that several hTAS2Rs, including hTAS2R8 and hTAS2R39, participate in the recognition of bitter peptides corresponding to their large structural diversity.

Interestingly, PR, as a substructure of FFPR, activated hTAS2R39 but did not activate hTAS2R8 (Fig. 2), although the response of hTAS2R8 was more sensitive to FFPR than that of hTAS2R39. hTAS2R8 and hTAS2R39 may probably recognize different substructures of FFPR. Since the amino acid sequences of hTAS2R8 and hTAS2R39 share only a 29% identity, it is thought that the structure of the ligand recognition site of each receptor was substantially different from the other. This is one reason why PR could activate hTAS2R39, but not hTAS2R8. Furthermore, the degree of hTAS2R39 activation by PR observed in our study is comparable with the threshold value obtained in a sensory test of *ca.* 3 mM.[4]

In contrast, neither hTAS2R8 nor hTAS2R39 was activated by FP which had a lower threshold than PR (*ca.* 1.5 mM). It is suggested that bitter peptides other than FFPR and PR could possibly be recognized by other hTAS2Rs. A number of hTAS2Rs are present in the human oral cavity and a great many bitter substances

are contained in foods, and there must thus be a variety of ligand-receptor combinations. Details await further experiments.

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