Chem. Senses 30 (suppl 1): i23–i24, 2005 doi:10.1093/chemse/bjh094

Regional Expression Patterns of T1r Family in the Mouse Tongue

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Key words: taste receptor, circumvallate papillae, fungiform papillae, umami, sweet, gustducin

Introduction

The *T1r* family is one of the receptor family belong to class C type of G protein coupled receptors, and comprised of three taste bud-specific receptors, *T1r1*, *T1r2* and *T1r3* (Nelson *et al.*, 2001). *T1r1* and *T1r2* are known to have distinctive patterns of regional expression, respectively (Hoon *et al.*, 1999). *T1r1* is expressed in taste buds in the fungiform papillae, but is rare in the taste buds of circumvallate papillae. In contrast, *T1r2* is rarely expressed in fungiform papillae but is expressed in all taste buds of the circumvallate papillae. *T1r3* is strongly expressed in both fungiform and circumvallate papillae (Kitagawa *et al.*, 2001; Nelson *et al.*, 2001) and forms an amino-acid (umami) receptor and a sweet receptor in combination with *T1r1* and *T1r2*, respectively (Nelson *et al.*, 2001; Li *et al.*, 2002). These expression patterns suggest that taste cells in circumvallate papillae receive the sweet taste substances through the heterodimer of *T1r2* and *T1r3* (*T1r2*/*T1r3*), and those in fungiform papillae receive the umami substances through the heterodimer of *T1r1* and *T1r3* (*T1r1*/*T1r3*). However, physiological studies in mice showed that taste receptor cells in the anterior as well as in the posterior parts of the tongue were sensitive to both sweet and umami substances (Ninomiya *et al.*, 1993). This contradiction may suggest an inadequacy of information about the expression of *T1r* family.

We compared *T1r* receptors (*T1rs*) expression pattern between circumvallate and fungiform papillae in mice using double-colored *in situ* hybridization. Furthermore, we examined the expression patterns of *T1rs* and the taste cell specific G protein, *gustducin*, in order to compare taste signal transductions in circumvallate and fungiform papillae.

Results and discussion

Comparison of *T1rs* expression in circumvallate and fungiform papillae

The expression patterns of *T1rs* were examined by double-colored *in situ* hybridization using all combinations of *T1rs* cRNA probes to obtain further information about the regional expression patterns of *T1rs*. In circumvallate papillae, we found that *T1r1* was expressed in larger numbers of taste cells than *T1r3*, and that the majority of *T1r3*-positive cells were included among the *T1r1*-positive cells (Figure 1). This result raised the possibility that *T1r3*-positive cells not only mediate sweet taste through *T1r2*/*T1r3* as reported previously, but also madiate umami taste through *T1r1*/*T1r3* in the circumvallate papillae. This novel finding is consistent with the nerve recording data (Ninomiya *et al.*, 1993), but is not consistent with the data of *T1r3* knock out mouse (Damak *et al.*, 2003). Therefore, we could not determine the role of *T1r1* in the circumvallate papillae. We also found that the signal intensity of *T1r1* was lower than those of *T1r2* and *T1r3*, suggesting that *T1r1* expression in circumvallate papillae was weaker than *T1r2* and *T1r3*. Taking this result and the

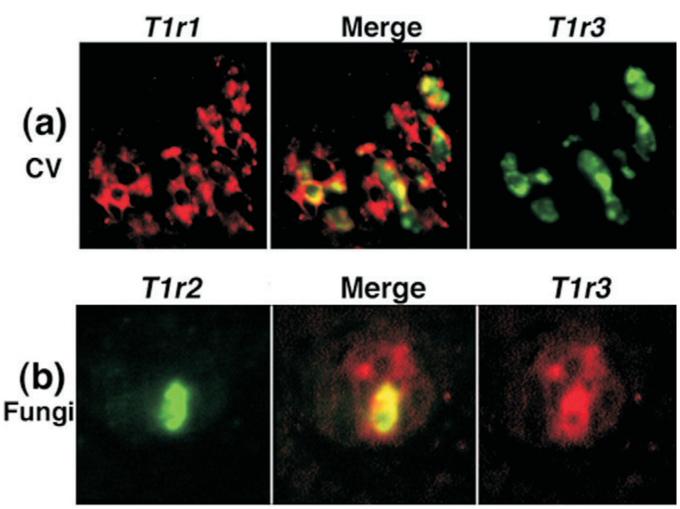

Figure 1 Expression patterns of *T1r*s in circumvallate and fungiform papillae. **(a)** *T1r3*-expressing cells were included among the *T1r1*-expressing cells in circumvallate papillae. **(b)** *T1r2*-expressing cells were included among the *T1r3*-expressing cells in fungiform papillae.

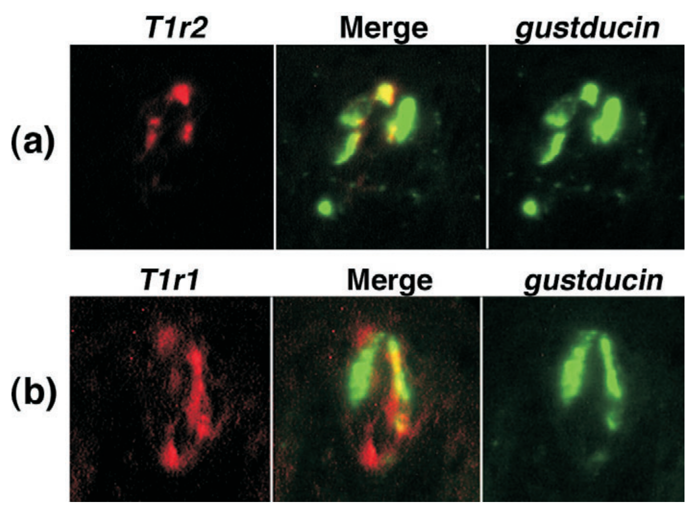

Figure 2 Co-expression patterns of *T1rs* and *gustducin* in fungiform papillae. **(a)** *T1r2*-expressing cells were included among the *gustducin*-expressing cells. **(b)** A part of *T1r1*-expressing cells co-expressed *gustducin*.

data of *T1r3* knock out mouse into consideration, *T1r1*/*T1r3* might be related to the restricted part of the umami taste signal transduction, and other receptors such as mGluR1 and mGluR4 might play central roles in this transduction. In fungiform papillae, we found that *T1r2* was expressed in restricted taste cells (Figure 1). Double-colored *in situ* hybridization showed that about half of *T1r3*-positive cells expressed *T1r2*, and *T1r2*-positive cells were included among the *T1r3*-positive cells (Figure 3). We also observed that the expression of *T1r1* and *T1r2* partly overlapped each other. Because almost all *T1r2*-positive cells were observed to express *T1r3*, *T1r1* and *T1r2*-positive cells were considered to express *T1r3*. These data suggest that sweet and umami taste signal transduction in fungiform papillae are mediated by *T1r1*/*T1r3* and *T1r2*/*T1r3*, respectively, corresponding to the nerve recording data in which chorda tympani (CT) nerves innervating the fungiform papillae respond to both sweet and umami stimuli (Ninomiya *et al.*, 1993).

Expression patterns of *T1rs* with *gustducin* in circumvallate papillae and fungiform papillae

Gustducin, a taste cell-specific G protein, has been considered to play roles in bitter, sweet and umami taste signal transduction, based on behavioral and electrophysiological studies using *gustducin*-null mutant mice (Wong *et al.*, 1996; Ruiz *et al.*, 2003). However, histological studies in taste buds of circumvallate papillae indicated that *gustducin* and the sweet receptor *T1r2*/*T1r3* were expressed separately (Nelson *et al.*, 2001), while bitter taste receptors, *T2rs* were co-expressed with *gustducin* (Adler *et al.*, 2000). Moreover, there is little information about the co-expression pattern of *T1r1* and *gustducin*. Accordingly, it was hard to understand how *gustducin* was associated with the sweet and umami taste receptors. We considered that the confusion might be due to differences in the taste cells tested in behavioral and histological experiments. The data of the two bottle choice tests using *gustducin*-null mutant mice (Wong *et al.*, 1996; Ruiz *et al.*, 2003) reflect the total responses of all taste cells on the tongue. On the other hand, the results from histological experiments using sections of circumvallate papillae show the expression profile of taste cells in the circumvallate papillae only (Adler *et al.*, 2000; Nelson *et al.*, 2001). Therefore, we hypothesized that the role of *gustducin* in the fungiform papillae were different from that in the circumvallate papilla, and then compared the expression patterns of *gustducin* with *T1rs* in the fungiform papillae.

In fungiform papillae, *T1r2*-positive cells were included among the *gustducin*-positive cells (Figure 2). As mentioned above, *T1r2*-positive cells were also included among *T1r3*-positive cells, therefore, every *T1r2*- positive cell co-expressed *T1r3* and *gustducin* in the fungiform papillae (Figure 3). Moreover, a part of *T1r1*-positive cells co-expressed both *T1r3* and *gustducin* (Figures 2 and 3). These results raise the possibility that taste cells in fungiform papillae respond to sweet and/or umami substances through *T1r2*/*T1r3* and/or *T1r1*/*T1r3*, and then transduce the signals by *gustducin*. This speculation is in agreement with the physiological and behavioral data of *gustducin*-null mouse (Wong *et al.*, 1996; Ruiz *et al.*, 2003).

To summarize, we observed different expression patterns of *T1rs* and *gustducin* in circumvallate and fungiform papillae. These findings suggested for the first time that *gustducin* might be involved in different taste signal transductions in the circumvallate papilla and the fungiform papillae, and might play a role in sweet and umami taste signal transduction in fungiform papillae, from the view point of gene expression.

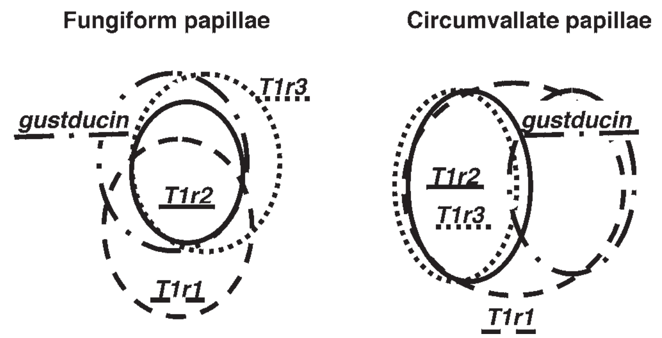

Figure 3 Diagrams of expression patterns of *T1rs* and *gustducin* in fungiform and circumvallate papillae. These diagrams are based on the results of double-colored *in situ* hybridization and supplemented with our speculation. The areas of circles designate the numbers of cells expressing *T1r1*, *T1r2*, *T1r3* or *gustducin*. The overlapped area represents the co-expression ratio.

Acknowledgements

This work was supported by Bio-Oriented Technology Research Advancement Institution and Japan Society for the Promotion of Science. We thank Ms Hashimoto, Ms Okano, Ms Kato and Ms Tagami for technical help, and thank Mr Matsushita for providing the experimental mice.

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