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Expression of Phospholipase C-β4 in Rat Circumvallate Taste Buds

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Introduction

The heteromer of T1R1 and T1R3 and taste-mGluR4 function as receptors for glutamate (umami) taste sensation (Chaudhari *et al.*, 2000; Nelson *et al.*, 2002). Metabotropic glutamate receptor type 1 (mGluR1α) is expressed in taste receptor cells in rat gustatory papillae (Toyono *et al.*, 2003). It has been known that mGluR1α couples preferentially to an α-subunit of the Gαq family, leading to activation of phospholipase C-β (PLC-β) and the consequent mobilization of intracellular Ca^{2+} levels in the central nervous system (Hermans and Challiss, 2001). The inositol tri-phosphate (IP_3) pathway is involved in taste transduction for glutamate in mouse fungiform papilla (Ninomiya *et al.*, 2000). Applications of glutamate and the mixture of GMP and glutamate to rat taste cells increase intracellular Ca^{2+} levels (Lin *et al.*, 2003). Thus, mGluR1α may be a candidate for another type of umami receptors because mGluR1α plays some roles in IP_3 pathway and the mobilization of intracellular Ca^{2+}.

Recent studies have provided evidence that the members of Gαq family, Gαq, Gα14, Gα15 and PLC-β2, are expressed in rat taste buds (Kusakabe *et al.*, 1998; Rössler *et al.*, 1998). Further, PLC-β2 is known to co-expressed with IP_3 type III receptor (IP_3R3) in rodent circumvallate taste buds (Clapp *et al.*, 2001). PLC-β2 generates IP_3, which then activates IP_3R3 of intracellular Ca^{2+} stores in taste cells. In this context, it is conceivable that there may exist a similar signaling cascade via mGluR1α in umami taste sensation. However, no mention has so far been made of the expression patterns of mGluR1α and these signaling molecules in rat taste bud cells.

There are four different PLC-β isoforms (PLC-β1–4) that have been cloned (Rhee and Bae, 1997). They are all regulated by heterotrimeric G proteins and there is evidence suggesting that different isoforms may be involved in a variety of signaling circuits. PLC-β can be activated by both the Gα subunits of the Gαq family and by the βγ subunits generated by a number of different heterotrimeric G proteins. On the other hand, PLC-β4 can be activated by Gαq but not by βγ-subunits of G-proteins (Jiang *et al.*, 1994). The major molecular cascade from mGluR1 to PLC-β is considered to be mGluR1–Gαq–PLC–β4 in Purkinje cells (Hirono *et al.*, 2001). In view of these respects, we deduced that PLC-β4 might contribute the mGluR1-mediated signal transduction in taste sensation. However, a search of the literatures fails to reveal the expression of PLC-β4 in rat taste tissues.

In the present study, we examined for the first time the expression patterns of mGluR1α and taste signaling molecules, Gαq and PLC-β2 in rat circumvallate papillae. In addition, we examined the expression PLC-β4 mRNA and its protein in gustatory papillae and taste buds by using reverse transcription–polymerase chain reaction (RT–PCR) and immunohistochemistry.

Materials and methods

The methods used in this study were approved by the Institutional Animal Care and Use Committee of Kyushu Dental College.

In situ hybridization

Adult SD rats were anesthetized with chloral hydrate (350 mg/kg) and transcardially perfused with a fixative containing 4% paraformaldehyde (PFA) in 0.1 M phosphate buffer (pH 7.3) for 15 min. The areas of circumvallate papillae were dissected out and rinsed overnight with 0.1 M phosphate buffer containing 30% sucrose. Tissues were then embedded in Tissue-Tek and snap-frozen in a dry ice-isopentane mixture. *In situ* hybridization analysis for mGluR1 were performed as described previously (Toyono *et al.*, 2003).

Immunohistochemistry

The frozen sections were prepared in similar manners to as described above. Sections were washed in PBS, and incubated for 12 h at room temperature in a solution containing rabbit polyclonal antibody against rat PLC-β4 (Santa Cruz Biotechnology) at a dilution of 1:1500. Alexa™488-conjugated goat anti-rabbit IgG (1:200; Invitrogen) was used as a secondary antibody. Negative controls to immunofluorescence staining were performed by replacing the primary antibodies with PBS or by pre-incubating of primary antibodies with cognate peptides. After immunostaining for PLC-β4, double-labelled experiments were performed with Alexa Fluor 546-labelled PLC-β2 rabbit polyclonal antibody (Santa Cruz biotechnology). This labelled antibody was prepared with the Zenon Rabbit IgG Labeling Kits (Invitrogen).

After *in situ* hybridization, some sections were analyzed for co-expression of mGluR1-mRNA and Gαq-protein, or of mGluR1-mRNA and PLC-β2-protein by using immnohistochemistry.

RT–PCR

RT–PCR analyses for *PLC-β4* were performed as described previously (Toyono *et al.*, 2003). Primer sequences for the PCR were as follows: *PLC-β4*, 5′-ATCGTGGCCCAGTATGACAAG-3′ (forward) and 5′-ATCTGCTGCATCTCCTTCGC-3′ (reverse); product size, 590 bp. PCR amplifications were performed under the following conditions: 94°C for 30 s, 55°C for 1 min, 72°C for 1 min for a total of 40 cycles and elongation step at 72°C for 10 min for *PLC-β4* and *GAPDH*.

Results and discussion

The expression patterns of mGluR1 and signaling molecules were examined by *in situ* hybridization and immunohistochemistry in rat circumvallate taste buds. A subset of mGluR1-expressing taste bud cells co-expressed PLC-β2. According to the study by Zhang *et al.* (2003), PLC-β2 deficient mice abolished sweet, amino acid and bitter taste perception. Their study suggested that mGluR1α might play some roles in umami taste-signaling cascades through PLC-β2. We also examined the expression of Gαq in *mGluR1* expressing cells in rat taste bud cells. Almost all *mGluR1* expressing cells co-expressed Gαq. Further, double immunolabeling experiments for Gαq and PLC-β2 showed that almost all PLC-β2 expressing cells co-expressed Gαq. In their study on the group I metabotropic glutamate receptors, Hermans and Challiss (2001), found that mGluR1α couples

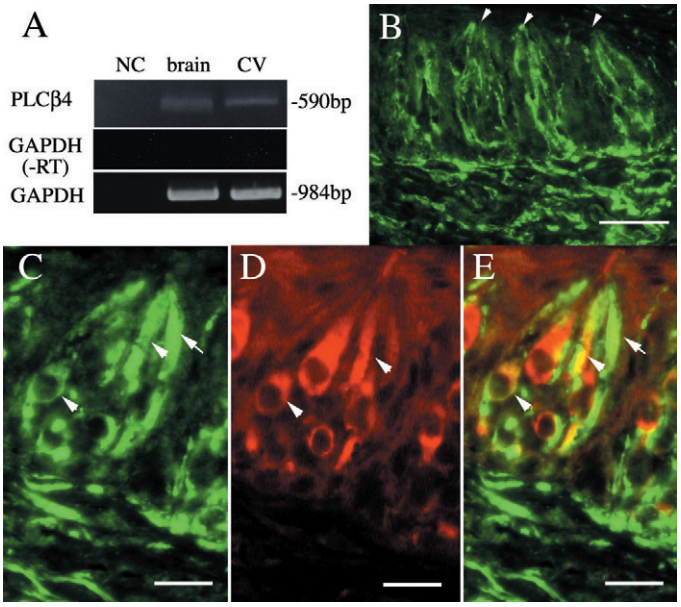

Figure 1 Expression patterns of PLC-β4 in rat circumvallate papillae. RT–PCR analyses for PLC-β4 in rat circumvallate papillae **(A)**. PCR was performed using total RNAs prepared from circumvallate papillae (CV), whole brain (brain) and distilled water (NC), amplification products of *PLC-β4* (590 bp) and *glyceraldehyde-3-phosphate dehydrogenase* (*GAPDH*, 984 bp) were obtained with each specific primer sets. The reverse transcriptase step was omitted in controls to confirm removal of all genomic DNA. PLC-β4 (green) produces intense labeling of the subset of taste bud cells and intragemmal and subgemmal nerve fibers **(B)**. Arrowheads show the taste pores. Double immunostaining for PLC-β2 (red) and PLC-β4 (green) **(C, D, E)**. PLC-β4 produces labeling of the subset of taste bud cells and intragemmal and subgemmal nerve fibers (C). PLC-β2 produces intense labeling of the subset of taste bud cells (D). The fluorescent image of PLC-β4 immunoreactivities is overlaid on the fluorescent image of PLC-β2 immunoreactivities to illustrate the co-expression in a subset of taste bud cells (arrowheads) (E). Arrow shows the cell expressing only PLC-β4. Scale bars: 25 μm (B); 10 μm (C, D, E).

preferentially to an α subunit of the Gαq family, leading to activation of PLC-β in the central nervous system. Taken together with our results it seems that, as well as the central nervous system, mGluR1α may couple with Gαq which consequently, may activate PLC-β2 in umami taste transduction in taste bud cells.

RT–PCR assay showed that PLC-β4 -mRNA expressed in circumvallate papillae (Figure 1A). In fungiform, foliate and circumvallate papillae, the antibody against PLC-β4 gave labelling of the subset of taste bud cells and intragemmal and subgemmal nerve fibers (Figure 1B). Double labeled experiments showed that a subset of PLC-β4 expressing cells also co-expressed PLC-β2 (Figure 1C–E). In the central nervous system of the mouse, despite the existence of four PLC-β isoforms (PLC-β1–4), only one or two of them is expressed in each neuron and glial cell (Watanabe *et al.*, 1998). PLC-β3 and PLC-β4 are major isoforms with lower levels of PLC-β1 in Purkinje cells. Similar to the results obtained from mouse central nervous system, PLC-β2 and PLC-β4 are expressed in a subset of taste bud cells and these isoforms may form a functional IP_3 signaling cascade, playing some roles in the taste signal transductions. PLC-β4 is known to work through mGluR1 in the mouse cerebellum, and PLC-β4-deficient mouse is reported to show ataxia (Kim *et al.*, 1997). A clue to understand the role of PLC-β4 in taste transduction may be gain from the analysis of the PLC-β4-null mouse.

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