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IP_3 receptor type 3 and PLCβ2 are co-expressed with taste receptors T1R and T2R in rat taste bud cells

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Abstract

The Ca^{2+} signaling cascade has been reported to be activated by many tastants in vertebrate taste systems. Recently we have shown that G_{i2} and phospholipase Cβ2 (PLCβ2) are co-expressed in a subset of taste bud cells and are possibly involved in Ca^{2+} triggering of taste signaling in rats. We report here that, as a component downstream of PLCβ2, the type 3 isoform of the inositol 1,4,5-trisphosphate (IP_3) receptor (IP_3R3) is specifically expressed in the same cells as PLCβ2 in rat taste buds. We also show that cells expressing rT2R9, a probable cycloheximide receptor, are included among PLCβ2- and IP_3R3-positive cells, as in the case of rT1R2, a different type of taste receptor. Our findings indicate that PLCβ2 and IP_3R3 co-localize together with G_{i2} as downstream components of two different types of taste receptors, T1R and T2R, in taste bud cells.

Introduction

In the taste buds of vertebrates the Ca^{2+} signaling cascade of PLC followed by inositol 1,4,5-trisphosphate (IP_3)-dependent Ca^{2+} release from intracellular stores has been reported to be activated by various tastants, including many bitter tastants (Akabas *et al.*, 1988; Hwang *et al.*, 1990; Spielman *et al.*, 1994; Ogura *et al.*, 1997), some non-sugar sweeteners (Bernhardt *et al.*, 1996) and several amino acids (Bryant and Leftheris, 1991). In our most recent report (Asano-Miyoshi *et al.*, 2000) we showed that a subset of taste bud cells contain a taste transduction pathway comprising rT1R2 as a G protein-coupled receptor, G_{i2} as a G protein and PLCβ2, although the ligand of rT1R2 is unknown.

Recently another type of G protein-coupled receptor, T2R, was isolated and shown to be specifically expressed in a subset of taste bud cells (Alder *et al.*, 2000; Matsunami *et al.*, 2000) independent of T1R2-expressing cells. It was shown that cells expressing T2Rs are gustducin-positive cells (Alder *et al.*, 2000) and that one type of T2R, mouse mT2R5, responds to a bitter tastant, cycloheximide, and induces an increase in intracellular Ca^{2+} concentration in a heterologous expression system with G_{15} (Chandrashekar *et al.*, 2000). However, the native signaling pathway downstream of these receptors is not yet known.

On the other hand, the inositol 1,4,5-trisphosphate receptor (IP_3R) is a ligand-gated ion channel that releases Ca^{2+} from internal stores and directly causes an increase in intracellular Ca^{2+} concentration as one of the components downstream of PLC. Three subtypes of IP_3R, IP_3R1–IP_3R3, have been identified and each shows a distinct tissue distribution (Newton *et al.*, 1994), but it not known which types of IP_3R are expressed in taste tissues.

In this study we have identified a type of IP_3 receptor, IP_3R3, a probable component downstream of PLCβ2, in rat taste bud cells. In addition, we examined the correlation between the expression of calcium signaling components such as PLCβ2 and IP_3R3 and that of two different types of taste receptors in taste bud cells.

Materials and methods

RNA preparation and reverse transcription–PCR (RT–PCR)

The poly(A)$^+$ RNA of the circumvallate and foliate papillae containing many taste buds was isolated as described previously (Asano-Miyoshi *et al.*, 1998). The cDNA fragments encoding rat IP_3Rs were amplified using the poly(A)$^+$ RNA and degenerate primers:

5′-GA(AG)TA(CT)TG(CT)CA(AG)GG(GATC)CC-
(ATC)TG(CT)CA(TC)GA(AG)AA(CT)CA-3′

and

5′-AA(AG)(CT)A(GATC)A(AG)(AG)TA(AG)TGC-CACAT(AG)TT(AG)TG(CT)TC-3′

corresponding to the amino acids sequences EYCQGP-CHENQ and EHNMWHYLCF, respectively, conserved among the three types of rat IP_3Rs. Each cDNA fragment was subcloned into the pBluescript SK(–) vector, sequenced and subjected to further analyses.

In situ hybridization

RNA probes labeled with digoxigenin-UTP were synthesized with T3 or T7 RNA polymerase from the cDNA fragments encoding rat IP_3Rs obtained above, the full-length cDNA of rT1R2 (Hoon *et al.*, 1999), a gift from Dr C.S. Zuker, and a partial cDNA corresponding to the open reading frame of rT2R9 (Alder *et al.*, 2000), which was obtained by PCR using a rat genomic DNA as template (GenBank accession no. AF227146).

The circumvallate papillae of the tongue of a 5-week-old rat (Wistar) were excised and 4-μm-thick frozen sections were prepared to be used for *in situ* hybridization as described previously (Asano-Miyoshi *et al.*, 2000; Yasuoka *et al.*, 1999). In the double labeling analysis a signal amplification method using two tylamide fluorogenic substrates was adopted according to the method described previously (Asano-Miyoshi *et al.*, 2000). Fluorescent images were obtained under a fluorescence microscope (Olympus BX60) using a MicroMax cooled CCD camera system (Nippon Roper) and analyzed with MetaMorph imaging software (Universal Imaging Corp.).

Immunohistochemistry

The circumvallate papillae of the tongue of a 5-week-old rat (Wistar) were excised, and 10 μm thick frozen sections were prepared in the same way as for *in situ* hybridization. The sections were post-fixed in 4% paraformaldehyde for 10 min. After washing three times in phosphate-buffered saline (PBS), the sections were blocked with 3% normal goat serum and 1% bovine serum albumin (BSA) in PBS for 1 h at room temperature. Then, the sections were incubated with a mixture of the primary antibodies, a monoclonal IP_3R3 antibody (Transduction Laboratories) and either a polyclonal PLCβ2 antibody (Q-15) (Santa Cruz Biotechnology) or a polyclonal Ggust antibody (I-20) (Santa Cruz Biotechnology), each diluted 1:200 in 1% normal goat serum and 1% BSA at 4°C overnight. After washing three

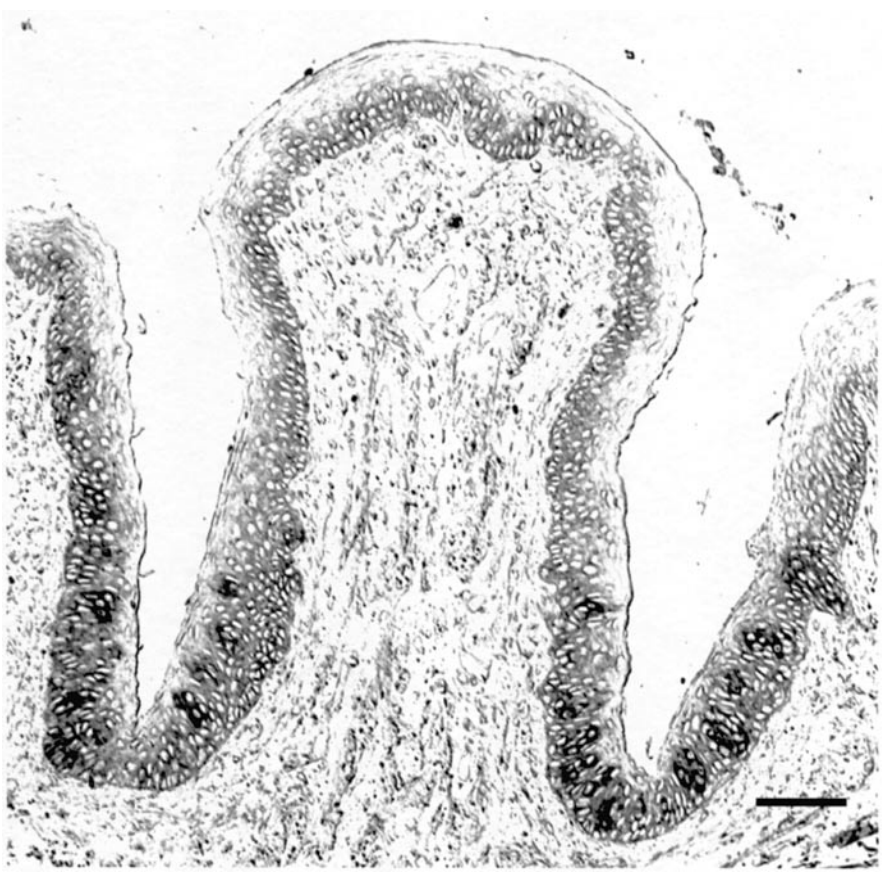

Figure 1 Cellular localization of IP_3R3 in taste buds. The result of an *in situ* hybridization experiment on rat circumvallate papillae with an antisense RNA probe for IP_3R3 is shown. Bars represent 50 μm.

times in PBS, the sections were incubated in the secondary antibodies, Texas Red-labeled donkey anti-mouse IgG and FITC-labeled donkey anti-rabbit IgG (Jackson Immuno Research), each diluted 1:100 in 1% normal goat serum and 1% BSA at room temperature for 1 h. After washing three times in PBS, the sections were mounted with Gel/Mount (Biomeda). Fluorescent images were obtained as described above.

Results

We first tried to identify IP_3R isoforms expressed in rat taste tissues by RT–PCR using the degenerate primers corresponding to the amino acid sequences conserved among the three types of rat IP_3Rs (see Materials and methods). As a result, two of types of IP_3R cDNA, IP_3R1 and IP_3R3 (GenBank accession nos A36579 and A46719), were obtained. Next, *in situ* hybridization experiments were performed with these IP_3R subtypes. The results clearly indicated that IP_3R3 is highly expressed in a subset of cells in all taste buds with a frequency similar to that of PLCβ2 (Rosseler *et al.*, 1998; Asano-Miyoshi *et al.*, 2000) (Figure 1). No significant signals were observed when IP_3R1 was used as probe (data not shown).

Next, we examined the correlation between expression of PLCβ2 and IP_3R3 by *in situ* hybridization (Figure 2). The expression profiles of PLCβ2 and IP_3R3 were compared in longitudinal (Figure 2a) and transverse (Figure 2b) serial sections of taste buds of the circumvallate papillae, where each cell would be divided into two or more adjacent sections. In this analysis when a certain cell expressed two genes, probes for the two genes should both give positive signals in the same cell divided between two serial sections (Asano-Miyoshi *et al.*, 2000). As a result, it was revealed that PLCβ2 and IP_3R3 are expressed in the same cells. We also carried out double labeling *in situ* hybridization experiments using digoxigenin- and FITC-labeled probes, which gave a clearer indication of the correlation (Figure 2c). The results of overlaid images of a single section were essentially the same as that obtained with the serial sections.

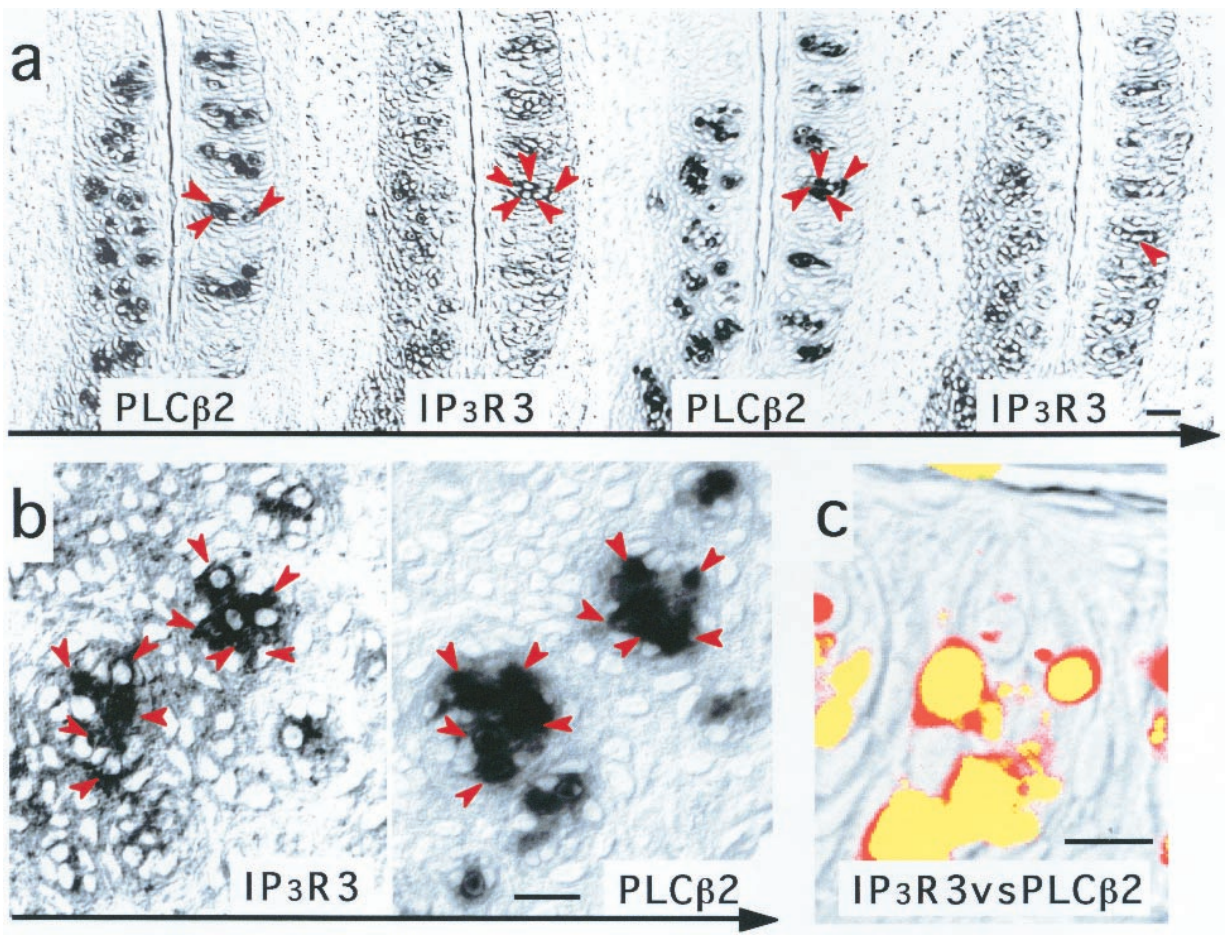

Figure 2 Co-expression of PLCβ2 and IP3R3. *In situ* hybridization experiments on 4 μm thick longitudinal **(a)** and transverse **(b)** serial sections of rat circumvallate papillae with antisense RNA probes for PLCβ2 and IP3R3 were performed. The serial sections in each panel are aligned along the arrow below the panel. The red arrowheads show cells positive to both probes. **(c)** The result of double label fluorescent *in situ* hybridization with PLCβ2 (green) and IP3R3 (red). Cells positive for both probes show yellowish signals as a result of overlap of the green (FITC) and red (Cyanine 3) signals. Bars represent 10 μm.

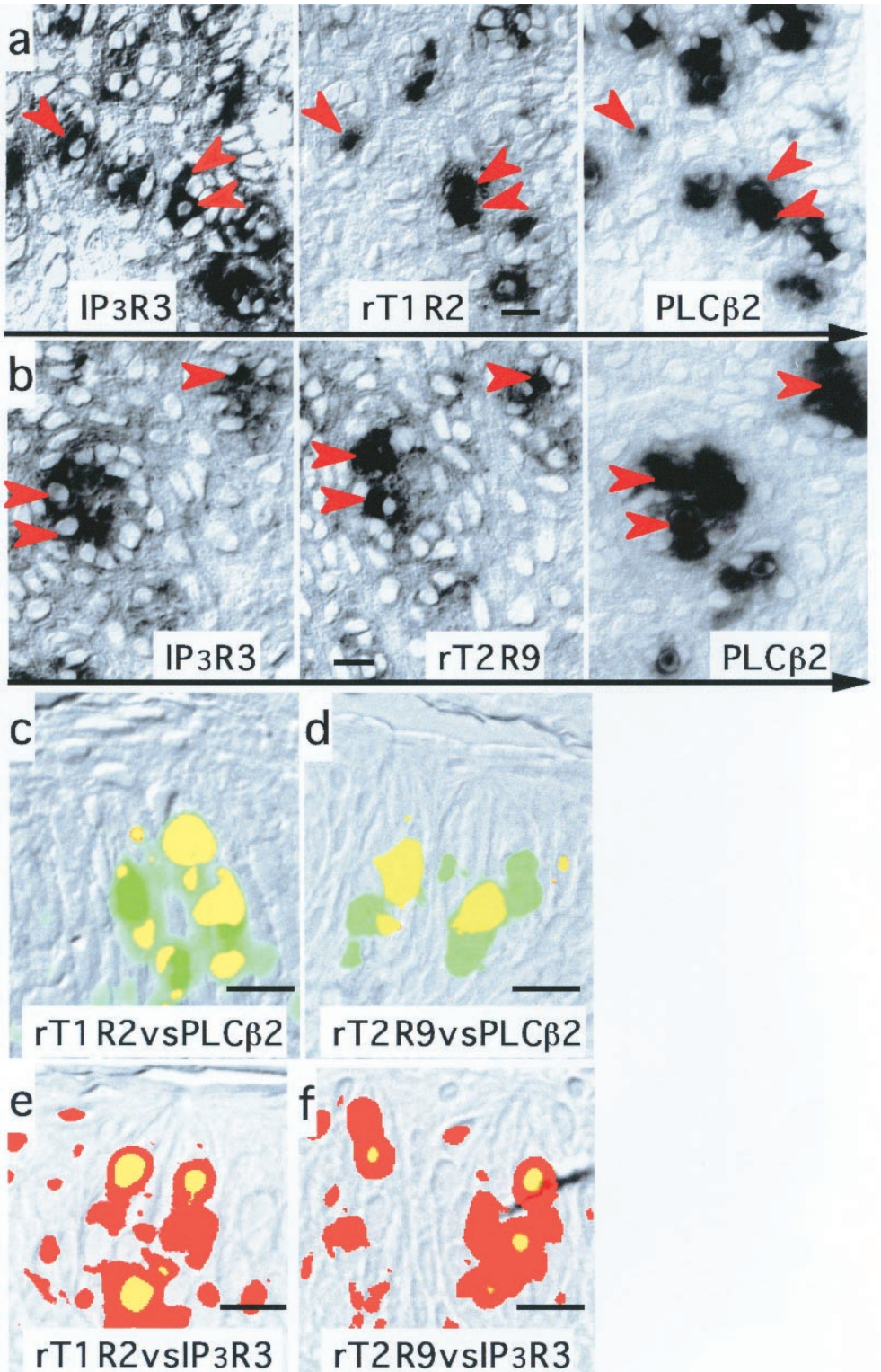

Figure 3 Correlation between the expression of taste receptors and calcium signaling components. *In situ* hybridization experiments on 4 μm thick transverse serial sections of rat circumvallate papillae with antisense RNA probes for the taste receptors rT1R2 **(a)** or rT2R9 **(b)**, PLCβ2 and IP_3R3 were performed. The serial sections in each panel are aligned along the arrow below the panel. The red arrowheads show the cells positive for both probes. **(c–f)** The results of double label fluorescent *in situ* hybridization with **(c)** PLCβ2 (green) versus rT1R2 (red), **(d)** PLCβ2 (green) versus rT2R9 (red), **(e)** IP_3R3 (red) versus rT1R2 (green) and **(f)** IP_3R3 (red) versus rT2R9 (green). Cells positive for both probes show yellowish signals as a result of overlap of the green (FITC) and red (Cyanine 3) signals. Bars represent 10 μm.

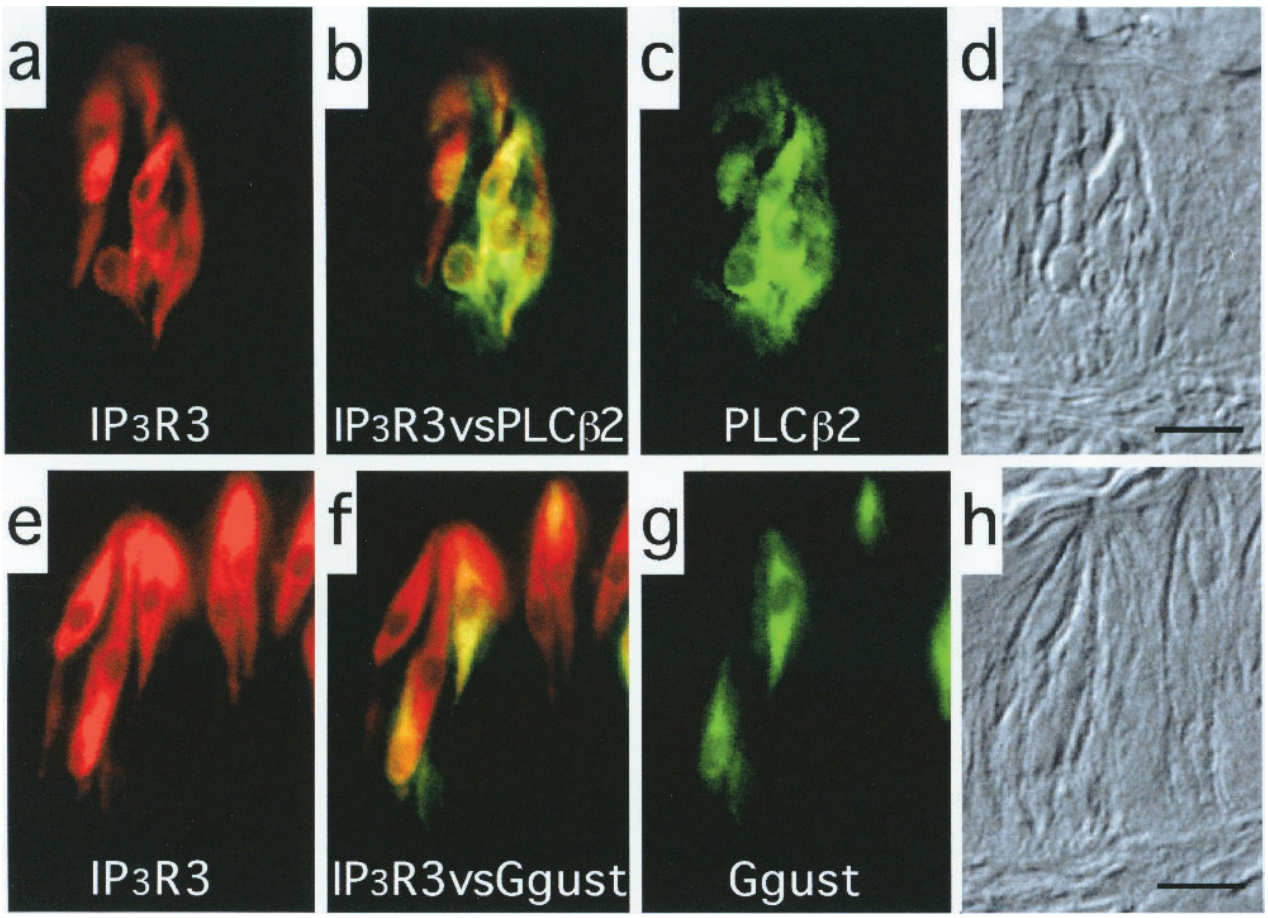

Figure 4 Correlation of the expression of IP_3R3, PLCβ2 and gustducin proteins. Double labeling immunofluorescense stainings on 10 μm thick sections of rat circumvallate papillae with **(a–d)** anti-IP_3R3 Ab **(a)** and anti-PLCβ2 Ab **(c);** and **(e–h)** anti-IP_3R3 Ab **(e)** and anti-gustducin Ab **(g)** were performed. Co-distributed signals for both proteins appear yellowish as a result of overlap of the green (FITC) and red (Texas Red) signals on each composite image **(b, f)**. The bar on each transparent view **(d, h)** represents 10 μm.

It is thus revealed that a certain subtype of IP_3 receptor, IP_3R3, is expressed in the same cells that express PLCβ2.

To gain further insight into the molecular process of the taste transduction pathway, we next examined the correlations between these cells and cells expressing two types of taste receptors, including a recently identified T2R, rT2R9, a rat homolog of the mouse cycloheximide receptor (mT2R5) (Alder *et al.*, 2000), in taste buds of rat circumvallate papillae (Figure 3).

In order to examine the relationship between expression of taste receptors and calcium signaling components, we performed *in situ* hybridization experiments using 4 μm thick transverse serial sections of rat circumvallate papillae in the same way as described for Figure 2b. As shown in Figure 3a,b, all rT1R2- and rT2R9-positive cells expressed PLCβ2 and IP_3R3. Next, we also carried out double labeling *in situ* hybridization experiments, which again revealed that all of the receptor-positive signals were in PLCβ2- (Figure 3c,d) and IP_3R3-expressing (Figure 3e,f) cells. Consequently, the results suggest that both types of taste receptor can transduce the taste signal to activation of PLCβ2, leading to activation of IP_3R3, and induce IP_3-dependent Ca^{2+} release from intracellular stores.

Finally, we examined the existence of PLCβ2 and IP_3R3 proteins by double labeling immunofluorescense staining (Figure 4a–d). The results show the same correlation as those of the *in situ* hybridization experiments (Figure 2), i.e. cells expressing IP_3R3 protein and those expressing PLCβ2 protein were identical. On the other hand, gustducin-positive cells were also IP_3R3 positive (Figure 4e–h).

Discussion

Although many reports have described the physiological features of Ca^{2+} signaling cascades in vertebrate taste systems (Akabas, 1993; Lindemann, 1996), there is little information concerning the molecular components. Our *in situ* hybridization results show that in taste buds a certain subtype of IP_3R, IP_3R3, is highly expressed in the same subset of cells that express PLCβ2 as one of two downstream signaling molecules generated by PLCβ2, IP_3 and diacylglycerol. In addition, double labeling immunostaining

showed that IP_3R3 protein and PLCβ2 protein co-existed in the same cells, which included gustducin protein-containing cells, although further investigations are needed to determine their functional linkage in terms of subcellular localization, because it might be altered during experimental procedures, including fixation and permeabilization. These observations strongly suggest that these components mediate Ca^{2+} responses induced by various taste stimuli. It is thus confirmed that Ca^{2+} release from internal stores triggered by IP_3 has an important function in taste transduction.

IP_3R3 is a subtype that is expressed predominantly in gastrointestinal tissues, such as in the apical region of villous enterocytes in the jejunum and pancreatic acinar cells, as well as kidney, lung, testis and brain (Blondel *et al.*, 1993). Its activation is considered to result in a single transient, but global, increase in the concentration of cyto- solic Ca^{2+} (Hagar *et al.*, 1998), as observed in isolated taste bud cells (Akabas *et al.*, 1988; Spielman *et al.*, 1994; Ogura *et al.*, 1997). Thus, IP_3R3 is better suited to signal initiation in taste cells than other types of IP_3Rs, whose properties are ideal for Ca^{2+} oscillation.

The finding that both rT1R2- and rT2R9-positive cells express PLCβ2 and IP_3R3 (Figure 3) suggests that taste stimuli received by these receptors probably induce the calcium response mediated by PLCβ2 and IP_3R3. In our most recent report (Asano-Miyoshi *et al.*, 2000) we found that PLCβ2 and G_{i2} are co-expressed in the same subset of cells and that gustducin-positive cells are also included in this subset. It is thus possible that the βγ subunits of G_{i2} are the general upstream components activating taste cell PLCβ2 and that those of gustducin might also act in a limited subpopulation of PLCβ2-positive cells. Since no correlation has been observed between expression of rT1R2 and gustducin (Hoon *et al.*, 1999; Asano-Miyoshi *et al.*, 2000), rT1R2 appears to transduce the taste signal of its unknown ligand through a pathway comprising $G_{i2}(G_{\beta\gamma})$, PLCβ2 and IP_3R3. On the other hand, T2R-positive cells have been reported to be included among gustducin-positive cells and the βγ subunits ($G_{\beta1\gamma13}$) of gustducin have been shown to mediate IP_3 responses to a bitter tastant, denatonium (Huang *et al.*, 1999). Therefore, cycloheximide, a ligand of rT2R9, probably causes the Ca^{2+} response in taste bud cells through a pathway comprising rT2R9, gustducin ($G_{\beta1\gamma13}$) and/or $G_{i2}(G_{\beta\gamma})$, PLCβ2 and IP_3R3.

In summary, a significant subset of taste bud cells have a Ca^{2+} signaling pathway involving PLCβ2 and IP_3R3 and these cells are taste cells showing IP_3 responses to several of the tastants reported in previous physiological studies. In a subpopulation of Ca^{2+}-signaling PLCβ2- and IP_3R3-positive taste cells both types of taste receptors, T1R and T2R, might receive some specific taste stimulus, such as cycloheximide for rT2R9, and might transduce the signals through the Ca^{2+} signaling pathway. Identification of downstream components following Ca^{2+} release are needed, together with further physiological studies using native Ca^{2+} signaling components, taste receptors and their specific ligands.

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References

Akabas, M.H. (1993) *The molecular biology of chemotransduction*. In Simon, A. and Roper, S.D. (eds), Mechanisms of Taste Transduction. CRC Press, Boca Raton, FL, pp. 175–200.

Akabas, M.H., Dodd, J. and **Al-Awqati, Q.** (1988) *A bitter substance induces a rise in intracellular calcium in a subpopulation of rat taste cells*. Science, 242, 1047–1050.

Alder, E., Hoon, M.A., Mueller, K.L., Chandrashekar, J., Ryba, J.P. and **Zuker, C.S.** (2000) *A novel family of mammalian taste receptors*. Cell, 100, 693–702.

Asano-Miyoshi, M., Kusakabe, Y., Abe, K. and **Emori, Y.** (1998) *Identification of taste-tissue specific cDNA clones from a subtraction cDNA library of rat circumvallate and foliate papillae*. J. Biochem., 124, 927–933.

Asano-Miyoshi,M., Abe, K. and **Emori, Y.** (2000) *Co-expression of calcium signaling components of vertebrate taste bud cells*. Neurosci. Lett., 283, 61–64.

Bernhardt, S.J., Naim, M., Zehavi, U. and **Lindemann, B.** (1996) *Changes in IP_3 and cytosolic Ca^{2+} in response to sugars and nonsugar sweeteners in transduction of sweet taste in the rat*. J. Physiol. Lond., 490, 325–336.

Blondel, O., Takeda, J., Janssen, H., Seino, S. and **Bell, G.I.** (1993) *Sequence and functional characterization of a third inositol trisphosphate receptor subtype, IP_3R-3, expressed in pancreatic islets, kidney, gastrointestinal tract, and other tissues*. J. Biol. Chem., 268, 11356–11363.

Bryant, B.P. and **Leftheris, K.** (1991) *Structure/activity relationships in the L-alanine taste receptor system of channel catfish,* Ictalurus punctatus. Physiol. Behav., 49, 891–898.

Chandrashekar, J. Mueller, K.L., Hoon, M.A., Alder, E., Feng, L., Guo, W., Zuker, C.S. and **Ryba, N.J.** (2000) *T2Rs function as bitter taste receptors*. Cell, 100, 703–711.

Hagar, R.E., Burgstahler, A.D., Nathanson, M.H. and **Ehrlich, B.E.** (1998) *Type III InsP3 receptor channel stays open in the presence of increased calcium*. Nature, 396, 81–84.

Hoon, M.A., Adler, E., Lindemeier, J., Battey, J.F., Ryba, N.J. and **Zucker, C.S.** (1999) *Putative mammalian taste receptors: a class of taste-specific GPCRs with distinct topographic selectivity*. Cell, 96, 541–551.

Huang, L., Shanker, Y.G., Dubauskaite, J., Zheng, J.Z., Yan, W., Rosenzweig, S., Spielman, A.I., Max, M. and **Margolskee, R.F.** (1999) *Gγ13 colocalizes with gustducin in taste receptor cells and mediates IP_3 responses to bitter denatonium*. Nature Neurosci., 2, 1055–1062.

Hwang, P.M., Verma, A., Bredt, D.S. and **Snyder, S.H.** (1990) *Localization of phosphatidylinositol signalling components in rat taste cells: role in bitter taste transduction*. Proc. Natl Acad. Sci. USA, 83, 7395–7399.

Lindemann, B. (1996) *Taste reception*. Physiol. Rev., 76, 719–766.

Matsunami, H, Montmayeur, J.-P. and **Buck, L.B.** (2000) *A family of candidate taste receptors in human and mouse*. Nature, 404, 601–604.

Newton, C.L., Mignery, G.A. and **Südhof, T.C.** (1994) *Co-expression in vertebrate tissues and cell lines of multiple inositol 1,4,5-trisphosphate (InsP3) receptors with distinct affinities for InsP3*. J. Biol. Chem., 269, 28613–28619.

Ogura, T. Mackay-Sim, A. and **Kinnamon, S.C.** (1997) *Bitter taste transduction of denatonium in the mudpuppy* Nectrurus maculosus. J. Neurosci., 17, 3580–3587.

Rossler, P., Kroner, C., Freitag, J., Noe, J. and **Breer, H.** (1998) *Identification of phospholipase C beta subtype in rat taste cells*. Eur. J. Cell Biol., 77, 253–261.

Spielman, A.I., Huque, T., Nagai, H., Whitney, G. and **Brand, J.G.** (1994) *Generation of inositol phosphates in bitter taste transduction*. Physiol. Behav., 56, 1149–1155.

Yasuoka, A., Endo, K., Asano-Miyoshi, M., Abe, K. and **Emori, Y.** (1999) *Two subfamilies of olfactory receptor genes in medaka fish,* Oryzias latipes*: genomic organization and differential expression in olfactory epithelium*. J. Biochem., 126, 866–873.

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