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# Taste Receptor Cells Express Voltage-Dependent Potassium Channels in a Cell Age–Specific Manner

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## Abstract

Two voltage-dependent potassium channels, KCNQ1 and KCNH2, are expressed in the taste buds and were identified as strong candidates involved in the repolarization of taste receptor cells expressing phospholipase C-β2 and TRPM5 (β2/M5-TRCs). In cell type–specific expression, KCNQ1 was expressed in most taste bud cells, including β2/M5-TRCs, whereas KCNH2 was expressed in a subset of β2/M5-TRCs with no correlation with their taste modality, such as sweet or bitter taste reception. Expression of KCNH2 was restricted to young β2/M5-TRCs. These results suggest that taste bud cells other than β2/M5-TRCs are depolarized by some stimuli and also that β2/M5-TRCs have cell age–dependent molecular mechanisms of repolarization.

**Key words:** cell age, Kv channel, taste bud

## Introduction

Food intake stimulates the sense of taste. In mammals, this involves activation of taste receptor cells (TRCs) in the taste buds, which are distributed mainly on the surface of the tongue epithelium, by the food chemicals. T1Rs and T2Rs identified as sweet/umami and bitter receptors, respectively, are expressed in a mutually exclusive manner (Hoon et al. 1999; Adler et al. 2000; Chandrashekar et al. 2000; Matsunami et al. 2000; Nelson et al. 2001, 2002). TRCs expressing T1Rs and T2Rs share intracellular signaling molecules such as phospholipase C-β2 (PLC-β2) (Asano-Miyoshi et al. 2001; Zhang et al. 2003). TRPM5, another signaling molecule, is involved in the depolarization of TRCs after the reception of food chemical ligands (Hofmann et al. 2003; Liu and Liman 2003; Prawitt et al. 2003).

Taste buds arise from the local epithelium and are maintained by cell turnover with a cycle of about 10–14 days (Farbman 1980; Stone et al. 1995). Some molecules, such as T1Rs and T2Rs, are expressed in specific subsets of taste bud cells, whereas others, such as group IIA PLA2, are expressed in a cell age–dependent manner in TRCs expressing PLC-β2 and TRPM5 (β2/M5-TRCs) (Oike et al. 2006). Thus, elucidation of the functions of the molecules in β2/M5-TRCs requires determination of when and where the molecules are expressed.

Electrophysiological studies on isolated taste bud cells have suggested the existence of voltage-dependent potassium (Kv) channels (Bigiani et al. 2002; Medler et al. 2003), although it is still unclear whether these cells are the β2/M5-TRCs themselves. Kv channels in general are involved in the repolarization of action potentials, and the cells depolarized in the taste bud should be β2/M5-TRCs. Therefore, it is reasonable to assume that Kv channels, if any, are expressed in β2/M5-TRCs to repolarize them after reception of tastant. However, it remains to be identified what molecule plays a role in repolarizing the membrane potentials of depolarized β2/M5-TRCs.

Here, we report the identification of 2 Kv channels, KCNQ1 and KCNH2, expressed specifically in taste buds by DNA microarray and subsequent histochemical analyses. We also report expression profiles indicating the existence of 2 types of β2/M5-TRCs: one with both KCNQ1 and KCNH2 and another with only KCNQ1, correlated with the age of β2/M5-TRCs.

## Materials and methods

### Animals

Male Wistar rats weighing 150–250 g were sacrificed under deep anesthesia with sodium pentobarbital (60 mg/kg body weight, intraperitoneally) or by cervical dislocation to allow dissection of fresh circumvallate papillae (CVPs) from the

tongue. The experimental protocols were approved by the Institutional Animal Care and Use Committee of the University of Tokyo and carried out in accordance with the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals.

### Preparation of total RNA

Taste buds from CVPs were isolated as described previously (Kishi et al. 2001). About 2 mg/ml of collagenase (type I; Sigma, St Louis, MO) was injected beneath the epithelium surrounding the CVP of the dissected tongue and incubated in $Ca^{2+}$-free Ringer's solution for 30 min at room temperature. Tongue epithelium containing the CVP was peeled off and incubated with collagenase type I (2 mg/ml). Taste buds were sucked out with glass capillaries, approximately 50 μm in inner diameter, and collected in microtubes. The residual tissue was divided into CVP epithelium (Cvp-epi) and non-papillal tongue epithelium (Np-epi) excluding Cvp-epi. Total RNA was extracted from the taste buds using TRIzol LS (Invitrogen, San Diego, CA) and purified by additional DNase I treatment with an Absolutely RNA Nanoprep kit (Stratagene, La Jolla, CA). Total RNAs were extracted from Cvp-epi and Np-epi and purified using an RNeasy Mini kit (Qiagen, Hilden, Germany).

### DNA microarray analysis

DNA microarray experiment with Rat Genome 230 2.0 (Affymetrix, Santa Clara, CA) was performed according to the manufacturer's protocol as described in the Expression Analysis Technical Manual (Affymetrix). Briefly, biotinylated cRNA was obtained from 10 ng of total RNA using a Two-Cycle Target Labeling and Control Reagents kit (Affymetrix). The cRNA was fragmented and hybridized with a DNA microarray. After hybridization, the microarray was washed and labeled with streptavidin-phycoerythrin. The resultant fluorescence was scanned to analyze the data using GeneChip Operation software version 1.1 (Affymetrix). All data were normalized relative to the signal intensity of glyceraldehyde-3-phosphate dehydrogenase (NCBI accession no. NM_017008) to 7000.

### The cDNA cloning

The cDNA fragments of KCNQ1 and KCNH2 were obtained by reverse transcriptase–polymerase chain reaction from kidney and brain, respectively, and those of TRPM5, T1R3, and gustducin were from CVP. cDNA fragments were cloned into a plasmid vector and sequenced using an automated DNA sequencer (310A; Applied Biosystems Inc., Foster City, CA).

### In situ hybridization

Digoxigenin- and fluorescein-conjugated antisense RNAs were synthesized using RNA labeling mix (Roche Diagnostics, Indianapolis, IN) and RNA polymerase (Stratagene) and used for hybridization after fragmentation to about 150 bases under alkaline conditions. In situ hybridization was performed essentially as described previously (Braissant and Wahli 1998; Matsumoto et al. 2001). For single labeling, signals were developed using alkaline phosphatase–conjugated antidigoxigenin antibody (Roche Diagnostics) and 4-nitro blue tetrazolium chloride/5-bromo-4-chloro-3-indolyl-phosphate as chromogenic substrates. Stained sections were observed under an Olympus BX-51 microscope (Olympus, Tokyo, Japan). For fluorescent double labeling, a horseradish peroxidase–conjugated anti-fluorescein antibody (Roche Diagnostics) and an alkaline phosphatase–conjugated anti-digoxigenin antibody (Roche Diagnostics) were used in combination with TSA biotin system (Perkin Elmer, Norwalk, CT), Alexa488-conjugated streptavidin (Molecular Probes, Eugene, OR), and HNPP Fluorescent Detection Set (Roche Diagnostics). Fluorescent images were observed under a confocal laser scanning microscope (LSM510; Carl Zeiss, Oberkochen, Germany).

### BrdU labeling and cell age determination

CVP was dissected from the tongue 1–4 days after intraperitoneal injection of 5-Bromo-2-deoxyuridine (BrdU; Sigma) into rats at 50 mg/kg body weight and fixed with 10% formalin in phosphate-buffered saline overnight at room temperature. Fixed CVP embedded in paraffin was cut into sections of 6 μm thick using a microtome (ROM-380; Yamato Kohki Ind., Saitama, Japan), and the sections were attached to silanized slides (Matsunami Glass Ind., Osaka, Japan). After deparaffinization and hydration, sections were treated with proteinase K (30 μg/ml), postfixed with 4% paraformaldehyde, and acetylated with acetic anhydride. In situ hybridization was carried out as described above using digoxigenin-labeled RNA, alkaline phosphatase–conjugated anti-digoxigenin antibody, and HNPP Fluorescent Detection Set. Before the development of fluorescent signals of mRNA expression, BrdU incorporated into the nucleus was detected using a BrdU Labeling and Detection kit II (Roche Diagnostics) and Alexa488-conjugated anti-mouse immunoglobulin G (IgG) antibody (Molecular Probes). Nuclei were counterstained with TO-PRO-3 (Molecular Probes). Fluorescent images were observed under a confocal LSM (LSM510; Carl Zeiss), recorded with LSM image browser (Carl Zeiss) to count the numbers of cells with mRNA expression signals and BrdU signals in 4–6 sections from 2 CVPs.

### Immunohistochemistry after in situ hybridization

For detection of KCNH2 mRNA and SNAP-25-like immunoreactivity (LI), fresh-frozen sections were used. Before developing fluorescent signals of KCNH2 mRNA expression using HNPP Fluorescent Detection Set, signals of SNAP-25-LI were detected using anti-SNAP-25 monoclonal antibody (1:500, Chemicon, Temecula, CA) and

Alexa488-conjugated anti-mouse IgG antibody. Fluorescent images were observed with a confocal LSM (LSM510; Carl Zeiss).

## Results

### Identification of Kv channels expressed in taste buds

To identify Kv channels expressed in β2/M5-TRCs, we first carried out differential screening by DNA microarray analysis. Gene expression data for 3 tissue samples, taste buds, Cvp-epi, and Np-epi, showed that most genes known to be specifically expressed in the taste buds in the tongue epithelium, such as T1R3 and PLC-β2, were predominantly expressed in the taste buds and that their levels of expression in the taste buds were over 4-fold high compared with those in Cvp-epi and Np-epi (Table 1). Thirty-one Kv genes were included in the DNA microarray used, and 7 of these were expressed in the taste buds, with 2 of the 7 genes showing over 4-fold higher expression levels in the taste buds than in either epithelium excluding the taste buds (Table 1). In situ hybridization analysis revealed that both Kv channels were specifically expressed in the taste buds in the tongue epithelial layer: KCNQ1 (KvLQT1) in most of the taste bud cells (Figure 1A) and KCNH2 (ERG1) in a subset of taste buds (Figure 1B).

### Molecular characteristics of the cells expressing KCNQ1 and KCNH2 in the taste buds

Next, we investigated the cells in which each Kv channel is expressed. For this, a correlation was examined between Kv channels and other molecules known to be expressed in the taste buds. As expected from the results of single staining for KCNQ1 shown in Figure 1A, the expression of KCNQ1 was observed broadly in the taste bud cells, and TRPM5-expressing cells were a subset of KCNQ1-expressing cells. The result indicates that KCNQ1 is expressed in both β2/M5-TRCs and taste bud cells other than β2/M5-TRCs (Figure 2A–C). The signals of KCNH2 appeared only in a subset of β2/M5-TRCs (Figure 2D–F), but the signals partially overlapped with those of T1R3 (Figure 2G–I) and gustducin (data not shown). These observations indicate that KCNH2 is expressed in a subset of β2/M5-TRCs irrespectively of their modality, such as sweet and bitter reception.

As described above, the expression of both Kv channels identified in the present study was not correlated with any specific taste modality, suggesting that some common molecular mechanism of regulating the membrane potential by Kv channels exists in β2/M5-TRCs, although there are differences in terms of KCNH2 expression in β2/M5-TRCs. KCNQ1 is also expressed in taste bud cells other than β2/M5-TRCs.

**Table 1** Microarray data of voltage-dependent potassium channel (6TM/1P Kv channel)

| Gene product | | NCBI accession no. | Probe set | Signal | | | TB/Cvp-epi | TB/Np-epi |
|---|---|---|---|---|---|---|---|---|
| | | | | TB* | Cvp-epi* | Np-epi* | | |
| Taste receptors | | | | | | | | |
| T1R3 | | NM_130818 | 1369397_at | 35.2 | (8.1) | (5.1) | 4.35 | 6.90 |
| T2R4 | | NM_023995 | 1387628_at | 60.1 | (6.7) | (2.5) | 8.97 | 24.04 |
| Signal transduction | | | | | | | | |
| Ggust | | X65747 | 1388223_at | 4074.1 | 241.3 | (7.2) | 16.88 | >100 |
| PLC-β2 | | NM_053478 | 1387717_at | 176.6 | (22.5) | (12.3) | 7.85 | 14.36 |
| Others | | | | | | | | |
| PLA2-IIA | | NM_031598 | 1368128_at | 3444.8 | 114.4 | (1) | 30.11 | >100 |
| SNAP-25 | | NM_030991 | 1387073_at | 72.9 | (10.7) | (8.1) | 6.81 | 9.00 |
| Kv channels | | | | | | | | |
| KCND1 | Kv4.1 | AA957975 | 1388173_at | 29 | (7.8) | (8.2) | 3.72 | 3.54 |
| KCNG3 | Kv6.3 | NM_133426 | 1387845_at | 23.1 | 26.7 | 10.7 | (0.87) | 2.16 |
| **KCNQ1** | **Kv7.1** | **NM_032073** | **1368371_at** | **576.8** | (19.4) | (17) | **29.73** | **33.93** |
| KCNS3 | Kv9.3 | NM_031778 | 1368751_at | 205.5 | 84.9 | 286.9 | 2.42 | (0.72) |
| KCNH1 | Kv10.1 | NM_031742 | 1368061_at | 31.5 | (12) | 61.1 | 2.63 | (0.52) |
| **KCNH2** | **Kv11.1** | **NM_053949** | **1368343_at** | **126.4** | (15) | (8.7) | **8.43** | **14.53** |
| KCNH3 | Kv12.2 | NM_017108 | 1368634_at | 120.8 | (37.2) | 63.4 | 3.25 | 1.91 |

*TB, Cvp-epi, Np-epi indicate tase bud, CVP epithelium excluding taste bud, non-papillal epithelium, respectively. Signal values indicated in parenthesis are the genes determined as "not expressed" by GeneChip Operation Software. Bold letters indicate the data of genes analyzed by *in situ* hybridization.

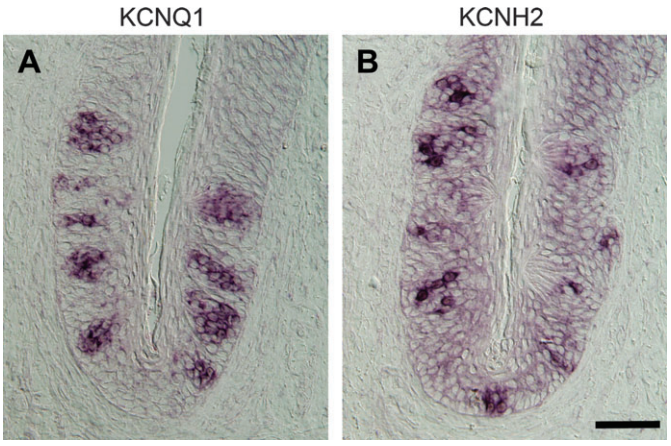


**Figure 1** Expression of KCNQ1 and KCNH2 in the taste buds. In situ hybridization analysis was carried out for CVPs from adult male Wistar rats using cryosections 10 μm thick. Signals of KCNQ1 mRNA were clustered in the taste buds **(A)**, whereas those of KCNH2 mRNA were scattered **(B)**. The bar in panel (B) represents 50 μm.

This raises the possibility that these cells without expression of taste receptors identified so far could be depolarized.

### Cell age–dependent expression of KCNH2 in TRCs

In our recent study, we showed that expression of PLA2-IIA restricted to a subset of β2/M5-TRCs is not correlated with any specific taste modality but with cell age (Oike et al. 2006). Therefore, we investigated whether the expression of KCNH2 restricted to a subset of β2/M5-TRCs could be correlated with cell age. Counting the number of BrdU-incorporating cells with the expression of KCNH2 and calculating the ratio to the total number of KCNH2-expressing cells, we investigated when and how much KCNH2 can appear in β2/M5-TRCs. The signals of BrdU were observed in the nuclei of cells with those of KCNH2 from 1 to 4 days after BrdU injection (Figure 3A–D and Table 2). The ratio of BrdU/KCNH2 double-positive cells to KCNH2-positive cells began to increase rapidly in 3 days after BrdU injection and then tended to decrease (Figure 3I). On the other hand, the ratio of BrdU/gustducin double-positive cells to gustducin-positive cells began to increase gradually in 4 days after BrdU injection, and the ratio was about one-third of that of BrdU/KCNH2 double-positive cells to KCNH2-positive cells 3 days after BrdU injection (Figure 3I and Table 2). These results suggest that KCNH2 is expressed in young β2/M5-TRCs. This also suggests that the expression of KCNH2 is distinct from that of molecules expressed in older β2/M5-TRCs such as PLA2-IIA and SNAP-25 in terms of cell ages (Oike et al. 2006). We then investigated a correlation, if any, between the expression of KCNH2 and PLA2-IIA or SNAP-25. As shown in Figure 4, most of the signals of KCNH2 mRNA were distinct from the signals of SNAP-25-LI, although we observed some overlapping signals (arrowheads in Figure 4). Similar results were obtained when we carried out double-labeling immunohistochemistry for KCNH2 and SNAP-25 and for KCNH2 and PLA2-IIA (data not shown). These observations indicate that KCNH2 is expressed only in young β2/M5-TRCs, whereas the expression ceases in older β2/M5-TRCs. This suggests that cellular functions of β2/M5-TRCs can be regulated by cell age–dependent gene expression.

## Discussion

In this study, we identified 2 Kv channels—KCNQ1 and KCNH2—specifically expressed in the taste buds in the tongue epithelium. The expression patterns show 2 interesting and important points: the one is that the expression of KCNQ1 is not restricted to β2/M5-TRCs where the depolarization can be induced by reception of tastants and the other is that KCNH2 is expressed only in young β2/M5-TRCs.

KCNQ1 and KCNH2 are well-known crucial molecules for 2 components of delayed rectifier potassium current ($I_K$) in the heart, slow $I_K$ and rapid $I_K$, and inherited mutations in these Kv channels cause cardiac arrhythmia and long QT syndrome (Curran et al. 1995; Wang et al. 1996). They show partial subcellular colocalization but predominantly have differential subcellular localization, and thus, yield different currents in cardiomyocytes (Rasmussen et al. 2004). It is still unclear whether KCNQ1 and KCNH2 show subcellular colocalization, but they can be involved in the regulation of β2/M5-TRC membrane potential by repolarization. Possibly, therefore, the mode of repolarization differs between young β2/M5-TRCs expressing both KCNQ1 and KCNH2 and older β2/M5-TRCs that possess only KCNQ1. Heterologous expression of KCNQ1 and KCNH2 in Chinese hamster ovary cells showed their subcellular colocalization (Ehrlich et al. 2004). It is difficult to think that, unlikely to cardiomyocytes, different potassium currents yielded by KCNQ1 and KCNH2 are necessary for only young β2/M5-TRCs. For these reasons, KCNH2 may play a role in altering the properties of KCNQ1 rather than in producing different types of potassium currents. For details, we should await further electrophysiological analyses.

Taste buds are maintained by cell turnover, and therefore, they contain cells in various stages of differentiation/maturation. The expression of some molecules, such as cytokeratins and gustducin, begins at different cell ages (Zhang et al. 1995; Cho et al. 1998). However, following expression in a given stage, the molecules maintain their expression thereafter. In contrast, the expression of KCNH2 distinctly decreased 4 days or more after BrdU administration, and most of the signals of KCNH2 mRNA were distinct from that of SNAP-25 or PLA2-IIA in aged β2/M5-TRCs. KCNH2 is thus the first well-defined gene that makes their expression limited to young β2/M5-TRCs, putatively immature β2/M5-TRCs. Possibly, there are genes that are expressed temporally during young stages. It will be important to investigate the time courses of expression of various genes to elucidate their functions in β2/M5-TRCs, and the

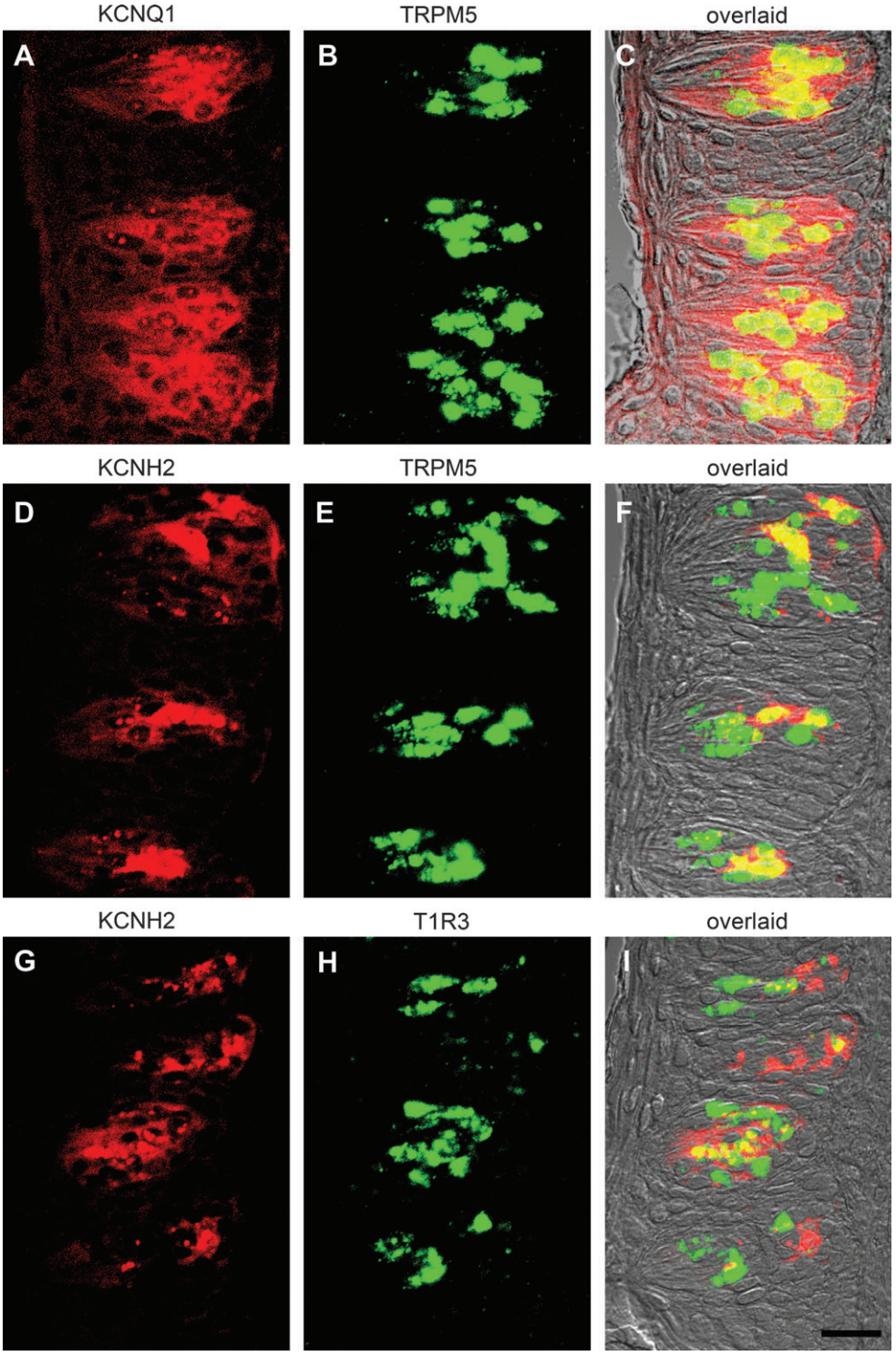


**Figure 2** Molecular features of cells expressing KCNQ1 and KCNH2 in the taste buds. Double-labeled in situ hybridization analysis was carried out to determine the characteristics of taste bud cells expressing KCNQ1 and KCNH2 using cRNA of Kv channel and TRC genes as probes. Signals of TRPM5 mRNA were partially included among those of KCNQ1 **(A–C)**, whereas they included those of KCNH2 in part **(D–F)**. Signals of T1R3 mRNA partially overlapped with those of KCNH2 **(G–I)**, although T1R3 is expressed in a subset of β2/M5-TRCs (Asano-Miyoshi et al. 2001; Nelson et al. 2001; Zhang et al. 2003) as well as KCNH2 (F). The bar in panel (I) represents 20 μm.

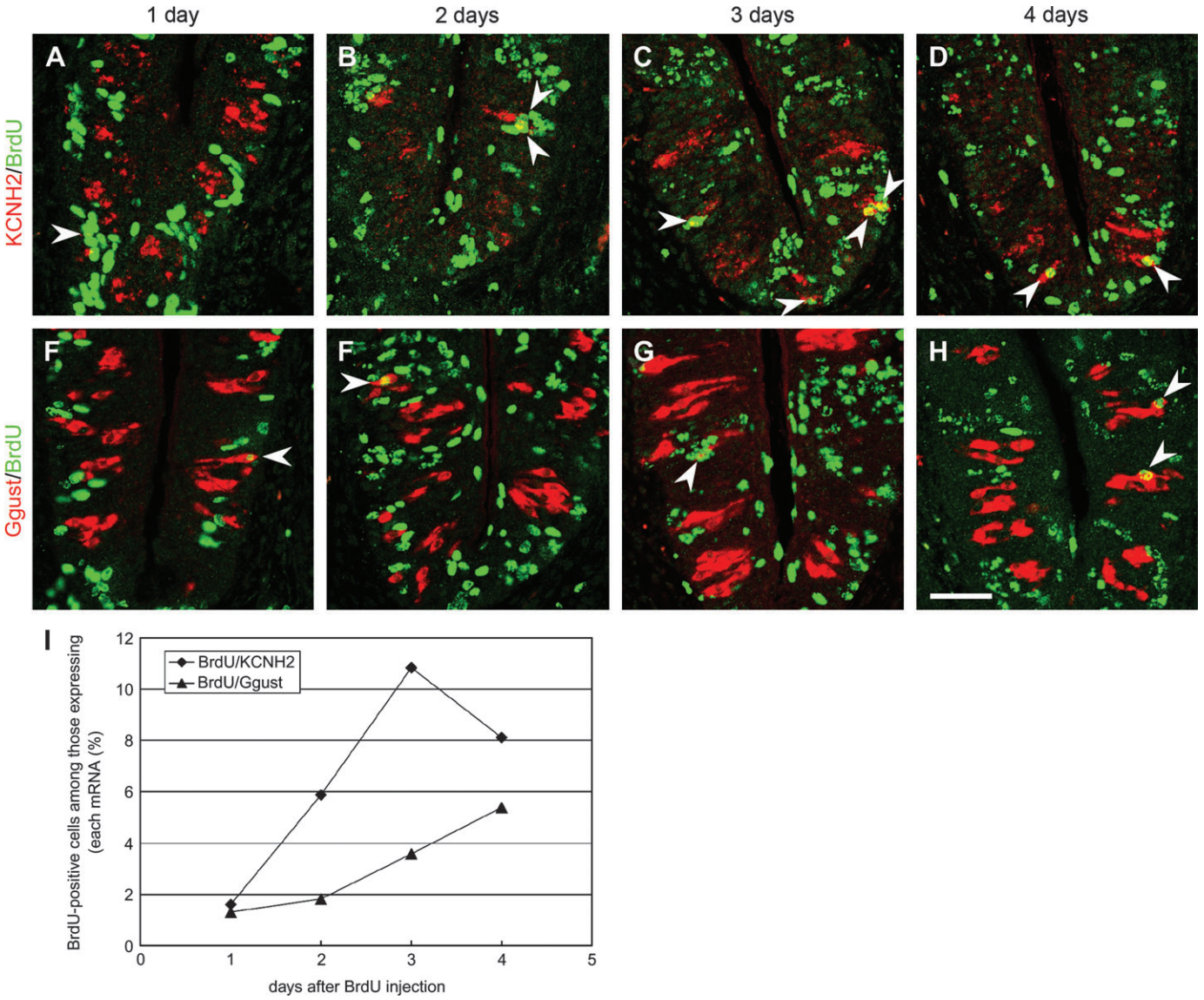


**Figure 3** Relationship between KCNH2 expression and age of TRCs. In situ hybridization was performed to detect the mRNA of KCNH2 **(A–D)** or gustducin **(E–H)** in conjunction with immunohistochemical detection of BrdU integrated in the nuclei. Red and green signals represent KCNH2 or gustducin and BrdU, respectively. White arrowheads indicate double-positive cells with signals for BrdU and KCNH2 (BrdU/KCNH2) or gustducin (BrdU/gustducin). The numbers of cells with signals for KCNH2, gustducin, BrdU/KCNH2, and BrdU/gustducin were counted, and the ratios of BrdU/KCNH2 double-positive cells to KCNH2-positive cells and BrdU/gustducin double-positive cells to gustducin-positive cells were calculated (details are shown in Table 2). The changes of these ratios with cell age represented by days after BrdU injection are depicted in **(I)**. The white bar in panel (H) represents 50 μm.

**Table 2** BrdU-positive cells among KCNH2- and Ggust-expressing cells in rat CVPs

| Days after BrdU injection | $BrdU^+$ in $KCNH2^+$ | $KCNH2^+$ | $BrdU^+/KCNH2^+$ to $KCNH2^+$ (%) | $BrdU^+$ in $Ggust^+$ | $Ggust^+$ | $BrdU^+/Ggust^+$ to $Ggust^+$ (%) |
|---|---|---|---|---|---|---|
| 1 | 5 | 311 | 1.6 | 8 | 613 | 1.3 |
| 2 | 22 | 374 | 5.9 | 12 | 659 | 1.8 |
| 3 | 36 | 332 | 10.8 | 18 | 503 | 3.6 |
| 4 | 38 | 468 | 8.1 | 30 | 557 | 5.4 |

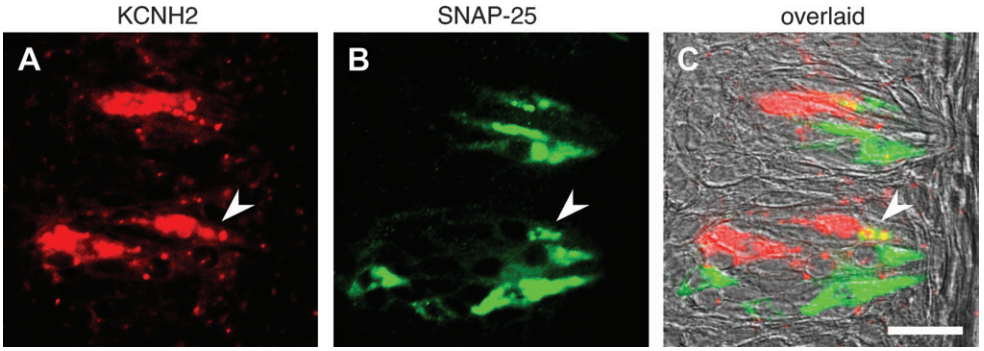


**Figure 4** Relationship of expression between KCNH2 and SNAP-25. KCNH2 mRNA **(A)** and SNAP-25 protein **(B)** were simultaneously detected in the taste buds of cryosections of CVP. **(C)** overlaid image of (A) and (B) on bright field. Most of the signals of KCNH2 mRNA were distinct from the signals of SNAP-25 protein. In some cells, signals of KCNH2 mRNA and those of SNAP-25 protein were overlapped (arrowhead). The bar in panel (C) represents 20 μm.

analysis of the resultant data would contribute to promoting our understanding of a whole aspect of the mechanisms behind the differentiation and/or maturation of β2/M5-TRCs.

At present, taste bud cells expressing molecules such as PLC-β2 and TRPM5 are categorized as sweet/umami and bitter TRCs, whereas neither PLC-β2 nor TRPM5 is essential for either sour or salty taste signaling (Zhang et al. 2003). Although it is still unclear whether sour and/or salty tastes are received by these β2/M5-TRCs, it is reasonable to note that taste bud cells are depolarized when activated by taste stimuli and that taste bud cells activated by taste stimuli can express Kv channels for repolarization. Therefore, it is possible that there are some additional populations of TRCs besides β2/M5-TRCs. In the present study, we have shown that KCNQ1 is expressed in taste bud cells in addition to β2/M5-TRCs. If the expression of KCNQ1 is functional in taste bud cells, the occurrence in cells other than β2/M5-TRCs suggests the existence of another type of TRCs, although taste bud cells other than β2/M5-TRCs are not well defined at present. Our finding of the cells expressing KCNQ1 in distinction from β2/M5-TRCs would be significant, and we need to clarify their properties by further experimentation.

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