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Type II and III Taste Bud Cells Preferentially Expressed Kainate Glutamate Receptors in Rats

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Glutamate-induced cobalt uptake reveals that non-NMDA glutamate receptors (GluRs) are present in rat taste bud cells. Previous studies involving glutamate induced cobalt staining suggest this uptake mainly occurs via kainate type GluRs. It is not known which of the 4 types of taste bud cells express subunits of kainate GluR. Circumvallate and foliate papillae of Sprague-Dawley rats (45～60 days old) were used to search for the mRNAs of subunits of non-NMDA GluRs using RT-PCR with specific primers for GluR1-7, KA1 and KA2. We also performed RT-PCR for GluR5, KA1, PLCβ2, and NCAM/SNAP 25 in isolated single cells from taste buds. Taste epithelium, including circumvallate or foliate papilla, express mRNAs of GluR5 and KA1. However, non-taste tongue epithelium expresses no subunits of non-NMDA GluRs. Isolated single cell RT-PCR reveals that the mRNAs of GluR5 and KA1 are preferentially expressed in Type II and Type III cells over Type I cells.

Key Words: Taste bud, Kainate receptor, RT-PCR, Single isolated cell

INTRODUCTION

Taste is a vital sense for preventing consumption of poisonous foods as well as searching for nutrients. Taste sensation is transduced in secondary sensory cells in taste buds, a specialized structure in the oral cavity. The taste signals from taste cells project to the taste area in brain.

Taste buds consist of several cell types, including supporting cells, sensory cells, synaptic cells, and basal cells. The interactions between taste bud cells play important roles in the perception of taste. Numerous neurotransmitters such as glutamate (Caicedo et al., 2000; Kim et al., 2001), serotonin (Herness and Chen, 1997; Huang et al., 2005), norepinephrine (Herness et al., 2002) and ATP (Finger et al., 2005) are present in rat taste bud cells.

Taste buds are mainly present in the tongue epithelium, though a small number of taste buds are also present in the posterior part of the soft palate and the upper part of the esophagus. Fungiform papilla in the anterior part of the tongue and circumvallate and foliate papilla in the posterior third of the tongue comprise most of the taste buds in rats.

Taste buds consist of 30 to 100 cells in rats. They can be separated into 4 groups based on shape and function (Farbman, 1965). Spindle shaped cells include type I, II, and III whereas type IV cells are cuboidal shaped. Type I cells are supporting cells that act similarly to glial cells (Bigiani, 2001), type II cells are taste sensory cells that respond to taste substances, and type III cells have synaptic connections with taste sensory nerves (Farbman, 1965; Pumplin et al., 1997; DeFazio et al., 2006). Type II cells also express Gα-gustducin, a taste specific G-protein, phospholipase Cβ2 (PLCβ2), and several taste receptor proteins (DeFazio et al., 2006). Type III cells express neural cell adhesion molecules (NCAMs) and a synaptosomal associated protein of 25 kD (SNAP 25). They also act as output cells to taste nerves (Yang et al., 2000; DeFazio et al., 2006).

Glutamate is a ubiquitous neurotransmitter in the human nervous system. Glutamate acts via metabotropic and inotropic glutamate receptors (mGluR and iGluR). iGluRs are divided into 3 groups based on ligand specificity and the structure of the subunit protein: N-methyl-D-aspartate (NMDA) receptors, α-amino-3-hydroxy-isoxazole-propionic acid (AMPA) receptors, and kainate receptors. AMPA GluRs contain either heteromeric or homomeric tetramers of GluR1～4 whereas kainate GluRs contain either homomeric or heteromeric tetramers of GluR5～7, KA1, or KA2 (Dingledine and Conn, 2000).

AMPA and kainate GluRs play different roles in the central nervous system. AMPA GluRs transmit fast excitatory signals (Song and Huganir, 2002) and modulate the size of synapses (Passafaro et al., 2003). The length of excitatory

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ABBREVIATIONS: PLCβ2, phospholipase Cβ2, NCAM; neural cell adhesion molecule; SNAP 25, synaptosomal associated protein of 25 kD; NMDA, N-methyl-D-aspartate; AMPA, α-amino-3-hydroxy-isoxazole-propionic acid; RT-PCR, reverse transcription-polymerase chain reaction; EGTA, ethylene glycol tetraacetic acid.

postsynaptic currents (EPSCs) elicited by kainate receptors is longer than that elicited by AMPA receptors (Lerma, 2003). Slow and small EPSCs modulated by kainate receptors have long time windows and play an important role in the integration of information (Lerma, 2003). Moreover, the gating mechanisms of kainate and AMPA receptors are also different. In kainate receptors, ions in the extracellular environment modulate the amplitude and velocity of EPSCs (Bowie, 2002).

Functional kainate GluRs are expressed in subsets of taste bud cells (Caicedo et al., 2000; Kim et al., 2001; Chung et al., 2005, Lee et al., 2008). However, the function of kainate GluRs in taste cells are not known, and neither are the types of taste bud cells that express kainate GluRs. In this study, we measured the differential expression patterns of kainate GluRs in taste buds using RT-PCR techniques.

METHODS

All experimental protocols were approved by the Animal Care and Use Committee, College of Dentistry, Gangneung-Wonju National University. The experimental protocol was adopted from procedures published previously (Kim et al., 2001). Male Sprague-Dawley rats, 45～60 days old (Semtaco, Korea) were used for these experiments.

RT-PCR in taste epithelium

The tongues of rats were excised after sacrifice under general anesthesia. A collagenase (0.5 mg/ml, Sigma, USA)-dispase (type II, 5 mg/ml, Roche, Germany) enzyme cocktail solution was injected beneath the tongue epithelium. After a 30-min incubation in N-2-hydroxy-ethylpiperazine-N-2-ethane-sulfonic acid (HEPES) buffered-Tyrode solution (in mM; NaCl 140, KCl 5, $MgCl_2$ 1, $CaCl_2$ 1, Glucose 5, HEPES 10, Pyruvate 5, pH 7.3) at 36°C. The tongue epitheliums, including circumvallate and foliate papilla, were peeled off of the subcutaneous tissues under a surgical microscope. The epithelia were homogenized by a tissue homogenizer (Wheaton, USA) and ultrasonic tissue disruptor (Vibra cell, Sonics & Material Inc., USA). RNA from the homogenized epithelium was extracted with a Corezol total RNA preparation kit (CoreBio System, Korea). Non-taste tongue epithelium and the hippocampus were used as negative and positive controls, respectively. One hundred ng of extracted RNA and 10 μM primer were mixed in an Accupower RT/PCR Premix (Bioneer, Korea) for reverse transcription and PCR. The Gene Amp PCR system 9700 (PE Applied Biosystems, USA) was used for RT-PCR. RT-PCR protocols included 90 min of reverse transcription at 50°C and 35 cycles at 45 seconds. Primers for the nine subunits of non-NMDA GluRs, GluR1-7, KA1, and KA2 are listed in Table 1. In several experiments, reverse transcription procedures were omitted as a negative control. All RT-PCR procedures include RT-PCR of Glyseraldehyde-3-phosphate dehydroxygenase (GAPDH) as a positive control.

RT-PCR in isolated single taste bud cells

The circumvallate and foliate papillae were excised from peeled epithelium. The papilla were incubated in a Ca^{2+} free Tyrode solution (in mM; NaCl 140, KCl 5, EGTA 2, Glucose 5, HEPES 10, Pyruvate 5, pH 7.3) for 30 min. Mechanical agitation released single taste bud cells from the papilla. Under the inverted microscope, isolated single taste bud cells were picked up in micropipettes of 50 μm opening. cDNA was obtained from reverse transcription of lysed cells with M-MLV reverse transcriptase (Promega, USA) for 90 min. PCR of GluR5, KA1, PLCβ2, NCAM/SNAP25, and GAPDH was performed with smart Taq premix (Solgent, Korea). In the procedure, 10～15 cells were collected from each rat. A total of 157 taste bud cells from 12 rats were used in this procedure. The PCR protocol included 3 cycles of 5 min polymerization and 57 cycles of

Table 1. DNA sequences, annealing temperatures, and expected product sizes of specific primers of subunits of non-NMDA glutamate receptors used in RT-PCR

Name		Sequence	Annealing temperature (°C)	Expected product size (bp)
GluR1	Sense	GCT TCA TGG ACA TTG TA	40	623
	Antisense	ATC TCA AGT CGG TAG GAG TA		
GluR2	Sense	ATT GTA GAC TAC GAT GAT TC	40	643
	Antisense	AAT AGT CAG CTT GTA CTT GA		
GluR3	Sense	AAA CGA TAC TTG ATT GAC TG	40	655
	Antisense	GCT GAT TTG TTG ATC TGA GA		
GluR4	Sense	CCA CTG CTA GAA GAG CTT GA	40	612
	Antisense	CAT ATC TTG AAT CAA GAC TA		
GluR5	Sense	GCC CCT CTC ACC ATC ACA TAC	52	208
	Antisense	ACC TCG CAA TCA CAA ACA GTA		
GluR6	Sense	TTC CTG AAT CCT CTC TCC CCT	52	260
	Antisense	CAC CAA ATG CCT CCC ACT ATC		
GluR7	Sense	TGG AAC CCT ACC GCT ACT CG	52	356
	Antisense	CCG CAA GCC ACT GGT TTT GTT		
KA1	Sense	AGC GTT ATG TCA TGC CCA GAC CAG	52	316
	Antisense	GGG GAG GAT CTG ACA CAT		
KA2	Sense	TGC CCC GTG TCC TCA ACT CA	52	398
	Antisense	CAC CGA CAC CTC CTC AGA CT		

Table 2. DNA sequences, annealing temperatures and expected product sizes for specific primers used in RT-PCR to test specific markers for cell type in taste buds (positive control GAPDH)

Name		Sequence	Annealing temperature (°C)	Expected product size (bp)
PLCβ2	Sense	CTG GAG GCT GAA GTA AAG GAG	40	623
	Antisense	GCC CCT GCA TGT ATG TTA GG		
NCAM	Sense	TTG TGG GCA TCC TCA TTG TC	52	643
	Antisense	TGT CAG TGG TGT GGT CTC GT		
SNAP25	Sense	GGC AAT AAT CAG GAT GGA GTA G	58	612
	Antisense	AGA TTT AAC CAC TTC CCA GCA		
GAPDH	Sense	TGG GGT GAT GCT GGT GCT GA	60	398
	Antisense	CGC CTG CTT CAC CAC CTT CT		

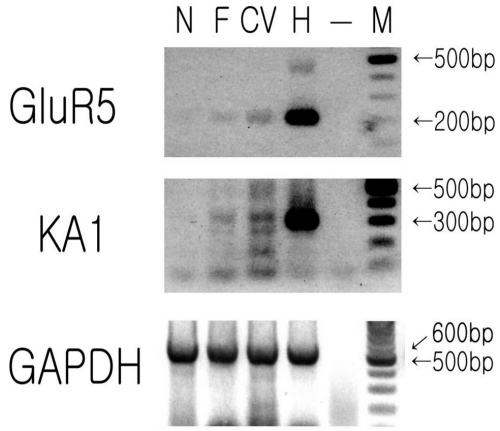

Fig. 1. Results of tissue RT-PCR of mRNA obtained from tongue epithelium and hippocampus. N, non-taste epithelium; F, epithelium including foliate papilla; CV, epithelium including circumvallate papilla; H, hippocampus as positive control; −, no tissue as negative control; M, size marker.

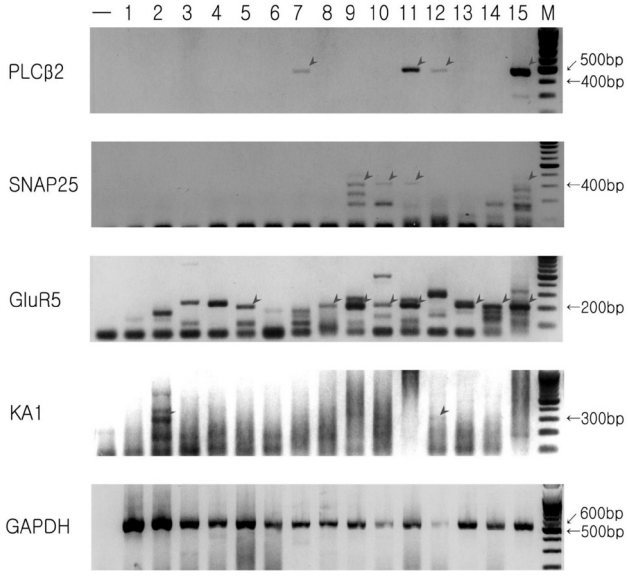

Fig. 2. Representative isolated single cell RT-PCR results. Data is from 15 cells obtained from a single rat. Data from lanes 11 and 15 were discarded because both PLCβ2 and SNAP25 are expressed. Two cells express PLCβ2, another 2 cells express SNAP 25, 6 cell express GluR5, and 2 cells express KA1. Even cells in lanes 11 and 15 do not express KA1. However, GAPDH is expressed in all lanes except the negative control.

30 sec polymerization. The sequences and expected product sizes for specific primers are listed in Table 2. GAPDH was used as a positive control of RT-PCR. Data from RT-PCR of single taste bud cells was analyzed by ANOVA. Reagents not indicated above were obtained from Sigma (USA).

RESULTS

Expression of subunits of non-NMDA GluRs; RT-PCR of tissues

RT-PCR was performed on taste epithelium, including circumvallate and foliate papilla. Epithelia without taste buds were used as a negative control. Hippocampus tissue in the brain was used as a positive control. All samples except the negative control show a band corresponding to the expected product of GAPDH (Fig. 1). The result of the RT-PCRs of nine subunits of non-NMDA GluRs reveal that GluR5 and KA1 are expressed in taste epithelium and in the hippocampus. When reverse transcription procedures were omitted, no PCR product was observed (data not shown). All nine subunits are seen in the hippocampus sample. However, none of nine subunits are expressed in non-taste epithelium (data not shown).

Expression of GluR5 and KA1 in isolated single taste bud cells

RT-PCR was performed for GluR5, KA1, PLCβ2, and NCAM/SNAP25 using 157 cells from 12 rats. Representative RT-PCR data from one rat is shown in Fig. 2. In this rat, PLCβ2 and SNAP25 are simultaneously detected in lanes 11 and 15. This indicates that more than one cell was collected, so that these data were excluded from the data analysis (DeFazio et al., 2006). In 11 of the 157 samples, PLCβ2 and NCAM/SNAP25 were expressed simultaneously. Data from 146 samples were analyzed and are shown in Table 3 and Fig. 3. PLCβ2, a marker for type II taste bud cells, is expressed in 42 samples (31.5%). NCAM/SNAP25, a marker of type III taste bud cells, is expressed in 19 sam-

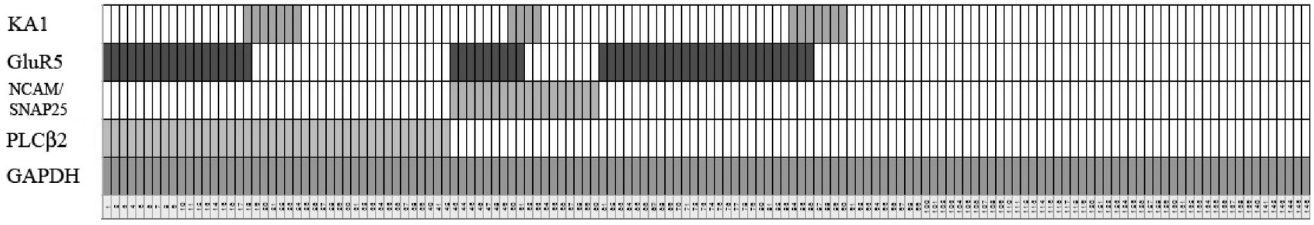

Fig. 3. Results of isolated single cell RT-PCR for taste bud cells. Each column represents data from an individual taste cell. Each row indicates expression of different mRNAs.

Table 3. Expression of GluR5, KA1, PLCβ2 and NCAM/SNAP25 in 146 isolated single taste bud cells from12 rats

	PLCβ2	NCAM/ SNAP25	None	Total
GluR5	17	7	27	51
KA1	6	2	4	12
GluR5 & KA1	1	2	3	6
None	18	8	51	77
Total	42	19	85	146

ples (13.0%) (Table 3). GluR5 and KA1 are expressed in 57 samples (39.0%) and 18 samples (12.3%), respectively (Table 3). GluR5 and KA1 subunits of GluRs are expressed preferentially in type II and type III cells over type I cells ($p < 0.05$).

DISCUSSION

RT-PCR experiments on taste epithelium and isolated taste cells reveal that GluR5 and KA1 subunits are expressed in taste bud cells and preferentially expressed in PLCβ2 or NCAM/SNAP25 expressing cells.

GluR6 vs. GluR5

Non-NMDA GluRs in taste bud cells were observed by cobalt staining techniques (Caicedo et al., 2000). Immunohistochemistry and pharmacological experiments suggested that those receptors were kainate type GluRs (Chung et al., 2005). The RT-PCR procedure in this experiment reveals that GluR5 is expressed in taste bud cells instead of GluR6, which is detected by immunohistochemistry (Chung et al., 2005; Lee et al., 2008). This discrepancy may be explained by antibody cross reactivity. GluR5 and GluR6 have 88% identical amino acid sequences and share ligand specificity (Mayer, 2005). For this reason, the polyclonal antibody against GluR6 may cross react with GluR5. Another possibility is that our primer for GluR5 produced the same size PCR products as the mRNAs of GluR6. Although having 88% identical amino acid sequences is unusual, it is unlikely to produce PCR products of similar size (Mezei and Store, 1994). Both GluR5 and GluR6 are components of the kainate GluR (Mayer, 2005). Expression of kainate GluRs in taste bud cells is supported by previous reports (Chung et al., 2005; Lee et al., 2008).

Differential expression of kainate GluRs in taste bud cells

There are 4 morphologically different types of taste bud cells in mammals (Murray, 1974), and each plays a different role in taste transduction. Type I cells are the supporting cells of taste buds, and function like glial cells in the nervous system (Bigiani, 2001). Type II cells express bitter and sweet taste receptor proteins as well as several taste signal transduction proteins, such as Gα-gustducin (Boughter et al., 1997) and PLCβ2 (DeFazio et al., 2006). Type II taste bud are the sweet and bitter sensing and transducing cells (Roper, 2006). Type III cells have characteristics similar to neural cells and express proteins relating to synaptic transmission, such as NCAM and SNAP25 (Yang et al., 2000; DeFazio et al., 2006). They have synaptic connections with taste afferent nerves (Murray, 1986) and export taste signals to the nervous system.

In the RT-PCR experiment on isolated cells, only 11 of 157 samples (7.0%) expressed PLCβ2 and NCAM/SNAP25 simultaneously. This data is similar to others (DeFazio et al., 2006). There were 42 PLCβ2 expressing type II cells (31.5%) and 19 NCAM/SNAP25 expressing type III cells (13.0%). These data also agree with previous reports (Lindemann, 1996; DeFazio et al., 2006). In type II and type III taste bud cells, 61.9% (26 of 42) and 57.9% (11 of 19) of cells expressed mRNA for kainate GluRs. However, only 27.0% of type I cells, which did not express PLCβ2 or NCAM/SNAP25, expressed those mRNAs. The GluR5 and KA1 subunits of GluRs were expressed preferentially in type II and type III cells over type I cells in this experiment ($p < 0.05$).

Kainate GluRs in taste buds

Glutamate is a ubiquitous neurotransmitter in the mammalian nervous system. Glutamate acts on mGluRs or iGluRs. iGluRs, classified according to ligand specificity and receptor structure, consist of NMDA, AMPA, and kainate GluRs. AMPA and kainate GluRs are also called non-NMDA GluRs. According to Caicedo et al. (2000) and Kim et al. (2001), non-NMDA GluRs in taste bud cells are synaptic receptors rather than taste receptors. Chung et al. (2005) reports that these non-NMDA GluRs are kainate GluRs.

The function of AMPA GluRs is different than kainate GluRs. AMPA GluRs are involved in fast synaptic transmission and synaptic plasticity, whereas kainate GluRs exhibit much slower EPSCs, thus integrating excitatory inputs over large time windows or modulating synaptic activity

(Lerma, 2003; Mayer, 2005). Receptors containing GluR6 play an important role in temporal summation at postsynaptic sites (Castillo et al., 1997). Receptors with GluR5 modulate the presynaptic release of neurotransmitters (Chittajallu et al., 1996). GluR5 in taste bud cells, preferentially expressed in type II and type III cells, may modulate the release of neurotransmitters at synapses between taste bud cells or between taste bud cells and taste nerves.

Where does glutamate originate?

Axons of primary gustatory neurons are immunoreactive for glutamate (Jain and Roper, 1991) and release glutamate at their central projections in solitary tract nuclei (Bradley et al., 1996). Although there is no direct evidence that taste buds have efferent innervation from the central nervous system, efferent inputs are found in other sensory organs such as the cochlea, which is needed for the clear perception of sound information (Raphael et al., 2003). Axons of primary gustatory neurons are possible sources of glutamate.

ATP (Finger et al., 2005) or serotonin (Huang et al., 2005) are the major neurotransmitters between gustatory neurons and taste bud cells. Moreover, information exchange between taste bud cells may play an important role in the modulation of taste perception (Roper, 2006). Nagai et al. (1996) reported that subsets of taste bud cells might release glutamate and modulate gustatory information.

Possible roles for kainate GluRs in taste buds

In the central nervous system, kainate GluRs exhibit much slower EPSCs and integrate excitatory inputs over large time windows (Lerma, 2003). They can mediate excitatory synaptic signals, but are also involved in modulating the presynaptic release of neurotransmitters, and therefore in regulating the strength of synaptic connections (Madden, 2002). The GluR5 and KA1 subunits of GluRs are expressed in subsets of taste bud cells. Type II and type III cells preferentially express kainate receptors over type I cells. This suggests that kainate GluRs in taste bud cells might be involved in information transmission from type II cells to type III cells, or that efferent glutamatergic control from the central nervous system might modulate communication between taste cells and/or afferent nerve fibers. Moreover, the presence of kainate GluRs in subsets of type I taste bud cells suggests that those cells may be involved in taste transduction procedures, though further investigation is needed. Experiments with knock out animals may elucidate more specific roles for kainate GluRs in taste bud cells.

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