Chem. Senses 26: 179–188, 2001

Immunocytochemistry of Glutathione *S*-Transferase in Taste Bud Cells of Rat Circumvallate and Foliate Papillae

Tomoko Nishino, Hideaki Kudo, Yoshiaki Doi, Masanobu Maeda[1,4], Kunshige Hamasaki[3], Miyako Morita[2] and Sunao Fujimoto

Departments of Anatomy and [2]Dentistry and Oral Surgery, School of Medicine, [1]Department of Systems Physiology, Institute of Industrial Ecological Sciences, [3]Department of Clinical Pathology, School of Health Sciences, University of Occupational and Environmental Health, Kitakyushu 807-8555, Japan

[4]Present address: Department of Physiology, Wakayama Medical College, Wakayama 641-8509, Japan

Correspondence to be sent to: Tomoko Nishino, Department of Anatomy, University of Occupational and Environmental Health, School of Medicine, 1-1 Iseigaoka, Yahatanishi-ku, Kitakyushu 807-8555, Japan. e-mail: 24no-tom@med.uoeh-u.ac.jp

Abstract

Immunocytochemistry was used to investigate the distribution of cells reacting with specific antibodies against glutathione *S*-transferase (GST) μ and π in rat circumvallate and foliate taste buds; the findings were confirmed by Western blotting. Double immunofluorescence staining for protein gene product (PGP) 9.5 and GST subunits allowed the classification of taste bud cells of both papillae into: (i) cells immunoreactive to either PGP 9.5 or GST subunit antibody; (ii) cells immunoreactive to both antibodies; and (iii) cells that did not react with either of these antibodies. Immunoelectron microscopy revealed that most GST subunit-immunoreactive cells seemed to be either type II or type III cells based on their ultrastructure. Since PGP 9.5 is now widely used as a marker for type III cells in mammalian taste buds, it seems reasonable to believe that most GST subunit-immunoreactive cells are type II cells. Whether cells immunoreactive for both PGP 9.5 and GST subunits consitute a small subpopulation of type III cells or whether they are intermediate forms between type II and III cells is under investigation. No type I cells reacted with antibodies against GST subunits in the present study. GST subunits in taste bud cells may participate in xenobiotic metabolism of certain substances exposed to taste pits, as already shown for olfactory epithelium.

Introduction

Cells comprising mammalian taste buds have been divided into 'dark' and 'light' cells by classic light microscopists. Using ultrastructural criteria, several researchers (Murray and Murray, 1967; Murray *et al.*, 1969; Fujimoto and Murray, 1970; Takeda and Hoshino, 1975; Takeda, 1976; Yamasaki *et al.*, 1984; Farbman *et al.*, 1985) have classified mammalian taste bud cells into type I (dark) cells, which have supporting and secretory function, type II (light) cells of unknown function and type III (light) cells, which have a chemoreceptive function. Type III cells are characterized by afferent synaptic specializations to intragemmal nerve endings including increased density of the plasma membrane along the nerve and aggregations of synaptic vesicles, and by the presence of cored vesicles of 80–150 nm in diameter. Recent studies using immunoelectron microscopy have shown that immunoreactivity for protein gene product (PGP) 9.5, a marker of certain types of neurons and sensory paraneurons (Iwanaga *et al.*, 1992; Astbäch *et al.*, 1995), is found exclusively in type III cells of the rat circumvallate taste bud (Kanazawa and Yoshie, 1996). Type II cells are broader in shape like type III cells and display the lowest electron density resulting in the clearest appearance of the nucleoplasm and cytoplasm among the above cell types. Type II cells do not appear to possess afferent synaptic specializations as in type III cells in spite of extensive contact with nerve terminals. Thus, the involvement of this cell type in taste transduction has been suspected. Due to the presence of subsurface cisternae, both along and close to the nerve contact area, several studies have proposed that type II cells may receive efferent projections, although the chemoreceptive function of these cells was not denied by these researchers (Fujimoto and Murray, 1970; Takeda, 1976). However, Idé and Munger (Idé and Munger, 1980) indicated that type II and III cells are associated with

different nerve terminals by means of the subsurface cisterna and conventional afferent synapse. Kinnamon and associates (Kinnamon *et al.*, 1985, 1988; Royer and Kinnamon, 1988) have argued that all of these cell types could be chemoreceptive, since all appeared to form synaptic connections on analysis of serial sections.

Taste bud cells have a limited life span and are constantly replaced by stem cells (Beidler and Smallman, 1965). It has long been debated whether the above cell types originate separately from a single less-differentiated form as stem cells (basal or type IV cells) with a different function, which matures and degenerates without transforming into other cell types (Fujimoto and Murray, 1970; Zahm and Munger, 1983; Farbman *et al.*, 1985; Toyoshima and Tandler, 1986) or whether they represent different developmental stages with ultrastructural variations reflecting functional changes of a single cell line (Scalzi, 1967; Pumplin *et al.*, 1997). In this regard, several groups have proposed the existence of intermediate forms between each cell type (Kinnamon *et al.*, 1985, 1988; Delay *et al.*, 1986; Roper, 1989).

Glutathione *S*-transferase (GST; EC 2.5.1.18) constitutes a superfamily of xenobiotic metabolizing enzymes that bind various ligands and catalyze the nucleophilic addition of glutathione to diverse electrophilic substrates in cytoplasm and/or nuclei of various tissues (Jakoby, 1978; Salinas and Wong, 1999). Based on its biochemical characteristics, cytosolic GST is divided into α, μ, π and θ isoforms (Mannervik and Danielson, 1988; Meyer *et al.*, 1991). Recent studies have shown high expression of GST proteins and/or mRNAs in the rat olfactory epithelium (Banger *et al.*, 1993; Ben-Arie *et al.*, 1993). Subsequent immunocytochemical studies reported that GST subunits were expressed on cells constituting rat sensory organs such as supporting cells of the olfactory epithelium (Banger *et al.*, 1994; Rama-Krishna *et al.*, 1994) and hair cells of the organ of Corti (El Barbary *et al.*, 1993). In addition, based on the widely accepted notion that zinc deficiency induces olfactory and gustatory dysfunction (Russell *et al.*, 1983; Tomita, 1990), Kudo *et al.* (Kudo *et al.*, 2000), using an *in situ* hybridization technique, revealed that zinc deficiency in rats was associated with a marked reduction in the expression of GST mRNAs in supporting cells of the rat olfactory epithelium. The above groups proposed that GST subunits may be involved in xenobiotic metabolism of various substances exposed to sensory epithelia such as odorants and toxicants as well as debris from dying cells.

The presence of GST subunits in mammalian taste buds and the exact cell type or cell types that contain this enzyme remain to be fully investigated. In the present study, we used immunocytochemistry to investigate the presence of GST subunits on taste buds of circumvallate and foliate papillae. Our study included double immunofluorescent labeling of PGP 9.5 and GST subunits on taste bud cells of both papillae. Our results may enhance our understanding of the function of type II cells in mammalian taste buds.

Materials and methods

Animals

Male Wistar rats aged 8 weeks and weighing 250 ± 30 g (Seac Yoshitomi, Fukuoka, Japan) were provided for the present study. The care and use of animals followed 'The Guiding Principles for the Care and Use of Animals', approved by our university in accordance with the principles of the Declaration of Helsinki. Forty rats were deeply anesthetized with an intraperitoneal injection of 5 mg of pentobarbital per 100 g body weight and used for the following experiments.

Western blot analysis

Circumvallate and foliate papillae were isolated and quickly washed in ice-cold phosphate-buffered saline (PBS). Specimens were homogenized in ~1.5 vol of PBS and centrifuged at 10 000 *g* for 10 min. The supernatant fluid was stored at –30°C and used for gel electrophoresis. Samples containing 50 μg protein, as determined by Lowry's method (Lowry *et al.*, 1951), were heated at 100°C for 2 min in 3% sodium dodecyl sulfate (SDS) with a reduction with 10% 2-mercaptoethanol. They were separated using 15% SDS–polyacrylamide gel electrophoresis (SDS–PAGE, pH 8.8) and the separated proteins were stained with 0.1% Coomassie brilliant blue R-250 in a mixture of 0.5% ethanol and 5% acetic acid. Protein mol. wt markers (low mol. wt set) were obtained from Pharmacia (Uppsala, Sweden). Protein samples were resolved by SDS–PAGE and transferred to a polyvinylidene difluoride membrane (Millipore, Bedford, MA) with a Semidry Trans-blot SD (Bio-Rad, South Richmond, CA). The membrane was incubated for 1 h with 5% skimmed milk in Tris-buffered saline (TBS) pH 7.4 to reduce non-specific protein absorption and allowed to react overnight with a rabbit polyclonal antibody against GSTα, GSTμ or GSTπ (Novocastra Laboratories, Newcastle upon Tyne, UK) at a dilution from 1:300 to 1:2000 in 5% skimmed milk in TBS. After rinsing with TBS, the membrane was incubated with a horseradish peroxidase (HRP)-conjugated goat anti-rabbit IgG (Bio-Rad) at a dilution of 1:1000 in TBS for 3 h, developed in 0.06% 4-chloro1-naphthol in TBS containing 0.01% H_2O_2 for 3–5 min and air-dried.

Light microscopy and immunocytochemistry

Animals were perfused with physiological saline from the left ventricle, followed by a solution of 2% paraformaldehyde (PFA) in 0.1 M phosphate buffer (PB; pH 7.2) for 5 min each. After perfusion, the circumvallate and foliate papillae were isolated. Both papillae were immersed with 4% PFA in 0.1 M PB for 72 h at 4°C, rinsed with 0.1 M PB containing 10% sucrose, dehydrated through a graded ethanol series and embedded in paraffin (Histosec; Merck, Darmstadt, Germany). Serial sections (~5 μm thick) were prepared using a microtome, mounted on glass slides (MAS

coated Superfrost; Matsunami, Osaka, Japan), air-dried at 4°C and used for Delafield's hematoxylin and eosin staining or immunostaining. Deparaffinized and hydrated sections were blocked with 0.1% H_2O_2 in methanol for 20 min to remove endogenous peroxidase and rinsed with PBS. They were then incubated with 10% normal goat serum for 20 min and incubated in a humid chamber with the above rabbit anti-GSTμ and rabbit anti-GSTπ polyclonal antibody at a dilution from 1:500 to 1:1000 in PBS, or rabbit anti-PGP 9.5 polyclonal antibody (UltraClone, Cambridge, UK) at a dilution of 1:1000 in PBS for 16 h at 4°C, respectively. After rinsing in PBS, the labeled streptavidin–biotin complex (LSAB) method (LSAB kit; DAKO, Carpinteria, CA) was used for the following immunostaining. The peroxidase complex was visualized by treatment with a freshly prepared 0.1 mg/ml diaminobenzidine tetrahydrochloride (DAB) solution with 0.01% H_2O_2 for 8 min. The specificity of the above immunoreactivities was confirmed by replacing the primary antibodies with either normal rabbit sera or PBS.

Double immunofluorescence microscopy

To examine the correlation between GST subunit- and PGP 9.5-immunoreactive cells in the same taste buds, deparaffinized sections were irradiated with 600 W microwaves (Micromed T/T microwave equipment; Milestone, Sorisole, Italy) for 20 min. Antigen-retrieved sections were treated with 10% normal goat serum in PBS for 30 min, first immunoreacted with rabbit anti-GSTμ or anti-GSTπ polyclonal antibody at a dilution of 1:400 and then with mouse anti-PGP 9.5 monoclonal antibody (UltraClone) at a dilution of 1:200 each for 18 h at 4°C. Sections were reacted with Alexa Fluor™ 488 goat anti-rabbit IgG conjugate (Molecular Probes, Eugene, OR) at a dilution of 1:100 for 2 h each at room temperature. Alexa Fluor™ 546 goat anti-mouse IgG conjugate was applied at a dilution of 1:100 to PGP 9.5 for 2 h at room temperature. All incubations were carried out in a humid chamber. Sections were coverslipped with 90% nonfluorescent glycerol containing 2 mg/ml 1,4-diazabicyclo[2.2.2]octane (DABCO) and 7.5 μg/ml of 4′,6-diamino-2-phenylindole dihydrochloride (DAPI) in PBS, and examined on a Carl Zeiss LSM 410 confocal laser scanning microscope. The digitally captured images were superimposed using software of the equipment in the LSM410. Final processing (to adjust size, brightness and contrast) was performed with Adobe Photoshop software (Adobe Systems Inc., San Jose, CA). The specificity of the above double-labeling immunofluorescence was confirmed by replacing the anti-GST subunit antibody and/or anti-PGP 9.5 antibody with PBS.

To determine the number of cells that were immunoreactive towards antibodies against GSTs and PGP 9.5, the circumvallate papillae from four randomly chosen rats were evaluated histologically. We counted DAPI-labeled nuclei and each immunoreactive cell in complete sets of serial longitudinal sections of two randomly selected taste buds in each papilla on confocal images at a magnification of 1400×. When the same cells were recognized in serial sections, they were counted as one cell.

Immunoelectron microscopy

Both papillae were fixed in a mixture of 1% glutaraldehyde (GA) and 2% PFA in 0.1 M PB for 16 h at 4°C. Approximately 40-μm-thick sections were prepared on a Microslicer DTK-3000W (Dosaka EM, Kyoto, Japan) and then incubated with either rabbit anti-GSTμ or rabbit anti-GSTπ polyclonal antibody at a dilution from 1:500 to 1:800 in PBS for 12 h at 4°C. After a brief rinse with PBS, sections were immunostained according to the LSAB method. Sections were fixed in 0.1% GA in 0.1 M PB, developed in a mixture of 0.05% DAB and 0.01% H_2O_2 in 0.05 M Tris–HCl buffer (pH 7.6), postfixed in 0.1% osmium tetroxide in 0.1 M PB for 1 h at 4°C, dehydrated in graded series of acetone and embedded in epoxy resin. Ultrathin sections were prepared using an LKB ultramicrotome and examined under a JEM 1200 EX Electron Microscope (JEOL, Tokyo, Japan) without counterstaining. The specificity of the above immunoreactivities was confirmed by substitution of the primary antiserum for PBS.

Results

Soluble extracts from the rat circumvallate and foliate papillae were resolved by SDS–PAGE as shown in lanes 2 and 3 of Figure 1. In Western blot analysis, anti-GSTμ (lanes 4 and 5) and anti-GSTπ (lanes 6 and 7) antibodies recognized a band of molecular mass 26.5 and 24 kDa, respectively. However, the analysis did not detect positive immunoreactivity for anti-GSTα antibody (lanes 8 and 9) in either of the papillae.

Analysis of serial sections of circumvallate (Figure 2) and

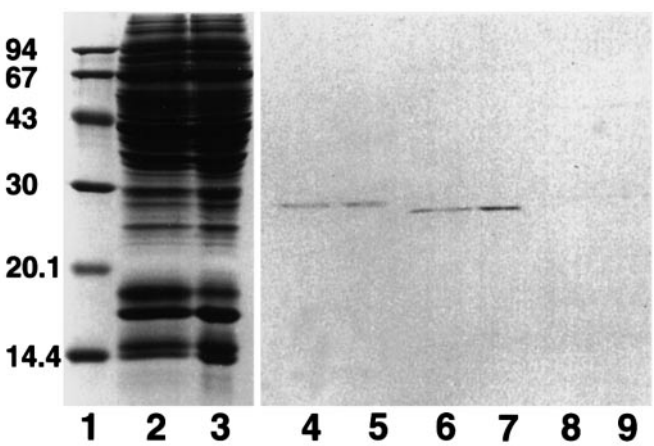

Figure 1 SDS–PAGE (lanes 1–3) and Western blotting with antibodies against GSTμ (lanes 4 and 5), GSTπ (lanes 6 and 7) and GSTα (lanes 8 and 9) of soluble extracts from rat circumvallate (lanes 2, 4, 6 and 8) and foliate papillae (lanes 3, 5, 7, and 9). Positions of low molecular mass markers (lane 1), expressed in kDa, are indicated on the left of the figure.

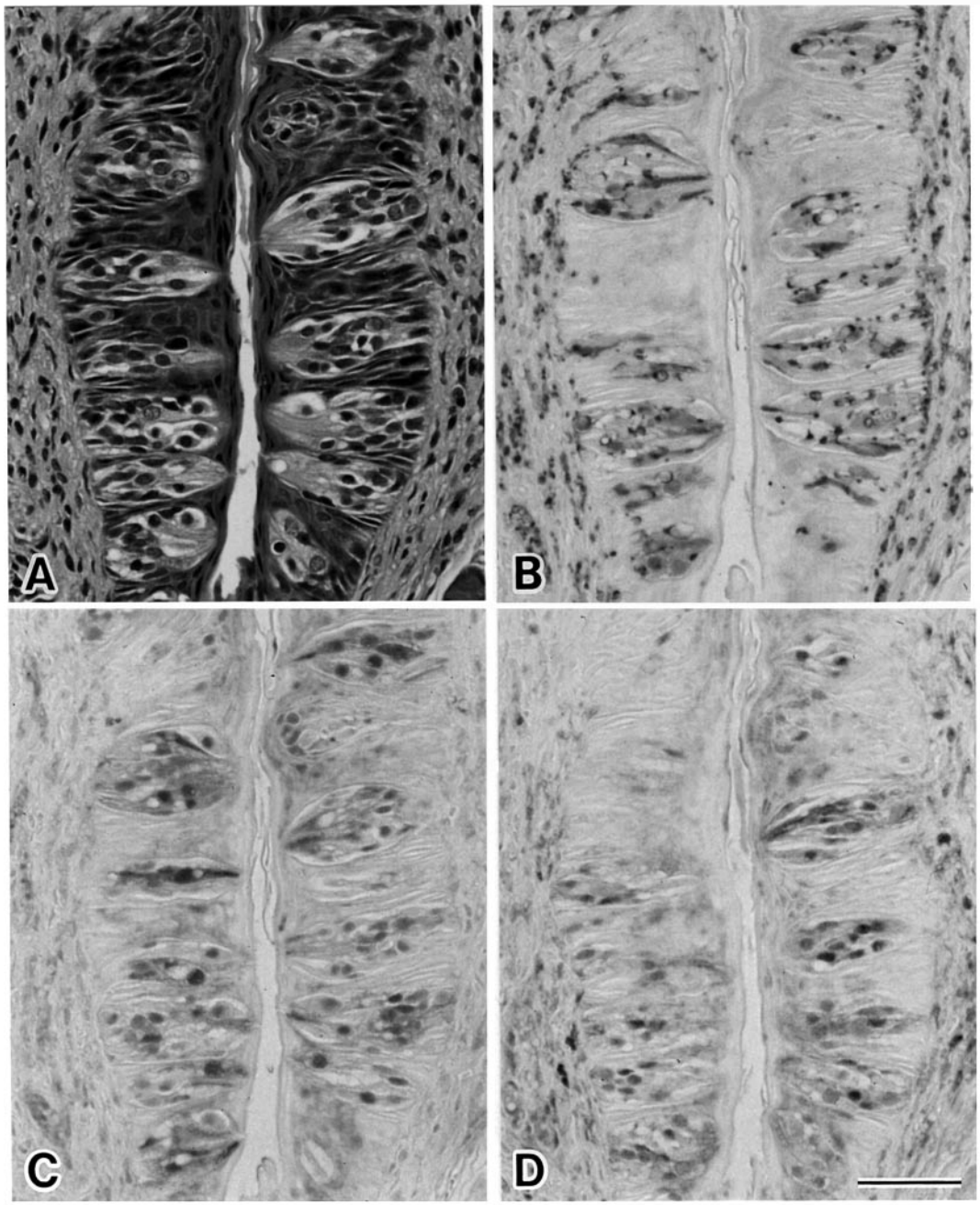

Figure 2 Serial sections of rat circumvallate papilla. **(A)** Delafield's hematoxylin and eosin staining. **(B)** Immunoreactivity for PGP 9.5 in subepithelial nerve fibers, intragemmal nerve fibers and taste bud cells. **(C)** Immunoreactivity for GSTμ in taste buds. **(D)** Immunoreactivity for GSTπ in taste buds. Bar: 50 μm.

foliate (Figure 3) papillae showed immunoreactivity for PGP 9.5 (Figures 2B and 3B) in bundles of nerve fibers forming subepithelial networks, intragemmal nerve fibers which extended throughout the taste buds and a certain population of taste bud cells that were occasionally associated with the immunoreactive nerve fibers. Immunoreactivity for GSTμ (Figures 2C and 3C) and for GSTπ (Figures 2D and 3D) was also found in another taste bud cell group but was undetectable in nerve elements. A few taste bud cells appeared not to be immunoreactive with the antibodies used. Taste bud cells that were immunoreactive to GSTα were not detected.

Double immunofluorescence labeling of PGP 9.5 and GSTμ in the same section of a single circumvallate taste bud showed immunoreactivity in a certain population of taste bud cells for either PGP 9.5 (red signals in Figure 4A) or GSTμ (green signals in Figure 4B). Only a few cells were immunoreactive to both antibodies (yellow signals in Figure 4C). Based on the differential interference contrast image (Figure 4D), some taste bud cells did not express any signals.

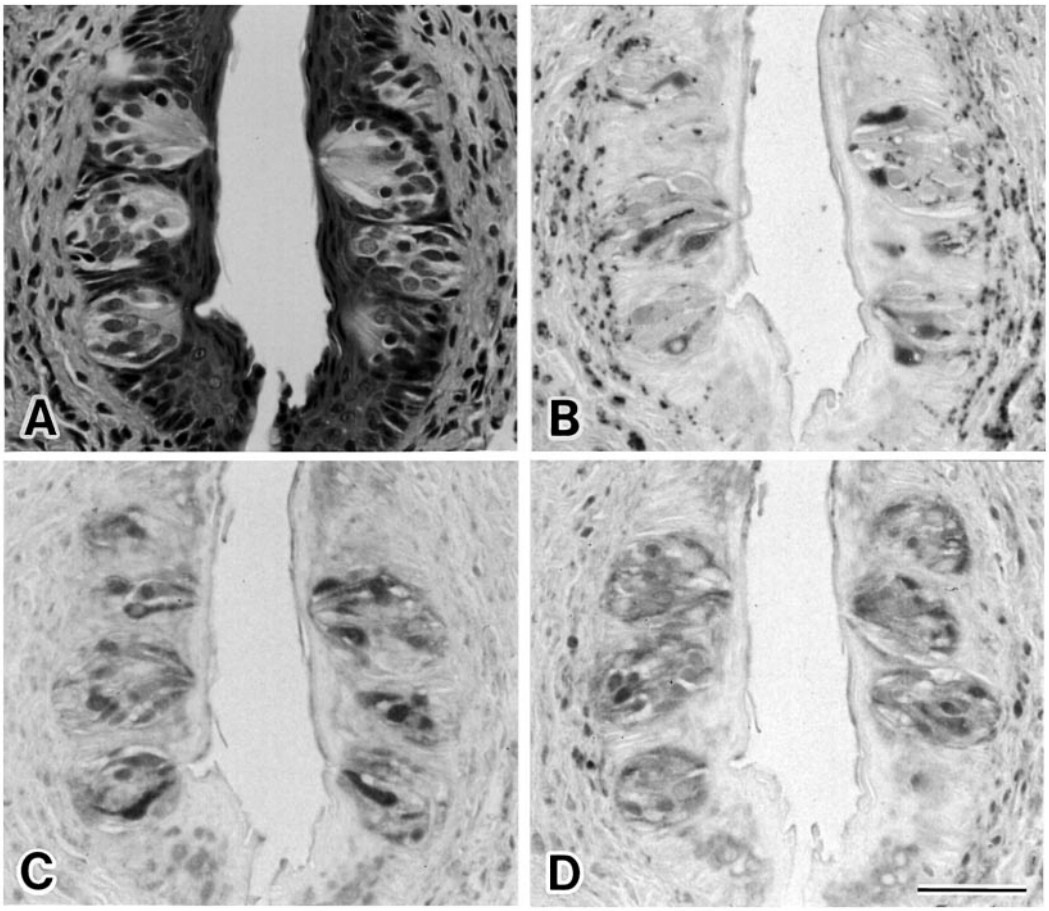

Figure 3 Serial sections of rat foliate papilla. **(A)** Delafield's hematoxylin and eosin staining. **(B)** Immunoreactivity for PGP 9.5 in subepithelial nerve fibers, intragemmal nerve fibers and taste bud cells. **(C)** Immunoreactivity for GSTμ in taste buds. **(D)** Immunoreactivity for GSTπ in taste buds. Bar: 50 μm.

Figure 5 demonstrates immunoreactive cells for PGP 9.5 and GSTπ in a single circumvallate taste bud by the same double immunofluorescence labeling technique. We found no fundamental inconsistencies in immunoreactivities for PGP 9.5 and GST subunits between circumvallate and foliate taste buds. In Figures 4 and 5, fluorescent granular structures occasionally appeared throughout the taste buds. These are considered to be non-specific large chemical complex, because such structures were not detected by HRP-labeling immunocytochemistry as shown in Figures 2 and 3. Table 1 summarizes populations of cells showing PGP9.5-immunoreactive cells among GSTμ- or GSTπ-immunoreactive cells, type IV (basal) cells, and immunonegative cells in circumvallate taste buds. There were no significant differences in the percentage and number of those cells between GSTμ and GSTπ ($P < 0.01$).

Immunoelectron microscopic examination based on the preembedding method identified cells immunoreactive for GSTμ (Figure 6A) and GSTπ (Figure 6B), which were characterized by the lightly appearing nucleus and broader cytoplasm. The latter contained abundant dilated cisternae of smooth endoplasmic reticulum and evenly dispersed mitochondria. Cored vesicles of ~100 nm, unique inclusions found near the nucleus of type III cells, were not found in GST subunit-immunoreactive cells. Immunoreactivities for both subunits were preferentially seen in amorphous cytoplasmic areas throughout the cells but were not conspicuous on the plasma membrane and limiting membrane of organelles such as mitochondria and smooth endoplasmic reticulum. The slender cytoplasm, which included dark granules of 100–300 nm in diameter, a unique feature of type I cells in the apical part near the taste pit, did not react with antibodies against either of the GST subunits (Figure 6A and B). Loss of immunoreactivity for GSTs in the nuclei in our immunoelectron micrographs is thought to be due mainly to a reduction in the sensitivity of detection caused by the use of fixative such as glutaraldehyde in preembedding methods.

Discussion

Western blot analysis in the present study revealed a single band of 26.5 kDa (with the anti-GSTμ antibody) and 24 kDa (with the anti-GSTπ antibody) in both circumvallate and foliate papillae. These molecular masses are identical to

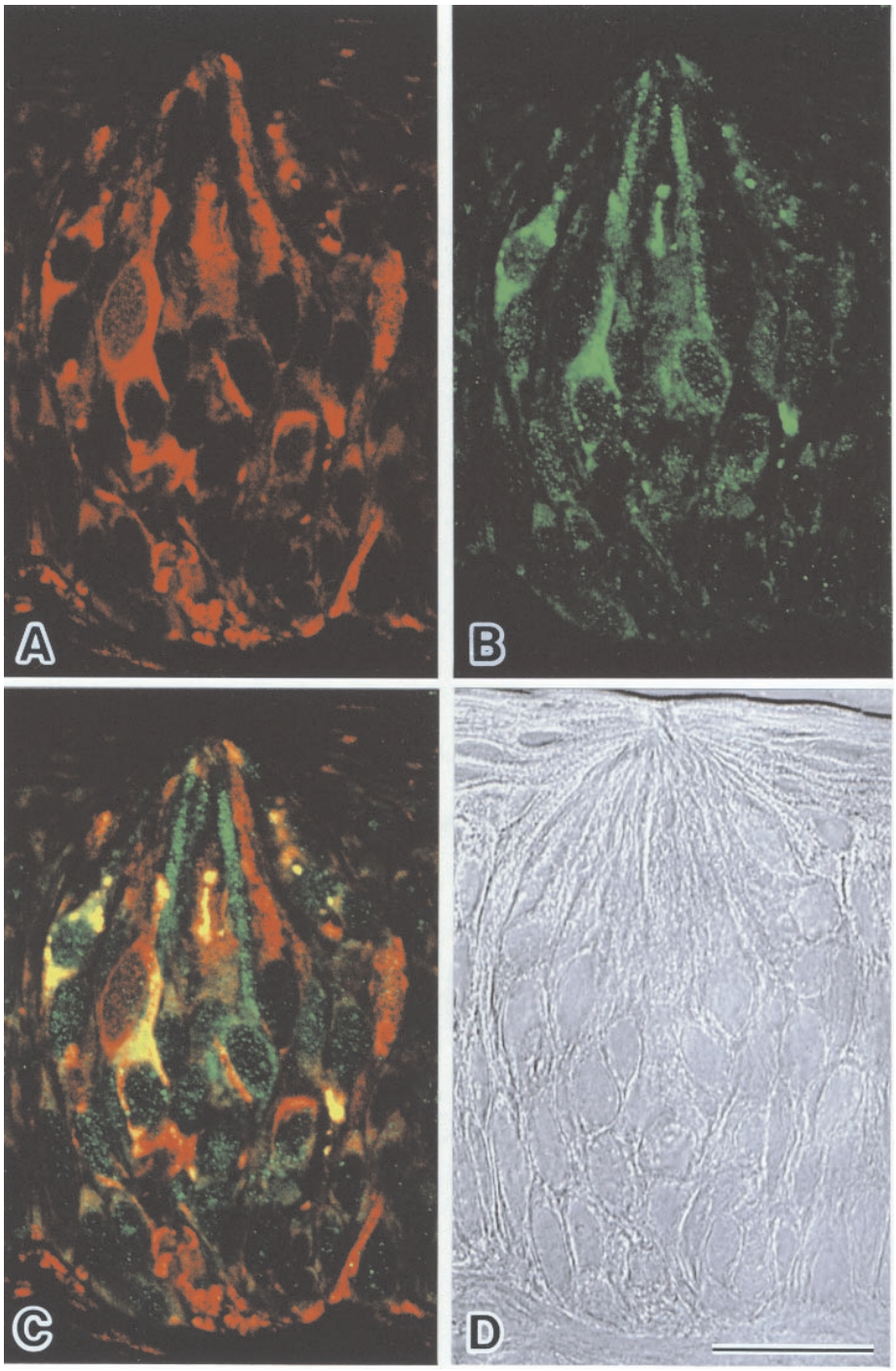

Figure 4 Double immunofluorescence labeling of PGP 9.5 and GSTμ in a rat circumvallate taste bud. **(A)** Immunoreactivity for PGP 9.5 (red signal). **(B)** Immunoreactivity for GSTμ (green signal). **(C)** Combined double-labeled image of PGP 9.5 and GSTμ shown in (A) and (B). The yellow signal reflects overlapping of immunoreactivities for PGP 9.5 and GSTμ. **(D)** Differential interference contrast image. Bar: 20 μm.

those of rat GST subunits found in other tissues (Suguoka *et al.*, 1985; Ding *et al.*, 1986). Our results thus indicate the usefulness of these antibodies for immunocytochemical detection of GSTμ and π subunits in taste bud cells. In contrast, no band was detectable with the anti-GSTα antibody in the present analysis, indicating that the GSTα subunit is not present in either type of papilla or is present but below the detection limit.

Our immunocytochemical study demonstrated that a proportion of taste bud cells in circumvallate and foliate papillae reacted with antibodies against GSTμ and GSTπ. Analysis of PGP 9.5- and GSTμ or π-immunoreactive cells

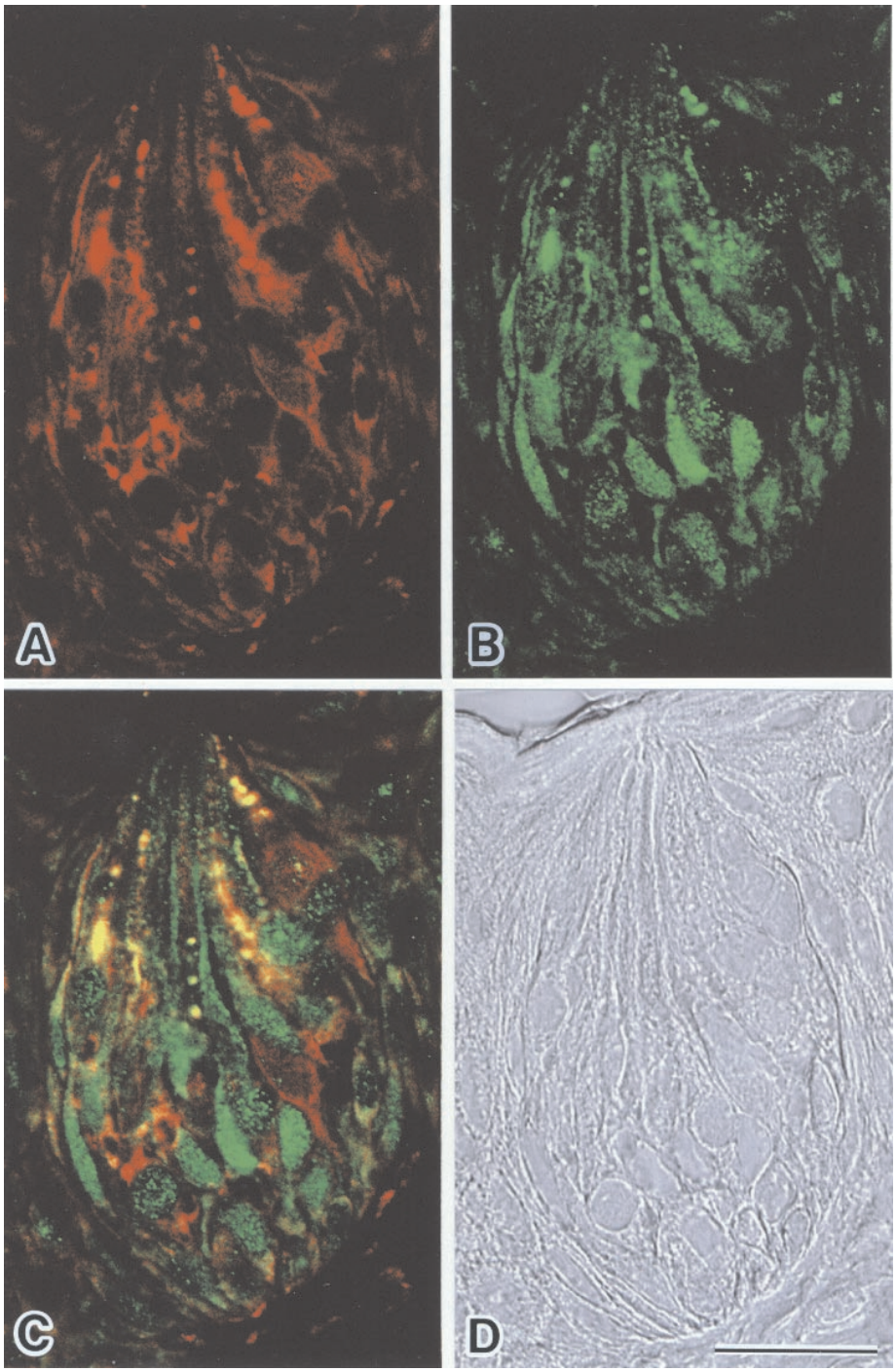

Figure 5 Double immunofluorescence labeling of PGP 9.5 and GSTπ in a rat circumvallate taste bud. **(A)** Immunoreactivity for PGP 9.5 (red signal). **(B)** Immunoreactivity for GSTπ (green signal). **(C)** Combined double-labeled image of PGP 9.5 and GSTπ shown in (A) and (B). The yellow signal reflects overlapping of immunoreactivities for PGP 9.5 and GSTπ. **(D)** Differential interference contrast image. Bar: 20 μm.

by double immunofluorescence labeling in both papillae revealed that cells comprising these taste buds could be divided into three groups based on their distinct immunoreactivities: (i) cells immunoreactive for either PGP 9.5 or GST subunits; (ii) cells immunoreactive for both PGP 9.5 and GST subunits; and (iii) cells that were not immunoreactive for PGP 9.5 or GST subunits. Since previous immunoelectron microscopic studies have shown that type III cells in the rat circumvallate taste bud are uniquely immunoreactive to PGP 9.5 (Kanazawa and Yoshie, 1996), we believe that PGP 9.5-immunoreactive cells, which were occasionally associated with immunoreactive nerve fibers

Table 1 Proportion of PGP 9.5-immunoreactive cells among GSTμ or GSTπ-immunoreactive cells, type IV (basal) cells and immunonegative cells in circumvallate taste buds[a]

	% of each cell					Total cell no.[g]
	GST[b]	PGP[c]	PGP + GST[d]	Type IV[e]	Immunonegative[f]	
GSTμ	18.43 ± 1.611	13.94 ± 2.380	7.097 ± 1.620	18.00 ± 1.098	56.73 ± 2.406	82.00 ± 4.520
GSTπ	19.23 ± 1.509	15.14 ± 1.402	8.226 ± 0.782	13.01 ± 1.369	60.84 ± 1.271	78.38 ± 6.772

[a]Values are means ± SEM.

[b]Number of GSTμ- or GSTπ-immunoreactive cells per taste bud.

[c]Number of PGP 9.5-immunoreactive cells per taste bud.

[d]Number of cells reacting with antibodies against PGP 9.5 and GSTs per taste bud.

[e]Number of type IV (basal) cells per taste bud.

[f]Number of cells that did not react with antibodies against PGP 9.5 or GSTs (excluding type IV cells) per taste bud.

[g]Number of DAPI-labeled nuclei per taste bud.

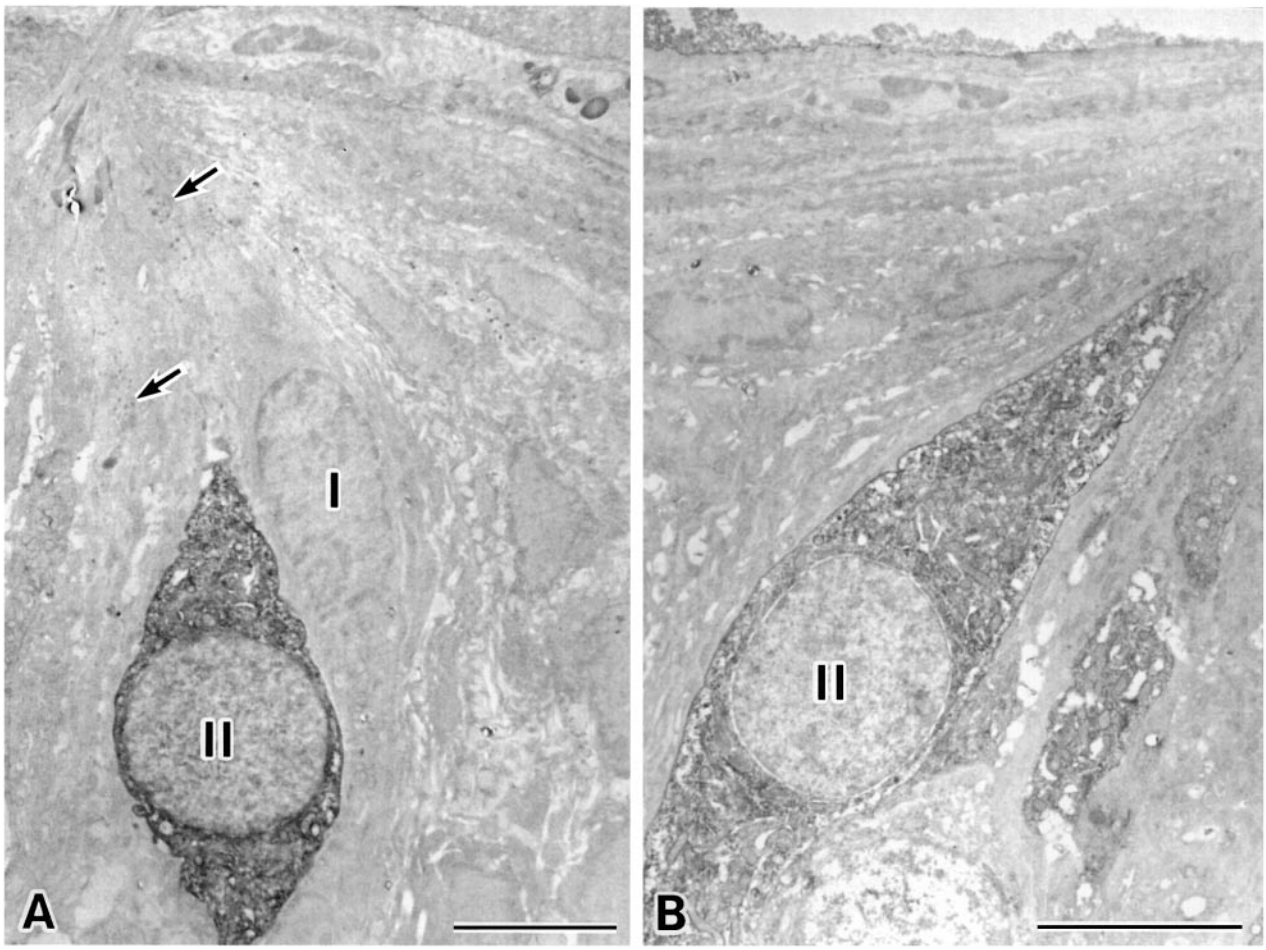

Figure 6 Immunoelectron micrograph showing immunoreactive cells for GST subunits in circumvallate taste bud. **(A)** GSTμ-immunoreactive type II cell (II). The type I cell (I) did not react with the antibody. The apical cytoplasm containing dark granules (arrows) of type I cells is not immunoreactive. **(B)** GSTπ-immunoreactive type II cell (II). Bars: 5 μm.

(see Figures 2B and 3B), are type III cells. Furthermore, cells that did not react with these antibodies are considered to represent type I cells, judging from the distribution of cell types shown in Table 1, which are almost consistent with type I cells in mouse and rabbit taste buds (Murray, 1969; Delay *et al.*, 1986).

The immunoelectron micrographs shown in Figure 6 demonstrate that the nuclei of GST subunit-immunoreactive cells are characterized by loose chromatin networks, which are characteristic of type II or III cells. Other ultrastructural features of type II cells include abundant smooth endoplasmic cisternae, and mitochondria evenly dispersed in the cytoplasm. From these results together with the data from double immunofluorescence studies, we infer that most GST subunit-immunoreactive cells represent type II cells. In the present study, reactivity with antibodies against GST subunits was not detected in type I cells which uniquely contain dark granules in the apical cytoplasm (see Figure 6).

Expression of GST subunits in supporting cells of the rat olfactory epithelium has been found previously (Banger *et al.*, 1994; Rama-Krishna *et al.*, 1994; Genter *et al.*, 1995). These groups proposed that the GST subunits may be involved in xenobiotic metabolism of various substances such as odorants, olfactory toxicants and cellular debris from dying cells, suggesting that xenobiotic enzymes including GST subunits may terminate the odorant response by removing the above substances. Furthermore, Kudo *et al.* (2000) demonstrated, in zinc-deficient rats that exhibited olfactory dysfunction, a reduced expression of GSTμ at both protein and mRNA levels in supporting cells of the rat olfactory epithelium. These results suggest that GST subunits in the supporting cells are essential for the maintenance of olfactory cell function.

Although GST subunits in type II cells may be involved in tasks similar to those proposed for the olfactory epithelium, the exact role of GST subunits in this cell type is difficult to explain at present. It has been suggested that type I cells expressing carbonic anhydrase isozyme II (CAII) are involved in regulating intracellular pH in responses to specific forms of taste stimuli in rat taste buds (Daikoku *et al.*, 1999). Thus, it seems likely that enzymes such as CAII in type I cells and GSTs in the type II cells studied here may be involved in homeostasis of the chemical environment, which is essential for maintaining the chemoreceptive function of type III cells. Conventional electron microscopy has demonstrated that type II cells occasionally contain coated vesicles derived from invaginations of the plasma membrane at the microvillus-specialized areas in the taste pit (Fujimoto, 1973). Thus, various substances, which are taken up by type II cells, may ultimately be exposed to GST subunits, although this is entirely speculative at present.

Our double immunofluorescence labeling study indicates that a small proportion of taste bud cells react with antibodies against PGP 9.5 and with those against GST subunits. Further studies are now in progress in our laboratory to investigate whether such cells represent a small subpopulation of type III cells, or whether they reflect intermediate forms between type II and type III cells of a single cell line.

Acknowledgements

We thank Toyono Nobukuni and Itsumi Ban from the Department of Anatomy for excellent secretarial assistance, and Mitsuru Yokoyama from the Electron Microscope Laboratory for preparation of photographs.

References

Astbäch, J., Arvidson, K. and **Johansson, O.** (1995) *Neurochemical markers of human fungiform papillae and taste buds*. Regul. Pept., 59, 389–398.

Banger, K.K., Lock, E.A. and **Reed, C.J.** (1993) *The characterization of glutathione S-transferase from rat olfactory epithelium*. Biochem. J., 290, 199–204.

Banger, K.K., Foster, J.R., Lock, E.A. and **Reed, C.J.** (1994) *Immunohistochemical localisation of six glutathione S-transferases within the nasal cavity of the rat*. Arch. Toxicol., 69, 91–98.

Beidler, L.M. and **Smallman, R.L.** (1965) *Renewal of cells within taste buds*. J. Cell. Biol., 27, 263–272.

Ben-Arie, N., Khen, M. and **Lancet, D.** (1993) *Glutathione S-transferases in rat olfactory epithelium: purification, molecular properties and odorant biotransformation*. Biochem. J., 292, 379–384.

Daikoku, H., Morisaki, I., Ogawa, Y., Maeda, T., Kurisu, K. and **Wakisaka, S.** (1999) *Immunohistochemical localization of carbonic anhydrase isozyme II in the gustatory epithelium of the adult rat.* Chem. Senses, 24, 255–261.

Delay, R.J., Kinnamon, J.C. and **Roper, S.D.** (1986) *Ultrastructure of mouse vallate taste buds: II. Cell types and cell lineage.* J. Comp. Neurol., 253, 242–252.

Ding, G.J.-F., Ding, V.D.-H., Rodkey, J.A., Bennett, C.D., Lu, A.Y.H. and **Pickett, C.B.** (1986) *Rat liver glutathione S-transferase. DNA sequence analysis of a Yb2 cDNA clone and regulation of the Yb1 and Yb2 mRNAs by phenobarbital*. J. Biol. Chem., 261, 7952–7957.

El Barbary, A., Altschuler, R.A. and **Schacht, J.** (1993) *Glutathione S-transferases in the organ of Corti of the rat: Enzymatic activity, subunit composition and immunohistochemical localization.* Hearing Res., 71, 80–90.

Farbman, A.I., Hellekant, G. and **Nelson, A.** (1985) *Structure of taste buds in foliate papillae of the rhesus monkey,* Macaca mulatta. Am. J. Anat., 172, 41–56.

Fujimoto, S. (1973) *On the Golgi-derived vesicles in the rabbit taste bud cells: an electron microscopy and related cytochemistry.* Kurume Med. J., 20, 133–148.

Fujimoto, S. and **Murray, R.G.** (1970) *Fine structure of degeneration and regeneration in denervated rabbit vallate taste buds*. Anat. Rec., 168, 393–414.

Genter, M.B., Owens, D.M. and **Deamer, N.J.** (1995) *Distribution of microsomal epoxide hydrolase and glutathione S-transferase in the rat olfactory mucosa: relevance to distribution of lesions caused by systemically-administered olfactory toxicants*. Chem. Senses, 20, 385–392.

Idé, C. and **Munger, B.L.** (1980) *The cytologic composition of primate laryngeal chemosensory corpuscles*. Am. J. Anat., 158, 193–209.

Iwanaga, T., Han, H., Kanazawa, H. and **Fujita, T.** (1992) *Immunohistochemical localization of protein gene product 9.5 (PGP 9.5) in sensory paraneurons of the rat*. Biomed. Res., 13, 225–230.

Jakoby, W.B. (1978) *The glutathione S-transferases: a group of multifunctional detoxification proteins.* Adv. Enzymol. Relat. Areas Mol. Biol., 46, 384–414.

Kanazawa, H. and **Yoshie, S.** (1996) *The taste bud and its innervation in the rat as studied by immunohistochemistry for PGP 9.5*. Arch. Histol. Cytol., 59, 357–367.

Kinnamon, J.C., Taylor, B.J., Delay, R.J. and **Roper, S.D.** (1985) *Ultrastructure of mouse vallate taste buds. I. Taste cells and their associated synapses.* J. Comp. Neurol., 235, 48–60.

Kinnamon, J.C., Sherman, T.A. and **Roper, S.D.** (1988) *Ultrastructure of mouse vallate taste buds: III. Patterns of synaptic connectivity*. J. Comp. Neurol., 270, 1–10.

Kudo, H., Doi, Y., Nishino, T., Nara, S., Hamasaki, K. and **Fujimoto, S.** (2000) *Dietary zinc deficiency decreases glutathione S-transferase expression in the rat olfactory epithelium*. J. Nutr., 130, 38–44.

Lowry, O.H., Rosebrough, N.J., Farr, A.L. and **Randall, R.J.** (1951) *Protein measurement with the folin phenol reagent*. J. Biol. Chem., 193, 265–275.

Mannervik, B. and **Danielson, U.H.** (1988) *Glutathione transferase-structure and catalytic activity.* Crit. Rev. Biochem., 23, 283–337.

Meyer, D.J., Coles, B., Pemble, S.E., Gilmore, K.S., Fraser, G.M. and **Ketterer, B.** (1991) *Theta, a new class of glutathione transferases purified from rat and man.* Biochem. J., 274, 409–414.

Murray, R.G. (1969) *Cell types in rabbit taste buds*. In Pfaffmann, C. (ed.), Olfaction and Taste, Vol. 3. Rockefeller University Press, New York, pp. 331–344.

Murray, R.G. and **Murray, A.** (1967) *Fine structure of taste buds of rabbit foliate papillae*. J. Ultrastruct. Res., 19, 327–353.

Murray, R.G., Murray, A. and **Fujimoto, S.** (1969) *Fine structure of gustatory cells in rabbit taste buds.* J. Ultrastruct. Res., 27, 444–461.

Pumplin, D.W., Yu, C. and **Smith, D.V.** (1997) *Light and dark cells of rat vallate taste buds are morphologically distinct cell types*. J. Comp. Neurol., 378, 389–410.

Rama-Krishna, N.S., Getchell, T.V. and **Getchell, M.L.** (1994) *Differential expression of alpha, mu, and pi classes of glutathione S-transferase in chemosensory mucosae of rats during development.* Cell Tissue Res., 275, 435–450.

Roper, S.D. (1989) *The cell biology of vertebrate taste receptors.* Annu. Rev. Neurosci., 12, 29–53.

Royer, S.M. and **Kinnamon, J.C.** (1988) *Ultrastructure of mouse foliate taste buds: synaptic and nonsynaptic interactions between taste cells and nerve fibers.* J. Comp. Neurol., 270, 11–24, 58–59.

Russell, R.M., Cox, M.E. and **Solomons, N.** (1983) *Zinc and the special senses.* Ann. Intern. Med., 99, 227–239.

Salinas, A.E. and **Wong, M.G.** (1999) *Glutathione S-transferases—a review.* Curr. Med. Chem., 6, 279–309.

Scalzi, H.A. (1967) *The cytoarchitecture of gustatory receptors from the rabbit foliate papillae.* Z. Zellforsch., 80, 413–435.

Suguoka, Y., Kano, T., Okuda, A., Sakai, M., Kitagawa, T. and **Muramatsu, M.** (1985) *Cloning and the nucleotide sequence of rat glutathione S-transferase P cDNA*. Nucleic Acids Res., 13, 6049–6057.

Takeda, M. (1976) *An electron microscopic study on the innervation in the taste buds of the mouse circumvallate papillae*. Arch. Histol. Jpn., 39, 257–269.

Takeda, M. and **Hoshino, T.** (1975) *Fine structure of taste buds in the rat.* Arch. Histol. Jpn., 37, 395–413.

Tomita, H. (1990) *Zinc in taste and smell disorder*. In Tomita, H. (ed.), Trace Elements in Clinical Medicine. Springer-Verlag, Tokyo, pp. 15–37.

Toyoshima, K. and **Tandler, B.** (1986) *Dividing type II cell in rabbit taste bud.* Anat. Rec., 214, 161–164.

Yamasaki, H, Kubota, Y, Takagi, H. and **Tohyama, M.** (1984) *Immunoelectron-microscopic study on the fine structure of substance-P-containing fibers in the taste buds of the rat.* J. Comp. Neurol., 227, 380–392.

Zahm, D.S. and **Munger, B.L.** (1983) *Fetal development of primate chemosensory corpuscles. I. Synaptic relationships in late gestation.* J. Comp. Neurol., 213, 146–162.

Accepted October 10, 2000