FULL PAPER *Anatomy*

Glycoconjugate Residues in a Subpopulation of Feline Taste Cells

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ABSTRACT. There have been a number of studies which have categorized cells of feline taste buds: Types I, II, III and IV; however, few studies have examined whether feline taste bud cell types differ from each other histochemically. The goal of the present study is to figure out what kinds of glycoconjugates correspond to the four different types of cells in the taste bud. We have detected glycochains by lectin histochemistry. We have also identified Types II and III by immunohistochemistry. Then, we combined lectin histochemistry and immunohistochemistry to determine which types of cells have which glycochains. In addition, we have compared these reactions in different papillae in the oral cavity: circumvallate papillae, fungiform papillae and epiglottises. Our results demonstrated that glycoconjugates showed a variety of distributions among cells in these papillae, although immunopositive reactions of the proteins involved in the taste transduction showed similar distributions in the taste buds in these papillae. Amongst all, N-acetyllactosamine was the most prominently detected glycoconjugate residue in a subpopulation of Type II (receptor) cells and Type III (pre-synaptic) cells. Our findings suggest that 1) Different localization of glycol-residues in taste buds might be owing to the possibility that different types of cells need different types of glycoconjugates, possibly for the function of cells in the taste buds, and 2) N-acetyllactosamine might play some roles in taste sensation perception and their transfer by Type II and III cells.

KEY WORDS: feline, glycoconjugate, presynaptic, receptor, taste.

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Four types of cells compose taste buds, which are distributed on the tongue, palate, epiglottis and other areas of the lingual and pharyngeal epithelia [18]. It has been reported that taste buds express variety of glycoconjugates, consisting of glycochains (sugar chains) chemically bound to fat or protein [9, 21]. However, their precise localizations, and their relationship to the cell types in cat taste buds, have not yet been fully clarified and still being controversial [5, 19]. In this study, first, we investigate cat taste buds lectin histochemically [24], and compared the results taken from different locations in the oral cavity: the circumvallate papillae (CV), the fungiform papillae (FG) and the epiglottis (EP). Second, we endeavored to determine which types of glycoconjugates are localized in the taste bud cells of the domestic cat, and which glycoconjugates are present in Type II and in Type III cells, because, among the four cell types, these two types play the central role in receiving and transferring the sensation of taste [14]. The proteins necessary for taste transduction, such as phospholipase Cβ2 (PLCβ2) [15] and inositol 1,4,5-triphosphate receptor type 3 (IP_3R3) [1, 3], are expressed in Type II cells. Type III cells contain various proteins related to synthesizing biogenic amines, such as tryptophan hydroxylase (TPH) [6] and serotonin (5HT). It has been reported that protein gene product 9.5 (PGP 9.5) is also localized in a transitional type of cell, namely one that is in transition from Type II to Type III [23, 25]. Thus, we immunohistochemically identified cell types and examined the relationship with localizations of glycoconjugates.

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MATERIALS AND METHODS

Animals: Eight healthy domestic Japanese cats of both sexes with an age range of 1–3 years and weighing 2–3 kg each were utilized. The cats were donated by the Laboratory of Infectious Diseases, School of Veterinary Medicine, Kitasato University, after being anesthetized with pentobarbital sodium (25.0 mg/kg body weight, i.v.) and euthanized. All the procedures were performed according to the guidelines for animal experiments of Kitasato University and the experimental plan was approved by the committee. The taste buds were fixed in 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4) (PB), embedded in paraffin, and cut to make serial sections. Some of them were stained with hematoxylin and eosin (HE). The numbers of certain types of cells and nuclei were counted and the percentages of each cell types per taste bud were calculated (average ± standard deviation).

Lectin histochemistry: Endogenous peroxidase activity was blocked by immersing the sections in 0.26% hydrogen peroxide in methanol at RT for 25 min. The sections were incubated with 10% normal goat serum to block non-specific binding (Histofine SAB Kit, Nichirei, Tokyo, Japan) at RT for 25 min, followed by incubation with one of 23 types of biotinylated lectins (Vector Laboratories, Peterborough, U.K.) at 4°C, overnight [21]. Subsequently, the sections were incubated with horseradish peroxidase (HRP)-conjugated streptavidin (Histofine SAB Kit, Nichirei) at RT for 50 min as previously reported by Taniguchi *et al.* [21]. Then, the sections were incubated with hydrogen peroxide conjugated 3–3'-diaminobenzidine tetrahydrochloride (DAB) (Histofine Simple Stain DAB Solution; Nichirei). Finally, the sections were counterstained with hematoxylin.

After each step, the sections were washed in PB containing either 0.05% Tween 20 or 0.05% Triton X100. Reactions were observed with a bright field microscope (BH-2 microscope, Olympus, Tokyo, Japan).

Immunohistochemistry: After blocking endogenous peroxidase activity and non-specific binding, some sections were incubated, at 4°C, overnight, with one of the following antibodies: rabbit anti-PLCβ2 antibody (Santa Cruz Biotechnology, Santa Cruz, CA, U.S.A.), rabbit anti-PGP 9.5 antibody (Acris, Herford, Germany), or goat anti-IP_3R3 antibody (Santa Cruz Biotechnology). The sections were further incubated with secondary antibody, biotinylated goat anti-rabbit antibody or rabbit anti-goat antibody (Histofine SAB Kit, Nichirei), at RT for 1 hr. The other sections were incubated with 10% normal rabbit serum, followed by incubation with anti- tryptophan hydroxylase (TPH) monoclonal antibody (Enzo Life Sciences, Plymouth Meeting, PA, U.S.A.) at 4°C, overnight. They were then incubated with biotinylated rabbit anti-mouse antibody (Histofine SAB Kit, Nichirei) at RT for 1 hr. Negative control reactions were prepared by incubating the sections with PB solution which did not contain any primary antibody. All control reactions tested negative.

Double-labeling for PLCβ2 and TPH immunohistochemistry: After blocking non-specific binding, deparaffinized sections were incubated at 4°C, overnight, with rabbit anti-PLCβ2 antibody. Then, the sections were reacted with Alexa Fluor 568-conjugated goat anti-rabbit antibody (Invitrogen, Paisley, U.K.) at RT for 1–2 hr, followed by incubating with anti-TPH antibody at 4°C, overnight. The sections were then reacted with Alexa Fluor 488-conjugated goat anti-mouse antibody (Invitrogen), at RT for 1–2 hr. Finally, the sections were observed with a fluorescent microscope.

Double-labeling by immunohistochemistry and lectin histochemistry: After blocking non-specific binding, some sections were incubated with either rabbit anti-PLCβ2 antibody or mouse anti-TPH antibody. Those sections incubated with anti-PLCβ2 antibody were then reacted with Alexa Fluor 568-conjugated goat anti-rabbit antibody, and those incubated with anti-TPH antibody were done with Alexa Fluor 568-conjugated goat anti-mouse antibody (Invitrogen) at RT for 1–2 hr. After observation, all sections were incubated with biotinylated PHA-L at 4°C, overnight, followed by staining with fluorescein isothiocyanate-conjugated streptavidin (Invitrogen) at RT for 1–2 hr, and observed with a fluorescent microscope.

RESULTS

Lectin histochemistry: Out of 23 types of lectins examined, 14 types showed positive reactions in the cat taste buds as summarized in Table 1. Four patterns of lectin distribution were observed as follows: (1) Reactions bound to UEA-1, DBA, GSL-1, SBA, WFA, ECL and WGA were distributed in the majority of cells in the CV, FG and EP (Fig. 1a–f); (2) Reactions bound to SJA, LEL and STL were seen in many cells in the FG and EP but not in the CV; (3) Reactions

Table 1. Lectin histochemical reactions of the feline taste bud cells in circumvallate papillae (CV), fungiform papillae (FG) and epiglottises (EP)

Lectins	Specific sugars	Abbreviations	CV	FG	EP
Ulex europeus agglutinin-1	α-L-Fuc	UEA-1	++	++	++
Dolichos biflorus agglutinin	α-D-GalNAc	DBA	++	++	++
Griffonia simplicifolia-1	α-D-Gal, α-GalNAc	GSL-1	++	++	++
Soybean agglutinin	α-, β-GalNAc-	SBA	++	++	++
Wisterial flotibunda agglutinin	α-, β-GalNAc	WFA	++	++	++
Vicia villosa simplicifolia	D-GalNAc-3GalNAc	VVA	+/–	+/–	+/–
Sophora japonica agglutinin	β-D-GalNAc	SJA	+/–	++	++
Erythrina cristagalli lectin	β-D-Gal(1-4)D-GalNAc	ECL	++	++	++
Racinus communis agglutinin 1	β-D-Gal-4GlcNAc	RCA–1	+/–	+/–	+/–
Jacalin	Galactocyl (β-1,3)GalNAc	Jacalin	+/–	+/–	+/–
Peanut agglutinin	Galactocyl (β-1,3)GalNAc	PNA	+/–	+/–	+/–
Griffonia simplicifolia-2	D-GlcNAc	GSL-2	+/–	++	+/–
Griffonia simplicifolia-1B4	α-GalNAc	GSL-1B4	+/–	+/–	+/–
Succinylated wheat germ agglutinin	$(GlcNAc)_n$	S-WGA	+/–	+/–	+/–
Datura stramonium lectin	$(GlcNAc)_2$	DSL	+/–	++	+/–
Lycopersicon esculentum lectin	$(GlcNAc)_{2–4}$	LEL	+/–	++	++
Solanum Tuberosum lectin	$(GlcNAc)_{2–4}$	STL	+/–	++	++
Wheat germ agglutinin	$(GalNAc)_n$, Neu5Ac	WGA	++	++	++
Lens culinaris agglutinin	α-D-Man	LCA	+/–	++	+/–
Pisum sativum agglutinin	α-D-Man	PSA	+/–	+/–	+/–
Concanavalin A	α-D-Man, α-D-Glc	ConA	+/–	+/–	+/–
Phaseolus vulgaris agglutinin-L	Oligosaccharide	PHA-L	++/–	++	++
Phaseolus vulgaris agglutinin-E	Oligosaccharide	PHA-E	+/–	+/–	+/–

++: Intense reaction, ++/–: Intense reaction in some of the taste bud cells, +/–: Negative or weak reaction, CV: circumvallate papilla, EP: epiglottis, FG: fungiform papilla, Gal: D-Galactose, GalNAc: N-Acetyl-D-galactosamine, Glc: D-Glucose, GlcNAc: N-Acetyl-D-glucosamine, Fuc: L-Fucose, Man: D-Mannose, Neu5Ac: N-Acetylneuraminic acid.

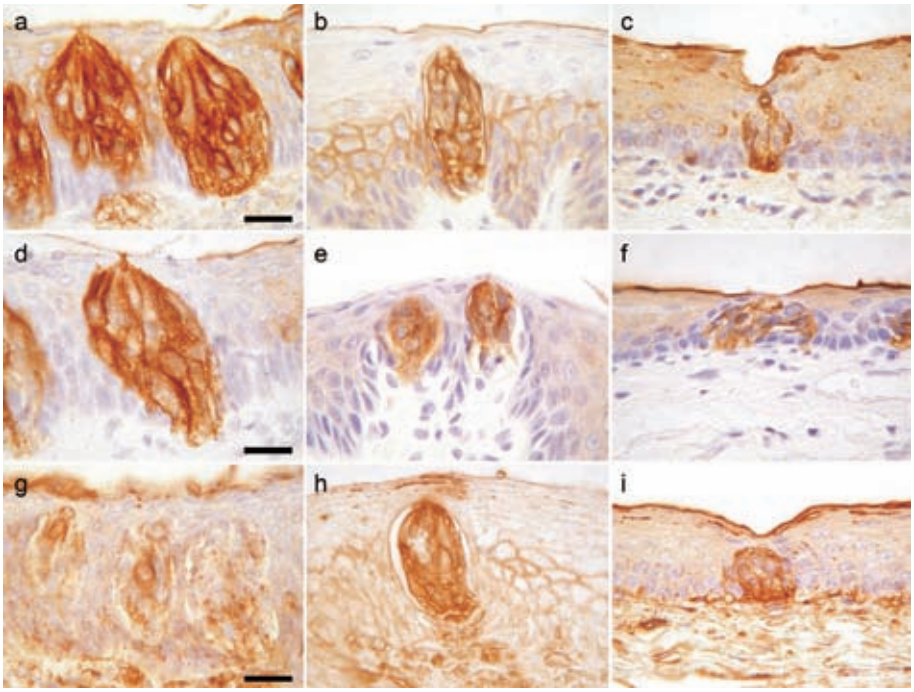


Fig. 1. Lectin histochemical reactions of UEA-1 (a–c), DBA (d–f) and PHA-L (g–i) in the CV (a, d, g), FG (b, e, h) and EP (c, f, i). UEA-1 and DBA intensely react to the cellular membrane and weakly label in the cytoplasm of the majority of taste bud cells in the CV, FG and EP. PHA-L reacts to a few taste bud cells with an oval-to-round nucleus in the CV (g) but it binds to the majority of taste bud cells in FG (h) and EP (i). Bar = 2 μm.

bound to GSL-2, LCA and DSL were seen only in the FG; and (4) Reactions bound to PHA-L were distributed in the majority of the cells in the EG and EP, while only in the minority of the cells in the CV. Among all lectins examined, most impressive result was obtained by PHA-L. PHA-L-positive reactions were widely distributed in many cells; both the bipolar and basal cells in the FG and EP taste buds (Fig. 1h and 1i). In the CV, however, PHA-L-positive reactions were localized in only a few cells' cytoplasm in each taste bud (Fig. 1g). In the FG and EP, PHA-L-positive reactions were intense on the cell membrane but weak in the cytoplasm.

Immunohistochemistry: The immuno-positive products to either PLCβ2, IP_3R3 or PGP 9.5 were localized in the cytoplasm of the bipolar cells provided with a round or oval nucleus in the CV, FG and EP (Fig. 2a–c). PGP 9.5-positive reactivity was seen in 19, 34, and 40 % of the bipolar cells with slender, round and oval nuclei, respectively. PGP 9.5 immunoreactions were intense and granular in the apical cytoplasm, while weak and diffusable in the basal cytoplasm. TPH-positive reactivity was predominantly found in bipolar cells with slender nuclei (Fig. 2d). Of the majority of taste cells with a round-to-oval nucleus in the CV, FG and EP, most of the granular immuno-products were localized at the apical cytoplasm, and the minority in the basal cytoplasm.

Immunohistochemical double-labeling for PLCβ2 and

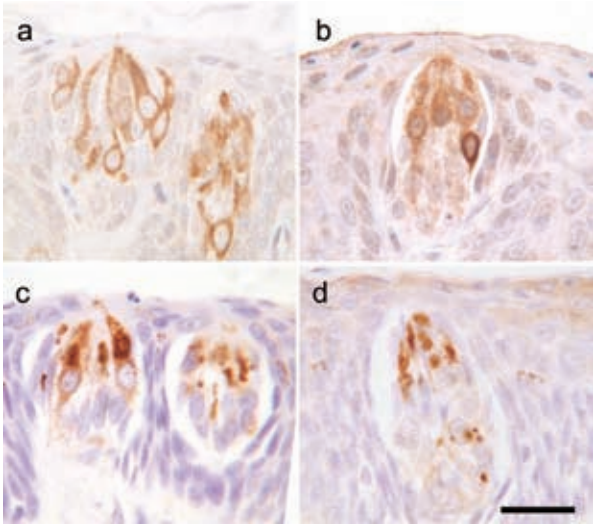


Fig. 2. Immunohistochemical reactions of PLCβ2 (a), IP_3R3 (b), PGP 9.5 (c) and TPH (d) in feline CV taste buds. The reactions of PLCβ2 and IP_3R3 are localized in the cytoplasm of the majority of taste bud cells with a round-to-oval nucleus. PGP 9.5 is localized in the cytoplasm of majority of taste bud cells with a round-to-oval nucleus. The granular reactions of PGP 9.5 and TPH are shown in the apical part of majority of the taste bud cells with a round-to-oval nucleus. Bar =20 μm.

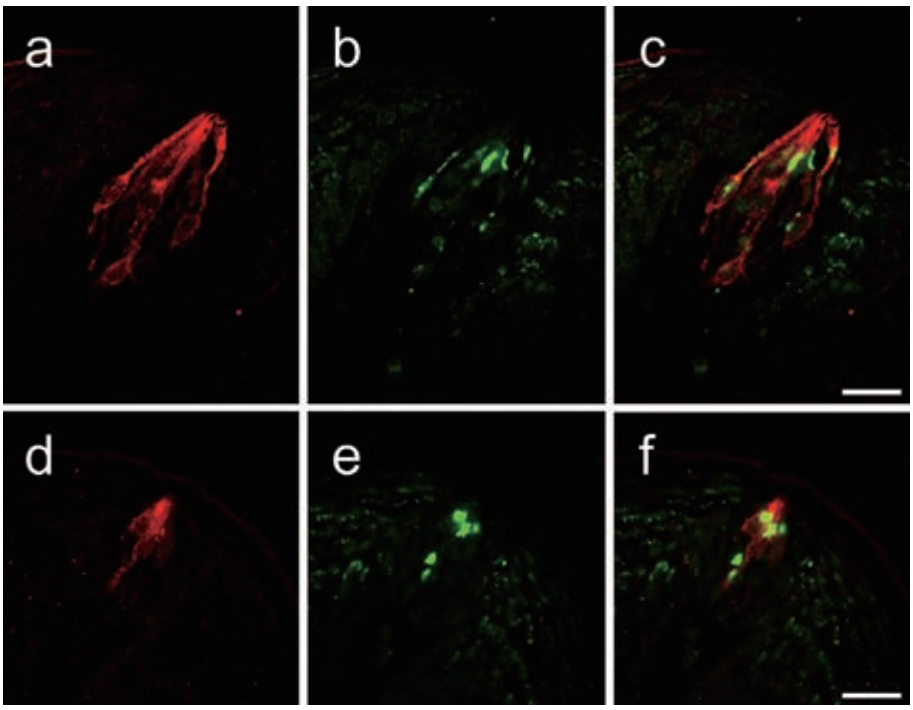


Fig. 3. Double-labeling reactions of immunohistochemistry for PLCβ2 (red) and TPH (green) in CV (a–f) and FG (g–m). PLCβ2 is intensely localized in the cytoplasm of taste bud cells with an oval-to-round nucleus in both of the CV (a, d) and FG (g, k). TPH-immunoreactions are shown as granular reactions at the apical cytoplasm of taste bud cells with an oval-to-round nucleus in both of the CV (b, e) and FG (h, i). Double-labeling images show co-localizations of reactions of PLCβ2 and TH in the many taste bud cells in CV (c, f) and FG (i, m). Bar =20 μm.

TPH: Some sections were double-labeled with anti-PLCβ2 polyclonal and anti-TPH monoclonal antibodies. The immuno-positive products of PLCβ2 and TPH were colocalized in many taste cells in the CV and FG (Fig. 3).

Immunohistochemical and lectin-histochemical double-labeling: PHA-L-binding reactions in the CV were colocalized in some cells which were immuno-positive to PLCβ2 (Fig. 4). PHA-L-binding reactions in the CV were colocalized to some cells which were immunopositive to TPH, too. The double-labeled cells were small in number (Fig. 5).

DISCUSSION

Our study has demonstrated that the distribution of the glycoconjugate residues varies among the papillae. Our results also reveal that certain types of glycoconjugates are localized in certain types of papillae (CV, FG and EP) and certain types of cells (Types I–IV). These results indicate that different glycoconjugates may play different roles in the formation or process of taste sensation and transmission. Moreover, our results show that feline glycoconjugates are different from those of other mammals reported as follows: (1) α-L-Fucose, detected by UEA-1 is distributed in the majority of the cells in the cat taste buds; while Ohmura *et al.* (1989), when studying Syrian hamsters, reported that UEA-1 was localized only in Type I (supporting) cells in the CV [11]; and (2) (GalNAc)n and Neu5Ac, are distributed the majority of cells which react positively to WGA in the cat. However, in the rat CV [22] and in the pig CV and FL, (GalNAc)n and Neu5Ac, which bind to WGA, have been reported to be localized only in a subpopulation of Type II (receptor) cells [16]; (3) (GlcNAc)2–4, to which LEL and STL specifically react, has been reported to be localized in a subpopulation of Type II (receptor) cells in the pig CV, FL, and EP [16]; and (4) Galactocyl (alpha-1,3)GalNAc, to which Jacalin and PNA specifically bind, were not detected in our cat observations, however, they are reportedly positive in Type IV (basal) cells in the rat CV, FL, FG, SP and nasoincisive papillae [20]. The discrepancy between the distributions of these lectins in our results and those of other researchers might be due to the difference in species of the research animals used.

Our immunohistochemical results have confirmed that all PLCβ2-, IP_3R3-, TPH- and PGP 9.5-immunopositive reactions are localized in bipolar elongated cells that contain a round-to-oval nucleus. Since it has been reported that bipolar cells with a round-to-oval nucleus are Type II or III cells [2, 4, 10], our results agree with previous reports showing PLCβ2, IP_3R3 in Type II cells, and TPH in Type III cells. Type III cells have an enzyme, TPH, for synthesizing sero-

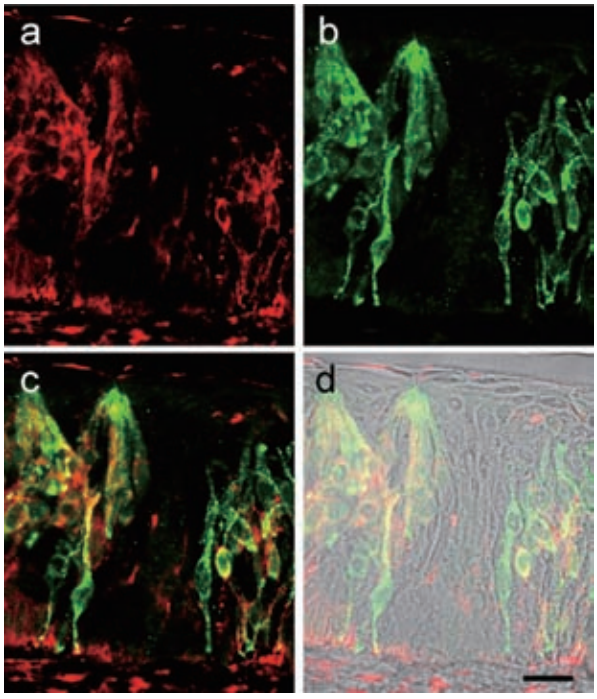


Fig. 4. Double-labeling reactions of lectin histochemistry for PHA-L and immunohistochemistry for PLCβ2 (a–d) in the feline CV. The reactions of PHA-L (a) are shown in the cellular membrane of lingual epithelial cells and cytoplasm of some of the taste bud cells. PLCβ2 (b) is localized in the cytoplasm of the taste bud cells with an oval-to-round nucleus. Some PHA-L-positive cells co-express PHA-L (c). The overlapped images of differential interference contrast (DIC) and fluorescence are shown in d. Bar =20 μm.

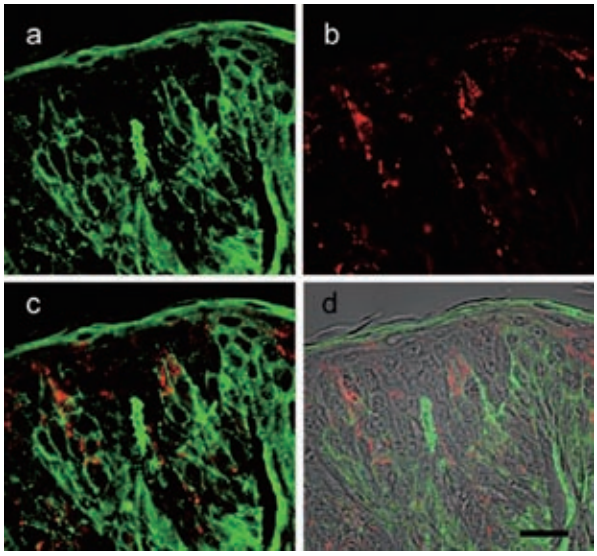


Fig. 5. Double-labeling reactions of lectin histochemistry for PHA-L and immunohistochemistry for TPH (a–e) in the feline CV. The reactions of PHA-L are positive in the cellular membrane of lingual epithelial cells and in the cytoplasm of some taste bud cells (a). TPH is shown as granule reactions at the apical cytoplasm of taste bud cells with an oval-to-round nucleus (b). The TPH immuno-positive granules in the taste bud are localized in some of the PHA-L-positive cells (c). The overlapped images of differential interference contrast (DIC) and fluorescence are shown in d. Bar =20 μm.

tonin [6, 7]; which is a neurotransmitter found in taste buds, and is released by Type III cells when they are stimulated by Type II cells to which ATP has been introduced [8]. PGP 9.5 is seen in either Type II or III cells. In one theory of cell lineage in taste bud cells, Type III cells differentiate into Type II cells [23], or Type II cells differentiate into Type III cells [12]. PGP 9.5 is a protein that is believed to be localized in the transitional stage of from Type II to III cells [25]. Our result shows that PLCβ2 and TPH-colocalized cells are about 46% of PLCβ2-immunopositive cells. These results may suggest that the transitional stage between Types II and III might abundantly exist in the cat taste buds.

In the present study of double-labeling of lectin-histochemistry and immuno-histochemistry, N-acetyllactosamine, the glycoconjugate residue detected by PHA-L, is localized in a small subset of PLCβ2-immunopositive cells and in a small subset of TPH-immunopositive cells of the cat CV, although N-acetyllactosamine is localized in the majority of the taste bud cells in the FG and EP. In the cat CV, this glycochain might play some important role associated with taste sensation. Since the number of the cells which are doubly positive to both PHA-L and PLCβ2 is small, the double-labeled cells can be only a subpopulation of the Type II cells but not all Type II cells. Similarly, the cells doubly positive to both PHA-L and TPH are small in number, the double-labeled cells can be only a subpopulation of the Type III cells but not all Type III cells. These results suggest the possibility that Types II and III cells are not uniform in their natures. Reichling *et al.* (2008) have recently suggested the importance of glycosylation in the maturation bitter receptor proteins [13]. Our results showing a variety of glycochains in the cells in the taste buds, including Type II cells, not only in the apical plasma membrane facing the taste pore but also in deeper portions of the taste buds, including cytoplasm, may support Reichling *et al.*'s suggestion that some glycochains might be involved in the maturation process of taste receptor proteins. Our finding on the glycoconjugate type in a small subset of cat taste bud cells could possibly be further utilized to understand the nature of a subpopulation of cat taste receptor cells and presynaptic cells.

In conclusion, our study has revealed the detailed distributions of glycoconjugates in feline taste buds and its relationship to cell types.

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REFERENCES

1. Asano-Miyoshi, M., Abe, K. and Emori, Y. 2001. IP_3 receptor type 3 and PLCβ2 are co-expressed with taste receptors T1R and T2R in rat taste bud cells. *Chem. Senses* **26**: 259–265.
2. Chandrashekar, J., Hoon, M. A., Ryba, N. J. and Zuker, C. S. 2006. The receptors and cells for mammalian taste. *Nature* **444**: 288–294.
3. Clapp, T. R., Yang, R., Stoick, C. L., Kinnamon, S. C. and Kinnamon, J. C. 2004. Morphologic characterization of rat taste receptor cells that express components of the phospholipase C signaling pathway. *J. Comp. Neurol.* **468**: 311–321.
4. Defazio, R. A., Dvoryanchikov, G., Maruyama, Y., Kim, J. W., Pereira, E., Roper, S. D. and Chaudhari, N. 2006. Separate populations of receptor cells and presynaptic cells in mouse taste buds. *J. Neurosci.* **26**: 3971–3980.
5. Dubový, P. 1988. A study of the dipeptidylpeptidase IV activity in cat fungiform papillae: light and electron microscope histochemistry. *Acta Histochem.* **83**: 173–184.
6. Dvoryanchikov, G., Tomchik, S. M. and Chaudhari, N. 2007. Biogenic amine synthesis and uptake in rodent taste buds. *J. Comp. Neurol.* **505**: 302–313.
7. Huang, Y. J., Maruyama, Y., Lu, K. S., Pereira, E. and Roper, S. D. 2005. Mouse taste buds release serotonin in response to taste stimuli. *Chem. Senses* **30** *Suppl.* 1: i39–i40.
8. Huang, Y. J., Maruyama, Y., Dvoryanchikov, G., Pereira, E., Chaudhari, N. and Roper, S. D. 2006. The role of pannexin 1 hemichannels in ATP release and cell-cell communication in mouse taste buds. *Proc. Natl. Acad. Sci. U.S.A.* **104**: 6436–6441.
9. Max, M., Shanker, Y. G., Huang, L., Rong, M., Liu, Z., Campagne, F., Weinstein, H., Damak, S. and Margolskee, R. F. 2001. Tas1r3, encoding a new candidate taste receptor, is allelic to the sweet responsiveness locus Sac. *Nat. Genet.* **28**: 58–63.
10. Murray, R. G. 1973. The ultrastructure of taste buds. pp. 1–81. *In*: The Ultrastructure of Sensory Organs (Friedemann, I. ed.), North Holland, Amsterdam.
11. Ohmura, S., Horimoto, S. and Fujita, K. 1989. Lectin cytochemistry of the dark granules in the type 1 cells of Syrian hamster circumvallate taste buds. *Arch. Oral. Biol.* **34**: 161–166.
12. Oike, H., Matsumoto, I. and Abe, K. 2006. Group IIA Phospholipase A2 is coexpressed with SNAP-25 in mature taste receptor cells of rat circumvallate papillae. *J. Comp. Neurol.* **494**: 876–888.
13. Reichling, C., Meyerhof, W. and Behrens, M. 2008. Functions of human bitter taste receptors depend on N-glycosylation. *J. Neurochem.* **106**: 1138–1148.
14. Roper, S. D. 2007. Signal transduction and information processing in mammalian taste buds. *Eur. J. Physiol.* **454**: 759–776.
15. Rössler, P., Kroner, C., Freitag, J., Noè, J. and Breer, H. 1998. Identification of a phospholipase C β subtype in rat taste cells. *Eur. J. Cell Biol.* **77**: 252–261.
16. Sotthibandhu, P., Taniguchi, K., Brand, G. J. and Mutoh, K. 2007. Scanning electron microscopic, immunohistochemical and lectin histochemical studies in swine gustatory papillae. The proceeding of the second conference of Asian Association of Veterinary Anatomist.
17. Stedman, H. M., Mistretta, C. M. and Bradley, R. M. 1983. A quantitative study of cat epiglottal taste buds during development. *J. Anat.* **136**: 821–827.
18. Stinson, A. W. and Calhoun, M. L. 1976. Digestive system. pp. 205–265. *In*: Textbook of Veterinary Histology, (Dellmann, H. and Brown, E. M. eds.), Lea and Febiger, Philadelphia.
19. Tanaka, Y., Yoshida, Y., Hirano, M., Morimoto, M. and Kanaseki, T. 1993. Distribution of SP- and CGRP-immunoreactivity in the cat's larynx. *J. Laryngol. Otol.* **107**: 522–526.
20. Taniguchi, R., Shi, L., Fujii, M., Ueda, K., Honma, S. and Wakisaka, S. 2005. Jacalin and peanut agglutinin (PNA) bindings in the taste bud cells of the rat: new reliable markers for type IV cells of the rat taste buds. *Arch. Histol. Cytol.* **68**: 243–250.
21. Taniguchi, K., Koida, A. and Mutoh, K. 2008. Comparative lectin histochemical studies on taste buds in five orders of mammals. *J. Vet. Med. Sci.* **70**: 65–70.
22. Wakisaka, S. 2005. Lectin histochemistry of taste buds in the circumvallate papilla of the rat. *Chem. Senses* **30** *Suppl*. 1: i46–i47.
23. Wakisaka, S. 2006. Development of cell lineage of taste bud cells. *J. Oral. Biosci.* **48**: 177–184.
24. Wu, A. M., Lisowska, E., Duk, M. and Yang, Z. 2009. Lectins as tools in glycoconjugate research. *Glycoconj. J.* **26**: 899–913.
25. Yee, C. L., Yang, R., Börttger, B., Finger, T. E. and Kinnamon, J. C. 2001. "Type III" cells of rat taste buds: immunohistochemical and ultrastructural studies of neuron-specific enolase, protein gene product 9.5, and serotonin. *J. Comp. Neurol.* **440**: 97–108.