FULL PAPER *Anatomy*

Comparative Lectin Histochemical Studies on Taste Buds in Five Orders of Mammals

Kazumi TANIGUCHI[1)]*, Ayumu KOIDA[1)] and Ken-ichiro MUTOH[1)]

[1)]*Laboratory of Veterinary Anatomy, School of Veterinary Medicine, Kitasato University, Towada 034–8628, Japan*

(Received 27 August 2007/Accepted 11 September 2007)

ABSTRACT. Although it has been reported that specific proteins are present to take charge in the gustation in the taste buds, there have been only a few reports on the distribution of glycoconjugates binding to glycoproteins on the cellular membranes of the taste cells. In the present study, therefore, binding patters of 24 biotinylated lectins were examined in the three types of lingual papillae in five species of mammals belonging to different orders: cow (artiodactyl), horse (perissodactyl), monkey (primate), dog (carnivore) and mouse (rodent). As the results, lectin binding patterns were different among circumvallate, foliate and fungiform papillae, among the cells of the taste buds, and among animal species. These findings suggest that the different binding patterns of the lectins in the taste papillae and taste bud cells may be involved in different sensitivities of taste among mammalian species.

KEY WORDS: glycoconjugate, lectin, lingual papillae, mammalian orders, taste bud.

J. Vet. Med. Sci. 70(1): 65–70, 2008

Taste molecules bind to the receptors on the cellular membrane of the taste cells in the taste buds [1, 12, 19]. Based on the molecular structures of these receptors, it has been postulated that at least some of the receptors are glycoproteins, exposed to the extra-cellular surface of taste cells [7], and supposed to have a role in perceiving taste sensations. On the other hand, although several lectin histochemical studies have been reported until now, all of them reported only on a single species, such as rabbits [15], frogs [3] and teleost [16–18] and rats [4, 14]; and no comparative studies have been made on different mammalian species in one report. In the present study, therefore, we lectin histochemically examined the cells of taste buds in the lingual papillae to determine and compare the types and compositions of glycoconjugates in five orders of mammalian species: artiodactyl, perissodactyl, rodent, carnivore and primate.

In addition, we investigated whether there are any differences in lectin binding patterns among different types of lingual papillae in these animals. It has been reported that the expression patterns of various molecules involved in taste perception are different depending on the region on the tongue [8]. The localization of glycoconjugate might reflect these differences.

MATERIALS AND METHODS

Animals: Four cows, three horses, one monkey, three dogs, and five mice were used in the present study. The cows (Holsteins) were supplied by the Department of Veterinary Internal Medicine, the horses (thoroughbreds) by the Department of Large Animal Gynecology, the monkey (Japanese macaque) by the Department of Veterinary Radiology, Kitasato University. The dogs (mongrels) were obtained from the Public Health Center, Towada City; and the mice (C57BL) were commercially purchased from Charles River Japan Inc. (Yokohama, Japan).

All animals except dogs were undergone euthanasia by bleeding after deeply anesthetized with xylazine and ketamine for the cows, horses and the monkey, or with diethyl ether for the mice. The dogs were already euthanized by the Public Health Center before being given. All the procedures were approved by the Kitasato University Animal Rights Committee.

Histological and lectin histochemical procedures: The tongue was removed from each animal and immediately immersed in the fixative containing 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.3). Under a dissection microscope, lingual tissues including taste papillae were observed semi-macroscopically to take pictures. Then, they were removed and further fixed in the same fixative for 24 hr at 4°C. They were washed, dehydrated with up-graded series of ethanol, cleared with xylene, embedded in paraffin, and cut into 3 μm. After removal of the paraffin, some sections were stained with hematoxylin and eosin (HE) or Periodic Acid Schiff (PAS) staining. The other sections were processed for lectin histochemistry: they were deparaffinized and put into methanol containing 0.3% hydrogen peroxide in order to eliminate endogenous peroxidase. Then, the sections were incubated in 10% normal goat serum (Histofine, Nichirei, Tokyo, Japan) at RT for 30 min to block non-specific bindings. The sections were incubated using one of the 24 types of biotinylated lectins (Vector Laboratories, Inc., Burlingame, CA, U.S.A.) at 4°C overnight. Optimal concentrations and sugar specificity of the lectins used are listed in Table 1. Peroxidase-conjugated streptavidin was applied onto the sections for 1 hr at RT. The peroxidase was visualized with the mixture of 0.01% 3–3' diaminobenzidine tetrahydrochloride (DAB) and 0.003% hydrogen peroxide in 0.05 M Tris-HCl buffer (pH 7.6) for

* CORRESPONDENCE TO: TANIGUCHI, K., Laboratory of Veterinary Anatomy, School of Veterinary Medicine, Kitasato University, Towada 034–8628, Japan.
e-mail: taniguch@vmas.kitasato-u.ac.jp

Table 1. Binding specificity of the lectins used in the present study

Lectin	Abbreviation	Concentration (μg/m*l*)	Binding Specificity
Concanavalin A	Con A	4	α-Man
Wheat Germ Agglutinin	WGA	3	GlcNAc
Dolichos biflorus agglutinin	DBA	16	(β-1,4) GlcNAc
Ulex europeaeus agglutinin-I	UEA-I	8	α-Fuc
Peanut agglutinin	PNA	8	galactocyl(β-1,3)GalNAc
Pisum satibum agglutinin	PSA	3	α-Manm B-acetylchitobiose-kinked α-Fu
Lens culinaris agglutinin	LCA	3	α-Man
Phaseolus vulgaris agglutinin-E	PHA-E	2	oligosaccharide(erythroagglutinin)
Phaseolus vulgaris agglutinin-L	PHA-L	2	oligosaccharide(erythroagglutinin)
Sophora japonica agglutinin	SJA	8	GalNAc, β-Gal
Succinylated wheat germ agglutinin	s-WGA	8	GlcNAc
Ricinus communis agglutinin-I	RCA-I	3	GalNAc
Griffonia simplicifolia lectin-I	GSL-I	6	α-,β-GalNAc
Griffonia simplicifolia lectin-II	GSL-II	6	α-,β-GalNAc
Vicia villosa agglutinin	VVA	4	α-,β-GalNAc,α-Gal
Erythrina cristagali lectin	ECL	6	galactocyl(β-1,3)GalNAc
Solanum tuberosum lectin	STL	3	GlcNAc
Soybean agglutinin	SBA	4	α-,β-GalNAc
Jacalin	Jacalin	4	galactocyl(β-1,3)GalNAc
Datura stramonium lectin	DSL	2	GlcNAc
Lycopersicon esculentum lectgin	LEL	2	GlcNAc
Soybean seedling agglutinin	SSA	5	sia-alpha-6G
Griffonia simplicifolia lectin-I-isoform B4	GSA-I-B4	10	β-GlcNAc
Wisteria Floribunda lectin	WFA	10	α-,β-GalNAc

Fuc: fucose; GlcNAc: N-acetylglucosamine; Gal: D-galactpse; Glc: D-glucose; Man: mannose; GalNAc: N-acetylgalactosamine: NeuAc: N-acetylneuraminic acid.

10 min. After each step, the sections were rinsed three times for 5 min each in 0.05 M phosphate buffered saline (pH. 7.3) containing 0.005% Triton X-100. Some sections were briefly counterstained with hematoxylin. Control stainings were performed by preadsorbing lectins with excess amount of specific sugar residues, or by replacing the lectin or streptavidin with PBS. No specific lectin stainings were observed in the control stainings (data not shown).

RESULTS

Semi-macroscopic observations: On the tongues of the horses, monkey and mice studied, three types of papillae, circumvallate, foliate and fungiform, were identified. In contrast, on the tongues of the cows and dogs, only two types of papillae, circumvallate and fungiform, were observed without foliate papillae. Circumvallate papillae were located on the posterior dorsum of the tongue close to the epiglottis. The number differed among animals; for example, a mouse had only one circumvallate on the mid-sagittal plane, while a cow had more than ten per tongue.

Lectin histochemical observations: Lectin histochemical findings in 5 mammalian species and the types of glycoconjugates detected in the lingual papillae are shown in Table 2 (circumvallate papillae), Table 3 (foliate papillae), and Table 4 (fungiform papillae.)

In the cows, six of lectins, PHA-L, UEA-I, GSL-II, LEL, STL and WGA, showed positive reactions in the circumvallate papillae (Fig. 1A). PHA-L was intensely positive in all taste bud cells in the circumvallate papillae. In particular, intensely positive reactions were localized on the taste hairs projecting into the lumen of the taste pores. UEA-I, LEL, STL, WGA and GSL-II were positive in some of the taste

Table 2. Lectin binding patterns in circumvallate papillae

	Cow	Horse	Monkey	Dog	Mouse
PHA-L	++	–	–	+	–
PHA-E	–	–	+	+	–
UEA-I	++	++	+	–	++
DBA	–	–	±	++	–
SBA	–	–	–	+	–
WFA	–	+	–	–	–
VVA	–	–	–	–	–
SJA	–	–	–	–	–
SSA	–	++	–	–	–
ECL	–	++	–	–	–
RCA-I	–	+	–	–	–
GSL-I	–	–	–	++	–
GSL-I-B4	–	–	–	–	–
PNA	–	–	–	–	–
Jacalin	–	–	±	–	+
GSL-II	++	–	–	–	–
s-WGA	–	–	+	–	–
LEL	+	+	+	++	–
DSL	–	–	–	+	–
STL	+	–	–	++	+
WGA	+	+	+	+	+
LCA	–	–	–	–	–
PSA	–	–	–	–	–
Con A	–	–	–	–	–

Table 3. Lectin binding patterns in foliate papillae

	Horse	Monkey	Mouse
PHA-L	–	+	–
PHA-E	–	+	–
UEA-I	+	±	++
DBA	–	+	–
SBA	–	–	–
WFA	–	–	–
VVA	–	–	–
SJA	–	–	–
SSA	–	–	–
ECL	+	–	–
RCA-I	–	–	+
GSL-I	–	–	–
GSL-IB4	–	–	–
PNA	–	–	–
Jacalin	–	+	+
GSL-II	–	–	–
s-WGA	–	–	–
LEL	+	–	++
DSL	–	–	++
STL	–	–	+
WGA	–	+	+
LCA	–	–	–
PSA	–	–	–
ConA	–	–	–

Table 4. Lectin binding patterns in fungiform papillae

	Cow	Horse	Monkey	Dog	Mouse
PHA-L	+	–	–	–	++
PHA-E	–	–	–	–	–
UEA-I	–	+	–	++	+
DBA	–	–	++	–	–
SBA	++	–	–	–	–
WFA	–	+	–	–	–
VVA	–	–	–	–	–
SJA	–	–	+	+	–
SSA	–	–	+	–	–
ECL	–	++	–	–	–
RCA-I	–	–	–	–	–
GSL-I	+	+	–	–	–
GSL-I-B4	+	–	–	–	–
PNA	–	–	–	–	–
Jacalin	–	–	–	–	+
GSL-II	–	–	–	–	–
s-WGA	–	++	–	–	–
LEL	–	+	+	+	–
DSL	+	+	+	–	+
STL	++	+	–	+	+
WGA	+	–	+	+	–
LCA	–	–	–	–	–
PSA	+	–	–	+	–
ConA	–	–	–	–	–

bud cells in the circumvallate papillae. Positive reactions for PHA-L, UEA-I, LEL, STL and WGA were evenly distributed throughout the cellular membranes of the taste bud cells, while positive reactions for GSL-II appeared granular in the taste bud cells. In the fungiform papillae, positive reactions were observed for the following lectins: PHA-L, SBA, GSL-I, GSL-I-B4, DSL, STL, WGA and PSA (Fig. 2A). STL showed granular localizations in the fungiform papillae. PHA-L was positive in a small number of cells in the taste buds, while SBA, GSL-I, GSL-IB4, DSL, STL and PSA were positive in many cells in the taste buds. WGA was weakly positive in many cells on the cellular membranes of the taste bud cells.

In the horses, seven lectins, UEA-I, SSA, ECL, WFA, RCA-I, LEL and WGA, were positive in the circumvallate papillae (Figs. 1B, 2B). UEA-I showed positive reactions evenly and were intensely positive on the cellular membranes in all cells of the taste buds and on the taste hairs in the circumvallate papillae. SSA was positive in many cells in the taste buds as well as on the taste hairs. ECL showed remarkably intense reactions on the taste hairs. ECL was positive in many cells in the taste buds. Positive reactions for WFA, RCA-I, LEL and WGA were localized on the cellular membranes. In the foliate papillae, UEA-I, ECL and LEL were also positive as in the circumvallate. Reactions for UEA-I and LEL were localized on the cellular membranes of many cells, while those of ECL appeared on the cellular membranes of a small number of cells in the taste buds. In the fungiform papillae, UEA-I, WFA, ECL, GSL-I, s-WGA, LEL, DSL and STL were positive. LEL was moderately positive in many taste bud cells, while UEA-I, WFA, GSL-I, s-WGA, DSL and STL were weakly positive in some taste bud cells. Reactions for ECL were distributed on the cellular membranes of many taste bud cells as well as on the taste hairs in the taste pores.

In the monkey, PHA-E, UEA-I, DBA, s-WGA, LEL and WGA were positive in the circumvallate papillae. Positive reactions for PHA-E, s-WGA, LEL and WGA were observed evenly on the cellular membranes in almost all taste bud cells; while those of UEA-I and DBA were granular in some cells. Jacalin showed weakly positive reactions on the cellular membranes of almost all cells in the taste buds. In the foliate papillae, PHA-L, PHA-E, UEA-I, DBA, Jacalin and WGA were positive. Intense to moderate reactions were detected in almost all cells in the taste buds for PHA-L, PHA-E, DBA, Jacalin and WGA, while weak reactions were detected for UEA-I. In the fungiform papillae, DBA, SJA, SSA, LEL, DSL and WGA showed positive reactions. These lectins were moderately to intensely positive in the cells of the taste buds.

In the dogs, the following lectins were positive in the circumvallate papillae: PHA-L, PHA-E, DBA, SBA, GSL-I, LEL, DSL, STL and WGA (Fig. 3). Evenly positive reactions were observed in many cells of the taste buds for PHA-L, LEL and WGA, and some cells for PHA-E and DSL. In contrast, granular reactions were distributed in some cells for DBA, SBA and STL. GSL-I was positive in the cells with dark slender nuclei in the circumvallate papilla (Fig. 3, upper left), but not in the fungiform papillae (Fig. 3, upper right). The binding pattern of LEL was different from that of GSL-I as shown in Fig. 3. In the fungiform papillae, UEA-I, SJA, LEL, STL, WGA and PSA showed positive

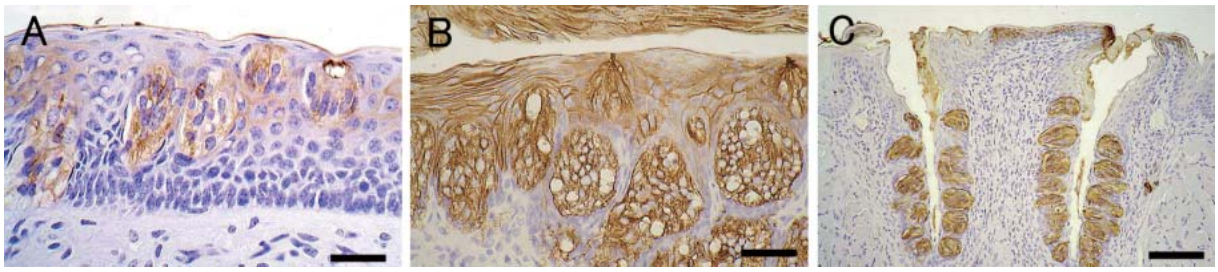

Fig. 1. Ulex europaeus agglutinin-I (UEA-I) in the circumvallate papillae in the cow (A), horse (B), and mouse (C). Bars in A and B=20 μm. Bar in C=80 μm.

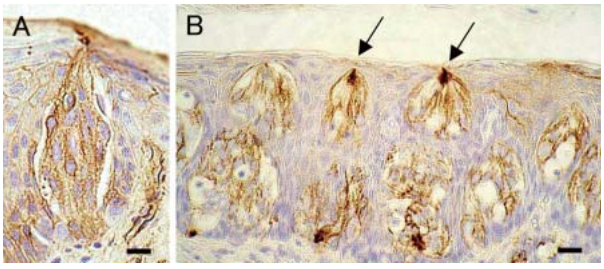

Fig. 2. Solanum tuberosum lectin (STL) in the fungiform papilla in the cow (A) and Erythrina cristagalli lectin (ECL) in the circumvallate papilla in the horse (B). Note intense reactions on the taste hairs in the equine circumvallate (arrows). Bar in A=10 μm. Bar in B=20 μm.

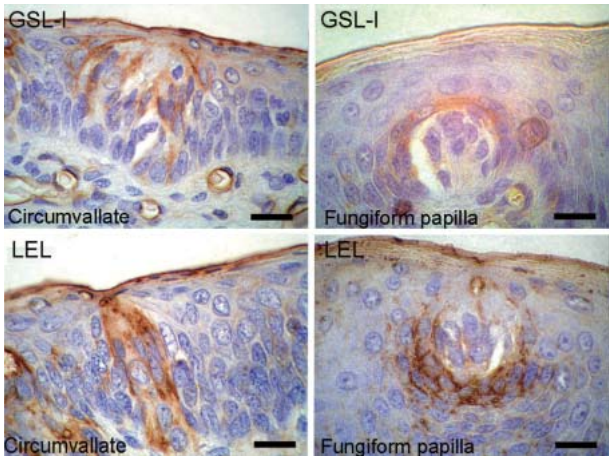

Fig. 3. Griffonia simplicifolia lectin-I (GSL-I) (upper two pictures) and Lycopersion esculentum lectin (LEL) (lower two pictures) in the circumvallate papilla (left) and fungiform (right) papillae in the dog. The distributions of these two lectins differ in the circumvallate and in the fungiform papillae. Bars=20 μm.

reactions. The positive reaction products for UEA-I and SJA were localized in the cytoplasm of taste cells, while those for LEL, STL, WGA and PSA were distributed in some taste bud cells, but not all of them.

In the mice, UEA-I, Jacalin, STL and WGA were positive in the circumvallate papillae. UEA-I was evenly and intensely positive on the cellular membranes in all cells of the taste buds and on the taste hairs (Fig. 1C). Jacalin was weakly positive in the cellular membranes of some taste bud cells. STL and WGA were positive on the cellular membranes of some taste bud cells. In contrast to the circumvallate papillae, the following lectins showed positive reactions in the foliate papillae: UEA-I, RCA-I, Jacalin, LEL, DSL, STL and WGA. UEA-I, LEL and DSL were intensely positive in some taste bud cells. RCA-I and STL were moderately to intensely positive in many cells in the taste buds, while Jacalin and WGA were weakly positive in some taste bud cells. In the fungiform papillae, PHA-L, UEA-I, Jacalin, DSL and STL were positive. PHA-L was intensely positive in almost all taste bud cells. Meanwhile, UEA-I and STL were positive in some taste bud cells, and Jacalin was positive in a small number of cells in the taste buds. Weak DSL reactions were seen in some taste bud cells.

DISCUSSION

In the present study, we examined the localization of various types of glycoconjugates in the three types of taste papillae on the tongue in five orders of mammals: artiodactyl (cow), perissodactyl (horse), primate (monkey), carnivore (dog) and rodent (mouse). Our examination revealed a wide variety of binding patterns of lectins among animals and among types of taste papillae. We used 24 lectins and revealed 21 of them as positive at least in some of the taste buds, but VVA, PNA and LCA were completely negative in the taste buds. The present results suggest that the taste buds contain a wide variety of glycoconjugates: oligosaccharides, alpha-fucose, alpha-, beta-N-acetylgalactosamine, alpha-mannose and alpha-D-glucose. Several lectins showed different binding patterns among animals and among types of lingual papillae. To our best knowledge, the present study is the first extensive comparative study using lectin histochemistry on the taste buds in different orders of mammals. Although several lectins are supposed to bind to the same types of carbohydrate epitopes (ex. WGA, s-WGA, LEL and STL bind to beta-N-acetylglucosamine), the binding patters of these lectins differed greatly among animals and/or taste papillae. As it has been postulated that lectins identify three-dimensional structures of glycoconjugates [4], the present results suggest the differences in the spatial molecular structures of glycoconjugates that even when

cells in the taste buds show the same distribution patterns of lectin bindings.

Specific bindings to certain types of cells have been reported in the tongue [11, 13]; for example, Taniguchi, *et al.* (2005) reported that PNA bound to type IV cells of the rat taste buds [13]. In the present study, however, the distributions of PNA were not restricted to type IV cells in the 5 mammals examined, suggesting the binding of PNA to rat Type IV cells can be species-specific.

The present observations on the taste buds from various papillae among the five mammals studied revealed differential characteristics of lectin bindings among the mammals, as summarized in Tables 2–4. One of the common characteristics is the frequent detection of positive reactions for UEA-I, WGA, STL and LEL in the taste buds of the five mammalian species. These lectins bind to alpha-fucose and beta-N-acetylglucosamine. Since beta-N-acetylglucosamine is ubiquitously localized in all types of papillae in all mammals examined, it might play an essential role in the taste bud function. As for alpha-fucose, the present results positively correlated with the previous reports on the localizations of glycoconjugates in the rat [4], rabbit, frog and fish [3, 4, 14–18]. In contrast, certain glycoconjugates were unique in some but not all types of papillae, such as alpha-N-acetylgalactosamine, alpha-mannose and alpha-D-glucose.

It has been reported that regional expression patterns of taste receptors varies in the regions of the murine tongue [5]. Kano *et al.* (2001) suggested the possibility that nerves innervating the taste papillae might have some effect on the types of glycoconjugates [4]. The taste buds located in the anterior two-thirds of the tongue are innervated by the chorda tympani nerve, while the taste buds located in the posterior one-third of the tongue are innervated by the glossopharyngeal nerve [2, 9]. Our results could not deny the possibility that the variations in the lectin binding patterns are caused by the differences in innervations. However, we failed to find any rules determining the different patterns of lectin bindings.

Since our results showed wide varieties of lectin binding patterns, they might be caused by the differences in the dietary habits differences among mammals. The five species of mammals used in the present study belong to three categories: herbivores (cows and horses), carnivores (dogs), and omnivores (mice and monkey). The present results showing the different patterns of lectin bindings among the animals examined might be partly due to their differences in the dietary habit. According to the present results shown as in Table 2, even cows and horses, representatives of herbivores, display different lectin binding patterns. Although both cows and horses eat plants, cows ruminate while horses do not. Cows strongly depend on rumens, reticulum, and omasum when they digest plants, while horses rely on the colon. These factors might reflect differences in the taste preferences between them. Physiological and taste preference tests showed that animals have different sensitivity to different taste substances [10]. For example, feline animals such as cats, lions and tigers do not prefer sweet tastes because they lack the ability to sense sweetness [6], while other types of carnivores, such as dogs, love sweetness. Although little is known about the taste preferences in the other animals, the variety in the lectin binding patterns demonstrated by the present study suggests that they might have their own unique ways to "enjoy" tastes even humans cannot ever imagine.

REFERENCES

1. Caicedo, A., Pereira, E., Margolskee, R. F. and Roper, S. D. 2003. Role of the G-protein subunit alpha-gustducin in taste cell responses to bitter stimuli. *J. Neurosci.* **23**: 9947–9952.
2. Chibuzo, G. A. and Cummings, J. F. 1981. The origins of the afferent fibers to the lingual muscles of the dog, a retrograde labelling study with horseradish peroxidase. *Anat. Rec.* **200**: 95–101.
3. Gonzalez-Elorriaga, M. A., Jaloveckas, D. and Salazar, de, Candelle, M. 1995. Lectin histochemistry of the Bufo marinus L. toad tongue. *Acta Cient. Venez.* **46**: 97–109.
4. Kano, K., Ube, M. and Taniguchi, K. 2001. Glycoconjugate in rat taste buds. *J. Vet. Med. Sci.* **63**: 505–509.
5. Kim, M.R., Kusakabe, Y., Miura, H., Shindo, Y., Ninomiya, Y. and Hino, A. 2003. Regional expression patterns of taste receptors and gustducin in the mouse tongue. *Biochem. Biophys. Res. Commun.* **312:** 500–506.
6. Li, X., Li, W., Wang, H., Bayley, D. L., Cao, J., Reed, D. R., Bachmanov, A. A., Huang, L., Legrand-Defretin, V., Beauchamp, G. K. and Brand, J. G. 2006. Cats lack a sweet taste receptor. *J. Nutr.* **136:** 1932S–1934S.
7. 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.
8. Ninomiya, Y., Kajiura, H., Ishibashi, T. and Imai, Y. 1994. Different responsiveness of the chorda tympani and glossopharyngeal nerves to L-lysine in mice. *Chem. Senses* **19**: 617–626.
9. Ninomiya, Y., Nakashima, K., Fukuda, A., Nishino, H., Sugimura, T., Hino, A., Danilova, V. and Hellekant, G. 2000. Responses to umami substances in taste bud cells innervated by the chorda tympani and glossopharyngeal nerves. *J. Nutr.* **130**: 950S–953S.
10. Norgren, R. 1983. The gustatory system in mammals. *Am. J. Otolaryngol.* **4**: 234–237.
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. Tabata, S., Wada, A., Kobayashi, T., Nishimura, S., Muguruma, M. and Iwamoto, H. 2003. Bovine circumvallate taste buds: taste cell structure and immunoreactivity to alpha-gustducin. *Anat. Rec.* **271**: 217–224.
13. 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.
14. Wakisaka, S. 2005. Lectin histochemistry of taste buds in the circumvallate papilla of the rat. *Chem. Senses (Suppl.)* **1**: i46–i47.

15. Witt, M. and Miller, I. J. Jr. 1992. Comparative lectin histochemistry on taste buds in foliate, circumvallate and fungiform papillae of the rabbit tongue. *Histochemistry* **98**: 173–182.
16. Witt, M. and Reutter, K. 1988. Comparative lectin histochemical investigation of the taste buds of different vertebrates. *Acta. Histochem. (Suppl.)* **36**: 405–408.
17. Witt, M. and Reutter, K. 1990. Electron microscopic demonstration of lectin binding sites in the taste buds of the European catfish Silurus glanis (Teleostei). *Histochemistry* **94**: 617–628.
18. Zeng, Q., Lawton, A. and Oakley, B. 1995. Glycoconjugates and keratin 18 define subsets of taste cells. *Histochem. J.* **27**: 997–1006.
19. Zhao, G. Q., Zhang, Y., Hoon, M. A., Chandrashekar, J., Erlenbach, I., Ryba, N. J. and Zuker, C. S. 2003. The receptors for mammalian sweet and umami taste. *Cell* **115**: 255–266.