FULL PAPER *Anatomy*

Glycoconjugate in Rat Taste Buds

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ABSTRACT. The taste buds of the fungiform papillae, circumvallate papilla, foliate papillae, soft palate and epiglottis of the rat oral cavity were examined by lectin histochemistry to elucidate the relationships between expression of glycoconjugates and innervation. Seven out of 21 lectins showed moderate to intense staining in at least more than one taste bud. They were succinylated wheat germ agglutinin (s-WGA), *Dolichos biflorus* agglutinin (DBA), *Bandeiraea simplicifolia* lectin-I (BSL-I), *Ricinus communis* agglutinin-I (RCA-I), peanut agglutinin (PNA), *Ulex europaeus* agglutinin-I (UEA-I) and *Phaseolus vulgaris* agglutinin-L (PHA-L). UEA-I and BSL-I showed moderate to intense staining in all of the taste buds examined. They strongly stained the taste buds of the epiglottis, which are innervated by the cranial nerve X. UEA-I intensely stained the taste buds of the fungiform papillae and soft palate, both of which are innervated by the cranial nerve VII. The taste buds of circumvallate papilla and foliate papillae were innervated by the cranial nerve IX and strongly stained by BSL-I. Thus, UEA-I and BSL-I binding glycoconjugates, probably α-linked fucose and α-D-galactose, respectively, might be specific for taste buds. Although the expression of these glycoconjugates would be related to the innervation of the cranial nerve X, the differential expression of α-linked fucose and α-D-galactose might be related to the innervation of the cranial nerve VII and IX, respectively.

KEY WORDS: glycoconjugate, innervation, lectin-histochemistry, rat, taste bud.

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The gustatory sense is one of four special senses in mammals. The gustatory sense is transmitted by the taste buds distributed in the oral cavity and laryngopharynx. The taste buds, composed of 40–70 cells with different cell types, have a complex structure and access to the oral cavity via the taste pore [2, 10, 12, 17, 23]. They can catch chemical materials in the solvent and transmit the signals to the central nervous system. In mammals, the taste buds are observed in fungiform papillae, circumvallate papillae, foliate papillae, soft palate and epiglottis where individual taste bud is innervated by only one of three kinds of the cranial nerves, VII, IX, and X. Taste buds of fungiform papillae, anterior region of foliate papillae and soft palate were innervated by chorda tympani of the cranial nerve VII [14]. Taste buds of circumvallate papilla and posterior region of foliate papillae were innervated by the cranial nerve IX and those of epiglottis were innervated by the cranial nerve X in the rat [14]. These cranial nerves have crucial roles for the development, maintenance and regeneration of the taste buds in the rat [3, 4, 11, 13, 15, 24].

The glycoconjugates on the surface of taste bud cells were involved in chemosensory regulation in olfactory cilia of the channel catfish [5]. The carbohydrate components in the taste buds of circumvallate papillae were detected by lectin histochemistry in the Syrian hamster [16]. In the tongue of various vertebrates, Witt *et al.* showed many differences of cell surface substances among several animals and associations between carbohydrate residues and chemoreception phenomena in the taste bud [19–22]. In the denervated adult rat tongue, the signals of UEA-I and BPA were reduced [25], which indicates that the expression of glycoconjugates of taste bud cells may be controlled by their local innervation in the rat.

The relationship between glycoconjugate expression and innervation of taste bud cells have not been reported in respective papillae, soft palate and epiglottis. In the present study, therefore, the lectin histochemistry using 21 kinds of biotinylated lectins was performed on the taste bud cells of the respective papillae, soft palate and epiglottis in the rat oral cavity to investigate the relationship between the expression of glycoconjugates and innervation.

MATERIALS AND METHODS

Animals and conditions: Male mature 21 rats (Wistar) of either sex were deeply anesthetized by intraperitoneal injection of nembutal sodium solution (60 mg/kg body weight)(Abbott, North Chicago, IL) and perfused with physiological saline followed by Bouin's solution without acetic acid. The tongue, soft palate and epiglottis were removed, and immersed in the same fixative overnight at room temperature. They were dehydrated in a graded series of ethanol, routinely embedded in paraffin, and cut at 5 μm for staining with 21 lectins.

Lectin histochemistry: The sections were deparaffinized with xylene, and processed for lectin histochemistry by the avidin-biotin complex (ABC) method with 21 biotinylated lectins in commercial lectin screening kits (Vector, Burlingame, CA, U.S.A.) according to the staining procedure as follows: 1) incubation with 1% bovine serum albumin (BSA) at 32°C for 30 min; 2) rinse in 0.02 M phosphate buffered saline (PBS; pH 7.25) for 15 min; 3) incubation with a biotinylated lectin at 4°C for 48 hr; 4) rinse in PBS for

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15 min; 5) incubation with ABC at 32°C for 30 min; 6) rinse in PBS for 15 min; 7) incubation with 0.05 M Tris-HCl (pH 7.6) containing 0.01% 3,3-diaminobenzidine tetrahydrochloride (DAB) and 0.003% hydrogen peroxide for 30 min; 8) rinse in distilled water. Working dilutions of 21 lectins were the same as in our previous report [18]. If the concentrations of lectins were higher than those in working dilutions as reported in our pervious report, lectins bound nonspecifically to all the histological structures in sections. In contrast, if their concentrations were lower, lectins bound to no histological structures at all. Therefore, the working dilutions were settled within ranges of concentrations providing specific staining of lectins. Their specific stainings were stable within these ranges of concentrations. Control lectin stainings were performed by the preabsorption of lectins with excess amounts of respective specific sugar residues or by the use of PBS to replace the biotinylated lectins or ABC. No specific lectin bindings were observed in these control stainings.

RESULTS

We used 21 kinds of biotinylated lectins to examine expression patterns of glycoconjugates in the taste buds in the rat (Table1). The taste bud papillae were positively stained by 7 out of 21 lectins (s-WGA, DBA, BSL-I, RCA-I, PNA, UEA-I, PHL-A). Staining pattern of these lectins in taste buds are summarized in Table 2. Among them, intense signals were observed with BSL-I and UEA-I lectins. UEA-I showed intense staining in the whole intragemmal cells of fungiform papillae, soft palate and epiglottis (Fig. 1A-C) and moderate staining in those of circumvallate papillae and foliate papillae. BSL-I showed intense staining in a few intragemmal cells of circumvallate papilla, foliate papillae and epiglottis (Fig. 2A-C) and moderate staining in those of soft palate. It was not easy to identify the cell type of taste bud by light microscope, but these BSL-I positive cells are speculated as type I cells in all taste buds examined. In circumvallate papilla and foliate papillae, the moderate stain-

Table 1. Bindings specificities of lectins used

Lectin	Abbreviation	Concentration (mg/m*l*)	Binding specificity
Wheat germ agglutinin	WGA	5.0×10^4	GlcNAc
Succinylated wheat germ agglutinin	s-WGA	4.0×10^4	GlcNAc
Lycopersicon esuculentum lectin	LEL	2.0×10^3	GlcNAc
Solanum tuberosum lectin	STL	6.6×10^4	GlcNAc
Datura stramonium lectin	DSL	3.3×10^4	(β-1,4)GlcNAc
Bandeiraea simplicifolia lecitn-II	BSL-II	5.0×10^3	α-, β-GlcNAc
Dolichos biflorus agglutinin	DBA	4.0×10^2	α-GlcNAc
Soybean agglutinin	SBA	5.0×10^3	α-, β-GalNAc
Bandeiraea simplicifolia lectin-I	BSL-I	1.2×10^3	α-GalNAc, α-Gal
Vicia villosa agglutinin	VVA	2.0×10^3	α-, β-GalNAc
Sophora japonica agglutinin	SJA	1.0×10^2	GalNAc, β-Gal
Ricinus communis agglutinin-I	RCA-I	1.0×10^3	GalNAc
Jacalin	Jaca	1.0×10^2	galactocyl (β-1,3)GalNAc
Peanut agglutinin	PNA	2.0×10^3	galactocyl (β-1,3)GalNAc
Erythrina cristagalli lectin	ECL	5.0×10^3	galactocyl (β-1,4)GalNAc
Ulex europaeus agglutinin-I	UEA-I	2.0×10^3	α-Fuc
Concanavalin A	Con A	1.0×10^3	α-Man
Pisum sativum agglutinin	PSA	1.0×10^3	α-Man, N-acetylchitobiose-linked α-Fu
Lens culinaris agglutinin	LCA	1.0×10^3	α-Man
Phaseolus vulgaris agglutinin-E	PHA-E	2.0×10^3	oligosaccharide (erythroagglutinin)
Phaseolus vulgaris agglutinin-L	PHA-L	1.0×10^3	oligosaccharide (leucoagglutinin)

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

Table 2. Lectin-binding patterns in the rat taste bud

	Fungiform papillae	Circumvallate papilla	Foliate papillae	Soft palate	Epiglottis
s-WGA	±	++	++	±	±
DBA	±	++	++	–	–
BSL-1	++	+++	+++	++	+++
RCA-1	±	+	+	±	±
PNA	±	±	±	++	+
UEA-1	+++	++	++	+++	+++
PHA-L	±	+	+	++	+

–: Negative staining. ±: Faint staining. +: Weak staining. ++: Moderate staining. +++: Intense staining.

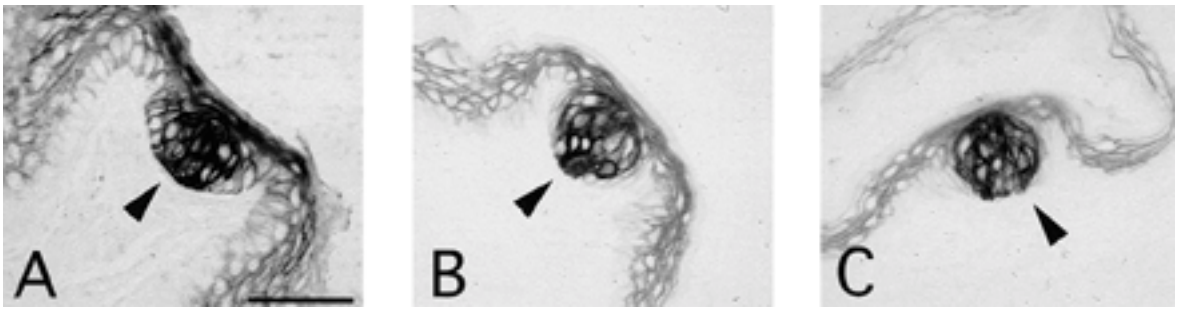

Fig. 1. Binding pattern of a lectin, UEA-1 in the rat taste buds in fungiform papillae (A), soft palate (B) and epiglottis (C). Arrowhead indicates a taste bud. Scale bar=50 μm.

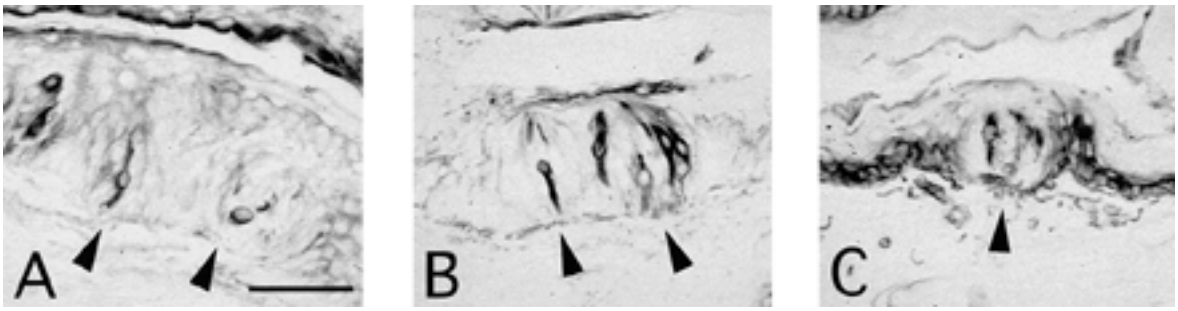

Fig. 2. Binding pattern of a lectin, BSL-1 in the rat taste buds in circumvallate papillae (A), foliate papillae (B) and epiglottis (C). Arrowhead indicates a taste bud. Scale bar=50 μm.

ing was observed with s-WGA and DBA in a few intragemmal cells, but faint staining in fungiform papillae, soft palate and epiglottis. Only in the soft paltate, PHA-L showed moderate staining in whole intragemmal cells of circumvallate papilla, foliate papillae and epiglottis.

DISCUSSION

Many investigators examined expression patterns of glycoconjugates in the taste bud cells of various animals by lectin histochemistry as a useful tool for classification of their cellular varieties and their differential innervation [16, 20–22]. However, the systematic survey of localization has not been reported on the glycoconjugates among the rat taste bud papillae in relation to innervation.

The present study shows that 7 (s-WGA, DBA, BSL-I, RCA-I, PNA, UEA-I and PHA-L) out of 21 lectins specifically stained the taste bud papillae to show various expression of glycoconjugates in rat taste bud cells (Table 2). According to the conjugate specificity of these lectins, N-acetylglucosamine, N-acetylgalactosamine, α-D-galactose, galactosyl (β-1,3) N-acetylgalactosamine and α-linked fucose would be candidate sugar residue glycoproteins expressed in the taste bud cells. Although the carbohydrate binding specificity is similar in several lectins (e.g. WGA, s-WGA, LEL, and STL for N-acetylglucosamine in this study), these lectins showed differential staining pattern in the rat. The variety of staining pattern might reflect some differences in the binding manner of individual lectins. For example, WGA preferentially binds to dimers or trimers of N-acetylgalactosamine. s-WGA does not bind to sialic acid residues, but it retains its specificity to N-acetylglucosamine.

In the rabbit, comparative lectin histochemistry was performed in foliate papillae, circumvallate papilla and fungiform papillae [21]. In foliate papillae and circumvallate papilla, SBA and PNA reacted with their taste bud cells, and in fungiform papillae DBA and HPA reacted with those in fungiform papillae. In all papillae, UEA-I and WGA reacted with their taste bud cells. These lectins recognized N-acetylgalactosamine (WGA), N-acetylglucosamine (DBA, SBA and HPA), galactosyl (β-1,3) N-acetylgalactosamine (PNA) and α-linked fucose (UEA-I). These sugars resemble to those expressed in rat taste buds. The similarity of sugar expression between rabbit and rat indicates that the components of glycoconjugates on the surface of the taste bud cells might be highly conserved among mammals.

Taste bud cells were distinguished into two groups, the intragemmal cells and the perigemmal cells, by the variety of keratin [7], and furthermore, morphologically subdivided into 4 types [6, 12]. In our study, it was not able to distinguish the cell types of the taste bud stained by lectins, but the taste bud cells stained by BSL-I were speculated as the type I cells from their population, shape and localization in all taste buds examined.

Among several lectins showing positive staining in this study, BSL-I and UEA-I lectins showed intense stainings in

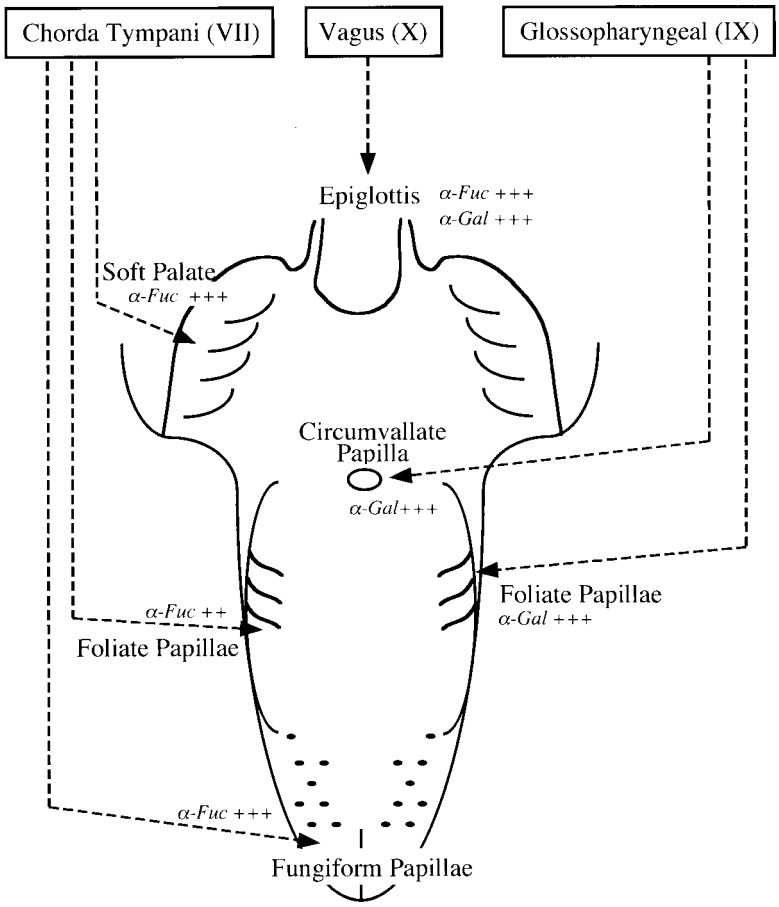

Fig. 3. Schematic model of the relation of expressed glycoconjugates and the innervation in the taste bud cells of individual papillae. α-fuc: α-linked fucose, α-Gal: α-D-galactose.

the intragemmal cells. UAE-I specific to α-linked fucose, reacted with the whole intragemmal cells in all papillae. The intragemmal cells of the taste buds in fungiform papillae, soft palate and epiglottis may have α-linked fucose in common in the rat oral cavity. BSL-I, spcific to alpha-linked N-acetylgalactosamine and α-D-galactose, reacted with a few taste bud cells of the intragemmal cells in circumvallate papilla, foliate papillae and epiglottis. Among lectins specific to N-acetylgalactosamine, SBA, VVA, SJA and RCA-I did not stain any of the taste bud cells examined, although RCA-I showed weak staining in foliate papillae and circumvallate papilla. Therefore, α-D-galactose, but not alpha-linked N-acetylgalactosamine, would be expressed on the surface of the taste bud cells.

Soft palate, foliate papillae and fungiform papillae are innervated by the cranial nerve VII, circumvallate papilla and foliate papillae by the cranial nerve IX and epiglottis by the cranial nerve X [1, 8, 9] (Fig. 3). The present study suggests that α-linked fucose and α-D-galactose might respectively be specific sugars in the taste bud cells of the rat. Although the expression of these glycoconjugates would be related to innervation of the cranial nerve X in the epiglottis, the differential expression of α-linked fucose and α-D-galactose might be related to the innervation of the cranial nerve VII and IX in fungiform papillae, circumvallate papilla, foliate papillae and soft palate, respectively (Fig. 3). There is a report showing cell type-specific lectin staining in relation to their innervation in the particular taste bud [25]. We, however, report here for the first time the relationship between the specificity of lectin staining and the innervation in the taste bud cells. Foliate papillae, whose anterior and posterior regions are innervated by the cranial nerve VII and IX, respectively, were stained intensely by BSL-I and moderately by UEA-I (Table 2). Double staining of foliate papillae by BSL-I and UEA-I or their detailed topographical lectin staining would reveal the differential staining in regions of respective innervation. In order to confirm the relationship between the observed lectin staining specificity and the innervation in the taste bud cells, some experiments, such as lectin staining after denervation of the relevant nerve, might be necessary.

In the epiglottis, the intense stainings for both UEA-I and

BSL-I lectinis are observed. Despite the difference of innervation in the epiglottis taste bud cells from that of other papillae, it is very interesting to observe similar expression pattern of glycoconjugates among these papillae. The candidate glycoproteins, α-linked fucose and α-D-galactose, might have some important roles to transmit gustatory signals in the taste bud cells in common.

In circumvallate papilla and foliate papillae, innervated by the cranial nerve X, the moderate staining for both s-WGA and DBA are observed in a few intragemmal cells, but faint staining in fungiform papillae, soft palate and epiglottis (Table 2). It may reflect the relationship with the innevation and the glycoconjugates.

In conclusion, our data suggested α-linked fucose and α-D-galactose respectively might be specific sugars in the taste bud cells of the rat. Although the expression of these glycoconjugates would be related to the innervation of the cranial nerve X, the differential expression of α-linked fucose and α-D-galactose might be related to the innervation of the cranial nerve VII and IX, respectively.

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