Localization of *Ulex europaeus* agglutinin-I (UEA-I) in the developing gustatory epithelium of the rat*

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Summary. To understand the development of the gustatory structures necessitates a reliable marker for both immature and mature taste buds. It has been reported that the intragemmal cells within the taste buds of adult rats were bound to *Ulex europaeus* agglutinin-I (UEA-I), a specific lectin for α -linked fucose, but it has not been determined whether immature taste buds, i.e. taste buds without an apparent taste pore, are labeled with UEA-I. The present study was conducted to examine the UEA-I binding pattern during the development of the rat gustatory epithelium. In adult animals, UEA-I bound to the membrane of taste buds in all examined regions of the gustatory epithelium. Within the individual taste buds, UEA-I labeled almost all intragemmal cells. The binding of UEA-I was occasionally detected below the keratinized layer of the trench wall epithelium but could not be found in the lingual epithelium of the adult animal. During the development of circumvallate papilla, some cells within the immature taste buds were also labeled with UEA-I. The developmental changes in the UEA-I binding pattern in fungiform papillae were almost identical to those in the circumvallate papilla: both immature and mature taste buds were labeled with UEA-I. The

present results indicate that UEA-I is a specific lectin for the intragemmal cells of both immature and mature taste buds and, thus, UEA-I can be used as a reliable marker for all taste buds in the rat.

Introduction

In mammals, taste buds contain 50-80 elongated epithelial cells (taste cells) as well as proliferative basal cells (progenitor cells). Taste buds are localized mostly within two regions: the lingual epithelium and palatal epithelium. In the lingual epithelium of the rat and mouse, taste buds are localized in three lingual papillae; fungiform, foliate and circumvallate papillae. In the palatal epithelium, taste buds are present in the nasoincisor papilla and soft palate. In addition, few taste buds are present along the laryngeal surface of the epiglottis. Ultrastructurally, taste cells have been classified into three types; dark, light and basal cells. Dark cells have an electron-dense cytoplasm, are thought to be supporting cells, and are known as type I cells. Light cells are characterized by the presence of an electron-lucent cytoplasm, are thought to be receptor cells, and can be further subdivided into two types based on the presence of synaptic vesicles. Light cells without apparent synaptic vesicles are called type II cells, while those with synaptic vesicles are known as type III cells. Basal cells are located at the basal portion of the taste buds, and are known as type IV cells, which are thought to be progenitor cells. Several histochemical studies have demonstrated the expression of bioactive substances in specific cell types of taste cells, but there are few general markers for all the taste buds. Oakley and his colleagues examined the expression of various cytokeratins and found that cytokeratins 7, 8, 18, 19 and 20 are localized in taste buds (Wong *et al.*, 1994; Knapp *et al.*, 1995; Zeng *et al.*, 1995; Zhang *et al.*, 1995). Our previous

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study has revealed that human blood antigen type H (AbH), previously thought to be a specific marker for type I or dark cells, was localized at the cell membranes of all types of taste buds of the rat. However, the immunoreaction for AbH was not present in the membrane of all intragemmal cells in the circumvallate papillae of the adult rat (Ueda *et al.*, 2003).

Lectins are often used as markers for several types of epithelium or mesenchymal cells to identify particular cell populations based on their different abilities for binding with high specificities to different glycohydate epitopes of the cell membrane. Several lectin histochemical studies have been conducted to examine the overall distribution of various kinds of lectin in the gustatory epithelia of various species (Witt and Reutter, 1988; Witt and Miller, 1992; Zeng *et al.*, 1995; Ohishi *et al.*, 2000; Kano *et al.*, 2001). Among several lectins in the taste buds, *Ulex europaeus* agglutinin-I (UEA-I), a lectin specific for α -linked fucose, labeled the surface of almost all intragemmal cells in the rat (Zeng *et al.*, 1995; Ohishi *et al.*, 2000; Kano *et al.*, 2001). However, to our knowledge, the developmental changes in the binding pattern of UEA-I in the taste buds have not been reported. The availability of a specific marker for both immature and mature taste cells within all subpopulations of taste buds is necessary in order to trace the developmental changes of the gustatory structures. The present study was thus designed to examine the localization of UEA-I in the gustatory epithelium of rats during its development.

Materials and Methods

Animals and tissue preparation

Sprague-Dawley rats at various pre- and postnatal days were purchased from Nihon Dobutsu (Osaka). For the pre-natal study, the day on which a vaginal plug was identified was designated as "embryonic day (E) 0". Pregnant animals at E18, 19 and 20 were killed by an overdose injection of chloral hydrate (800 mg/kg), and the fetuses were removed. Tongues were dissected out, and immersed in 4% paraformaldehyde in 0.1M phosphate buffer (PB; pH 7.4) for 2–3 days. For the postnatal study, the day of birth was designated as "postnatal day (PN) 0". Animals at various postnatal stages (PN0, 1, 3, 5, 7, 14, 21, 28 and adult (PN 70) were perfused with 0.02% phosphate-buffered saline (PBS; pH 7.4), and then with a mixture of 4% paraformaldehyde and 0.05% glutaraldehyde in 0.1M PB. The lingual papillae, soft and hard palates, and nasoincisor papilla were dissected out and fixed in 4% paraformaldehyde for additional 2–3 days. Specimens were dehydrated through a graded series of ethanol, embedded

in paraffin, and cut at a thickness of 4–5 μ m. Some adult animals were perfused with a mixture of 1% paraformaldehyde and 1.5% glutaraldehyde in 0.1M PB. Circumvallate papillae were dissected out, sectioned at a thickness of 50 μ m with a vibratome (Leica VT1000S, Germany), collected in cold PBS, and treated as floating sections.

For whole mount preparation, the anterior portions of the tongue were removed from animals at E20, PN0, PN1, PN2, PN3 and PN70. A small volume of 2% collagenase (Wako, Osaka) was injected into the tongue musculatures, and the tongues were further incubated with 2% collagenase for 1–2 h at 37°C. Lingual epithelia were gently peeled off and rinsed in PBS several times, and fixed in 4% paraformaldehyde in 0.1M PB overnight. All animal experiments were reviewed and approved by the Intramural Animal Use and Care Committee at Osaka University Graduate School of Dentistry prior to the experiment (Project #03-002).

Lectin histochemistry and immunohistochemistry

Paraffin sections were deparaffinized with xylene, and processed for lectin histochemistry. Sections were incubated with biotinylated UEA-I (Vector Laboratories, Burlingame, CA, USA) for 14–16 h at room temperature at a concentration of 0.5 μ g/ml. Following several rinses, the sections were incubated with Alexa Fluoro488-conjugated streptavidin (1:500; Molecular Probes, Eugene, OR, USA) for 90 min at room temperature. The sections were coverslipped with Vectashield (Vector) and examined with a fluorescein microscope (Axioskop 2 plus, Carl Zeiss, Germany). Fluorescent images were captured with a CCD camera (AxioCam, Carl Zeiss), and transferred to Adobe Photoshop (Ver.5.0). After observation, the coverslips were carefully removed and stained with hematoxylin eosin for general histological observations. In some cases, double labeling for UEA-I and AbH was performed. The protocol of immunohistochemistry for AbH was identical to that of our previous study (Ueda *et al.*, 2003). Briefly, after lectin histochemistry for UEA-I as mentioned above, sections were incubated with monoclonal anti AbH (1:200; clone 92-FR-A2, Dako, Copenhagen, Denmark) for 14–16 h at room temperature, and subsequently with Cy3-conjugated anti-mouse IgM (1:100; Molecular Probes) for 90 min at room temperature. The sections were then coverslipped with Vectashield and viewed with a fluorescein microscope with an appropriate exciting filter.

Whole mount sections from the anterior portion of the tongue were incubated with biotinylated UEA-I for two days, and subsequently with Alexa Fluoro488-conjugated streptavidin for 90 min. Prior to the incubations, the sections were treated with 0.3% Triton X-100/PBS for 30 min.

Fig. 1–5. UEA-I binding patterns in the circumvallate (**Fig. 1**) and foliate papillae (**Fig. 2**), anterior portion of the tongue (**Fig. 3**), nasoincisor papilla (**Fig. 4**), and the border between hard and soft palates (**Fig. 5**) of the adult rat.

Fig. 1. **A:** Low magnification of UEA-I in the circumvallate papilla. All taste buds are labeled with UEA-I. **B:** Cross-section of the taste buds. Cell membranes of all intragemmal cells are labeled with UEA-I. **C:** UEA-I binding is completely abolished following preincubation of L-fucose (10 mM). **D** and **E:** Paired photographs of UEA-I (**D**) and AbH (**E**) of the circumvallate papilla of the adult rat. Both UEA-I binding (**D**) and AbH immunoreactivity (**E**) are present in the cell membranes. Some UEA-I-labeled membranes are devoid of immunoreactivity for AbH, while all AbH-immunoreactive membranes are bound to UEA-I. Scale bars: 50 μ m (**A**, **C**), 20 μ m (**B**, **D**, **E**)

Fig. 2. The UEA-I binding pattern in the foliate papillae is almost identical to that in circumvallate papilla. Some debris in the trench shows strong bindings of UEA-I. Scale bar: 50 μ m

Fig. 3. **A:** Whole mount preparation of the anterior portion of the tongue. UEA-I binding sites are restricted to the center of fungiform papillae (arrows), but no UEA-I binding is detected in the piliform papillae. Scale bar: 200 μ m. **B** and **C:** Cross (**B**) and horizontal (**C**) views of fungiform papilla. UEA-I binds the membranes of all cells within the taste buds. Scale bars: 20 μ m

Fig. 4. **A:** UEA-I labels all taste buds at the ductural epithelium of the nasoincisor papilla. **B:** Same section stained with HE. Scale bar: 50 μ m

Fig. 5. Two taste buds at the border between hard and soft palates are labeled with UEA-I. Scale bar: 50 μ m

Fig. 6 and 7. UEA-I binding pattern of the circumvallate papilla (**Fig. 6**) and fungiform papillae (**Fig. 7**) of the developing rat.

Fig. 6. UEA-I in circumvallate papilla at E20 (**A**), PN1 (**B**), PN3 (**C**) and PN5 (**D**). HE stained sections are shown in Figures 6A'–D'. **A, A'**: A spindle-shaped cell at the trench wall epithelium (arrow) is labeled with UEA-I at E20 (**A**). The labeled cell (arrow) is not localized in a taste bud-like structure (**A'**). **B, B'**: Immature taste buds (arrow) bind to UEA-I at PN1. Cells at the granular layer are also labeled with UEA-I. **C, C'**: Number of labeled taste buds (arrows) increase at PN3. **D, D'**: Sizes of taste buds increase. All taste buds (arrows) bind to UEA-I. Scale bars: 30 μ m

Fig. 7. UEA-I in the anterior portion of the tongue at E20 (**A**), PN0 (**B**), and PN1 (**C**). **A**: At E20, cores of most fungiform papillae have UEA-I binding sites. **Inset**: Horizontal section of fungiform papilla observed by a confocal laser scanning microscope. UEA-I binding sites show a mesh-like appearance. **B**: At PN0, the fungiform papillae increase in size, and all of them contain UEA-I binding sites. **C**: At PN3d, all fungiform papillae show UEA-I labeling in their cores. The periphery of their cores does not show UEA-I binding. Scale bars: 200 μ m for all, except 20 μ m for 7A inset.

They were then mounted onto gelatin-subbed glass slides and examined with a fluorescein microscope or confocal laser scanning microscope (LSM510; Carl Zeiss).

Floating sections were treated for 30 min with PBS containing 0.03% H_2O_2 to inactivate endogenous peroxidase activity, and then incubated with biotinylated UEA-I for 14–16 h at 4°C. Following incubation with ABC complex (Vector) for 90 min, horseradish peroxidase was developed with diaminobenzidine with 0.003% H_2O_2 . The sections were further fixed with 1% OsO_4 reduced by 1.5% potassium ferrocyanide, dehydrated through an ascending series of ethanol, transferred to propylene oxide, and finally embedded in Epoxy resin. Semithin sections at a thickness of 1 μm were prepared and slightly stained with toluidine blue.

For control of the lectin staining, biotinylated UEA-I was preabsorbed with an excess amount of L-fucose, and applied to the section of circumvallate papillae from adult animals.

Results

UEA-I in the gustatory epithelium of the adult rat

In the circumvallate papilla of the adult animal, UEA-I bound to all the taste buds (Fig. 1A, D) but was lacking in the trench wall epithelium. Cross-sections revealed that UEA-I labeled the cell surface of all individual cells in the taste buds (Fig. 1B). Sections incubated with UEA-I preabsorbed with L-fucose showed no bindings in the cell membrane (Fig. 1C). The combined lectin histochemistry of UEA-I with the immunohistochemistry of AbH revealed cell membranes of all intragemmal cells to be labeled with UEA-I (Fig. 1D) while some UEA-I labeled cells lacked the AbH immunoreactivity in their membranes (Fig. 1E). In the foliate papillae, the binding pattern of UEA-I was almost identical to that in the circumvallate papilla (Fig. 2). Whole mount preparations of the anterior portion of the tongue showed the distinct binding pattern of UEA-I (Fig. 3A). The binding sites of UEA-I were restricted to the core of the fungiform papillae, while the peripheral areas lacked the UEA-I binding. UEA-I did not bind to the piliform papillae (Fig. 3A). Cross- and horizontal sections showed that the membranes of all cells within the taste buds reacted with UEA-I (Fig. 3B,C). In the taste buds of the nasoincisor papillae (Fig. 4) and the border between the hard and soft palates (Fig. 5), the membranes of all cells within the taste bud bound to UEA-I.

UEA-I binding pattern in the developing circumvallate papilla

At E18 and 19, no distinct labeling of UEA-I could be detected in trench wall epithelium. At E20, UEA-I bound to the membrane of a single spindle-shaped cell, extending from the basal portion to the apical portion of the trench wall epithelium (Fig. 6A, A'). Occasionally two or three solitary oval cells labeled with UEA-I were observed nearby. No taste bud-like structures were found in these specimens. At PN1, UEA-I binding was seen to coincide with the taste bud-like structures recognized at the trench wall epithelium. These taste bud-like structures, i.e. immature taste buds, did not have any apparent taste pores (Fig. 6B,B'). At PN 3, the taste buds increased in number, and some contained distinct taste pores. The surface of all intragemmal cells bound to UEA-I (Fig. 6C,C'). The number of pored taste buds increased gradually at PN5, and the membranes of cells within the taste buds were labeled with UEA-I (Fig. 6D,D'). Cells at the granular layer of the trench wall epithelium were occasionally labeled with UEA-I at early postnatal stages (Fig. 7B–D). At PN7, the binding pattern of UEA-I in the taste buds was almost identical to that at PN5, but the binding of UEA-I in the granular layer of trench wall epithelium became weak. The binding of UEA-I in the granular layer could not be detected at PN14 and thereafter (data not shown).

UEA-I binding in the developing fungiform papillae

At the anterior portion of the rat tongue of animals at E20, an apparent array of fungiform papillae was established, and many piliform papillae were detected among the fungiform papillae (Fig. 7A). Most fungiform papillae had binding sites of UEA-I; meanwhile, piliform papillae lacked the labeling (Fig. 7A). Horizontal sections revealed that the binding sites of UEA-I formed a mesh-like appearance (Fig. 7A; inset). Immediately after birth, i.e. PN0, the diameters of fungiform papilla became larger than those at E20. The cores of all fungiform papillae were labeled with UEA-I (Fig. 7B). At PN3, the fungiform papillae further increased in size, and their cores reacted with UEA-I (Fig. 7C). At PN7, the binding sites of UEA-I were found at the core of all fungiform papillae as observed in the adult animals (data not shown).

Discussion

The present study clearly demonstrates the binding pattern of UEA-I in the adult gustatory epithelium as well as the developing circumvallate papilla and fungiform papillae. UEA-I bound to the membranes of all intragemmal cells of the circumvallate papilla of the adult rat, a finding which agrees with previous reports on the rat gustatory epithelium (Zeng *et al.*, 1995; Ohishi *et al.*, 2000; Kano *et al.*, 2001). However, some species differences in the binding patterns of UEA-I have been reported. The UEA-I was absent from the taste disc of the frog (Witt and Reutter, 1988). In the rabbit, UEA-I was present in the cytoplasm of spindle-shaped cells of the taste buds (Witt and Reutter, 1988; Witt and Miller, 1992). Our preliminary study on the adult guinea pig revealed that UEA-I bound to the cell membranes at the granular cell layer of the trench wall epithelium, but the taste buds lacked any binding of UEA-I (our unpublished observations). The differences in binding patterns of UEA-I in the taste buds between several species may account for the different conformation of fucose-containing glycoproteins. Zeng *et al.* (1995) and Kano *et al.* (2001) reported that UEA-I also bound to the ordinary lingual epithelium of the adult rat. In the present study, however, we could not find any apparent labeling of UEA-I in the ordinary lingual epithelium of the adult animals, but the binding of UEA-I was occasionally detected in trench wall epithelium of circumvallate and foliate papillae during postnatal developmental stages. We cannot explain the precise reasons for this discrepancy.

We also examined the colocalization between UEA-I binding and AbH immunoreactivity—because both UEA-I and the antibody against AbH are specific for α -linked fucose and for human blood group antigen H type 2 (Smith *et al.*, 1994)—and found a partial colocalization between them. Our previous study showed that AbH immunoreactivity was present in cell membranes of all types of intragemmal cells, but some individual cells lacked the immunoreactivity (Ueda *et al.*, 2003) while the cell membranes of all intragemmal cells bound to UEA-I (compare Fig. 1C of Ueda *et al.*, 2003 and Fig. 1B of the present study). We once more cannot offer definitive explanations for this.

The development of taste buds in circumvallate papilla has been examined by several researchers (Hosley and Oakley, 1987; Harada *et al.*, 2000; El-Sharaby *et al.*, 2001), and it is accepted that mature taste buds, i.e. taste buds with an apparent taste pore, appear after birth. In the present study, most taste buds at PN1 had no apparent taste pores, these being first recognized around PN3, which is in agreement with previous studies (Hosley and Oakley, 1987;

Harada *et al.*, 2000; El-Sharaby *et al.*, 2001). At E20, a weak labeling of UEA-I was detected in the membrane of spindle-shaped or oval cells in the trench wall epithelium, but these UEA-I-labeled cells did not form taste bud-like structures. We cannot determine whether or not these UEA-I-labeled cells which appeared at E20 are associated with the development of taste buds. Previous immunohistochemical studies revealed that some solitary oval to spindle-shaped cells showed an immunoreactivity for α -gustducin, a taste-related G protein around birth (Sbarbati *et al.* 1999, El-Sharaby *et al.*, 2001). A further analysis is required to elucidate the correlation between UEA-I binding and the expression of α -gustducin around birth. Apparent taste buds were detected postnatally; most of them lacked distinct taste pores around PN1, but pored taste buds were recognized around PN3 and increased in number thereafter. Our results showing that all membranes of cells within the taste buds at PN1 and PN3 reacted with UEA-I indicate that UEA-I labels all cells associated with taste buds—both immature and mature taste buds.

In the fungiform papillae, the taste buds are formed during the prenatal stage, but the maturation of taste buds, i.e. the appearance of apparent taste pores, occurs after birth (Mistretta, 1972; Harada *et al.*, 2000). Harada *et al.* (2000) reported that approximately 12% of taste buds in the fungiform papillae contained taste pores at birth, and approximately 75% of taste buds contained taste pores at PN7. A scanning electron microscopical study revealed that very few taste buds with apparent taste pores were detected at PN1, and that approximately 72% of taste buds in the fungiform papilla at PN12 possessed taste pores (Mistretta, 1972). Based on these reports, most taste buds at E20 did not have taste pores, while some taste buds at PN3 have distinct taste pores. The present whole mount analysis of the anterior portion of the tongue clearly showed that cores of all fungiform papillae were labeled with UEA-I at E20 and immediately after birth, indicating that both immature and mature taste buds in the fungiform papillae bind to UEA-I.

In conclusion, lectin histochemistry of UEA-I has revealed that the membranes of almost all intragemmal cells react with UEA-I. A developmental study of circumvallate papilla and fungiform papillae showed that taste buds without an apparent taste pore also bound to UEA-I. Thus, we conclude that UEA-I is a reliable marker for labeling all taste buds—both immature and mature taste buds—of the rat.

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