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Lectin Histochemistry of Taste Buds in the Circumvallate Papilla of the Rat

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In mammals, taste buds are localized mostly within two regions, i.e. lingual and palatal epithelium. In the lingual epithelium, taste buds are localized in three lingual papillae; fungiform, foliate and circumvallate papillae. In the palatal epithelium, taste buds are present in the nasoincisor papillae and soft palate. In addition, few taste buds are recognized at the laryngeal surface of epiglottis. Taste buds contain 50–80 elongated specialized epithelial cells (taste cells) and proliferative basal cells (progenitor cells). Ultrastructually, taste cells have traditionally been classified into two types of cells; dark and light cells. Dark cells or type I cells have an electron-dense cytoplasm and thought to be supporting cells. Light cells are characterized by the presence of electron-lucent cytoplasm and 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 termed as type III cells. Progenitor cells are located at the basal portion of the taste buds. There are at least two theories for cell lineage of the taste buds; each type of cells in taste buds has individual progenitor cells (multi cell line theory) and all types of cells are derived from the same progenitor cell (one cell line theory). Several histochemical studies to demonstrate the expression of bioactive substances in specific cell type of taste cells for the analysis of cell lineage of the taste buds.

Lectins are useful for histochemistry and have been used in several areas to identify particular cell populations on the base of different ability to bind with high specificity to different carbohydrate epitopes of cell membrane. Although several lectin histochemical studies have been carried out to the taste buds to map the overall distribution of different kinds of lectins in the different location of taste buds in various species (Witt and Reutter, 1988; Witt and Miller, 1992; Zeng *et al.*, 1995; Ohnishi *et al.*, 2000; Kano *et al.*, 2001), few studies are conducted on the relation between lectin binding patterns and specific cell types within the taste buds. The present presentation focused on the lectin binding pattern of rat circumvallate papilla with special references to the relation with type II cells, i.e. α-gustducin-immunoreactive cells.

Ulex europaeus agglutinin-I (UEA-I) reacts strongly with α(1, 2)-linked fucose. In the taste buds of the circumvallate papilla, UEA-I was found to bind with the cell membrane of taste buds (Figure 1A, compared with Figure 1C). Within the taste buds, UEA-I bound to almost all individual cells in the taste buds. Most blood group H type 2 antigen (AbH) immunoreactive cells co-expressed UEA-I (Figure 1B). In addition to the taste buds, UEA-I binds occasionally to the keratinized layer of the trench wall of the circumvallate papilla and in the lingual epithelium. Although lectin from *Lotus tetragonolobus* is also specific for L-fucose, it did not, however, bind to the taste bud cells.

Peanut agglutinin (PNA) is specific for terminal β-galactose and binds to the membrane of rounded-shaped cells at the basal portion of taste buds. Spindle-shaped cells within the taste buds were devoid of PNA binding. In the trench wall epithelium, PNA strongly labeled the membrane of cells from basal cell layer to granular cell layer. Cells at keratinized cell layer lack PNA bindings. In the lingual epithelium, strong bindings for PNA were also detected from basal cell layer to granular cell layer.

Jacalin is purified from jackfruit and specific for D-galactose. Similar to PNA, jacalin binds the membrane of cells located from basal cell layer to granular layer in the trench wall epithelium. Keratinized cells lacked in jacalin binding. Some rounded cells at the basal portion of the taste buds were also found to bind to jacalin. PNA bound to membrane of some rounded-shaped cells that does not react with jacalin.

Within the taste buds, some spindle-shaped cells reacted with both wheat germ agglutinin (WGA) and succinyl WGA. The number of cells binding to WGA is slightly smaller than that binding to succinyl WGA. Many cells binding to WGA and succinyl WGA displayed immunoreactivity for α-gustducin; Not all cells lebeled with WGA or succinyl WGA showed α-gustducin immunoreactivity and vise versa.

Dolichos bifforus agglutinin (DBA) binds to the mostly apical portion of the spindle-shaped cells within the taste buds of circumvallate papilla. Occasionally, DBA also binds to the cytoplasm of the basal portion of taste buds. The number of cells binding to DBA is low compared to cells binding to WGA or succinyl WGA. Most of cells binding with DBA did not show immunoreactivity for α-gustducin.

The present lectin histochemical analysis of taste cells showed that some lectins bind specifically to certain types of cells in the rat circumvallate papilla.

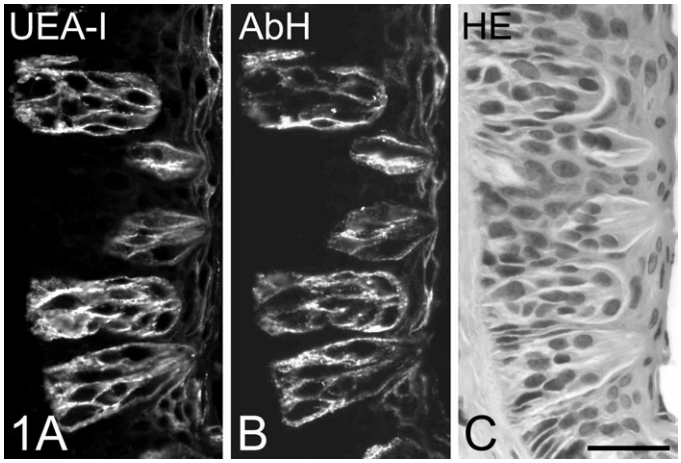

Figure 1 Paired photographs of UEA-I binding pattern **(A)**, AbH immunoreactivity **(B)** and HE staining **(C)** in the circumvallate papilla of the adult rat. UEA-I labels the cell membrane of almost all intragemmal cells (A), while AbH-immunoreactivity is localized in the cell membrane of some cells within taste buds (B). All figures were taken from the same section. Scale bar = 30 μm.

The pattern of UEA-I bindings in the taste buds of the rat circumvallate papilla is in agreement with previous studies in rat (Zeng *et al.*, 1995; Kano *et al.*, 2001). Zeng *et al.* (1995), however, reported that UEA-I also bound to the cells at the lingual and trench wall epithelium, while Kano *et al.* (2001) showed no UEA-I binding were detected in the lingual epithelium that coincide with our present study. The UEA-I binding pattern is almost identical to that of AbH. It is reasonable that binding pattern of UEA-I is almost comparable to the distribution of AbH immunoreactivity since UEA-I is specific with blood group H type 2 antigen and the antibody against AbH also recognizes fucose (Smith *et al.*, 1994).

It is interesting that both PNA and jacalin labeled the membrane of rounded cells at the basal portion of the taste buds. On the base of their location and morphology, these cells are likely type IV cells. Although most rounded cells bound with both PNA and jacalin, few cells reacted with PNA only, indicating heterogeneous characteristics of glycoproteins in the membrane of type IV cells. Although the expression pattern of PNA and jacalin was slightly different in type IV cells, they are considered as specific markers for type IV cells as well as sonic hedgehog (Shh) (Miura *et al.*, 2001). In addition to the type IV cells, both jacalin and PNA also bind to the cell membrane from basal cell layer to granular cell layer of adjacent stratified epithelium of the trench wall. It is known that type IV cells are differentiated from cells at stratified epithelium around the taste buds. Thus it is speculated that type IV cells have similar cytochemical property to the neighbor cells of the stratified epithelium.

Both WGA and succinyl WGA labeled some spindle-shaped cells in the taste buds. Combined immunohistochemistry revealed that most of cells binding WGA and succinyl WGA were immunopositive for α-gustducin; a specific marker protein for type II cells. In contrast, DBA was localized mostly in the apical portion of the spindle-shaped cells within the taste buds and those cells were devoid of immunoreactiviy for α-gustduin. Thus, cells labeled by DBA belong to either subpopulation of α-gustducin immunonegative type II cells, type III cells, or type I cells. Further analysis is required to determine the type of cells that react with DBA.

In conclusion, the present lectin histochemistry revealed that some lectins specifically bind to certain type of the cells. Although the usage of lectin histochemistry in the gustatory epithelium is an old subject, yet it provides a great validity for the detection of cell lineage of taste buds.

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