Intermediate Filaments in Mouse Taste Bud Cells*

Masako TAKEDA, Nobuko OBARA and Yuko SUZUKI

Department of Anatomy, Higashi Nippon Gakuen University School of Dentistry, Tobetsu, Hokkaido, Japan

Received August 6, 1987

Summary. The intermediate filaments in mouse taste bud cells were studied by immunocytochemistry using antikeratin antibodies, and by conventional electron microscopy. Taste bud cells (types I, II, and III) possessed less densely aggregated bundles of intermediate filaments than the surrounding epithelial cells. Type III cells, however, contained more densely aggregated bundles than type I or II cells. Basal cells in the taste buds showed aggregations of filaments as dense as those seen in the epithelial cells, although their bundles were more slender than those of the epithelial cells. The antibodies to keratins from the bovine muzzle and human stratum corneum stained all types of the taste bud cells as well as the surrounding epithelial cells. PKK2 antibody reacted with the surrounding epithelial cells, but did not react with the taste bud cells.

These results show that keratins are present in both taste bud and surrounding epithelial cells, although the keratin subtype differs between those cells. This finding has led us to the supposition that all cell types comprising the taste buds—including type III (receptor) cells—originate from the epithelial cells surrounding the taste buds. It is also suggested that both keratin subtypes and aggregation patterns of intermediate filament bundles change during differentiation from surrounding epithelial cells to taste bud cells, and from basal cells in the taste buds to types I, II, or III cells.

In taste bud cells, intermediate-sized (7–10 nm in diameter) filaments are distributed throughout the cytoplasm, and terminate at the desmosomes. They are aggregated into conspicuous bundles, or are assembled in loose, parallel arrays. Similar filaments in the epithelial cells of other tissues are generally called tonofilaments, and composed of a specific subset of keratin polypeptides whose isoelectric pH values range from 5 to 8 and whose molecular weights vary from 40,000 to 70,000 (BRYSK et al., 1977; FRANKE et al., 1978, 1979; SUN et al., 1979; SCHLEGEL et al., 1980; DENK et al., 1982; MOLL et al., 1982; MURASE et al., 1986). Keratin composition varies depending on epithelial cell type (DENK et al., 1982; MOLL et al., 1982; BANKS-SCHLEGEL, 1983; REGAUER et al., 1985) and changes during embryonic development (BANKS-SCHLEGEL, 1982) and during the transition of epidermal cells progressing from the basal through the spinous and granular layers to the outer cornified layer (WOODCOCK-MITCHELL et al., 1982; SKERROW and SKERROW, 1983; EICHNER et al., 1984, 1986).

It has been reported that rat taste bud cells are continuously replaced by cells differentiating from the surrounding epithelial cells (BEIDLER and SMALLMAN, 1965). KIM et al. (1986) have detected keratin using antihuman keratin antiserum in certain taste bud cells of rats, suggesting that most taste bud cells, if not all, originate from epithelial cells. However, GROVER-JOHNSON and FARMBAN (1976) have suggested that the basal cells of the catfish taste buds, which have synapse-like contacts with nerve terminals, do not originate from the epidermis but rather from the neural crest.

The aim of the present study was to examine the characteristics of intermediate filaments of taste bud cells by immunocytochemistry using antikeratin antibodies and by conventional electron microscopy in an attempt to reevaluate the differentiation of taste bud cells from surrounding epithelial cells in the mouse tongue.

*This study was supported by a grant-in-aid for scientific research from the Ministry of Education, Science and Culture, Japan.
MATERIALS AND METHODS

Electron microscopy

Adult dd-mice were anaesthetized with chloroform, and perfused through the left ventricle with a cold mixture of 2% gluteraldehyde and 2% paraformaldehyde in cacodylate buffer. Small blocks of the tongues, containing circumvallate, foliate and fungiform papillae, were excised and immersed in the same fixative overnight and then postfixed in 1% osmium tetroxide in cacodylate buffer for 2 h. After embedding the tissue in Epon 812, thin (60–100 nm) and thick (0.3–1.0 μm) sections were cut and stained with uranyl acetate followed by lead citrate. These thin and thick sections were observed in a Hitachi electron microscope at accelerating voltages of 75 kV, and 125 kV, respectively.

Immunocytochemistry

The following antibodies to keratin polypeptides were used: a) Polyclonal antibody against bovine muzzle, which strongly reacts with 58, 56, and 52 kD and weakly with 60, 51, and 48 kD subunits (DAKO Corporation); b) monoclonal antibody (EAB-902) against human hepatocellular carcinoma cells, which reacts with a 54 kD subunit (Enzo Biochem. Inc.); c) monoclonal antibody (EAB-903) against human stratum corneum, which reacts with 57 and 66 kD subunits (Enzo Biochem. Inc.); d) monoclonal antibody (EAB-904) against human squamous epithelium, which reacts with 66 kD subunit (Enzo Biochem. Inc.); e) monoclonal antibody (PKK1) against the pig kidney epithelial cell line, which reacts with 40, 45, and 52.5 kD subunits (Labsystems); and f) monoclonal antibody (PKK2) against the pig kidney epithelial cell line, which reacts with 40, 46, 48, and 54 kD subunits (Labsystems).

The adult dd-mice were anaesthetized with chloroform, and the tongues were excised. Small blocks containing circumvallate, foliate, and fungiform papillae were cut out and fixed in 3% formalin in 0.1 M phosphate buffer (pH 7.4) for 2 h. After washing in the same buffer overnight, the tissue blocks were then dehydrated and embedded in paraffin. Deparaffinized sections were treated with 100 ml of 0.05 M Tris-HCl buffer (pH 7.6) containing 0.1 g trypsin and 0.1 g CaCl₂, stained by the peroxidase-antiperoxidase (PAP) method using a PAP kit from the DAKO Corporation, and observed with a light microscope.

Some pieces of the tongues were frozen and cut on a cryostat, and the sections were examined by the indirect immunofluorescence method.

As controls, normal rabbit serum and normal mouse ascites were used instead of polyclonal and monoclonal antibodies, respectively.

RESULTS

Electron microscopy

Mammalian taste bud cells have been classified into distinct type I, II, and III cells, which extend from the basal lamina to the apical taste pore, and basal (immature or stem) cells localized at the basal portion (MURRAY, 1973; TAKEDA and HOSHINO, 1975; TAKEDA and KITAO, 1980; FARBMAN et al., 1985; TAKEDA et al., 1985). The apical cytoplasm of type I (secretory) cells possesses many dense granules which are assumed to be secreted into the taste pore by exocytosis. Type II cells are characterized by an abundance of variously sized vesicles and a smooth-surfaced endoplasmic reticulum (Fig. 7). Type III (receptor or gustatory) cells contain scattered dense-cored vesicles (80–100 nm in diameter) throughout their cytoplasm and make afferent synaptic contacts with the nerve terminals (Figs. 1, 7).

The intermediate-sized (7–10 nm) filaments in all types of taste bud cells were aggregated into bundles in the region around the nucleus and in the periphery of the cytoplasm, and were anchored by looping into desmosomes (Fig. 2). Types I, II and III cells possessed less densely aggregated bundles of filaments than the surrounding epithelial cells (Fig. 3). Small cross-bridges were infrequently observed between such filaments in the taste bud cells (Fig. 2). Only basal cells in the taste buds showed the dense
Figs. 1-3. Legends on the opposite page.
Figs. 4-6. Legends on the opposite page.
osmiophilic aggregations of filaments seen in the surrounding epithelial cells, although the bundles of the former were more slender than those of the epithelial cells (Figs. 1, 4). Electron micrographs of thick (0.3-1.0 μm) sections observed at an accelerating voltage of 125 kV clearly showed that filaments in the type III (receptor) cells formed more densely aggregated bundles than those in the types I or II cells (Figs. 5, 6). As a case in point, filaments in the types I and II cells were assembled in relatively loose parallel arrays. Type II cells, however, contained a greater abundance of bundles of filaments than types I and III cells (Figs. 5, 6). All layers of epithelial cells surrounding taste buds in the trench wall of circumvallate and foliate papillae possessed great numbers of dense filament bundles (Figs. 3, 6-8). Similar dense bundles were also observed in the epithelial cells surrounding taste buds of fungiform papillae. A dense cement-like material filled the spaces between those filaments. Desmosomes between adjacent taste bud cells were small and sparse in com-

Fig. 7. Electron micrograph of the apical portion of a taste bud in a circumvallate papilla from a thin section. Large and numerous desmosomes (D) are seen between adjacent epithelial cells. Desmosomes (d) between adjacent taste bud cells are small. 1 Type I cell, 2 type II cell, 3 type III cell. ×19,000

Figs. 4-6. Electron micrographs of thick (0.3-0.5 μm) sections observed at an accelerating voltage of 125 kV, from taste buds in circumvallate papillae. Fig. 4. A basal cell (B) in a taste bud contains dense osmiophilic aggregations of filaments (arrows) as seen in the surrounding epithelial cells (E), although their bundles are more slender than those of epithelial cells. 1 Type I cell. ×19,000. Fig. 5. The filament bundles (arrow) in a type III cell (3) form more densely aggregated bundles than those (arrowhead) in a type I cell (1). E epithelial cell. ×21,000. Fig. 6. A type II cell (2) contains an abundance of filament bundles (arrows). E epithelial cell. ×19,000
parison with those between adjacent epithelial cells, although apical parts of the taste bud cells contained relatively more desmosomes (Fig. 7). The keratinized epithelium of the dorsal surface contained fewer electron-dense bundles of filaments than the nonkeratinized epithelium of the trench wall, because of the absence of any dense cement-like material between the filaments (Fig. 8).

Immunocytochemistry

Polyclonal antibodies against keratin generated from the bovine muzzle stained all taste bud and epithelial cells in the circumvallate, foliate, and fungiform papillae (Figs. 9, 10). Control experiments exhibited no detectable staining in corresponding tongue sections. The monoclonal antibody (EAB 903) against human stratum corneum also stained all taste bud and epithelial cells in each papilla (Figs. 11, 12).

PKK1 monoclonal antibody exhibited no staining of taste bud and epithelial cells in the trench wall epithelium of the circumvallate and foliate papillae, which comprise nonkeratinized epithelium (Fig. 14). Similarly, taste bud cells on the dorsal surface of fungiform papillae were not stained by PKK1 (Fig. 13). In the keratinized epithelium of the dorsal surface of circumvallate, foliate, fungiform and filiform papillae, PKK1 antibody gave no staining of basal cell layers; however, spinous and granular layers were stained (Figs. 13, 14). PKK2 monoclonal antibody reacted with all layers of the trench wall epithelium in the circumvallate and foliate papillae, but not with taste bud cells (Fig. 15). In the fungiform papillae, epithelial cells surrounding the taste buds reacted similarly with PKK2, but the taste bud cells themselves did not react (Fig. 16). In contrast to PKK1, PKK2 reacted with basal cell layers in the keratinized epithelium of each papilla; it did not with spinous and granular layers (Figs. 15, 16). Control specimens using mouse ascites exhibited no detectable staining in epithelial and taste bud cells of any type of papilla (Fig. 17).

With immunofluorescence microscopy, neither monoclonal antibody (EAB 902) against human hepatocellular carcinoma cells nor that (EAB 904) against human squamous epithelium significantly stained any of the epithelial and taste bud cells in each type of papilla (Fig. 18).
DISCUSSION

MOLL et al. (1982) have distinguished 19 different human keratin polypeptides. Detailed analysis of the keratins in various epithelia has shown that only 2–10 keratins are expressed in any individual tissue, but at least one basic and one acidic subfamily are required for keratin filament constitution (LEE and BÄDEN, 1976; STEINERT et al., 1976; TSENG et al., 1982; HATZFELD and FRANKS, 1985; EICHNER et al., 1986). The immunohistochemical reaction using monoclonal antibody (EAB 903 and 904) against human stratum corneum and squamous epithelium revealed that at least a 57-kD keratin must exist in all taste bud and epithelial cells in each papilla of the mouse tongue. The reaction using polyclonal antibody to keratins from bovine muzzle showed that some additionnal 52, 56 and 58 kD keratins occurred within those cells. Therefore, it is likely that all cell types comprising the taste buds—including type III (receptor) cells—originate from the epithelial cells surrounding the taste buds and course along a differentiation pathway distinct from that of the epithelial cells.

Monoclonal antibody EAB 902 normally reacts with almost all simple epithelium, but fails to recognize stratified epithelium (GOWN and VOGEL, 1982). However, in this study, EAB 902 gave no staining of the taste bud cells—which are not stratified epithelium. On the other hand, LANE (1982) has reported that monoclonal antibodies generated against cytoskeleton extracts from PtK, cells, which demonstrate the presence of a simple epithelium antigenic determinant, react with the taste bud cells in the rat and mouse, but not with the surrounding epidermis. PKK2 antibody did not react with the taste bud cells in any type of papilla, though it did react with the surrounding epithelial cells. These results show that the keratin subtypes present in taste bud cells are different from those of the surrounding epithelial cells. KIM et al. (1986) reported that most basal cells and some of the elongated cells in rat taste buds were immunoreactive, staining with anti-human keratin serum; however, there were some nonimmunoreactive taste bud cells. Thus, it is likely that keratin composition also differs among the cell types of the taste buds.

The bundles of intermediate-sized filaments were assembled in loose parallel arrays in types I and II cells, in denser arrays in type III cells, and in the densest arrays in immature basal cells. The epithelial cells surrounding the taste buds contained dense bundles of filaments as seen in the basal cells, although their bundles were more abundant than those of the basal cells. The positive reaction of the taste bud cells with antikeratin antibodies suggests that the intermediate filaments in those cells contain keratins. Thus, the range of aggregation patterns of the filament bundles may reflect differences in keratin subtypes between epithelial cells and taste bud cells, or among the cell types of the taste buds. Also, the dense bundles of surrounding epithelial cells may play the role of providing a structural framework for the maintenance of the oval outline of the taste buds.

In conclusion, this study has led to the supposition that both keratin subtype and aggregation pattern of intermediate filament bundles change during differentiation from surrounding epithelial cells to taste bud cells, and from basal cells in the taste buds to types I, II or III cells.

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<td>52, 56, 58 kD</td>
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Figs. 9-18. Immunocytochemical staining of sections of mouse tongues with various kinds of antikeratin antibodies, paraffin sections stained with the PAP technique (Figs. 9-17), and frozen sections with the indirect immunofluorescence technique (Fig. 18). Figs. 9, 10. Circumvallate (Fig. 9) and fungiform (Fig. 10) papillae, polyclonal antibody to bovine muzzle. ×160. Figs. 11, 12. Foliate (Fig. 11) and fungiform (Fig. 12) papillae, monoclonal antibody (EAB 903) to human stratum corneum. ×160. Note the staining of all taste bud (arrows) and epithelial cells (Figs. 9-12). D duct of Ebner’s gland. Figs. 13, 14. Circumvallate (Fig. 14) and fungiform (Fig. 13) papillae, PKK1 monoclonal antibody. ×160. Note the preferential staining of spinous and granular cell layers and lack of staining of basal cell layers (B) in the keratinized epithelium. Taste bud cells (arrows) and all layers of the trench wall epithelium (T) are not stained. Figs. 15, 16. Foliate (Fig. 15) and fungiform (Fig. 16) papillae, PKK2 monoclonal antibody. Fig. 15: ×325, Fig. 16: ×650. Note the staining of all layers of the trench wall epithelium (T) and basal cell layers (B) of the keratinized epithelium. Taste bud cells (arrows) are not stained, but epithelial cells surrounding taste buds (E) in the fungiform papilla are stained. Fig. 17. Circumvallate papilla, control specimens using mouse ascites. ×80. Staining is not detected in the epithelial and taste bud cells. Fig. 18. Circumvallate papilla, monoclonal antibody (EAB 902) to human hepatocellular carcinoma cells. ×160. No fluorescence occurs in any epithelial and taste bud cells.

REFERENCES


Dr. Masako TAKEDA  
Department of Anatomy  
School of Dentistry  
Higashi Nippon Gakuen University  
Tobetsu, Ishikari, Hokkaido  
061-02 Japan

武田 正子  
061-02北海道石狩郡当別町金沢1757  
東日本学園大学歯学部
解剖学第二講座