Structure of Bovine Fungiform Taste Buds and Their Immunoreactivity for Gustducin

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ABSTRACT. The taste buds of bovine fungiform papillae were studied by light and electron microscopy using both histological and immunohistochemical methods. The taste buds existed in the epithelium of the apical region of the papillae. By electron microscopy, two types of taste cells, namely type I and type II cells, could be classified according to the presence of dense-cored vesicles, the cytoplasmic density and the cell shape. Type I cells were thin, had an electron-dense cytoplasm containing dense-cored vesicles, and possessed long thick apical processes in the taste pore. Type II cells were thick, had an electron-lucent cytoplasm containing many electron-lucent vesicles, rather than dense-cored vesicles, and possessed microvilli in the taste pore. Immunohistochemical staining with an antiserum against gustducin was investigated by both light and electron microscopy using the avidin-biotin complex (ABC) method. Some, but not all, of the type II cells exhibited gustducin immunoreactivity, whereas none of the type I cells showed any immunoreactivity.

KEY WORDS: bovine, fungiform papilla, gustducin, structure, taste bud.

Gustducin, a G-protein specific for taste cells [7], has been reported to be involved in not only bitter but also sweet taste transduction [5–7, 9, 14]. Our previous study of bovine circumvallate papillae demonstrated that type II taste cells exhibit immunoreactivity for a gustducin antiserum [12]. In the bovine tongue, two types of papillae, namely the circumvallate and fungiform papillae, contain taste buds. The present study investigated the structures of the taste cells in bovine fungiform papillae and their immunoreactivities for the gustducin antiserum.

MATERIALS AND METHODS

After collecting bovine tongues from a local slaughterhouse, the fungiform papillae were removed and fixed with 2% glutaraldehyde and 2% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4) for the histological study or 0.1% glutaraldehyde and 4% paraformaldehyde for the immunocytochemical study. For the histological study, the fungiform papillae were placed into the above-mentioned fixatives for 4 hr on ice. The specimens were then washed in the phosphate buffer overnight, and post-fixed in 1% OsO₄ in the same phosphate buffer for 1 hr on ice. After dehydration in an ascending series of ethanol and absolute acetone, the specimens were embedded in epoxy resin. Semi-thin sections were cut with glass knives, stained with toluidine blue and observed with a Nikon Eclipse E 800 microscope. Ultra-thin sections were cut with a diamond knife, mounted on formvar-supported one-slot grids, stained with lead citrate and uranyl acetate and observed with a Hitachi H-600 transmission electron microscope.

The avidin-biotin complex (ABC) visualization method was carried out as described previously [11, 12]. Briefly, the fungiform papillae were fixed in a phosphate buffer containing 0.1% glutaraldehyde and 4% paraformaldehyde for 4 hr on ice, and then cut into 50 µm sections with a microslicer (Dosaka, Kyoto, Japan). Next, the sections were treated with 1% sodium borohydride for 2–3 min, incubated in phosphate-buffered saline (PBS) containing 1% bovine serum albumin, 1% goat serum and 0.05% Triton to block non-specific binding, and then incubated with an antiserum against gustducin (Santa Cruz Biotechnology Inc., Santa Cruz, CA) diluted 1:1,000 in PBS overnight at 4°C. To prevent endogenous peroxidase reactivity, specimens were treated with periodic acid according to a previously described method [2]. Subsequently, the specimens were incubated with a biotinylated goat anti-rabbit secondary antibody (Vector Laboratories Inc., Burlingame, CA ; 1:1,000 dilution in the blocking solution without Triton) for 4 hr at 4°C. Washed with PBS, and reacted with an avidin-biotin-horseradish peroxidase (HRP) conjugate (Vector Laboratories Inc., Burlingame, CA) overnight at 4°C. To prevent endogenous peroxidase reactivity, negative controls were treated with periodic acid according to a previously described method [2]. After sequential washing with PBS and the phosphate buffer, the sections were treated with 0.05% diaminobenzidine (DAB) solution for several minutes. Next, 0.0018% H₂O₂ in DAB solution was added and incubated for several minutes. The specimens were then washed with the phosphate buffer, fixed in 1% glutaraldehyde solution, washed again with the phosphate buffer, post-fixed with 1% OsO₄, dehydrated in a graded series of ethanol and absolute acetone, and embedded in epoxy resin on glass slides. After viewing the specimens by light microscopy, the semi-thin sections on glass slides were embedded in gelatin capsules filled with epoxy resin for transmission electron microscopy. After splitting the specimens from glass slides, ultrathin sections were cut with a diamond knife and viewed using a Hitachi H-600 transmission electron microscope. In the present study, a negative
control that omitted the primary antibody was carried out in the same manner. No specific immunoreactivity was observed in the control specimens.

RESULTS

There were many fungiform papillae on the anterior part of the bovine tongue. The papillae were dome-shaped and approximately 1 mm in diameter (Fig. 1-a), and the secondary papillae showed deep invagination into the epithelial tissue (Fig. 1-a, arrowheads). The taste buds were located in the epithelium of the apical region of the papillae (Fig. 1-a, arrows), and columnar or spindle-shaped (Fig. 1-b, arrows) with a height of 100–150 µm and a width of 20–40 µm. Thin epithelial cells encircled the taste buds (Fig. 2-a, arrowheads). Melanin-like granules were observed in the cytoplasm (Fig. 2-a, small arrows). Two types of cells had microvilli or cytoplasmic processes in the taste pore. Type I cells contained small dense-cored vesicles in an apical cytoplasm (Fig. 2-b, arrows), while type II cells had no or a few dense-cored vesicles in the cytoplasm, and instead contained electron-lucent vesicles in the apical cytoplasm (Fig. 2-c, arrowheads). The nucleus of type I cells was elongated, while that of type II cells was oval (Fig. 2-a). The perinuclear region of the cytoplasm in type I cells was thinner and more electron-dense than that of type II cells (Fig. 2-d). The cytoplasmic processes of type I cells in the taste pores were thick and long (Fig. 2-b, arrow), while the cell processes or microvilli of type II cells in the taste pore were thin and short (Fig. 2-c, arrows).

A subset of taste cells exhibited intense immunoreactivity for the gustducin antiserum (Fig. 3-a, arrow). Since the immunopositive cells had a thick cytoplasm without any dense-cored vesicles, the cell were considered to be type II cells (Fig. 3-b, asterisk). The reaction products were diffusely distributed in the cytoplasm (Fig. 3-c). Some, but not all, of the type II cells showed immunoreactivity for gustducin, while no reactivity was observed in any of the type I cells (Fig. 3-b).

DISCUSSION

Taste cells that extend cytoplasmic processes into taste pores are generally classified into two or three types. According to the criteria by Kinnamon [3], type I and type II taste cells correspond to dark and light taste cells respectively. For example, mouse and rat taste cells are usually classified into two types, designated type I and type II cells [4, 15], whereas rabbit taste cells are classified into three types, designated type I, type II and type III cells [8]. Our previous study demonstrated that bovine circumvallate taste cells could be classified into two types [12]. The present investigation has further revealed that two types of taste cells exist in the bovine fungiform papillae. The structural features of the bovine fungiform taste cells are summarized as follows. Type I cells have a thin cytoplasm and an elongated nucleus, and the cytoplasm of their perinuclear region is more electron-dense than that of type II cells. Type I cells also contain many dense-cored vesicles in the apical cytoplasm, and have thick long cytoplasmic processes in the taste pore. On the other hand, type II cells have a thick cyto-

Fig. 1. a: Photomicrograph of a bovine fungiform papilla. The secondary papilla shows a deep invagination into the epithelial tissue (arrowheads). Taste buds only exist in the epithelium of the apical region of the papilla (arrows). b: Higher magnification photomicrograph of the papilla. The taste buds (arrows) are columnar or spindle-shaped, 100–150 µm in height and 20–40 µm in width. Scale bars indicate 200 µm in (a) and 50 µm in (b).

Fig. 2. a: Electron photomicrograph of a taste bud in a bovine fungiform papilla. Thin epithelial cells (arrowheads) encircle the taste bud. The taste pore (large arrow) opens into the oral cavity. Type I cells (I) have a thin cytoplasm and an oval nucleus. Melanin-like granules are seen in the cytoplasm (small arrows). b: Apical region of a bovine fungiform taste bud. Type I cells (I) contain many dense-cored vesicles (arrowheads). The cells extend long thick cytoplasmic processes into the taste pore (arrow). c: Type II cells (II) contain many electron-lucent vesicles in the apical cytoplasm. The cells have short thin cytoplasmic processes or microvilli (arrow). d: Higher magnification photomicrograph in (a). The perinuclear region of the cytoplasm in type I cell (I) was thinner and more electron-dense than that of type II cell (II). Scale bars indicate 5 µm in (a), 1 µm in (b) and (c) and 2 µm in (d).
Fig. 3.  a: Light photomicrograph of a bovine fungiform papilla treated with a gustducin antiserum. A subset of spindle-shaped cells show immunoreactivity (arrow). b: Electron photomicrograph of a fungiform taste bud treated with a gustducin antiserum. Since the immunopositive cells (asterisk) have a thick cytoplasm and do not contain any dense-cored vesicles, they are considered to be type II cells. Type I cells (I) show no immunoreactivity. Some of the type II cells (II) do not show any immunoreactivity. c: Higher magnification photomicrograph in (b). The reaction products were diffusely distributed in the cytoplasm of the immunoreactive cell (asterisk). The type I cell (I) shows no immunoreactivity. The type II cell (II) does not show any immunoreactivity. Scale bars indicate 50 µm in (a), 5 µm in (b) and 2 µm in (c).
plasm and an ovoid nucleus. They have no or a few dense-cored vesicles in the cytoplasm, and instead contain electron-lucent vesicles in the apical cytoplasm. Moreover, type II cells have short and thin cytoplasmic processes or microvilli in the taste pore.

Gustducin is a taste cell-specific G-protein that functions as a bitter and possibly also a sweet taste transducer [5–7, 9, 14]. Our previous study demonstrated that the gustducin reaction band at 40 kDa was displayed in the bovine taste tissue sample of Western blot [12]. Immunoreactivity for gustducin has previously been observed in humans [13], rats [10, 15], hamsters [1] and bovine circumvallate papillae [12]. The present immunohistochemical investigation also demonstrates that a subset of taste cells in the bovine fungiform papillae exhibit immunoreactivity for gustducin. Some, but not all, of the type II cells showed such immunoreactivity, whereas the type I cells did not. These results for bovine fungiform papillae are consistent with previous results for rat [10, 15] and bovine [12] circumvallate papillae. It is reported that the remaining type II cells of rat taste buds show the immunoreactivity against PGP 9.5 but the gustducin immuno-positive cells do not [16]. The reason why only some of the type II cells show the immunoreactivity against gustducin is still unclear. From the present and our previous [12] studies, the structure and immunohistochemical property of taste cells are not different between anterior and posterior parts of the bovine tongue.

REFERENCES