Postnatal Development of the Mouse Volatile Papilla Taste Bud Cells

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ABSTRACT. In the present study, we examined specific markers for taste bud cells in the mouse and the postnatal development of volatile papilla taste bud cells in ddY mice. We examined the immunoreactivity of 4 types of carbonic anhydrase isoenzymes, CA I, CA II, CA III and CA VI, as specific markers for taste bud cells, and K8.13 cytokeratin antibody as a specific marker for the lingual epithelial cells. Of the carbonic anhydrase isoenzymes, only CA III immunoreactivity was clearly detected in the spindle shaped gustatory cells. CA VI immunoreactivity was detectable in suspentacular cells. CA I and CA II antibodies did not recognize any taste bud cell specifically. K8.13 cytokeratin immunoreactivity was detected in the lingual epithelial cells, but not in taste bud cells. At 7 days after birth, the suckling phase, very small taste buds developed from the anaplastic gustatory cells. At 14 days after birth, the taste buds showed larger size than those at 7 days after birth. At 21 days birth, after the weaning phase, taste bud structure approximated the mature structure. These results demonstrate the specificity of anti-CA III and anti-CA VI for gustatory cells and suspentacular cells, respectively. These markers should be useful for an analysis of taste bud development in mice.

KEY WORDS: carbonic anhydrase, gustatory cell, mouse.

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Taste cells express taste receptors for several gustatory senses, including salty, sweet, bitter, etc. Taste disorders, including taste blindness, are an important symptom in patients undergoing antibiotic and other medical treatments [11] and those suffering from zinc deficiency disorders [6]. The taste bud has been reported to consist of three or four different cell types. These include the gustatory cells, suspentacular cells, and basal cells [4, 12], or type I, type II, type III and basal cells [9]. The gustatory cells or type I and II cells have a spindle shape with a taste receptor. The suspentacular cells or type III cells support the gustatory cells or type I and type II cells. Basal cells are stem cells that generate other taste bud cells. The taste buds are located mainly in the taste papillae, volatile papilla, foliate papilla and fungiform papilla, other oral mucosa and palate epithelium.

Carbonic anhydrase is a zinc-containing protein. Eleven isoenzymes of carbonic anhydrase have been reported [7, 13]. Human carbonic anhydrase isoenzyme I and II are useful as specific markers of gustatory cells in rat volatile and foliate papillae [3], but not in mouse. Only the use of enzyme histochemical methods has demonstrated the presence of carbonic anhydrase activities in the mouse gustatory bud cells [2]. Cytokeratin is a specific marker of developing epithelial cells, and the K8.13 antibody detects type 8 and 18 cytokeratin in the mouse [8]. These cytokeratins are localized in oral epithelial cells [5]. In this study, we examined the specificity of antibodies against carbonic anhydrase isoenzymes and antibodies against cytokeratins for use in investigating the development of taste bud cells in the mouse.

As the experimental animals, the ddY strain mice were

maintained in an air-conditioned room, and fed laboratory standard food pellets and water *ad libitum*. Pro-estrous female mice were mated with male mice overnight. Gestation was confirmed by the presence of a vaginal plug at the next morning, and this time was designated as 0.5 days post coitus (dpc). Tongue samples were collected from 5 animals that were sacrificed by cervical dislocation at 7 days, 14 days, 21 days and 6 months of age. Weaning was started at 21 days after birth. Only four ages are listed with the 5 animals used. Dissected tissues were fixed in Bouin's solution for microscopic examination. The experimental procedures and care of animals were in accordance with the guidelines of the Animal Care and Use Committee of Nippon Veterinary and Animal Science University.

After fixation, histological and immunohistochemical samples were embedded in paraffin, and serially cut at 6 μ m parasagittally. Adjacent sections were stained with hematoxylin-eosin for histological examination. Anti equine CA I, CA II and CA III polyclonal antibodies, anti bovine CA VI antibody (working dilution is 1: 2,000, cross-reactivity was confirmed by the western blotting; data not shown) [1] and anti cytokeratin antibody K8.13 (working dilution is 1: 200; ICN Biomedical Inc., California, U.S.A.) were used in this study. The primary antibodies were detected using Histofine Simplestain PO MULTI kit (Nichirei Co., Tokyo, Japan), and visualized using 3,3'-diaminobenzidine (DAB; Wako Pure Chemicals, Co., Tokyo, Japan). Stained sections were assessed and photographed using light microscopy.

In compatibility of marker for the taste buds cells, at 6 months after birth, the expression of CA I and CA II was not specific to the taste bud cells, and CA II was expressed in

Fig. 1. At 6 months after birth, the taste bud structure is mature and consists of large numbers of taste bud cells (1a; HE stain; Right square is shown enlarged taste buds.). CA III and VI immunoreactivity is clearly detectable in gustatory cells and suspentacular cells, respectively (CA III; 1b and CA VI; 1c). Cytokeratin K8.13 antibody reacts strongly to lingual epithelial cells (1d). Bar: 10 μ m. Arrowhead: taste bud. Arrow: spindle shaped gustatory cells. Doublearrow: suspentacular cells.

Fig. 2. At 21 days after birth, the taste bud consists of a smaller number of taste bud cells than observed in the 6 month old mouse (2a; HE stain; Right square is shown enlarged taste buds.). CA III and VI immunoreactivity is detectable in gustatory cells and suspentacular cells, respectively (CA III; 2b and CA VI; 2c). Cytokeratin K8.13 antibody recognizes epithelial cells (2d). Bar: 10 μ m. Arrowhead: taste bud. Arrow: spindle shaped gustatory cells. Doublearrow: suspentacular cells.

Fig. 3. At 7 days after birth, taste bud structure was not clear and consisted of anaplastic taste bud cells (3a; HE stain; Right square is shown enlarged taste buds.). CA isoenzyme antibodies do not recognize any taste bud cells (CA III; 3b and CA VI; 3c), but cytokeratin K8.13 antibodies detect lingual epithelial cells (K8.13; 3d). Bar: 10 μ m. Arrowhead: very small anaplastic taste bud.

Table 1. Expressional patterns of CA I, CA II, CA III, CA VI and K8.13 in the lingua and taste bud at 7 days, 14 days, 21 days and 6 months after birth mice

		CA I	CA II	CA III	CA VI	I. 8.13
6 months	Taste bud cells	—	—	+++	++	±
	Lingual epithelial cells	—	++	—	—	+
	Glandular cells	—	+	+	+	—
	Ductal cells of Ebner's gland	+	+	+	+	+
	Muscle cells	—	+	+	+	—
21 days	Taste bud cells	—	—	++	+	—
	Lingual epithelial cells	±	+	—	—	+
	Glandular cells	—	—	—	+	—
	Ductal cells of Ebner's gland	+	+	+	+	+
	Muscle cells	—	+	+	+	—
14 days	Taste bud cells	—	—	+	+	—
	Lingual epithelial cells	±	±	—	—	+
	Glandular cells	—	—	—	—	—
	Ductal cells	+	±	—	+	+
	Muscle cells	—	+	+	—	—
7 days	Taste bud cells	—	—	—	±	—
	Lingual epithelial cells	±	±	—	—	+
	Ductal cells of Ebner's gland	—	—	—	—	—
	Ductal cells	—	±	—	±	+
	Muscle cells	—	—	+	—	—

+++ : Very strong positive reaction. ++ : Strong positive reaction. + : Moderate positive reaction.

± : Trace reaction. — : Negative reaction.

tongue epithelium and muscle (Table 1). The expression of CA III was clearly detected in spindle shaped gustatory cells (Fig. 1b), lingual muscle and Ebner's gland (Table 1). The expression of CA VI was detected in suspentacular cells (Fig. 1c), lingual muscle and Ebner's gland (Table 1). Cytokeratin K 8.13 was strongly expressed in epithelial cells, but not in taste bud cells (Fig. 1d). Rat gustatory cells expressed immunoreactivity to anti-human CA I and CA II antibodies and anti-alpha-gustducin antibody [3, 10]. These results show that CA III is very clearly detectable in the mouse gustatory cell and CA VI recognizes the suspentacular cells.

In postnatal development of volatile papilla taste bud cells, at 7 days after birth, the structure of volatile papillae taste bud (Fig. 3a), foliate papilla and fungiform papilla taste buds (data not shown) was immature. They consisted of very small numbers of anaplastic taste cells (Fig. 3a), which showed no immunoreactivity for CA III and CA VI (Fig. 3b, 3c). Volatile papilla taste buds showed the mature structure at 14 days and 6 months after birth (Figs. 1a and 2a). CA I and CA II were not expressed in the taste bud cells, and CA II was expressed only in tongue epithelium and muscle (Table 1). The expression of CA III was strongly detected in spindle shaped gustatory cells at 14 days and 6 months after birth (Figs. 1b, 2b), but not at 7 days after birth (Fig. 3b, Table 1). The expression of CA VI was also strongly detected in suspentacular cells at 14 days and 6 months after birth (Figs. 1c, 2c), but not at 7 days after birth (Fig. 3c). Cytokeratin K 8.13 was expressed in lingual epithelial cells at all stage examined, but not in taste bud cells (Figs. 1d, 2d, 3d). All of these results are shown in Table 1.

The present study shows that anti-equine CA III and anti-

bovine CA VI isoenzyme antibodies recognize mouse gustatory cells and suspentacular cells, respectively. Using these antibodies, we demonstrated that volatile papilla taste bud cells develop and increase their cell number from 14 days after birth. Our results suggest that these antibodies are useful for the clarification of function, development, turnover mechanisms and disorder mechanisms in mouse taste bud cells.

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