〔ORIGINAL〕

Histochemical Localization of Carbonic Anhydrase in the Taste Buds of the Mouse and Goldfish

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Abstract

The activity of carbonic anhydrase in the taste buds of the mouse and goldfish was examined by enzyme histochemistry. An intense reaction of carbonic anhydrase was observed in the middle and basal regions of the taste buds in the mouse circumvallate papillae. Under the electron microscope, the reaction product was found in the cytoplasm of type-I cells which are characterized by the presence of ribosomes and rough endoplasmic reticulum. In other cell types (type-II and type-III cells), no reaction was detected. In the fungiform papillae, the reactivity was similar to that in the circumvallate ones ; however, only a few cells were positively stained. In the goldfish, the receptor cells, which are characterized by the presence of tubular system, showed strong carbonic anhydrase activity. The results suggest that the activity of carbonic anhydrase, which is usually associated with H^+ or HCO_3^- transport, is present in a specific type of taste bud cells.

Key words : carbonic anhydrase, taste buds, circumvallate papillae, fungiform papillae, tubular cells

Introduction

Carbonic anhydrase is an enzyme responsible for the hydration of carbon dioxide, and plays a role in secretory processes and ion transport in various organs[1, 2]. Histochemical study on the taste buds of rat circumvallate papillae has shown that carbonic anhydrase is localized in the taste bud cells[3]. Recent immunohistochemical study using antibodies to various carbonic anhydrase isozymes has shown that antisera directed against CA-I and CA-II yield a similar pattern of reactivity in the rat taste bud cells and that CA-I/II reactivity is present in most

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taste cells of the circumvallate and foliate papillae. The CA-I/II reactivity was also observed in a few cells of the fungiform papillae. Moreover, CA-IV reactivity was found in a few cells of the fungiform, foliate, and circumvallate papillae[4]. These studies have clarified the presence of carbonic anhydrase in the taste buds, however, little is yet known about the subcellular localization of the enzyme in them. Under the electron microscope, taste buds of rat, mouse, monkey, rabbit, and dog are recognized to contain four types of cells, type I, type II, type III, and basal cells[5-11]. The type I, II and III cells are spindle-shaped and extended from basal region to the apical taste pore region, whereas the round-shaped basal cells are located in the basal region. The type-I cells contain dense granules that are secreted into the taste pore, and possess rough endoplasmic reticulum and ribosomes in their cytoplasm; the type-II cells contain smooth endoplasmic reticulum in their cytoplasm; and the type-III cells are characterized by the presence of afferent synaptic contacts of nerve terminals. Moreover, each type of cell originates from the basal cells[8].

The taste buds of teleost fish consist of receptor cells, supporting cells, and basal cells[12, 13], and it is not known whether these cell types possess carbonic anhydrase activity or not.

The aim of present study was to examine the localization of carbonic anhydrase in the taste bud cells. We examined histochemically the taste buds of circumvallate papillae and fungiform papillae of mice and in taste buds of goldfish for the presence of carbonic anhydrase activity.

Materials and methods

Adult ddy mice were anethetized with Nembutal (Abbott Lab., North Chicago, IL, U. S. A.) and perfused intracardially with 4% glutaraldehyde in 0.1% phosphate buffer (p.H. 7.4). Tongues including circumvallate papillae and fungiform papillae were excited and kept in the same fixative for 1 hr at 4°C. Tongues of goldfish, *Carassius auratus*, were removed and cut into small pieces and fixed with 2.5% glutaraldehyde in 0.1% phosphate buffer (p.H. 7.4) for 1 hr at 4°C. Next, the specimens were rinsed in phosphate buffer containing 25% sucrose overnight. The tissues were then frozen in a spray freezer (Oken, Japan) at -20°C, sectioned sagittally at 10 μm in a cryostat and mounted on gelatin-coated slides. The sections were incubated for 20-30 min with constant agitation in an incubation medium consisting of 1.75mM $CoSO_4$ 11.7mM KH_2PO_4, 157mM $NaHCO_3$, and 53mM H_2SO_4at p.H. 6.5[14]. After incubation, the slides were rinsed in phosphate buffer and immersed in 0.5% ammonium sulphide for 2 min. For electron microscopy, the slides, after incubation in the reaction medium, were postfixed in phosphate-buffered 2% OsO_4 for 1 hr, dehydrated and embedded in Epon 812. Ultrathin sections were cut, stained with uranyl acetate followed by lead citrate, and examined under a Hitachi H7000 electron microscope. Controls were performed either in the presence of 10^{-6} M acetazolamide or in the absence of $NaHCO_3$ in the medium.

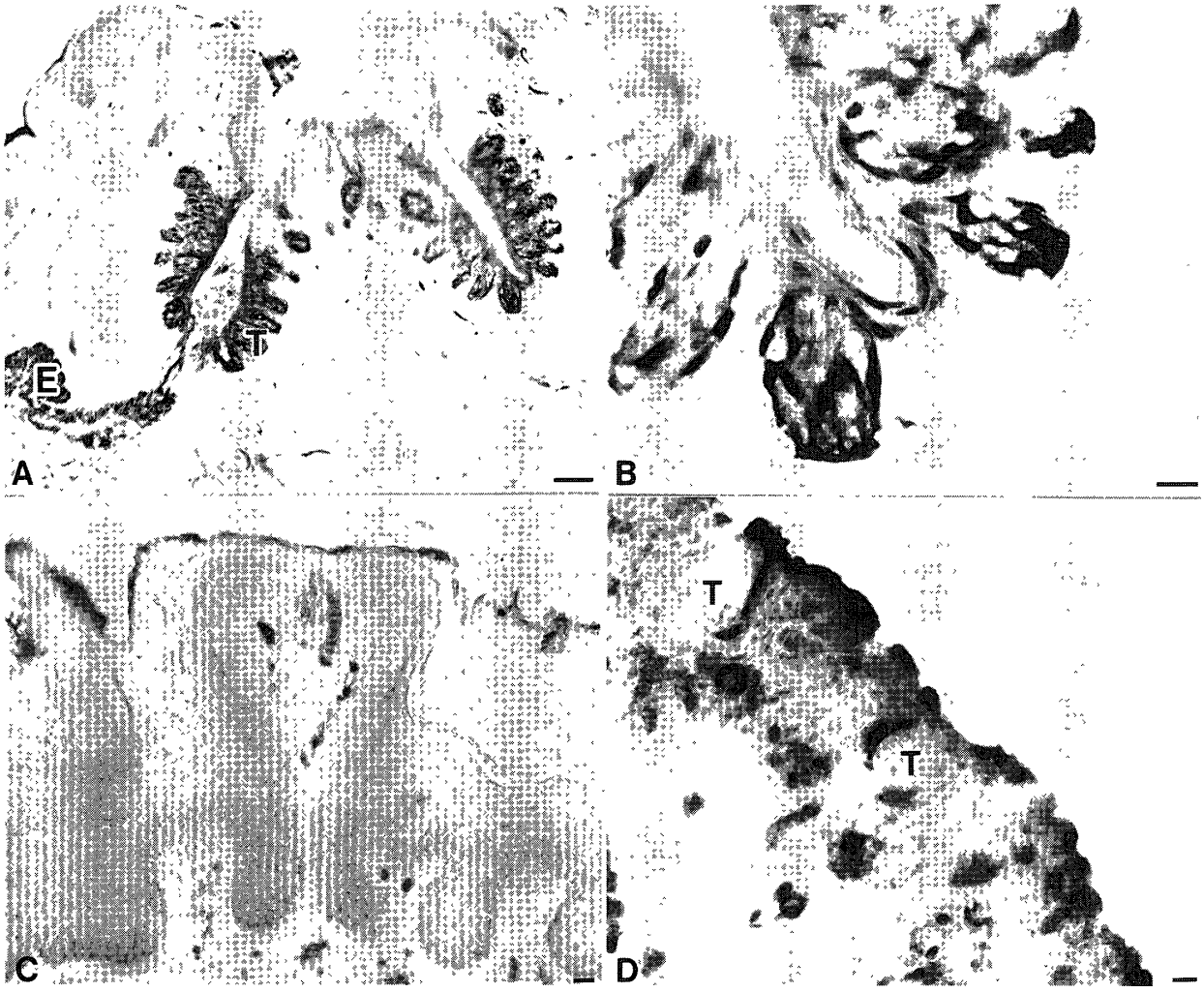

Fig. 1. Light micrographs of mouse circumvallate papillae (A, B), fungiform papillae (C), and goldfish tongue (D), showing the carbonic anhydrase reaction. A : Positive reactions are seen in the taste buds (T), von Ebner's glands (E), and blood vessels. B : Higher magnification of "A", showing the localized reaction in the taste buds. C : Positive reactions are seen in a few cells in the taste buds. D : Intense reaction is evident in the surface of the lingual epithelium and in a few cells in the taste buds (T). Scale bars, 100 μm in A, 20 μm in B, C, and D.

Results

Light microscopy

In the circumvallate papillae, a dark brown reaction product was found in the trench wall containing taste buds. The reaction was absent in the surface epithelium of circumvallate papillae. The blood vessels in the connective tissue and cells in von Ebner's glands also showed a strong positive reaction (Fig. 1A). At higher magnification, the cells in the taste buds showed a strong reaction also, and the reaction products were seen in the basal and middle regions (Fig. 1B). The reaction for carbonic anhydrase in the taste buds of fungiform papillae was weak and observed in only a few cells of taste buds (Fig. 1C). In goldfish, the reaction for carbonic anhydrase was observed in the surface and bottom of the lingual epithelium and in a few cells of the taste buds (Fig. 1D).

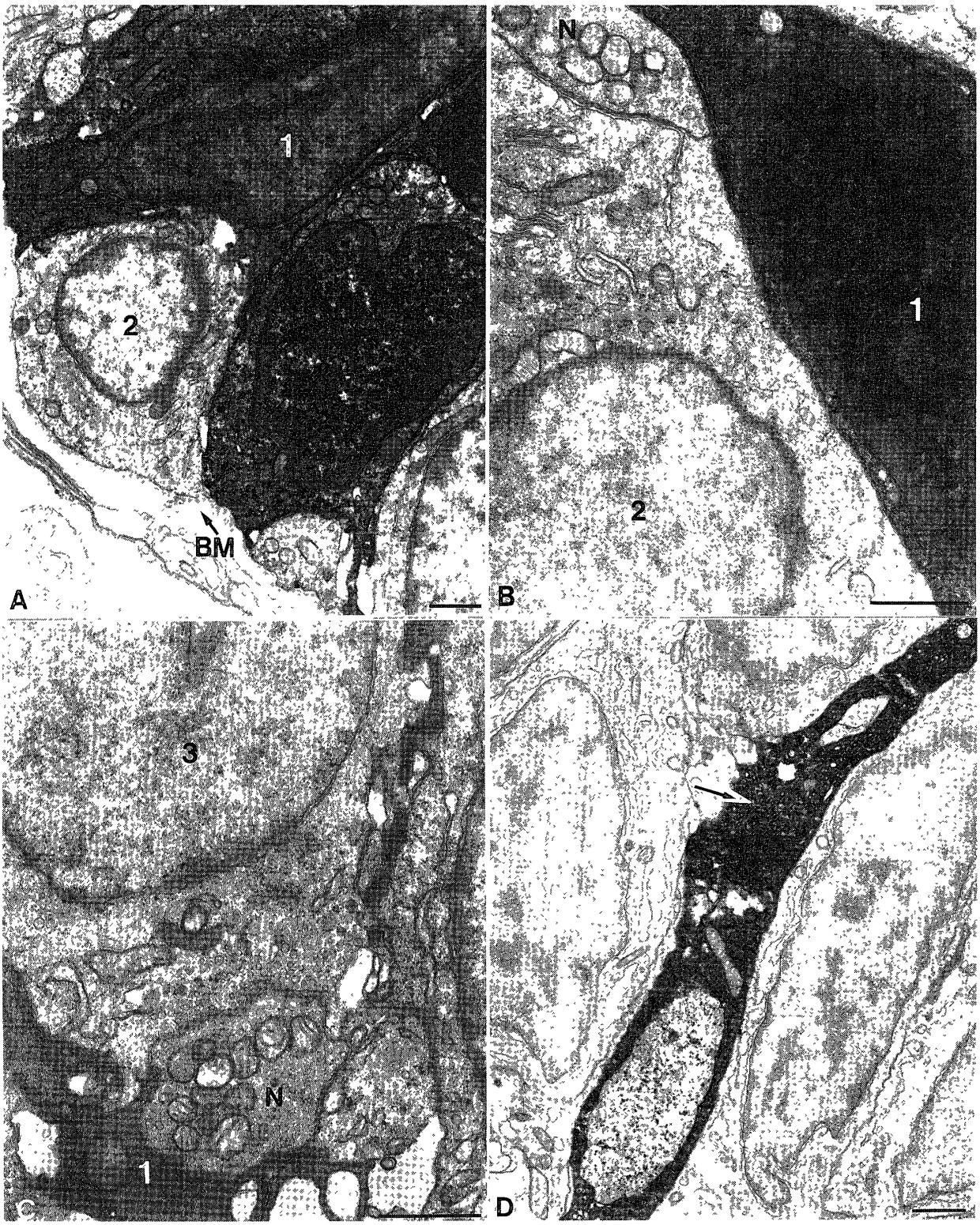

Fig. 2. Electron micrographs of the taste buds of mouse circumvallate papillae (A, B, C) and goldfish lingual epithelium (D) A : Basal region Strong reactions are observed in the cytoplasm and nuclei of type-I (1) cells, which are characterized by the presence of rough ER. The round cells are also positive. 2 · Type-II cells. BM : Basement membrane. B . Middle region The reactions are seen type-I (1) cells and not in type-II (2) ones. Smooth ER in type-II cells from a parallel alignment with nerve fibers (N) C : Middle region Type-III (3) cells, which contain small clear vesicles that form afferent synapses with nerve terminals (N), are negatively stained 1 : Type-I cells D . Positive reactions are seen in the tubule-rich cells (arrow) of the taste buds of the goldfish Scale bars, 1 μm

Electron microscopy

In the taste buds of circumvallate papille, the reaction product was mainly observed in the cytoplasm and nuclei of type-I cells. In the basal region, type-I cells, which were spindle-like in shape and were characterized by the presence of rough endoplasmic reticulum (ER) and ribosomes in their cytoplasm, showed a strong reaction indicating carbonic anhydrase activity. The reaction product was seen also in round-shaped cells, which were presumed to be type-I cells or basal cells. Type-II cells, which contained much smooth ER, showed a negative reaction. Nerve fibers were also negatively stained (Fig. 2A). In the middle region, type-I cells showed a strong reaction (Fig. 2B). The reactions were absent in both type-II and type-III cells, although type-III cells are regarded as gustatory cells since they form synapses with the nerve terminals (Fig. 2C). The apical end of type-I cells, bearing microvilli, projected into the taste pore, however, the reaction was not observed in the apical cytoplasm. In the taste buds of fungiform papillae, the reaction product was seen in type-I cells, but the number of positive cells was smaller than that in the circumvallate papillae.

In the goldfish taste buds, the carbonic anhydrase activity was found in the receptor cells, which contained many tubules (Fig. 2D). In the supporting cells and basal cells, no reaction was detected. Moreover, the reaction was also observed in the surface cells of the lingual epithelium.

The controls, performed either by inhibiting the enzyme activity with 10^{-6}M acetazolamide or by omitting the substrate, did not show any reaction product in any structures of the lingual tissues.

Discussion

The main finding of the present study is that type-I cells in the mouse taste buds and the receptor cells in goldfish taste buds showed strong activity of carbonic anhydrase. Type-I cells are known to secrete a substance into the taste pore and to have the function of phagocytosing dead cells[6], thus they are supportive in nature[7]. However, little is yet known about the enzyme-related ion transport in type-I cells. The general function of carbonic anhydrase is to catalyze the hydration of carbon dioxide, resulting in the formation of a proton and a bicarbonate ion. Consequently, this enzyme plays an important role in such diverse physiological phenomena as acid secretion by the kidney and stomach, removal of carbon dioxide produced glycolysis, and transport of carbon dioxide across erythrocyte membranes[15]. Accounting for its diverse function, several isozymic forms of the enzyme have evolved. CA-I is found in the colon, cecum, renal tubules, collecting tubules, parietal cells, adipocytes, parotid glands, submadibular glands, sublingual glands, and lacrimal glands[1,16,17]. CA-II constitutes the majority of carbonic anhydrase in erythrocytes and is the isozyme of carbonic anhydrase found in the nervous system[18, 19]. Immunohistochemical study has shown that most taste bud cells are positive for CA-I and II[4]. It is likely that carbonic anhydrase play a role

in ion transport from the basal region of type-I cells to the blood vessels in the lamina propria. The activity of carbonic anhydrase in von Ebner's glands is suggested to have a function of ion transport and production of saliva containing carbonic anhydrase, the latter of which is reported to occur in major salivary glands[16].

The difference in carbonic anhydrase activity between the circumvallate papillae and the fungiform papillae as revealed by the present histochemical method is in accord with the findings of an earlier immunohistochemical study[4]. In our study, as smaller number of taste bud cells of the fungiform papillae showed carbonic anhydrase activity than did those in circumvallate papillae. Moreover, the reaction in the fungiform papillae was weak. In the immunohistochemical study, most taste bud cells in the circumvallate and foliate papillae were reactive with anti-CA antibodies; but only a few cells were positive in the fungiform papillae[4]. Perhaps, the lower enzyme content in the taste buds of fungiform papillae might be related to their exposed location on the surface of the tongue.

The cells possessing a tubular structure in fish taste buds have been regarded as receptor cells; and fiber-rich cells, as the supporting cells[12]. The taste buds in the teleost fish are exposed to a dilute ionic environment; thus, active transport of ions through the tubular system in the receptor cells is speculated to be involved in the perception of sapid substances[13]. Carbonic anhydrase in these receptor cells may mediate such an active transport of ions.

The precipitation of reaction product in the nuclei is a common observation with the cobalt deposition technique of Hansson[14]. In the present study, which utilized this method, the reaction product was observed in both the cytoplasm and nuclei of type-I cells. However, no reaction was detected in the nucleus by immunohistochemical means[4, 16-18]. Moreover, the goldfish specimens in the present study showed less deposition of reaction product in the nucleus. We suggest that the deposits in the nucleus are due to non-specific reactions, as reported by Brown et al[3].

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