The Fine Structural Localization of Adenosine Triphosphatase Activity on the Taste Bud in the Fungiform Papillae of the Rat

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Summary. The localization of Mg++ activated adenosine triphosphatase (ATPase) activity in rat fungiform taste buds was demonstrated by electron microscopic histochemistry. Reaction product was found on the cell membrane of the taste cells, but not within their cytoplasm. Especially strong reaction occurred in the taste pore. Besides, enzymes were observed in axon-Schwann cell and axon-type I cell interspace, but not in the contacts of the nerve fiber and type II cell. The taste bud could well be distinguished from the surrounding epithelium by the existence of the reaction product. ATPase activity was absent at the synaptic portion where type III cells faced nerve fibers. No reaction product was seen along the basal lamina. In fungiform buds, the nerve fibers were frequently shown to make contact with each other, and these sites were devoid of deposits for ATPase.

Since the work of Engström and Rytzner (1956) who first applied the electron microscope to a study of the taste bud, many reports on the ultrastructure of mammalian taste buds have been published (Murray, 1973).

Taste buds are numerous on the vallate, foliate and fungiform papillae of the tongue. Some ultrastructural differences have been observed among these. On the basis of their location, morphology and the relation between intraepithelial nerve processes, Farbman (1969) classified the rat fungiform buds into four cell types: peripheral, basal, type I (dark) and type II (light) cells.

Murray, Murray and Fujimoto (1969) have reported the occurrence of type III cells in the taste buds of rabbit foliate papillae. This cell type most resembles the light cell in morphology and location, but differs from the latter in aggregation of clear (so called synaptic vesicles) and cored vesicles in the synaptic region of its cytoplasm. The type III cell, which has been shown also in the taste bud of the fungiform papillae of the rat, has typical chemical synapses which act as gustatory transmission. At present, it is thus accepted that five cell types compose the taste bud: peripheral, basal, type I, II and III cells.

Many investigators have demonstrated that Mg++ATPase activity was localized on the plasma membrane of various cells: neurons and glia (Torack and Barrnett; 1963), intestine, kidney and liver (Ashworth, LuiBel and Stewart, 1963), retina (Scarpelli and Craig, 1963), sperm (Nagano, 1965), ganglia and peripheral nerve (Novikoff et al., 1966), adrenal medulla (Wood, 1967) and anterior pituitary gland (Pelletier and Novikoff, 1972).

Some reports on electron microscopic histochemical study of the taste bud have been published (Scalzi, 1967; Iwayama, 1970; Jahnke, 1972; Fujimoto, 1973; Takeda and Hoshino, 1975). Previous light microscopic histochemical observations on the bud indicated that Mg++ATPase occurred in the subgemmal nerve plexus and taste bud.
Both light and electron microscopic observations lay stress on the fact that Mg\textsuperscript{++} ATPase was localized on the taste pore, but did not mention the relationship between the localization of enzyme activity and the cell types.

The present work was carried out in order to investigate the difference in enzyme activity in each cell type composing the bud.

**Material and Method**

Young adult rats of Sprague-Dawley strain were used in this experiment. Some rats, under anesthesia, were perfused through the ascending aorta 20 min with 2\% glutaraldehyde in buffered cacodylate. After perfusion, tissue blocks were cut from the tongue and fixed by immersion with the same fixative for 40 min.

In other animals the tongue was cut into small blocks and fixed by immersion in 4\% paraformaldehyde-5\% glutaraldehyde mixture for 1 hr according to KARNOVSKY's (1965) method.

The fixed tissues were cut at 30-50\(\mu\) thickness with a Vibratome (Scientific Product, INC.). In the attempt to preserve the fine structure of the taste bud and to obtain good localization of reaction products, this procedure was performed with unfrozen sections. The Vibratome sections were incubated in WACHSTEIN-MEISEL medium adjusted to pH 7.2 for 15-20 min at room temperature. Following incubation, tissues were postfixed in 1\% osmium tetroxide for 1 hr, dehydrated in a graded series of ethanol, infiltrated in propylene oxide and embedded in Epon 812.

As controls, the following incubation media were used:
(a) containing MgCl\textsubscript{2} as activator
(b) containing CaCl\textsubscript{2} in place of MgCl\textsubscript{2}
(c) substrate free
(d) full medium to which 0.1 or 0.01 mM ouabain was added
(e) with ATP replaced by equimolar concentrations of sodium beta glycero-phosphate.

**Observation**

The taste buds of the mammalian tongue are open to the oral environment through the pore. The fungiform buds contact the oral fluid directly through the taste pore, but the foliate and circumvallate buds indirectly by the deep furrow. Electron microscope observation confirms that microvilli of fungiform buds are shorter in length and deeper within the pore than foliate and circumvallate buds. The pore cavity of fungiform buds is filled with many vesicles, but dense and amorphous substances as observed in circumvallate buds are not seen.

Intragemmal nerve fibers in fungiform buds frequently branch and make contact with each other. The plasma membranes at the contact portions are symmetrically dense and synaptic vesicles are not aggregated there. So, it is assumed that these contact areas are not synaptic but mere cellular attachments. Taste cells are in contact with a large number of nerve fibers. Especially, type III cell shows a morphologically synaptic relation with the nerve axon. Two kinds of vesicles in the cytoplasm of type III cells were present in large numbers. One is a cored type (500-700\(\AA\)), the other is similar to the synaptic vesicle described at synapses in the nervous system (GRAY and GUILLERY, 1966). Morphologically, type III cells thus are accepted as
Fig. 1. Membranes of the taste bud (TB) surrounded by lingual epithelium (LE) show ATPase activity. ×5,000

Fig. 2. Higher magnification of the taste bud cells in Figure 1. Enzymatic activity is restricted to cell membranes such as axolemma and plasmalemma. A nerve axon. ×10,000
the taste receptor. On the other hand, no evidence of a synaptic nerve supply has been found in type I or II cells.

The localization of precipitate for ATPase activity was restricted to the cell surface of the taste buds, whereas no activity was detected on the surrounding epithelial cells of the tongue (Fig. 1, 2). Intensive reaction product was detected at the taste pore. Lead salts, showing ATPase activity, were deposited on the surface of microvilli and around the pore vesicles (Fig. 8). The reaction product filled not only the intercellular space between type I cells and the nerve axon, but also covered the membrane of type I cells. In contrast, the contact space between type II or III cells and the nerve axon were devoid of precipitates (Fig. 7). A synaptic portion where the nerve axon faced a type III cell presented no ATPase activity (Fig. 5, 6). Intragemmal nerve fibers of fungiform buds, where they make contact with each other, showed no ATPase activity. The contact sites were characterized ultrastructurally by the thickening of the cell membrane. It was revealed that the enzymatic activity was absent at desmosomes and tight junctions within the buds. The nerve fibers penetrating the lingual epithelium showed marked ATPase activity. Reaction product was seen in the narrow space between the nerve axon and epithelial cells (Fig. 3).

The distribution of ATPase activity in the subgemmal nerve plexus was similar to that in the intraepithelial nerve fibers. Enzyme activity was detected on the axolemma of unmyelinated nerve fibers and the mesaxon. The reaction product was seldom found on the axolemma of myelinated nerve fibers.

The results obtained from the specimens incubated with full medium according to WACHSTEIN-MEISEL, were reported above. After the use of the medium with beta
Fig. 4. An electron micrograph of a synaptic portion in the fungiform bud. This section is unincubated preparation. ×19,000

Fig. 5. A nerve axon (A) is juxtaposed with a type III cell. Note that no ATPase activity is found in the synaptic region indicated by arrows. N nucleus. ×33,000
glycerophosphate, no reaction product was detected anywhere in the tissue. In the absence of ATP as substrate, no deposit was observed in the taste buds. In the case where Ca++ was added instead of Mg++, the distribution of the reaction product was similar. With full medium to which 0.1 or 0.01 mM ouabain was added, ATPase activity was not inhibited.

Discussion

ATPase which hydrolyzes ATP is well known to associate intimately with cell membranes. The present study demonstrated not Na+-K+ dependent and ouabain sensitive ATPase but Mg++ activated membrane ATPase by the WACHSTEIN-MEISEL method that is inhibited by the presence of lead ion in the incubation medium and fixation procedure (ROSENTAL et al., 1966). Besides, 0.1 or 0.01 mM ouabain added to full medium does not inhibit enzyme activity. The exact relationship between Na+-K+ATPase and Mg++ATPase has not yet been clarified. Two suggestions (SKOU and HILBERG, 1965; MARCHESI and PALADE, 1967) have been made in this regard: (1)
Fig. 7. Lead salts of reaction product fill the intercellular space between the dark cell (D) and nerve axon (A), but are absent between the light cell (L) and nerve axon (A). ×7,900

Fig. 8. An electron micrograph showing the taste pore. Enzymatic activity exists prominently in the microvilli (MV) of the taste cells (T). ×23,000
these enzymes do act independently of each other. (2) these enzymes are considered as representing two different functional stages of a single enzyme. So, the opinions concerning these enzymes are controversial. However, it appears that the localization of reaction product of Mg\(^{++}\)ATPase is similar to that of Na\(^+-\)K\(^+\)ATPase (MARCHESI and PALADE, 1967).

The histochemical localization of Mg\(^{++}\)ATPase fits well with the site of transport of inorganic phosphate, thus this enzyme is presumed to mediate the energy necessary for various functional and synthetic activities of cells. The occurrence of reaction product in the taste pore suggests that Mg\(^{++}\) ATPase might be related to the early stage of taste transmission.

Only type III cells are extensively associated with the nerve ending in synaptic association. No evidence of typical chemical synapses between the nerve ending and type I or II cells has been found. However, the possibility exists that the nerve ending would be efferent to the type II cell. Despite the absence of the membrane differentiation, small vesicles containing a few cored and many empty types were seen in the axoplasm. The nerve ending contacts are broader with type II cells, and a cisterna of endoplasmic reticulum in type II cells parallels the apposed surfaces of the nerve ending.

In view of the possible functional and morphological implication of such relation as type II cells and the nerve ending, further study will be needed. The present study indicates that the junction of type II cells and the nerve ending may be regarded as having functional specificity of the cell membrane.

Nerve fibers were related to type I cells in much the same manner as the latter were Schwann cells. Type I cells completely envelop the intragemmal nerve fibers, but type II cells incompletely envelop them. In cellular function, type I cells may be supporting cells such as oligodendrocytes in the central nervous system. The oligodendrocyte is considered to possess greater metabolic activity, and Mg\(^{++}\)ATPase was demonstrated on its entire surface membrane (TRACK and BARRNETT, 1963). The distribution of reaction product in the present study is similar to the findings of their previous work. The relationship of type I and II cells to the nerve endings are thus ultracytochemically clear, but it remains functionally obscure.

One of the most interesting findings in the present study is the absence of Mg\(^{++}\) ATPase activity at synaptic spaces. Although Mg\(^{++}\)ATPase activity is shown at synaptic clefts in the rat cerebral cortex (HIRANO, 1968), in the rat adrenal medullary cells the enzyme activity is reduced or absent at synaptic clefts (YOKOYAMA, 1974). Further studies on the distribution pattern of ATPase activity must be made not only from morphological interest but also from the viewpoint of functional specialization of cells and their components.

ラット茸状乳頭味蕾における Mg\(^{++}\)ATPase 活性の局在について

明 坂 年 隆 と 織 田 正 豊

ラット茸状乳頭味蕾における Mg\(^{++}\) 活性型 アデノシン-3-リン酸分解酵素の局在について 電子顕微鏡による観察を行なった。反応産物は味蕾細胞の細胞膜に局在し、これによ
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り 周囲の舌上皮と容易に識別することができる。
味蕾部味毛に強い反応がみとめられた。また 味蕾内神経と各型味蕾細胞との間には、
活性の局在について特異性がみとめられた。すなわち I 型細胞と神経線維との間隙には
反応がみとめられるが、II 型と III 型細胞との間隙にはみとめられなかった。明瞭な求心性
シナプス構造が III 型細胞のみに見いだされるが、そこには酵素活性が欠如することが注
目される。味蕾内神経どうしの間に接触がみとめられるが、その部には反応が存在しない。
また細胞間接着部にも活性がみとめられなかった。

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