

## PROCEEDING

# Receptosecretory nature of type III cells in the taste bud

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**Abstract :** Type III cells in taste buds form chemical synapses with intragemmal afferent nerve fibers and are characterized by the presence of membrane-bound vesicles in the cytoplasm. Although the vesicles differ in shape and size among species, they are primarily categorized into small clear (40 nm in diameter) and large dense-cored (90-200 nm) types. As such vesicles tend to be closely juxtaposed to the synaptic membrane of the cells, it is reasonable to consider that the vesicles include transmitter(s) towards the gustatory nerve. In the guinea-pig taste bud, stimulation with various taste substances (sucrose, sodium chloride, quinine hydrochloride, or monosodium L-glutamate) causes ultrastructural alterations of the type III cells. At the synapse, the presynaptic plasma membrane often displays invaginations of 90 nm in a mean diameter towards the cytoplasm, which indicates the dense-cored vesicles opening into the synaptic cleft by means of exocytosis. The vesicles are also exocytosed at the non-synaptic region into the intercellular space. These findings strongly suggest that the transmitters presumably contained in the vesicles are released to conduct the excitement of the type III cells to the nerves and also to exert their paracrine effects upon the surroundings, such as the Ebner's salivary gland, acting as local hormones. *J. Med. Invest.* 56 Suppl. : 205-208, December, 2009

**Keywords :** *gustatory stimulation, taste bud, synapse, dense-cored vesicle, guinea pig*

## INTRODUCTION

The taste bud is a chemoreceptive sensory organ functioning primarily for gustatory sensation. This organ existing in the oral region of all vertebrate classes is composed of specialized epithelial cells and nerve fibers. Extensive, morphological studies have revealed that taste buds comprise gustatory cells to form synapses with afferent sensory fibers along with other non-synapsing cells (1-4). The synapsing cells contain membrane-bound vesicles in the cytoplasm. Although these vesicles are variable in appearance and size among species, they are

fundamentally categorized into small clear and large dense-cored types. As both of the vesicles are accumulated around the synaptic zone of the cytoplasm, the vesicles are considered to contain a transmitter or transmitters for the nerve.

Our previous taste-stimulation study on the guinea pig-taste bud revealed that the umami substance, monosodium L-glutamate, makes the type III cells discharge the contents of the dense-cored vesicles into not only the synaptic cleft but also the intercellular space (5). The present study extends to other basic tastes to clarify whether different qualities of stimuli cause variable responses of the type III cells in guinea pigs.

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## MATERIALS AND METHODS

Five male guinea pigs were used. After being

anesthetized by an intraperitoneal injection of sodium pentobarbital (50 mg/kg), the dorsal surface of each tongue was flashed in situ with one of the following stimulants for 20 sec.: 0.5 M sucrose (sweet), 0.1 M citric acid (sour), 10 mM quinine hydrochloride (bitter), 0.3 M sodium chloride (salty), and 20 mM monosodium glutamate (umami). Immediately after stimulation, the circumvallate papillae were removed, cut into small pieces, and immersed overnight in 2.5% glutaraldehyde in 0.1 M cacodylate buffer at pH 7.3. They were then post-fixed in 2% osmium tetroxide in the buffer for two hrs. After dehydration through an ascending ethanol series and propylene oxide, the tissue blocks were embedded in Epon 812. Ultrathin sections were double-stained with uranyl acetate and lead citrate, and examined with a JEOL 1200EX transmission electron microscope under an accelerating voltage of 80 kV.

## RESULTS

In response to each stimulus the type III cells exhibited definite ultrastructural modifications. Any other types of cells and the nerves distributed in the taste buds remained unaffected. Before describing the changes of the type III cells in detail, the ultrastructural features of the non-stimulated gustatory cells will be referred briefly (6).

The type III cells in guinea pigs are characterized by the presence of numbers of round, dense-cored vesicles which have a mean diameter of 90 nm and contain a moderately electron-dense material. The vesicles are in close association with the Golgi apparatus, scatter in the perinuclear cytoplasm, and gather to the synaptic zone. Two types of vesicles occur in the synaptic area: small clear vesicles (40 nm in the mean diameter) and dense-cored vesicles. As for their localizations at the synapse, the dense-cored vesicles tend to be closely juxtaposed to the synaptic membrane, whereas the small clear vesicles are located at a distance from the membrane.

Although every stimulant was effective in causing response of the type III cells, not all the cells responded to each stimulus. Moreover, the responsive cells displayed common changes for all the stimuli as follows. The synaptic membrane of certain type III cells invaginated towards the cytoplasm. Most invaginations were empty but some contained a dense material comparable of that appearing in the dense-cored vesicles. Hence, these structures

indicate the dense-cored vesicles being exocytosed towards the nerves. Probably as a result of their rapid release, the dense-cored vesicles have decreased in number at the synapses. The small clear vesicles, another component at the synapses, increase more or less in number to the contrary, but exhibited no exocytotic signs.

In addition to their release at the synapses, the dense-cored vesicles were discharged also at non-synaptic sites into the intercellular space. None of the small clear vesicles appeared at those sites.

No significant structural changes were recognized in any other organelles, e. g., the endoplasmic reticulum and Golgi apparatus of the responding cells as well as the non-responding cells.

## DISCUSSION

This study shows that the guinea pig-type III cells, but not all, release the contents of the large dense-cored vesicles to both the synaptic cleft and the intercellular space, and also accumulate the small clear vesicles at the synapses in response to every stimulus. With regard to the umami taste, the present data confirm our previous findings (5).

Although the sense of taste has a wide spectrum, sweet, sour, bitter, salty, and umami sensations are generally accepted as basic taste modalities. Indeed, electrical responses have been recorded from mouse gustatory cells stimulated with the corresponding taste substances (7). Individual gustatory cells reportedly were variable in the manner of response: certain cells responded to single basic taste stimuli and others to two or more stimuli. The gustatory cells receiving more than two stimuli showed no regularity in the response modalities. These electrophysiological data signify that all the gustatory cells do not always respond to each basic taste stimulus and, accordingly, coincide with the present results.

The present findings that type III cells respond to different stimuli in the same way, *i.e.*, exocytotic discharges of the dense-cored vesicles, indicate that identical transmitter substance(s) might be involved in the transduction of different taste information.

Any type III cells in submammalian and mammalian species comprise intrinsically dense-cored vesicles, which resemble in appearance those contained in autonomic neurons and other paraneuronal cells (e. g., Merkel cells, small granule-containing cells in the adrenal medulla, chief cells in the carotid body, and pinealocytes) (8). In certain neurons and

paraneurons, furthermore, the dense-cored vesicles have been demonstrated to include bioactive peptides (8-10). Hence, it is most probable that in the type III cells such peptides are contained in the vesicles to transduce the excitement of the cells to the nerves.

Our preliminary immunohistochemical examination indicates the occurrence of cells immunoreactive for Met-enkephalin-Arg<sup>6</sup>-Gly<sup>7</sup>-Leu<sup>8</sup>, a component peptide of preproenkephalin A (11), in the taste buds of the guinea pig and some other mammalian species (Yoshie, *et al.*, unpublished data). This peptide has been demonstrated to be located in the granules or vesicles of adrenomedullary cells (12), chief cells in the carotid body (13), and Merkel cells (14). Thus, enkephalins are likely candidates for the transmitters of the type III cells.

Although small clear vesicles have been reported to predominate in the non-stimulated type III cells in the rabbit, rat and some other species (4, 15-18), our observation in the guinea pig indicate that they accumulate densely at the synapses of the type III cells only after stimulation. Moreover, the present examination failed to demonstrate any evidence of their exocytotic opening. In spite of these enigmatic findings, it seems reasonable to consider that the clear vesicles are also synaptic vesicles containing transmitter(s). Further studies are required to disclose how the small clear vesicles participate in the taste transduction.

Another conspicuous feature of the responding gustatory cells is the release of the dense-cored vesicles other than at the synaptic site. This action of the type III cells possibly causes paracrine effects of the messenger substances, as local hormones, upon the surroundings. The Ebner's salivary gland may be considered as one of the targets (19).

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