Exocytotic Release of Neurotransmitter Substances from Nerve Endings in the Taste Buds of Rat Circumvallate Papillae

Yasuhisa Endo

Department of Applied Biology, Kyoto Institute of Technology, Kyoto, Japan

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Summary. Exocytotic release of neurotransmitters from nerve endings was demonstrated ultrastructurally in the taste buds of rat circumvallate papillae by stimulation of high K⁺ and Ca²⁺ Ringer perfusion and application of tannic acid-Ringer incubation (TARI) method. Omega-shaped images of large cored vesicles and small clear vesicles, indicating exocytotic release of their contents, were found only in the non-synaptic sites. Occasionally exocytosis occurred at sites facing other nerve fibers. Many coated pits were also seen, which presumably represent membrane retrieval at a later stage of exocytosis. It is likely that the taste buds receive more than one type of innervation.

It is generally accepted that the gustatory cells of taste buds possess a synaptic contact with the sensory nerve endings: Both gustatory cells and nerve endings contain not only large cored vesicles but also synaptic clear vesicles (cf. Murray, 1973; Fujita et al., 1988). An early immunohistochemical work (Lundberg et al., 1979) indicated the presence of substance P in some nerve fibers in the taste buds. A later immunoelectron microscopic study (Yamasaki et al., 1984), however, showed that the substance P-containing nerve fibers have no synaptic contact with taste bud cells. Thus, the nature and functions of nerves supplying taste buds are complex. Although many electron microscopic studies have been done on the innervation apparatus and synapse in the taste buds (Murray et al., 1969; Takeda and Hoshino, 1975; Takeda, 1976; Kinna-mon et al., 1985, 1988; Royer and Kinnamon, 1988), the destiny of neurotransmitters in the nerve endings has not been established.

The aim of this study is to clarify whether or not neurotransmitters in the nerve endings are released in the taste buds in situ. Generally, the rate of encountering the exocytotic images in the nerves to taste buds is very low, compared with that in endocrine cells (cf. Nagasawa, 1977). In order to stimulate the nerve endings and efficiently collect exocytotic images under the electron microscope, the perfusion of Ringer solution containing high K⁺ and Ca²⁺ and modified TARI (tannic acid-Ringer incubation) method (Buma et al., 1984; Endo, 1988) were applied in the present study.

MATERIALS AND METHODS

Three male Wistar rats (about 100 g in body weight) were anesthetized by an intraperitoneal injection of sodium pentobarbital (50 mg/kg body weight). The animals were fixed by a modified TARI method (Buma et al., 1984; Endo, 1988). A Ringer solution (100 ml of 145 mM NaCl, 5.6 mM KCl and 2.3 mM CaCl₂) was first perfused through the left ventricle, followed by a second perfusion of a Ringer solution containing high K⁺ and Ca²⁺ (100 ml of 30 mM NaCl, 120 mM KCl, 6 mM CaCl₂ and 0.5% tannic acid) and by a third perfusion of a fixative solution (2.5% glutaraldehyde and 0.5% tannic acid in 0.1 M phosphate buffer, pH 7.4). The circumvallate papillae were removed and immersed in the fixative solution for 2–12 h at 4°C. After being rinsed in a 0.1 M phosphate buffer, the tissues were postfixed with 1% osmium tetroxide for 1 h at 4°C, dehydrated in an ethanol series, passed through propylene oxide and embedded in Epon 812 resin. Thin sections were stained with uranyl acetate and lead solution, and examined under an electron microscope (JEM 100C) at 80 kV.
Fig. 1. Nerve endings (N) in the taste bud of rat circumvallate papilla. Nerve endings have small clear vesicles and large cored vesicles. Some nerve endings are rich in mitochondria. Taste bud cells (T) also contain small clear vesicles and large cored vesicles. Nu nucleus of taste bud cell. ×24,000

Fig. 2. Synaptic contact between a nerve ending (N) and a taste bud cell (T). a. At the presynaptic site, a dense amorphous substance (arrows) and synaptic vesicles are present. b. The arrow indicates thickening in the postsynaptic membrane. a: ×39,000, b: ×49,000
Fig. 3. Exocytotic figures (arrowheads) of large cored vesicles in the nerve endings (N). Exocytosis occurs at sites facing not only the taste bud cells (T) (a, b), but also other nerve fibers (c). A cisterna of smooth-surfaced endoplasmic reticulum (a, arrow) is seen in the taste bud cell facing the exocytosis. a: ×45,000, b: ×57,000, c: ×63,000
RESULTS

Many nerve fibers were found in the taste buds of circumvallate papillae (Fig. 1). Most of them contained small clear vesicles and large cored vesicles. Some nerve endings were rich in mitochondria. The frequency of encountering an apparent synaptic contact with a gustatory cell was relatively low. At the presynaptic sites of gustatory cells, small clear vesicles, large cored vesicles and dense amorphous substances (Fig. 2a) were found. Another type of synaptic contact was also present, which involved a thickening of the membrane of the nerve ending (Fig. 2b, arrow).

The frequency of encountering exocytotic figures of synaptic vesicles was low, in spite of the stimulation by high K⁺ and Ca²⁺ and the application of TARI method. Nevertheless, five omega-shaped images of large cored vesicles (Fig. 3) and two similar ones of small clear vesicles (Fig. 4) were found, indicating the exocytotic release of their contents. In addition, more than ten images of empty omega figures bounded with a coated membrane were obtained (Fig. 5). These coated pits were presumed to represent the images of membrane retrieval at a later stage of the exocytosis. These exocytotic figures could not be recognized at those places which deserved to be called synaptic sites. Some exocytotic figures were found at sites facing other nerve fibers (Figs. 3c, 4b). The cisternae of smooth-surfaced endoplasmic reticulum were sometimes found in gustatory cells facing the exocytotic figures (Fig. 3a).

Exocytotic figures of vesicles in the gustatory cells could not be demonstrated.

DISCUSSION

The present electron microscope study demonstrates...
images suggesting that the nerve endings in the taste buds release their neurotransmitters, although the artificial depolarization induced by high K⁺ and Ca²⁺ is a rather violent condition. The present results indicate that the innervation of the taste bud is not simple, and that more than one type of fiber supplies the taste bud. This coincides with a recent immunoelectron microscopic study by YAMASAKI et al. (1984), who found that some nerve fibers contained substance P and had no synaptic contact with the taste bud cells. Although the significance of nerve fibers having a possible efferent nature remains to be established, it may be proposed that they are involved in paracrine regulation of their functions and that they maintain or induce differentiation of the cells.

Recently, the phenomenon of non-synaptic release of neurotransmitters has been reported by a few authors using several different organs. The discovery of this phenomenon was mainly owing to the improvement of TAGO (tannic acid-glutaraldehyde-osmium tetroxide) (ROUBOS and WAL-DIVENDAL, 1980) and TARI methods, by which the exocytotic processes have been clearly visualized under an electron microscope in various endocrine cells and neurosecretory cells.

In the authentic neurons, using a conventional method, NITSCH and RINNE (1981) found exocytotic figures

Fig. 5, a–c. Empty omega figures (arrowheads) in nerve endings (N), suggestive of a later stage of the exocytotic process. Nu nucleus of taste bud cell, T taste bud cell. a: ×34,000, b: ×80,000, c: ×62,000
of large cored vesicles at only the synaptic sites in the hippocampus mossy fibers of the rabbits being symptomatic of epileptiform seizures by the administration of methoxy pyridoxine. On the other hand, using the TARI method, BUMA and ROUBOS (1986) demonstrated the non-synaptic release of large cored vesicles in the central nervous system of snails, insects and rats. In the peripheral nervous system, GOLDFING and POW (1987) found in the adrenal chromaffin glands that the exocytosis of large cored vesicles in the nerve terminals occurred mainly at the non-synaptic sites, in spite of the apparent presence of synaptic contact with the adrenal chromaffin cells. The present study demonstrated a similar phenomenon in the nerve terminals of the taste bud.

As shown in the present study, exocytosis occurred at the sites facing not only the taste bud cells but also other nerve fibers. Similar events were also demonstrated in the enteric nervous system of rats in my previous work (ENDO, 1988). In the smooth muscle coat of the small intestine, exocytotic release of neurotransmitters occurred at the sites not only facing the smooth muscle cells but also facing Schwann or other neuronal processes. These findings suggest the possibility that the nerve terminals may not always release their chemical messengers only in sites directly facing the target cells.

REFERENCES


