A Possibility of Efferent Innervation of the Gustatory Cell in the Rat Circumvallate Taste Bud

Sumio Yoshie1, Hiroaki Kanazawa2 and Tsuneo Fujita1

Department of Histology1, Nippon Dental University School of Dentistry at Niigata; and Department of Anatomy2, Niigata University School of Medicine, Niigata, Japan

Received July 8, 1996

Summary. A transmission-electron microscope study of the rat circumvallate taste bud revealed that occasionally one and the same gustatory or Type III cell received innervation associated with subsurface cisterns as the Type II cell did, in addition to the ordinary afferent synapse.

The subsurface cistern, a flattened, smooth-surfaced saccular membrane system, hugged the plasma membrane of the gustatory cell along the boundary against the nerve terminal. The nerve terminal attaching to the cell was occupied with mitochondria and synaptic type vesicles.

As these structural features resemble those of efferent synapses in certain other sensory cells including the inner ear hair cells, the gustatory cells dually innervated as demonstrated in the present study are presumably involved not only in the afferent but also in the efferent projection of nerves.

The taste bud is a richly innervated epithelial organ of gustatory sensation which appears in all the vertebrate classes. Early electron-microscopic studies classified mammalian taste bud cells as Type I or dark, Type II or light, and basal cells (Murray and Murray, 1960, 1967; Farbman, 1965a, b; Scalzi, 1967; Uga, 1969). In the meantime, Murray et al. (1969) first distinguished an additional cell population (Type III cell) in the foliate taste bud of rabbits. The Type III cells specifically formed afferent synapses with intragemmal nerves and contained characteristic vesicles in their cytoplasm which were gathered towards the synaptic zones (Murray et al., 1969).

Supporting Murray and his associates (Murray et al., 1969), most later workers divided mammalian taste bud cells into four types: Type I, Type II, Type III, and basal or Type IV cells (e.g., in man: Paran et al., 1975; in the rat: Takeda and Hoshino, 1975; in the mouse: Takeda, 1976; in the monkey: Farbman et al., 1985; in the guinea pig: Yoshie et al., 1990; in the dog: Kanazawa, 1993; see also Murray, 1986). The Type I, Type II, and Type III cells are all spindle in shape and reach apically to the taste pit, whereas the basal cell resides at the basal region of the taste bud without extending to the taste pit.

Concerning the functions of these types of cells, it is generally accepted that the Type I, Type III, and basal cells are sustentacular, gustatory, and undifferentiated precursory in nature, respectively. As for the Type II cell, however, its functional significance remains obscure.

The Type II cells usually make broad contact with intragemmal nerve fibers and possess subsurface cisterns in the cytoplasm just beneath the contacting zone (Fujimoto and Murray, 1970; Murray, 1971, 1986; Takeda, 1976; Akisaka, 1980; Kondo, 1983; Yoshie et al., 1990; Kanazawa, 1993). Owing to these intimate and specialized relationships between the Type II cells and the nerves, the Type II cells are postulated to receive an efferent innervation (Fujimoto and Murray, 1970; Takeda, 1976; Akisaka, 1980; Yoshie et al., 1990; Kanazawa, 1993).

The gustatory (Type III) cells contain vesicles in their cytoplasm which vary in appearance and size among species. As these vesicles tend to gather to the synaptic zones of the cells, they are considered synaptic vesicles containing transmitters acting upon the nerves. Indeed, our experiment in guinea pigs demonstrated that stimulation of the taste buds with different taste substances in the gustatory cells induced exocytosis of the dense-cored vesicles into the synaptic clefts (Yoshie et al., 1991, 1994).

Our recent studies in the rat demonstrated that the antiserum against PGP (protein-gene product) 9.5, a neuron-specific protein, is a useful marker to detect...
not only nerve fibers but also a part of the cells in the taste buds (Iwanaga et al., 1992); we recently characterized the immunoreactive cells as gustatory cells (Kanazawa and Yoshie, 1996). To extend this line of studies to other animals, we proceeded to examine the taste buds of rats by conventional transmission electron microscopy. In the course of the examination, we encountered certain gustatory cells receiving innervation with subsurface cisterns in addition to the ordinary afferent synapse. The present paper describes this new type of innervation, which has previously been reported in the taste buds of a few species (in the rhesus monkey: Ide and Munger, 1980 and Zahm and Munger, 1983; in the mouse: Royer and Kinnamon, 1988; in the rabbit: Royer and Kinnamon, 1991) but, so far as we are aware, has not been documented in the rat (Takeda and Hoshino, 1975).

**MATERIALS AND METHODS**

Details of the tissue preparation for transmission electron microscopy have already been described elsewhere (Kanazawa and Yoshie, 1996). In brief, rat tongues were perfusion-fixed in 2.5% glutaraldehyde in 0.1 M phosphate buffer at pH 7.4. Circumvallate papillae were then post-fixed in osmium tetroxide in the same buffer and embedded in Epon 812 resin. 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**

During observation of electron micrographs of rat taste buds, it was noticed rather frequently that a gustatory cell received a nerve supply in a manner different from the ordinary afferent synapse. A typical case is given in Figures 1 and 2, which illustrate a gustatory cell attaching to three profiles of nerve terminals showing either of two different kinds of innervation patterns.

In two of the nerve fiber profiles, this gustatory cell formed afferent synapses of the ordinary type (Fig. 2). The synaptic membranes facing each other were separated by a cleft of 15–20 nm in width. They both were thickened by deposit of a dense material on the plasmic sides. The synaptic areas of the gustatory cell gathered predominantly small clear vesicles (30 nm in diameter) intermingled with a few large dense-cored ones (50 nm in diameter). The nerve varicosity synapsing onto the gustatory cell contained an abundance of mitochondria and a moderate number of vesicles mainly of a small clear type (for further details of the gustatory cell, see Kanazawa and Yoshie, 1996).

Along the boundary against the other nerve profile, the same gustatory cell revealed a flattened, smooth-surfaced saccular structure, the subsurface cistern (Fig. 2). The cistern contained a narrow space and extended while keeping a distance of 5–15 nm from the plasma membrane. No other conspicuous organelles such as vesicles or mitochondria could be recognized in its vicinity. Neither of the plasma membranes facing each other exhibited a thickening. The cleft between the membranes measured 15–20 nm in width. On the other hand, the nerve attaching to the cell formed a swelling, which was occupied with mitochondria and synaptic-type vesicles (Fig. 2). These vesicles measured 30–55 nm in diameter and were predominantly clear in appearance.

Type II cells displayed an electron-lucent appearance of the cytoplasm and very often were associated with intragemmal nerve fibers (Fig. 3). The structural relationship between the Type II cell and the nerve was identical with the latter innervation type of the gustatory cell depicted above, being characterized by the presence of subsurface cisterns in the cytoplasm and by the accumulation of mitochondria and synaptic vesicles in the nerve terminal (Fig. 2).

---

**Fig. 1.** Part of a taste bud in the rat circumvallate papilla. A gustatory cell (G) is associated with three nerve profiles (N). Nu nucleus of the gustatory cell. × 22,000

**Fig. 2.** Closer view of the gustatory cell and associated nerves shown in Figure 1. The gustatory cell forms afferent synapses with two nerves (N1, N2) and contacts the other nerve (N3), being equipped with a subsurface cistern (small arrows) in the cytoplasm subjacent to the plasma membrane. In the afferent pre-synaptic areas, small clear and large dense-cored vesicles are gathered (large arrows). Note that all the nerves include clear and dense-cored vesicles as well as numerous mitochondria. × 59,000

**Fig. 3.** Type II cell and an associated nerve. The Type II cell (II) places a subsurface cistern (arrows) in the cytoplasm along and close to the nerve-contacting plasma membrane. The nerve (N) contains vesicles and mitochondria. × 74,000
Figs. 1-3. Legends on the opposite page.
DISCUSSION

There have been some reports describing the occurrence of a dual innervation of mammalian gustatory cells (Ide and Munger, 1980; Zahn and Munger, 1983; Royer and Kinnaman, 1988, 1991). Ide and Munger (1980) identified two types of innervation of the laryngeal taste bud in the rhesus monkey. An identical gustatory cell often was associated with different nerve terminals, being independently equipped with the conventional structure for afferent chemical transmission and with subsurface cisterns (Ide and Munger, 1980; see also Zahn and Munger, 1983), in accordance with the present study’s finding in the rat. Because of the presence of these two types of nerve-cell specializations, these authors postulated a reciprocal function for nerves supplying the monkey gustatory cell (Ide and Munger, 1980; Zahn and Munger, 1983). Royer and Kinnaman (1988) also demonstrated gustatory cells showing these two nerve terminals in foliate taste buds of the mouse; using a method of computer-assisted, three-dimensional reconstruction, they depicted the same nerve fiber associated with a gustatory cell forming both types of contact specializations at different sites in the cell.

These authors, however, were of a passive opinion concerning the efferent-projection hypothesis and, instead, proposed such other possible functions of the subsurface cistern as the synthesis of synaptic membrane components, modification of the electrical or adhesive properties of the gustatory cell membrane, and exchange of trophic factors with nerves (Royer and Kinnaman, 1988).

Type II cells of taste buds in mammalian species generally make broad contact with the intragemmal nerve swellings and dispose subsurface cisterns in the cytoplasm immediately beneath the nerve-associating zones (e.g., Fujimoto and Murray, 1970; Takeda, 1976; Akisaka, 1980; Yoshie et al., 1990; Kanazawa, 1993; for review, see also Reutter and Witt, 1993). It is well known that certain mechanosensory cells such as the outer hair cell in the cochlea are innervated with both afferent and efferent fibers (Smith and Sjöstrand, 1961; Saito, 1980; Saito and Hama, 1984; Simmons and Liberman, 1988). At the efferent synapse, the sensory cells exhibit subsurface cisterns along the synaptic membranes.

Considering the common appearance of the subsurface cistern in the hair cells and taste bud-Type II cells and, furthermore, the accumulation of mitochondria and synaptic vesicles in the nerve terminal apposed to the subsurface cistern in the Type II cells, an efferent function is suggested for this type of cell.

It could then be hypothesized that the Type III or gustatory cells provided with subsurface cisterns as demonstrated in the present study might also receive an efferent projection additional to an afferent one from nerves (see Ide and Munger, 1980; Zahn and Munger, 1983).

Apart from the reciprocal projection of the dually innervated gustatory cell dealt with in this paper, it seems worthwhile to mention that the classic “afferent” synapse of the cell implies its reciprocal nature, as the axoplasm of the nerve forming this synapse usually reveals clear and dense-cored vesicles (in the snake and turtle: Uchida, 1980; in the guinea pig: Yoshie et al., 1990). This view was originated by Hirata (1966) based on his early electron microscopic study of teleostean taste buds. The bilateral occurrence of synaptic vesicles suggesting a reciprocal synapse seems to be a common feature among sensory paraneurons (review: Fujita et al., 1988), including neuro-epithelial body cells in the lung (Lauwersyns and Cokelaere, 1973; Rogers and Haller, 1978; Lauwersyns and Lommel, 1982), chief cells in the carotid body (McDonald and Mitchell, 1975; Osborne and Butler, 1975), and Merkel cells in the skin (English, 1977; Munger, 1977; Mihsara et al., 1979). Further studies are needed to clarify the functional significance of this essentially universal phenomenon in the sensory synapse.

REFERENCES


Dr. Sumio YOSHIE
Department of Histology
Nippon Dental University
School of Dentistry at Niigata
1-8, Hamaura-cho, Niigata
951 Japan

吉江　紀夫
951 新潟市浜浦町1-8
日本歯科大学新潟歯学部
第二口腔解剖学教室