Fine Structure of Taste Buds in the Tongue, Palatal Mucosa and Gill Arch of the Axolotl, *Ambystoma mexicanum*

By

Kuniaki TOYOSHIMA, Kenji MIYAMOTO and Akitatsu SHIMAMURA

Department of Oral Anatomy, Kyushu Dental College
Kokurakita-ku, Kitakyushu 803, Japan

—Received for Publication, January 10, 1987—

Key Words: Taste bud, Palatal mucosa, Gill arch, Ultrastructure

Summary: The fine structure of taste buds in the tongue, palatal mucosa and gill arch of the axolotl, *Ambystoma mexicanum*, was examined by means of electron microscopy. The taste buds consisted of three types of cells: light, dark and basal cells. The light cell was characterized by the presence of microtubules in the supranuclear cytoplasm and two kinds of vesicles, dense-cored and synaptic vesicles, in the basal cytoplasm. This cell type had afferent synaptic contacts with nerve fibers. The supranuclear cytoplasm of the dark cell was filled with secretory granules. Membrane fragments were always associated with microvilli of the dark cells. The basal cell, located only at the basal part of the taste bud, contained dense-cored vesicles mainly in the basal cytoplasm. Neither the synaptic vesicle nor the synaptic contact were found in this cell type.

Since the first study on the ultrastructure of taste buds in rabbit foliate papillae by Engström and Rytznér (1956), taste buds are increasingly being examined by morphologists attempting to address several basic problems dealing with the cell types and their functional significances. Among these investigations, amphibian taste buds have been the subject of a number of ultrastructural (Fährmann, 1967; Farbman and Yonkers, 1971; Graziadei and DeHan, 1971; Stensaas, 1971; Düring and Andres, 1976; Whitear, 1976; Toyoshima and Shimamura, 1982; Toyoshima et al., 1984) as well as electrophysiological studies (Kusano and Sato, 1957; Rappuzzi and Casella, 1965).

In contrast to the adult anurans which usually lack the gill, the axolotl continues to have it even in adult stage. For a comparative view point the ultrastructural study

of taste buds in the axolotl has been limited to a small number (Fährmann, 1967; Whitear, 1976), and little is known about the morphology of the taste buds in the gills of this animal when compared to the studies on this organs in teleost gills (Hossler et al., 1979; Hossler and Merchant, 1983).

The purpose of the present communication is to describe further observations on the fine structure of taste buds in the gill arch as well as in the tongue and palatal mucosa of the axolotl.

Materials and Methods

Six neotenus axolotl, *Ambystoma mexicanum* (Black variety) were anesthetized with ether. The tongue, palatal mucosa and gills were surgically removed and were fixed immediately in half-strength Karnovsky's

(1965) fixative buffered with phosphate. After 3 hr in this fixative, the tissues were postfixed for 2 hr in phosphate-buffered 2% osmium tetroxide. The specimens were then dehydrated in ascending concentrations of ethanol, passed through propylene oxide, and embedded in Epon-Maraglas (Tandler and Walter, 1977). Thick sections for light microscopy were stained with toluidine blue and examined in an ordinary light microscope. Thin sections for electron microscopy were stained with uranyl acetate followed by lead citrate and examined in a JEM 100C electron microscope. For scanning electron microscopy, intact tongue, palatal mucosa and gills, fixed as described above, were dehydrated in ethanol, immersed in isoamyl acetate, critical point dried in liquid CO₂ and sputter-coated with gold-palladium to be examined in a Hitachi T-500 scanning electron microscope.

Results

Taste buds are always found as a bud in shape in the epithelium of all places examined. Since the light and electron microscopic structures of taste buds are essentially indistinguishable irrespective of their locations, they are considered together here.

Taste buds are composed of three characteristic cell types, the light, dark and basal cells as well as nerve elements (Fig. 1). The light and dark cells extend throughout the entire thickness of the epithelium and directly contact with oral environment. The basal cell, on the other hand, is located only at the basal part of the taste bud.

The light cell terminates in a few thick microvilli, measuring approximately 1–1.2 μm long and 0.4–0.5 μm wide (Figs. 2–4). The contents of the microvilli show a parallel pattern of numerous filaments which penetrate deeply into the cytoplasm and make rootlets. The light cell has a large

number of microtubules, about 40 nm in diameter, in the supranuclear cytoplasm. They run almost parallel to the long axis of the cell. Mitochondria, small vacuoles and amorphous dense granules are also the components of the supranuclear cytoplasm of this cell type. The most significant feature of the light cell is the presence of a number of dense-cored vesicles of about 140–180 nm in diameter and synaptic vesicles of about 60–80 nm in diameter. These vesicles are most numerous in the basal cytoplasm (Figs. 7 and 8). The density of the contents of dense-cored vesicles is variable. These two types of vesicles tend to accumulate in the cytoplasm facing the junction with nerve fibers, and peculiar contacts suggesting afferent synapses are observed in this region (Fig. 8).

The dark cell is characterized by the presence of a number of secretory granules, ranging from 0.2 to 0.4 μm in size, in the supranuclear cytoplasm. The secretory granules display a spectrum of electron density. At the cell apex, microvilli of the dark cells are longer but thinner, measuring approximately 2–2.5 μm long and 0.2 μm wide, and more numerous than in nearby light cells. At higher magnification it becomes evident that there exists a number of membrane fragments as well as a surface coat on the plasma membrane of microvilli of this cell type (Fig. 6). These fragments are composed of double layers of electron-dense leaflets sandwiched the electron-lucent gap. The thickness of these laminae is estimated to be about 9 nm. These membrane fragments orient almost perpendicularly toward the microvillar plasma membrane. In addition, fragments showing concaved appearance are occasionally seen among leaf-like laminae. These fragments are always observed in association with microvillar surface of dark cells and never seen in relation to microvilli of light cells.

The basal cells are located at the basal

part and do not reach to the free surface of the taste bud (Fig. 9). Basal cells contain characteristic dense-cored vesicles, about 120–160 nm in diameter, which tend to distribute mainly in the cytoplasmic area facing the basal lamina. The electron density of these vesicles is variable. Neither the synaptic vesicle nor the synaptic contact are found in this cell type.

Discussion

This study has revealed the fine structural details of taste buds in the tongue, palatal mucosa and gill arch of the axolotl. Three types of cells are distinguished in the taste buds, irrespective of their locations.

The light cells contain two types of vesicles. These are dense-cored vesicles and synaptic vesicles. In addition, the light cell makes synaptic contacts with nerve fibers, which are characterized by the densification of plasma membrane and the aggregation of both synaptic vesicles and dense-cored vesicles. It seems likely that the light cell may function as a gustatory transducer cell.

The characteristic dense-cored vesicles of light cells of the axolotl are similar in appearance to those of gustatory cells in other amphibians (Uga and Hama, 1967; Graziadei and DeHan, 1971; Farbman and Yonkers, 1971; Düring and Andres, 1976; Toyoshima and Shimamura, 1982; Toyoshima et al., 1984). In contrast to the light (gustatory) cells of the axolotl and *Necturus*, which possess synaptic vesicles in addition to dense-cored vesicles, anuran gustatory cells contain dense-cored vesicles solely in the cytoplasm even in the synaptic contact regions.

The functional significance of the vesicles is still unknown, but several points seem worthy of mention. Insofar as synaptic vesicles are concerned, it seems reasonable to consider that these vesicles may contain chemical transmitters.

A clue to the nature and function of dense-cored vesicles may be gained from the pioneer work of Reutter (1971), who showed monoamine-containing cells in the taste bud of the catfish barbel. After that, there have been several reports on the occurrence of monoamine-containing cells in taste buds of fishes (Nada and Hirata, 1977; Reutter, 1978; Toyoshima et al., 1984), frogs (Hirata and Nada, 1975; Hirata and Nada, 1977; Esakov et al., 1984) and mammals (Nada and Hirata, 1975; Nada and Hirata, 1976; Nada and Hirata, 1977, Takeda and Kitao, 1980).

Many of these investigators are of the opinion that monoamines may be stored in dense-cored vesicles of a certain cell type in the taste bud. Recently, Nada and Komatsu (1983) demonstrated serotonin-like immunoreactivity in the rabbit taste buds, and Uchida (1985) identified serotonin-like immunoreactivity in dense-cored vesicles as well as cytosol in the gustatory cells of the mouse by immuno-electron microscopy.

In contradistinction to the fact that monoamine-containing cells in the taste buds of the rabbit and the mouse can be followed from the basal lamina to the taste pore, this cell type in amphibians is located exclusively in the basal part and fail to reach the taste pore. DeHan and Graziadei (1973), who classified taste bud cells of the frog into two types, the sensory (gustatory) and the associate (supporting) cells, suggested that the sensory cell contains monoamine and may be involved in taste sensation as chemical transmitters. Düring and Andres (1976), on the other hand, distinguished additional cell type, the basal cell, besides these two cell types. They described that this type of cells also contained dense-cored vesicles. Further it possessed cytoplasmic processes, which were reminiscent of the characteristics of the Merkel cells. Thus the possibility is raised from these morphological view points that monoamine-containing

cells in amphibian taste buds may correspond to the basal cells, and monoamines may be stored in dense-cored vesicles of this cell type. No transformation from one cell type to another was observed in the present axolotl and other amphibian taste buds. This may suggest independent functions for these cell types.

In regard to the membrane fragments being associated with microvilli of the dark cells, Whitear (1976) have suggested in her excellent paper that these were secreted lipo-protein materials derived from dark cells, which might be involved in cleansing process of the taste pore. Similar membrane fragments are also described in *Necturus* taste buds (Farbman and Yonkers, 1971) and in polyvillous and oligovillous cells in the epidermis of lamprey skin (Whitear and Lane, 1983-a, b). Why the membrane fragments fails to occur in other amphibian and in mammalian taste buds is unknown.

References

- 1) DeHan, R.S. and Graziadei, P.P.C.: The innervation of frog's taste organ. "A histochemical study". *Life Sci.*, **13**: 1435-1449 (1973).
- 2) Düring, M.v. and Andres, K.H.: The ultrastructure of taste and touch receptors of the frog's taste organ. *Cell Tissue Res.*, **165**: 185-198, (1976)
- 3) Engström, H. and Rytzner, C.: The fine structure of taste bud and taste fibers. *Ann. Otol. (St. Louis)*, **65**: 361-375 (1956).
- 4) Esakov, A.I., Solovieva, N.A. and Krokhina, E.M.: Effects of tryptophan and 5-hydroxytryptophan on the number of monoamine-containing cells in the frog taste buds. *Chem. Senses*, **9**: 303-309 (1984).
- 5) Fährmann, W.: Licht- und Elektronenmikroskopische Untersuchungen an der Geschmacksknospe des neotenen Axolotls (*Siredon mexicanum* Shaw). *Z. mikr.-anat. Forsch.*, **77**: 117-152 (1967).
- 6) Farbman, A.I. and Yonkers, J.D.: Fine structure of the taste bud in the mud puppy, *Necturus maculosus*, *Am. J. Anat.*, **131**: 353-370 (1971).
- 7) Graziadei, P.P.C. and DeHan, R.S.: The ultrastructure of frog's taste organs. *Acta anat.*, **80**: 563-603 (1971).
- 8) Hirata, K. and Nada, O.: A monoamine in the gustatory cell of the frog's taste organ. A fluorescence histochemical and electron microscopic study. *Cell Tissue Res.*, **159**: 101-108 (1975).
- 9) Hirata, K. and Nada, O.: Cytoarchitecture of monoamine-containing cells in the frog's gustatory epithelium. *Experientia*, **33**: 1223-1224 (1977).
- 10) Hossler, F.E., Ruby, J.R. and Mucllwain, T.D.: The gill arch of the mullet, *Mugil cephalus*. 1. Surface ultrastructure. *J. Exp. Zool.*, **208**: 379-398 (1979).
- 11) Hossler, F. E. and Merchant, L.H.: Morphology of taste buds on the gill arches of the mullet *Mugil cephalus*, and the killifish *Fundulus heteroclitus*. *Am J. Anat.*, **166**: 299-312 (1983).
- 12) Karnovsky, M.J.: A formaldehyde-glutaraldehyde fixative of high osmolality for use in electron microscopy. *J. Cell. Biol.*, **27**: 137A (1965).
- 13) Kusano, K. and Sato, M.: Properties of fungi-form papillae in frog's tongue. *Jap. J Physiol.*, **7**: 324-338 (1957).
- 14) Nada, O. and Hirata, K.: The occurrence of the cell type containing a specific monoamine in the taste bud of the rabbit's foliate papilla. *Histochemistry*, **43**: 237-140 (1975).
- 15) Nada, O. and Hirata, K.: Pharmacohistochemical studies on a specific monoamine in the gustatory epithelia of the rabbit. *Histochemistry*, **50**: 12-17 (1976).
- 16) Nada, O. and Hirata, K.: The monoamine-containing cell in the gustatory epithelium of some vertebrates. *Arch. Histol. Jpn.*, **40**: (Suppl.): 197-206 (1977).
- 17) Nada, O. and Komatsu, K.: Immunohistochemical observations on the serotonin-containing cell in the taste bud of the rabbit. *Acta Anat. Nippon.*, **59**: 311A (1983).
- 18) Rapuzzi, G. and Casella, C.: Innervation of fungiform papillae in the frog tongue. *J. Neurophysiol.*, **29**: 154-165 (1965).
- 19) Reutter, K.: Die Geschmacksknospen des Zwergwelses *Amiurus nebulosus* (Lesueur). Morphologische und Histochemische Untersuchungen. *Z. Zellforsch.*, **120**: 280-308 (1971).
- 20) Reutter, K.: Taste organ in the bullhead

- (Teleostei). Adv. Anat. Embryol. Cell Biol., **55**: 1-98 (1978).
- 21) Stensaas, L.J.: The fine structure of fungi-form papillae and epithelium of the tongue of a South American toad, *Calyptocephalella gayi*. Am. J. Anat., **131**: 443-462 (1971).
 - 22) Takeda, M. and Kitao, K.: Effect of monoamines on the taste buds in the mouse. Cell Tissue Res., **210**: 71-78 (1980).
 - 23) Tadler, B. and Walter, R.J.: Epon-Maraglas embedment for electron microscopy. Stain Technol., **52**: 238-239 (1977).
 - 24) Toyoshima, K. and Shimamura, A.: Comparative study of ultrastructures of the lateral-line organs and the palatal taste organs in the African clawed toad, *Xenopus laevis*. Anat. Rec. **204**: 371-381 (1982).
 - 25) Toyoshima, K., Honda, E., Nakahara, S. and Shimamura, A.: Ultrastructural and histochemical changes in the frog taste organ following denervation. Arch. Histol. Jpn., **47**: 31-42 (1984).
 - 26) Toyoshima, K., Nada, O. and Shimamura, A.: Fine structure of monoamine-containing basal cells in the taste buds on the barbels of three species of teleosts. Cell Tissue Res., **235**: 479-484 (1984).
 - 27) Uchida, T.: Serotonin-like immunoreactivity in the taste bud of the mouse circumvallate papilla. Jap. J. Oral Biol., **27**: 132-139 (1985).
 - 28) Uga, S. and Hama, K.: Electron microscopic studies on the synaptic region of the taste organ of carps and frogs. J. Electron Microsc., **16**: 269-276 (1967).
 - 29) Whitear, M.: Apical secretion from taste bud and other epithelial cells in amphibians. Cell Tissue Res., **172**: 389-404 (1976).
 - 30) Whitear, M. and Lane, E.B.: Polyvillous cells of the dermis: transportive elements of lamprey skin. J. Zool., **199**: 345-358 (1983).
 - 31) Whitear, M. and Lane, E.B.: Oligovillous cells of the epidermis: sensory elements of lamprey skin. J. Zool., **199**: 359-384 (1983).

Explanation of Figures

Plate I

- Fig. 1. Light micrograph of a taste bud located in the gill arch. The taste bud consisted of three cell types, the light, dark and basal cells. X500.
- Fig. 2. Scanning electron microscopic view of palatal mucosa. Taste pores (arrows) are easily identified. X 140.
- Fig. 3. A higher magnification picture of a taste pore region of the gill taste bud. Tufts of microvilli extend beyond the taste pore. X 5,000.

Plate II

Fig. 4. Electron micrograph showing an apical part of a taste bud in the tongue. Light cells(L) terminate in a few thick microvilli. X 8,300.

Fig. 5. Supranuclear cytoplasm of the light cell contains a number of microtubules which run almost parallel to the long axis of the cell. X 15,000.

Fig. 6. View at a higher magnification showing microvilli of light and dark cells in the taste bud of the gill arch. In addition to the network of a surface coat, membrane fragments are observed in close association with microvilli of the dark cell.

Inset: Bilaminar fragments oriented vertically to the microvillar plasma membrane are seen.
X 25,000. X 63,000.

Plate III

- Fig. 7. Electron micrograph showing a basal part of a taste bud. The dense-cored vesicles are discernible in the cytoplasm of the light cell (L). X 6,800.
- Fig. 8. The light cell contains dense-cored vesicles and synaptic vesicles, and makes synaptic contact with nerve fiber(N). X 19,000.
- Fig. 9. The basal cell(BC) is located exclusively in the basal region of the taste bud. The dense-cored vesicles can be recognized in the cytoplasm. Inset: View at a higher magnification showing dense-cored vesicles in the basal cell. X 7,500., X 22,000.