A Scanning and Transmission Electron Microscope Study of the Premetamorphic Papillae: Possible Chemoreceptive Organs in the Oral Cavity of an Anuran Tadpole (Rana japonica)*

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Summary. Premetamorphic papillae of an anuran tadpole (Rana japonica) were studied by scanning and transmission electron microscopy. Premetamorphic papillae of several shapes are seen in and around the oral cavities of tadpoles during late larval and early metamorphic stages. These papillae are composed of three parts: the apical cellular part, the underlying connective tissue core and the epithelial covering. In the apical cellular part, two populations of cells are observed: apical and basal cells. The apical cell has a slender cytoplasmic process whose apical surface is exposed in the oral cavity. The basal cell is located at the basal portion of the apical cellular part of the papilla without reaching the oral cavity. A long solitary microvillus and a bunch of short microvilli are seen on the apical surface of each apical cell. The apical cells contain dense-cored vesicles of 100 nm diameter and make synaptic contacts at their basal membranes with terminals of nerve fibers. Possible chemoreceptive function of the premetamorphic papilla is discussed.

It was reported that the fungiform papillae on the tongue of the adult frog are chemoreceptors (Pumphrey, 1935; Kusano and Sato, 1957; Rapuzzi and Casella, 1965), and that chemoreception in the tadpole is performed with the lateral-line organ distributing on the skin (Onoda and Katsuki, 1972). The amphibian tadpoles, however, feed actively at larval stages before the end of metamorphosis. A question, then, may arise: Are there no chemoreceptive organs in the oral cavity of the tadpole until the fungiform papillae are differentiated? Helff and Mellingker (1941a) found four bluntly-pointed, finger-like papillae on the floor of the mouth of Rana sylvatica at premetamorphic stages. These papillae were named "premetamorphic papillae" as they undergo regression during the metamorphic period. Helff and Mellingker, however, failed to find light microscopically any nerve elements within the coria of the premetamorphic papillae. In the present study attempts were made to re-examine the possibility that the premetamorphic papillae may possess chemoreceptive function.

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MATERIAL AND METHODS

The fertilized eggs of the frog, *Rana japonica*, were collected from rice fields in the suburbs of Hiroshima City. The developing embryos were reared in aquaria filled with dechlorinated tap water at 18–21°C. Boiled spinach was fed once a day. Developmental stages of the larval and metamorphic periods were determined according to SHUMWAY (1940) and TAYLOR and KALLROS (1946), respectively; each of the larval and metamorphic periods was divided into 25 stages. The mandibular and maxillary portions were excised separately, placed immediately in cold 3% glutaraldehyde in 0.1 M phosphate buffer (pH 7.3) for 1 hr, and postosmicated with cold 2% OsO₄ in 0.1 M phosphate buffer (pH 7.3) for 2 hrs.

For transmission electron microscopy, the materials were cut into small blocks under a dissection microscope during the fixation in glutaraldehyde. After fixation, the tissue blocks were dehydrated in graded ethanol series and embedded in Epon 812. Thick and thin sections were made on a Porter-Blum MT–II Ultramicrotome for both light and transmission electron microscopy. Thin sections were placed on grids coated with Neoplane W, stained with saturated uranyl acetate and 0.3% lead citrate, and examined in a Hitachi HS–8 Electron Microscope operated 50 kV.

For scanning electron microscopy, the materials were dehydrated in amyl acetate after dehydration in ethanol series, dried by the critical-point method using liquid carbon dioxide, and set on specimen-stands. They were then coated in vacuo with gold and carbon particles and examined in a JSM–U3 scanning electron microscope operated at 15 kV.

OBSERVATIONS

Scanning electron microscopy

At metamorphic stage 5, when tadpoles swim and feed actively, four finger-like papillae measuring 50 µm in proximal diameter and 350–400 µm in length are observed in the area close to the anterior margin of the oral floor as shown in Figure 1a. Two of these papillae are branched into two or three branchlets at their tips (Fig. 1a). These four papillae are recognizable at larval stage 22 as four small protuberances in the anterior portions of the oral floor where the tongue anlage is to appear in later stages of metamorphosis. In addition to these four finger-like premetamorphic papillae, many premetamorphic papillae of several shapes are seen in and around the oral cavity of the tadpole at metamorphic stage 5: finger-like papillae measuring 40–150 µm in proximal diameter and 50–400 µm in length, and cone-like papillae measuring 50 µm in proximal diameter and 50 µm in length are seen in the lateral and posterior portions of the oral floor (Fig. 1a, b, 2a). The average number of papillae is 20 ± 4 in four tadpoles.

Fig. 1. a. Oral lip (OL) and anterior region of oral floor (OF) in a frog tadpole at metamorphic stage 5. Two palm-like (PPP) and many finger-like (FPP) premetamorphic papillae are seen. × 40. b. Posterior region of the same oral floor (OF) as shown in Figure 1a. Cone-like (CPP) and finger-like (FPP) premetamorphic papillae are seen. Some of the finger-like papillae branch at their tips (arrows). ×40
Fig. 1. Legend on the opposite page.

Fig. 2. a. A cone-like premetamorphic papilla (CPP) on the oral floor. Arrows point to apical portions of the papilla. ×750. b. The apical region of a finger-like papilla (FPP). Solitary thick microvilli (arrows) and bunches of slender microvilli (arrow heads) are seen. ×2,900

Fig. 3. a. The apical region of a cone-like papilla. Solitary thick microvilli (arrows) and bunches of slender microvilli (arrowheads) are also seen. ×3,000. b. High power scanning electron micrograph of the apical region of a finger-like premetamorphic papilla. Arrows point to solitary thick microvilli, and arrow heads indicate bunches of slender microvilli. ×15,000
finger-like papillae is 29 and that of cone-like papillae is 15. Palm-like papillae are observed on the oral lip (Fig. 1a). The whole extent of the oral palate is also covered with finger-like, cone-like and rampart-like premetamorphic papillae. All of these papillae appear around larval stage 23 and grow up fully by metamorphic stage 5. All premetamorphic papillae are capped with two kinds of microvilli: solitary long, thick microvilli measuring 0.5 μm in diameter and 2 μm in length, and bunches of 10–25 short, slender microvilli measuring 0.15 μm in diameter and 1.5 μm in length (Fig. 2b, 3a, b). In each papilla, the number of solitary long microvilli and that of bunches of short microvilli are approximately equal.

The premetamorphic papillae begin to degenerate at mid-metamorphic stages (stage 15–16) and disappear completely by metamorphic stage 23.

**Light microscopy**

Finger-like papillae are composed of three parts: the apical cellular part, loose connective tissue core, and epithelial covering which is continuous to the covering epithelium of the oral cavity. In the apical cellular part, two kinds of cellular components are observed: apical and basal cells. The apical cell has an oval-shaped nucleus at the basal portion, protrudes its slender dark cytoplasm upward and bears many microvilli at the free surface (Fig. 4a). The basal cell is located at the basal portion of the apical cellular part of the papilla without exposing its cytoplasm to the oral cavity. The loose connective tissue core, which is capped with the apical cellular part of the papilla and covered at the lateral aspects with two layers of
flattened epithelial cells, contains nerve elements, blood vessels and other mesenchymal cells (Fig. 4a, b).

Structures of other premetamorphic papillae are principally the same as those of finger-like papillae. Connective tissue core, however, is lacking or only poorly developed in the cone-like papillae, whereas it is well developed in the palm-like and rampart-like papillae.

**Transmission electron microscopy**

In each apical cell of the finger-like premetamorphic papilla, a large ellipsoidal nucleus with dispersed chromatin granules is located at the basal portion. Cytoplasm of the apical cell is electron-dense and contains well-developed smooth-surfaced endoplasmic reticula and mitochondria running parallel to the axis of the cell body (Fig. 5, 6). Many dense-cored vesicles with a diameter of 100 nm are seen in the areas surrounding the nucleus (Fig. 7, 8). A solitary long, thick microvillus and a bunch of short, slender microvilli protrude from the apex of the apical cells, and these microvilli contain microfilaments measuring 10 nm in diameter and running parallel to their long axis (Fig. 5).
In the connective tissue core of each papilla, a nerve fascicle consisting of several unmyelinated nerve fibers is accompanied by Schwann cells (Fig. 8). The nerve fascicle appears to run upward in the connective tissue core to reach the basal lamina. The nerve fibers further pass through the basal lamina to distribute within the apical cellular part of the papilla, where they appear electron-lucent and are not accompanied by Schwann cells (Fig. 7, 8). Mitochondria, dense bodies, clear and dense-cored vesicles of various sizes are seen within these nerve fibers. In the epithelial cells covering the lateral aspects of the papillae, mucous granules are lined up as a single layer just under the surface membrane of the cells.

Nerve terminals distributing in the apical cellular part of the papillae are often observed to make contacts with the basal membranes of the apical cells. At the contact area, intercellular space measures 25 nm in width and membrane thickening is seen in the apical cell as well as in the nerve terminals (Fig. 9a–c). The dense-cored vesicles in the apical cells tend to accumulate in the regions close to the contact area (Fig. 9a–c). Omega-shaped structures continuous to the cell membrane of the apical cell are also observed at the contact area (Fig. 9d).
Light and transmission electron microscopic findings of the finger-like premetamorphic papillae are summarized diagrammatically in Figure 10. Ultrastructures of other papillae are essentially the same as those of finger-like papillae.

**DISCUSSION**

The finger-like premetamorphic papillae observed on the anlage of the tongue in *Rana japonica* correspond in their shape, size, number and location to the "premetamorphic papillae" described by HELFF and MELICKER (1941a, b) in *Rana sylvatica*. In *Rana japonica* at the early metamorphic period, however, many other papillae of several shapes are also seen on the oral lips and in the oral cavity. In the present study, all of these papillae observed in and around the oral cavity of tadpoles before
the end of metamorphosis are included in the premetamorphic papillae.

The main cellular components of the premetamorphic papillae are the apical cells which are characterized by their apical process with microvilli, by their dense-cored vesicles and by their basal synaptic contacts with nerve terminals. Each apical cell is equipped with two kinds of microvilli; a long and thick, solitary microvillus and a bunch of short, slender microvilli. Two kinds of microvilli similar to these have been described also in the receptor cells of the terminal buds on the barbel of the teleost. In the receptor cells on the barbel, however, a thick microvillus projects from the dark cell and a bunch of slender ones from the light cell (HIRATA, 1966; GROVER-JOHNSON and FARBMAN, 1976; OVALLE and SHINN, 1977). The sensory hair cell in the lateral-line organ of lower aquatic vertebrates is equipped with a kinocilium and many stereocilia (HAMA, 1965; JANDE, 1966). In mammalian taste buds, both dark and light cells are capped by a brush of slender microvilli of one sort (MURRAY, 1973).

Although the dense-cored vesicles contained within the apical dark cells of the premetamorphic papillae differ in their size and electron opacity from synaptic vesicles observed in the sensory cells of mammalian taste buds (TRUJIRO-CENÓZ, 1957; MURRAY and MURRAY, 1960), lateral-line organs of lower aquatic vertebrates (HAMA, 1965; JANDE, 1966; YAMADA and HAMA, 1971) and Corti organ (SMITH and SJÖSTRAND, 1961), they are quite similar to those seen in synaptic areas of the taste cells of the adult frog (DeHan and GRAZIADEI, 1971; GRAZIADEI and DeHan, 1971; HIRATA and NADA, 1975). The dense-cored vesicles in the taste cells of the adult frog have been advocated to contain serotonin (HIRATA and NADA, 1975) or noradrenalin (DeHan and GRAZIADEI, 1973). The dense-cored vesicles contained in the apical cells of the premetamorphic papillae tend to accumulate in the regions close to the thickenings of the cell membrane where contacts between the apical cells and nerve terminals are made. These features resemble chemical synapses; the nerve terminals contacting upon the basal portions of the apical cells are reasonably suggested to receive sensory inputs from the cells. The omega-shaped images of the granules described above are believed to represent exocytotic

![Fig. 10. Schematic drawing of the finger-like premetamorphic papilla. The apical cells (AC) with ellipsoidal nuclei at their basal portions bear two kinds of microvilli (Mv) at the apical surface. Nerve fibers (Ne) running upward through the connective tissue core (CT) make synaptic contacts (arrows) with the apical cells. BC basal cell.](image-url)
release of the granule contents into the synaptic cleft.

The premetamorphic papillae of tadpoles degenerate completely by the late metamorphic period when fungiform papillae are fully differentiated (Nomura et al., 1979). The fungiform papillae of the adult frog were reported to be chemoreceptive as well as mechanoreceptive (Kusano and Sato, 1957), and it was assumed that, while chemoreception of the fungiform papillae is performed with the taste cells bearing microvilli, mechanoreception is carried out by Merkel cells located in the basal portions of the papillae (During and Andres, 1975). On the other hand, in the premetamorphic papillae only possible sensory cells are the apical cells bearing microvilli; no Merkel cells are found. Thus, the premetamorphic papillae are considered most likely to serve as chemoreceptive organs in tadpoles during late larval and early metamorphic stages.

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