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Actin Filaments of Taste Buds in the Goldfish and Parrot

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Abstract

The filaments in the apical region of the taste bud cells of both goldfish and parrot were examined by fluorescence histochemistry and electron microscopy. The apical cytoplasm of goldfish taste buds terminated in long, slender processes and microvilli, and contained thin and straight filaments composed of f-actin, as detected by fluorescein-labeled phalloidin binding. In the parrot, the apical cytoplasm of taste buds terminating in microvilli also showed phalloidin fluorescence. The result suggests that the apex of taste buds of most vertebrate species is composed of actin filaments.

Key words : actin filament, taste bud, goldfish, parrot

Introduction

It is generally accepted that the core filaments in the microvilli are composed of actin¹⁾. Studies on the mammalian taste buds have shown that the apical cytoplasm of taste bud cells terminates in microvilli that project into the taste pore. Evidence of actin filaments in the microvilli in mouse taste buds has been obtained by immunohistochemistry using anti-actin antibody²⁾. In the rabbit foliate papillae, the fluorescence of phalloidin has been observed in the microvilli of the taste buds, indicating the presence of f-actin³⁾. In the frog, the apical cytoplasm of taste cells terminates in microvilli and rod-shaped processes. We observed actin filaments in both microvilli and these rod-shaped processes by histochemistry using phalloidin⁴⁾. Although taste buds are widely distributed among the vertebrate species, there is no report about the filaments in the apical region in fishes, reptiles, and birds.

In the present study, we examined the cytoskeletal structures in the apical region of taste buds of a goldfish and parrot by fluorescence histochemistry and by conventional electron

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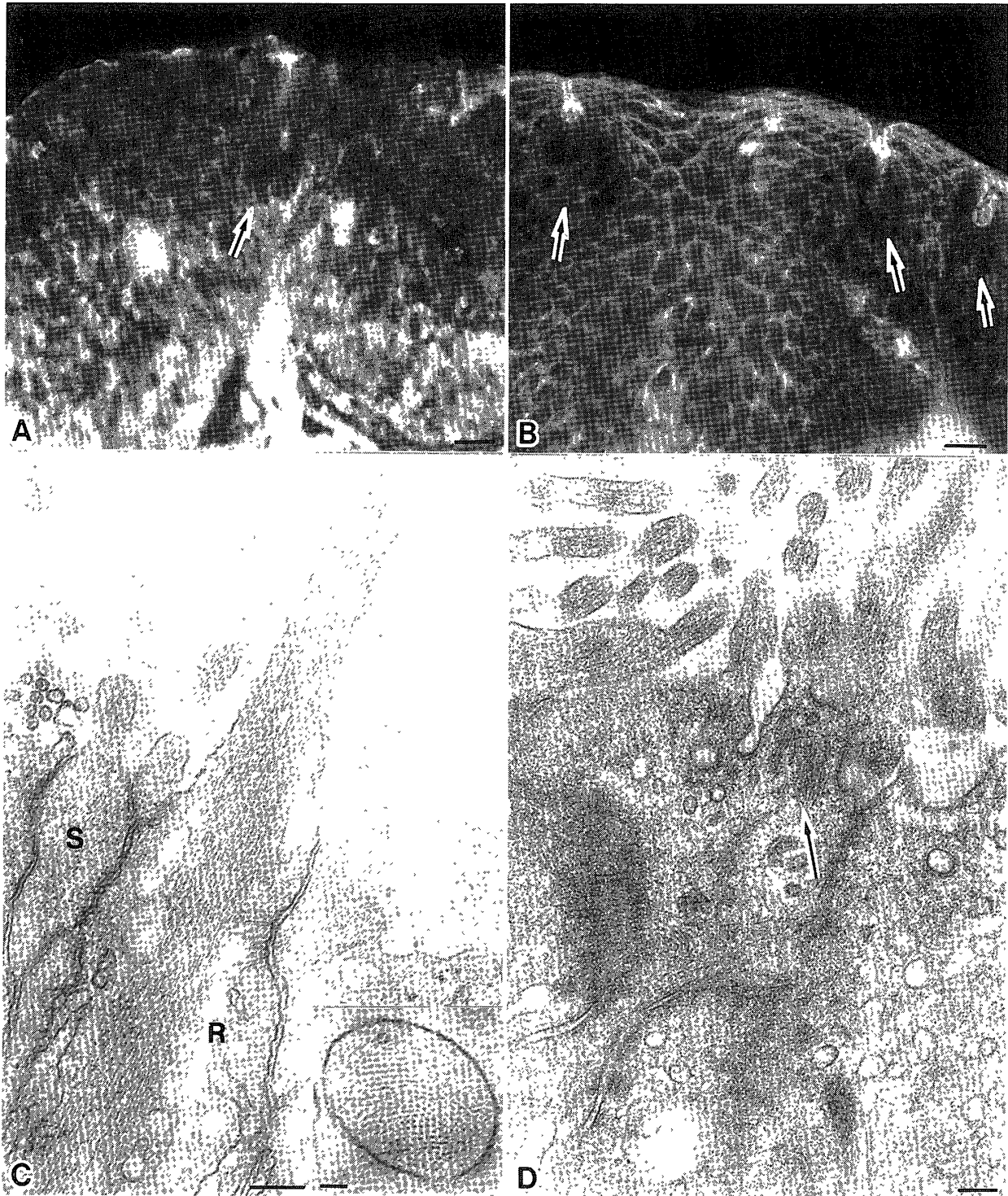


Fig. 1. A. B. Fluorescence micrograph of lingual epithelium of goldfish (A) and parrot (B) after the application of FITC-labeled phalloidin. The reactions are seen in the apex of taste buds (arrows). C. D. Electron micrograph of the apical region of taste buds of goldfish (C) and parrot (D). C: Note the bundles of filaments of 6-nm diameter in the long processes of receptor cells (R) and microvilli of supporting cells (S) in the goldfish epithelium inset: A transverse section of a long process, showing the alignment of filaments. D: Filaments of 6-nm diameter are seen in the microvilli of taste bud cells and extend toward the junctional complex in the apical cytoplasm (arrow). Scale bar, A, B = 20 μm , C, D = 0.2 μm , inset C = 0.05 μm

microscopy

Materials and Methods

The goldfish (*Carracius auratus*) and parrot (*Melopsittacus undulatus*), both of which are commercially available, were used. The tongues were removed and cut into small blocks. For fluorescence microscopy, the tissues were fixed in 4% paraformaldehyde dissolved in phosphate-buffered saline (PBS) for 1 hr at 4°C, and rinsed overnight at 4°C in PBS containing 10% sucrose. The tissues were then frozen in a spray freezer (Oken, Japan) at -20°C, sectioned sagittally at 10 μ m in a cryostat and mounted on gelatin-coated slides. The sections were treated with FITC-labeled phalloidin (Sigma, 33 ng for each section) for 30 min at room temperature. They were then rinsed in PBS, mounted with glycerin, and examined under a fluorescence microscope. For electron microscopy, the tissues were fixed in a phosphate-buffered (pH 7.4) solution containing 2% glutaraldehyde and 1.6% paraformaldehyde. They were postfixed in phosphate-buffered (pH 7.4) 1% OsO₄, dehydrated, and embedded in Epon 812. Ultrathin sections were cut, stained with uranyl acetate followed by lead citrate, and examined under a Hitachi H7000 electron microscope.

Results

After the application of FITC-labeled phalloidin, intense fluorescence was observed in the apical region of the taste buds of both goldfish and parrot specimens. In the goldfish tongue, fluorescence was observed also in the cells of the lingual epithelium and in muscle fibers in the lamina propria (Fig 1A, B). Under the electron microscope, the apical cytoplasm of receptor cells of goldfish taste buds terminated in a single tapering process, which protruded outward from the taste pore. The apical cytoplasm of supporting cells terminated in microvilli. Straight and thin filaments (6 nm in diameter) filled both the tapering processes and microvilli. The filaments, in parallel alignment in the tapering processes, were bound to the cell membrane at one end, the other end extended downward about 5 μ m into the apical cytoplasm of the taste bud cells (Fig 1C). In transverse section of the processes, many filaments were packed in regular alignment (Fig 1C, inset). In the parrot, the apical cytoplasm of taste bud cells terminated in microvilli and thin and straight filaments were observed in them. At the base of microvilli, these filaments extended downward to the level of the zonula adherens of the junctional complex (Fig 1D).

Discussion

In both the goldfish and parrot taste buds, we regard the 6-nm filaments in the apical cytoplasm of taste bud cells to be f-actin, judging from the characteristic binding of FITC-labeled phalloidin there. In the goldfish specimens, a greater number of filaments existed in the long processes of receptor cells than in the microvilli. A similar distribution pattern of

actin filaments has been observed between rod-shaped processes of taste cells and microvilli of supporting cells in frog taste organ⁴⁾ This suggests that the actin filaments are related to the support of the taste cell membrane, where the taste stimulus may occur In fact, in the frog inner ear, actin filaments in the sensory hairs of receptor cells have been found to exhibit considerable stiffness, as determined by micromanipulation of sensory hairs where the membrane has been removed by treatment with Triton-X-100⁵⁾ In other species of teleost fishes, e.g., silurid fish, the apex of receptor cells terminates in the long tapering processes, in which thin filaments with parallel alignment are seen⁶⁾ In chicken and canary, the apical end of taste bud cells consists of microvilli and thin filaments were also observed in them^{7,8)} We did not examine the taste buds in reptiles in the present study, but it is reported that the apical processes of cells in the taste buds of tortoises, lizards, and snakes terminate in microvilli⁹⁾ The apexes of taste buds in the silurid fish, chicken, canary, tortoise, lizard, and snake are likely to be composed of actin filaments Therefore, the apical ends of taste buds or taste organ throughout the vertebrates are suggested to be composed of actin filaments, which may afford a rigid structure to taste-sensing region of the taste cell membrane

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