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RESEARCH ARTICLE

Histochemical studies of the olfactory epithelium of brackish-water cichlid fish, *Etroplus suratensis* (Bloch)

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Abstract. The localization and detection of silver stain for axons, the chemical nature of mucopolysaccharides, protein, lipid, alkaline phosphatase, and adenosine triphosphatase on the olfactory epithelium of *Etroplus suratensis* (Bloch) were studied by employing different histochemical techniques. Silver stain was used to detect the occurrence and distribution of different types of axons, if any, in the epithelium and in various layers in the lamella. The chemical nature of acid and neutral mucins in the various regions of the olfactory epithelium was identified by employing the PAS-AB histochemical test. The histochemical localization of basic protein and lipid were recorded in the various cells of the olfactory epithelial lining as well as in the central core. The localization and detection of alkaline phosphatase (ALPase) and adenosine-tri-phosphatase (ATPase) in the different cells lining the olfactory epithelium were discussed with the functional significance of the fish concerned.

Keywords: axon, mucopolysaccharides, protein, lipid, enzymes, olfactory epithelium, *Etroplus suratensis*

Introduction

The study of the olfactory organ in fish is of paramount importance, because this organ is essentially a chemoreceptor and plays a meaningful role not only in locating food, predators, and reproductive synchrony, but also in detecting the presence of odoriferous substances and contaminants in the surrounding aquatic environment. Olfaction is achieved by the stimulation of the sensory receptor cells in the olfactory organ, which is innervated by the olfactory nerve. The distribution and organization of various cells lining the olfactory epithelium of teleosts have been studied by many researchers using light microscopes (Ojha and Kapoor 1973, Bandyopadhyay and Datta 1996, Mandal et al. 2005, Ghosh and Chakrabarti 2009, Chakrabarti and Ghosh 2010, 2011). However, histochemical approaches of the olfactory organ in fishes have been applied by only a few researchers (Ojha and Kapoor 1972, Datta Munshi and Singh 1975, Pandey and Mishra 1984, Belanger et al. 2003, Chakrabarti 2005, Ghosh and Chakrabarti 2012) to evaluate the chemical nature of various cells in the epithelial lining. No enzyme histochemical investigation has been done to correlate the functional significance of various cells in the olfactory epithelium of brackish water teleosts. The aim of the present study is to determine the precise chemical constitution and functional aspects of different cell types on the olfactory epithelium of brackish water pearl spot, *Etroplus suratensis* (Bloch).

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Materials and methods

Living, mature *E. suratensis* (13 to 15 cm in length) were collected from the Junput brackish water fish farm in Purba Midnapore, West Bengal, India. The fish were anesthetized with tricaine methone-sulphonate (MS 222; Sigma Chemical Co.) solution (100 mg l^{-1}) and sacrificed following the guidelines of the Institutional Ethical Committee. The olfactory rosettes were excised from the nasal chamber and immediately processed for the histochemical studies.

Olfactory tissues were fixed in 10% neutral formalin for 18 h. Fixed tissues were washed repeatedly in 70% ethanol and dehydrated properly through ascending series of ethanol. Then the tissues were cleared with xylene and embedded in paraffin wax of 52-54°C in a thermostat vacuum paraffin-embedding bath for a period of 40 min. Serial sections were cut at 8 μm thick using a rotary microtome (Weswox) and were subjected to various histochemical techniques: the silver impregnation method (SIM) for detecting axons (Marsland et al. 1954); periodic Schiff's reaction (PAS) in combination with alcian blue (AB) (PAS-AB) for detecting neutral and acid mucins (Mowry 1956); the mercury-bromphenol blue (MBPB) method for detecting basic proteins (Bonhag 1955); and the Sudan black B (SB) method for detecting bound lipid (Berenbaum 1958). Some tissues were fixed quickly in cold absolute acetone for 16 h at 4°C for the enzyme histochemical study. The tissues were dehydrated in absolute acetone, cleared in benzene, and embedded in paraffin wax at 48°C in a vacuum medium for 30 min. The tissues were sectioned serially at an 8 μm thickness. The adapted calcium-cobalt method for detecting alkaline phosphatase (ALPase) (Gomori 1951) and the calcium method for detecting adenosine triphosphatase (ATPase) were applied (Padykula and Herman 1955).

Results

Detection and localization of axons

The degrees of silver deposition differed in the various layers of the olfactory epithelium of *E. suratensis*. The intense reaction of the silver stain was well marked in the knob and dendrite process of primary receptor cells (Figs. 1 and 2). The intensity of the reaction was also discernible in the synaptic contact in between primary and secondary receptor cells. The blood cells and nerve fiber networks in the central core of the lamella were also positively stained with the silver reaction (Figs. 1 and 2).

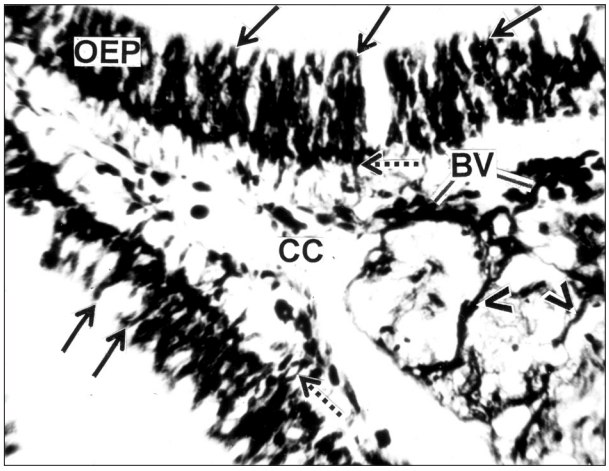

Figure 1. Intense silver reaction in primary receptor cells (RC) with knobs (arrows) of the olfactory epithelium (OEP) of *E. suratensis* and moderate reaction in the nerve fibers (N) (arrow heads) and blood cells (BV) of the central core (CC). Broken arrows indicate synaptic contact in between primary and secondary RC (SIM) × 400.

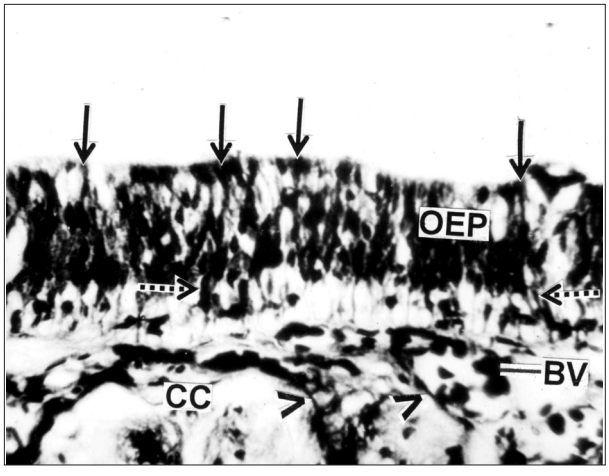

Figure 2. Localization of strong silver reaction in the dendrite process of primary RC (solid arrows) and synapse with secondary receptor cells (RC) (broken arrows) of the olfactory epithelium (OEP) of *E. suratensis*. Note silver reaction in N (arrow heads) and BV of CC (SIM) × 400.

Detection and localization of mucopolysaccharides

The combined PAS-AB reaction resulted in a purple-bluish color of varying intensities in accordance with the neutral and acid mucin content of several mucous cells in the olfactory epithelium. This combined test imparted a bright purple color from PAS for neutral mucin and a glossy blue color from the AB reaction in the presence of acid mucin exclusively. In *E. suratensis*, the intensity of bluish-purple color was discernible at its maximum in the secretory mucous cells at the epithelial border, confirming the presence of a mixture of acid and neutral mucin in different proportion (Figs. 3 and 4). In some areas, the secretory mucous cells, which were vacuolated and coarsely reticulated and situated mainly at the apical region of olfactory lamellae, took on a bluish-purple color from the PAS-AB reaction (Fig. 4). Secreted epithelial mucins also reacted positively with this test.

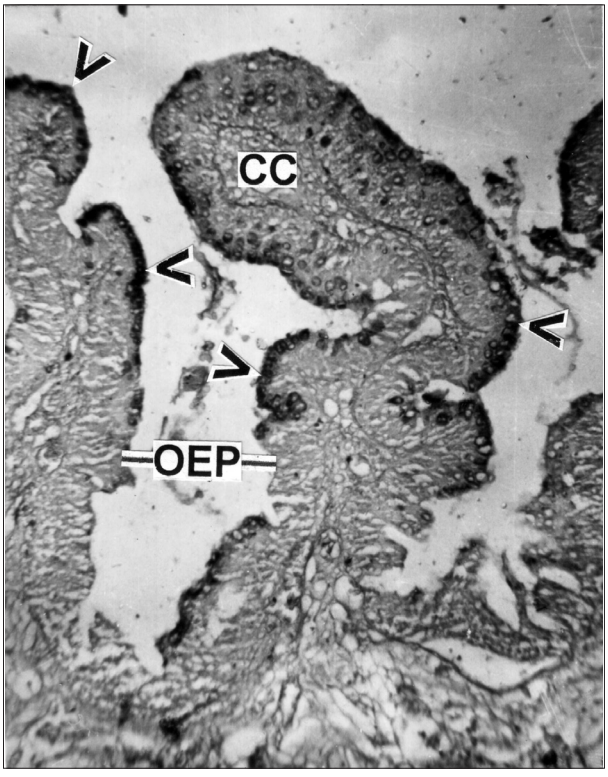

Figure 3. Presence of mixture of acid and neutral mucin (ANM) in the mucous cells (MC) (arrow heads) of the olfactory epithelium (OEP) of *E. suratensis*. CC displays moderate reaction (PAS-AB) × 100.

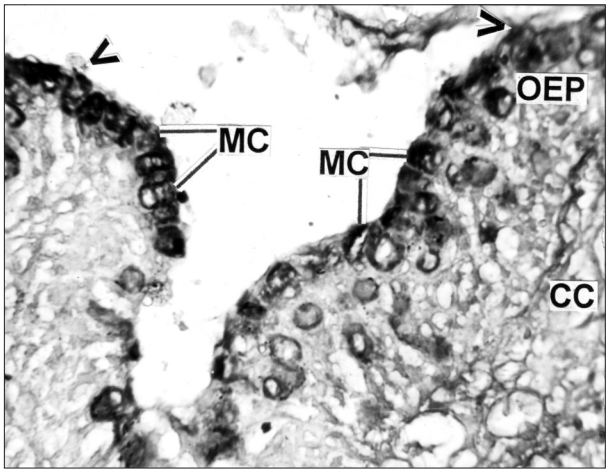

Figure 4. Intense acid and neutral mucin (ANM) in the secretory mucous cells (MC) and moderate PAS-AB reaction in the central core (CC). Note – arrow heads indicate luminal secretion (PAS-AB) × 400.

The connective tissues and nerve fibers in the central core exhibited moderate to weak reactions (Figs. 3 and 4). The receptor cells, however, displayed a negative reaction to the PAS-AB test.

Detection and localization of basic protein

The mercury-bromphenol blue histochemical test was used to detect protein material associated with the different cells of the olfactory epithelium. An intense reaction was discernible in the receptor cells of the surface epithelium (Fig. 5). Moreover, microvillous cells and basal cells were also positive to this test; however, the maximum protein reaction was observed in the blood cells and connective tissue of central core region (Fig. 5).

Detection and localization of bound lipid

Sudan black B only stained the areas of olfactory epithelium with bound lipids. Higher amounts of lipid was found to be associated with the receptor cells along with terminal knobs at the surface epithelium (Fig. 6). Moreover, the blood cells of the central core also showed considerable reaction with Sudan black B staining (Fig. 6).

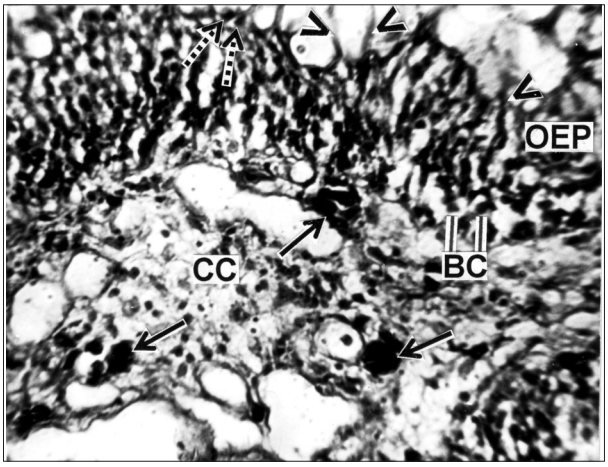

Figure 5. Intense localization of protein in receptor cells (RC) (arrow heads), microvillous cells (MV) (broken arrows), and basal cells (BC). Note strong reaction in BV (solid arrows) of CC (MBPB) × 400.

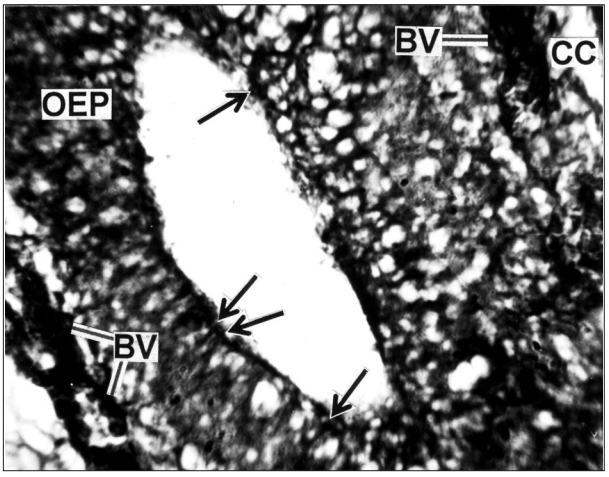

Figure 6. Lipid content in receptor cells (RC) (solid arrows) on free border of olfactory epithelium (OEP) of *E. suratensis*. Note maximum reaction in BV of CC (SB) × 400.

Detection and localization of alkaline phosphatase (ALPase)

Intense ALPase activity was discernible in the extended dendrite processes of the olfactory epithelial surface and in the distal part of receptor axons (Fig. 7). Positive ALPase activity was also found in the basal cells of the olfactory epithelium (Fig. 7). Weak activity was observed in the mucous cells. Moderate localization of this enzyme was also observed in the blood cells of the central core (Fig. 7).

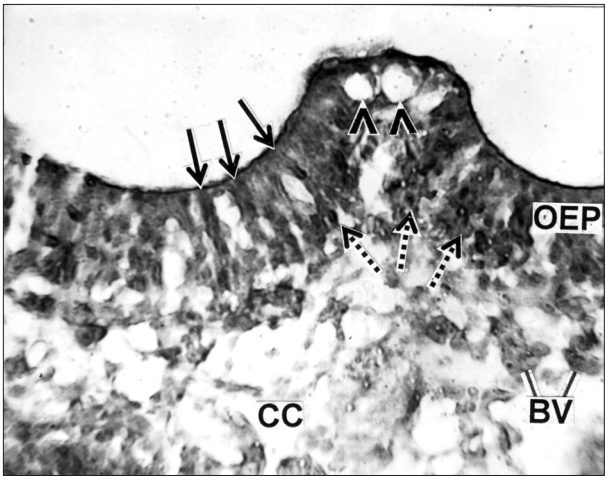

Figure 7. ALPase activity strictly localized to the receptor cells (RC) (solid arrows) and BC (broken arrows) of the olfactory epithelium (OEP) of *E. suratensis*. Note mild to moderate enzyme activity in MC (arrow heads) and BV of CC (ALPase) × 400.

Detection and localization of adenosine triphosphatase (ATPase)

The histochemical location and distribution of ATPase were assigned in the different cells lining the olfactory epithelium. Maximum ATPase activity was found in the extended dendrite processes of the receptor cells and the nuclei of receptor cells (Fig. 8). The intense activity of this enzyme was also evidenced in the microvillous cells. Moderate localization of this enzyme was observed in the basal cells provided with dense granules in the deeper portion of the olfactory epithelium (Fig. 8).

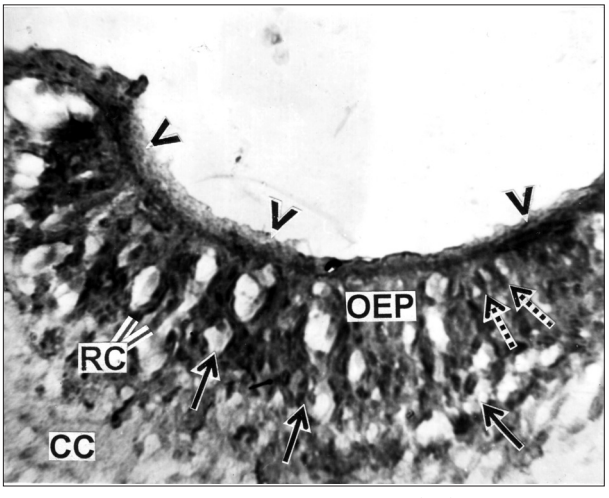

Figure 8. ATPase activity in receptor cells (RC) along with extended dendrite processes (arrow heads), the nuclei of MV (broken arrows) and BC (solid arrows) of OEP of *E. suratensis* (ATPase) × 400.

Discussion

In the present study, the intense localization of silver reaction in the olfactory epithelium of *E. suratensis* provided evidence of the synaptic connection of primary and secondary neurons as well as the orientation of dendrites of the receptor cells along the most superficial layer of the olfactory epithelium. This is consistent with the findings of Ojha and Kapoor (1973) and Ghosh and Chakrabarti (2012) in the olfactory epithelium of *Labeo rohita* (Hamilton) and *Cyprinus carpio* L. Acute localization of silver staining in the knob-like structure of the primary receptor cells in *E. suratensis* was linked to the transmission of various nerve impulses to the dendrites of the secondary receptor cells. In some places of the olfactory epithelium, intense silver deposition in the axons of the primary receptor cells made synaptic contacts with the dendrites of the secondary neurons and the axons of the secondary neurons which enter in the central core and were also intensely stained with the silver reaction. This indicated clearly that the impulses received by the dendrites of primary receptor cells ultimately send impulses to the central core for the final transduction of impulses to the brain. Further study is needed to fully describe the morphology of the neurons and their connection with the central core and olfactory bulb.

The conspicuous mucous cells, which differ in shape and stages of maturation, discharge their secretory product through the opening of the extracellular surface coat. The histochemical nature of mucous cells in the olfactory epithelium of *E. suratensis* was examined by employing the PAS-AB histochemical test to establish its chemical nature and the importance of its secretion with olfaction in fish. Chemically, mucins are hexoseamine-containing polysaccharides which are covalently bonded with varying amounts of proteins. The histochemical nature of mucous secreting cells and their secretory products have been studied by various authors in the olfactory epithelium of different fishes (Datta Munshi and Singh 1975, Chakrabarti 2005). According to them, mucins secreted by the mucous cells of the olfactory epithelium are of mucoprotein or glycoprotein in nature. It was noted during the present study that the content of the mucous cells in the various regions of the olfactory epithelium in *E. suratensis* varies greatly in chemical nature and distribution. The mucous cells situated at the surface of the olfactory epithelium contained a mixture of neutral and acid mucins as was confirmed by the PAS-AB histochemical test. The secretion of a mixture of acid and neutral mucopolysaccharides from the mucous cells probably helps to prevent friction against microscopic debris and also helps the smooth the flow of water in the olfactory chamber. This is consistent with the findings of Rahmani and Khan (1980) regarding the olfactory mechanism of *Anabas testudineus* (Bloch) and Chakrabarti (2005) regarding the olfactory epithelium of *Barbonymus gonionotus* (=*Puntius javanicus*) (Bleeker). Zalewsky and Moody (1979) also reported the heterogenous nature of mucus and its secretion as a mosaic of neutral and acidic mucopolysaccharides in canine gastric mucosa. The intense bluish-purple color of the PAS-AB reaction in the empty mucous cells in *E. suratensis* was because of the presence of a profuse amount of heparin, which is thought to cause fluctuations in the production of mucus in the olfactory mucosa. Moulton and Beidler (1967) reported that the terminal mucus film in the olfactory mucosa is believed to be an important factor in olfactory process that influences variations in olfactory sensitivity.

The receptor, microvillous, and basal cells of the olfactory epithelium of *E. suratensis* probably exhibit intense reactions to protein for various metabolic and physiological activities. The occurrence and localization of sudanophilic lipid material in the dendrite process of receptor cells and nerve fibers in the central core recorded in *E. suratensis* could indicate myalinated sheaths in the axons of receptor cells and probably help in the impulse transduction process. The concentration of lipid material in blood cells of the central core is needed as a source of endogenous energy including its involvement in physiological activities.

Intense ALPase activity in the dendrite process of receptor cells of *E. suratensis* could be associated with the transport of various nerve impulses in the

olfactory transduction mechanism. ALPase activity in the mucous cells in the olfactory epithelium could be related to the synthesis of neutral mucopolysaccharide. Weinreb and Bilstad (1955) reported that ALPase activity and the occurrence of neutral mucopolysaccharides occupy the same sites in the digestive tract of rainbow trout. ALPase activity of mucous cells in *E. suratensis* unequivocally suggests its secretory nature. The dendrite process of the receptor cells displayed strong ALPase reaction was probably because of its positive role in the transport of various chemicals and nerve impulses from the olfactory epithelium to the central core. Further, the ALPase activity in the nuclei of basal cells in the olfactory epithelium could be involved in the degradation of nucleotides and could be coupled with some kind of metabolic processes. Cuschieri (1974) suggested this enzyme might be linked with active transport processes across the base of the epithelium. ALPase could somehow alter the permeability of the olfactory axons and facilitate the conduction of the sense of smell (Shantha and Nakajima 1970). Banerjee and Mittal (1975), from a study on the histochemistry of giant cells located in the skin epidermis of *Clarias batrachus* (L.), reported that the perinuclear areas of the aforementioned cells are metabolically active. The intense ALPase activity in the blood capillaries of the central core of the olfactory epithelium in *E. suratensis* probably helped the transportation of various macromolecules. Moog (1946), however, confirmed the role in transportation of ALPase in the blood vessels of the ovulatory follicles in the domestic duck.

During the course of the present investigation, the deposition of ATPase reaction product in the various cells present in the olfactory epithelium of *E. suratensis* was observed. However, intense ATPase activity in the basal cells could be involved in the process of mitotic activity for the replacement of regenerating receptor and other cells of the olfactory epithelium. Andres (1966, 1969) also suggested that basal cells are the precursors of regenerating receptor cells. Evans et al. (1982) also observed increased mitotic figures in the basal region in a constituting epithelium after degeneration. ATPase activity in the receptor cells and microvillous cells, including their dendrite processes, of the fish in the present study was related to the transmission of various nerve impulses. Morozov and Khramtsov (1979) described the role of ATPase in the secretory activity of the cells of various tissues in different animals. Shantha and Nakajima (1970) reported that the presence of ATPase in the olfactory cell axons of the olfactory mucosa of monkey are possibly involved in the process of olfactory sensations elicited by the contact of odor particles with these receptor cells.

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Author contributions. P.C. and S.K.G. designed and performed the research; P.C. wrote the paper.

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