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A STUDY ON OLFACTORY NEUROEPITHELIUM OF *PSEUDAPOCRYPTES LANCEOLATUS* (BLOCH AND SCHNEIDER): MEDICAL ANALOG X-RAY BASED ANATOMY AND TRANSMISSION ELECTRON MICROSCOPICAL INTERPRETATIONS

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ABSTRACT

The olfactory neuroepithelium of *Pseudapocryptes lanceolatus* (Bloch and Schneider) has been studied under light microscope (LM) and transmission electron microscope (TEM). The olfactory apparatus of *P. lanceolatus* comprises of olfactory lamella on either side of the head along with accessory nasal sacs (*viz.*, ethmoidal sac and lachrymal sac). Medical analog X-ray radiograph reveals that this structure is present at the ethmoid region of head. Microanatomically the olfactory lamella is externally lined by pseudostratified olfactory epithelium which is associated with several types of cell (*viz.*, sensory receptor cells, supporting cells, goblet cells, basal cells, blood cells, *etc.*). Among the epithelial cells, sensory receptor cells are bipolar neuron in nature. Three types of sensory receptor cells has been identified within the olfactory epithelium *i.e.*, crypt cell, microvillous sensory receptor cell and ciliated sensory receptor cell. Under transmission electron microscope (TEM), the apical structures of the dendron *i.e.*, olfactory knob shows clear demarcations among the sensory receptor cells. Possibly these sensory epithelial components are related with the various life functions in this air – breathing (Teleostean: gobiid) coastal fish.

KEYWORDS: Pseudapocryptes lanceolatus, gobiid, olfactory epithelium, microanatomy, crypt cell, etc.

INTRODUCTION

Olfaction is an important type of chemoreception of fish (Hara, 1975). This sense is mediated through olfactory apparatus. Burne (1909) first described the anatomy of the olfactory apparatus in some teleostean fishes. The olfactory system is usually developed from an ectodermal thickening, called olfactory placode (Whitlock and Westerfield, 2000). The olfactory apparatus of fish generally comprises of olfactory chambers, olfactory rosette, accessory nasal sacs, olfactory bulbs, olfactory nerve tracts and brain (Hamdani and Døving, 2007). The major components of the olfactory apparatus show wide ranges of variation among the different teleostean groups (Kleerekoper, 1969). Different research groups have studied the various biological aspects viz., gross anatomy (Kapoor and Ojha, 1972; Datta et al., 1983; De and Sarkar. 2009). physiology (Nevitt, 1991). electrophysiology (Caprio, 1978; Kang and Caprio, 1995), biochemical studies (Cagan and Zeiger, 1978; Rhein and Cagan, 1983), basic ultrastructural work (Bannister, 1965; Bertmer, 1973; Fisher et al., 1984; Hansen and Finger, 2000; Hansen et al., 2003; Sarkar and De, 2011), molecular aspects (Firestein, 2001; Lastein et al., 2006) etc. to understand the mechanism of the reception of chemical cues through olfactory system. The initial events in olfactory processing occur at the apical surfaces of the olfactory receptor neurons (Buck and Axel, 1991). The information regarding specific distribution pattern of these important olfactory sensory receptor cells along with other type of epithelial cells in most of the Indian teleostean fishes is still not properly studied. P. lanceolatus is a common teleostean gobiid fish of Bengal (Fig. 1). It is a mud-skipper and generally found in the intertidal zone of coastal area of the South-East Asia. The present study dealt with the anatomy of the olfactory apparatus along with microscopical *i.e.*, light microscopical (LM) and transmission electron microscopical (TEM) studies of olfactory neuroepithelium of *P. lanceolatus* to unfold the distribution pattern of sensory and non-sensory cellular components and their possible roles in olfaction.

MATERIALS AND METHODS

Adult, live, sex-independent, disease-free P. lanceolatus were collected from the local markets of South 24 Parganas and east Midnapore districts, West Bengal and brought to the laboratory for anatomical and microscopical studies (LM and TEM). Specimens were acclimatized with the laboratory conditions and then anaesthetized with MS-222 (100-200mg./lit.). Anaesthetized specimens having (15 - 20) cm. body length were selected for medical analog X-ray radiograph study. For microanatomical and transmission electron microscopical study, olfactory tissues were procured by dissecting the dorsal side of the head and fixed in 2.5% gluteraldehyde in 0.1 (M) phosphate buffer (pH. 7.2-7.4) for 1hour at 4°C for primary fixation. After completion of the primary fixation, tissues were then further fixed in 1% osmium tetraoxide (OsO₄) in 0.1 (M) phosphate buffer (pH. 7.2-7.4) for 1hour at 4⁰ C for secondary fixation. Dehydrated with graded ethanol and embedded in araldite mixture. For microanatomical study, semithin sections (1µm) were cut by ultramicrotome (Leica ultracut), followed by staining with 0.1% toluidene blue (1% sodium borate) and examined under light microscope. For transmission

electron microscopical study, ultrathin sections (70 – 90nm) were cut by ultramicrotome and stained with uranyl acetate and lead citrate and viewed under transmission

Fig.1. *Pseudapocryptes lanceolatus* (Bloch and Schneider), a teleostean:gobiid fish of South-East Asia.



Fig. 3 – The dissected anterior-lateral part of the head in *P. lanceolatus* shows olfactory apparatus. The photograph marks olfactory lamella (OL), ethmoidal sac (ES), olfactory chamber (OC), olfactory nerve tract (ON), olfactory bulb (OB), brain (B) and eye (E).

electron microscope (TEM: MORGAGNI – 268D), operated at 40kV.

Fig. 2 – The X-ray radiograph shows the ethmoid region of head (dorsal view) in *P. lanceolatus*. This region includes olfactory chambers (oc) and guarded by different type of bones *viz.*, lacrimal (l), maxilla (m), neurocranium (n), premaxilla (pm), parietal (p), post parietal (pp), *etc.*



Fig. 4 – The photomicrograph indicates pseudostratified olfactory epithelium (oe) resting on basal lamina and enclose the entire nasal cavity (nc). Beneath the basal lamina fila olfactoria (fo) is also marked.





RESULTS

The olfactory apparatus of P. lanceolatus is present at the ethmoid region of the head (Fig. 2). Anatomically the olfactory apparatus is comprises of single olfactory lamella on either side of the head and associated with accessory nasal sacs (viz., lachrymal and ethmoidal sac) (Fig. 3). The olfactory lamella is a tube like structure. The ethmoidal sac is connected with the olfactory lamella at the ventro-caudal region where as the lachrymal sac is well marked at the dorso-caudal region of the lamella. The olfactory nerves are originated from the base of the olfactory lamella and passes through the olfactory chambers. This paired nerve is connected with olfactory bulbs of the brain (Fig. 3). The X-ray radiograph shows that olfactory apparatus is guarded by different types of bones viz., maxilla, lachrymal, ethmoidal, etc. (Fig. 2). Each olfactory lamella is externally covered by olfactory epithelium. The olfactory epithelium of P. lanceolatus is a pseudostratified structure (Fig. 4) and it is composed of the following cell types - sensory receptor cells, supporting cells, goblet cells and basal cells (Fig. 4).



Sensory receptor cells are bipolar neuron and may conveniently divide into three regions: dendron, perikaryon and axon (Fig. 4). Dendron of the sensory receptor cells runs towards the nasal cavity (Fig. 4). Three distinct types of sensory receptor cell have been identified histologically under light microscope viz., crypt cells, microvillous sensory receptor cells and ciliated sensory receptor cells (Fig. 5). The tip of the dendron bulges to form olfactory knob. The olfactory knob of crypt cell shows microvilli and sunken cilia (Fig. 6) but the olfactory knob of microvillous sensory receptor cells possesses several microvilli (Fig. 7). In ciliated sensory receptor cells, the olfactory knob bears 3-6 numbers of cilia. The transverse section of cilia shows (9+2) arrangements of microtubules. The cilia possess well developed basal body associated with centrioles and neurofilaments (Fig. 8). The length of the dendron differs among the sensory receptor cells *i.e.*, crypt cells with very short dendron, microvillous sensory receptor cells with moderate length of dendron and ciliated sensory receptor cells with very long dendron (Fig. 5). Thus the perikaryons of the different type of

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sensory receptor cells *i.e.*, crypt cell, microvillous sensory receptor cells and ciliated sensory receptor cells are present at the different layer (*viz.*, apical part, middle part and lower part) of the olfactory neuroepithelium respectively (Fig. 5). Apparently crypt cells are very rare within olfactory epithelium of *P. lanceolatus*. The sensory receptor cells possess deeply stained oval shaped nucleus at the lower extremity of the perikaryon. Granulated cytoplasm is present in the perikaryon of sensory receptor cells (Fig. 5). Supporting cells are columnar in nature and serially distributed within the olfactory neuroepithelium of *P. lanceolatus*. Two types of supporting cells are present within the olfactory epithelium *i.e.*, ciliated and microvillous supporting cells (Fig. 5). The apical surfaces

Fig. 5 – The distribution of the olfactory epithelial cellular components *viz.*, basal cell (bc), basal lamina (bl), blood vessel (bv), crypt cell (cc), ciliated supporting cell (csc), goblet cell (gc), microvillous supporting cell (msc), perikaryon of microvillous sensory receptor cell (P₁), perikaryon of ciliated sensory receptor cell (P₂), *etc.* within the different depth of olfactory neuroepithelium of *P. lanceolatus*.



of the supporting cells are flat. The nucleus which is round in shape, present at the central portion of the supporting cells and the cytoplasm is moderately granulated (Fig. 5). Goblet cells are ovoid in shape. Nucleus is round in shape and placed at the distal part of the cell (Fig. 5). Basal cells are loosely arranged in the deeper part (*viz.*, basal part) of the olfactory epithelium just above the basal lamina. Beneath the basal lamina, fila olfactoria region is present (Fig. 5). A thin layer of connective tissue and blood capillaries with different type of blood cells *viz.*, erythrocytes, leukocytes, macrophages, *etc.* are also marked. Aggregations of axons of different type of sensory receptor cells are also evident in the fila olfactoria region (Figs. 4 and 5).

Fig. 6 – The transmission electron micrograph shows crypt cell (cc) with several microvilli (mv) and sunken cilia (c) in the olfactory epithelium of *P. lanceolatus*. Mag. X 3500]



Fig. 7 – The apical tip *i.e.*, olfactory knob of the microvillous sensory receptor cell possess several microvilli (arrowheads) and neurofilaments (nf).

[Mag. X 5500]



Fig. 8 – The apical zone of the olfactory neuroepithelium shows olfactory knobs of the ciliated sensory receptor cells. Prominent cilia (c) protrude from the olfactory knob of ciliated sensory receptor cells. Cilia are associated with basal body (b) centrioles (ce) and neurofilaments (nf). The transverse section of cilia indicates (9+2) arrangement of microtubules (arrowhead). [Mag. X 3500]



DISCUSSION

The olfactory apparatus is one of the important chemosensory organs of fish (Hara, 1992). This organ analyzes the chemical nature of the surroundings environment by reception of different chemical cues. The olfactory epithelium is generally raised from the floor of the olfactory chamber and often folded to form lamellae (Hara, 1971). In P. lanceolatus, single olfactory lamella is present instead of olfactory rosette. The nasal cavity is an integral part of the olfactory lamella. The nasal cavity is a tube-like structure. The nasal cavity is extended ventrally from the anterior tip of the lamella to the posterior part and may acts as an avenue for water ventilation. Water circulation is created by pumping mechanism in which volume changes occur synchronously with respiratory movements, provide a passive, rhythmic irrigation of the olfactory system (Burne, 1909, Kleerekoper and Van Erkel, 1960; Yamamoto, 1982). A mechanism by which jaw protrusion is linked to water suction into the olfactory chamber is proposed by Nevitt (1991). In P. lanceolatus, several bony structures *i.e.*, premaxilla, maxilla, mandible etc. are well marked and probably helps in jaw protrusion during water ventilation. The nasal cavity is completely lined by olfactory epithelium and does not show any epithelial folding. Epithelial folding is evident in multilamellar olfactory apparatus such as in Channa punctatus (Mandal et al., 2005), Cyprinus carpio (Chakrabarti and Hazra Choudhury, 2007), etc. The olfactory epithelium including P. lanceolatus consists of sensory receptor cells, supporting cells and basal cells. Apart from these epithelial cells, some species such as Heteropneustes fossilis possesses goblet cells (Datta and Bandopadhyay, 1997), Mugil parsia possesses mast cells and labyrinth cell (Chakrabarti, 2005) in their olfactory epithelium. Labyrinth cells and mast cells are not properly explored in other teleostean species including P. lanceolatus. The distinct types of olfactory epithelial cells are present in the olfactory epithelium of P. lanceolatus. The functional difference of ciliated and microvillous receptor cells indicate a relation with the length of dendron (Hamdani et al., 2001). According to Hamdani et al. (2001)microvillous sensory receptor cells with comparative short dendrite, mediates the feeding behaviour in Crucian carp (Carassius carassius) where as ciliated sensory receptor cells having long dendrites, participates in alarm reaction elicited by pheromone (Hamdani and Døving, 2002). Another type of sensory receptor cell *i.e.*, crypt cell has described by Hansen and Finger (2000). In the olfactory epithelium of P. lanceolatus, crypt cells are also marked. Ciliated sensory receptor cells and microvillous sensory receptor cells are dominant over crypt cells in the olfactory epithelium of *P*. lanceolatus. Crypt cells may responsible for the reproductive behaviour of fish (Hansen et al., 2003; Hamdani and Døving, 2006). The presence of these different olfactory receptor cells with different morphoanatomical structures in the olfactory epithelium of P. lanceolatus are indicative of the involvement of olfactory apparatus in different behavioural functions such as feeding, alarming, reproduction, etc.. Supporting cells are present within the olfactory neuroepithelium of P. lanceolatus, to provide the mechanical support to the

sensory receptor cells. The ciliary movement of the ciliated supporting cell may help in the water ventilation process over the neuroepithelium of P. lanceolatus. Goblet cells secrete mucus to produce a thin mucus coating over the olfactory epithelium. This mucus coating protects the olfactory epithelium from various mechanical injuries and deleterious effect of pathogens. Mucus also may play an important role in the initiation of olfaction process. Odorants stimulate olfactory sensory receptor cells by first absorbed into the mucous layer and bound to specific protein carrier (Bigneiti et al., 1988). The protein is known as G- protein (Reed, 1990). Probably this step may initiate a multiple reaction cascade in olfaction process (Getchell, 1986). Another interesting cell is basal cells found in the basal region of the olfactory epithelium of P. lanceolatus. The basal cells are supposed to be the progenitors of the sensory receptor cells and supporting cells (Yamamoto, 1982; Zeiske et al., 1992). Perhaps the cellular integration of olfactory epithelium in *P. lanceolatus* is well organized to perceive the chemical cues from the external environment during water ventilation over the olfactory neuroepithelium.

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