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ACETYLCHOLINE ESTERASE (AChE) ACTIVITY IN CILIATED OLFACTORY NEURON OF A TELEOSTEAN: GOBIID [*PSEUDAPOCRYPTES LANCEOLATUS* (BLOCH AND SCHNEIDER, 1801)]

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ABSTRACT
The acetylcholine esterase (AChE) activity in ciliated olfactory sensory receptor neurons (cOSRN) of *Pseudapocryptes lanceolatus* (Teleostean: Gobiid) has been studied under light and electron microscope respectively (using acetylthiocholinne iodide as substrate). Positive reaction of acetylcholine esterase (AChE) activity at perikaryon of ciliated olfactory sensory receptor neurons (cOSRN) is clearly identified under light microscope. No dendritic reactions were marked. Electron microscopical study revealed intense reactions of actylcholine esterase (AChE) in dense core vesicles (diameter: 30nm. to 40nm.) that are distributed within the perinuclear cytoplasm and accumulated in axoplasm of ciliated olfactory sensory receptor neurons (cOSRN). Therefore the perikaryon of ciliated olfactory sensory receptor neurons (cOSRN) within the olfactory neuroepithelium of *P. lanceolatus* may be a primary site of acetylcholine esterase (AChE) synthesis that plays a pivotal role in termination of olfactory signal transduction.

KEYWORDS: *Pseudapocryptes lanceolatus***,** olfactory, vesicles, acetylcholine.

INTRODUCTION

Olfaction or sense of smell mediates several behavioural responses through perception of variety of chemical odorants from the external environment (Firestein, 2001). In fish, this sense is mediated through a distinct anatomical structure *viz.*, olfactory apparatus. Despite the anatomical variation, this structure is specialized for recognition of water soluble chemical odorants during water ventilation through the nostrils (Hansen *et al.*, 2003; Sarkar *et al.*, 2014). Three different morpho-types of sensory receptor cells (*i.e.*, ciliated sensory receptor cell, microvillous sensory receptor cell and crypt cell) have been identified within the olfactory neuroepithelium that are responsible for perception of different chemical cues during olfaction (Hansen and Zielinski, 2005). The cytology based neural event of olfactory signal transduction is still less studied in fish chemosensory system. Recently, De and Sarkar (2014) has reported cytosketal structure mediated vesicular transport in ciliated olfactory sensory receptor neuron (cOSRN) to explain olfactory signal transduction in *Pseudapocryptes lanceolatus* (Bloch and Schneider, 1801). The present study is emphasized on histochemical analysis of acetylcholine esterase (AChE) activity within ciliated olfactory sensory receptor neuron (cOSRN) in olfactory neuroepithelium of *P. lanceolatus* under light microscope (LM) and transmission electron microscope (TEM) to signify its role in olfactory signal transduction.

MATERIALS & METHODS

Live, healthy specimens of *P. lanceolatus* (total length: 15cm. - 20cm.) were collected from the local markets of South 24 Parganas, India and brought to the laboratory for acclimatization in laboratory conditions for 72 hours [temperature: 20˚C to 25˚C, humidity: >40%, *etc.*]. The living specimens were anaesthetized by MS-222 (dose: 100 mg /L – 200 mg /L). The olfactory apparatus of *P. lanceolatus* were dissected out and fixed in 2.5% glutaraldehyde and 4% paraformaldehyde (1:1) in 0.1 M phosphate buffer (pH. 7.2-7.4) at 4°C for 2 hours. The tissue were washed in same buffer and incubated in the medium described by Karnovsky and Roots (1964). The incubated tissue were then rinsed in DAB solution and processed for LM and TEM study. For LM study, the tissues were transferred into gradient of sucrose solution (15% and 30%) in 0.1 M phosphate buffer (pH 7.2-7.4) for cryoprotection. The frozen sections (thickness: 20µm-30µm) were cut; rinsed in the same buffer; counter stained by haematoxylin and observed under trinocular light microscope (Primo Star; Carl Zeiss Microscpy, GmbH, Germany). For electron microscopical study, the processed tissues were re-fixed in 1% osmium tetroxide in 0.1 M phosphate buffer (pH 7.2-7.4) for 1 hour at 27°C. The tissue was then rinsed in the same buffer; dehydrated in graded chilled acetone and embedded in Araldite mixture for 48 hours at 60°C. The sections (thickness: 70nm – 80nm) were cut by using ultramicrotome (Leica Ultracut – UCT), collected on copper grids and viewed under transmission electron microscope (Morgagni 268D) operated at 80kV.

RESULTS

The acetylcholine esterase (AChE) positive ciliated olfactory sensory receptor neurons (cOSRN) are distinctly identified under light microscope (LM) and transmission electron microscope (TEM) (Figs. 1, 2 and 3). The egg-

shaped perikaryon of this type of cell shows positive reaction against acetylthiocholine iodide as substrate. The dendroplasm does not show any positive reaction in ciliated olfactory sensory receptor neurons (cOSRN) of neuroepithelium (Fig. 1). The electron micrographs are also showing intense reaction within the perinuclear cytoplasm of ciliated olfactory sensory receptor neuron. The vesicles having diameter (30nm. to 40nm.) are acetylcholine esterase (AChE) positive (Fig. 2). These vesicles are observed within perinuclear cytoplasm, close to Golgi apparatus (Fig. 2). The aggregation of these vesicles is largely noted at the terminal part of axon in ciliated olfactory sensory receptor neuron (Figs. 3).

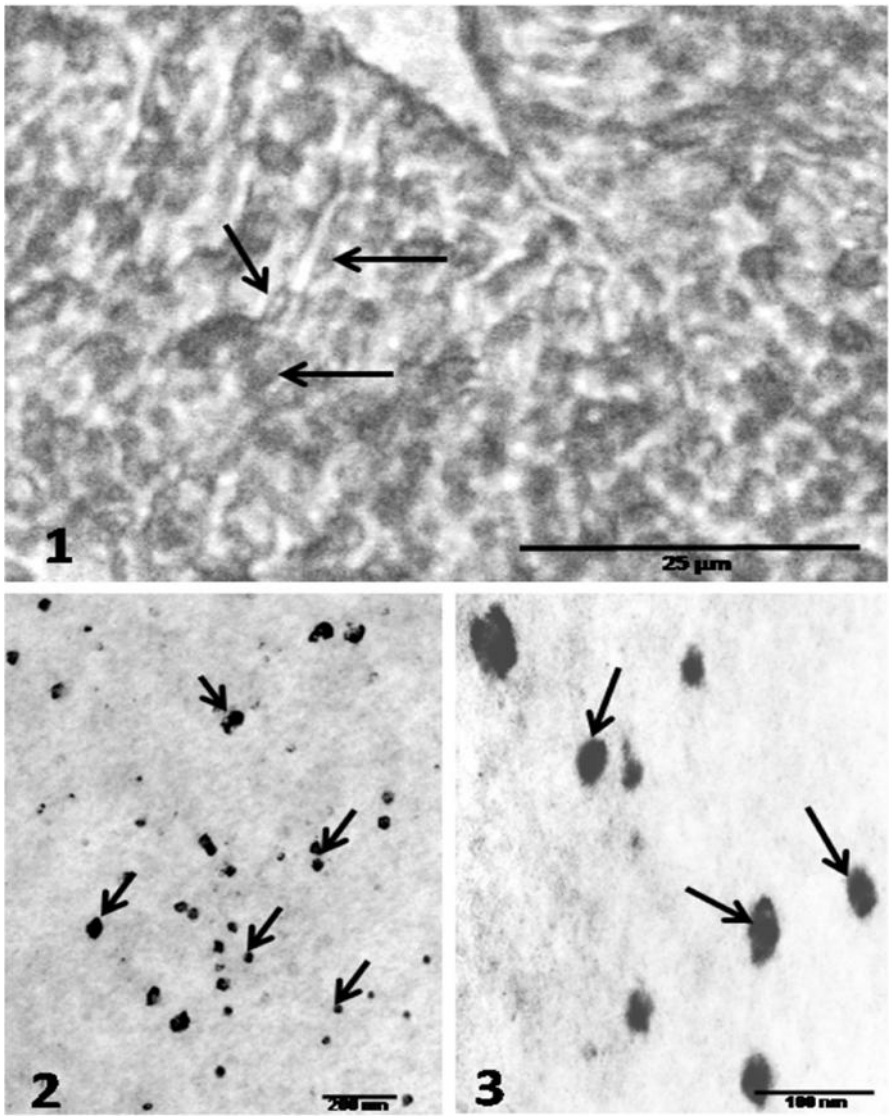

FIGURE1: The acetylcholine esterase (AChE) activity within the ciliated olfactory sensory receptor neuron (arrows) is prominently marked under light microscope (LM).
FIGURE 2: The electron micrograph shows a part of perinuclear cytoplasm. Dense core vesicles (diameter 30nm. – 40nm.) are showing positive reaction (arrows) using acetylthiocholine esterase as substrate.
FIGURE3: Accumulation of dense core vesicles (diameter 30nm. – 40nm.) is marked under TEM at the terminal axoplasm of ciliated olfactory sensory receptor neuron in *P. lanceolatus*.

DISCUSSION

Acetylcholine (ACh) serves an important role in olfactory perceptions (Barrett *et al.*, 2010; Devore and Linster, 2012). It is also involved in specific neural functions like modulation of learning and memory (Brennan and Keverne 1997; Cools *et al.*, 2008). The processing of olfactory signals is begins at the neuroepithelium that consist of olfactory sensory receptor neuron (Firestein, 2001). Odorants binds with the olfactory receptors (OR) those are present in the kinocilia of ciliated olfactory sensory receptor neuron and subsequently release acetylcholine as neurotransmitter towards synaptic cleft. The neural information then transmitted to the second order neuron in olfactory neural circuit (Gilbert *et al.*, 2001). The perceived neural signal is conveyed to the olfactory bulb of brain through olfactory nerves (Murphy

et al., 2004). Acetylcholine esterase (AChE) is an enzyme that generally hydrolyzes Acetylcholine (ACh) into choline and acetate; terminates the synaptic transmission (Ćolović *et al.*, 2013). In *P. lanceolatus*, the olfactory sensory receptor neuron shows numerous vesicles at different subcellular compartments (De and Sarkar, 2014). The vesicle having diameter (30nm. to 40nm.) is only shows positive reaction for acetylcholine esterase (AChE). The aggregation of these vesicles at perinuclear cytoplasm and axoplasm of olfactory sensory receptor neuron in *P. lanceolatus* is significant. These vesicles are transported towards the terminal part of axons by neurofilaments and microtubules (De and Sarkar, 2014). Therefore this study denotes that the perikaryon of the olfactory sensory receptor neuron is a primary site of acetylcholine esterase (AChE) synthesis. Current experimental studies indicate that the use of acetylcholine esterase (AChE) inhibitors is helpful for treatment of neurodegenerative disease (Rees and Brimijoin, 2003). Thus, the detail knowledge on acetylcholine esterase (AChE) activity may be convenient for diagnosis as well as drug design for neurodegenerative disorders like Alzheimer's diseases, Parkinson disease, *etc*. (Ćolović *et al.*, 2013).

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