Original Article

Histophysiological and surface ultrastructural studies of the saccus vasculosus of *Notopterus chitala* (Hamilton)

Padmanabha Chakrabarti*, Rubina Khatun

Fisheries Laboratory, Department of Zoology, The University of Burdwan, Burdwan 713104, West Bengal, India

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A B S T R A C T

We investigated the cytoarchitecture and surface ultrastructural characteristics of different cells lining the epithelium of the saccus vasculosus of *Notopterus chitala* (Hamilton, 1822). The saccus vasculosus, the richly vascularized reddish, sac-like organ is situated on the ventral aspect of the diencephalon just behind the pituitary gland. Histologically, the saccus vasculosus consisted of several loculi lined with a heterogeneous population of a large number of specialized coronet cells and a smaller number of alternately arranged supporting cells. The loculi were densely surrounded by blood sinusoids. The loculi opened into several collecting channels that united and ultimately drained into the third ventricle of the brain. The coronet cells possessed central or basal nuclei and an apical globular protrusion. Under scanning electron microscopy, the coronet cells were variable in shape with prominent nuclei. The supporting cells were triangular in shape and placed in between the coronet cells. The apical parts of the coronet cells had different shapes of globular protrusions with distinct stalks. Intense silver stain was noted in the terminal end of the coronet cells and nerves were attached with blood vessels. Under scanning electron microscopy observation, nerve fibers of different caliber were attached to the coronet cells and blood vessels. The various cells of the saccus vasculosus in *N. chitala* were correlated with their functional significance.

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1. Introduction

The saccus vasculosus is a specialized vascular and sac-like diverticule, and occupies a centrocaudal portion of the diencephalic infundibular just behind the hypophyseal complex. The cavity of this organ is continuous with the third ventricle of the brain [1]. The vascularized neuroepithelium of the saccus vasculosus consists of characteristic coronet cells and supporting glial cells with interspersed liquor-containing neurons [2,3]. This specialized ependymal organ is mainly concerned with a secretory function [4] and works for an umbilical conjoin between cerebrospinal fluid and the blood vascular system [5]. The structure and function of the saccus vasculosus has been studied by various workers. According to them, the shape, structural characterization, distribution, and organization of various cells lining the saccus epithelium of teleosts vary among different fishes. Comprehensive explorations and knowledge on the microstructure and function of the saccus vasculosus of different fishes using light and electron microscopy are also documented [7–10].

The aim of the present work was to perform histological and scanning electron microscopic studies of various cells lining the epithelium of saccus vasculosus of *Notopterus chitala*.

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* Corresponding author. Fisheries Laboratory, Department of Zoology, The University of Burdwan, Burdwan 713104, West Bengal, India.

E-mail address: dr.pchakrabarti@yahoo.in (P. Chakrabarti).

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2. Materials and methods

Live mature fishes of *N. chitala* 48–50 cm in length were procured from the River Ganga near Katwa, Burdwan District, West Bengal, India. Fishes were killed following the guidelines given by the Institutional Ethical Committee, The University of Burdwan. The brain including the saccus vasculosus was exposed from the ventral side of the head and was initially fixed in situ with 10% neutral formalin. After 30–45 minutes, the saccus vasculosus along with the rest of the brain were carefully removed from the cranium and fixed in aqueous Bouin’s fluid for 18 hours for routine histology and 10% neutral formalin for silver impregnation studies. The tissues were subsequently dehydrated through an ascending series of ethanol, cleared in benzene, embedded in paraffin wax (56–58°C), and cut serially at 4 μm thickness. The dewaxed sections were stained with Delafield’s hematoxylin and eosin, Mallory’s triple and chrome alum hematoxylin phloxin stains. Some sections were cut at 10 μm thickness and stained with silver impregnation [11] for detection of axons.

For the purpose of scanning electron microscopy (SEM), the brain mass including the saccus vasculosus was immediately exposed and removed. The tissues were fixed in 2.5% glutaraldehyde, buffered with cacodylate (pH 7.4) for 6 hours, and post-fixed in 1% osmium tetroxide for 2 hours. Then, the tissues were dehydrated through ascending concentrations of acetone followed by amyl acetate and subjected to critical point drying with liquid carbon dioxide. The dried tissues were mounted on metal stubs, coated with gold palladium with a thickness of ~20 nm and scanned in a Hitachi, S-530 scanning electron microscope (HITACHI, Tokyo, Japan).

3. Results

The saccus vasculosus of *N. chitala* was notably capacious and situated on the ventral aspect of the diencephalon just behind the pituitary complex. The saccus was detached from the pituitary by an empty interspace (Figures 1 and 2). In histological sections, the saccus vasculosus was separated from the pituitary by a narrow space. The saccus vasculosus comprised several loculi draining into channels (Figure 2). These loculi were surrounded by blood sinusoids/vessels. The lumen of the saccus loculi through intercommunicating system of tubes and channel and all the terminal or distal branches united to form a few large collecting channels, which ultimately fused and ended in a large duct that led into the diacoel (Figure 3). In *N. chitala*, the loculi were lined by a heterogeneous population of characteristic coronet and supporting cells. These cells were distributed along the basal membrane, beneath which were the vascular elements (Figure 4). Each coronet cell possessed an apical globular protrusion projecting into the saccus lumen. The size of these protrusions varied from cell to cell (Figure 4). The predominant coronet cells were round or oval with centrally placed conspicuous nuclei. In some areas, the elongated coronet cells with cytoplasmic projections at the apex had large ovoid nuclei in the basal

![Fig. 1. Scanning electron micrograph (SEM) of the whole saccus vasculosus (SV) of Notopterus chitala adjacent to the pituitary (PT) on the ventral side of the brain. (SEM, × 25.)](image-url)
region (Figure 5). The supporting cells were numerous and scattered among the coronet cells (Figure 5).

According to SEM, the round or oval loculi of the saccus were composed of dense populations of coronet cells with prominent nuclei interspersed with supporting cells that were almost triangular in outline (Figure 6). These cells were distributed along the basal membrane beneath which the blood vessels were present (Figure 6). The luminal surface of the saccus vasculosus was composed of closely packed coronet cells leaving deep furrows of blood vessels and basal supporting cells (Figure 7). SEM revealed that the apical part of the coronet cells had different shapes of globular protrusions, including differences in physiological activity of the cells (Figure 8). In certain areas, the basal part of the coronet cells probably shows the nerve fiber indicated by arrowhead (Figure 8).

Some of the elongated coronet cells had basal nuclei with prominent luminar secretion and these cells were attached to blood vessels (Figure 11). The saccus epithelium in N. chitala was connected to nerve terminals, as demonstrated by the intense reaction of Marsland, Glees, and Erikson [11] technique. Some of the nerve terminals had a clear synaptic structure (Figure 9). Under SEM observation, the saccus vasculosus of N. chitala represented the most efficient elaborate system of blood vessels containing...
numerous blood cells (Figure 11). This system increased the surface area of the saccus epithelium many fold. The nerve fibers with different calibers in the basal part of the coronet cells were also connected to blood vessels (Figure 10).

4. Discussion

The saccus vasculosus is generally situated posterior to the pituitary gland, although in some teleosts, it is opposed to the hypophysis [12]. In the present study, the saccus vasculosus in N. chitala was a sac-like protuberance on the ventral side of the brain and detached from the pituitary by an interspace, although it seemed to have no functional connection with the gland. In the present study, the terminals of the simple and branched loculi of the saccus vasculosus united to form a large collecting channel that ended in a larger duct with an open connection with the third ventricle of the brain. This may be because some secretory product was conveyed to the brain from the saccus vasculosus. Kamer [13] noted that the saccus vasculosus was a gland of the brain and assigned it a secretory role of unknown function. In N. chitala, the saccus vasculosus consists of several loculi composed of a heterogeneous population of coronet cells and supporting cells lining the saccus epithelium. The coronet cells are pear-shaped or tall columnar cells with central or basal conspicuous nuclei. The apical portion of the coronet cells has globular protrusions projecting into the saccus lumen, which varies from cell to cell. The apical globules may have a role in the production of some

Fig. 5. The arrangement of coronet cells (CC) of Notopterus chitala within the saccus loculi connected with blood vessels (BV). Note apical secretion of CC (arrow heads). Broken arrows indicate supporting cells. (Photomicrograph, × 600; stain: chrome alum haematoxylin phloxin.) SV = succus vasculosus.

Fig. 6. Cut surface of locula in higher magnification showing coronet cells (CC) of Notopterus chitala with prominent nucleus (arrows). Note triangular shape supporting cells in between CC (arrow head). (Scanning electron micrograph, × 20,000.) BV = blood vessels.

Fig. 7. Luminal surface of succus vasculosus of Notopterus chitala showing closely packed coronet cells (CC) leaving deep furrows of blood vessels. (Scanning electron micrograph, × 5000.)

Fig. 8. Different shapes of apical globular protusions (broken arrows) of coronet cells (CC) of Notopterus chitala. Arrowhead points the secretion. (Scanning electron micrograph, × 5000.)
secretory products from coronet cells that pass into the saccus lumen and ultimately through locular ducts to the third ventricle. The granular content of the globules may be released into the lumen of the saccus vasculosus, while some coronet cells form new globules to compensate for the loss of existing globules. Different phases of coronet cells have also been found in rainbow trout [9]. According to Shimada [6] in the cytoplasm of coronet cells, the smooth endoplasmic reticulum is composed of reticular connecting tubules in the zone near the basal process. These tubules in the coronet cells must open into the saccus lumen to discharge their contents. Lanzing and van Lennep [14] have demonstrated acid mucopolysaccharide in the apical protrusions of the coronet cells in teleosts, which was first stored in the globules and ultimately secreted into the ventricle of the brain. Saksena [15] opined that the saccus vasculosus acts as a storage site of carbohydrates in the brain. By converting glycogen to acid mucopolysaccharides, the coronet cells are involved in glycogen metabolism. In the present investigation, the supporting cells were almost triangular in outline, less numerous, and scattered among the coronet cells. However, their structural organization did not indicate the morphological signs of intense activity that may be correlated with secretory function. Singh and Sathyanesan [16] noticed coronet and supporting cells alternating with each other in the saccus epithelium of Mystus vittatus, Callichthys pabda, and Bogarius bagarius.

In present observation, the saccus vasculosus of *N. chitala* represented an elaborate system of capillaries and sinusoids. This system probably increased the surface area of the saccus to facilitate the secretion process. The coronet cells in the experimental teleosts are in contact with nerve terminals. Some of the terminals show a clear synaptic structure, suggesting cholinergic nerves. We suggest that the coronet cells probably function as chemoreceptors and maintain the composition of the cerebrospinal fluid (CSF). Coruo et al [9] emphasized that CSF-containing neurons are observed in the saccus epithelium. Ryohi and Keiji [17] during their electron microscopic study in *Cyprinus carpio* noted that the terminals on the coronet cells contained only clear synaptic vesicles, suggesting cholinergic nerves, and emphasized that metabolism of the CSF by the coronet cells is controlled by the cholinergic nerves. The present study indicates that the coronet cells of the saccus vasculosus of *N. chitala* are metabolically active and probably involved in both secretory and sensory functions.

In conclusion, the saccus vasculosus of *N. chitala* comprises an elaborate system of capillaries and sinusoids. The apical globular protrusions loaded with secretory materials emptied their product into the saccus lumen. The large collecting channels ultimately lead into the third ventricle of the brain. The coronet cells are also in contact with nerve terminals and probably function as chemoreceptors and monitor the composition and secretion of CSF.
Conflicts of interest

All authors declare no conflicts of interest.

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