Zoologischer Anzeiger 262 (2016) 1–9

Contents lists available at ScienceDirect

Zoologischer Anzeiger

journal homepage: www.elsevier.com/locate/jcz

Zoologischer Anzeiger

Research paper

Structure of the main heart of the articulate brachiopod *Hemithiris psittacea*: Morphological evidence of dual function

Tatyana V. Kuzmina[a,*], Elena N. Temereva[a], Vladimir V. Malakhov[a,b]

[a] *MV Lomonosov Moscow State University, Vorobjevi Gory, Moscow 119991, Russia*
[b] *Far Eastern Federal University, ul. Sukhanova 8, Vladivostok 690600, Russia*

ARTICLE INFO

Article history:
Received 17 May 2015
Received in revised form 20 February 2016
Accepted 23 March 2016
Available online 25 March 2016

Keywords:
Brachiopod anatomy
Blood system
Heart
Ultrastructure
Podocyte-like cells
Excretion
Hemithiris psittacea

ABSTRACT

Brachiopods have a complex blood system that includes a main central heart and several accessory hearts. Recent ultrastructural studies suggested that the accessory hearts have dual functions: blood propulsion and ultrafiltration. In the current study, the main heart of the brachiopod *Hemithiris psittacea* was studied by electron microscopy and confocal laser scanning microscopy. In addition, the structure and function of the main heart were compared with those of the accessory hearts. The main heart is located on the dorsal side of the stomach posterior of the gastroparietal mesentery. It is a diverticulum of the dorsal vessel that passes along the stomach surface. The main heart consists of a short stalk that attaches to the dorsal vessel and a wide sac-like part that projects into the perivisceral coelom. The heart wall is composed of an outer coelomic epithelium, an extracellular matrix (ECM), and amoebocytes that adhere to the inner surface of the ECM. The coelomic epithelium consists of two types of cells: myoepithelial and peritoneal. The myoepithelial cells resemble podocytes, strongly stain with phalloidin, but show a weak immunoreactivity reaction against α-tubulin. These cells form long basal muscle processes, which run in different directions, branch, interweave, and form a strong muscle coating of the heart. The muscle processes give rise to the secondary thin pedicels interdigitating with each other. The peritoneal cells exhibit a strong α-tubulin-like immunoreactivity, do not stain for phalloidin, and form basal protrusions that contain abundant microtubules and that extend between the muscle processes of the myoepithelial cells. The peritoneal cells contain well-developed rough endoplasmic reticulum and numerous inclusions, and apparently have storage and secretory functions. Amoebocytes form the inner layer of the main heart but do not form a true endothelium because they lack desmosomes and a basal lamina. In summary, the main and the accessory hearts are sites in the blood system with dual functions: propulsion of blood in the ramified vessels and sinuses, and ultrafiltration of liquid that moves from the blood system to the perivisceral coelom.

1. Introduction

Brachiopods are marine invertebrates that have been intensively studied by paleontologists but much less intensively studied by zoologists. The microscopic anatomy and ultrastructure of extant brachiopods are still poorly known. Brachiopods have a complex blood system, the general anatomy of which was studied in the late nineteenth and early twentieth centuries (Hancock, 1859; Blochmann, 1892, 1900; Schaeffer, 1926). The brachiopod blood system contains a main heart and, in some taxa, also accessory hearts (Hancock, 1859; Foster, 1974; Williams et al., 1997). Detailed study of the ultrastructure of the accessory hearts (Kuzmina and Malakhov, 2015) revealed that they may perform dual functions, i.e., they may function in blood propulsion and in ultrafiltration. That they might function in ultrafiltration is indicated by the accessory heart wall, which consists of myoepithelial podocyte-like cells. However, there has been only one investigation of the fine structure of the brachiopod main heart of *Hemithiris psittacea* (Martynova and Chaga, 1997). According to the cited study, the main heart is unlike the accessory hearts in that it contributes only to propulsion and not to ultrafiltration.

A first objective of this work was to use electron microscopy and confocal laser scanning microscopy to describe the microscopic anatomy, ultrastructure and cytochemistry of the main heart of the articulate brachiopod *H. psittacea*. A second objective was to compare the structure and possible function of the main heart with those of the accessory hearts.

* Corresponding author.
E-mail address: kuzmina-t@yandex.ru (T.V. Kuzmina).

http://dx.doi.org/10.1016/j.jcz.2016.03.012
0044-5231/

2. Materials and methods

H. psittacea was collected in August 2011 at the White Sea Biological Station of Moscow State University (Kandalakshskii Bay of the White Sea). The samples were collected at a depth of 9 m. The blood system anatomy was studied in detail by manual dissection of collected specimens. The dissected tissues were contrasted by methylene blue staining and photographed in the laboratory with Panasonic DMC-TZ10 digital camera mounted on a binocular light microscope.

For transmission electron microscopy (TEM), the main hearts of two specimens (volume per heart = 1 mm^3) were fixed in 2% cacodylate-buffered glutaraldehyde (pH 7.2) with saccharose (100 mM) for 2 h at 4 °C. After fixation, the hearts were rinsed in cacodylate buffer. After postfixation in 1% cacodylate-buffered osmium tetroxide for 30 min at 20 °C, the hearts were rinsed in distilled water. The tissue was then dehydrated through an ascending ethanol series and embedded in resin (Epoxy Embedding Medium Kit, Fluka, Switzerland). Semithin sections (2 μm in thickness) were cut with a diamond knife on a Reichert Ultracut E ultramicrotome and stained with methylene blue, and were then viewed and photographed with a Zeiss Axioplan 2 imaging photomicroscope. Ultrastructure was studied on short series consisting of 10 ultrathin silver sections. Ultrathin sections were cut with a diamond knife on a Reichert Ultracut E ultramicrotome, mounted on copper grids, stained with uranyl acetate and lead citrate, and examined with Jeol Jem 100 V and Jeol-1011 80 kV transmission electron microscopes (TEMs).

For confocal laser scanning microscopy (cLSM), the main hearts were dissected, fixed overnight in a 4% paraformaldehyde solution in filtered sea water, and washed two times in phosphate buffer (pH 7.4) (Fisher Scientific, Pittsburgh, PA, USA) with Triton X-100 (0.3%) (Fisher Scientific) for a total of 12 h. Non-specific binding sites were blocked with 10% normal donkey serum (Jackson ImmunoResearch, Newmarket, Suffolk, UK) in phosphate buffer (PBS) with Triton X-100 (PBT) overnight at 4 °C. The hearts were then placed in PBT containing primary antibody: anti acetylated α-tubulin-mouse (1:700) in PBT for 24 h at 4 °C. Specimens were washed for 24 h at 4 °C in PBT and then exposed to the secondary antibody, which was 635-Alexa-Mouse (1:2000) (Invitrogen, Grand Island, NY, USA) in PBT for 24 h at 4 °C. Then, the specimens were washed in PBT and incubated in rhodamine-conjugated phalloidin (1:50) (Fisher Scientific, Pittsburgh, PA, USA) for 2 h at room temperature in the dark. They were subsequently washed in PBS (three times x 60 min), mounted on a cover glass covered with poly-L-lysine (Sigma-Aldrich, St. Louis, MO, USA), and embedded in Murray Clear. Specimens were examined with a Nikon Eclipse Ti confocal microscope (Moscow State University, Moscow, Russia). Z-projections were generated using the program Image J version 1.43.

3. Results

The main heart is located on the dorsal side of the stomach posterior of the gastroparietal mesentery (Fig. 1A–B). It is a diverticulum of the dorsal vessel that passes along the stomach surface. The main heart attaches to the dorsal vessel via a short stalk (Figs. 1 A, 6 A). The remaining part of the heart, which is wide and sac-like, projects into perivisceral coelom and bends backwards (Figs. 1 A, 6 A). During diastole, the heart enlarges greatly and becomes 1.2 mm long with a diameter of 0.5 mm in its sac-like part. During systole, the heart shrinks and becomes 0.6 mm long with a diameter of 0.2 mm in its sac-like part.

We had to study the ultrastructure of the main heart wall during systole because fixation resulted in contraction (Figs. 1 C–D, 2 A). The main heart wall is about 40 μm thick and consists of an outer coelomic epithelium, an extracellular matrix (ECM), and amoebocytes that adhere to inner surface of the ECM (Fig. 1D).

The outer coelomic epithelium facing the perivisceral coelom consists of two types of cells: myoepithelial cells and peritoneal cells (Figs. 2 A, 3 A). The myoepithelial cells have apical cytoplasmic and basal contractile parts (Fig. 3A, C). The apical cytoplasmic part contains the nucleus and most of the cell organelles. The nucleus, which has a clear nucleolus, usually has an irregular shape (Fig. 3E). There are numerous mitochondria proximal of the nucleus. Large phagosomes with heterogeneous contents, electron-dense granules, and lipid droplets occur in the cytoplasmic parts of the myoepithelial cells (Fig. 3F). The cytoplasm includes apical electron-lucent areas without obvious presence of cytoplasmic organelles (Fig. 3F). Microtubules occur in the cytoplasm around the nucleus (Fig. 3C). A single cilium arises from a deep pouch in the middle of the cell (Fig. 3F). A cilium originates from one of the two centrioles. The two centrioles are always underlain by a Golgi complex and are connected by short rootlets. A striated rootlet is present deep in the cell (Fig. 4F).

The myoepithelial cells form a complex system of basal muscle processes that pass along the heart wall (Figs. 2 and 4). The muscle processes (about 2 μm in diameter) contain myofilaments, which run parallel to the process axis and form smooth muscles. The myofilaments may run in different directions in the same process (Fig. 4B). The cytoplasm of this process displays a high electron-density. In those parts lacking myofilaments several microtubules are evident, and these usually run parallel to the process' axis (Fig. 4B). Groups of mitochondria occur peripherally (Fig. 4B). The processes are attached to the basal lamina by serial hemidesmosomes (Fig. 4B, E). The basolateral aspects of these processes are joined by 1–5 belt desmosomes, which may be arranged in a row (Fig. 4D). The contractile processes extend in different directions, branch, and interweave, thus forming a strong muscle cover of the heart. The basal muscle processes give rise to thin branches that resemble podocyte pedicels of other invertebrates (Fig. 4C, E–F). These pedicels (about 0.12 μm in diameter) interdigitate with each other (Fig. 4E–F). In addition to the contractile processes, the myoepithelial cells form cytoplasmic non-muscular processes (from 0.4 to 2.5 μm in diameter) (Figs. 3 A, 4 A). They occur in the cytoplasmic part of the cell above the muscle processes and usually contain microtubules and sometimes groups of electron-lucent vesicles (about 0.03 μm in diameter). The cytoplasm of these processes is usually electron-lucent, but regions with a matrix of moderate electron density may also occur (Fig. 4A). These cytoplasmic processes penetrate into the bases of the neighbored myoepithelial as well as peritoneal cells. In cross section these processes appear to be enclosed by two membranes: an inner membrane is a plasmalemma of the process, while an outer membrane is a plasmalemma of the penetrated cell (Fig. 4A).

The second cell type of the outer coelomic epithelium is the peritoneal cells. The cytoplasm of these cells contains well-developed rough endoplasmic reticulum and numerous inclusions including large phagosomes, electron-dense granules, and lipid droplets (Figs. 2 A, 3 A–D). A single cilium originates in a shallow pouch in the median part of each cell (Fig. 3D). Microtubules run through the cells in the cytoplasm near the nucleus (Fig. 3C). The cells form basal, thin, digitiform processes (Fig. 3B–C). These processes run in different directions: some of them run perpendicular to the heart surface extending into the ECM (Fig. 3B–C) while the others are parallel to heart surface (Fig. 3C). These processes sometimes enclose the muscle processes of the myoepithelial cells and interdigitate with each other and with the pedicels of the myoepithelial cells.

Myoepithelial and peritoneal cells are usually interspersed among each other (Fig. 3A). Firm adhesion is maintained by desmosomes between basolateral interspaces of these cells (Fig. 3B–C). In both types of cells, some areas of cytoplasm are filled with rosette-