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ULTRASTRUCTURAL LOCALIZATION OF ACID AND ALKALINE PHOSPHATASES, GLUCOSE-6-PHOSPHATASE AND SODIUM-POTASSIUM ATPase ENZYMES IN THE NEUROSECRETORY AND OESOPHAGEAL CELLS OF EARTHWORM

By

SHOAA AL YOUSUF and ZEINAB EL-GOHARY

Department of Zoology, Faculty of Science, University of Qatar, Doha, Qatar

التركيب الدقيق لمواقع إنزيمات مختلفة في الخلايا العصبية الإفرازية وخلايا المريء المحيطة بالعقدة المخية لدودة الأرض

شعاع اليوسف و زينب الجوهري

إشتمل البحث الحالي على دراسة التركيب الدقيق لمواقع إنزيمات الفوسفاتيز الحمضي والفوسفاتيز القاعدي وجلوكوز سداسي الفوسفاتيز وكذا إنزيم صوديوم بوتاسيوم ثلاثي فوسفات الادينوزين في قطاعات رقيقة من العقدة المخية للخلايا العصبية الافرازية في دودة الأرض أبريكتودا كاليجينوسا .

وقد أوضحت الدراسة أن تفاعل معظم هذه الانزيمات يظهر في صهاريج الشبكة الاندوبلازمية الخشنة والأغشية النووية وكذلك في أجهزة جولجي .

Key Words: Earthworm, Enzymes, Neurosecretory cells, Oesophageal cells.

ABSTRACT

In the present study we have localized acid and alkaline phosphatases, glucose-6-phosphatase and sodium-potassium ATPase (Na-K-ATPase) in ultra-thin sections of cerebral neurosecretory cells and oesophageal cells of the earthworm, Aporrectodea caliginosa. The majority of the enzymes studied were located in the rough endoplasmic reticulum (RER), nuclear envelope and Golgi apparatus.

INTRODUCTION

Acid phosphatase is known to be associated with lysosomal activity [1, 2] and protein reabsorption [3]. Glucose-6-phosphatase is associated with oxidative metabolism. The functional significance of alkaline phosphatase is more questionable; however, it is generally accepted that this enzyme takes part in reabsorption processes [4]. Active transport of sodium and potassium (e.g. in renal tubules) is generally driven by Na-K-ATPase [5].

The intracellular localization of acid phosphatase in Guinea pig testicular interstitial cells was investigated by incubating nonfrozen thick sections. The reaction product was seen in the inner cisternae of Golgi elements [6]. In rat aortic smooth muscle cells, acid phosphatase activity was found in tubular structures which may have been lysosomes [7]. Other structures with acid phosphatase activity included mitochondria and, some membrane profiles were regarded as residual bodies [2, 8].

Alkaline phosphatase reactivity has been noted in the cytoplasm and nucleus of some types of neurons of the earthworm at the level of light microscopy [9]. Alkaline phosphatase activity in rat hepatocytes was localized in rough endoplasmic reticulum (RER), nuclear envelope, Golgi apparatus and tubulo-vesicular organelles, and other the entire plasma membrane [10].

Glucose 6-phosphatase (G6Pase) is a multifunctional enzyme [11]. Therefore, it is possible that G6pase acts as the phosphohydrolytic enzyme in the phosphorylative pathway in various tissues. However, the exact role of this enzyme in a variety of cell types in other organs is not quite clear.

The presence of Na-K-ATPase activity localized in the plasma membrane is well documented in a variety of animal cells. Data on sites of Na-transport have been obtained form stop-flow, micropuncture and microperfusion studies, whereas information on Na-K-ATPase distribution is derived from microassay of renal tubule fragments of different mammalian species and from histochemical identification of basolateral membranes of the epithelial cells of the proximal and distal convoluted tubules [5].

The aim of the present study was to show the intracellular localization of these enzymes in neurosecretory cells of the earthworm cerebral ganglion as well as in the oesophageal cells around the ganglion.

MATERIALS AND METHODS

The animal used in the present study is the earthworm, *Aporrectodea caliginsa.* Fifty mature earthworms (about 8-10 cm in length) were collected from Roudat Al-Faras, State of Qatar. Localization of acid phosphatase, alkaline phosphatase, glucose-6-phosphatase and Na-K-ATPase was histochemically investigated in the cerebral neurosecretory cells and in the surrounding oesophageal cells of earthworms.

Control: Tissue sections from ten specimens were incubated in the media described below without appropriate substrates.

Cytochemical localization of acid phosphatase

Ten specimens were fixed in the following medium: 0.8% glutraldahyde in 0.1 M cacodylate buffer for 45 min. Sections 20 μm thick were incubated for 60 min. in B-glycerophosphate/ serum media according to Robinson and Karnovsky [7].

Cytochemical localization of alkaline phosphatase and glucose-6-phosphatase and Na-K-ATPase Na-K-ATPase

Ten specimens were used for the demonstration of alkaline phosphatase by the lead precipitation method described by Mayahara *et al.* [12]. Another ten specimens were used for localization of glucose-6-phosphatase which was achieved by using the method described by Kanai *et al.* [13]. Ten additional specimens were used for demonstration of Na-K-ATPase, using the method of Milhorat *et al.* [14].

Following each incubation, all specimens were washed in buffer, postfixed in 1% osmium tetroxide, dehydrated and embedded in epon-araldite. Ultrathin sections were cut with Nova ultramicrotome, doubly stained with uranyl acetate and lead citrate and examined by electron microscopy, (100 KV Hitachi: H-300), Department of anatomy -Kyote University, Japan.

RESULTS

Figure 1 demonstrates the distribution of neurosecretory cells within the control cerebral ganglion in 1 μm section by light microscope. These cells are characterized by their position, shape and size.

The cytochemical localization of acid and alkaline phosphatase, glucose- 6-phosphatase and Na-K-ATPase in the cerebral-neurosecretory and oesophageal cells of the earthworm were investigated by light and electron microscopy. The localization of these several enzymes are illustrated in Figures 2-12.

In the cerebral neurosecretory cells, positive reactions of acid phosphatase activity was found in lysosomes which measure about 0.2-0.4 μm in diameter (Fig. 2). The reaction was also found in autophagic vacuoles (0.5-0.8 μm in diameter) which contain several inclusions in various stages of degeneration (Fig. 2). Acid phosphatase activity was also found in some elongated narrow cisternae which are regarded as parts of RER. Moreover, weak reaction was observed within the outer membrane of the nucleus, which is connected with RER cisternae (Figs. 3, 4). Some reaction is also present in the oesophageal cells. In addition, a strong reaction appeared within granules around Golgi bodies (Fig. 5).

Alkaline phosphatase reaction was positive in the RER associated with the nuclear envelope (Fig. 6) and also within granules membranes of neurosecretory cells. Some reactions were found in cytoplasmic areas around free ribosomes (Fig. 7). In the oesophageal cells, the reaction was localized within the ribosomes and RER. The reaction appears also within the plasma membranes of goblet cells (Figs. 8, 9).

The reaction product for the activity of glucose-6-phosphatase was localized in RER cisternae and the nuclear envelope of neurosecretory cells (Fig. 10). In the oesophageal cells the reaction was positive within the outer membranes of RER and ribosomes (Fig. 11).

Na-K-ATPase was demonstrated in the apical cell surface of the neurosecretory cells. Within the oesophageal cells the reaction was localized in the basolateral plasma membrane and in the brush border (Fig. 12).

DISCUSSION

Accurate preservation of cells structure and chemical identification of defined constituents are major goals of the ultrastructural-cytochemical studies. The preservation of cell structure required for examination by electron microscopy is traditionally achieved by means of aldehyde fixatives. This fixation can be gentle enough to permit certain enzymes to retain a portion of their activity yet strong enough to stabilize cell structure to a large degree. Thus, cytochemical localization of these enzymes is possible.

Our results are consistent with the findings of Frank and Christenses [6] and Parenti *et al.* [15] who demonstrated localization of acid phosphatase in the inner cisternae of Golgi elements and in the plasma membrane. The reaction product of acid phosphatase was earlier seen in the lysosomes and the autophagic vacuoles of neurosecretory cells [1, 2]; the present results are in agreement. Furthermore, acid phosphatase reaction products are associated with the ascending microvilli, which traverse the cuticle and the septate zone of the intercellular junctions [16]. Since a correlation between phosphatase distribution and absorptive function occurs in many endoparasites, it is postulated that molecules could diffuse through the earthworm cuticle matrix and enter the epidermal cytoplasm along the microvilli and also by a paracellular route at the level of the septate zone [16].

Waheed *et al.* [17] have demonstrated more recently that lysosomal acid phosphatase is synthesized as a transmembrane protein which is transported to the cell surface and subsequently internalized. It is estimated that each acid phosphatase molecule cycles more than 15 times between the endosomal compartment and the plasma membrane before it enters the lysosomes [18].

Alkaline phosphatase is a membrane-associated glycoprotein that enhances the hydrolysis of monophosphate esters at an alkaline pH [19]. In the present study the cytochemical localization of alkaline phosphatase in the plasma membrane of neurosecretory and oesophageal cells of the earthworm is consistent with the findings of Groothuis *et al.* [20] and Watanabe *et al.* [21, 22] who demonstrated

Figure 1: Semithin section (1 μm) of epoxy-resin showing part of the cerebral ganglion (CG) which contain neurosecretory cells. Note also cells of the oesophagus (arrows) around the ganglion. X 4000

Figure 2: Acid phosphatase localization within a neurosecretory cell. The reaction product is localized in lysosomes (L) and in possible autophagic vacuoles. Note also the negative reaction of mitochondria (M) X 33,000

Figure 3: Acid phosphatase localization within a neurosecretory cell. The reaction product is localized within the rough endoplasmic reticulum (RER) adjacent to the nucleus (N). X 22,000

Figure 4: High magnification of figure 3 demonstrating acid phosphatase reaction in ribosomes attached to the rough endoplasmic reticulum (RER) adjacent to the nucleus (N). X 55,000

Figure 5: Acid phosphatase localization within an oesophageal cell. The reaction product is localized within the nuclear membrane which represents part of the rough endoplasmic reticulum (RER). Note reaction of granules around Golgi body (G). X 22,000.

Figure 6: Alkaline phosphatase localization within a neurosecretory cell. The reaction product is localized in nuclear envelope and ribosomes (arrows). X 22,000.

Figure 7: Alkaline phosphatase localization within granules membranes of the neurosecretory cell. The reaction product is also localized within cytoplasmic areas mainly around ribosomes (arrows). X 55,000

Figure 8: Alkaline phosphatase localization within an oesophageal cell. The reaction product is localized in the apical goblet cell. (arrows). X 22,000.

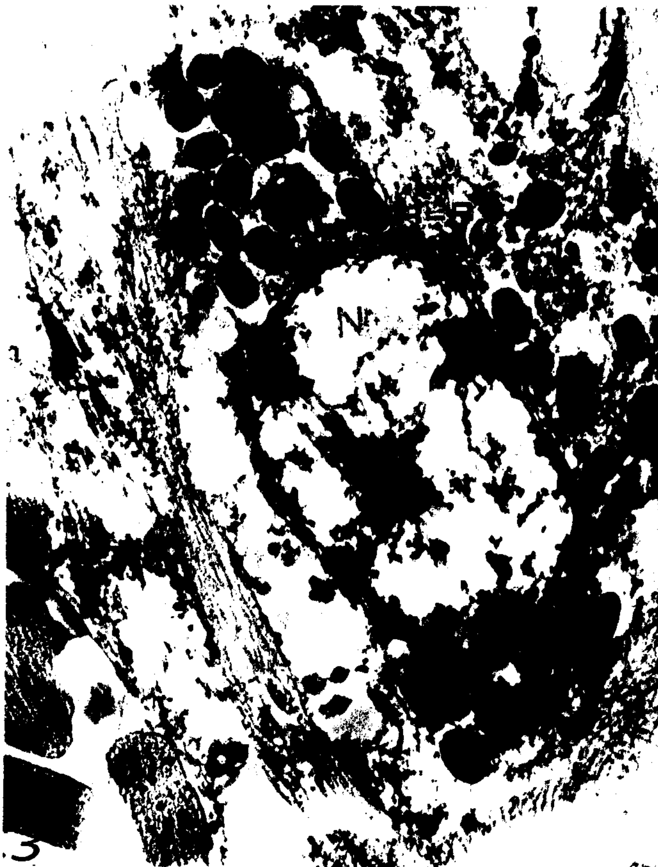
Figure 9: Alkaline phosphatase localization within an oesophageal cell. The reaction product is mainly localized in the apical plasma membrane of the goblet cell. X 55,000

Figure 10: Glucose-6-phosphatase localization within a neurosecretory cell. The reaction product is localized in the nuclear envelope (N) and all parts of RER cisternae (arrows). X 22,000.

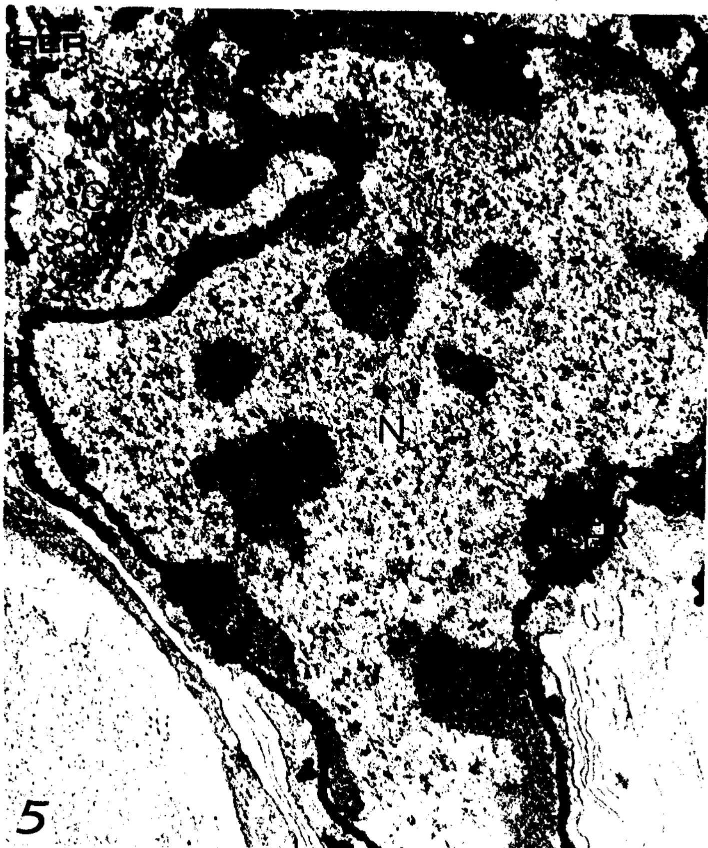
Figure 11: Glucose-6-phosphatase localization within an oesophageal cell. The reaction product is found only on the outer membrane of RER and ribosomes. X 33,000.

Figure 12: Na-K-ATPase localization within an oesophageal cell. The reaction product is found at the brush border of the apical cell surface (arrows). X 55,000

L
L
M
M
L
L
L
L
2


N
3

RER
N
4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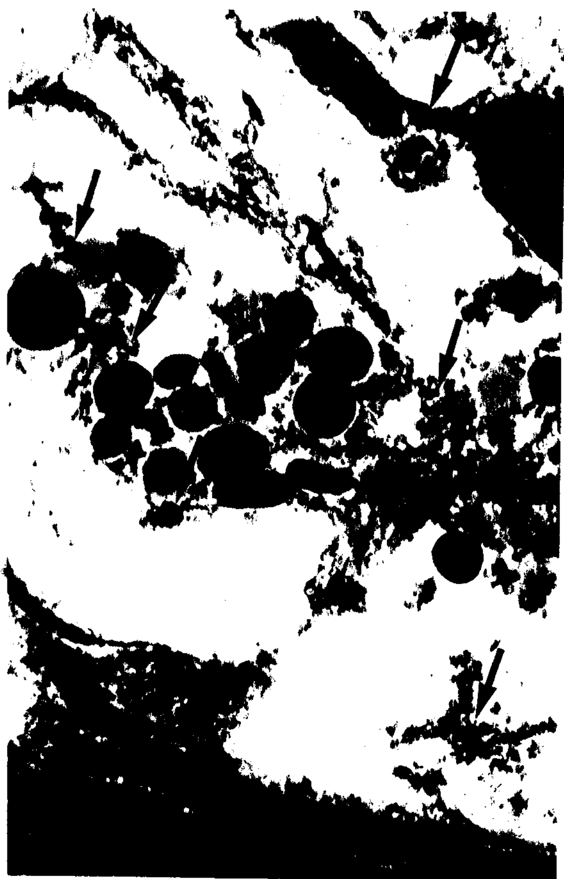

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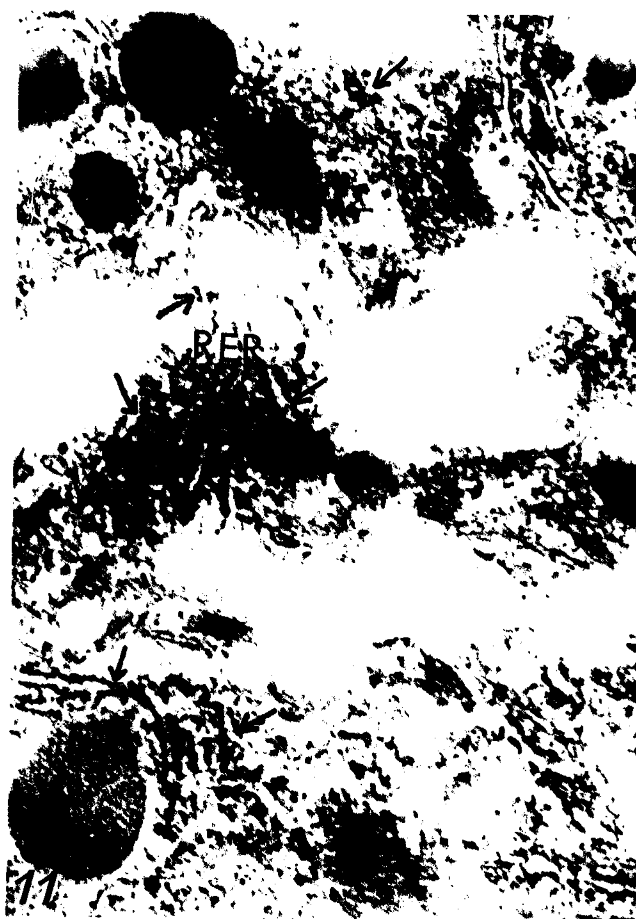
RER
11

12

high alkaline phosphatase activity in all regions of the plasma membrane of the adult hepatocyte in rat and mouse. Also, the present results are consistent with the finding of Kwan and Ito [23] who reported that alkaline phosphatase activity was associated mainly with the membrane component of smooth muscle of the main blood vessels. Furthermore, our results well with those of Safadi *et al.* [24] who detected the enzyme bound to the membrane of sacrolemma of striated muscle and to the membranes of endothelial cells in adjacent capillaries.

Glucose-6-phosphatase (G6Pase) is a soluble cytoplasmic enzyme found in all cells that employ the pentose phosphate pathway. A high level of G6Pase is related to the presence of glucose or fructose in various organs or cells [13, 22]. The physiological role of G6Pase in the liver and kidney is to release glucose into the blood by hydrolyzing glucose-6-phosphate produced via glycogenolysis and gluconeogenesis. G6pase also hydrolyses glucose-6-phosphate [11]. The cytochemical reaction product of G6Pase activity has been observed in the terminal cisternae of sarcoplasmic reticulum and in the nuclear envelope of the skeletal muscle cells of the mouse [25]. Also, the reaction product has been demonstrated in RER and nuclear envelope in endothelial cells of capillaries in smooth muscle of the mouse.

G6Pase was found in the skeletal muscle cells of the mouse [25], in the ciliary body of the rabbit [26], and in the neural tissue of the rat [27]. In the present study, the staining reaction of G6Pase was weak and localized mainly in the nuclear membranes and RER. The present results are consistent with the findings of Asaka *et al.* [26] who reported that the staining reaction of glucose-6-phosphatase was localized mainly in the RER and nuclear envelope of the ciliary body of rabbit. Because the neurosecretory cells of the earthworm are not among the cells that release either glucose or fructose into the blood or into other fluids [2] therefore, a weak reaction is expected.

According to the basis of the reaction, *in situ* localization of ATPase activity was performed by direct observation of lead deposits in areas where optimal concentration of phosphate appeared. These might correspond to sites where ATPase activity was localized.

Na-K-ATPase was demonstrated on the plasma membrane especially on basolateral membrane of different animal cells of both vertebrates [28 & 29] and invertebrates [30 & 31]. These results also parallel those of Chandra *et al.* [32] who found that intracellular potassium and sodium were homogeneously distributed throughout the animal cell and the fact that plasma membranes of all animal cells show Na-K-ATPase activity.

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