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MYOARCHITECTURE AND ANGIOARCHITECTURE OF THE ATRIO-AURICULAR COMPLEX RELATED TO SECRETION AND DELIVERY OF THE ATRIAL NATRIURETIC PEPTIDE (ANP) IN GUINEA PIG

[1]Eliane Florencio Gama, [2]Edson A. Liberti, [2]Claudio A Ferraz de Carvalho, [3]Natalie Souza de Andrade, [1]Romeu Rodrigues de Souza

[1]Department of Anatomy, São Judas Tadeu University, SP, Brazil,
[2]Department of Anatomy, São Paulo University, SP, Brazil,
[3]Santa Mônica Hospital, SP, Brazil

ABSTRACT

Atrial natriuretic peptide (ANP) is a polypeptide hormone that has diuretic, natriuretic and vasorelaxant effects. It is secreted primarily by atrial and auricular myoendocrine cells, where it is stored within secretory granules. ANP has been characterized by morphological methods in a number of vertebrate species, but little is known of the secretion, delivery and activation mechanisms of ANP. It is known that they are related to the atrial myoarchitecture and angioarchitecture. The objective of the present study was to analyze the myoarchitecture and the angioarchitecture of the guinea pig atria and auricles, focusing at their role in the ANP secretion, storage and delivery. For the study of the muscle fiber bundles arrangement of the atrial-auricle wall their cavities were widely opened and observed with a Zeiss stereoscopic microscope (X4, X40). For the study of the angioarchitecture of the atrial-auricle walls, a methacrylate resin (*Mercox*) was injected through the aorta and after the tissue corrosion; the resulting molds were metalized and examined with a scanning electron microscope Jeol JSM-6100. ANP granules were present in the cardiocytes from the four regions studied. ANP-granules were localized principally in the perinuclear region of the cells. The atrial myoarchitecture is formed by small muscle bundles, uniformly organized in defined directions, generally parallel to the transverse axis. The muscle bundles in the auricles are concentrated as multidirectional bundles. Smaller perpendicular oriented bundles come from these larger bundles and coming out from the small ones there are other bundles even smaller, resulting in a bundle arrangement ordered in a three main magnitude levels. The arterial arrangement in the atria, showed a vascular net very elongated and relatively uniform and dense, with the presence of more or less wide elongated spaces between the vessels. In the auricle wall, the arterial net is denser, more irregular, and tortuous. Therefore, the architecture of the auricles in the guinea pigs would represent besides a mechanical role an important morphological basis for the ANP release.

Key words: Atrio-auricular complex, myoarchitecture, angioarchitecture, atrial natriuretic peptide, guinea pig

INTRODUCTION

Previous studies from the fifties [17] in guinea pig hearts have shown the presence of specific atrial granules which has been functionally considered as an activator of sodium and water excretion and, consequently, blood pressure reduction [9,11,16,25,30]. Those granules, which are present in the atria and auricles, secrete a peptide hormone called atrial natriuretic peptide (ANP). The secretion, delivery and activation mechanisms of ANP are related to the atrial myoarchitecture and angioarchitecture, as the atrial and auricle walls distension under conditions of hypervolemia [29] or blood pressure increase would promote an increase in the circulating ANP.

Several publications have been performed in order to clarify the cellular kinetics related to the ANP. However, up to the moment, some aspects of the structure of the atria and auricle that could be related to the secretion of ANP have not yet been completely studied

[21]. The present study has been proposed to analyze the myoarchitecture and the angioarchitecture of the guinea pig atria and auricles, focusing at their role in the ANP secretion, storage and delivery.

MATERIAL AND METHODS

Guinea pigs (C*avia porcellus)*, of both sex, weighing 500-700 grams were used.

Ultrastructure

Three animals received anesthesia with sulfuric ether and their ventricles were perfused with heparinized saline solution (1mg/300ml of solution). The atria and the auricles were reduced to fragments of about 1 mm^3. Some fragments were placed in glutaraldehyde fixative solution at 5% in cacodylate buffered solution (0.2M, pH 7.3) and the others in Karnovsky's solution (paraformaldehyde 2%, glutaraldehyde 2.5%, in cacodylate buffer, 0.2M, pH 7.2 plus $CaCl_2$ 25mg) during 3 hours. The fragments were washed out 3 times with cacodylate buffer (0.1M, pH 7.3) for 5 minutes each time, and then placed in a fixative solution of osmium tetroxide at 1% and at 2% in cacodylate buffered solution 0.1M, pH 7.3 during 2 hours. The fragments were left overnight in uranyl acetate at 0.5% with sucrose (540mg/100ml) and after being washed out with cacodylate buffer they were also dehydrated in increasing series of alcohols (alcohol 70º, alcohol 70º + uranyl at 1%, alcohol 95º, alcohol 100º and propylene oxide) and embedded in resin *Epon*[1], with previous embedment in an 1:1 resin plus propylene oxide solution during 8 hours under rotation. Following that, they were embedded in resin alone during 5 hours and finally left in the same resin at 60 ºC during 5 days. Ultra thin sections were obtained with a diamond knife, on an ultramicrotome[2], and after contrasting with uranyl acetate and lead citrate they were analyzed on a Philips EM 400 transmission electron microscope at the Clinical Investigation Laboratory at the Medical School at the University of São Paulo.

Atrial myocardium architecture

Five (5) animals have been anesthetized with sulfuric ether. After opening the thorax, exposing the heart and sectioning the caudal vena cava, the atria and ventricles were washed out with saline solution (NaCl 0.9%) through the ventricles. One animal was injected with *Jeltrate* dental impression material in the atria, with the purpose of maintaining the atrial cavities form. The hearts were then placed in a solution of formaldehyde at 10% in sodium phosphate buffered (0.1M, pH 7.3) during 5 days. After removal of the ventricles through a section right below the coronary sulcus, *Jeltrate* was removed from the atrial cavity; the atria were stained as a whole mount preparation with a solution of Azocarmine B (1g:200ml of water) in order to evidence the muscle fibers, followed by the dehydration in alcohols 95 to 100º and posterior clarification with benzol. For the study of the muscle fiber bundles arrangement of the atrial-auricle wall their cavities were widely opened and observed with a Zeiss stereoscopic microscope (X4, X40) and photographed with a digital system for image analysis (Kontron - 300, Zeiss, Germany).

Atrial angioarchitecture

For the study of the angioarchitecture of the atrial-auricle walls, 5 hearts of anesthetized animals were perfused with heparinized phosphate buffered solution (1mg: 300ml). A methacrylate resin (*Mercox*) was injected through the aorta. The block containing the heart and the lungs was removed and placed in water at 40-50º C during 2 hours for resin polymerization. The pieces were then immersed in KOH solution at 20% until complete tissue corrosion, and the resulting mold was washed out with water. After drying at room temperature, the molds were placed on metal supports and metalized with gold using the Balzers-CSD-240 equipment. The examination was performed with a scanning electron microscope Jeol JSM-6100.

RESULTS

Ultrastructure

The electron micrographs of the atrial myocytes showed the presence of very electron dense granules, with sparsely granular and homogeneous content, coated with a double membrane. Among those there are others less dense, but in a smaller quantity. Most of their granules are located near the nuclear poles, among mitochondria, Golgi complex cisterns and rough endoplasmic reticulum, microtubules and myofilaments. In addition to the great number of ANP granules near

[1] DMP-30 2, 4, 6 (Tridimethylaminomethyl/phenol), Nadic Methyl Anhydride (Methyl Nadic Anydride), Eppoxy hardener – Polisciences, Inc.
[2] Sorvall MT-2 Ultramicrotome

the perinuclear region there is a small number near the plasma membrane (figs. 1a, b). Granule content released into the extra-cellular space (extrusion), as it can be observed in fig. 1c, is rarely seen. The plasma membranes of the granules are fused and the amorphous material, apparently derived from the granule, is observed in the intercellular space.

Myoarchitecture

The atrial myoarchitecture considerably differs from the auricular myoarchitecture, with the first one formed by small muscle bundles uniformly organized in defined directions, generally parallel to the transverse axis (Fig. 2a). In some regions they are sparse, making apparently possible a contact between the sub-epicardial and the sub-endocardial tissue.

A more complex arrangement of the muscle bundles can be observed in the auricles which are concentrated as multidirectional bundles. Smaller perpendicular or bent over oriented bundles come from these larger bundles and coming out from the small ones there are other bundles even smaller, resulting in a bundle arrangement ordered in a three main magnitude level (Fig. 2b). The disposition of bundles in the third level is similar to paintbrushes, with the most distal portion, at some sites, crossing over other bundles from the same level originating from close bundles. From this type of construction result ordered spaces in two or three orders of sizes, progressively smaller and frequently intercommunicated.

Angioarchitecture

The arterial arrangement shows an individual disposition, showing differences between atria and auricles walls. For the atria, the vascular net is very elongated and relatively uniform and dense, with the presence of more or less wide elongated spaces between the vessels (Fig. 3a). In the auricle wall, the arterial net is denser, more irregular, and tortuous (Fig. 3b). In the atrial auricle transition, the vessels are also tortuous, however, with a more or less uniform direction, thus constituting a transition between the two arrangements (Fig. 3c). Arterio-luminal communications have been observed mainly in the auricles (Fig. 3d).

DISCUSSION

The presence of mitochondrias associated with Golgi complex lamellae, numerous granules and translucent vesicles, in the atrial myocytes confirmed the data described in other species [4].

The distribution of specific atrial granules in the cardiac chambers is an issue which raises controversies. Thus, in the present study, a greater amount of marked granules were observed in the auricles than in the atria, a finding which is in accordance with others [6,22]. Those authors admit that the amount of marked granules decrease from the right to the left auricle and from the right to the left atrium. On the other hand, it was observed [3], a greater amount of marked cells in the left atrial-auricular complex in relation to the right one in guinea pig hearts. The granules distribution pattern in normal animals was similar in both right and left atrial-auricular complex [1,26] but the right auricle is functionally more active than the left one [26,29].

There is greater ANP secretion during the walls dilatation of the atrial-auricular complex [23]. Studies on the effects of pressure increase in the atrial-auricular complex in rats verified a marked release of such

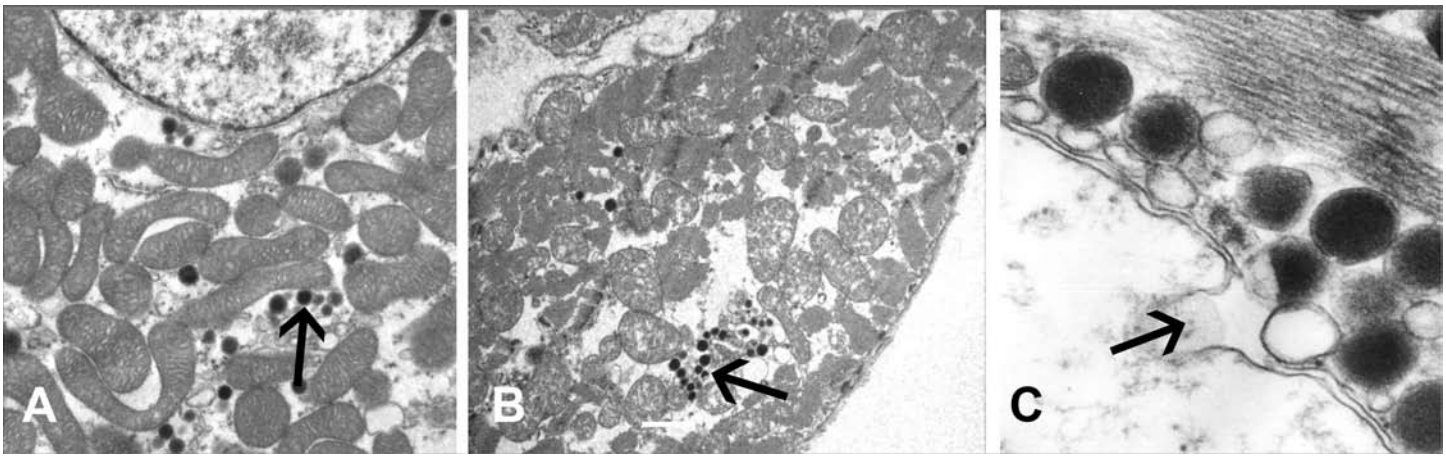

Figure 1- Electron micrographs of atrial and auricular cardiocytes in the guinea pig. A - A great number of ANP granules (arrows) can be seen near the perinuclear region and there are a small number of granules near the plasma membrane (B). Granule content (arrow) released into the extra-cellular space (extrusion), can be observed in C. A – x12,000, B – x5,000 and C – x60,000.

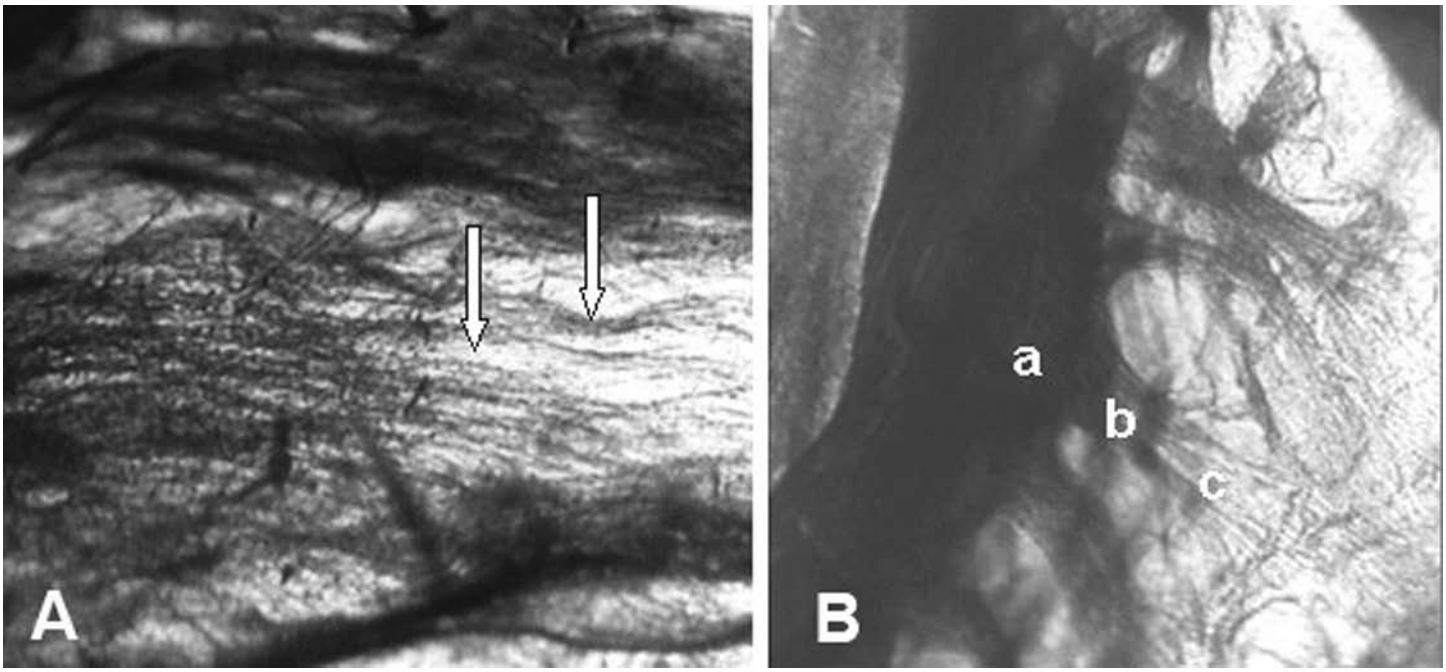

Figure 2 – Whole-mount preparation from the right atrium (A) showing small muscle bundles, uniformly organized in defined directions, generally parallel to the transverse axis. B – Right auricular wall. A more complex arrangement of the muscle bundles can be observed in the auricles. The muscle fibers are concentrated as multidirectional bundles resulting in a bundle arrangement ordered in a three main magnitude levels (a, b, c). The disposition of bundles in the third level (c) is similar to paintbrushes. A, B, C - x40.

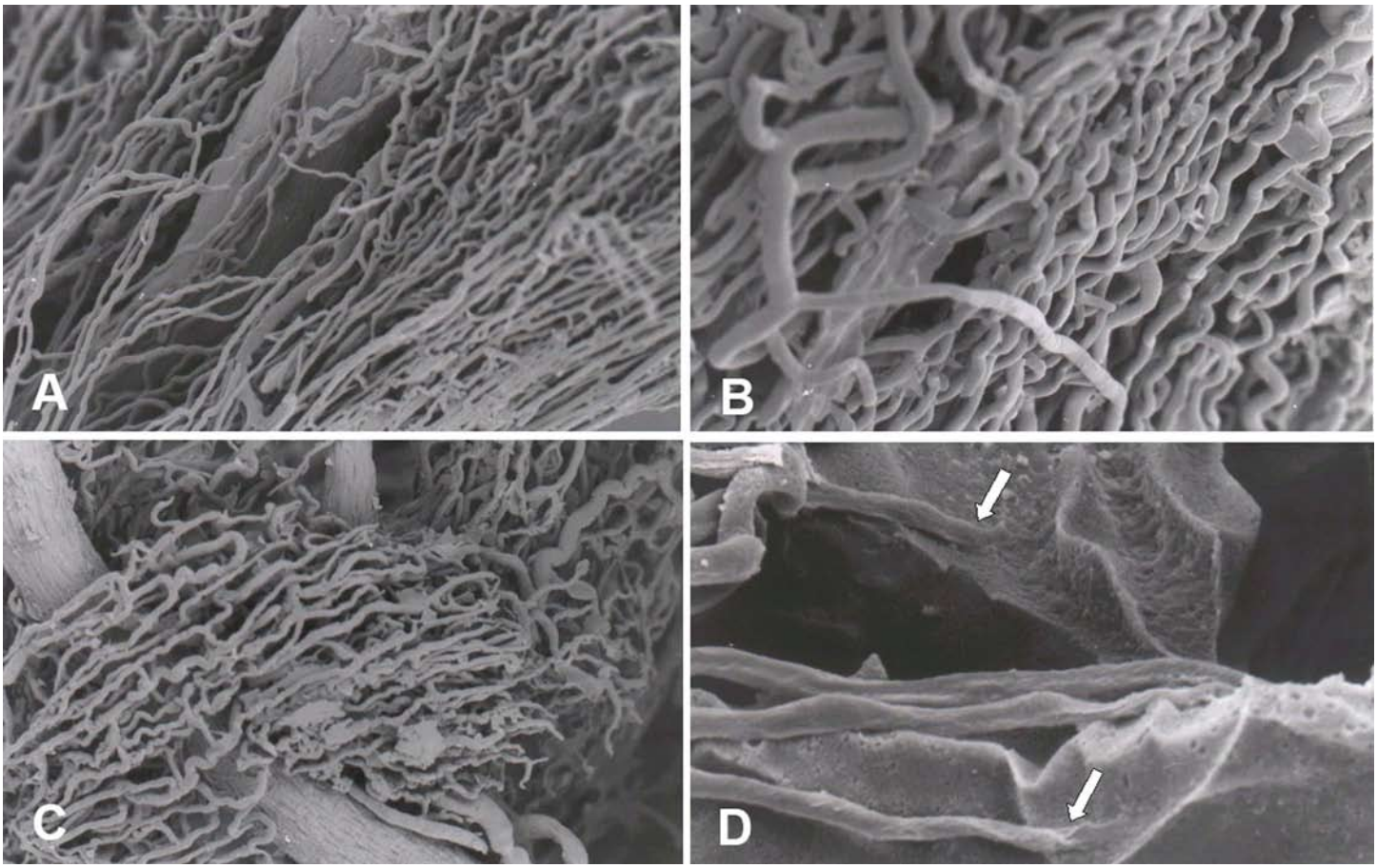

Figure 3 – Methacrylate resin (*Mercox*) corrosion molds examined with the scanning electron microscope, showing in A the vascular net from the atrial wall very elongated and relatively uniform and dense. B - Auricle wall, with the arterial net denser, more irregular, and tortuous. C - In the atrial auricle transition, the vessels are also tortuous, however, with a more or less uniform direction. D - Arterio-luminal communications can be observed in the auricles (arrows). A- x250; B- x500; C- x250 and D – x1800.

peptide as a result of changes in the properties of this complex wall dilatation–contraction process [24]. According to those authors [23,24] the amount of ANP released, should be analyzed together with the myoarchitecture as we did in this research. As observed, the atrium presents an arrangement that considerably differs from that of the auricle. A disposition of small and sparse bundles arranged in a uniform and parallel way to the transversal axis has been described for the atria, while for the auricles the arrangement is denser and more complex, formed by multidirectional bundles or in a net-like shape. Therefore, it can be concluded that the auricles in the guinea pigs would represent besides a mechanical role an important morphological basis for the ANP release; both contraction and dilatation would them take place in different directions; It is added to those facts the auricle larger inner surfaces in relation to the atria. Some authors [7] assessed the atria pressure, atrial wall dilatation and contraction frequency, both in humans [28] and in rabbits [7] and suggested that the major stimulus for ANP release is the frequency increase of cardiomyocytes shortening.

Methacrylate-injected arterioles have been evidenced, ending in the atrial-auricular complex cavity. With respect to the data cited above, it should be outlined that the corrosion technique employed in this study is a valuable method for the study of tri-dimensional images to be obtained from the microvasculature of several tissue types, offering many advantages in relation to other methods of vascular perfusion. Thus, the marked irregularities of the auricle vessels probably represent an adaptation of those structures to the dilatation – contraction process which occurs in those cavities walls. In addition, the presence of vessels showing greater caliber next to the endocardial surface and the dense net of vessels with small caliber paralleled located in relation to the epicardial surface constitute an arrangement similar to that verified in other structures, as described in the esophagogastric transition, where the vessels showing greater caliber in the esophageal mucosa and sub-mucosa play specific functions [10].

According to the present results, the approaches used and the analysis methods applied for the guinea pigs atrial-auricular complex study have demonstrated the morphological and functional complexity of these heart regions. The muscular and mainly the vascular arrangement resemble the architecture of organs showing dense glandular structures, such as the small intestine.

The morphofunctional findings with respect to the atrial-auricular complex are becoming very useful, since it has been observed in studies related to cardiac surgeries [8,19,30] an increasing concern regarding the acute and chronic effects of the ANP release reduction and consequently abnormalities in the renal function related to body fluid control arising from cardiac surgeries where removal of the auricles is carried out.

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