ULTRASTRUCTURAL AND IMMUNOHISTOCHEMICAL LOCALIZATION OF ANP-PRODUCING CELLS IN THE HEART OF RABBITS

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ABSTRACT

Cardiomyocytes of vertebrates combine contractile and endocrine functions. They synthesize and secrete atrial natriuretic peptide (ANP), which is localized in specific granules. In the present study, the ultrastructural features and the immunohistochemical localization of ANP-producing cells in the different regions of the rabbit's heart including both atria and ventricles was investigated. Most of the cardiocytes within the right atrial myocardium had a well-developed endocrine secretory apparatus composed of scarce rER, prominent Golgi and a considerable number of electron-dense ANP-immunoreactive granules within their cytoplasm. Therefore, most of the right atrial cardiocytes were considered to be ANP-producing cells. In comparison to the right atrial ANP-producing cells, the secretory activity of left atrial ANP-producing cells was not pronounced and the cells were classified as intermediate endocrine type. Three cell types were identified within the walls of both ventricles namely working, intermediate ANP-producing and conduction cells. No myoendocrine cells were identified within the left ventricular myocardium. The ventricular conduction cells showed a well-developed endocrine secretory apparatus with a considerable number of ANP-immunoreactive electron-dense granules within their cytoplasm. These findings demonstrate that in the rabbit's heart ANP-producing cells are present in the atrial and right ventricular walls and that the rabbit heart, similar to that of vertebrates, is a bifunctional organ.

INTRODUCTION

Cardiomyocytes of vertebrates combine contractile and endocrine functions **(Forssmann, 1998; Bystrova et al., 2002)**. These myoendocrine cells synthesize and secrete atrial natriuretic peptide (ANP), which is localized in their specific granules. The ANP has a well pronounced natriuretic, diuretic, and vasodilating activity **(DeBold et al., 1981; Rinne et al., 1986)**.

In previous studies, we had demonstrated the presence of ANP-producing cells in the hearts of camels **(Marei, 1994)**, tilapia **(Marei and Osman, 1996)**, Chicken **(Marei, 1996)** and rat **(Marei, 2002)**. Other studies have confirmed the presence of these cells in the heart of several mammalian and submammalian species **(Forssmann et al., 1983; Ackermann et al., 1984; Rinne et al., 1986; Mifune et al., 1991a, b,c; Mifune et al., 1992; Cerra and Gattuso, 1995; Richter et al., 1998; Bystrova et al., 2002)**.

In most vertebrates, ANP-immunoreactive cells were generally present in the atrial walls except for the sinoatrial node **(Forssmann et al., 1983; Rinne et al., 1986)**. In the ventricular walls, they were distributed in the impulse conducting system, particularly the left bundle branch, Purkinje fibers on the left side of the interventricular septum, and those in the chodre tendinae in the left ventricle, while they were sporadically seen in the atrioventricular node and bundle of His **(Back et al., 1986; Toshimori, et al., 1987; Toshimori et al., 1988 a,b)**. In the diseased human hearts, the positivity of ANP-containing cells increased significantly in conduction systems and in ventricular muscles **(Mochizuki et al., 1991)**. The increased regional production of ANP within the ventricular myocardium is induced by increased mechanical stretch of the cardiac myocytes, and this might contribute to the increased release of ANP in myocardial infarction **(Larsen and Saetersdal, 1993)**. Human ventricular muscle cells have retained the genetic ability to form specific ANP-containing granules in certain clinical disorders **(Lee and Lee, 1990)**. ANP is present in the ventricular impulse-conducting system of the human heart, and ANP is also present in the working ventricular cardiocytes in patients with dilated cardiomyopathy (DCM) as well as in human foetuses **(Jougasaki et al., 1989)**. Atrial natriuretic peptide receptors are localized on ventricular myocytes but not in the conduction system **(Hansson et al., 1997)**.

At the morphological level, few studies have been devoted to demonstrating the presence of ANP-producing cells in the heart of rabbits. Moreover, the distribution of these cells within the different compartments of the rabbit s heart is as yet unknown. Therefore, the present study was conducted primarily to give morphological evidence about the presence or absence of ANP-producing cells in the rabbit's heart. A second objective was to study their distribution within the different regions of the rabbit s heart. Such goals are crucial for completion of our comprehensive survey concerning confirmation of the presence and studying the distribution pattern of ANP-producing cells in the heart of different animal species. Confirming the presence of myoendocrine cells in the heart of different animal species is crucial for confirming the ANP hormone theory and will help to highlight the expected therapeutic use of ANP as a promising new candidate for controlling many disease conditions such as hypertension and congestive heart failure.

MATERIAL AND METHODS

The present study was conducted using ten hearts collected from apparently healthy white New Zealand rabbits that were obtained from a local farm. We followed the Guiding Principles for the Care and Use of Animals as set up by the University of Mansoura according to the principles of the Declaration of Helsinki and its revisions **(1964, 1975, 1983)**.

After scarifying, specimens were excised from the different compartments of the hearts including right atrium, right ventricle, left atrium and left ventricle. They were fixed in 2.5 % glutaraldehyde in 0.1M sodium cocodylate, pH 7.3 for 12 hs at room temperature. After rinsing in the same buffer, the tissues were post fixed in 1 % buffered osmium tetroxide for 2 hs at 4°C. After a second rinse in 0.1M cacodylate buffer, they were dehydrated in a graded ethanol series and cleared in propylene oxide, infiltrated in three stages of and embedded in Epon 812. Semithin sections of tissues were cut from each sample, stained with 1% azure and methylene blue (1:1) and examined by light microscopy for orientation study. Ultrathin sections were obtained using a diamond knife, mounted onto Formvar-coated 200-mesh copper grids and double stained with uranyl acetate and lead citrate and observed with an JEOL 100c electron microscope.

Immunoelectron Microscopy

Specimens were fixed in a mixture of 2% paraformaldehyde and 1% glutaraldehyde in 0.1M sodium cacodylate, pH 7.4 for 6 hrs. They were dehydrated in graded ethanol without post-fixation in osmium tetroxide, and embedded in L.R. white resin (London Resin Company, Basingstoke, UK). Ultrathin golden or silver sections (80 nm thick) were cut on an ultratome and picked up on uncoated 200-mesh nickel grids. Following a short rinse in distilled water, they were incubated in 10% heat inactivated normal goat serum **(Sigma, St. Louis, MO)** in Tris buffer pH 7.4 at 25°C for 30 minutes for blocking the non-specific binding. They were then incubated in a humid chamber with goat anti-α human ANP (position 1-28 amino acid residues) antibody (Sigma, St. Louis, MO); diluted 1:100 in 0.01 M phosphate buffer, pH 7.4, containing 0.15 M NaCl, 0.3% BSA, 0.1% Triton X 100, 0.001 M EDTA and 0.1% sodium azide for 16 hours at 25°C. After rinsing in the same dilution buffer, the grids were incubated in rat anti-goat IgG (10 nm gold-conjugate) (BioCell Research Laboratories) diluted to 1:50 in the same dilution medium as for the primary antibody. The incubation of the gold-labeled antibody lasted for 1.0 hour at 25°C. Sections were then washed, first with the same dilution buffer and then with distilled water. After drying, they were stained with a low concentration of uranyl acetate (0.5 %) for 30 seconds, and with lead citrate, for 5 minutes.

Controls :

Controls for immunostaining included processing tissue as above, but the primary antiserum

was pre-absorbed by prior incubation (24 hr) with synthetic ANP (**Sigma, St. Louis, MO.**). Substitution of the primary antiserum with normal goat serum and omission of the secondary antibody, were also used as controls.

RESULTS

Right atrium :

Ultrastructural examination of the right atrial myocardiocytes revealed the presence of well-developed contractile and secretory elements within the majority of the cells. Thus, the right atrial myocardial layer seemed to be composed entirely of myoendocrine cells. The right atrial myoendocrine cells were elongated with ovoid euchromatic nuclei with a distinct nucleolus and peripheral heterochromatin (Fig. 1). The major part of the cytoplasm was occupied by a large amount of striated contractile myofibrils (Fig. 1). A considerable amount of homogeneous non-fibrilar sarcoplasm was encountered especially in the perinuclear (Fig. 2), subsarcolemmal and interfibrillar regions (Fig. 1, 2).

The most distinctive ultrastructural feature of the myoendocrine cells cytoplasm was the presence of a well-developed endocrine secretory apparatus composed of few tubules of rER, prominent Golgi saccules (Fig. 3) and a considerable number of progranules and mature electron-dense granules (Fig. 3). Well-developed Golgi saccules were evident in most cells, located within the perinuclear areas (Fig. 3). In addition to the scarce rER tubules and the Golgi saccules, a considerable number of progranules and electron-dense granules were observed in the perinuclear sarcoplam (Fig. 1, 2, 3). The electron-dense granules were spherical and membrane-bound with an average diameter of 120 nm. Their electron dense homogenous contents were separated from their outer limiting membranes by a thin rim of electron-lucent materials (Fig. 1, 2, 3). The average number of electron-dense granules within the perinuclear cytoplasm ranged between 30-40, however in many cases these electron-dense granules were encountered also both within the interfibrillar (Fig. 4) and subsarcolemmal sarcoplasm (Fig. 5, 6). In comparison to the electron-dense granules, the progranules were smaller, membrane-bound, and their finely granulated matrix usually displayed a moderate electron density (Fig. 3). In many occasions, the progranules were observed to be located very close to or inside the saccules of Golgi apparatus (Fig. 3). As in the ordinary cardiac muscle cells, a considerable number of ovoid and elongated mitochondria were observed in the different cytoplasmic regions including the perinuclear (Fig. 1, 2), interfibrillar and subsarcolemmal (Fig. 5, 6) regions. Well-developed T tubules were encountered in most of the examined myoendocrine cells and in some cases a considerable number of electron-dense granules were located in close proximity to them (Fig. 6, 7).

Left atrium :

Based on their ultrastructural peculiarities three types of myocardiocytes were recognized within the left atrial myocardium: myoendocrine cells, working cells, and intermediate endocrine cells. The left atrial myoendocrine cells were similar to those of the right atrium (Fig. 8). The cytoplasm of the working cells was entirely occupied by closely packed myofibrils with a great reduction in the amount of non-fibrillar cytoplasm especially within the perinuclear, subsarcolemmal (Fig. 8) and interfibrillar areas. In comparison to the myoendocrine cells, the secretory apparatus was ill-developed with a very scarce rER tubules that were difficult to discern in most cells, a small Golgi and an entire absence of electron-dense granules and progranules. The intermediate endocrine cells shared common ultrastructural features between the myoendocrine and working cells. In comparison to the myoendocrine cell, intermediate endocrine cells had an ill-developed endocrine secretory apparatus composed primarily of scarce rER tubules and small Golgi that were primarily encountered within the perinuclear area. There was a great reduction in the number of electron-dense granules with an average number of 2 - 3 granules that were mainly located within the perinuclear areas (Fig. 9). Insinuation of the electron-dense granules within the interfibrillar or subsarcolemmal regions was not seen in any of the examined cells (Fig. 9).

Right ventricle :

Three cell types were identified within the right ventricular myocardium: working, myoendocrine, and conduction cells. The working cells constituted the great majority of the cells forming the right ventricular myocardium. Their ultrastructural features were similar to those previously described in the right atrial myocardium. The right ventricular myoendocrine cells were to a great extent similar to the myoendocrine cells of left atrium. They were encountered mainly as sporadic endocrine cells randomly distributed throughout the different myocardial regions. In comparison to the working cells, the cells had a recognizable endocrine secretory apparatus located mainly within the perinuclear areas, and composed of one or two rER tubules, small Golgi saccules and from 4-5 electron-dense granules. The third cell type that was identified within the myocardium of the right ventricle was the conduction cells. The major part of their cytoplasm was filled with non-contractile non-fibrillar elements with very few myofibrils concentrated mainly in the peripheral cytoplasmic areas (Fig. 10, 11). An interesting ultrastructure feature of these cells was the presence of a comparatively well-developed endocrine secretory apparatus similar to those previously described for the typical myoendocrine and intermediate endocrine cells. The endocrine secretory apparatus of the conduction cells was composed primarily of sparse rER tubules, prominent Golgi saccules and an average of 10-15 electron-dense granules and progranules that were mostly observed within the perinuclear cytoplasm (Fig. 10, 11).

Left ventricle :

Two cell types were identified within the left ventricular myocardium: working and conduction cells. The working cells constituted the great majority of the cells forming left ventricular myocardium. The ultrastructural features of left ventricular working and conduction cells were similar to those previously described in the right ventricular myocardium. No myoendocrine cells were identified among the myocardiocytes forming the left ventricular myocardium.

Treating sections with anti-α human ANP showed ANP immunoreactivity, as homogenous distribution of colloidal gold particles within the granule matrices of mature secretory granules of both atria (Fig. 12) and in the right ventricular conduction and myoendocrine cells. Very weak ANP immunoreactivity was encountered within the progranules.

DISCUSSION

In the present study, most of the right atrial myocardiocytes were considered to be myoendocrine cells. The endocrine secretory apparatus of the right atrial myoendocrine cells was composed of scarce rER tubules, prominent Golgi and a considerable number of membrane-bound progranules and electron-dense granules. In most mammalian species, the greatest number of myoendocrine cells are found in the atria **(Back et al., 1986; Reinecke et al., 1989; Forssmann et al., 1998; Marei, 1994; Marei, 2002)**. In rabbit, the atrial tissue levels of ANP peak near birth **(Thompson et al., 1988). Aoki et al. (1988)** reported that all atrial cells contain the genetic information to produce hormonal peptides. **Seul et al. (1992)** suggested that the right atrium is a predominant site in ANP secretion in rats. **Mifune et al. (1991a)** in mouse, rat and Mongolian gerbil demonstrated that ANP immunoreactivity was detected in the atria of all three species, and the most intensely reacting cardiocytes were localized in the right auricular part of the atrium. **Richter et al. (1998)** in equines identified auricular cardiocytes as the loci of ANP synthesis and suggested that equine ANP is produced in auricular cardiocytes and the predominant storage form of ANP in the auricle is the prohormone ANP-1-126.

The structure of the endocrine secretory apparatus in the rabbit's right atrial myoendocrine cells that was demonstrated in the present investigation correlated well with those previously described for the myoendocrine cells in several animal species **(Toshimori et al., 1988a,b; Mifune et al., 1991a,b,c; Marei, 1994; Marei, 1996; Marei and Osman, 1996)**. The morphological features of the progranules and the electron-dense granules that were demonstrated in the present study were to a great extent similar to the ANP-containing granules that have previously been demonstrated in the myoendocrine cells of many mammalian and submammalian species **(Rippegathr et al., 1987; Forssman et al., 1988; Herbst et al., 1988; Agnoletti et al., 1989;**

Forssmann et al., 1998; Marei, 1994; Marei, 1996; Marei and Osman, 1996; Marei, 2002). In addition, treating sections with anti-a human ANP showed ANP immunoreactivity as homogenous distribution of colloidal gold particles within the granule matrices of mature secretory granules. The close morphological resemblance of these granules to the ANP-containing granules as well as the positive reactivity with anti-α human ANP might lead us to suggest that these electron-dense granules contain a hormonal peptide of the same ANP family. In this respect, **Toshimori et al. (1988a,b)** reported that in the atrial cardiocytes the electron-dense granules were revealed to be storage sites of the peptide. The electron-dense material, thought to be the peptide, was found in the sarcoplasmic reticulum and Golgi saccules. In the present investigation, ANP-immunoreactive electron-dense granules were located in very close proximity to the Golgi saccules; moreover many progranules were identified as located within the Golgi saccules. These observations might indicate an active role of the Golgi saccules in cardiac hormone synthesis as was previously suggested by **Toshimori et al. (1991)**.

Within the myocardium of the rabbit's left atrium, three types of myocardiocytes were recognized, namely ANP-producing myoendocrine cells, working cells, and intermediate myoendocrine cells. The left atrial myoendocrine cells were similar to those of the right atrium and were recognized mainly by their well-developed endocrine secretory apparatus. In comparison to the ANP-producing myoendocrine cell, intermediate myoendocrine cells had an ill-developed endocrine secretory apparatus and small numbers of electron-dense granules located mainly within the perinuclear sarcoplasm. Whether the left atrial intermediate endocrine cells represent a distinctive cell type or they are a variant of typical myoendocrine cells that are subjected to condition of decreased hormonal synthesis and production is still a matter of speculations. **Aoki et al. (1988)** demonstrated that the functional heterogeneity among the amphibian myoendocrine cells; the small number of secretory granules seen during summer can be interpreted as the result of accelerated synthesis and intracellular processing of hormonal peptides, by-passing the packing step in the Golgi complex. This suggestion might further explain the ill-developed nature of the Golgi complex that was demonstrated in the present study. However, **Marei (1994)**, based on the presence of an ill-developed endocrine secretory apparatus in the intermediate endocrine cells, did not support the hypothesis concerning the accelerated synthesis and cellular processing and he suggested that these cells represent either inhibited myoendocrine cells or they are genetically programmed to produce less hormone. The present findings concerning the presence of fewer myoendocrine cells in the left atrial myocardium as compared to the right atrial ones were in accord with those of **Gutkowska and Nemer (1989) and Brand and Jockusch (1987)** who demonstrated that the left atrial cells were found to store less ANP than the right atrial ones.

Three cell types were identified within the right ventricular myocardium: working, ANP-

produeing myoendoerine, and conduction cells. The right ventricular myoendoerine cells were encountered mainly as sporadic endocrine cells randomly distributed throughout the different myocardial regions. They were to a great extent similar to intermediate endocrine cells of the left atrium with an average of 4-5 electron-dense granules mainly within the perinuclear sarcoplasm. Since the discovery of the endocrine nature of the heart by **Kisch (1956)**, the existence of myoendocrine cells among the ventricular eardiocytes has been the subject of many debates. Several investigators have asserted that some of the ventricular cardiocytes possess an endocrine potentiality and they serve as an important source of ANP hormones. This view could be appreciated by referring to the work of **Leak and Burke, 1964; Zivin et al., 1984; Nemer et al, 1986; Bloch et al., 1986; Gardner et al., 1986; Wei et al., 1987; Thibault et al., 1989; Albino-Teixeira et al., 1990; Marei, 1994; Klinger et al., 2001)**. In contrast to the results presented here, other investigators claimed that the ANP-producing myoendocrine cells were located only in the atrial wall **(Mckenzie et al., 1985; Moringa et al., 1985; Saper et al., 1985; Cantin et al.,1991)**. The increased expression of the ANP gene in right ventricles was reported in volume loaded and spontaneously hypertensive rats **(Lattion et al, 1986; Takayanagi et al., 1987)**. The right ventricle may beeome a major source for ANP synthesis and release during hypertension, and may play important roles in cardiae endoerine pathology and cardiac hypertrophy **(Gu et al., 1988)**. This suggests that the potential to produee ANP is activated by some stimuli such as wall stretch and high salt diet. **Gu and McGrath (1990)** suggest that human ventricular cardiocytes in and around aneurysm can convert to produce large amounts of the endocrine peptide ANP. This ventricular endocrine conversion was localized and was probably caused by physieal over-stretch of the cardiocytes. **Reinecke et al. (1985)** reported that ANP-myoendocrine cells in the heart of mammals, birds and reptiles were restricted to the atria, while in lower vertebrate classes such cells occur in considerable number in the ventricle as well. Thus, there seem to be a physiological trend toward the concentration of cardiac hormone producing cells in the atria with increasing complexity of the cardiovascular system. **Back et al., (1986)** suggested that the ventricular myoendocrine cells might represent a phylogenetic remnant rather than having a particular physiological role. In rat and golden hamster, ANP-immunoreactive granules first appear in the myocardial sleeve of the embryonic heart tube during the looping stages and the density of granules in ventricular myocytes decreases during further stages of fetal and neonatal development **(Navaratnam et al., 1989)**. **Gu et al. (1989)** suggested that the extent of ventricular ANP synthesis and release are regulated by intraventricular pressure, and the occurrence of ANP in overloaded ventricles may be a transient, immediately reversible phenomenon.

In the present study, a well-developed endocrine secretory apparatus similar to those for the

typical myoendocrine and intermediate endocrine cells was identified in most of the examined ventricular conduction cells. These observations might suggest that ventricular conduction cells are also involved in synthesis and secretion of hormone that might be related to the same cardiac hormone of ANP family. The present results disagreed with those of **Melax and Leeson (1970)** who stated that in rat conduction cells, no speeific secretory granules such as those in atrial myoendocrine cells were detected. However, our findings were in harmony with those of **Back et al., 1986; Forssmann et al.,1986; Gobel et al., 1986; Gardner et al., 1986; Thompson et al., 1988; Thoshimori et al., 1987; Thoshimori et al., 1988a,b; Marei, 1994**. In the rat, the entire intraventricular conduction system is constituted of endocrine cells producing ANP **(Cantin et al., 1989)**. The exact role of cardiac hormone produced by ventricular and conduction cells is still uncertain. It has been suggested that the ventricular conduction cells may only be involved in local endocrine regulation and the amount of cardiac hormone produced by these cells compared to that of atrial appendages is negligible **(Gobel et al., 1989)**.

In the present study, no myoendocrine cells were identified among the cardiocytes forming the myocardium of left ventricles. A similar conclusion was previously reported in the heart of camels **(Marei, 1994)**. The entire absence of ANP secretory activity within the left ventricular myocardiocytes seemed to be logical as the great pumping forces needed from the left ventricular myocardiocytes leave no room for other functional attributes.

Figure 1 : Electron micrograph of right atrium myoendocrine cells of rabbit showing nucleus (n), myofibrills (y), non-fibriller permuclear sarcoplasm (s), mitochondria (u) and electron-dense granules (e) X 13000.

Figure 2 : Right atrial myoendocrine cells of rabbit showing nucleus (n), non-fibriller permuclear sarcoplasm (s), mitochondria (u), myofibrills (y) and electron-dense granules (e) X 17000.

Figure 3 : Higher magnification of right atrial myoendocrine cells of rabbit showing a well developed Golgi (L) with electron-dense granules (e) and progranules (p). Note also mitochondria (u) X 36000.

Figure 4 : Right atrial myoendocrine cells of rabbit showing the presence of electron-dense granules (e) in the interfibriller regions X 36000.

Figure 5 : Right atrial myoendocrine cells of rabbit showing the presence of electron-dense granules (e) in the subsarcolemmal regions X 17000.

Figure 6 : Right atrial myoendocrine cells of rabbit showing the presence of a well-developed T-tubules (t) and the presence of electron-dense granules (e) in the subsarcolemmal region X 17000.

Figure 7 : Higher magnification to figure 6 showing a well-developed T-tubule (t) and its close proximity to the electron-dense granules (e) X 28000.

Figure 8 : Left atrium of rabbit showing the presence of working cell (w) and typical myoendocrine cell (m) with a considerable number of electron-dense granules (e) in the subsarcolemmal sarcoplasm X 17000.

Figure 9 : Left atrial intermediate myoendocrine cells of rabbit showing nucleus (n) and few electron-dense granules (e) within the perinuclear areas X 13000.

Figure 10 : General view of right ventricular conduction cell (v) of rabbit X 8000.

Figure 11: Higher magnification to fig 10 showing evidence of secretory activity for conduction cell. Note eccentric nucleus (n), few myofibrils (y) and electron-dense granules (e) X 13000.

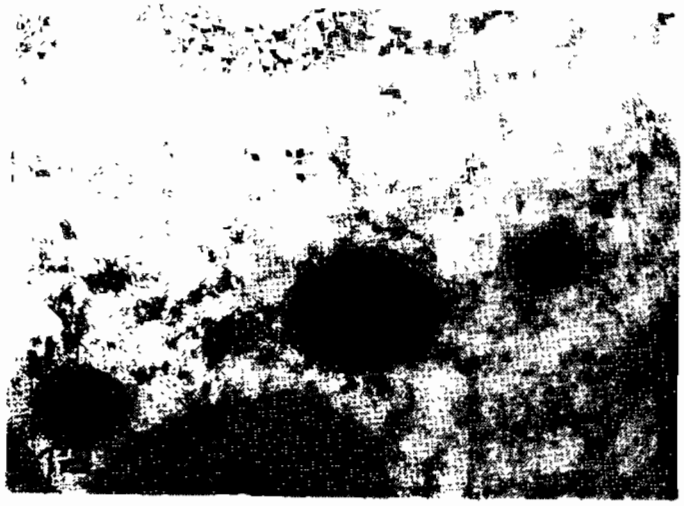

Figure 12 : Representative ultrastructural immunohistochemical labeling of the storage of ANP in the electron dense granules of the right atrial myoendocrine cells. Note the positive ANP reactivity as indicated by the presence of a considerable number of gold particles within the granule matrix X 72000.

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الملخص العربى

التركيب الدقيق وتحديد كيمياء الأنسجة المناعية للخلايا المنتجة لهرمون القلب (ANP) فى الأرانب

المشتركون فى البحث

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تتميز خلايا القلب فى الفقاريات بقيامها بوظائف إنقباضية وإفرازية حيث تقوم تلك الخلايا بتخليق وإفراز هرمون القلب (*ANP*) والذى يتركز فى الحبيبات الخاصة داخل سيتوبلازم تلك الخلايا. فى هذه الدراسة تم التركيز على دراسة التركيب الدقيق وتحديد أماكن كيمياء الأنسجة المناعية لهرمون القلب (*ANP*) فى المناطق المختلفة لقلب الأرانب مثل الأذينين والبطينين.

أوضحت الدراسة أن معظم الخلايا المكونة لجدار الأذين الأيمن تتميز بوجود جهاز إفرازى متطور تمثل فى وجود بعض أنابيب الشبكة الإندوبلازمية المحببة وجهاز جولجى والعديد من الحبيبات الإفرازية لذلك فقد أعتبرت كل الخلايا المكونة لجدار الأذينين الأيمن خلايا عضلية هرمونية لإنتاج هرمون القلب (*ANP*)، بالنسبة لجدار الأذين الأيسر فإن النشاط الإفرازى لخلاياه كان غير مميز بوضوح وبناء على ذلك تم تقسيم خلايا الأذين الأيسر إلى خلايا متوسطة النشاط بالنسبة للإفراز هرمون القلب.

كما تم التعرف على ثلاثة أنساع من الخلايا داخل جدار البطينين وهم الخلايا العاملة والخلايا المفرزة الوسيطة والخلايا الموصلة. لم يتم العثور على أى خلايا عضلية هرمونية فى جدار البطين الأيسر كما تم تميز وجود جهاز إفرازى متطور داخل سيتوبلازم الخلايا الموصلة المتواجدة فى جدار البطينين حيث تكون تلك الجهاز الإفرازى من العديد من الحبيبات الإفرازية والتى أعطت تفاعلاً موجباً مع هرمون القلب.

ومن هذه الدراسة تم إستنتاج وجود الخلايا العضلية الهرمونية المفرزة لهرمون القلب (*ANP*) فى كل من الأذينين الأيمن والأيسر، والبطين الأيمن، وبناء على ذلك أعتبر قلب الأرانب كما هوالحال بالنسبة لقلوب الكثيرة من الحيوانات الفقارية كعضواً ثنائى الوظيفة.