THE EFFECT OF MEXIDOL ON ATRIAL NATRIURETIC PEPTIDE IN LANGENDORF RAT HEART PREPARATION

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© **M.L. Bugrova,** PhD, Associate Professor, Head of Electron Microscopy Unit, Central Scientific Research Laboratory[1];
E.E. Kharkovskaya, Postgraduate, the Department of Neurodynamics and Neurobiology, the Biological Faculty[2];
E.I. Yakovleva, PhD, Senior Research Worker, Electron Microscopy Unit, Central Scientific Research Laboratory[1]

[1]Nizhny Novgorod State Medical Academy, Minin and Pozharsky Square, 10/1, Nizhny Novgorod, Russian Federation, 603005;

[2]Lobachevsky State University of Nizhni Novgorod — National Research University, Prospekt Gagarina, 23, Nizhny Novgorod, Russian Federation, 603950

Atrial natriuretic peptide (ANP) participating in fluid-and-electrolyte balance maintenance in body plays a certain role in pathogenesis of cardiovascular diseases. The effect of medications on its metabolism is understudied. In cardiology, Mexidol an antihypoxic agent of metabolic type, with a cardioprotective effect — has a widespread application. The effect of Mexidol on ANP was studied for the first time.

The aim of the investigation was to study the effect of Mexidol on the intensity of cardiomyocyte ANP accumulation and release in rat isolated perfused heart.

Materials and Methods. The experiments were carried out on 15 isolated hearts of male Wistar rats perfused according to Langendorf by Crebs-Henseleit solution with Mexidol administration. The intensity of ANP accumulation and release were assessed by the quantitative analysis of immunolabeled atrial myocyte granules under a transmission electron microscope.

Results. Mexidol administered at a dose of 25 mg/kg in a perfusion solution enhances ANP accumulation and release processes in atrial myocytes of a rat Langendorf isolated heart, and results in an additional cardioprotective effect. Slight hypoxia promotes ANP synthesis increase and has no impact on peptide release.

Conclusion. The analysis of immunolabeled granules showed a positive effect of Mexidol on ANP synthesis and release in a rat Langendorf isolated heart and proved a cardioprotective action of Mexidol. The study of the effects medications have on ANP will enable to show the possibilities of application of their pharmacological effects.

Key words: atrial natriuretic peptide (ANP); isolated heart; Mexidol.

Atrial natriuretic peptide (ANP) as a hemodynamics regulator has long been of interest to researchers in biology, pharmacology, and medicine [1–10]. The ANP has a broad spectrum of action in organs and tissues: it participates in the fluid-and-electrolyte balance and adipose tissue metabolism regulation, reduces the amount of water and sodium concentration in the bloodstream, etc. It is the antagonist of the renin-angiotensin-aldosterone system [3]. As patients with cardiovascular disease show an increased level of ANP in the plasma, it is used a clinical marker. Recently, researchers have been making attempts to introduce ANP in the complex therapy of the diseases accompanied by high blood pressure [4]. However, the data on the interaction of ANP with drugs is insufficient as of today.

In Russia in 1999 Mexidol was developed and implemented into clinical practice. It is a synthetic antihypoxic drug with antioxidant properties that does not have foreign equivalents and belongs to the drugs of metabolic action type (3-hydroxy-6-methyl-2-ethylpyridine succinate) [11]. It is known for its cardioprotective, neuroprotective and other effects. The protective effect of Mexidol in pathological conditions is caused by the antioxidant activity of 3-oxypyridins and the antihypoxic property of succinic acid. Succinate, when in the intracellular space, can oxidize by the respiratory chain in hypoxia. The membrane protecting effect of 3-oxypyridins

For contacts: Bugrova Marina Leonidovna, phone: (831)465-41-92, +7 903-849-12-38; e-mail: marysmir@mail.ru

derivatives is caused by the reduction of microviscosity, i.e. the stabilization of the membrane lipid component and inhibiting effect on the lipid peroxidation process [12, 13]. The Mexidol effect on various organs in pathology have been studied for quite a long time [14, 15]. However, the subtle mechanisms of the drug effect on the metabolism of biologically active substances have been understudied, thus conditioning the scientific interest in the study of the Mexidol effect on the ANP synthesis and release.

The aim of the investigation is to determine the presence or absence of the effect of Mexidol on the process of accumulation and release of atrial natriuretic peptide in a rat isolated perfused heart using morphometry of the secretory cardiomyocyte granules.

Materials and Methods. The present study was carried out on 15-male Wistar rats weighing 210–240 g in accordance with the guidelines of the European Convention for the protection of vertebrate animals used for experimental and other scientific purposes (adopted in Strasbourg 18.03.1986, and confirmed in Strasbourg, 15.06.2006). To create the model of a Langendorf isolated heart, the heparinized (500 IU/kg) rats under intraperitoneal debutalbum anesthesia (35 mg/kg) were subjected to the chest dissecting, isolating the heart, which was then connected to the perfusion installation with the saline Krebs–Henseleit solution of the following composition (mmol/L): NaCl — 130; KCl — 4; $NaH_2PO_4 \cdot 2H_2O$ — 1.1; $NaHCO_3$ — 24; $MgCl_2$ — 1; $CaCl_2 \cdot 2H_2O$ — 1.8; glucose — 5.6. The solution was saturated with Carbogen (95% O_2, 5% CO_2), with the pH of 7.3–7.4; the temperature of 37°C [16]. Two refrigerators were used to switch to perfusion with Mexidol: one with Krebs–Henseleit control solution, the other one with Mexidol in the dose of 25 mg/kg added. The therapeutic effects of Mexidol are identified in the doses ranging from 10 to 300 mg/kg: the drug in the dose of 25 mg/kg has a marked vasoprotective and cardioprotective effect [13]. Perfusion was conducted for an hour. The studied myocardium tissue was taken from three experimental groups: intact rats (n=5), isolated perfused hearts (n=5) and isolated perfused with Mexidol administration hearts (n=5).

The electron microscope analysis of the right atrium and left ventricle tissues was performed by standard methods [17]. The ANP localization was revealed on the ultrathin sections by the method of immunocytochemistry using polyclonal Rabbit anti-Atrial Natriuretic Factor antibodies (1-28) (rat) (Peninsula Laboratories, LLC, Bachem, USA) and Protein-A/Gold (15 nm) (EM Grade, Electron Microscopy Sciences, USA). The sections were contrasted with uranylacetate and lead citrate, analyzed in the electronic Morgagni 268D (FEI, USA) microscope. The quantitative analysis of the two types of granules with the peptide in atrial CMC (mature, reserving A-type and dissolving B-type) were counted by the technique within eyeshot (38×38 μm²) [18, 19].

Statistical treatment was performed in the Statistica 10.0 program with the application of the Mann–Whitney criterion ($p<0.05$).

Results. The quantitative analysis of the secretory granules of the right atrial cardiomyocytes of the isolated perfused heart, containing ANP-immunoreactive material, revealed a statistically significant increase in A-type granules by 35% and the total number of granules by 25% comparatively to this rate in the intact rats, i.e. the integral body (Fig. 1). The number of granules of B-type was not significantly different from the initial level.

The ultrastructural analysis of the myocardium of the right atrium and left ventricle of the isolated perfused heart showed two types of changes in the cardiomyocyte nuclei: some nuclei had smooth contours, contained nucleoli and euchromatin, the others were with no nucleoli, had a significant introsusception of karyolemma and heterochromatin. Moderate total spread of the perinuclear space was exhibited in several cardiomyocytes. Mitochondria were in the state of physiological norm. Myofibrils were clearly defined. The sarcoplasmatic reticulum was significantly expanded in 50% of the cells (Fig. 2). Lipid inclusions and a reduced in comparison with the intact animals' myocardium content of cytogranules were found in the sarcoplasm (Fig. 3). The sarcolemma preserved its structure, it sometimes forming folds. Moderate intercellular edema was detected.

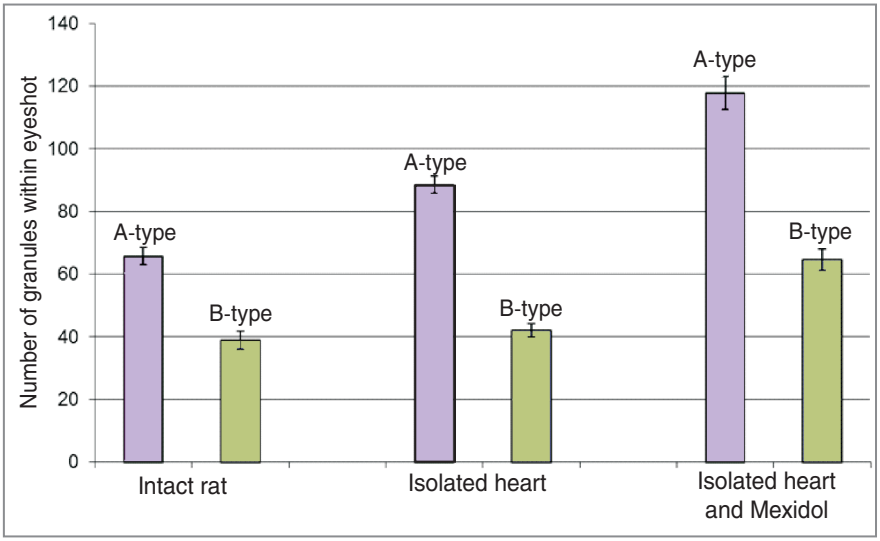

Fig. 1. Quantitative distribution of the granules with ANP in the intact rat hearts, isolated perfused hearts and isolated perfused hearts with Mexidol administration (according to Mann–Whitney test)

The morphometric analysis of the samples of an isolated perfused

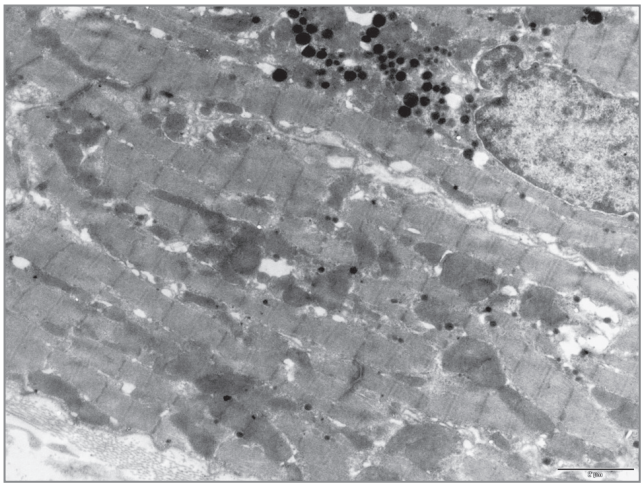

Fig. 2. The right atrial cardiomyocytes of a rat Langendorf isolated heart; ×5600

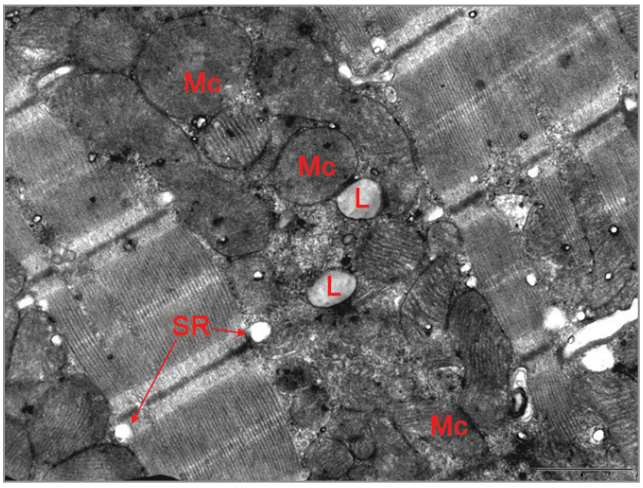

Fig. 3. Ultrastructure of the left ventricular cardiomyocyte in a rat Langendorf isolated heart: Mc — mitochondria; L — lipid drops; SR — expanded sarcoplasmatic reticulum; ×14 000

heart with Mexidol administration showed a statistically significant increase in all types of granules with ANP-immunoreactive material and their total number in comparison with the rates in the control series of isolated perfused hearts: the number of A-granules increased by 33%, B-granules by 53% and the total number by 39% (See Fig. 1).

The subcellular structure of the myocardium of rats isolated hearts (the group with Mexidol administration) showed the following changes. The cardiomyocyte nuclei contained nucleoli and chromatin, evenly distributed in the karyoplasm with a moderate aggregation on the nucleus periphery. The karyolemma in most cells was smooth or with slight invagination. The perinuclear space was not expanded. Most of the mitochondria were in an energized condition with a small increase in their area (See the Table) and parallel-oriented cristae. Myofibrils preserved their structure, the morphometric analysis revealed a statistically significant increase in the average length of sarcomeres in comparison with the rates in the control series (perfused hearts without Mexidol administration). The cisterns of the sarcoplasmatic reticulum were not expanded (Fig. 4). A significant number of cytogranules was found in the sarcoplasm (Fig. 5). The sarcolemma in most cells remained intact. Minor focal interstitial edema was identified.

Discussion. According to the authors [20], when a heart is connected to the isolated perfusion installation by Langendorf, the myocardium is subject to slight hypoxia, since the oxygen content in the blood due to the high sensitivity to hemoglobin is greater than in the oxygenized Krebs–Henseleit solution. The increased number of the "storing" forms of granules observed in the control series (without Mexidol administration) is associated with the increased ANP transcription due to HIF (hypoxia inducible factors) activation [19, 20]. Another factor of increasing ANP synthesis could

Size of the mitochondria and the length of sarcomeres in the left ventricular cardiomyocytes of a rat isolated heart (M±m)

Parameter	Isolated heart	Isolated heart with Mexidol administration
Sarcomere length, μm (n=65)	1.68±0.04	2.02±0.03*
Mitochondrion area, μm^2 (n=280)	1.61±0.02	0.78±0.03*

* — differences in the values are statistically significant ($p<0.05$).

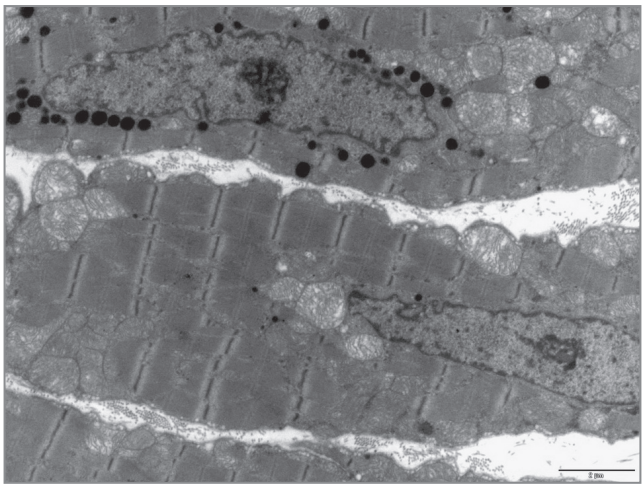

Fig. 4. The right atrial cardiomyocytes in a rat Langendorf isolated heart with Mexidol administration; ×5600

be the SK4 Ca^{2+}-dependent K^+ canal stimulation of the sarcoplasmatic reticulum, the cisterns of which were expanded, as well as in the rat cardiomyocytes with modeled total ischemia in the whole body in the

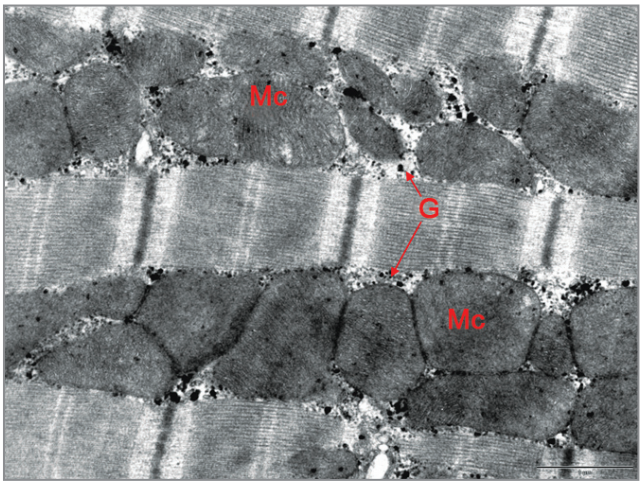

Fig. 5. Ultrastructure of the left ventricular cardiomyocyte in a rat Langendorf isolated heart with Mexidol administration: Mc — mitochondria; G — glycogen cytogranules; ×14 000

experiments we had carried out before [2, 3, 19]. The unchanged number of "dissolving" forms of granules in comparison with the rates in the intact animals indicates the lack of hypoxia effect on the release of ANP, localized in the atrial cardiomyocyte granules. In the experiments on isolated heart A.J. Baertschi et al. showed an increase in ANP release into the perfusate under short-term hypoxia [20]. In our experiments the degree of hypoxia, observed under heart perfusion by Langendorf, is presumably, a stimulus for ANP synthesis but it does not affect its release.

In the experimental group a dramatic increase in the A- and B-type granules indicated a beneficial effect of Mexidol on ANP formation and release in the isolated rat heart. Apparently, it was related to the cytoprotective effect of the drug, which manifested itself on the myocardium ultrastructure as a high content of glycogen cytogranules in the sarcoplasm and sarcoplasmatic reticulum without dilatation cisterns. The identified increase in the average value of the mitochondria area with the preservation of membrane structures and matrix indicated the energized state of the organelles that arise, according to the authors [21, 22], at media aeration, with oxidation substrates or ATP added. The average length of the sarcomere, increased compared to the control one, indicates a better myofibrils relaxation, which led to a positive inotropic effect shown by the researchers [12, 23] on isolated rat hearts with Mexidol administration with the use of electrophysiological techniques. The membrane protecting effect, improvement and preservation of high-energy compounds synthesis with Mexidol administration had a positive impact on energy-input processes of ANP formation and release.

According to the research data [24, 25] ANP introduced into the ANP perfusion solution has a cardioprotective effect on the cardiomyocytes of an isolated perfused heart. The ANP effect on the electrophysiological heart function is also known [26]. It is put into effect in two ways in the isolated heart: 1) directly, through the autonomic nervous system (according to the authors, ANP depresses the sympathetic and activates parasympathetic component of the autonomic nervous system; 2) through calcium canals: ANP weakens the calcium flow into the cell, inhibiting I_{CaL} canals. Herewith, the cyclic guanosine monophosphate (cGMP) activated by the peptide facilitates the performance of calcium ATPases which carries intracellular calcium into the sarcoplasmatic reticulum and reduces the risk of calcium overload. Besides, ANP is shown to [27] to prevent so-called electrical remodeling leading to atrial fibrillation. The identified effect of Mexidol on ANP synthesis and release expands the concept of the mechanisms of the drug's cardioprotective action, which enhances ANP synthesis and secretion.

Thus, the study of ANP properties on the model of a rat Langendorf isolated heart by the method of quantitative analysis of the immunometric granules of atrial cardiomyocytes enabled to detect a pronounced positive effect of Mexidol in the dose of 25 mg/kg on ANP synthesis and release and confirm the cardioprotective properties of this drug. The study makes a certain contribution to the investigation of the ANP interaction with drugs and can be recommended as a technique for the study of the efficacy of drugs used in cardiology.

Conclusion. Mexidol has an effect on the natriuretic peptide, significantly increasing its accumulation and excretion in the atrial cardiomyocytes of a rat Langendorf isolated heart, which causes additional cardioprotective effect of the drug in the myocardium.

Minor hypoxia contributes to an increase in the synthesis of peptide and has no effect on its release.

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Conflict of Interests. The authors have no conflict of interest.

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