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PARTICIPATION OF NPY_1-RECEPTORS IN DEVELOPING HEART REGULATION

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Abstract

In the present work, they studied the effect of the selective NPY_1 agonist of Leu (31), Pro (34) -NPY receptors of different concentrations on the myocardial contractility of neonatal and adult rats. During analysis of the myocardial strip isometric contraction, they found that the agonist causes a dose-dependent changes of the myocardial strip contraction strength. Leu31, Pro34NPY in the concentration range of 10^{-7}-10^{-5} M does not cause significant changes in the isometric contraction of the myocardial strips in the atria and ventricles among 7-day-old animals. Among 21-day-old animals, Leu31, Pro34NPY, at the concentration of 10^{-6} M, and the agonist (10^{-7} M) increases the force of contraction in the myocardium among 100-day-old animals.

Key words *:* Neuropeptide Y, NPY receptors, myocardial contractility, ontogenesis, rat.

Introduction

In 1982, they published the data on the isolated neuropeptide Y (NPY) in the pig brain consisting of 36 amino acids among 100-day-old animals for the first time (Tatemoto *et al.,* 1982). The highest concentration of this peptide is shown in the hypothalamus, postganglionic sympathetic fibers, megakaryocytes and platelets (Masliukov *et al.,* 2016; Masliuko *et al.,* 2017). The presence of NPY in the cytoplasm of cells, in the extracellular medium and in blood plasma is a confirmed fact nowadays (Tan *et al.,* 2018; Masliukov *et al.,* 2016). Now they showed that NPY and its analogues act as a new class of peptide regulatory compounds that have a great influence on various physiological functions. It is known that NPY is contained in the presynaptic vesicles of the sympathetic terminals together with norepinephrine and ATP, and it has the greatest latent period upon release (Protas *et al.,* 2003; Zverev *et al.,* 2016).

Currently, there are six types of metabotropic receptors sensitive to the neuropeptide Y (Y1-Y6), which are located in all organs and tissues of the body. In the nervous system, the receptors sensitive to NPY are localized both on pre - and postsynaptic membranes.

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NPY targets are membrane receptors conjugated to Gái- and Gáq proteins. Six NPY receptors were identified: Y1, Y2, Y4, Y5, Y6. Y6 receptor is not functional among rats and humans, and Y3 does not exist among mammals. The action of NPY on peripheral target organs among rats is realized mainly through postsynaptic Y1, Y5 receptors, as well as via Y2 receptors found on the pre- and postsynaptic membrane of the nerve endings of the sympathetic and parasympathetic nerves. Activation of Y1 receptors in the cardiovascular system promotes stimulation of the sympathetic nervous system, which enhances the effect caused by norepinephrine. In addition, activation of Y1 receptors in the heart leads to the development of a positive inotropic effect through the activation of riadin receptors located on the sarcoplasmic reticulum (McDermott & Bell, 2007). Therefore, Y1, Y2, and Y5 receptors are three main subtypes of NPY receptors that mediate the biological functions of the neuropeptide in humans and rats. It is known that the expression of Y1, Y2, Y5 receptors changes in early postnatal ontogenesis. The expression of Y1- and Y2-type increases since the 20th day of postnatal ontogenesis. Y5 type receptors have been present in the myocardium of the atria and ventricles since birth (Masliukov *et al.,* 2016; Moiseev, 2016).

However, the role of various types of receptors sensitive to NPY has not been established in myocardial contractile function. There is also no evidence of NPY receptor involvement in myocardial contractility, localized in various parts of the heart. The studies of NPY-ergic regulation of heart in the age aspect have not been conducted.

Y_1, Y_2, Y_3 and Y_5 receptors were found in the heart endocardium and myocardium. NPY is found together with norepinephrine in large synaptic vesicles and is released only at a high frequency stimulation. Reverse transcription immunohistochemistry methods have shown the density increase of NPY-containing nerve fibers, accompanied by changes in the density of various subtypes of NPY-receptors in a developing heart. The density of Y_5 receptors in the heart is the highest among 1 day old rats and then it is decreased in postnatal ontogenesis. In addition, NPY plays an important role in the processes of age-related development of regulatory influences. The action of NPY can be associated with NPY receptor activation and blockade. It is known that many blockers have their own effect on the studied functions, therefore, to differentiate the effects, it is necessary to conduct studies with both activation and blockade of different subtypes of NPY receptors.

NPY activates six transmembrane G-protein dependent receptors Y_1-Y_6, which leads to the activation of intracellular signaling pathways. These receptors can be used as potential therapeutic targets. Activation or blockade of receptors is used in the treatment of ischemia, myocardial hypertrophy and heart failure, stops the destruction of nerve cells. Over the past three decades, NPY has been associated with many physiological functions such as energy homeostasis, stress, circadian rhythm, neurogenesis, and the immune system regulation. The actions of NPY on the heart are extensive and are found on almost all types of heart cells. NPY and receptors sensitive to it are localized on the membrane of atypical and working cardiomyocytes, and the cells of the cardiac conduction system. The fibers containing NPY surround a large number of arteries in the heart. Y_1 receptors cause vasoconstriction and regulate protein metabolism and expression of the constitutive gene in cardiomyocytes.

To study the role of the selective NPY_1 agonist of Leu (31) Pro (34) NPY receptors in the regulation of myocardial contractility during postnatal ontogenesis.

Materials and Methods

White mongrel rats were used as an experimental animal model. The rats were kept under identical conditions, 5-7 animals per cage. All rats had free access to water and food. One ration was used for all rats. The experiments were conducted on three age groups of animals. According to the literature, 7-day-old cubs are characterized by a lack of sympathetic innervation in the heart, 21-day-old animals demonstrate the peak of heart rate and a high level of sympathetic influences. 100-day-old animals act as a biological model for the complete formation of sympathetic innervation on the heart. Thus, we consider the animals with a maximal, minimal, and fully morpho-functionally formed sympathetic department of the autonomic nervous system for cardiac activity (Sitdikov *et al.,* 2008).

To record the amplitude-time parameters of myocardial strip isometric contraction of the right atrium and ventricle, we chose the tensometric method. Isometric contraction of myocardial strips was performed using PowerLab (AD Instruments) device with a "MLT 050/D" force sensor (ADInstruments).

After the introduction of urethane, the animal was fixed on a well-illuminated operating table. They opened the chest below the xiphoid process in the direction of the right and left clavicle. The heart was fixed with tweezers and cut off with scissors from the main arteries. The extracted heart was placed in a glass with Tyrode's solution. Then the heart was transferred to a Petri dish, where strips of the myocardium of the right atrium and the right ventricle were prepared in accordance with the heart anatomy. The strips of the myocardium were immersed in a special reservoir where Tyrode's solution was supplied, which contains 7.54 g/l of NaCl, 0.3 g/l of KCl, 0.134 g/l of $CaCl_{12}$, 0.06 g/l of $MgSO_4$, 0.14 g/l of $NaH2PO_4$, 1.68 g/l of $NaHCO_3$ g/l, 0.9 g/l of glucose (Sigma-Aldrich, USA). The solution was enriched with oxygen throughout the experiment. The fixation of the strips to the force sensor and the support point was made with silk threads.

Mechanical isometric contraction was recorded on a personal computer (Chart 5.1 software).

Myocardial drugs were immersed in baths, where they were fed with Tyrode's solution. During the run-in period, the strips were given maximum tension. Then they recorded the initial value of the isometric contraction. The effect of NPY analogue was studied in increasing concentrations. After the effect study of one of the concentrations, the drug was washed with a Tyrode solution and the initial contraction was recorded for the subsequent concentration. The application of the agonist and blockers was carried out for 30 minutes.

The contraction amplitude was calculated from isoline level to the peak of the isometric contraction (gr.), the

contraction duration was determined from the beginning of contraction to its end (sec). The isometric contraction curve was processed using the "Chart 5.1" program. They used Leu (31) Pro (34) NPY (Sigma-Aldrich, USA) drug in the experiments. The significance of differences was calculated using the paired student criterion with the determination of the normal distribution ($p < 0.05$).

Results and Discussion

To determine the functional activity of NPY receptors, they conducted the series of experiments with Leu31 and Pro34NPY drug, which is an agonist of NPY_1 and Y_5 receptor types. It is known that Y_5 receptors are activated at nanomolar agonist concentrations.

They determined the dose-dependent effect of Leu31, Pro34 - NPY on atrial and ventricular myocardial contractility. Leu31, Pro34-NPY does not cause significant changes among 7-day-old animals (10^{-7} - 10^{-5} M).

Among 21-day-old animals, Leu31, Pro34NPY at the concentration of 10^{-6} M caused significant changes in the amplitude-time parameters of the isometric contraction in the atria and ventricles (Fig. 1). Selective agonist at the concentration of 10^{-6} M caused the increase in myocardial contraction force by 12.8% in the ventricles and by 7.6% in the atria ($p < 0.05$, $n = 13$). The increase (10^{-5}M) and decrease (10^{-7}M) did not cause significant changes in the amplitude-time indicators of isometric contraction (Fig. 2). The action of Leu31, Pro34NPY is associated with the activation of NPY_1 receptors, since its effect completely disappears after the agonist removal from the drug bath.

Leu31, Pro34 NPY (10^{-6} M) on the strength of atrial

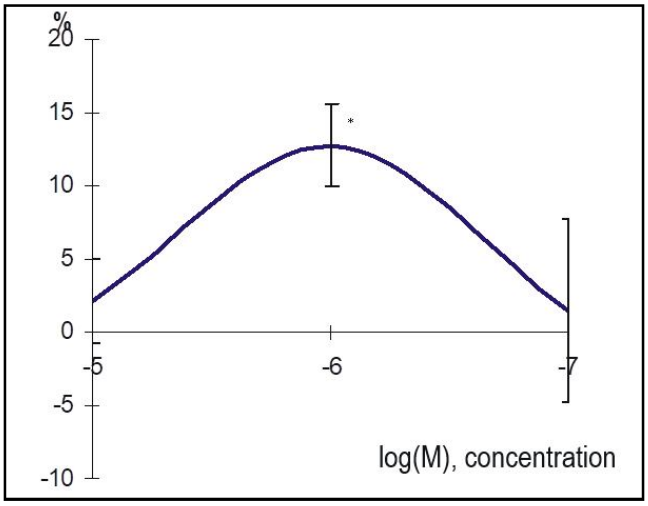

Fig. 1: The effect of different concentrations of Leu31, Pro34NPY on the amplitude of myocardial contraction among 21 day old rats.

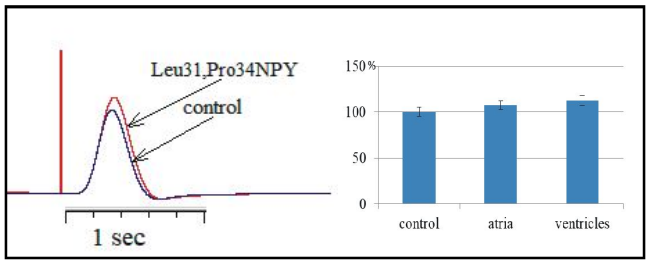

Fig. 2: The effect of Leu31, Pro34NPY (10^{-6} M) on the strength of atrial myocardial contraction among 21 day old rats (A - original record, B - the effect in percent).

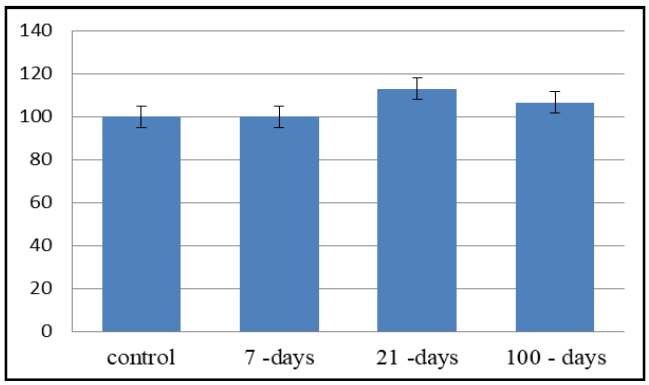

Fig. 3: The effect of Leu31, Pro34NPY on the strength of isometric atrial myocardial contraction. 7-day - 10^{-6} M, 21-day - 10^{-6} M, 100-day - 10^{-7} M.

myocardial contraction among 21 day old rats (A - original record, B - the effect in percent).

Among 100-day-old animals, Leu(31) Pro(34) NPY (10^{-7} M) leads to the development of a positive inotropic effect in the myocardium of the atria and ventricles. The largest increase of contraction amplitude relative to the initial values makes 6.6% ($p<0.05$, $n = 8$) and 7.7% ($p<0.05$, $n = 10$), respectively. The agonist causes a slight increase in the temporal parameters of the isometric contraction.

Leu(31) Pro(34) NPY at the concentration of $10^{-5, -6, -8, -9, -10}$ M among 100-day-old animals does not change the parameters of contractile activity. The duration of the isometric contraction of the myocardial strips is not changed significantly in the studied range of agonist concentration.

Summary

The force of isometric contraction is the main component of the heart operation, on which all hemodynamic parameters depend. The stronger the contraction, the faster the blood enters the body tissues. The most important factor regulating the force of contraction is cytosolic calcium increase. There are 2 mechanisms of calcium intake in cardiomyocytes: calcium entry from the extracellular medium through calcium

dependent L-type channels and calcium exit from the sarcoplasmic reticulum.

Neuropeptide Y, extracting together with norepinephrine and acetylcholine from synaptic vesicles, takes part in the regulation of the cardiovascular system through its own receptors, or by changing the activity of the main mediators. The action of neuropeptide Y in the heart of rats and humans can be realized through 3 main types of receptors: Y1, Y2 and Y5. Y1 and Y5 receptors are associated with the Gs protein, which stimulates the adenylate cyclase-cAMP protein kinase A cascade, which causes the entry of calcium ions into cardiomyocytes.

In our experiments, the selective Y1 receptor agonist caused the contraction force increase of myocardial strips among 21 and 100 day old rats. Thus, NPY1 receptors are involved in the development of a positive inotropic effect among 21- and 100-day old rats. NPY5 receptors can also be involved in a positive inotropic effect. The absence of significant changes in the amplitude-time characteristics among 7-day-old animals with the application of Leu31, Pro34NPY may be related to the fact that at this stage rats do not develop postnatal ontogenesis. L-type calcium channels have not been formed yet among newborn rats; in ventricular cardiomyocytes, T-type calcium channels are replaced by L-type Ca channels on the 21st day of animal life.

Conclusions

1. Leu (31) Pro (34) NpY increases atrial and ventricular myocardial contractility among 21 (10^{-6}M) and 100 (10^{-7}M) day old rats.
2. Leu (31) Pro (34) NpY does not affect rat myocardial contractility among 7 day old rats.

Conflict of Interest

The author confirms that the presented data do not contain a conflict of interest.

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