Research Article

Participation of neuropeptide Y_1 receptors in heart development regulation

Alekcei Anatolevich Zverev*, Tatiana Andreevna Anikina, Alevtina Vasilevna Krulova, Timur Lvovich Zefirov

ABSTRACT

Aim: Neuropeptide Y (NPY), containing 36 amino acid residues, belongs to the number of peptides widely distributed in the central and peripheral nervous system. NPY is released at high-frequency stimulation. The level of this peptide increases with circular shock, chronic stress, and heavy physical exercises. NPY and its receptors play a diverse role in the body. **Material and Methods:** The contractile activity of the myocardium in the experiment was studied on isolated strips of white 7-, 21-, and 100-day-old rat myocardium. All experiments were carried out according to the requirements of the World Society for the Protection of Animals and the European Convention for the Protection of Experimental Animals. The determination of myocardial contractile function reaction by Leu(31)Pro(34)NPY was carried out in three consecutively increasing concentrations on the "PowerLab" ("ADInstruments") unit with "MLT 050/D" force sensor ("ADInstruments"). **Result and Discussion:** Currently, six types of receptors for NPY (NPY1-6) have been found. Rat hearts have metabotropic NPY1, NPY2, NPY3, NPY4, and NPY5 receptors. The effect of NPY on the organs of a body is realized through post-synaptic receptors Y1, Y3, Y4, and Y5, and pre-synaptic receptors of Y2 type. They studied the effect of NPY receptor agonist on the myocardial contractility 7–100-day-old rats. During the analysis of myocardium strips isometric contraction among adult rats, it was established that an agonist at the concentration of 10−7 M causes a positive inotropic effect. Among 21-day-old rats, the increase in myocardial contractility is observed at the concentration of 10−6 M. **Conclusion:** Among 7-day-old animals, the addition of Leu(31) Pro(34)-NPY does not cause significant changes in isometric contraction rates. The obtained results indicate the functional activity of NPY1 receptors in the heart of 21- and 100-day-old rats.

KEY WORDS: Myocardial contractility, Neuropeptide Y, Neuropeptide Y receptors, Ontogenesis, Rat

INTRODUCTION

Neuropeptide Y (NPY), containing 36 amino acid residues, belongs to the number of peptides widely distributed in central and peripheral nervous system. It was established that NPY is released mainly at high-frequency stimulation of nerves (10-50 Hz). Other neurotransmitters are released at a lower frequency - <10 Hz.[1] The level of a man's NPY increases with a circular shock, chronic stress, and heavy physical exercises.[2]

It was established that the NPY is a cotransmitter and participates in a variety of vegetative functions, modulating the nerve transmission at the pre- and post-synaptic level.[3] NPY is found in large vesicles and can be caused by stressful situations.

NPY and its receptors are involved in the regulation of the cardiovascular system including frequency, cardiac contraction rate, and vascular tone.[4] The maturation of sympathetic and para-sympathetic regulatory influences on the heart, its receptor apparatus in ontogenesis occurs gradually.[5] An important role in the processes of heart age-related development is played by cotransmitters capable of increasing or weakening the effects produced by classical mediators, which must be taken into account during age and pathological process correction.

There are works that testify to the age-related changes in NPY-mediated cardiac innervation and regulation.[6,7] Hence, newborn animals had the lowest

Access this article online

Website: jprsolutions.info | ISSN: 0974-6943

Department of Man's Health Protection, Institute of Fundamental Medicine and Biology, Kazan Federal University, Kazan, Russia

***Corresponding author:** Alekcei Anatolevich Zverev, Institute of Fundamental Medicine and Biology, Kazan Federal University, 18 Kremlyovskaya Street, Kazan 420008, Russia. E-mail: Alekceii5@mail.ru

Received on: 18-07-2017; Revised on: 29-08-2017; Accepted on: 14-09-2017

density of NPY-containing sympathetic fibers and it increased during the first 20 days of life.[8]

NPY stimulates activation, migration, and proliferation of endotheliocytes in heart and vessels. It was established that NPY is required for the age-related development of L-type calcium channels in the myocardium.[3] There is evidence of the increase in the location of α- and β-adrenergic receptors in a cardiac muscle[9] under the influence of NPY,[9] which is important for the development of sympathetic regulatory influences on the heart.

The hearts of rats have metabotropic NPY_1, NPY_2, NPY_3, and NPY_5 receptors sensitive to pertussis. The most studied are the receptors of NPY_1- and Y_2-type.[1,10] The positive inotropic effect implementation of the selective NPY_1 agonist of receptor type is realized through Ca channels of L-type and the mobilization of calcium from the sarcoplasmic reticulum. At that the inhibition of adenylate cyclase and the decrease of production of cAMP production, as well as the stimulation of protein kinase C take place. NPY_1.and NPY_5 receptors are involved in the realization of this effect, which are capable of heterodimer development.[11] The receptors NPY_1 and NPY_5, as a rule, are located postsynaptic. The pre-synaptic effects of the NPY are take place mainly due to NPY_2 type receptors; nevertheless, there are also post-synaptic NPY_2 receptors, for example, in the myocardium. The stimulation of pre-synaptic NPY_2 leads to the suppression of various neurotransmitters release from the synaptic terminal: In the sympathetic nerves - noradrenaline, adenosine triphosphate, and the NPY itself, in parasympathetic - acetylcholine, in afferent - P substance, and peptide linked to the calcitonin gene.[1] The negative inotropic effect is associated with the activation of NPY_2 receptor type and the suppression of adenylate cyclase, which leads to the decrease of Ca_2+ current activity.[12]

The Aim of the Study

To study the functional activity of NPY_1 rat heart receptors in the regulation of myocardial contractility during postnatal ontogenesis.

MATERIALS AND METHODS

The contractile activity of the myocardium in the experiment was studied on isolated strips of white 7-, 21-, and 100-day-old rat myocardium. All experiments were carried out according to the requirements of the World Society for the Protection of Animals and the European Convention for the Protection of Experimental Animals. The determination of myocardial contractile function reaction by Leu(31)Pro(34)NPY was carried out in three consecutively increasing concentrations on the "PowerLab" ("ADInstruments") unit with "MLT 050/D" force sensor ("ADInstruments").

The chest was opened by the narcotized urethane; the heart was quickly removed and placed in a Petri dish with an oxygenated working solution with the connected "ESL-2" stimulator. The heart was quickly removed and placed in the bath with the working solution to which two stimulating electrodes were connected, and in accordance with the anatomical structure of the heart, the stripes of the myocardium were cut out from the right atrium and the right ventricle with the length of 2-3 mm and the diameter of 0.8-1.0 mm. The preparation was immersed in a separate 10 ml tank into which a working solution was supplied (μmol/L: NaCl - 119.8, KS1-5.4, $CaCl_2$-1.8, $MgCl_2$-1.05, NaH_2PO_4-0.42, and glucose - 5.05) with carbogen (95% of O_2 and 5% of CO_2). The pH was determined using a pH meter. To maintain the pH within 7.3-7.4, Trizma ("Sigma") base and acid buffers were added to the solution. The upper end of the drug was attached to a force sensor. The lower end was attached to a fixed base. The strips were stimulated through platinum electrodes with the frequency of 6 stimuli per minute and the duration of 5 ms.

The experiment was recorded on a personal computer using the "Chart 5.1" software. After the immersion of drugs in the reservoirs, the "run-in period" followed for 40-60 min, during which the optimal tension was gradually given to the muscle strips. Such a stretch point of the drug was considered an optimal tension, after which the contraction force of the drug began. At the end of the run-in, the initial reduction parameters were recorded for 10 min, then, for 20 min with one of the concentrations addition to the working solution Leu(31)Pro(34)NPY. At the end of Leu(31)Pro(34) NPY stimulation, the preparations were washed 3 times with the working solution for 20 min, then, the initial indices were recorded for each subsequent dose. The reaction of the force and the duration contraction was calculated in response to Leu(31)Pro(34)NPY as a percentage from the initial one.

The sympathetic nervous system plays an important role in heart regulation. The sympathetic innervation of the heart is not yet formed among 7-day-old rats. This age is characterized by its minimal presence in the heart. 21-day-old rats are characterized by the increase in the activity of sympathetic regulatory influences on the heart, and by high rates of heart contractions. The sympathetic nervous system is formed among 100-day animals. With this approach, in our opinion, it is possible to cover the main periods of rat development and to trace the formation of cardiac activity regulation in different stages of postnatal ontogenesis.[5]

The reliability of the differences was calculated according to the absolute values of the studied

indicators using the paired t Student test ($P < 0.05$). All used chemical reagents were produced by "Tokris" company.

RESULTS

To determine the functional activity of NPY receptors, a series of experiments was performed with the preparation Leu 31),Pro(34)NPY, which is the agonist of NPY_1 and Y_5-type receptors.

The dose-dependent effect of Leu(31) Pro(34) NPY on the contractility of the atrium and ventricle myocardium was determined. 7-day animals treated with Leu(31)Pro(34)NPY at the concentration of 10^{-5}-10^{-7} M did not have any significant changes in isometric contraction of myocardial striae within atriums and ventricles [Figure 1].

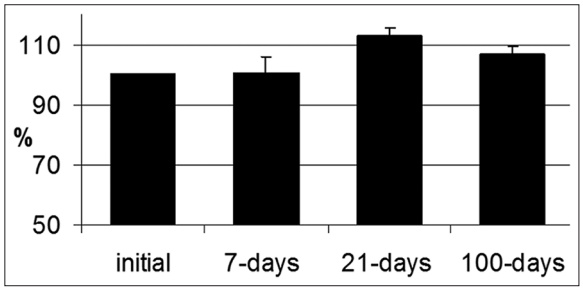

Figure 1: The effect of Leu(31)Pro(34)NPY on the force of atrial myocardium strips isometric contraction among 7-100-day-old rats

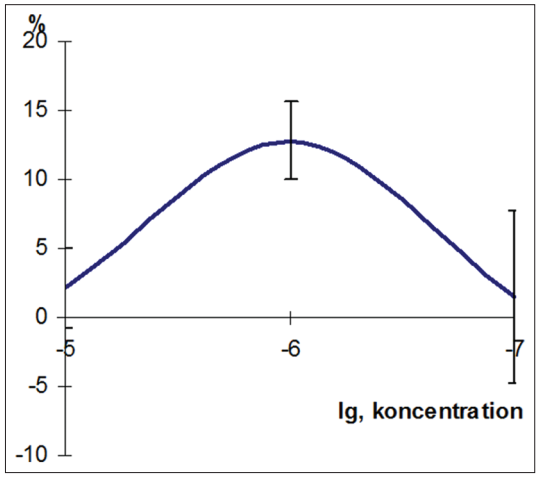

Figure 2: The dose-dependent effect of Leu(31)Pro(34)NPY on the contraction force of ventricle myocardium among 21-day-old rats

Among 21-day-old animals Leu(31)Pro(34) NPY, at the concentration of 10^{-6} M caused significant changes in the amplitude-time characteristics of myocardium striae isometric contraction within atria and ventricles. The concentration of the agonist 10^{-6} M caused the increase in the strength of the ventricular myocardium strip contraction by 12.8 + 2.8% ($P < 0.05$, $n = 6$) (Figure 2) and atria by 7.6 + 1.51% ($P < 0.05$, $n = 7$). The agonist at the concentration of 10^{-5} M and 10^{-7} M did not cause any significant changes in the strength of myocardium strip contraction both in atriums and ventricles (Figures 1-3). The duration of myocardial strip isometric contraction does not change significantly.

Leu(31)Pro(34) NPY among 100-day-old animals at the concentration of 10^{-7} M causes a positive inotropic effect in the myocardium of the atria and ventricles. The maximum increase of ventricular and atrial myocardium contraction force makes 6.6 ± 2.8% ($P < 0.05$, $n = 8$) and 7.7 ± 3.2% ($P < 0.05$, $n = 10$), respectively (Figure 1). The duration of isometric contraction of myocardial strips does not change significantly. There is no reliable increase in the rate of relaxation, contraction, and relaxation time, as well as the decrease in the rate of contraction, both in the ventricles and in the atria.

The agonist at the concentrations of $10^{-5, -6, -8, -9, -10}$ M among adult animals do not lead to a significant change of myocardial striae contractile activity. Among the concentrations under study, the most effective concentration was 10^{-6} M. The addition of the agonist leads to the increase of myocardial contractility in the ventricles 4.1% ($n = 8$), atria 3.6% ($n = 8$). The duration of isometric contraction of myocardial strips does not significantly change in the range of agonist concentration under study.

DISCUSSION

Thus, the results obtained by us show that the realization of the positive inotropic effect of Leu(31)

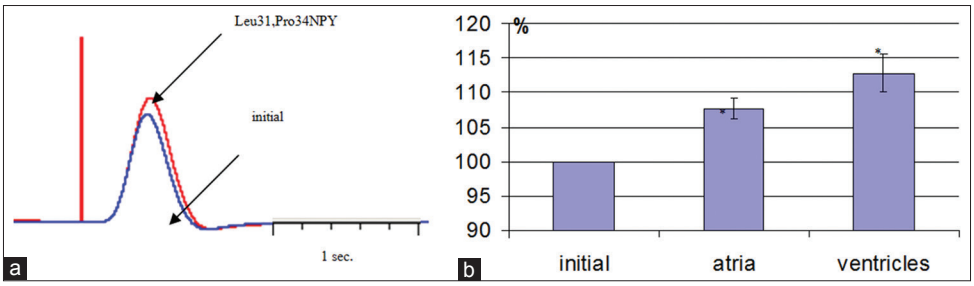

Figure 3: The effect of Leu(31)Pro(34)NPY on the force of atrial myocardium strip isometric contraction among 21-day-old rats (a) gr; (b) %

Pro(34) NPY among 21- and 100-day-old rats is carried out with the participation of NPY_1 receptors. In a positive inotropic effect, the participation of NPY_5 receptors is possible. This drug is able to activate a NPY_5 type of receptor in high concentration.[13]

The lack of significant changes in the amplitude-time characteristics among 7-day animals in the application of Leu(31)Pro(34)NPY may be related to the fact that at this stage of postnatal ontogeny rats did not develop the vegetative regulation of the heart. An increase in the concentration of the agonist causing the increase in myocardial contraction force from 100 to 7 days indicates a higher sensitivity of NPY receptors among adult animals.

CONCLUSIONS

1. Leu(31)Pro(34)NPY at the concentration of 10^{-6} M increases the contractility of 21- and 100-day-old rat myocardium within atria and ventricles.
2. Leu(31)Pro(34)NPY at the concentration of 10^{-5}-10^{-7} M does not alter the contractility of 7-day-old rat myocardium.

ACKNOWLEDGMENTS

The work is performed according to the Russian Government Program of Competitive Growth of Kazan Federal University, RFBR No. 15-04-05384, No. 17-04-00071.

REFERENCES

1. Hodges J, Jackson DN, Mattar L, Johnson JM, Shoemaker JK. Neuropeptide Y and neurovascular control in skeletal muscle and skin. Am J Physiol Regul Integr Comp Physiol. 2009;297:546-55.
2. Hökfelt T, Broberger C, Xu ZQ, Sergeyev V, Ubink R, Diez M. Neuropeptides-an overview. Neuropharmacology. 2000;39(8):1337-56.
3. Protas L, Qu J, Robinson RB. Neuropeptide Y: Neurotransmitter or trophic factor in the heart? News Physiol Sci. 2003;18:181-5.
4. Koroleva SV, Ashmarin IP. Neuropeptide Y: Diversity and apparent contradictoriness of functions. The analysis of possible mediated effects. Prog Physiol Sci. 2000;31(1):31.
5. Sitdikov FG, Anikina TA, Zverev AA, Bilalova GA, Khamzina EY. Purinergic regulation of rat heart function in ontogeny. Russ J Dev Biol. 2008;39(5):269-74.
6. Masliukov PM, Moiseev K, Emanuilov AI, Anikina TA, Zverev AA, Nozdrachev AD. Development of neuropeptide Y-mediated heart innervation in rats. Neuropeptides. 2016;55:47-54.
7. Zverev AA, Anikina TA, Maslyukov PM, Zefirov TL. Role of neuropeptide Y in myocardial contractility of rats during early postnatal ontogeny. Bull Exp Biol Med. 2014;157(4):421-3.
8. Maslyukov PM, Emanuilov AI, Bulibin AV, Zverev AA, Anikina TA. Morphological features of neuropeptide Y-ergic innervation of heart in postnatal ontogenesis. Morphology. 2014;146(6):46-50.
9. Rocha-Singh KJ, Matsuo R, Karliner JS. Prolonged incubation with neuropeptide Y upregulates beta-adrenoceptors yet does not cause supersensitivity of beta-adrenoceptor signaling. Eur J Pharmacol. 1995;288:349-53.
10. Michel MC, Beck-Sickinger A, Cox H, Doods HN, Herzog H, Larhammar D, *et al*. XVI. International union of pharmacology recommendations for the nomenclature of neuropeptide Y, peptide YY, and pancreatic polypeptide receptors. Pharmacol Rev. 1998;50:143-50.
11. Dinger MC, Bader JE, Kobor AD, Kretzschmar AK, Beck-Sickinger AG. Homodimerization of neuropeptide Y receptors investigated by fluorescence resonance energy transfer in living cells. J Biol Chem. 2003;278:10562-71.
12. Wahlestedt C. Effects of neuropeptide Y (NPY) on isolated guinea-pig heart. Acta Physiol Scand. 1987;129(4):459-63.
13. Cox HM, Tough IR. Functional characterization of receptors with affinity for PYY, NPY, [Leu31, Pro34]NPY and PP in a human colonic epithelial cell line. Br J Pharmacol. 1995;116(6):2673-8.