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NPY_1 Receptors Participate in the Regulation of Myocardial Contractility in Rats

P. M. Masliukov*, T. A. Anikina, A. A. Zverev, A. V. Krylova, K. Yu. Moiseev*, and T. L. Zefirov

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Selective agonist (Leu(31)Pro(34)NPY) and blocker (BIBP-3226) of NPY_1 receptors were used to determine the type of NPY receptors involved in myocardial contraction. Experiments with isometric contraction of myocardial strips from mature rats showed that the agonist produced the most potent effect in a concentration of 10^{-7} M. In this concentration, Leu(31)Pro(34)NPY showed the greatest positive inotropic effect on the contraction of the atria and ventricles. In contrast, selective blocker BIBP-3226 reduced the force of myocardial contractions. Pretreatment of myocardial strips with this blocker abolished the positive inotropic effect of Leu(31)Pro(34)NPY, which attested to important role of NPY_1 receptors in myocardial contraction.

Key Words: *neuropeptide Y; types of NPY receptors; myocardial contractility; rat*

Neuropeptide Y (NPY) regulates vascular tone, rate and force of cardiac contractions, produces trophic effects, and stimulates cell proliferation in the heart, blood vessels, and adipose tissue [2]. Despite a spectacular number of morphological studies demonstrating the presence of various types of NPY receptors in the heart, the role of many NPY receptors remains unclear.

Immunohistochemistry detected Y_1, Y_2, Y_3, and Y_5 receptors in rat endocardium and myocardium [4]. All types of NPY receptors can be pharmacologically discriminated using the appropriate blockers. This approach was especially fruitful in the study of Y_1 and Y_2 receptors. The postsynaptic action of NPY receptor agonists in the myocardium can manifest in negative or positive inotropic and chronotropic effects [1,3]. The positive inotropic effect is mediated via Y_1 receptors, L-type Ca^{2+} channels, and mobilization of calcium ions from the sarcoplasmic reticulum [6]. The negative inotropic effect is related to activation of Y_2 receptors and inhibition of adenylate cyclase resulting in suppression of Ca^{2+} current [7].

Our aim was to determine the type(s) of NPY receptors implicated in the regulation of contractile activity of atrial and ventricular myocardium in mature rats.

MATERIALS AND METHODS

Contractile activity of the myocardium was studied *in vitro* on myocardial strips isolated from outbred albino rats. All experiments were performed in strict adherence to the Regulations of World Society for the Protection of Animals *(*WSPA) and European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes (ETS No. 123). For evaluation of the changes in the myocardial contractile function to Leu(31)Pro(34)NPY, the agonist was applied in 3 increasing concentrations; PowerLab data acquisition system (ADInstruments) coupled to a MLT 050/D force transducer were used to measure and record the contractile force of myocardial strips. The data were analyzed using Chart 5.1 software.

Department of Anatomy, Physiology, and Human Health Protection, Kazan Federal University, Kazan; *Department of Normal Physiology and Biophysics, Yaroslavl State Medical University, Yaroslavl, Russia. ***Address for correspondence:*** alekcei5@rambler.ru. A. A. Zverev

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In urethane-anesthetized rats, the hearts had been rapidly removed after thoracotomy and placed into a Petri dish with oxygenated solution, where they were electrically stimulated with an ESL-2 generator. Myocardial strips were isolated and placed in a 10-ml chamber filled with oxygenated (95% O_2/5% CO_2) Krebs—Ringer solution containing (in mM): 119.8 NaCl; 5.4 KCl; 1.8 $CaCl_2$; 1.05 $MgCl_2$; 0.42 NaH_2PO_4; 5.05 glucose (pH 7.3-7.4) was adjusted with Trizma (Sigma) base and acid buffers. The strips were stimulated via platinum electrodes with 5-msec rectangular electric pulses at a rate of 6 min^{-1}.

After placing the strip into the chamber, it had been stimulated during the accommodation period lasting for 40-60 min used to select the optimal length and tension of the strip. After the end of this accommodation period, the initial (baseline) contractile parameters were recorded for 10 min; thereupon Leu(31)Pro(34)NPY in a selected initial concentration was added to the chamber, and the contractile parameters were recorded over 20 min. Then the strip was 3 times washed with Krebs—Ringer solution for 20 min; the baseline parameters were recorded for 10 min before addition of the next concentration of Leu(31)Pro(34)NPY. The effects of the agonist on myocardial strips pretreated with BIBP-3226 (selective blocker of NPY_1 receptors) were studied as follows: first, isometric contractions were recorded in the presence of the agonist; then, the strips were washed, the blocker was added to the chamber, and 12 min later, the agonist Leu(31)Pro(34)NPY was added to the bathing solution again. The force and duration of contractions induced under the action of agonist and/or antagonist were expressed in percentage to baseline levels. The absolute values were analyzed statistically using paired Student's *t* test at $p<0.05$. All reagents were from Sigma.

RESULTS

To confirm the presence of functionally active NPY receptors in the myocardium, the effect of selective NPY_1 receptor agonist Leu(31)Pro(34)NPY on myocardial contractility in 100-day rats were studied in a special series of experiments. This agonist in a concentration range of 10^{-10}-10^{-5} M induced dose-dependent contractions of atrial and ventricular strips.

In a concentration of 10^{-7} M, Leu(31)Pro(34)NPY induced a significant positive inotropic effect on atrial and ventricular myocardial strips, the respective increments of contractile force being 7.7±3.2% ($p<0.05$; $n=10$) and 6.6±2.8% ($n=8$). The duration of isometric contraction of both types of the strips did not change significantly. We observed an insignificant increase in contraction time, relaxation rate, and relaxation time

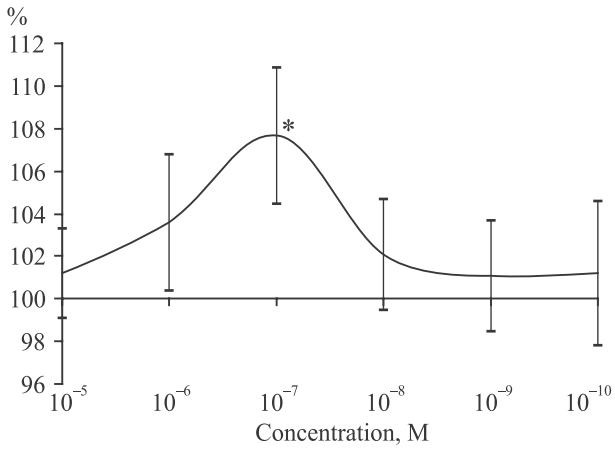

Fig. 1. Dose-dependent trend in the effect of NPY_1 receptor agonist Leu(31)Pro(34)NPY on isometric contractile force developed by atrial myocardial strips isolated from 100-day rats. Here and in Fig. 2: *$p<0.05$ in comparison with the control level.

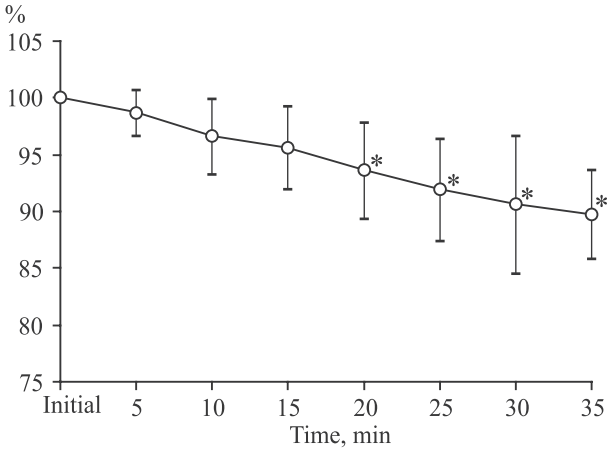

Fig. 2. Effect of NPY_1 receptor blocker BIBP-3226 (10^{-7} M) on isometric contractile force developed by atrial myocardial strips isolated from 100-day rats.

accompanied by a decrease in contraction rate in both the atrial and ventricular strips.

The agonist applied in concentrations of 10^{-10}, 10^{-9}, 10^{-8}, 10^{-6}, and 10^{-5} M produced no significant effect on the parameters of contractile activity of myocardial strips (Fig. 1). At this concentration range, Leu(31)Pro(34)NPY induced no significant changes in duration of isometric contraction of myocardial strips.

The effect of BIBP-3226 (a selective antagonist to NPY_1 receptors) on myocardial contractile activity is little known. Its effects on the amplitude-temporal parameters of myocardial contractility were observed for 35 min at a concentration of 10^{-5} M [5]. In our study, BIBP-3226 produced a negative inotropic effect in 10-12 min after its application to atrial and ventricular strips. In atrial and ventricular strips, the maximum negative inotropic effects were 10.3±3.9 % ($n=8$) and 9.8±3.9% ($p<0.05$, $n=8$), respectively. At this, duration of contraction in atrial and ventricular strips decreased

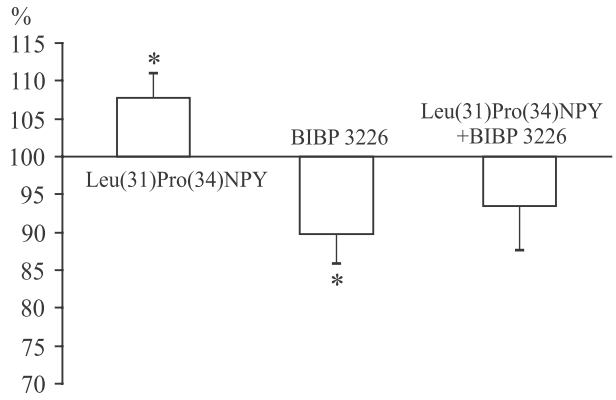

Fig. 3. Combined effects of NPY_1 receptor agonist Leu(31)Pro(34)NPY and antagonist BIBP-3226 on isometric contractile force developed by atrial myocardial strips isolated from 100-day rats.

by 12.4±7.2 and 9.1±6.3%, respectively. In addition, the blocker decreased the contraction rate of atrial strips by 9.3±7.2% ($p<0.05$, $n=8$) without any effect on their relaxation rate. In ventricular strips, BIBP-3226 induced insignificant increase of contraction and relaxation rates by 1.6 and 5.6%, respectively.

To determine the subtype of NPY receptors involved in cardiac inotropic effects, we studied the effect of Leu(31)Pro(34)NPY in most effective concentration (10^{-7} M) in the presence of selective NPY_1 receptor blocker BIBP-3226 (Fig. 2). Combined application of the agonist and antagonist of NPY_1 receptors produced no significant negative inotropic effect, which was observed after individual application of the blocker (Fig. 3).

The present findings showed that the positive inotropic effect of Leu(31)Pro(34)NPY is mediated predominantly via NPY_1 receptors, although NPY_5 receptors can be also implicated in this effect.

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