

Effect of Neuropeptide Y on Action Potential Generation in Working Cardiomyocytes of the Right Atrium in Rat Heart

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We studied the effect of neuropeptide Y in concentrations of 10^{-8} - 10^{-6} M on electrical activity of adult rat right atrial cardiomyocytes with preserved spontaneous activity. Neuropeptide Y was found to modulate the amplitude-time parameters of action potential: in concentrations of 10^{-7} and 10^{-6} M it reduced the membrane potential, increased the amplitude of action potential and duration of the repolarization phase, and reduced the frequency of action potential generation. In concentration of 10^{-6} M, neuropeptide Y produced stronger effect on the analyzed parameters, while in concentration of 10^{-8} M it produced no significant changes.

Key Words: *neuropeptide Y; action potential; membrane potential; cardiomyocytes; rat*

Neuropeptide Y (NPY) and its receptors are involved in various functions of the cardiovascular system. NPY affects HR, heart force, vascular tone, and produces trophic effects promoting proliferation of the myocardium cells, blood vessels, and adipose tissue [11]. Addition of NPY induces a 2-fold increase in the intracellular calcium concentration in working cardiomyocytes [11].

NPY is considered as the main co-transmitter of the sympathetic nervous system [1]. NPY is stored in synaptic vesicles of nerve endings and is released after electrical stimulation, acting through specific receptors. NPY acts through 6 known receptors (Y1-Y6) and is the most common neuropeptide in the heart [4,6,8,9]. All NPY receptors are metabotropic and their activity is mediated by G proteins: G_i , G_{s3} , and G_{so} . All effects in the heart are mediated by activation of Y1, Y2, and Y5 receptors. NPY has been identified in the myocardium, endocardium and cardiomyocytes [9]. About $2/3$ neurons in mammalian sympathetic ganglia contain this peptide beside norepinephrine [7]. In the

heart intramural nodes, NPY was detected in the vast majority (>80%) of neurons, 100% of NPY⁺ neurons also contain acetylcholine [12]. In rats, NPYergic heart innervation is present since birth [2].

The effect of NPY was studied in isolated cardiomyocytes [10], myocardial strips [8,13], isolated hearts [5]. We have not found any research on the effect of NPY on myocardial electrical activity.

The aim of our study was to investigate the effect of NPY on the parameters of electrical activity of rat right atrial preparations with their own rhythm.

MATERIALS AND METHODS

The study was carried out on 100-day white outbred laboratory rats ($n=23$), kept under identical conditions in the vivarium of Kazan Federal University.

The thorax was opened under urethane anesthesia, heart was quickly removed, placed in a Petri dish with oxygenated working solution, and the preparation of right atrial appendage with sinoatrial node, transverse crest, and fragments of the superior and inferior vena cava was prepared. The preparation was placed in a perfusion chamber with thermostated ($37\pm 1^\circ\text{C}$) solution (in mmol/liter): 129 NaCl, 4 KCl, 1.2 CaCl_2 , 0.5 MgSO_4 , 20.9 NaH_2PO_4 , 20 NaHCO_3 , and 5 glucose (95% O_2 and 5% CO_2). To maintain pH within 7.3-7.4,

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Trizma base and acid buffers (Sigma) were added to the solution.

Electrical activity of cardiomyocytes was studied using microelectrode leads on a right atrial preparation of rats with preserved sinoatrial node and spontaneous activity. All experiments were conducted in accordance with ethical standards. Electrical activity of cardiomyocytes after application of NPY in increasing concentrations (10^{-8} , 10^{-7} , and 10^{-6} M).

Membrane potential (MP) and action potential (AP) were recorded using glass microelectrodes (tip diameter $<1 \mu$, resistance 30-80 M Ω) made on the day of the experiment using a special horizontal puller P-1000 (Sutter Instruments). The signals were amplified and AP parameters were analyzed using an analog-to-digital converter E14-140 (L-Card). The records of myocardium electrical activity were analyzed using the custom-made software Elph 3.0. Data processing included evaluation of MP value, AP amplitude, AP depolarization phase duration, AP repolarization phase duration at the level of 20, 50, and 90% AP decay (APD20%, APD50%, and APD90%). AP amplitude was measured from the MP level to the AP peak. Duration of the depolarization phase was determined as the time from AP beginning to the AP peak. Duration of the repolarization phase was calculated as the time from the AP peak to the APD20%, APD50%, and APD90%. In addition, AP generation frequency was determined from the time interval between the neighboring AP peaks.

AP parameters were recorded on minutes 7 and 15 after NPY application. AP frequency prior to NPY administration was taken as the initial value (control). In the experiment, chemical reagents from Sigma and Tocris were used.

The data were processed statistically using PowerGraph Professional software (Disoft). Statistical significance was assessed using Student's *t* test.

RESULTS

NPY, a nonselective NPY receptor agonist, caused a concentration-dependent change in the electrical activity in rat right atrial myocardium. NPY in a concentration of 10^{-8} M did not significantly affect the studied parameters.

NPY in a concentration of 10^{-7} M markedly modulated electrical activity in rat atrial myocardium. In preparations with their own rhythm, MP value against the background of NPY application decreased from -75.2 ± 3.2 to -64.2 ± 3.4 mV, *i.e.* by 14% ($p < 0.05$; $n = 9$). By minute 15, depolarization phase duration decreased by 14%, from 0.21 ± 0.02 to 0.18 ± 0.02 msec ($p < 0.05$). The AP amplitude increased from 98.9 ± 13.2 to 120.6 ± 11.1 mV, *i.e.* by 22% ($p < 0.05$). By minute 15, APD20% increased from 4.3 ± 0.2 to 4.7 ± 0.2 msec,

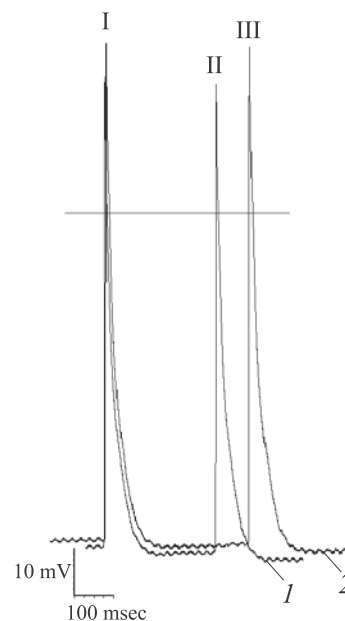


Fig. 1. Effect of NPY in a concentration of 10^{-7} M on AP generation frequency in cardiomyocytes in spontaneously contracting preparations of rat right atrium (original record). 1) Control (initial values), 2) NPY, 10^{-7} M.

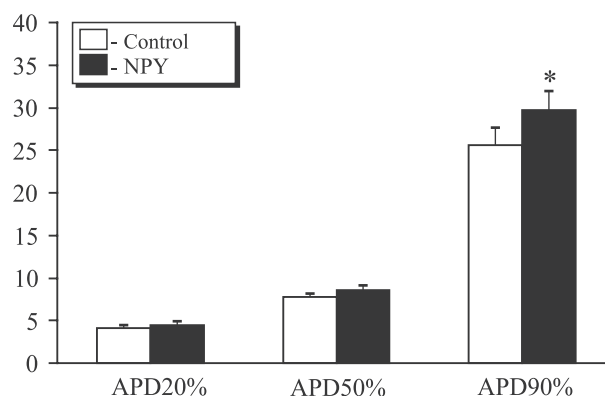


Fig. 2. APD20%, APD50%, and APD90% in the preparations of rat right atrium in control and upon exposure to 10^{-6} M of NPY. * $p < 0.05$ in comparison with the control.

APD50% increased from 7.6 ± 0.2 to 8.3 ± 0.3 msec, APD90% — from 24.9 ± 3.1 to 27.6 ± 4.1 msec ($p < 0.05$), *i.e.* by 9, 8, and 10% respectively (Fig. 1).

The increase in AP duration, *i.e.* AP repolarization phase delay was accompanied by spontaneous rhythm deceleration. By minute 7, AP frequency decreased from 302 ± 15 to 256 ± 12 min^{-1} ($p < 0.05$), which is 15% of the initial value. By minute 15, AP frequency was 309 ± 21 min^{-1} . Thus, in contrast to the MP and AP parameters, the increase in the frequency of spontaneous activity was transient and returned to the initial values by minute 15.

NPY addition in a concentration of 10^{-6} M reduced the MP value from -77.3 ± 2.5 to -68.1 ± 4.8 mV ($p < 0.05$; $n = 7$), *i.e.* by 12% of the original value. By

minute 15, the depolarization phase duration decreased by 18% of the initial value: from 0.22 ± 0.01 to 0.18 ± 0.02 msec ($p < 0.05$). AP amplitude increased from 101.2 ± 9.5 to 124.5 ± 10.3 mV, *i.e.* by 23% ($p < 0.05$). By minute 15, APD20% increased by 8%, APD50% — by 10% ($p < 0.05$), APD90% — by 14% ($p < 0.05$) (Fig. 2).

AP frequency changed unidirectionally, by minute 15 it decreased from 296 ± 37 to 183 ± 24 min⁻¹ ($p < 0.01$), *i.e.* by 38% of the initial value.

Thus, NPY receptor activation by NPY in the right atrial cardiomyocytes altered the MP value and the amplitude-time AP parameters.

It is known that the formation of cardiomyocyte AP involves numerous ion channels [3]. Our data on MP decrease in response to NPY can be explained by modification of the rest K channels. Altered amplitude-time AP parameters upon exposure to NPY can be explained by altered kinetics of the Na⁺ and Ca²⁺ channels of cardiomyocytes. The repolarization phase protraction is possibly related to altered kinetics of the potential-dependent K⁺ channels, which leads to a decrease in the total K⁺ current. Altered spontaneous rhythm frequency indicates that NPY also affects atypical cardiomyocytes, which is probably associated with modification of Ca²⁺ channels and If currents.

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