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Mechanisms of Modulation of Adrenergic Regulation of Spontaneous Activity Rate and Atrial Myocardial Contractility in Early Postnatal Ontogeny in Rats

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We studied combined effect of the $\beta_{1,2}$-adrenoreceptor agonist isoproterenol and the $Y_{1,5}$ receptor agonist [Leu31, Pro34]neuropeptide Y on the frequency of spontaneous activity and myocardial contractility in 21- and 100-day-old rats. Isoproterenol increased the frequency of spontaneous activity and reduced the main parameters of isometric contraction of the atrial myocardium. When [Leu31, Pro34]neuropeptide Y was added, the frequency of spontaneous activity and the negative inotropic and the positive chronotropic effects of isoproterenol were reduced in 100-day-old rats. In 21-day-olds rats, a tendency to a decrease in the effect of isoproterenol was observed.

Key Words: *frequency of spontaneous activity; NPY receptors; $Y_{1,5}$ receptor agonist; isoproterenol; contractility*

In the cardiovascular system, neuropeptide Y (NPY) is present in neurons, cardiomyocytes, and the endocardium and is involved in physiological processes, including myocardial contraction rate and force [1]. It is known that heart failure can manifest itself in increased activity of the sympathetic nervous system [2]. There is evidence for a relationship between NPY levels and the severity of heart failure. Plasma levels of NPY and norepinephrine increase during periods of sympathetic activity [3].

In the sympathetic nervous system, the main comediators are ATP and NPY [4,5]. NPY fully meets the neurotransmitter criteria because it is stored in synaptic vesicles, is released upon stimulation, and acts on receptors located on the cell membrane. The action of NPY in the heart is realized through different types of pre- and postsynaptic receptors. Cardiomyocytes are characterized by high density of NPY-sensitive receptors [6]. There are several types of NPY receptors in the heart: Y_1, Y_2, Y_3, and Y_5. NPY content in the atria is higher than in the ventricles [7]. NPY released by sympathetic nerves together with norepinephrine under physiological conditions or entering the extracellular environment from other sources is a component of the sympathetic regulatory system as well as a part of a previously unexplored mechanism of cardiac regulation. The activity of sympathetic regulatory influences on the heart changes during ontogenesis. Therefore, the physiological role of NPY in the developing heart requires further study.

The purpose of this work is to investigate the combined effect of the $\beta_{1,2}$-adrenoreceptor agonist isoproterenol and the $Y_{1,5}$ receptor agonist [Leu31, Pro34] NPY on the frequency of spontaneous activation and parameters of atrial isometric myocardial contraction in 21- and 100-day-old rats.

MATERIALS AND METHODS

We used 21-day-old (n=12) and 100-day-old (n=13) white outbred rats as an experimental model. In 21-day-old animals, the high level of sympathetic effects on the heart is accompanied by high HR. In 100-day-old rats, morphological and regulatory sympathetic

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influences on the heart are fully formed [8]. The experiments were performed in accordance with the Directive 2010/63/EU of the European Parliament and of the Council (September 22, 2010; On the Protection of Animals Used for Scientific Purposes).

To record the amplitude-time indices of isometric contraction of the atrial myocardium, a strain-measuring method was chosen. Isometric contraction of myocardial strips was performed on a PowerLab (ADInstruments) with an MLT 050/D force transducer (ADInstruments). The animals were anesthetized with 25% urethane solution (1.2 g/kg body weight; intraperitoneally) and fixed on a well-lit operating table, the thorax was opened below the xiphoid process toward the right and left clavicles, the heart was fixed with forceps and cut off from the main arteries with scissors. The extracted heart was placed in a beaker with Tyrode's solution, then transferred to a Petri dish, where strips of right atrial myocardium with preserved sinus node and spontaneous activity were prepared. Myocardial strips were immersed in a special tank and perfused with Tyrode's solution (in g/liter: 7.54 NaCl, 0.134 $CaCl_2$, 0.3 KCl, 0.06 $MgSO_4$, 0.14 NaH_2PO_4, 1.68 $NaHCO_3$, and 0.9 glucose; Sigma-Aldrich) saturated with carbogen throughout the experiment. The strips were fixed to the force sensor and fulcrum with silk threads. Mechanical isometric contraction was recorded on a personal computer (Chart 5.1 software).

Myocardial preparations were immersed in baths perfused with working Tyrode's solution. During the "running-in" period, the strips were maximally stretched. Then, the initial value of isometric contraction was recorded. We studied the effects of the $\beta_{1,2}$ adrenoreceptor agonist isoproterenol (10^{-5} M; Sigma-Aldrich) and the combined effects of isoproterenol and the $Y_{1,5}$ receptor agonist [Leu31, Pro34]NPY (10^{-7} M; Sigma-Aldrich) on isometric atrial myocardial contraction parameters in 21- and 100-day-old rats with preserved sinus node and spontaneous activity. To determine the possible effect of NPY on the effects of isoproterenol, the $Y_{1,5}$ receptor agonist [Leu31, Pro34]NPY (10^{-7} M) was added on the second minute after isoproterenol application.

The following parameters of isometric contraction were calculated: spontaneous activity rate (SAR, peak/min), amplitude of atrial myocardial contraction (g), and duration of isometric contraction (msec). The initial values of SAR and myocardial contractility after the "running-in" period before drug administration were used as a control.

The isometric contraction curve was processed using Chart 8.0 software. When analyzing the amplitude-time values, the values rounded to 2 and 4 decimal places were taken into account. The results were statistically processed using Microsoft Excel and SPSS Statistics 20 (IBM). Significance of differences was evaluated using the paired Student's test after verification of distribution normality at $p<0.05$. The data are presented as the $M\pm m$; n is the number of atrial myocardial preparations used.

RESULTS

In 21-day-old animals, isoproterenol caused a 107% ($p<0.001$, $n=5$) increase in SAR, a 51% decrease in contraction duration ($p<0.001$, $n=5$), and an 80% decrease in contraction amplitude ($p<0.001$, $n=5$) (Table 1). Application of isoproterenol and [Leu31, Pro34]NPY (Fig. 1) resulted in an 80% increase in SAR (from 171.1±20.8 to 308.0±25.2 peaks/min; $p<0.01$, $n=7$), decreased contraction duration by 46% (from 327.0±32.8 to 175.2±18.7 msec; $p<0.01$, $n=7$) and contraction amplitude by 70% (from 0.0080±0.0025 to 0.0011±0.0004 g; $p<0.01$, $n=7$). In 100-day-old rats, isoproterenol increased SAR by 214% ($p<0.001$, $n=5$) and decreased the duration of contractions by 49% ($p<0.01$, $n=5$) and their amplitude by 71% ($p<0.001$, $n=5$) (Table 1). Combined addition of isoproterenol and [Leu31, Pro34]NPY (Fig. 2) increased SAR by 89% (from 137.5±27.4 to 260.5±8.9 peaks/min; $p<0.001$, $n=8$) and reduced the duration of contraction by 47% ($p<0.001$, $n=8$) and contraction amplitude by 49% ($p<0.01$, $n=8$). Thus, in 100-day-old animals, SAR and negative inotropic and positive chronotropic effects of isoproterenol were reduced when coadministered with [Leu31, Pro34]NPY. In 21-day-old rats,

TABLE 1. Effect of Isoproterenol on Parameters of Isometric Contraction of Atrial Myocardium with Spontaneous Activity in 21- and 100-Day-Old Rats ($M\pm m$)

Parameter	21-day-old rats		100-day-old rats	
	control	isoproterenol	control	isoproterenol
PCA, peak/min	154.0±2.0	319.0±1.3***	76.2±7.9	239.7±19.3***
Duration of contraction, msec	331.2±4.6	163.9±1.1***	392.0±40.8	208.3±8.9**
Amplitude of contraction, g	0.0023±0.001	0.0019±0.0001***	0.0320±0.0037	0.0163±0.0028***

Note. **$p<0.01$, ***$p<0.001$ in comparison with the corresponding control.

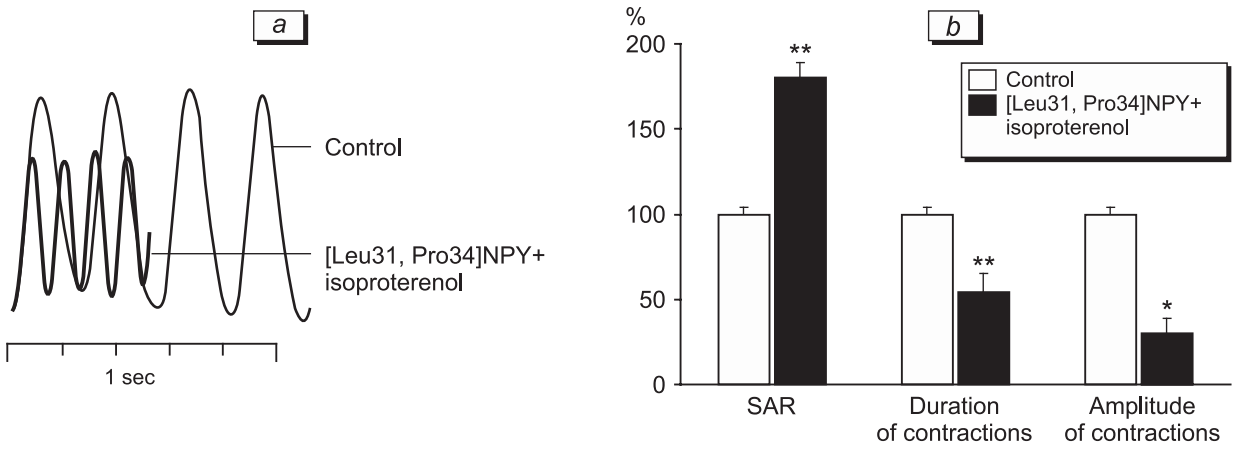

Fig. 1. Effect of isoproterenol and $Y_{1,5}$ receptor agonist [Leu31, Pro34]NPY on isometric contraction parameters of atrial myocardium with preserved sinus node and spontaneous activity in 21-day-old rats. *a*) Original record, *b*) changes in parameters in comparison with the control. *p<0.05, **p<0.01.

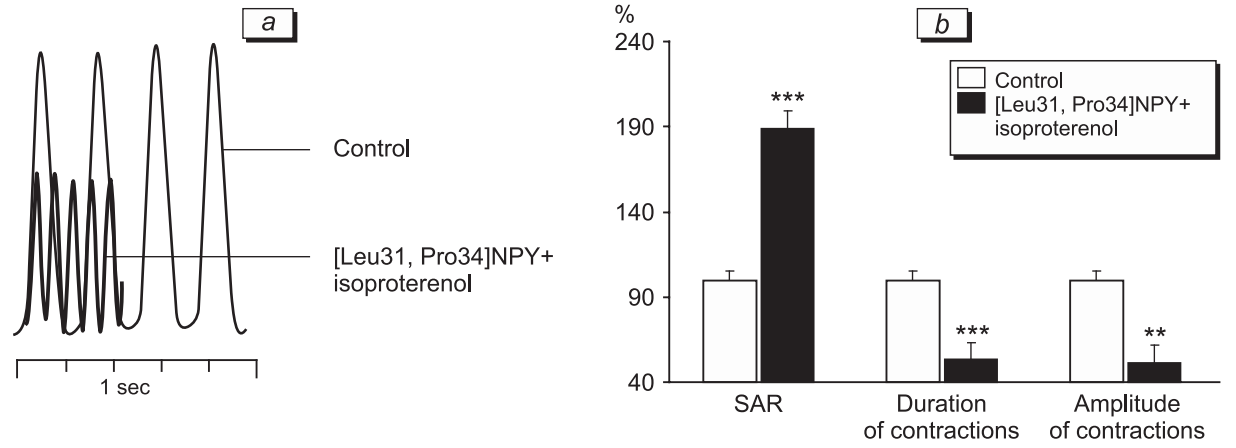

Fig. 2. Effect of isoproterenol and the $Y_{1,5}$ receptor agonist [Leu31, Pro34]NPY on isometric contraction parameters of atrial myocardium with preserved sinus node and spontaneous activity in 100-day-old rats. *a*) Original record, *b*) parameters in comparison with the control. **p<0.01, ***p<0.01.

a tendency to a decrease in the effect of isoproterenol was seen.

The results suggest the following mechanism of decreased SAR and atrial myocardial contractility under the combined effect of agonists. $NPY_{1,5}$ receptors in cardiomyocytes are conjugated to different G-proteins, such as Gi/o-proteins that inhibit adenylate cyclase, adenylate cyclase–cAMP–protein kinase A cascade and cause calcium channel inhibition [9]. Y_1 receptors are localized on atypical cardiomyocytes of the sinoatrial node, and NPY also can influence heart I_f currents and change SAR [10].

The effects of NPY in the right atrial myocardium with spontaneous activity against the background of isoproterenol are “inhibitory” and are expressed in the reduction of SAR and amplitude-time indices of atrial myocardial contractility.

The results allow us to hypothesize that the physiological role of NPY consists in supplementing the “inhibitory” effects on the heart, *i.e.*, it is “limiting” and appears to suppress the effects of peripheral adrenergic/sympathetic stimulation in 21- and 100-day-old rats. Probably, the modulating effect of β-adrenoreceptor stimulation by NPY depends on activity of the sympathetic regulatory influences on rat heart.

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