Blockade of Hyperpolarization-Activated Channels Modifies the Effect of β-Adrenoceptor Stimulation

T. L. Zefirov, N. I. Ziyatdinova, A. A. Gainullin, and A. L. Zefirov

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Experiments on rats showed that blockade of hyperpolarization-activated currents moderates tachycardia induced by β-adrenoceptor agonist isoproterenol and potentiates the increase in stroke volume produced by this agonist. Electrical stimulation of the vagus nerve against the background of isoproterenol treatment augmented bradycardia and increased stroke volume. Blockade of hyperpolarization-activated currents followed by application of isoproterenol moderated vagus-induced bradycardia and had no effect on the dynamics of stroke volume.

Key Words: *heart; hyperpolarization currents; sympathetic influences; rat; vagus*

According to classical concept sympathetic neurons stimulate and parasympathetic nervous system inhibits cardiac functions. The sympathetic cardiotropic effects are mainly explained by activation of β-adrenoceptors. Norepinephrine released from sympathetic terminals stimulates spontaneous activity of sinoatrial pacemaker cells by increasing calcium entry and shortening slow diastolic depolarization phase [11]. Despite some researchers detected no changes in the heart rate (HR) during blockade of β-adrenoceptors [5,8], most authors reported a decrease in HR after blockade of β-adrenoceptors in animals of different ages [1,2].

Recent studies showed that hyperpolarization-activated currents (Ih) play an important role in the regulation of cardiac function. In particular, these currents are involved in the genesis of pacemaker activity in the heart [9,12]. These non-selective inward cation currents depolarize the membrane of atypical cardiomyocytes in the sinoatrial node within the range of -60 to -40 mV [7]. Blockade of hyperpolarization-activated channels (H-channels) markedly decreases the frequency of pacemaker activity of the sinoatrial node by prolonging spontaneous diastolic depolarization phase [3,4].

Experiments with blockade of H-currents showed that the autonomic nervous system regulates HR by modulating activity of H-channels [6,14]. We previously studied the effect of Ih blockade on cardiac function during electrical stimulation and transection of the vagus nerves [3,4].

Here we studied the effects of isoproterenol and Ih blockade on parameters of cardiac function in mature rats.

MATERIALS AND METHODS

The study was carried out on 20-week-old albino rats (n=14). The rats were intraperitoneally anesthetized with urethane (1000 mg/kg, 25% solution). Ih blocker 4-(N-ethyl-N-phenylamine)-1,2-dimethyl-6 (methylamine) pyrimidine^{+++}chloride (ZD-7288, Tocris) was injected in doses of 0-0.7 mg/kg and β-adrenoceptor agonist isoproterenol in a dose of 0.1 mg/kg. For combined application ZD-7288 was injected 5 min before isoproterenol. The solutions were injected into right femoral vein. The vagus nerve was stimulated with an ESL-2 stimulator (5 V pulse amplitude, 1-12 msec duration, 0.2-0.4 msec delay, and 0.7-10 Hz repetition rate). Stimulation parameters were chosen individually for each rat and remained constant throughout the

Department of Anatomy, Physiology, and Human Health Protection, Kazan State Pedagogical University; Department of Normal Physiology, Kazan State Medical University. ***Address for correspondence:*** nafisa@kmu-mf.kcn.ru. Zefirov T. L.

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experiment. The right vagus nerve was stimulated before and after injection of ZD-7288. In experiments with vagotomized rats, the right vagus nerve (VN) was cut, and the left VN was cut 30 min later.

Parameters of cardiac function were recorded and processed on a computer. Original software processed 21 parameters of ECG and variational pulsogram and 7 parameters of volumetric and tetrapolar rheograms.

The results were analyzed statistically using Student's *t* and Wilcoxon tests.

RESULTS

Isoproterenol significantly decreased the mean cardiointerval (X_M) from 198.00±7.12 to 174.0±77.3 msec by the 15th min postinjection ($p<0.05$, Fig. 1). Standard deviation (δ) and variational range (ΔX) increased 1 min postinjection but then returned to the initial values. The maximum increase in parameters of variational pulsogram (mode amplitude, strain index, index of autonomic balance, index of autonomic rhythm, control conformity) was observed on minute 5 postinjection.

β-Adrenoceptors agonist isoproterenol reduced the duration of all intervals and waves on ECG, but the ratios of *P—Q/R—R*, *Q—T/R—R*, and *T—P/R—R* remained virtually unchanged. Stroke volume (SV) increased from 0.2370±0.0245 to 0.2890±0.0515 ml on minute 5 after injection of isoproterenol, but then this parameter decreased to 0.274±0.039 ml.

Stimulation of the right VN in isoproterenol-treated rats increased X_M to a greater extent than in control animals: from 183.4±10.7 to 555±87 msec ($p<0.05$, Fig. 2, *a*). Isoproterenol potentiated changes in the parameters of variational pulsogram induced by stimulation of the right VN. The dynamics of variability indices of the heart rhythm attests to potentiation of parasympathetic influences during vagal stimulation applied against the background of β-adrenoceptor stimulation.

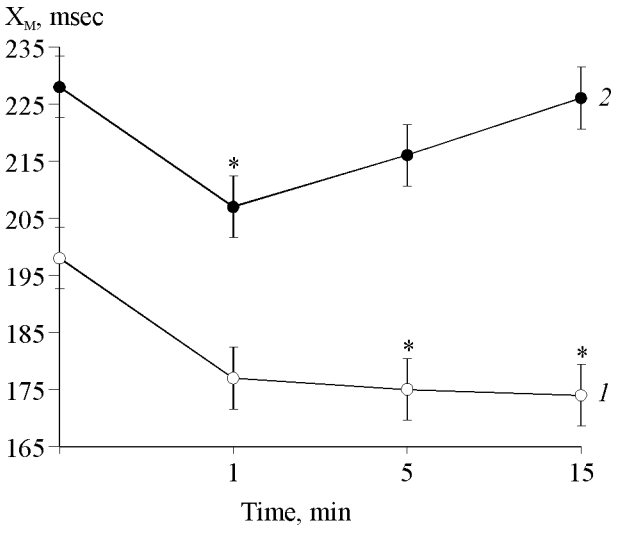

Fig. 1. Changes in mean cardiointerval (X_M) induced by isoproterenol alone (*1*) and isoproterenol+ZD-7288 (*2*). Here and in Fig. 2: *$p<0.05$ compared to the initial value.

The increase in X_M during vagal stimulation before and after injection of isoproterenol resulted primarily from lengthening of *T—P* interval (Fig. 2, *b*). *P—Q* interval decreased during vagal stimulation before and after injection of isoproterenol. Vagal stimulation before injection of β-adrenoceptor agonist increased SV from 0.209±0.044 to 0.260±0.353 ml, while after injection of isoproterenol SV increased more pronouncedly: from 0.2510±0.0303 to 0.5420±0.0415 ml ($p<0.05$).

Right-sided vagotomy in isoproterenol-treated rats decreased X_M, ΔX, and δ from 182.00±9.75 to 177.4±8.7 msec, from 6.20±0.96 to 4.40±0.68 msec, and from 1.72±0.19 to 1.44±0.25 msec, respectively, while the left-sided vagotomy increased these indices.

Transsection of the right and left vagus nerves increased the duration of *T—P* and *P—Q* intervals from 106.60±6.97 to 117.2±11.5 msec and from 61.20±0.73 to 66.40±5.35 msec, respectively. In addition, on minute 30 after right-sided vagotomy SV increased from 0.272±0.023 to 0.295±0.023 ml and then to 0.316±0.270 ml after the following left-sided vagotomy.

These findings suggest that activation of β-adrenoceptors significantly increases HR and slightly increases SV, the autonomic homeostasis been shifted towards the prevalence of sympathetic innervation. Stimulation of the right VN in isoproterenol-treated rats produces more pronounced bradycardia and increase in SV. These data agree with the theory of "accentuated antagonism" of sympathetic and parasympathetic systems [10,11], that says that stimulation of the sympathetic system potentiates the effect of stimulation of parasympathetic vagal fibers.

On minute 1 after injection of isoproterenol against the background of bradycardia caused by Ih blockade X_M significantly decreased from 227.8±4.6 to 206.6±3.4 msec ($p<0.05$), but then recovered. ΔX gradually increased from 14.8±4.9 to 38.8±21.8 msec on minute 15 after injection of isoproterenol (Fig. 1). Standard deviation, mode amplitude, and index of adequacy of regulation processes increased within 5 min after isoproterenol injection, but then these parameters slightly decreased. Other parameters of variational pulsogram (strain index, index of autonomic balance, and index of autonomic rhythm) slightly decreased immediately after injection of isoproterenol, increased on minute 5 postinjection, and then markedly decreased after 15 min.

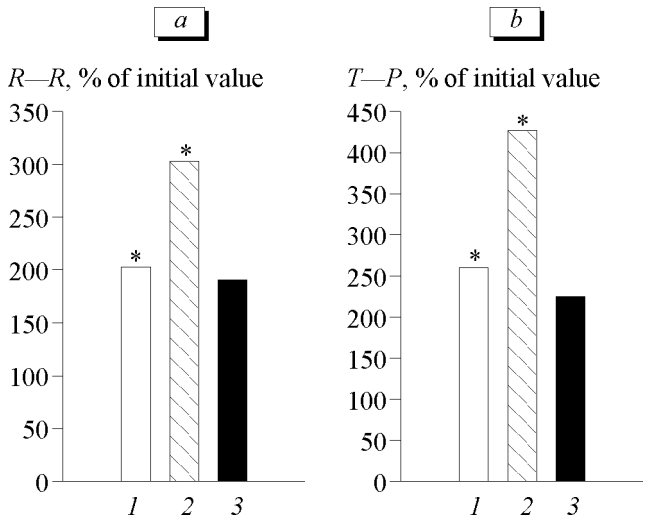

Fig. 2. Effect of right vagus nerve stimulation on *R—R* (*a*) and *T—P* (*b*) intervals. *1*) control rats; *2*) isoproterenol; *3*) ZD-7288+isoproterenol.

Isoproterenol injected after Ih blockade produced a significant increase in SV from 0.263±0.016 to 0.344±0.009 ml on minute 5 postinjection, but after 15 min this parameter decreased to 0.327±0.036 ml. This increase in SV (31%) was more pronounced than that observed after injection of isoproterenol alone (22%).

Stimulation of the right VN after injection of isoproterenol and ZD-7288 produced a less pronounced drop in HR (91%) in comparison with intact animals (115%), while injection of isoproterenol alone potentiated bradycardia caused by vagal stimulation. Changes in other parameters of variational pulsogram were different. For example, ΔX decreased, while the strain index increased. When the vagus nerve was stimulated before injection of test drugs, it increased *T—P* interval from 118.0±11.9 to 312.0±53.2 msec, while after injection it increased this interval from 149.6±7.3 to 336.0±90.9 msec (Fig. 2, *b*). It is noteworthy that the degree of SV increase caused by vagal stimulation was the same before and after injection of the drugs.

Right-sided vagotomy against the background of Ih blockade and stimulation of β-adrenoceptors decreased X_M from 231.0±11.5 to 218.6±9.3 msec on 5 min postinjection, but X_M recovered on minute 30. Left-sided vagotomy increased X_M to 240.0±8.9 msec. ΔX and δ decreased after right-sided vagotomy made against the background of injected chemicals, while they somewhat increased after the following left-sided vagotomy. In this paradigm, the right-sided vagotomy produced the initial increase and subsequent decrease of mode amplitude, strain index, and autonomic balance index, while the left-sided vagotomy increased mode amplitude ($p<0.05$) and autonomic balance index.

Right-sided vagotomy decreased *T—P* interval from 151.2±8.9 to 137.60±8.63 msec, while left-sided vagotomy increased it to 152.00±6.54 msec. Successive bilateral vagotomy increased *P—Q* interval from 62.8±4.6 to 70.00±6.61 msec and decreased SV from 0.304±0.033 to 0.284±0.031 ml.

These data attest to mutual modulating influences between activity of H-channels and activity of the parasympathetic and sympathetic systems. This is confirmed by different dynamics of HR and SV during individual and combined treatment with ZD-7288 and isoproterenol.

Of particular interest are opposite changes of cardiac indices produced by electrical stimulation of the vagus nerves in intact rats against the background of isoproterenol and ZD-7288+isoproterenol. It should be noted that ZD-7288 and isoproterenol did not modulate SV increase during vagal stimulation, which attests to peculiar mechanisms of regulation of chronotropic and inotropic properties of the heart in mature rats.

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