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Diadenosine Polyphosphates Suppress the Effects of Sympathetic Nerve Stimulation in Rabbit Heart Pacemaker

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The modulatory influence of diadenosine tetraphosphate (Ap4A) and diadenosine pentaphosphate (Ap5A) on the effect of intramural autonomic nerve stimulation in isolated rabbit sinoatrial node were examined. Electrical activity of the sinoatrial node was recorded intracellularly. Against the background of blockade of adrenergic effects with propranolol (3×10^{-6} M) or in preparations isolated 2 h after injection of reserpine (2 mg/kg), nerve stimulation induced short-term membrane hyperpolarization and diminished the sinus node firing rate. These phenomena were not affected by Ap4A or Ap5A (10^{-5} M). Under the action of atropine (3×10^{-6} M) that completely eliminated the cholinergic influences, nerve stimulation enhanced the sinus node firing rate by 17.30±3.45% from the initial rate. Both Ap4A and Ap5A moderated the stimulation-induced elevation of firing rate to 9.9±2.8 and 10.5±2.9%, respectively. The data suggest that diadenosine polyphosphates significantly modulate the sympathetic influences on the heart rhythm, but have no effect on the parasympathetic control over activity of sinoatrial node.

Key Words: *diadenosine polyphosphates; heart; pacemaker; action potential; sympathetic nervous system*

One of the promising avenues in physiology of the heart is the study of structural and functional organization of the sinoatrial (SA) node (*i.e.*, the cardiac pacemaker region) and its control mechanisms. Despite the fact that all cardiomyocytes in SA node possess some degree of automaticity in spike generation, the resulting SA node rhythm is a result of intricate interactions between cellular elements in this structure [4]. In the intact heart, the SA node is subjected to uninterruptible control from sympathetic and parasympathetic nervous systems [3,4]. The major neurotransmitters of the autonomic regulation in the SA node are norepinephrine and acetylcholine secreted by postganglionic sympathetic and parasympathetic nerve terminals, respectively. In parallel with major neurotransmitter, nerve terminals can secrete some auxiliary substances (cotransmitters) can to a certain extent modulate its effect. For instance, neuropeptide Y, ATP, and adenosine are well known cotransmitters of sympathetic postganglionic nerves [5].

At present, the role of sympathetic cotransmitters can be also given to diadenosine polyphosphates (Ap(n)A), representatives of a novel group of regulatory paracrinic factors. These molecules consist of two adenosine bases linked via several residues of phosphoric acid. It is established that Ap(n)A can be secreted from the nerve terminals with norepinephrine [3,13]. However, little is known about their potential role in the nervous regulation of the cardiovascular system in general and of the SA node, in particular. It was also reported that Ap(n)A decelerate automatic activity of the SA node [1].

This work was designed to elucidate the potency of Ap4A and Ap5A to modulate the effects of activation of the sympathetic and parasympathetic intramural nerves regulating automatic activity of rabbit SA node.

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MATERIALS AND METHODS

Experiments were performed on 45 male Soviet Chinchilla rabbits (n=20) aging 2.5-3 month and weighing 2.5-2.8 kg. The rabbits were narcotized with intravenous urethane (1.5 g/kg), the thorax was rapidly opened, the heart was extracted and washed with Tyrode solution containing (in mM): 133 NaCl, 4.69 KCl, 1.35 $NaH_2PO_4 \times 2H_2O$, 16.3 $NaHCO_3$, 1.18 $MgSO_4 \times 7H_2O$, 2.0 $CaCl_2 \times 2H_2O$, and 7.77 glucose and oxygenated with carbogen (95% O_2 and 5% CO_2). A fragment of the right atrium between the including SA node, openings of the upper and lower venae cavae, and the terminal crest was isolated. The preparations were mounted in a 3-ml chamber (endocardial side upwards) and superfused with Tyrode solution at 38°C and flow rate of 10 ml/min. The intramural nerves were stimulated with Teflon-coated silver electrodes positioned near the upper vena cava opening according to the routine Vincenzi—West method. The stimulation parameters were as follows: pulse train duration 300 msec, repetition rate 200 Hz, pulse width 100 μsec, and pulse amplitude 500 μA. The short duration of individual electric pulses secured excitation of the nerves only without provoking an extra excitation of the myocardium [2].

The action potentials (AP) were recorded via intracellular glass microelectrode with resistance of 25-50 MΩ. The signals were digitized by an E14-140 A/D convertor (L-Card) and recorded with PowerGraph 3.3 software (DiSoft). MiniAnalysis 3.0.1 software (Synaptosoft) was employed to determine the following parameters: maximum diastolic potential, AP amplitude, and cardiac interval used to calculate SA node firing rate.

Since stimulation of intramural nerves provokes both sympathetic and parasympathetic effects, they were pharmacologically separated in 3 series of experiments. In series I, the parasympathetic effects were eliminated *in vitro* with 3×10^{-6} M atropine. In series II, the sympathetic effects were blocked postsynaptically *in vitro* with β-adrenoblocker propranolol (3×10^{-6} M). In series III, the sympathetic effects were prevented *in vivo* by depletion of catecholamine depots in the presynaptic terminals with intravenous reserpine (2 mg/kg) injected 2 h prior to the experiment.

The data were analyzed statistically using SigmaPlot 12.5 software and non-parametric Wilcoxon's test. The latter was employed due to small sample size, which did not secure the normalcy of distribution.

RESULTS

In series I, the study assessed the modulatory effects of Ap4A and Ap5A on sympathetic stimulation in SA node preparation. In this series, the cholinergic effects were completely eliminated with atropine. It is a common knowledge that the effects of sympathetic stimulation in the SA node develop slower than the effects evoked by cholinergic stimuli. In our experiments, the effects of sympathetic stimulation attained maximum in 5-6 sec after the onset of stimulation (Fig. 1). The major effect of sympathetic stimulation was elevation of AP firing rate accompanied by slight decrease in AP amplitude. The maximum diastolic potential did not change significantly, although it demonstrated a depolarizing (positive) trend.

Application of Ap4A and Ap5A in a concentration of 10^{-5} M produced a small but significant drop in AP

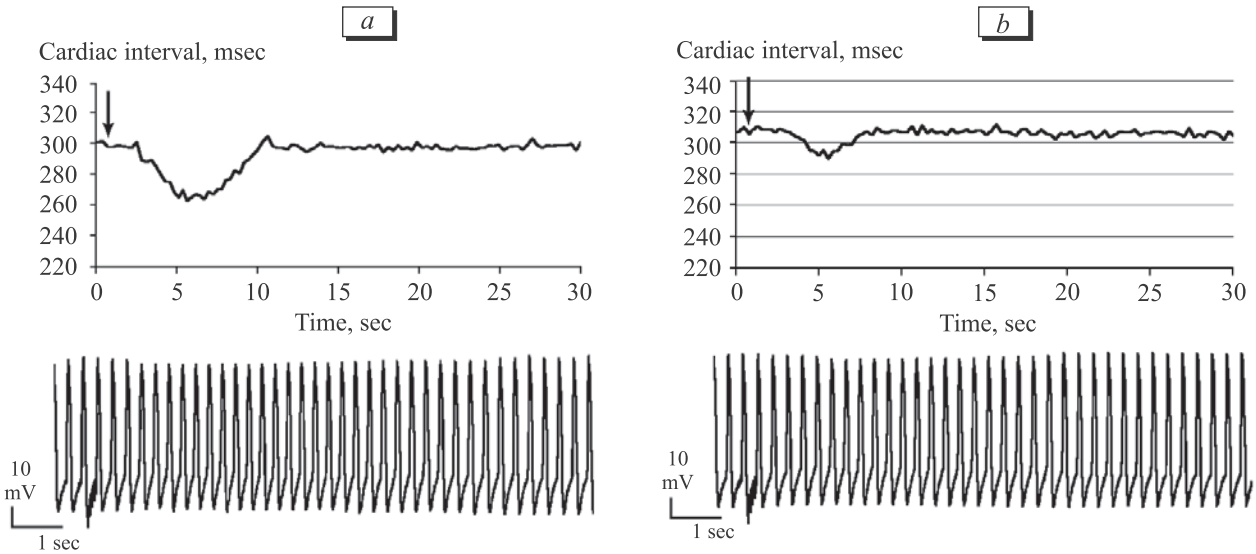

Fig. 1. Modulatory influence of Ap4A (10^{-5} M) on the effect of electrical stimulation of intramural nerve terminals in rabbit SA node under blockade of parasympathetic effects with atropine (3×10^{-6} M) in a typical experiment. Cardiac interval (at the top) and firing APs (at the bottom) under the control conditions (*a*) and during the action of Ap4A (*b*). The arrow indicates the onset of electric stimulation.

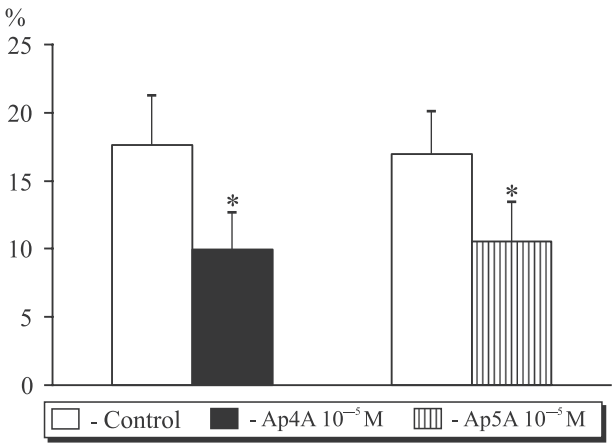

Fig. 2. Modulatory influence of Ap4A and Ap5A on the increment of SA node firing rate induced by electrical stimulation of intramural sympathetic nerve terminals in rabbit SA node. The increment is given in percentage of firing rate measured prior to stimulation. *p<0.05 in comparison with control. n=6.

firing rate by 4.2±0.9 and 6.4±1.2%, respectively (n=6, p<0.05). Ap4A diminished the positive chronotropic effect of sympathetic stimulation by 43.7% (the upper plots in Fig. 1, *a*, *b*). Similarly, Ap5A diminished this effect by 37.8% (Fig. 2). In control and under the action of both diadenosine polyphosphates, AP amplitude decreased by virtually the same value (by 8.3±2.3 and 7.7±1.3%, respectively), so these agents produced no significant effect on sympathetically-induced drop in AP amplitude.

The series II and III assessed the modulatory action of Ap4A and Ap5A on the effect of parasympathetic stimulation in SA node, where the sympathetic influences were eliminated with propranolol or reserpine at the post- or presynaptic levels, respectively. Depletion of catecholamine depots with reserpine secured elimination of the effects related to the action of secreted norepinephrine on α-adrenoceptors in cardiomyocytes or nerve terminals. In these series, parasympathetic stimulation induced virtually immediate membrane hyperpolarization accompanied with a decrease of AP amplitude to minimal level and diminished SA node firing rate (Fig. 3). These typical cholinergic effects [2] rapidly disappeared: the AP amplitude increased to initial level in poststimulation spikes 6-7, while the cardiac interval restored in poststimulation cycle 9-10. It should be stressed that in both series, the cholinergic effects were not accompanied with the following acceleration of the sine rhythm, which attests to complete blockade of the adrenergic influences in these experiments. Ap4A and Ap5A produced no modulatory effect on the action of parasympathetic stimulation, whereas they demonstrated a small intrinsic (in stimulation-free preparation) decelerating action on the SA nodal rhythm.

Recently it was shown that extracellular Ap(n)A and their derivatives decrease the tone of smooth muscles in vascular wall and in the hollow organs [9]. It was hypothesized that Ap(n)A are sympathetic cotransmitters that regulate the response to adrenergic stimulation in smooth muscles [6,12]. Some data indicate that Ap(n)A can play the regulatory role not only in smooth muscles, but also in cardiac tissue. It is not accidental that the fractions of cardiac tissues contain a pronounced amount of Ap(n)A [8]. While it is an established fact that Ap4A and Ap5A down-regulate contractility of the working myocardium in mammals [6,11], their role in autonomic nervous control of the heart was not examined. Here, we demonstrated ability of Ap4A and Ap5A to limit markedly the effects of sympathetic control over pacemaker in mammalian heart. In contrast, these agents produced no modulatory influence on cholinergic effects in SA node. It is

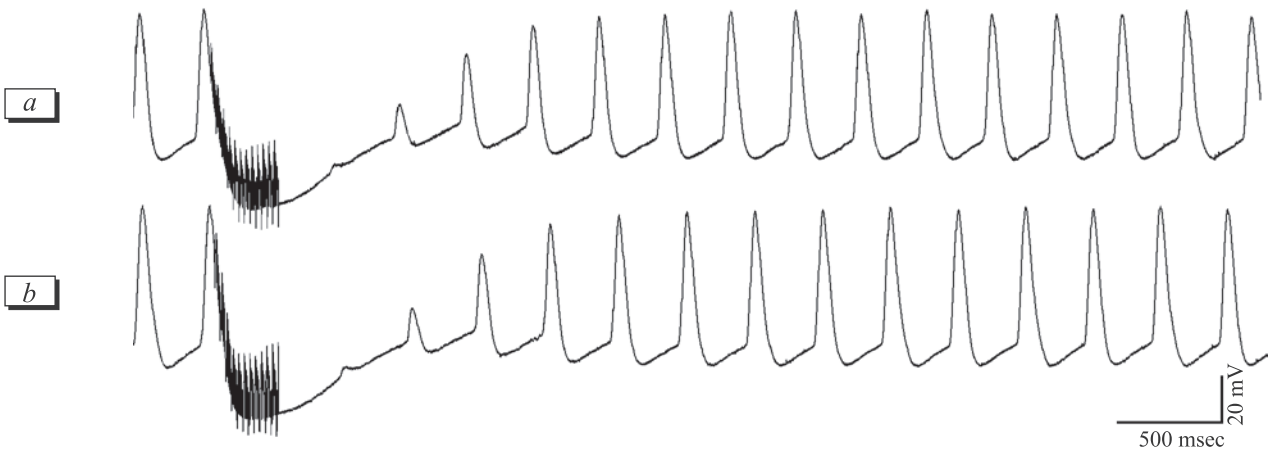

Fig. 3. Modulatory influence of Ap5A (10^{-5} M) on the effect of electrical stimulation of intramural cholinergic nerve terminals under blockade of sympathetic effects in rabbit SA node with propranolol (3×10^{-6} M) in a typical experiment. SA nodal firing under control conditions (*a*) and during the action of Ap5A (*b*). The arrow indicates the onset of electric stimulation.

interesting that Ap4A and Ap5A diminished the effect of sympathetic stimulation almost 2-fold, whereas they exerted a little effect on SA nodal firing when used without such stimulation. Therefore, the potential physiological role of Ap(n)A in the control of the heart can be inhibition of sympathetic influences, while their intrinsic cardiotropic effects are rather small.

The mechanisms underlying the modulatory inhibition of the effects of sympathetic stimulation are beyond the scope of this study. Since Ap(n)A belong to purines, it can be hypothesized that the modes of their action are similar to those demonstrated for such "classical" transmitter of the purinergic system as ATP mediating its effects via activation of membrane purinoceptors [5]. Some studies showed that the inhibitory effects of exogenous Ap(n)A in various tissues result from activation of purinoceptors P1 or P2 [7,10,11].

It should be noted that the inhibitory effect of Ap(n)A in SA node can be exerted both at the postsynaptic region (down-regulation of cardiomyocytic response to norepinephrine mediated via purinoceptors in their membrane) and in sympathetic terminals mediated via presynaptic purinoceptors. Since intrinsic effects of Ap(n)A on pacemaker activity are rather small, the latter hypothesis seems to be more favorable, although it should be tested in further detailed studies.

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