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Effects of Nicotinamide Adenine Dinucleotide (NAD^+) and Diadenosine Tetraphosphate (Ap4A) on Electrical Activity of Working and Pacemaker Atrial Myocardium in Guinea Pigs

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Effects of nucleotide polyphosphate compounds (nicotinamide adenine dinucleotide, NAD^+; diadenosine tetraphosphate, Ap4A) on the configuration of action potentials were studied in isolated preparations of guinea pig sinoatrial node and right atrial appendage (auricle). In the working myocardium, NAD^+ and Ap4A in concentrations of 10^{-5} and 10^{-4} M had no effect on resting potential, but significantly reduced the duration of action potentials; the most pronounced decrease was found at 25% repolarization. In the primary pacemaker of the sinoatrial node, both concentrations of NAD^+ and Ap4A induced hyperpolarization and reduction in the rate of slow diastolic depolarization, but significant slowing of the sinus rhythm was produced by these substances only in the concentration of 10^{-4} M. Moreover, AP shortening and marked acceleration of AP upstroke were observed in the pacemaker myocardium after application of polyphosphates. Comparative analysis of the effects of NAD^+ and Ap4A in the working and pacemaker myocardium drove us to a hypothesis on inhibitory effects of these substances on L-type calcium current accompanied by stimulation of one or several potassium currents, which induce enhancement of repolarization and hyperpolarization of membranes probably mediated by the activation of purine receptors.

Key Words: *nicotinamide adenine dinucleotide; diadenosine tetraphosphate; atrium; sinoatrial node; action potential*

Physiological role of a new group of regulatory paracrine factors, diadenosine polyphosphates (DAP), has recently attracted growing interest. DAP include two adenosine bases linked by phosphoric acid residues. DAP can be generated endogenously in several tissues by specific enzyme systems, which are present in cell cytoplasm and extracellular medium [5]. Well-known nucleotide polyphosphate substance NAD^+ can also be classified as DAP in which adenosine residue is substituted by nicotinic acid residue. NAD^+ is a neurotransmitter, because it is released from presynaptic nerve endings, metabolized outside the cell, and bind to membrane receptors [7,11,13].

DAP and NAD^+ can regulate blood flow and smooth muscle tone in vessels and hollow organs, platelet aggregation, and cell proliferation [8]. The effects of polyphosphates on the myocardium are little studied, but the well-known increase in the levels of these substances in the cardiac tissue accompanying ischemia-induced damage and cardiomyocyte death attests to a possible role of these substances in the pathogenesis of myocardial infarction. We have previously demonstrated that extracellular NAD^+ significantly accelerates repolarization phase of action potentials (AP), and suppresses contractive activity of the

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working myocardium of rat heart [1]. In addition, this substance affects automatic activity of the pacemaker in rat myocardium [2]. However, cardiotropic effects of DAP are poorly studied. Receptor mechanisms of DAP and ion activation pathways remain unclear, but it is suggested that the effects are mediated by the activation of P2X- and P2Y-subtypes of purine receptors [7,9,11]. It should be noted that all previous studies of electrophysiological effects of nucleotide polyphosphates were performed on rat myocardium, while guinea pig and rabbit cardiomyocytes are more similar to human myocytes by the expressed ion current repertoire [4].

Here we compared the effects of Ap4A and NAD^+ on electrical activity of the working atrial myocardium and central part of the sinoatrial node of guinea pigs.

MATERIALS AND METHODS

Experiments were performed on outbred mature male guinea pigs (n=12, age 2.5-3 months) weighing 420-480 g. The animals were narcotized with sodium pentobarbital (120 mg/kg, intraperitoneally), then the chest was opened, the heart was isolated and washed with Tyrode solution containing (in mmol/liter) 133.47 NaCl, 4.69 KCl, 1.35 $NaH_2PO_4 \times 2H_2O$, 16.31 $NaHCO_3$, 1.18 $MgSO_4 \times 7H_2O$, 2.0 $CaCl_2 \times 2H_2O$, and 7.77 glucose saturated with carbogen (95% O_2 and 5% CO_2). Then the samples of the right atrial appendage and intercaval myocardium containing the sinoatrial node, ostia of the superior and inferior vena cava, and the terminal crest were isolated. The samples were mounted in a 3-ml perfusion chamber (38°C, flow rate 10 ml/min) with the endocardium side up. Samples of the right atrial appendage were stimulated with the frequency of 3 Hz via silver electrodes.

AP were recorded using glass microelectrodes with resistance of 25-50 MΩ. The signal was digitalized using an E14-140 analog-to-digital converter (L-Card) and input in a computer using Powergraph 3.3 software (DiSoft). The data were analyzed using MiniAnalysis 3.0.1 software (Synaptosoft). On records obtained from the working myocardium, AP duration at 25, 50, and 90% repolarization levels (APD25%, APD50%, and APD90%, respectively), amplitude of AP, and resting potential were estimated. In the analysis of pacemaker bioelectric activity, the maximum diastolic potential, cardiac cycle duration, rate of slow diastolic depolarization, maximum AP upstroke rate (dV/dt_{max}), AP amplitude, and APD50% were evaluated.

Working concentrations of NAD^+ and Ap4A (Sigma) of 10^{-4} and 10^{-5} M were chosen based on our previous data [1,2]. Two concentrations of NAD^+ and Ap4a were tested on each sample.

Statistical analysis was performed using Statistica 6.0 software. Significance of differences was evaluated using Wilcoxon's test for related samples, and Mann–Whitney test for independent samples. The use of nonparametric test was stipulated by small size of samples, which could not provide normality of distribution.

RESULTS

Intracellular AP recording in the isolated right atrial appendage preparation showed that NAD^+ and Ap4A in the test doses significantly reduced AP duration (Fig. 1). The maximum effect of polyphosphates developed within 200-250 sec from the beginning of application, and therein after, only maximum effects of NAD^+ and Ap4A are discussed. The decrease in AP duration induced by NAD^+ and Ap4A in doses of 10^{-4} M at 25% repolarization was significantly higher than at 90% repolarization. It should be noted that NAD^+ and Ap4A did not significantly change AP amplitude in the dose of 10^{-5} M, but reduced AP amplitude in the dose of 10^{-4} M by 8.22±1.30 and 7.85±0.92%, respectively. Thus, nucleotide polyphosphates mostly affect the initial repolarization phase of AP in guinea pig heart corresponding to the plateau phase in cardiomyocytes of bigger animals.

In contrast to the working atrial myocardium, the sinoatrial node myocardium is characterized by high heterogeneity. The central and peripheral zones are distinguished. Few potential centers of automatism are found within the sinoatrial node. Normally, the rhythm for the whole sinoatrial node is determined by primary pacemaker cells located in its central area. Under the influence of some regulatory factors, this function can be taken by other parts of the sinoatrial node, normally overdriven, so-called latent pacemakers [3]. Thus, pacemaker shift occurs under these conditions. Activity of the primary pacemaker is characterized by relatively gradual transition from slow diastolic depolarization to AP depolarization and AP upstroke rate not exceeding 15 V/sec [12]. For estimation of NAD^+ and Ap4A effect, leads of electrical activity typical of primary pacemaker were used.

The test substances in doses of 10^{-4} M induced a significant hyperpolarization of the membrane: maximum diastolic potential increased by 3.8±0.7 and 3.10±0.74 mV under the influence of NAD^+ and Ap4A, respectively (Fig. 2, *a, b*). Polyphosphates in both tested concentrations significantly reduced the rate of slow diastolic depolarization (Fig. 2, *c*), but significant deceleration of the sinus rhythm was found only after application of NAD^+ and Ap4A in the concentration of 10^{-4} M (to 26.70±5.35 and 23.2±5.6%, respectively). It should be noted that NAD^+ and Ap4A

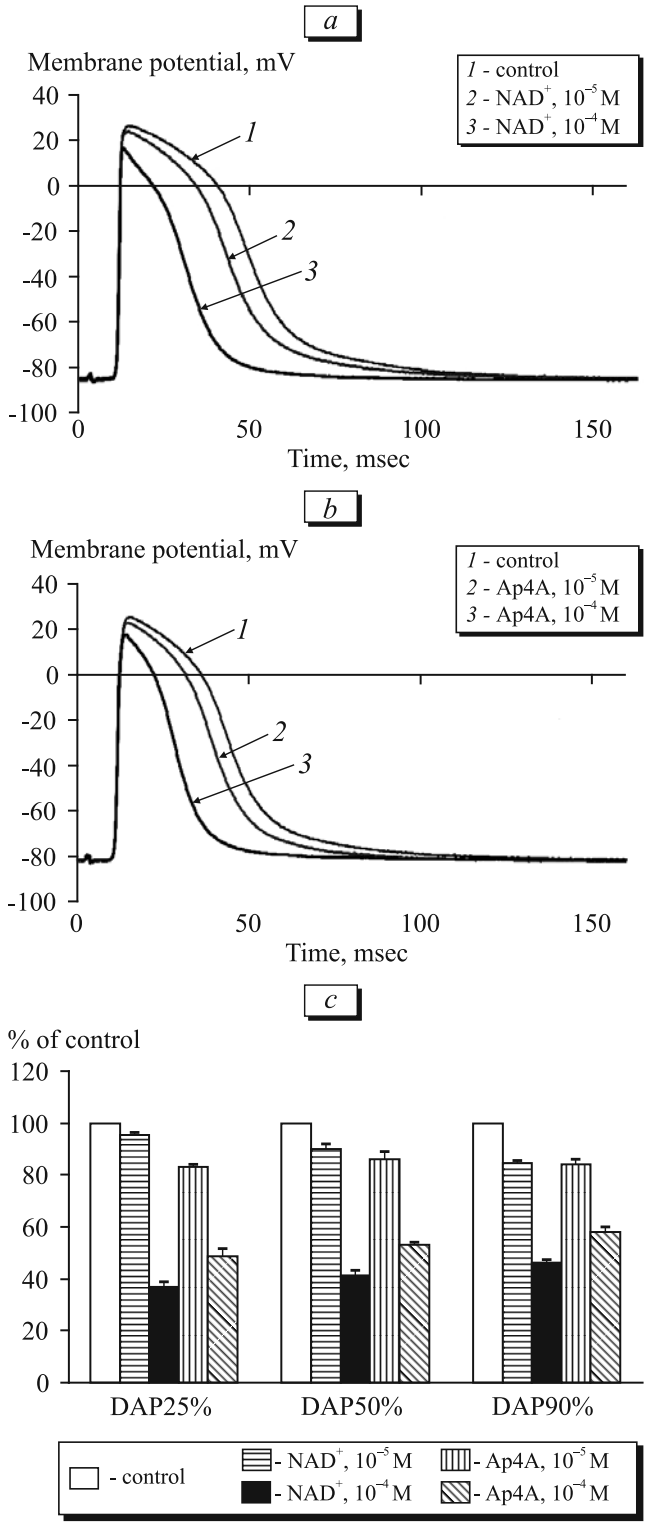

Fig. 1. Effects of NAD^+ and Ap4A on working atrial myocardium of guinea pigs. Original records of AP in the right atrial appendage under control conditions and during development of the maximum effects of NAD^+ (*a*) and Ap4A (*b*) are presented. Changes in AP duration (*c*) at 25, 50, and 90% repolarization under the influence of NAD^+ and Ap4A. Here and in Fig. 2, *c*: all effects of NAD^+ and Ap4A are significant in comparison with the control (n=6, p<0.05, Wilcoxon test).

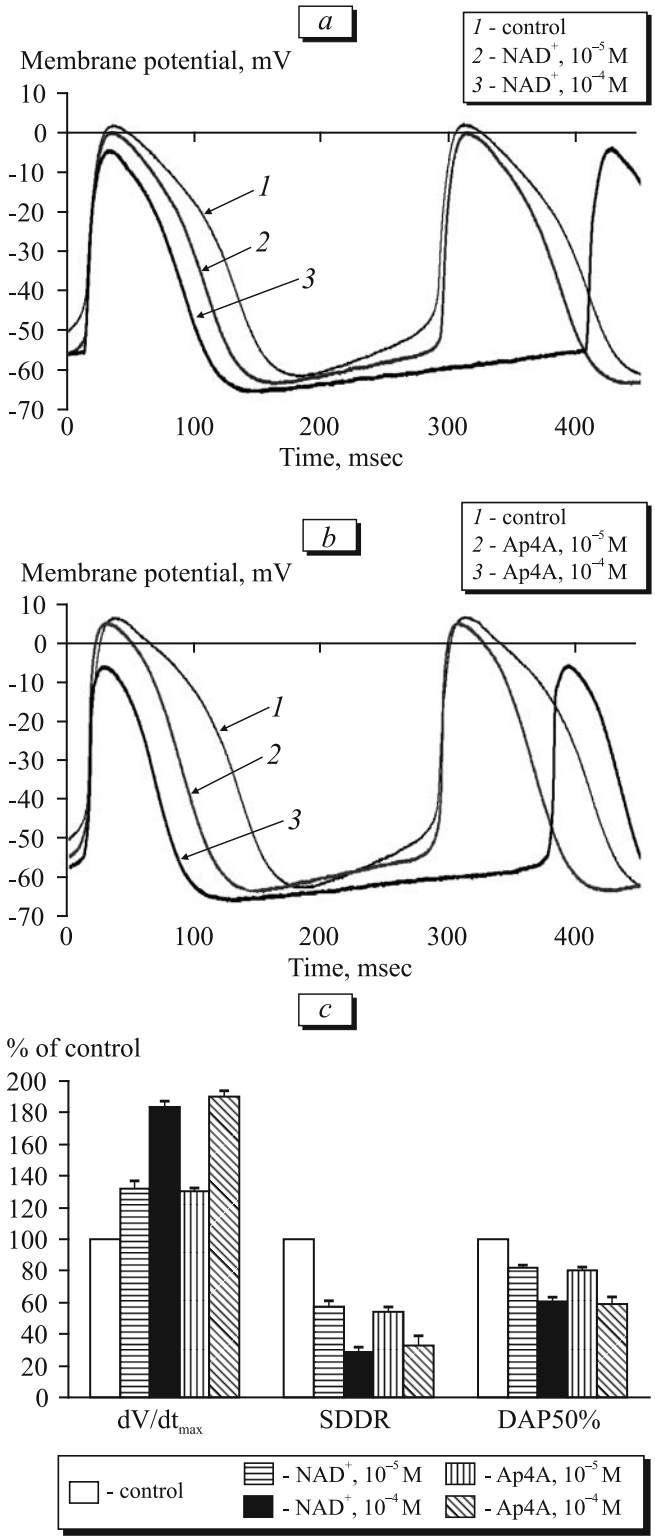

Fig. 2. Effects of NAD^+ and Ap4A on primary pacemaker in the sinoatrial node of guinea pigs. Original records of electrical activity in sinoatrial node specimen under control conditions and during the development of the maximum effects of NAD^+ (*a*) and Ap4A (*b*) are presented. Changes in the rate of AP upstroke, rate of slow diastolic depolarization, and AP duration at 50% repolarization under the influence of NAD^+ and Ap4A (*c*) are shown. SDDR: slow diastolic depolarization rate.

induced a switch of the pattern of electrical activity typical for a true pacemaker to electrical activity typical for a latent pacemaker (Fig. 2, *a, b*) characterized by fast transition from slow diastolic depolarization to AP upstroke, which is a typical sign pacemaker shift [3]. Pacemaker shift within the sinoatrial node can explain the absence of sinus rhythm deceleration after applications of NAD^+ and Ap4A in a concentration of 10^{-5} M that induced significant deceleration of slow diastolic depolarization in the area of recording.

Ap4A and NAD^+ did not induce significant changes in AP amplitude, but just a slight reduction in this parameter was found (Fig. 2, *a, b*). However, the polyphosphates significantly increased dV/dt_{max} (Fig. 2, *c*). It is known that the depolarization phase of AP in central cells of the node is normally stipulated by L-type calcium current [6]. At the periphery, sodium current mediated by myocardial isoforms of sodium channels Na_v 1.5 contribute to depolarization. However, cells of the central part express "neuronal" isoforms of sodium channels that can be activated during membrane hyperpolarization and contribute to AP depolarization phase [10]. The increase in dV/dt_{max} observed in our study is probably determined by involvement of sodium current into depolarization process due to polyphosphate-induced hyperpolarization. In turn, activation of sodium currentcontribute to maintenance of high AP amplitude during polyphosphate application.

Comparison of described changes induced by NAD^+ and Ap4A in the working and pacemaker myocardium of guinea pigs showed that the mechanism of polyphosphate effects can be based on suppression of L-type calcium current simultaneously with stimulation of one or few potassium currents that accelerate repolarization in both the sinoatrial node and working myocardium and promote membrane hyperpolarization in pacemaker cells. Realization of these effects is probably mediated by activation of P2-type purine receptors, but this assumption has to be experimentally proven. The hypothesis on the inhibitory effects of polyphosphates on L-type calcium current is based on the observed reduction in the amplitudes of AP and DAP25% in working myocardium. On the other hand, the inhibition of calcium current can contribute to significant shortening of AP in the sinoatrial node, but further experiments are required to prove this assumption.

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