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Effect of Exogenous Extracellular Nicotinamide Adenine Dinucleotide (NAD^+) on Bioelectric Activity of the Pacemaker and Conduction System of the Heart

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In rat sinoatrial node, NAD^+ (10 μM) reduced the rate of spontaneous action potentials, duration of action potentials, and the velocity of slow diastolic depolarization, but the rate of action potential front propagation increases. In passed rabbit Purkinje fibers, NAD^+ (10 μM) reduced the duration of action potentials. Under conditions of spontaneous activity of Purkinje fibers, NAD^+ reduced the firing rate and the rate of slow diastolic depolarization. The effects of extracellular NAD^+ on bioelectric activity of the pacemaker (sinoatrial node) and conduction system of the heart (Purkinje fibers) are probably related to activation of P1 and P2 purinoceptors.

Key Words: *sinoatrial node; Purkinje fibers; action potential; NAD^+*

NAD^+ is considered as a new, poorly known neurotransmitter, because it is released from presynaptic nerve endings, metabolized outside the cell, and binds to receptors on the cell membrane [6,12,14]. NAD^+ belongs to the family of purine compounds and can act as an agonist of P1 and P2 purinoceptors [12,14]. The effects of extracellular NAD^+ including those on the heart are poorly studied. It has been previously shown that extracellular NAD^+ substantially affects the action potentials (AP) and contractile activity of rat working myocardium [4]. However, the effects of NAD^+ on the pacemaker and conduction system of the heart of mammals remain unstudied.

Here we studied the effects of exogenous NAD^+ on bioelectric activity of the sinoatrial node and conduction system (Purkinje fibers) of the heart.

MATERIALS AND METHODS

The work was carried out on multicellular preparations isolated from the right atrium and including the sinoatrial node (SAN) and on preparations isolated from the heart ventricles and including fragments of Purkinje fibers (PF). Preparations of SAN were obtained from the heart of 3-5-month-old male rats weighing 300-350 g. Isolation and identification of conduction system elements in rat heart is very difficult, but in rabbit heart, some PF form loose network in the ventricular cavity and are easily available. The PF preparations were obtained from 4-5-month-old male rabbits weighing 2000-2500 g.

Rodent SANs were dissected, prepared, and perfused as described previously [1]. The animals were decapitated, the chest was opened, the heart was isolated, and SAN was dissected. The preparations were perfused at 37°C with a Tyrode solution containing (in mM): 129 NaCl, 4 KCl, 20.9 $NaH_2PO_4 \times 2H_2O$, 0.5 $MgSO_4$, 20 $NaHCO_3$, 1.2 $CaCl_2 \times 2H_2O$, and 5 glucose (pH 7.2-7.4). The solution was saturated with carbogen (95% O_2, 5% CO_2). SAN preparations were pinned (endocardial side up) in the perfusion chamber (3 ml). The isolated preparations of rat SAN showed automatism, *i.e.* generated AP (excited) with a certain frequency.

To isolate PF preparations, the rabbits were anaesthetized intravenously with urethane (0.5 g/kg), the chest was opened, the heart was removed, and

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transferred to a bath with a perfusion solution at room temperature. The heart was rinsed, the left ventricle was dissected along the interventricular septum to expose PF. To avoid damage, PF fragments were isolated together with the ventricular septum and placed in the perfusion chamber. Perfusion was performed at 37°C with adapted Tyrode solution containing (in mM): 133.47 NaCl, 4.69 KCl, 1.35 $NaH_2PO_4 \times 2H_2O$, 16.31 $NaHCO_3$, 1.18 $MgSO_4 \times 7H_2O$, 2.5 $CaCl_2 \times 2H_2O$, and 7.77 glucose (pH 7.2-7.4). The solution was saturated with carbogen. Two series of experiments with PF were performed. In series 1, the rhythm was paced; to this end, preparations were stimulated via the electrodes (2-msec duration, 3 Hz frequency). If PF preparations showed automatism like SAN (spontaneous excitation), they were not paced.

In SAN and PF, AP were recorded using conventional microelectrode techniques [2,3] via glass microelectrodes (20-30 MΩ) connected to an amplifier (A-M System 1600). The amplified signal was fed to an ADC module (E-154, L-Card), then stored in a computer. The signal was recorded and analyzed using PowerGraph 3.3 software (DISoft) and MiniAnalisys software (Synaptosoft).

In experiments with AP recording in rat SAN, AP duration at the level of 50% repolarization (APD50, msec), rate of spontaneous AP (frequency in Hz), rate of slow diastolic depolarization (V_{sdd}, V/sec), and the maximum velocity of AP front propagation (dV/dT_{max}, V/sec) were assessed.

In experiments with isolated PF, AP duration at the level of 50% (APD50) and 90% repolarization (APD90) (in paced preparations), frequency of spontaneous excitation, and V_{sdd} (in spontaneously beating preparations) were evaluated.

Before the experiment, the preparations were adapted in the perfusion chamber for 15-30 min (SAN) or 60-90 min (PF). After adaptation, SAN and PF preparations were perfused with a solution containing 10 μM NAD^+ (Sigma-Aldrich) for 10 min. NAD^+ at given concentration produced statistically significant effects on the working rat myocardium [4].

The obtained data were processed using the nonparametric Wilcoxon's *T* test. The differences were considered statistically significant at $p<0.05$. Values of the parameters of bioelectrical activity are presented as *M*±*SEM*, and (under the action of NAD^+) as the percentage of controls.

RESULTS

Extracellular NAD^+ significantly affected the duration of AP, rhythm, rate of slow diastolic depolarization, and the velocity of AP front propagation in rats. In control, the parameters of SAN were: APD50 50±3 msec, firing frequency 5.00±0.13 Hz, V_{sdd} 0.080±0.007 V/sec, and dV/dT_{max} 39.0±0.8 V/sec (n=6). NAD^+ significantly reduced APD50 and V_{sdd} (n=6) in the SAN. The velocity of AP front propagation increased under the influence of NAD^+ by ~16±4% (Fig. 1).

Extracellular NAD^+ changed bioelectrical activity in rabbit PF. In paced control preparations (n=5), APD50 and APD90 PF were 142±8 and 205±15 msec, respectively. Slow diastolic depolarization was absent under these conditions (Fig. 2, *a*). NAD^+ resulted in a significant decrease in APD50 and APD90 by 10±2 and 9±1%, respectively (Fig. 2, *b*).

Automatism was recorded in some PF preparations (n=3). In this case, the rate of spontaneous AP and V_{sdd} in the control were 1.45±0.20 Hz and 0.03±0.01 V/

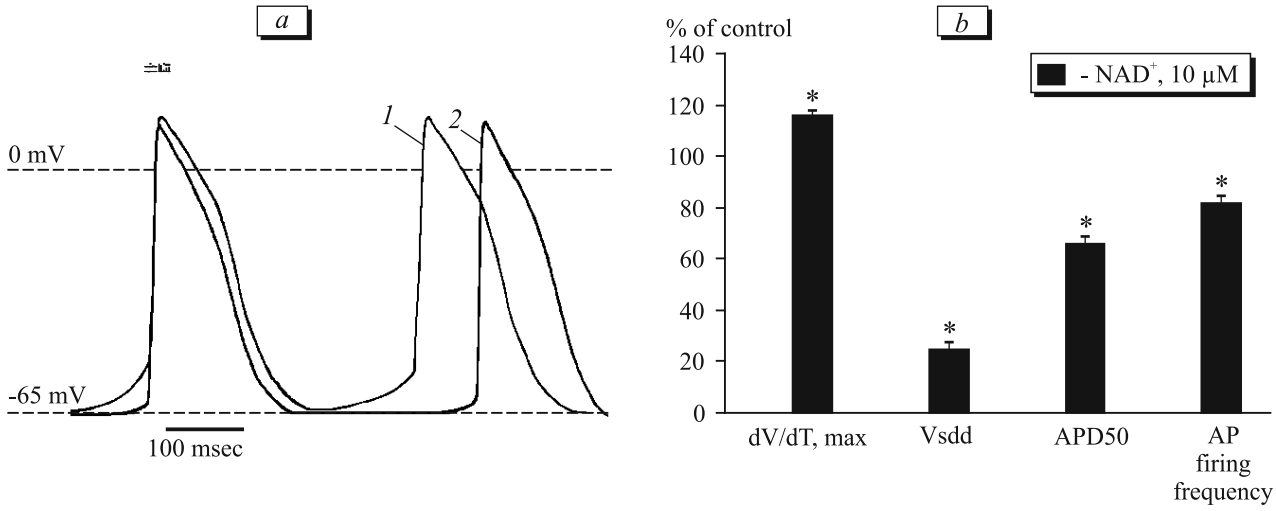

Fig. 1. Effect of NAD^+ on bioelectric activity of rat SAN. *a*) AP in the control (*1*) and at a time corresponding to the maximum effect of 10 μM NAD^+ (*2*); *b*) change in the maximum rate of depolarization and velocity of AP front propagation (dV/dT, max), rate of slow diastolic depolarization (V_{sdd}), AP duration at the level of 50% repolarization (APD50) and firing frequency. *$p<0.05$ in comparison with the control (100%).

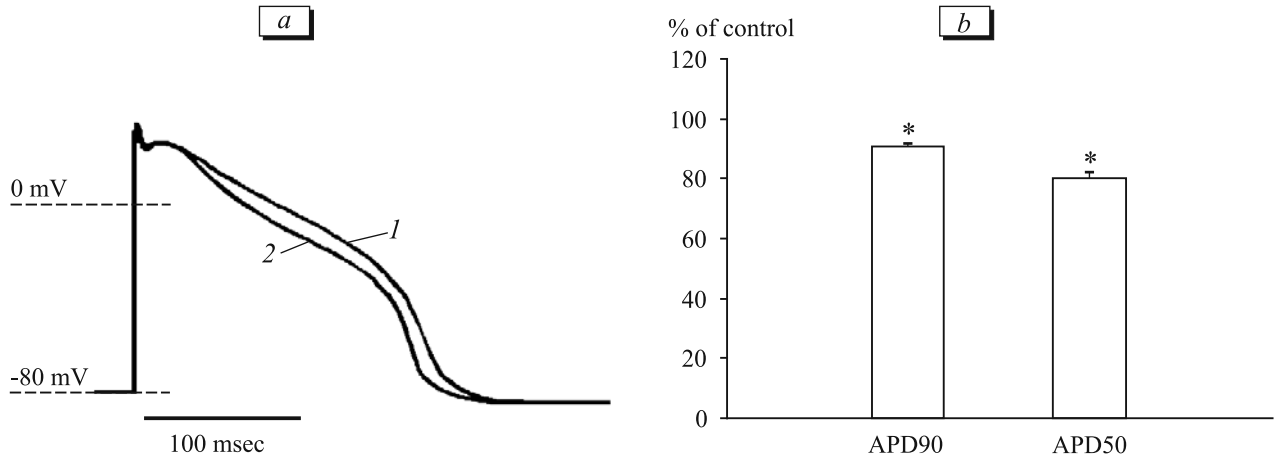

Fig. 2. Effect of NAD^+ on the duration of AP in the rabbit PF. *a*) AP in the control (*1*) and at a time corresponding to the maximum effect of 10 μM NAD^+ (*2*); *b*) reduction of AP duration (APD90, APD50) under the influence of NAD^+. *$p<0.05$ in comparison with the control (100%).

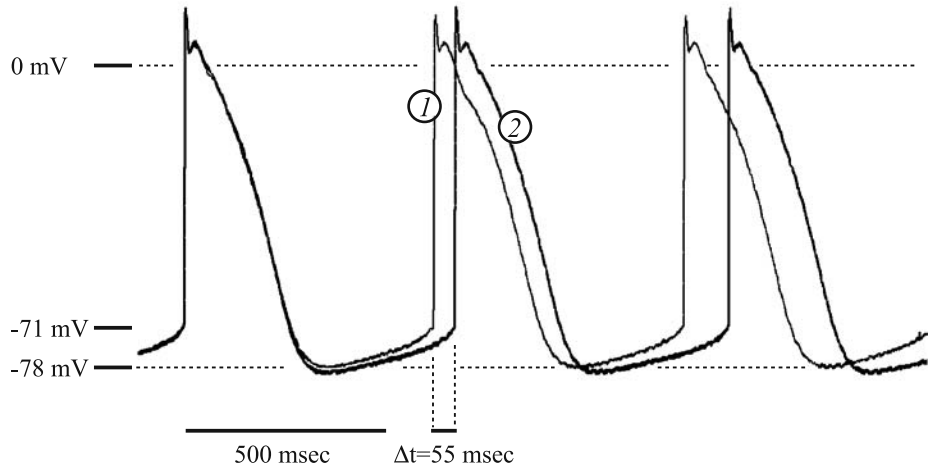

Fig. 3. Effect of NAD^+ on bioelectric activity of spontaneously active rabbit PF. Original record of AP. *1*) Controls, *2*) 10 μM NAD^+.

sec, respectively. NAD^+ reduced the rate of spontaneous AP to 1.3±0.2 Hz and V_{sdd} to 0.025±0.012 V/sec, but no significant shortening of AP was found (Fig. 3). Statistical significance of these changes cannot be assessed due to small number of the preparations with spontaneous activity.

Thus, extracellular NAD^+ in SAN and in PF produced effects of two types: shortening of AP duration and rhythm deceleration. The latter occurred under the influence of NAD^+ mainly due to the decrease in V_{sdd}, which led to an increase in the time required to achieve critical firing level and produce next AP (Figs. 1 and 3). Reduction of V_{sdd} was caused by activation repolarizing potassium currents, such as $IK_{Ach,Ado}$ (acetylcholine and adenosine-activated potassium current) and I_{K1} (current of anomalous rectification). The decrease in AP duration can be due to suppression of calcium current (I_{CaL}) or activation of delayed potassium current (I_K).

Activation of $IK_{Ach,Ado}$ and suppression of I_{CaL} are the classical effects observed in the heart under the influence of extracellular adenosine. Adenosine is the agonist of membrane A1 receptors that belong to the P1 purinoceptor family. Extracellular NAD^+ is cleaved by ectonucleotidases yielding adenosine and AMP [5]. It can be assumed that the effects of NAD^+ on the SAN and PF are caused by its metabolites (AMP and adenosine). However, it was previously shown that blockade of P1 receptors in the working myocardium did not abolish the effects of NAD^+ [4]. The effect of NAD^+ on SAN and PF can be determined, at least in part, by other mechanisms, such as activation of P2 purinoceptors.

ATP, an endogenous agonist of P2 receptors, enhances slow component of delayed rectifier current (I_{Ks}). This effect is observed in the atrial and ventricular cardiomyocytes of guinea pig [8,9]. Probably, NAD^+ as a purine compound activates P2Y purinoceptors coupled with $G_{q/11}$ protein, which potentiates the delayed rectifier potassium currents and reduces AP duration. Potentiation of delayed rectifier currents via

activation of P2Y receptors can be mediated by several mechanisms [7,13].

Nevertheless, the role of P2 receptors in the realization of NAD^+ effects in the pacemaker and conduction system of the heart remains unclear. It was previously shown that all subtypes of P2 purinoceptors are present in the rat SAN [10]. According to other studies [11], the effects of ATP in rabbit SAN are mediated exclusively by P1 receptors, because P2 receptors (P2Y1 and P2X1) were not found.

Shortening of duration of AP in spontaneously active rabbit PF under the effects of NAD^+ was not observed in our experiments, probably because NAD^+ can suppress only reinforced current, but not basal current I_{CaL} typical of low rhythm. In this case, NAD^+ would not reduce the AP duration in spontaneously active rabbit PF.

Thus, extracellular NAD^+ significantly affects bioelectric activity of the myocardial pacemaker (SAN) and cardiac conduction system (PF), probably due to activation purinoceptors. The release of NAD^+ from nerve endings and its accumulation in tissues can be a new mechanism of rhythm regulation and conduction of excitation in the heart.

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