BIOPHYSICS AND BIOCHEMISTRY

Effect of Nitric Oxide on Mechanoelectric Feedback in Rat Right Atrium

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In situ microelectrode examination of rat right atrium showed that in physiologically pre-stretched tissue, NO donor SNAP modifies the repolarization phase of cardiomyocyte AP in a "hump-like" way provoking the development of arrhythmia. Gadolinium both prevents and eliminates this effect attesting to involvement of stretch-activated channels in the development of NO-induced abnormalities. Elevation of SNAP concentration or further stretch of the tissue (presumably, it increases NO concentration) eliminated the hump depolarization induced by moderate SNAP stimulation. Thus, low NO opens the stretch-activated channels while high NO inactivates them.

Key Words: *stretch-activated channels; NO; cardiomyocytes; mechanoelectric feedback; microelectrode technique*

Mechanoelectric feedback in the heart is the cause of mechanically induced arrhythmias that quite often culminate in fibrillation [4,8]. The key players in the development of this phenomenon are the stretch-activated channels (SAC) opening in response to distention of the cell [3,5,6].

Recent studies showed that NO, a low-molecular endogenous compound with a wide range of physiological effects [11], can modulate SAC performance. The studies on isolated cardiomyocytes showed that NO donors activate SAC in unstretched cells, while the same agents inactivate SAC in stretched cells [1,9]. Binding of endogenous NO to receptors completely inhibits the currents flowing across SAC. It has been demonstrated that type 3 NO synthase (NOS-3) plays the major role in providing NO needed for generation of cardiomyocyte response to stretching [2,9]. However, despite pronounced effects obtained in isolated cells by using "patch-clamp" technique, there are no data on the effect of NO on bioelectric activity of cardiomyocytes at the tissue level and on the involvement of NO in realization of mechanoelectric feedback in the heart during appearance of mechanically induced arrhythmias.

Our aim was to examine *in situ* effects of NO on bioelectrical activity of cardiomyocytes in rat right atrium.

MATERIALS AND METHODS

Experiments were performed *in situ* on the right atrium preparations containing the sinus node isolated from male Wistar rats weighing 250-300 g. The preparation was perfused with oxygenated (100% O_2) physiological solution containing in mM: 137.0 NaCl, 5.4 KCl, 1.0 $CaCl_2$, 0.5 $MgCl_2$, 5.0 HEPES, 5.5 glucose (pH

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7.4, 37°C). Standard "floating" microelectrodes were used to recorder membrane potential during spontaneous contractions of the preparation and during its stretching [7]. Simultaneously, the force developed by the preparation was measured. In all experiments, standard physiological pre-stretching of the preparations by applying the force of 1 mN was performed. The additional stretch was produced with an extra force exceeding this value by no more than 2 mN [10].

NO donor S-nitroso-N-acetylpenicillamine (SNAP) was applied in concentrations of 3×10^{-4} and 6×10^{-4} M. Gadolinium (Gd^{3+}, Sigma) was applied in concentration of 40 μM to block SAC [3].

RESULTS

In series I, we examined the effect of SNAP (NO donor) on bioelectrical activity in pre-stretched atrial tissue. In 20 min after the start of perfusion, this agent changed the shape of repolarization phase of cardiomyocyte action potentials (AP) producing so-called "hump-like" depolarization (Fig. 1, *b*) followed by persistent paroxysms alternating with normal or abnormal AP (Fig. 1, *c*, *d*). Thus, NO elevation was accompanied with pronounced changes in the shape of AP generated by cardiomyocytes provoking the development of arrhythmias, which agrees with published data on pathophysiological role of NO in the heart [12].

In series II, a specific SAC blocker was used to examine whether NO-induced hump-like depolarization is related to activation of these channels. Inactivation of SAC with Gd^{3+} either prevented the appearance of SNAP-induced abnormal bioelectrical activity (Fig. 2, I, *c*) or eliminated it when the blocker was applied to SNAP-treated preparation (Fig. 2, II, *c*). Therefore, NO-induced "hump-like" depolarization and arrhythmia are Gd^{3+}-dependent and controlled by SAC.

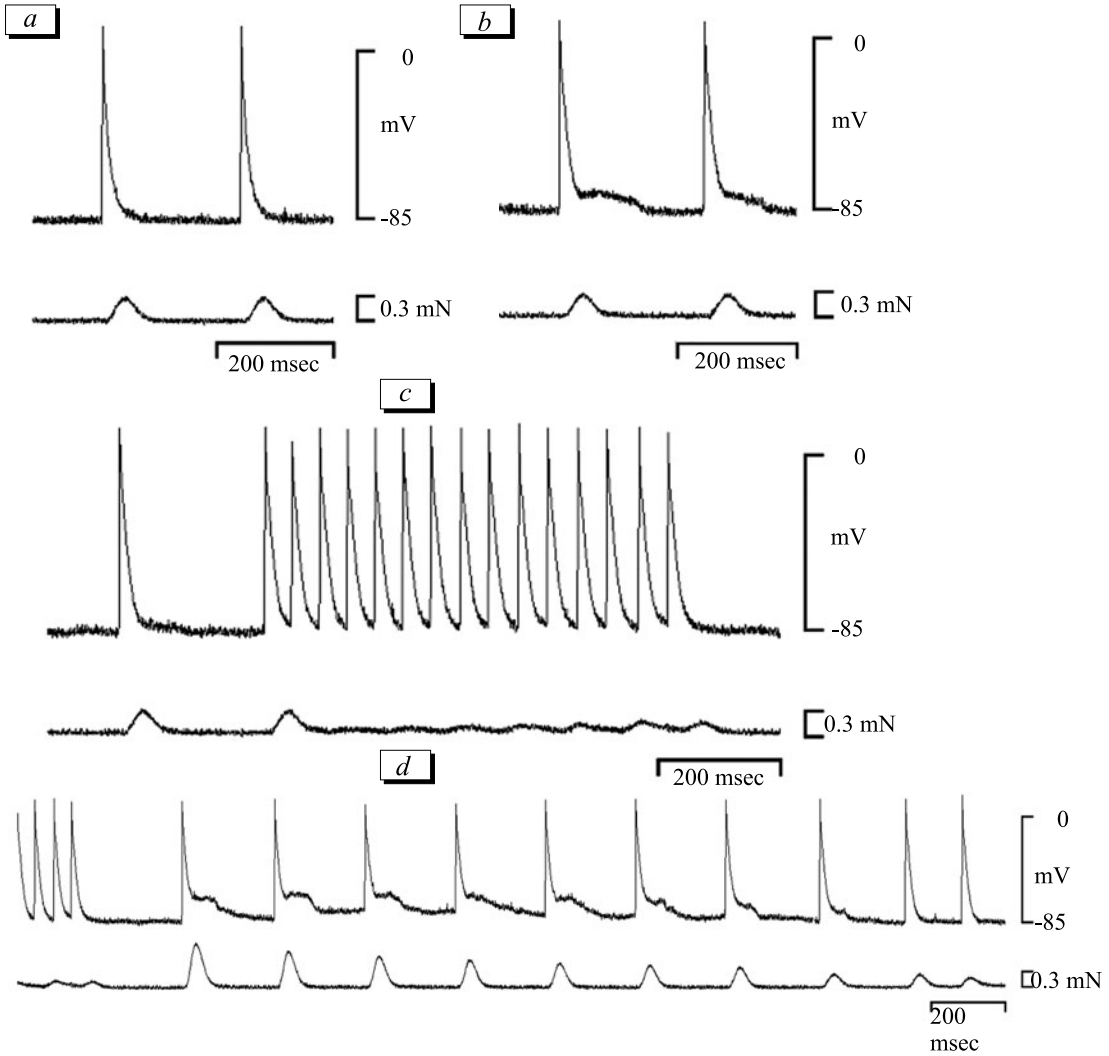

Fig. 1. Effect of SNAP on bioelectric activity of cardiomyocytes in the rat right atrium. *a*) control; *b-d*) SNAP perfusion for 20 min. The top and bottom curves are original records of membrane potential and mechanogram, respectively.

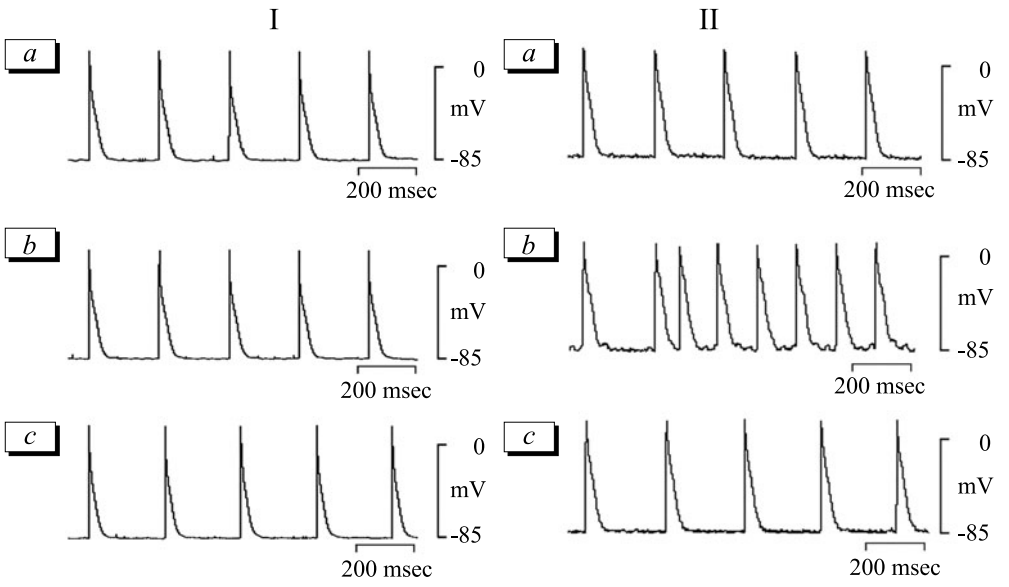

Fig. 2. Effect of Gd^{3+} ions on SNAP-induced abnormalities in bioelectric activity of cardiomyocytes in rat right atrium. I) application of SNAP to Gd^{3+}-perfused preparation: *a*) control; *b*) 10 min perfusion with Gd^{3+}; *c*) 20 min perfusion of SNAP+Gd^{3+}. II) application of Gd^{3+} to SNAP-perfused preparation: *a*) control; *b*) 20 min perfusion with SNAP; *c*) 10 min perfusion of Gd^{3+}+SNAP. The presented plots are original traces.

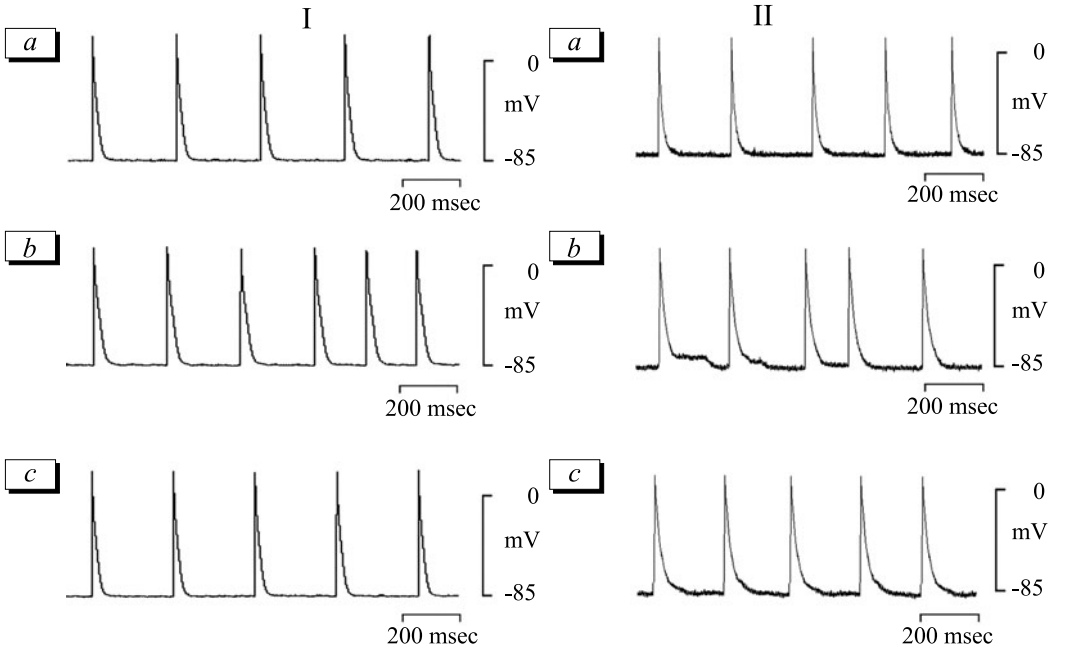

Fig. 3. Effect of extra SNAP application (I) and extra stretch of the preparation (II) on SNAP-induced abnormalities in bioelectric activity of cardiomyocytes in the rat right atrium. I: *a*) control; *b*) 20 min perfusion with SANP (3×10^{-4} M); *c*) 20 min perfusion with enhanced SANP concentration (6×10^{-4} M). II: *a*) control; *b*) 20 min perfusion with SANP (3×10^{-4} M); *c*) stretching the preparation during perfusion with SANP (3×10^{-4} M). The presented plots are original traces.

Further experiments were performed to examine the dose-dependence of hump-like depolarization on NO level. It is important that NO-induced changes in bioelectric activity observed at SNAP concentration of 3×10^{-4} M (Fig. 3, I, *b*) were eliminated with the same agent applied in a higher concentration of 6×10^{-4} M (Fig. 3, I, *c*). It is noteworthy that sustained myocardial stretch also elevates endogenous NO concentration via activation of NO-synthases [12]. In our experiments, the SNAP-induced bioelectrical abnormalities (Fig. 3, II, *b*) were completely eliminated by extra stretch of the atrium (Fig. 3, II, *c*). Thus, low NO is an activation factor for SAC in cardiomyocytes, while high NO (resulting from activation of NO-synthases) inactivates these channels. These inferences agree with the data previously obtained on

isolated cardiomyocytes with patch clamp technique [1,2,9].

The present study revealed involvement of NO in the control of mechanoelectric feedback in the heart and attested to possible role of NO in the development of mechanically induced arrhythmias, which is important to elaborate the novel approaches to pharmacotherapy of this pathology.

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