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Short Communication

Effects of interleukin-6 on the bio-electric activity of rat atrial tissue under normal conditions and during gradual stretching

V.M. Mitrokhin[a,*,1], M.I. Mladenov[a,b,**,1], A.G. Kamkin[a]

[a] Department of Fundamental and Applied Physiology, Russian National Research Medical University, Ostrovitjanova 1, Moscow 117997, Russia
[b] Faculty of Natural Sciences and Mathematics, Institute of Biology, "Ss. Cyril and Methodius" University, P.O. Box 162, 1000 Skopje, Macedonia

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ABSTRACT

Using the micro-electrode technique we studied the effects of interleukin-6 on bio-electric activity of rat atrial tissue under normal conditions and after gradual stretching. It was shown that IL-6 caused increasing of the duration of the action potential at the levels of 25, 50, and 90% re-polarization. The hump-like depolarization at APD90 appeared 7–10 min after initial stretching and transformed into single extra-potentials after tension removing. Perfusion with IL-6 for more than 20 min led to the appearance of atrial fibrillation even with the application of slight tension. Close observation of the IL-6 induced mechanisms and stretch induced APD alteration, confirmed the existence of a tight link between examined cytokine and stretch induced mechanisms.

Introduction

Previous studies have demonstrated that interleukin-6 (IL-6), along with tumor necrosis factor-α, is known as one of the main pro-inflammatory cytokines, especially during acute myocardial infarction. IL-6 exerts acute cardiac negative ionotropic action without detectable reduction in Ca^{2+} currents (I_{Ca}), under basal conditions. It is well known that nitric oxide (NO), affect L-type Ca^{2+} channels and sarcoplasmic reticulum (SR) in cardiac myocytes through signaling pathways such as cyclic guanosine monophosphate (cGMP), and/or by direct modification of the proteins (by S-nitrosylation/oxidation) (Campbell et al., 1996; Hare, 2003; Massion et al., 2003). It is also well known that the myocardial effect of NO is complex and controversial, probably due to the distinct concentration dependent sensitivities of its targets and distinguishable signaling mechanisms. Studies in adult ferrets ventricular myocytes showed that SIN-1, a NO donor, induces biphasic and bimodal changes in basal I_{Ca} (Campbell et al., 1996), which are not observed in guinea-pigs (Wahler and Dollinger, 1995) or rat (Abi-Gerges et al., 2001) ventricular myocytes. Additionally, Yu et al. (2003), have shown that the increase in NO production primarily is a result of expression/activation of inducible nitric oxide synthase (i-NOS) that is mediated via IL-6 induced Janus kinase 2/signal transducer and activator of transcription 3 (JAK2/STAT3) activation. The same author also confirmed that inhibition of iNOS/NO abolished IL-6 induced decrease in SR function (Yu et al., 2003). Accordingly, transient elevation of IL-6 observed in plasma or ventricular muscles of patients with different cardiac diseases, can induce prolonged effects on the contractility. In the same direction Janssen et al. (2005), have shown that *in vivo* IL-6 administration caused heart failure in a dose-dependent manner. Based on all mentioned above, we reasoned that, IL-6 might play an important role in the pathogenesis of different heart diseases.

Although experimental and clinical data mentioned above suggest that IL-6 exerts negative inotropic effect on the heart, detailed mechanisms underlining IL-6 involvement in myocardial dysfunction are not yet completely elucidated. The fact that, the negative ionotropic effect of IL-6 is mediated through myocardial NOS, from one and direct regulatory activity of NO on mechanically gated channels (MGCs) (Kazanski et al., 2011), from the other side, foster us to hypothesize the possible direct IL-6 induced MGCs alteration

Abbreviations: SIDs, stretch induced depolarizations; APs, action potentials; MGCs, mechanically gated channels; IL-6, interleukin-6; RP, resting potential; APA, AP amplitude; APD25, AP durations to 25% of repolarization; APD50, AP durations to 50% of repolarization; APD90, AP durations to 90% of repolarization; RAP, right atrial preparation.

* Corresponding author. Tel.: +7 095 131 4863; fax: +7 095 131 4863.
** Corresponding author at: Department of Fundamental and Applied Physiology, Russian National Research Medical University, Ostrovitjanova 1, Moscow 117997, Russia. Tel.: +7 095 131 4863; fax: +7 095 131 4863.
E-mail addresses: vmitrohin@gmail.com (V.M. Mitrokhin), m.mitko@gmail.com (M.I. Mladenov).
[1] These authors contributed equally as senior authors to this paper.

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and its participation in the regulation of mechano-electrical feedback. Keeping in mind that, modulation of the cardiac electrical activity by a cardiac mechanical environment (mechanoelectric feedback) (Lab, 1996), depends to a large extent on the variable filling pressure of the heart, the aim of this study was to investigate the influence of IL-6 on bio-electric activity of rat atrial cardiomyocytes under normal conditions and in stretched myocardium.

Methods and materials

Animals and experimental design

Male Wistar rats ($n = 28$) were used for all protocols and were maintained on a 12:12 light:dark cycle and fed with standard rat chow and water, *ad libitum*. All experimental procedures were conducted in accordance with the Guiding Principles for Care and Use of Laboratory Animals approved by the Russian Center for Bioethics. All protocols were approved by the Animal Bioethics Committee at the Russian National Research Medical University, in accordance with the International Guiding Principles for Biomedical Research Involving Animals published by the US National Institute of Health (NIH Publication No. 85-23, revised 1996). The animals were killed by decapitation without preliminary anesthesia, the chest was immediately opened, and the heart quickly excised.

Recordings of APs and contractile activity in control and stretched RAPs

Action potential (AP) recordings of right atrial preparation (RAPs) are described in detail in the study published by Kamkin et al. (2000). Briefly, APs (spontaneously occurring) were recorded from the endocardial surface of the auricle. Changes in the resting potential (RP), AP-amplitude (APA), and AP-durations to 25 (APD25), 50 (APD50) and 90% of repolarization (APD90) were analyzed. Further, in order to simulate changes in right atrial pressure, the stretch was applied in steps with a duration of about 5 s. Since the relationship between the stretch force and length changes differs among right arterial preparations (RAPs), we used an increasing isometric force amplitude (ΔAF), to achieve standardization of the mechanical test stimulus (Abramochkin et al., 2013).

Solutions and drugs

PSS had the following composition (in mM): NaCl, 137; KCl, 5.4; $CaCl_2$, 1.0; $MgCl_2$, 0.5; Na-HEPES, 5.0; Glucose, 5.5, bubbled with O_2 and pH adjusted to 7.4 with NaOH. IL-6 was dissolved in a perfusion solution at a concentration of 50 ng/ml. The MGC inhibitor gadolinium ($GdCl_3$) in a concentration of 40 μmol was used to block mechano-sensitive ionic channels. Rat recombinant IL-6 were obtained from Invitrogen (Carlsbad, CA, USA). The MGC inhibitor Gd^{3+}, and all other compounds in PSS were purchased from Sigma–Aldrich (St. Louis, MO, USA). O_2 (99.9% pure), was from Technical Gases (Moscow, Russian Federation).

Statistical analysis

Data is presented as means ± standard error (SE). Multiple comparisons were made using analysis of variance (ANOVA) with the post hoc test of Bonferroni, performed in selected instances to evaluate further differences between group pairs. The correlation between atrial fibrillation length cycle and APD was assessed by Pearson's test. Only 2-tailed probabilities were used for testing statistical significance. Probability values <0.05 were regarded as statistically significant. All analysis were performed with Graph Pad Prism 4.0 (San Diego, CA, USA).

Table 1
Effects of IL-6 on APA; RP and heart rate.

Parameter	Control	IL-6, 35 min	*p*
APA ($n = 14$)	114.6 ± 1.8 mV	119 ± 1.5 mV	ns
RP ($n = 14$)	−87 ± 1.1 mV	−87 ± 1.6 mV	ns
Heart rate ($n = 14$)	3.45 ± 0.0003 Hz	3.33 ± 0.001 Hz	<0.01

APA – action potential amplitude; RP – resting potential.

Results and discussion

Bio-electrical activity of right atrial preparations under control and conditions of stretch

We demonstrated that 60-min perfusion of the RAPs in control conditions did not modulate bio-electric and mechanical activity in the tissue ($n = 14$). Neither abnormalities in AP shape nor alterations of the sinus rhythm were detected under controled conditions.

The effect of stretch on the bio-electrical activity of RAPs was described in detail in our previous study (Abramochkin et al., 2013). Briefly, the stretch induced irregular episodes of AP elongation considered as stretch-induced depolarizations (SIDs), were observed in 65% of the preparations at 90% repolarization level and in 35% of the preparations at 50% repolarization level. In several experiments, SIDs were transformed into humplike SIDs. Only humplike SIDs occurring at the level of APD90 were regularly associated with extra APs (see Fig. 2A). The humplike SIDs and an episode of arrhythmia appears after the third step of stretch and every following step (data are shown in Abramochkin et al., 2013). The subsequent removal of stretch restored normal electrical activity (for details, see Kamkin et al., 2000, 2005). Since SIDs and humplike SIDs lead to extra AP generation only when occurring at the level of APD90, further, we discussed only the abnormalities appearing in this level. The dependence of bio-electrical activity from each step of stretch was shown in detail in Abramochkin et al. (2013).

The effect of Gd^{3+} on stretch-induced electrical activity

In order to check whether stretch-induced electrical activity is result of MGCs or not, we performed separate experiments with the employment of Gd^{3+} as a specific antagonist of MGCs. The effect of Gd^{3+} on stretch-induced electrical activity was described in detail in our previous studies (Kamkin et al., 2000, 2005; Abramochkin et al., 2013). Briefly, application of Gd^{3+} at a concentration of 40 μM, completely abolished all SIDs, humplike SIDs, and extra APs after 10 min, without significant alteration of contractile activity ($n = 14$). Adding Gd^{3+} in a perfusion solution before stretching did not produce any substantial alterations of basal electrical and mechanical activities, but prevented induction of electrical abnormalities during the subsequent stretch (data are shown in Abramochkin et al., 2013). Hence, we may say, that under our experimental conditions, stretching caused activation of mechano-gated channels in cardiomyocytes and led to an occurrence of hump-like depolarization, which is consistent with earlier obtained data (Kamkin et al., 2000, 2005).

The effects of IL-6 on bio-electrical and stretch-induced electrical activity

Analysis of electrical activity of the RAPs showed that IL-6 did not change the resting potential and the amplitude of action potentials (−87.0 ± 1.1 and 114.6 ± 1.8 mV before the start of perfusion, 87.0 ± 1.6 and 119.0 ± 1.5 mV after 35-min perfusion, respectively; $n = 14$). Also, there were no abnormalities in AP shape while the heart rate was significantly decreased (Table 1). Our results showed that, APD at all levels significantly increased in 85.71% cases within