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Effects of interleukin-2 on bioelectric activity of rat atrial myocardium under normal conditions and during gradual stretching

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ABSTRACT

Using micro-electrode technique we studied the effects of interleukin-2 (50 ng/ml) on bio-electric activity of rat atrial myocardium under normal conditions and after gradual stretching of the tissue. It was shown that interleukin-2 caused increasing in the duration of action potential at the levels of 25, 50, and 90% re-polarization. Perfusion with interleukin-2 resulted in appearance of frequent rhythm patterns followed by smooth transient fragments of paroxysmal tachyarrhythmia pacing into normal rhythms. In the presence of interleukin-2, stretching of the tissue by 1.7 mN led to appearance of abnormal bio-electrical activity, predominantly in the lengthening of the duration of action potential at the levels of 90% re-polarization. Close observation of both interleukin-2 induced action potential duration to 90% of re-polarization, hump-like depolarization and stretch induced hump-like alteration, indicate existence of a link between the interleukin-2 and stretch induced mechanisms.

1. Introduction

Interleukin-2 (IL-2), is a multifunctional cytokine with pleiotropic effects on several cells of the immune system. IL-2 was originally discovered as a T cell growth factor, but it was also found to have actions related to B cell proliferation and cytolytic activity of natural killer cells [1].

Compelling evidence now exists that shows that circulating IL-2 is actively involved in the pathophysiology of myocarditis and idiopathic dilated cardiomyopathy [2,3]. Clinical data showed that IL-2 therapy depresses cardiac contractility in patients with heart diseases [4]. Several experiments reported that IL-2 has a negative inotropic effect in a variety of cardiac muscle preparations including the whole heart, isolated papillary muscles, and myocytes [5–7]. It was also published that IL-2 has an effect similar to class I anti-arrhythmic inhibitors with extracellular concentration which is usually observed in patients having therapy with high doses of IL-2 [8]. Moreover, recent studies have shown that IL-2 has an inhibitory effect on Na^+ channels in skeletal muscles within concentrations observed in patients with inflammatory neuronal diseases [9]. Actually, in skeletal muscles, IL-2 acts directly on Na^+ channels without the involvement of secondary messengers [9]. Further, we have shown that IL-2 blocks voltage gated Na^+ channels by transferring the channel to a state of rapid inactivation (Mitrokhin et al., unpublished data). It is also believed that IL-2 decreases the intracellular cyclic adenosine monophosphate (cAMP) concentration and increases the sarcoplasmic reticulum calcium ATPase (SERCA) activity [4]. IL-2 stimulation induces the activation of the Janus family tyrosine kinases 1 and 3 (JAK1) and (JAK3), respectively. These kinases in turn phosphorylate IL-2R and induce a tyrosine phosphorylation of STATs (signal transducers and activators of transcription) and various other downstream players [10]. The conclusion arising from these findings is that signaling induced by IL-2 may be related to a substantial alteration in a wide range of signal players.

Although experimental and clinical data mentioned above suggest different IL-2 effects on the heart, detailed mechanisms underlining IL-2 involvement in myocardial dysfunction are not clear yet. In this direction, special attention has been given to the studies pointing out the influence of IL-2 on cardiac electrical activity. The studies for negative ionotropic effects of the IL-2 [5–7],

Abbreviations: APs, action potentials; APA, action potential amplitude; APD25, action potential durations to 25% of repolarization; APD90, action potential durations to 50% of repolarization (APD50) and action potential durations to 90% of repolarization; cAMP, cyclic adenosine monophosphate; IL-2, interleukine 2; MGCs, mechanically gated channels; RP, resting potential; RAP, right atrial preparation; SERCA, sarcoplasmic reticulum calcium adenosine triphosphate-ase; SIDs, stretch induced depolarizations.

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lay down the basis for possible IL-2 involvement in mechanogated channels (MGCs) signaling and its participation in the regulation of mechano-electrical feedback. Knowing that, modulation of the cardiac electrical activity by a cardiac mechanical environment (mechanoelectric feedback) [11], 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-2 on bio-electric activity of rat atrial cardiomyocytes under normal conditions and in stretched myocardium.

2. Methods and materials

2.1. Animals and experimental design

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 Institutes of Health (NIH Publication No. 85-23, revised 1996). Male Wistar rats weighing 230–250 g were used in the study ($n = 29$). The animals were killed by decapitation without preliminary anesthesia, the chest was immediately opened, and the heart quickly excised.

2.2. Intracellular recordings of APs in isolated right atrial preparations (RAPs)

APs recordings of RAPs are described in detail in the study published by Kamkin and Cow. [12]. Briefly, after isolation, the heart was immediately immersed in a physiological salt solution (PSS), pH 7.4. The preparations of the right atria, including the auricle, the crista terminalis, the intercaval region, and the central node were isolated and pinned to the bottom of an experimental chamber supplied with PSS at 10 ml min^{-1} (37 °C). APs (spontaneously occurring) and 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.

2.3. Tissue stretch and recording of contractile activity

The RAPs were fixed horizontally with two tungsten clips connected to a force transduction system (Plugsys 603, Hugo Sachs Elektronik, Germany) and an electric programmable micromanipulator (Sutter MP 285, Novato CA, USA). The micromanipulator was used for the adjustment of preload and stretch. Developed active forces (AFs), were measured at a baseline preload of 1 mN, about 0.30 ± 0.01 mN. A preload of 1 mN (further called resting force-RF), corresponded to the lengthening of the RAPs (approximately 6% of its initial length). To simulate changes in right atrial pressure, we applied stretch in steps, at approximately 5 s. Since the relationship between the stretch force and length changes differs among RAPs, we used the increasing in isometric force amplitude (ΔAF), to achieve standardization of the mechanical test stimulus [12].

2.4. Solutions and drugs

Freshly prepared 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-2 was dissolved in a perfusion solution at a concentration of 50 ng/ml. The MGCs inhibitor gadolinium (Gd^{3+}) in a concentration of 40 μmol was used to block mechanosensitive ionic channels. Rat recombinant IL-2 was obtained from Invitrogen (Carlsbad, CA, USA). The MGCs 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).

2.5. Statistical analysis

Results are presented as mean ± standard error (SE), for n experiments; (n is the number of RAPs). Multiple comparisons were made using analysis of variance (ANOVA) with the post hoc Tukey test. Probability values <0.05 were regarded as statistically significant. All analyses were performed with Graph Pad Prism 4.0 (San Diego, CA, USA).

3. Results

3.1. Bio-electrical activity of RAPs under control and conditions of stretch

The mean control values of the measured parameters are shown in Table 1. There was no significant change during the first hour of the experiment. Neither abnormalities in AP shape nor alterations of the sinus rhythm were detected under control conditions.

The effect of stretch on the bio-electrical activity of RAPs is shown in Table 2. The stretch provoked a gradual increase in APD90, under control conditions (Table 2; Fig. 1.C). Precisely in 66% of APD90 and 34% of APD50, irregular episodes of AP prolongation were observed. These alterations in the APs waveforms were considered as stretch induced depolarisations (SIDs), which in several cases were transformed into hump-like SIDs. There was no generation of extra APs by the hump-like SIDs that appeared at the level of APD50, while SIDs appeared at the level of APD90 were permanently associated with extra APs. The first step of stretch did not alter the APs configurations, the second one slightly increased APD90, the third one and every consecutive step induced hump-like depolarizations resulting with an arrhythmic episode (for details see [13]). Restored normal electrical activity was achieved by subsequent removal of the stretching. Since SIDs and hump-like SIDs led to extra APs generation only when occurring at the level of APD90. Further, we will discuss only the abnormalities occurring at this level.

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

In order to check whether stretch-induced electrical activity is a result of MGCs or not, we performed separated 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 [12–14]. Briefly, application of Gd^{3+} at a concentration of 40 μM, completely abolished all SIDs, hump-like SIDs,

Table 1
Mechanical and electrophysiological parameters of rat RAPs during 60 min of perfusion under normal conditions.

Parameter (unit)	Number (n)	Time from the start of recording	
		5 min	60 min
AF (mN)	14	0.31 ± 0.09	0.32 ± 0.01
RP (mV)	14	-87.1 ± 1.4	-86.6 ± 2.1
AP (mV)	14	114.2 ± 2.5	113.8 ± 2.8
APD25 (ms)	14	5.51 ± 0.94	5.54 ± 1.01
APD50 (ms)	14	9.03 ± 1.67	9.17 ± 1.52
APD90 (ms)	14	27.38 ± 4.68	27.59 ± 4.66

AF—active force; RP—resting potential; AP—action potential amplitude; APD25—action potential duration at 25% of repolarization; APD50—action potential duration at 50% of repolarization; APD90—action potential duration at 90% of repolarization; no significant differences for the respective parameters at 5 min and 60 min were observed.