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Interleukin-6 induced activation of a non-selective outward cation conductance in human cardiac fibroblasts

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ABSTRACT

Background: It has been demonstrated that cardiac fibroblasts of the human heart have several myocyte-like features, induced by inflammation.
Objectives: This study analyzed the changes of the expressed currents in the basal condition and in the presence of interleukin-6 in cultured human cardiac fibroblasts.
Methods: Human cardiac fibroblasts were cultured as monolayers from earlier passages (2–4). Whole-cell voltage clamp experiments were performed on single culture human cardiac fibroblasts.
Results: The cultured human cardiac fibroblasts had a membrane resistance of R_m of 412 ± 91 MΩ, and a resting membrane potential of −68.1 ± 3.2 mV. Among different cells, we have been analyzed these at which depolarizing clamp steps induced outward currents that reached peak within approx. 20 ms and then slowly decayed. Gd^{3+} decreased the current amplitudes at depolarizing steps. Superfusion with interleukin-6 caused increasing of the outward membrane currents. The changes in the membrane currents continued up to 6 min of interleukin-6 perfusion, by reaching their maximum at 3 min and slowly decreasing to the level of control recordings at 6 min. In the presence of 8 μmol/l Gd^{3+}, interleukin-6 does not modify the membrane currents.
Conclusion: The involvement of mechano sensitive channels in interleukin-6 induced electrical property of fibroblast was proposed. This report presents one particular model of action of interleukin-6, that can open new insights for a deeper understanding of the relationships between interleukin-6 and different ion channels into the fibroblast.

1. Introduction

Fibroblasts, encompassing 75% of all cells, are the most abundant cell type, by number in the myocardium [1].

Fibroblasts modulate cardiomyocyte electrical activity by depolarizing or hyperpolarizing cardiomyocytes, depending on the relative values of cardiomyocyte vs. fibroblast transmembrane potential. They also display a variety of ion channels that show voltage and time dependent conductance [2]. Recently published data demonstrated that a big conductance Ca^{2+}-activated K^+ current (BK_{Ca}), a delayed rectifier K^+ current (I_{KDR}), a non-selective cation channel current and an inward rectifier K^+ current (I_{Kir}) with either fast or slow inactivation, were detected in cultured human cardiac fibroblasts (CHCF) [3]. Also, Kiseleva with cow reported for changes in the fibroblast membrane potential, described by operation of the mechano-sensitive channels (MSCs) [4], and by oscillations in the intracellular Ca^{2+} concentration [5]. All above mentioned currents have different distribution and properties compared to those in the human cardiomyocytes [6].

In response to pathological conditions, such as inflammatory stimuli, fibroblasts proliferate, migrate and undergo phenotypic changes involving differentiation into myofibroblasts [7]. Myofibroblasts play a pivotal role in the fibrotic process by producing growth factors, cytokines, chemokines, extracellular matrix proteins and proteases [7,8]. Cytokines produced by myofibroblasts have been reported to exert ionotropic action upon their neighboring cells (mainly cardiomyocytes and fibroblasts) [9,10]. Interleukin-6 (IL-6), as one of the main pro-inflammatory cytokines, exerts acute cardiac negative inotropic action under basal conditions [10]. In spite of the data about its inotropic action, the literature data regarding its relation to ion channels, matching electrical features of the inflamed human cardiac fibroblast (HCF) are obscure.

Concerning signaling, it was shown that IL-6 was involved in

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cellular nitric oxide (NO) production primarily via the expression/activation of inducible NO synthase (i-NOS), which was initiated via IL-6 induced Janus kinase 2/signal transducer and activator of transcription 3 (JAK2/STAT3) activation [8]. Based on that, it was hypothesized that the regulation of these IL-6 induced MSCs mechanisms could ascertainably be utilized as an operating tool in the human cardiac fibroblasts (HCF) membrane influenced by NO. Acknowledging that the CHCF expressed different currents in the basal condition [11,12], different variations in their channel's activities in the presence of IL-6, could be expected. For that reason, in this research, CHCFs were patch-clamped in order to analyze the influence of IL-6 upon the CHCFs membrane conductance.

2. Materials and methods

2.1. Cell cultures

Human cardiac fibroblasts (adult ventricles, Catalog # 6310) were purchased from Scien Cell Research Laboratory (San Diego, CA). The cells were cultured as monolayers in completed DMEM, containing 10% fetal bovine serum (Invitrogen, Hong Kong) and antibiotics (100 U/ml penicillin G and 100 mg/ml streptomycin) at 37 °C in a humidified atmosphere of 95% air, 5% CO_2. The cells used in this study were from the early passages (2–4) in order to limit the possible variations in functional ion channel currents and gene expression. The cells were harvested for electrophysiological recording via trypsinization [12].

2.2. Whole-cell patch-clamp experiments

Whole-cell voltage clamp experiments were performed on single CHCF. A small aliquot of the solution containing cardiac fibroblasts was placed in an open perfusion chamber (1 ml) mounted on the stage of an inverted microscope. The cells were allowed to adhere to the bottom of the chamber for 15–20 min. During the experiment, Tyrode solution flowed continuously through the bath chamber. Drugs were usually added to the bath solution. The voltage clamp mode of the whole-cell patch-clamp technique was employed. The *I-V* relations were obtained by 16 pulses with a duration of 140 ms, which started at a holding potential of −45 mV and went to −100, −90, −80, −70, −60, −50, −40, −30, −20, −10, 0, 10, 20, 30, 40, and 50 mV. Membrane currents flowing at the end of the pulse ('late current' I_L) were plotted versus the respective clamp step potential. All experimental procedures, applied techniques and used materials are described in detail in our previous studies [12,13].

2.3. Mechanical stretching of CHCF

CHCF were mechanically stretched between patch and stretch pipettes (PP and SP). The PP was used for whole-cell clamp and served as a fix point. The SP-cell attached pipette was laterally displaced in regard to the PP, thereby stretching the cell. We demonstrate the reaction of the CHCF membrane to mechanical stretch, applied from the center of the cell.

2.4. Solutions

Tyrode solution for electrophysiological studies contained (in mmol/L): 140 NaCl, 5.0 KCl, 1.0 $MgCl_2$, 1.8 $CaCl_2$, 10 glucose, and 10 HEPES. pH was adjusted to 7.4 with NaOH. The solution in the recording pipette contained (in mmol/L): 140 KCl, 4.5 $MgCl_2$, 4.0 Na_2ATP, 10 HEPES; pH was adjusted to 7.4 with KOH. At the end of the experiments 8 μmol/L $GdCl_3$ was added to Tyrode solution. IL-6 was added to the Tyrode solution (final concentration 5 ng/ml). The dose of IL-6 used in this study (5 ng/mL) is within the physiological/pathophysiological range for circulating IL-6 concentrations *in vivo*. It has been shown that isolated cardiomyocytes in culture produce up to 6 ng/ml IL-6 in the culture supernatant [14] which is in the same range as the concentrations used in this study. Furthermore, interstitially reached concentrations *in vivo* are likely to be much higher. Postoperatively, in systemic inflammatory response syndrome following coronary artery bypass grafting, IL-6 concentrations achieve approximately 640 pg/ml, and 14 ng/ml in sepsis [15].

NaCl, KCl, $MgCl_2$, $CaCl_2$, glucose, HEPES, NaOH, KOH, $GdCl_3$, Na_2ATP, IL-6 and NO-synthase inhibitor L-N^G-nitroarginine methyl ester (L-NAME hydrochloride), were purchased from Sigma-Aldrich (Natick, MA, USA).

2.5. Statistical analysis

Currents were expressed in pA and plotted as functions of the potential applied to obtain data suitable for statistical analysis. Values are given as mean ± SD. The mean values for the changes caused by IL-6 or Gd^{3+} were calculated from currents recorded from the same cell before and after perfusion with the appropriate compound. The significance of differences between means was assessed using the Bonferroni multiple comparison test with $p < 0.05$ regarded as significant.

3. Results

3.1. Control currents

CHCF has a membrane resistance R_m of 412 ± 91 MΩ, and a resting membrane potential of −68.1 ± 3.2 mV (n = 48). The amplitude of the currents during both depolarizing and hyperpolarizing steps are demonstrated in Fig. 1A. The depolarizing steps induced outward currents that reached the peak within approx. 20 ms and then slowly decayed.

The experiments were finished by the addition of 8 μmol/l $GdCl_3$ (Gd^{3+}), to the perfusing solution. Gd^{3+} shifted the holding current at −45 mV to more positive values (beginning of the traces in Fig. 1B). Further, Gd^{3+} significantly decreased current amplitudes during the depolarizing clamp steps ($p < 0.05$; Fig. 1B).

The *I-V* curves of the late currents from control and Gd^{3+} perfused CHCF intersected the zero current axis at different values. Fig. 1D (empty circles) demonstrates a typical example of E_0 = −68.1 ± 3.2 mV, value which reflects the resting potential of the control currents. Gd^{3+} shifted the intercept of the *I-V* curve rightward on the voltage axis, indicating insignificant very small depolarization and E_0 shift from −68.1 ± 3.2 mV in the control condition (n = 48) to −65.7 ± 3.7 mV in the presence of Gd^{3+} (n = 13). The addition of Gd^{3+} (Fig. 1D, filled diamonds) shifted E_0 during the first 7 min. During the following time (10 min), Gd^{3+} induced additional very small depolarizations of E_0 toward more positive values than −65.7 ± 3.7 mV, −64.3 ± 2 mV; n = 7, respectively).

3.2. Stretch reduces G_{NS}

Fig. 2 shows membrane currents recorded in control (Fig. 2A), during 2 μm lateral stretch (Fig. 2B) and 7 min after addition of 8 μmol/l $GdCl_3$ (applied during continuous stretch) (Fig. 2C). Stretch shifted the holding current at −45 mV to more positive values (beginning of the traces in Fig. 2B). Stretch significantly reduced ($p < 0.05$), the amplitude of the currents during the pulses at depolarizing clamp steps (Fig. 2D). The experiment was finished by the addition of 8 μmol/l Gd^{3+} to the superfusing solution. Fig. 2B demonstrates a typical example of the stretch induced changes in the net membrane currents in CHCF. Starting from −68.1 ± 3.2 mV, a 2 μμm stretch changed E_0 to −73.3 ± 5.4 mV (n = 12). When Gd^{3+} was applied for 7 min, E_0 was shifted to −75.2 ± 4.4 mV (n = 8). The addition of Gd^{3+} significantly reduced the membrane conductance below the reduction obtained by mechanical stretch ($p < 0.05$; Fig. 2C).