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Original Article

Transfection by eukaryotic expression vector pcDNA3-HERG inhibits the cultured neonatal rabbit ventricular myocyte hypertrophy induced by phenylephrine

Yonghui Zhao [a], Yu Xu [a,*], Changcong Cui [b], Yu Li [b], Yanjun Zeng [c,*]

[a] *Department of Cardiology, Henan Provincial People's Hospital, Zhengzhou 450003, PR China*
[b] *Department of Cardiology, First Affiliated Hospital, Ion Channel Disease Laboratory, Key Laboratory of Environment and Genes Related to Diseases, Ministry of Education, Medical College of Xi'an Jiaotong University, Xi'an 710061, PR China*
[c] *Biomechanics and Medical Information Institute, Beijing University of Technology, Beijing 100022, PR China*

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Abstract

Objective: Prolonged action potential and decreased outward K^+ currents are consistent findings in hypertrophic myocardium. The relation between action potential prolongation and myocyte hypertrophy has remained unclear. The present study investigated the temporal relation between action potential prolongation and myocyte hypertrophy, and the effect of enhancing repolarization on myocyte hypertrophy induced by phenylephrine. **Methods:** Neonatal rabbit ventricular myocytes were cultured and treated with 10 μmol/l phenylephrine. At 6 and 48 h after phenylephrine stimulation, myocyte hypertrophic parameters (including myocyte volume, total protein content, and membrane capacitance), action potential duration (APD), and calcineurin activity were measured; meanwhile, the effect of human-ether-a-go-go–related gene (HERG; encoding the αsubunit of rapidly activating delayed rectifier potassium channel) transfection on the above parameters at 48 h of phenylephrine stimulation was also measured. **Results:** At 6 h after phenylephrine treatment, APD at 90% repolarization of neonatal rabbit ventricular myocytes was prolonged by 14.3% ($P<.05$), but myocyte hypertrophy was not found. At 48 h after phenylephrine stimulation, APD at 90% repolarization of neonatal rabbit ventricular myocytes was furthermore prolonged by 18.8% ($P<.05$); at the same time, myocyte volume, total protein content, membrane capacitance, and calcineurin activity were increased by 40.0%, 41.8%, 36.4%, and 124.1%, respectively ($P<.01$). Neonatal rabbit ventricular myocytes transfected by pcDNA3-HERG overexpressed $I_{HERG,tail}$ current, which was about fourfold higher than I_{Kr} (rapidly activating delayed rectifier K^+ current) of neonatal rabbit ventricular myocytes without transfection of HERG. HERG overexpression could accelerate repolarization and shorten APD at 90% repolarization prolonged by phenylephrine and partially inhibit myocyte hypertrophy and calcineurin activation. **Conclusions:** During the myocyte hypertrophy induced by phenylephrine, prolongation of APD at 90% repolarization is not secondary to but precedes myocyte hypertrophy. HERG overexpression could enhance the repolarization and inhibit the calcineurin activation and myocyte hypertrophy induced by phenylephrine.

Keywords: Action potentials; Calcium; Myocytes; Hypertrophy; Rapidly activating delayed rectifier K^+ current

1. Introduction

Hypertrophy represents the common heart response to a variety of intrinsic or extrinsic physiological or pathological stimuli [1]. The hypertrophied heart shows remodeling of its metabolic, biological, and electrophysiological properties (electrical remodeling), and is an independent risk factor for ventricular arrhythmias and sudden death. The most consistently electrophysiological change in hypertrophied myocytes

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* Corresponding authors. Yu Xu is to be contacted at the Department of Cardiology, Henan Provincial People's Hospital, Zhengzhou 450003, PR China. Yanjun Zeng, Biomechanics and Medical Information Institute, Beijing University of Technology, Beijing 100022, PR China.

E-mail addresses: xuyu1957@126.com (Y. Xu), yjzeng@bjut.edu.cn (Y. Zeng).

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is a prolongation of action potential duration (APD) as a result of an imbalance of repolarizing and depolarizing ionic currents [2–4]. Usually, alteration of ionic channels is thought to be secondary to structural hypertrophy resulting from fetal gene reprogramming. However, temporal dissociation of electrical remodeling and structural hypertrophy has been recently reported [5,6]. Moreover, an experimental study using a transgenic mouse model has demonstrated that up-regulation of L-type Ca^{2+} channel triggers cardiac hypertrophy [7]. In cultured neonatal ventricular rat myocytes, Zobel et al. [8] found that phenylephrine (PE)-mediated hypertrophy required transient outward K^+ current (I_{to}) reduction and APD prolongation associated with increased Ca^{2+} influx through voltage-dependent L-type Ca^{2+} channel as well as calcineurin (CaN) activation. Accordingly, electrical remodeling might not be secondary to but precede structural remodeling during the development of myocyte hypertrophy [5]. Furthermore, the implications of APD prolongation on cardiac hypertrophy need to be elucidated in other species.

The activation of the sympathetic nervous system plays a major role in the whole-body neuroregulation of hypertrophic remodeling. In particular, adrenergic signals stimulate cardiac hypertrophy mainly through the α_1 adrenoceptor subgroup, whereas β-receptors participate more in the regulation of cardiac function [9]. Stimulation of α_1 adrenoceptors can prolong APD in rat and rabbit ventricular myocytes by alterations of many ionic currents including the reductions in I_{to} and the delayed rectifier potassium current (I_K) [8,10]. Many of the electrophysiological and pharmacologic properties of endogenous currents in rabbit myocytes have been characterized [11]. Different from rodents', rabbit myocytes present a long plateau similar to human. Moreover, as compared to I_{to}, I_{Kr} contributes more to AP repolarization in rabbit myocytes. I_{Kr} terminates the plateau phase of the AP and initiates repolarization. The human-ether-a-go-go–related gene (HERG) encodes the voltage-gated potassium channel α-subunit underlying I_{Kr}. Reduction in cardiac I_{Kr} due to mutations in HERG produces chromosome 7-linked congenital long QT syndrome [12]. Left ventricular hypertrophy is a commonly acquired long-QT syndrome due to the down-regulation of several K^+ currents responsible for repolarization [13].

The α_1 adrenoceptor agonist PE has been commonly used to induce hypertrophy in cultured myocytes. In the present study, we observed the electrical characteristics of cultured neonatal rabbit ventricular myocytes at different stages after PE treatment. Moreover, the hypothesis was tested that overexpression of HERG could enhance repolarization, decrease activity of CaN, and therefore prevent myocyte hypertrophy induced by PE.

2. Materials and methods

2.1. Isolation and culture of neonatal ventricular myocytes

The investigation conforms to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996). The study protocol for the use of rabbit was approved by the Ethics Committee of Henan Provincial People's Hospital. Ventricular myocytes were isolated and cultured from the hearts of New Zealand White rabbits (either sex, 2 days old) by using a method previously described [14]. In brief, pups were killed by cervical dislocation and the hearts removed quickly into filter-sterilized buffer (pH 7.5). With the use of an aseptic technique, atria and blood vessels were removed and the ventricle apexes minced. Ventricular tissue was dissociated at room temperature (22–24°C) with trypsin (1 mg/ml in buffer) and gentle mechanical agitation. Cells liberated over the first 1 h were discarded. Thereafter, cells were collected in 5-ml aliquots every 5 min. Freed cells were collected in fetal bovine serum (10% FBS, Gibco). When dissociation was complete, the cell suspension was centrifuged at 1000 rpm at 4°C for 5 min, washed once, and resuspended in culture medium (DMEM, Gibco). Nonmyocytes were removed by differential adhesiveness, and myocytes were plated at a density of 2×10^5 viable cells per milliliter in culture medium supplemented with 5-bromo-2-deoxyuridine (0.1 mmol/l, Sigma) during the first 48 h. After 48 h in culture, the medium was replaced by 1% FBS in order to unite cell growth to G0 phase in the subsequent 24 h. Cells were incubated at 37°C in a humidified atmosphere of 5% CO_2 and 95% air. Cardiomyocytes' purity was verified by mouse monoclonal α-sarcomeric actin antibody.

2.2. DNA constructs and transient transfection of cardiomyocytes

HERG wild-type cDNA was subcloned into *Bam*HI/*Eco*RI sites of the pcDNA3 vector (Invitrogen, Carlsbad, CA, USA), as described previously [15]. Ventricular myocytes maintained in culture for 72 h were transiently transfected using the Lipofectamine 2000 method as recommended by the manufacturer (Life Technologies, Rockville, MD, USA). Transient transfection was performed with 4 μg HERG cDNA or pcDNA3 vector (mock transfection) per 35-mm culture dish. For visual identification of transfected cells and patch clamp experiments, green fluorescent protein (pRK-GFP) was coexpressed with a cDNA ratio of 1:5 to serve as an indicator. PE in a concentration of 10 μmol/l was added at 6 h after transfection. Myocytes without transfection were treated or not with PE as described above for control. All cells were continually incubated for 48 h in 1% FBS at 37°C until the following measurements.

2.3. Measurement of cardiomyocyte volume

At 48 h, myocytes were washed three times with Hanks' balanced salt solution (HBSS) without Ca^{2+} and Mg^{2+}, and digested by 0.25% trypsinase for 10 min. Then digestion was terminated by 10% FBS. Myocytes were harvested and plated on the coverslips with spherical morphology. The diameter of single cell was measured by the BI2000