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Enhanced effect of VEGF165 on L-type calcium currents in guinea-pig cardiac ventricular myocytes

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ABSTRACT

The mechanisms of vascular endothelial growth factor 165 (VEGF165) on electrical properties of cardiomyocytes have not been fully elucidated. The aim of this study is to test the hypothesis that VEGF165, an angiogenesis-initiating factor, affects L-type calcium currents ($I_{Ca,L}$) and cell membrane potential in cardiac myocytes by acting on VEGF type-2 receptors (VEGFR2). $I_{Ca,L}$ and action potentials (AP) were recorded by the whole-cell patch clamp method in isolated guinea-pig ventricular myocytes treated with different concentrations of VEGF165 proteins. Using a VEGFR2 inhibitor, we also tested the receptor of VEGF165 in cardiomyocytes. We found that VEGF165 increased $I_{Ca,L}$ in a concentration-dependent manner. SU5416, a VEGFR2 inhibitor, almost completely eliminated VEGF165-induced $I_{Ca,L}$ increase. VEGF165 had no significant influence on action potential 90 (APD90) and other properties of AP. We conclude that in guinea-pig ventricular myocytes, $I_{Ca,L}$ can be increased by VEGF165 in a concentration-dependent manner through binding to VEGFR2 without causing any significant alteration to action potential duration. Results of this study may further expound the safety of VEGF165 when used in the intervention of heart diseases.

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1. Introduction

VEGF 165 (isoform of VEGF A), is an angiogenetic cytokine that stimulates the formation of new blood vessels and has great potential to enhance cardiac repairment and treat devastating heart failure [1,2]. Although current genetic or cell-based research does not provide obvious evidence that VEGF165 has profound effects on ischemic heart disease (IHD) and heart failure (HF) which are the leading causes of morbidity and mortality worldwide [3,4], more studies are needed to understand the mechanisms of action of this important growth factor and explore its therapeutic potentials.

The potency of VEGF 165 is mainly mediated by VEGF type-2 receptor (VEGFR2) that is a highaffinity transmembrane tyrosine kinase receptor [1,5]. Studies have shown that VEGF165 exhibits multitudinous effects not just induces neovascularization in the heart [6] Protecting cardiomyocytes from apoptosis demonstrated the association between this growth factor and myocardium [7,8]. It has been known that calcium cycling is a complicated process in cardiac myocytes. Calcium current (I_{Ca}) through L-type calcium channels (LTCCs) is of great importance to cardiac excitation-contraction coupling (EC coupling) by initiating calcium-induced calcium release (CICR) and contributes to action potential plateau [9]. Under normal conditions or at a pathological state, L-type calcium currents ($I_{Ca,L}$) in cardiomyocytes are regulated and its dysfunction may contribute to the generation of arrhythmia and other heart diseases [10–13].

Given the regulatory role of VEGF165 in cardiac myocytes, we wonder if VEGF165 affects $I_{Ca,L}$ on cell membrane and cardiac

Abbreviations: VEGF, vascular endothelial growth factor; LTCC, L-type calcium channel; VEGFR2, VEGF type-2 receptor; $I_{Ca,L}$, L-type calcium currents; AP, action potential; APD, action potential duration; EC coupling, excitation-contraction coupling; IV, current-voltage.

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electrical properties which have not been studied. Therefore, we investigated the effects of different concentrations of VEGF165 on $I_{Ca,L}$ in isolated guinea-pig ventricular myocytes and their effects on action potential (AP) to provide useful information in exploring the therapeutic potentials and safety profiles of VEGF165.

2. Materials and methods

2.1. Myocytes isolation

Animal protocals used in this study were approved by the Institutional Animal Care and Use Committee of Zhengzhou University for Medical Research. Single ventricular myocytes were isolated from female Hartley guinea-pig heart following the method as previously described [14,15]. Guinea-pigs (weight 350–400 g) were anaesthetized by intraperitoneal injection of 2% pentobarbital sodium (30 mg/kg) and heparine (1000 u/ml). When deep anesthesia was achieved, the heart and sufficient length of aorta were excised gently. The heart was washed briefly in ice-cold Ca^{2+}-free perfusion buffer (in mmol/L: NaCl 136, KCl 5.4, $K_2HPO_4 \cdot 2H_2O$ 0.33, $MgSO_4 \cdot 7H_2O$ 1.0, Glucose 10 and HEPES 10; pH adjusted to 7.3–7.4 with 1 mol/L NaOH) to rinse out blood. Then the heart was cannulated through the aorta, secured with tie and perfused with a modified Langendorff apparatus. The heart was initially perfused with Ca^{2+}-free perfusion buffer saturated with 95% O_2/5% CO_2 at 37 °C at a rate of 7 mL/min by a constant flow pump to completely flush out blood. The heart was then perfused circularly with 50 mL enzyme solutions (Ca^{2+}-free perfusion buffer 50 mL, Collagenase Type II 25 mg, Pronase E 10 mg, BDM 25 mg, Carnitine 20 mg, Aurine 31 mg and L-Glutamic Acid 20 mg) for about 20 min. The left ventricle was cut down and minced in a small beaker with 5 mL KB solutions (in mmol/L: KOH 80, KCl 30, L-Glutamic Acid 50, $MgCl_2$ 1, HEPES 10, Glucose 10, KH_2PO_4 20, Taurine 20 and EGTA 0.5; pH adjusted to 7.3-7.4 with 1 mol/L KOH). If the rod-shaped myocytes were visible under the microscope, the isolation had been worked well. The myocytes were aspirated up and down gently, and the cell suspension was filtered through 200 μm nylon mesh. The calcium concentration was gradually restored to 1.0 mmol/L after dispersion and was restored at 10 °C. Cells were used for experiments within 8 h after isolation and only quiescent rod-shaped cells showing clear cross-striations were used.

2.2. $I_{Ca,L}$ recording

Patch pipettes were fabricated with a micropipette puller (model P97, Sutter Instruments, USA) and had a resistance of 4–5 MΩ when filled with pipette solutions (in mmol/L: CsCl 120, $MgCl_2$ 1, HEPES 10, Mg-ATP 4, EGTA 10 and Na_2-GTP 0.3, pH was adjusted to 7.2 with CsOH). Aliquots of cell-containing solution (0.2 mL) were added to a 2 mL bath chamber on the stage of an inverted microscope (Olympus IX71, Japan). The bath solutions (in mmol/L: TEA-Cl 140, $MgCl_2$ 2, $CaCl_2$ 1.8, HEPES 10 Glucose 5; pH was adjusted to 7.4 with TEA-OH) flowed into the chamber *via* a gravity-fed solution delivery system and removed by vacuum suction. The electrophysiological recordings were obtained under visual control under microscope. The amplifier EPC10 (Heka, Germany) was used for the recording of the electrophysiological signal. Offset potentials were nulled directly before formation of the membrane–pipette seal and no leak subtraction was made. Fast capacitance (in pF) compensation was made after high seal achieved. Cell capacitance (in pF) compensation was made from whole-cell capacitance compensation after the whole-cell mode was achieved. $I_{Ca,L}$ traces were elicited using a 500-ms step depolarizing pulse from −60 to 60 mV with 10 mV increments at a frequency of 1 Hz (holding potential was −60 mV). The data were stored and analyzed with Patchmaster (Heka, Germany) and Igor Pro (WaveMetrics Inc, USA). All experiments were performed at room temperature (23–25 °C).

2.3. Drug application

Nifedipine (1 μM in the bath solution of voltage clamp experiments) was used to verify the L-type calcium channels. In dose-response experiments, recombinant human VEGF165 proteins (Sino Biological Inc, China) of varying concentrations (10, 40, 100, and 300 ng/mL) were included in the medium for 10 min. In some experiments, solated cardiomyocytes were pretreated with an VEGFR2 inhibitor (20 μM SU5416, Sigma) for 1 h before addition of VEGF165 (100 and 300 ng/mL) to determine whether the interaction between VEGF165 and VEGFR2 contributes to the VEGF165-induced enhancement of voltage-gated L-type calcium currents. The concentrations of VEGF165 used in this study were based on published effective concentrations for myocardial angiogenesis [16,17].

2.4. Single myocyte action potential recording

Whole-cell patch clamp recording in current clamp mode was conducted to measure action potentials. Cells were superfused with a bath solution containing the following (in mmol/L): NaCl 140, KCl 3.5, $MgCl_2$ 1, $CaCl_2$ 2, Glucose 10, HEPES 10 NaH_2PO_4 1.25 (pH was adjusted to 7.4 with NaOH). Micropipettes contained 5 mM NaCl, 140 mM K-Gluconat, 10 mM HEPES, 1 mM $MgCl_2$, 1 mM EGTA, 2 mM Mg-ATP and 0.1 mM $CaCl_2$ (pH was adjusted to 7.2 with KOH). Only cells with no spontaneous activity and with resting potentials lower than −70 mV were used. The cells were continuously stimulated by a 5-ms suprathreshold pulse. All experiments were performed at room temperature (23–25 °C).

2.5. Statistical analysis

Statistical analysis was performed using SPSS 19.0 (SPSS Inc.). Data are expressed as mean ± SEM and were analyzed using the Student's *t* test or 1-way analysis of variance, followed by Bonferroni analysis for multiple comparisons. $P < 0.05$ was considered statistically significant.

3. Results

3.1. Effect of nifedipine on $I_{Ca,L}$

There are two classes of voltage-dependent calcium channels (L- and T-types) existing in cardiac myocytes. Ca^{2+} current (I_{Ca}) generally refers to the L-type because T-type I_{Ca} is inappreciable in most ventricular myocytes [9,18]. In order to investigate the electrophysiological effect of VEGF165 on L-type calcium current ($I_{Ca,L}$), the $I_{Ca,L}$ (Fig. 1A) in isolated guinea-pig ventricular myocytes was measured by whole-cell patch clamp in the presence and absence of nifedipine (1 μM) which is a L-type calcium channel blocker to confirm the voltage clamp protocol for $I_{Ca,L}$. The normalized current was −0.04 ± 0.02 in the presence nifedipine ($P < 0.001$ versus control group), comfirming that the current we recorded was $I_{Ca,L}$.

3.2. Effects of VEGF165 on $I_{Ca,L}$

In single cardiac ventricular cells, $I_{Ca,L}$ was recorded with the steady-state activation protocol in control and in the presence of VEGF165 (Fig. 2A). Compared with control group, the amplitude of normalized $I_{Ca,L}$ in cardiac myocytes treated with 10, 40, 100 and 300 ng/mL VEGF165 were −0.967 ± 0.029, −1.070 ± 0.049,

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