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EMF protects cardiomyocytes against hypoxia-induced injury via heat shock protein 70 activation

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ABSTRACT

Intracellular calcium ($Ca^{2+}{}_i$) overload induced by chronic hypoxia alters $Ca^{2+}{}_i$ homeostasis, whereas ameliorating calcium homeostasis is believed to be responsible for cardioprotection. We hypothesize that cardioprotection by electromagnetic fields (EMF) exposure may restore $Ca^{2+}{}_i$ homeostasis altered by hypoxia insults. Cardiomyocytes isolated from neonatal Sprague-Dawley rats were exposed to chronic hypoxia (1% O_2, 5% CO_2, 37 °C). We observed that cardiomyocytes injury and hypertrophy were alleviated in hypoxic cardiomyocytes exposed with EMF preconditioning. Compared with hypoxic cardiomyocytes, the diastolic $[Ca^{2+}]_i$ was decreased, the amplitude of $Ca^{2+}{}_i$ oscillations was recovered when cardiomyocytes exposed with EMF. In addition, we also found that EMF exposure significantly increased heat shock protein 70 (HSP70) mRNA expression in hypoxic cardiomyocytes. However, treatment with HSP70 blocker KNK437, almost completely inhibited the EMF induced-cardioprotection and the beneficial effects of Ca^{2+} oscillation in hypoxic cardiomyocytes. These results suggest that EMF preconditioning ameliorates $Ca^{2+}{}_i$ homeostasis through activating HSP70, thereby producing the cardioprotective effect and reduction in hypoxic cardiomyocytes damage.

1. Introduction

Hypoxia is the natural consequence of some environments (e.g., high altitude, diving), it is also a common feature of many clinical diseases (e.g., sleep apnea syndrome, chronic obstructive pulmonary disease, heart failure, vascular diseases, stroke, sepsis, metabolic myopathies). Hypoxia is generally associated with cardiovascular diseases, and it elicits a variety of functional responses in cardiomyocytes, including cell proliferation [1], hypertrophy [2] and death [3]. Many responses of cells on hypoxia are mediated by Ca^{2+} signals [4,5]. Intracellular calcium ($Ca^{2+}{}_i$) plays a central role on regulating contractility, gene transcription, energy balance, hypertrophic growth, and apoptosis in the heart [6–8]. In many instances, one of the constant responses to hypoxia is an increase in intracellular calcium concentration ($[Ca^{2+}]_i$) via the activation of various plasma membrane Ca^{2+} conductances, such as voltage-gated Ca^{2+} channels, ligand-operated Ca^{2+} channels, and non-specific cation channels [9]. $Ca^{2+}{}_i$ homeostasis is very critical to cell survival and hypoxia can induce cell death by increasing $[Ca^{2+}]_i$ [10]. Cardiomyocytes Ca^{2+} loading is recognized as a major factor in acute hypoxia pathology, and then promotes cell death, contractile dysfunction and arrhythmogenic activity [5]. Dysregulation of $Ca^{2+}{}_i$ homeostasis can lead not only to loss of normal physiological control mechanisms but also to pathological changes in cell growth. If a tool to manipulate $Ca^{2+}{}_i$ was available, it might be a promising approach for protection of cardiomyocytes against hypoxia-mediated injury.

Since extremely low frequency electromagnetic fields (ELF-EMF) can penetrate into tissues, ELF-EMF affects cellular functions, such as RNA transcription, DNA synthesis, protein expression, protein phosphorylation, microvesicle motility, proliferation, differentiation and apoptosis. As early as 1977, pulsed electromagnetic fields (PEMF) have been successfully used to treat chronic non-union bone fracture [11]. The Food and Drug Administration of USA has approved EMF as a safe and effective mean for treatment of osteoporosis and bone non-unions [12]. In the past two decades, effects of EMF exposure on the cardiovascular system have also been investigated. Dicarlo et al. showed that ELF-EMF induced stress responses that protect chick embryo myocardium from anoxia damage [13]. Barzelai et al. revealed that an EMF of 80 nT at 15.95–16.00 Hz protected against coronary artery occlusion [14].

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Albertini et al. found that exposure to a 3-mT 75-Hz magnetic field for 18 h significantly reduced necrotic area in rats subjected to acute myocardial infarction [15]. These results suggested that EMF exposure may be an effective and noninvasive way to treat cardiovascular-related diseases, although the mechanisms are not clear.

Calcium signaling is a possible target of ELF-EMF on biological systems according to a hypothesized ion-protein interaction [16]. Numerous studies showed that EMF changed $[Ca^{2+}]_i$ levels in rat pituitary cells, osteoblasts and cardiac cells, even though the mechanisms underlying these effects have not been fully understood [16–19]. Furthermore, our previous studies have found that ELF-EMF can regulate $Ca^{2+}{}_i$ oscillations via affecting Ca^{2+} associated protein activities in cardiomyocytes [20]. It suggests that EMF exposure may be one of the tools to manipulate the $Ca^{2+}{}_i$ handling under pathological conditions.

However, the effects of EMF exposure on $Ca^{2+}{}_i$ handling and mechanisms involved in its cytoprotective effects during myocardial injury induced by hypoxia remain largely unknown. The present study examined the protective effect of EMF exposure and the mechanism underlying this protection against hypoxia-induced injury in neonatal rat cardiomyocytes.

2. Materials and methods

2.1. Animals and primary cultures of neonatal rat ventricular cardiomyocytes

This investigation conformed 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) and was approved by the Animal Administrative Committee of Xi'an Jiaotong University (Xi'an, Shaanxi, China). Animals were supplied by the Animal Center of the Fourth Military Medical University (Xi'an, Shaanxi, China).

Neonatal ventricular myocytes were isolated from 1-day-old Sprague-Dawley rats as described previously [10,20]. In brief, neonatal rats were anesthetized with isoflurane (1.5–2.0% vol/vol in air) and disinfected with 75% ethanol and then decapitated under aseptic conditions. The heart was quickly removed and placed in ice-cold phosphate-buffered saline (PBS) containing 100 U/ml penicillin and 100 μg/ml streptomycin. The ventricles were minced into approximately 1 mm^3 fragments and dissociated with 0.1% collagenase II (GIBCO, Grand Island, NY, USA) for 30 min at 37 °C. After the cells were precipitated by centrifugation (at room temperature, 50xg for 5 min) and the collagenase solution was removed, the pellet of cells was resuspended in Kraft-Brühe (KB) solution (composed of (in mM): KCl 85, K_2HPO_4 30, $MgSO_4$ 5, EGTA 1, Na_2ATP 5, pyruvate 5, creatin 5, taurin 20, and glucose 20; titrated to pH = 7.3 with KOH) at room temperature for 15 min. Cardiomyocytes were then plated on a 22 × 22 mm^2 glass coverslip (Fisher Scientific, Pittsburgh, PA, USA) and incubated in Dulbecco's modified Eagle medium (DMEM; Invitrogen Corporation, Grand Island, NY, USA) supplemented with 15% fetal calf serum (FCS; Hyclone, Logan, UT, USA) in 5% CO_2 at 37 °C for 24 h. The medium was replaced with a serum-free medium before the cells were exposed to hypoxia.

2.2. EMF exposure

An EMF exposure system that provided a relatively uniform electromagnetic field for cells exposure as described previously was used [20]. Briefly, the exposure consists of a waveform generator, amplifier and solenoid. The waveform generator was an extremely-low frequency function generator, which provided rectangular waveforms. After being amplified, the signals were output to the solenoid. A cylindrical solenoid (25 cm long and 5 cm diameter) made of 1000 turns of 1 mm diameter copper wire on a plastic tube. The intensity of EMF at the position of the coverslip was measured with a Model 455 DSP Gaussmeter (Lakeshore, Westerville, OH, USA). Frequencies (pulse-width and interpulse-interval in msec) of the ELF-EMF used in the experiments were 15 Hz. The flux density was 2 mT. Culture plates were placed on the plexiglass shelves in the center of solenoid within an incubator. The cells were perpendicular to the long axis of the solenoid. At the same time, control plates were placed in an identical incubator on the plexiglass shelves with unpowered solenoid. The CO_2 concentration, humidity, and temperature of the control, treatment incubators were totally the same and were not affected by EMF. The EMF exposure system was checked daily with an oscilloscope.

During hypoxia culture, cells were placed in a hypoxic (1% O_2, 5% CO_2, 37 °C) incubator (Galaxy oxygen control incubator, RS Biotech, Irvine, UK) for 12 h. Control (Normoxia group) were incubated for equivalent periods under normoxic conditions (21% O_2, 5%CO_2, 37 °C). EMF exposure cells (EMF + Hypoxia group) were exposed with ELF-EMF (15 Hz, 2mT) for 30 min before hypoxic conditions. KNK437 (N-formyl-3,4-methylenedioxy-benzylidene-γ-butyrolactam; Sigma, St. Louis, MO, USA), a heat shock protein 70 (HSP70) inhibitor, was added to the culture medium 1 h before EMF exposure (EMF + Hypoxia + KNK437). The final concentration of KNK437 was 100 μM in EMF + Hypoxia + KNK437 group, the same concentration of DMSO was used as control in Hypoxia group and EMF + Hypoxia group. The final concentration of DMSO in each culture medium was 0.25% (v/v), and this concentration DMSO had no effect on results.

2.3. Cardiomyocytes morphological analysis

Cardiomyocytes images were captured with a CCD (600ES-CU; Pixera, Los Gatos, CA, USA) camera fixed to an inverted microscope (Leica DMIRB, Leica Microsystems, Wetzlar, Germany). From each coverslip, 30 regular myocytes were selected at random and viewed at 200 × magnification. Cardiomyocytes were outlined and the cell surface area (CSA) was measured with a Simple PCI software (High Performance Imaging Software, Compix, Cranberry, PA, USA).

2.4. Protein content

Cultured cardiomyocytes were treated with the three conditions for 12 h. The cells were washed with PBS and then treated with 10% trichloroacetic acid (Sigma, St. Louis, MO, USA) at 4 °C for 1 h to precipitate the protein. The precipitates were dissolved in 0.15 M NaOH. Thereafter, the protein content was determined using a BCA protein assay kit (Beyotime, Haimen, Jiangsu, China).

2.5. Cytotoxicity assay

Cell death was assessed by the trypan blue exclusion assay performed in each treated group. Trypan blue stain (Sigma, St. Louis, MO, USA) was prepared fresh as a 0.4% solution in 0.9% sodium chloride. The cells were washed in PBS twice, and then suspended in 0.25% trypsin (Sigma, St. Louis, MO, USA) for 5 min and centrifuged at 50xg for 5 min. Supernatants were removed and pellets were resuspended in 100 μL 0.4% trypan blue solution and incubated for 5 min at room temperature. Cells were microscopically counted in a hemocytometer, and the cell death rate was expressed as a percentage of the trypan blue-positive cells.

Cell viability was assessed by MTS (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethylphenyl)-2-(4-sulfophenyl)-2H-tetrazolium) assay (CellTiter 96® AQueous One Solution Cell Proliferation