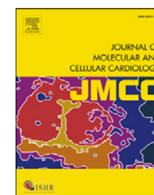
Journal of Molecular and Cellular Cardiology 81 (2015) 54–61

Contents lists available at ScienceDirect

Journal of Molecular and Cellular Cardiology

journal homepage: www.elsevier.com/locate/yjmcc



Original article

Obligatory role of neuronal nitric oxide synthase in the heart's antioxidant adaptation with exercise

Steve R. Roof [a], Hsiang-Ting Ho [a], Sean C. Little [a], Joseph E. Ostler [a], Elizabeth A. Brundage [a], Muthu Periasamy [a], Frederick A. Villamena [a], Sandor Györke [a], Brandon J. Biesiadecki [a], Christophe Heymes [b], Steven R. Houser [c], Jonathan P. Davis [a], Mark T. Ziolo [a,*]

[a] Department of Physiology & Cell Biology, Davis Heart and Lung Research Institute, The Ohio State University, Columbus, OH, USA
[b] Institut des Maladies Métaboliques et Cardiovasculaires, Institut National de la Santé et de la Recherche Médicale, Toulouse, France
[c] Department of Physiology, Cardiovascular Research Center, Temple University, Philadelphia, PA, USA

ARTICLE INFO

Article history:
Received 22 January 2014
Received in revised form 18 December 2014
Accepted 6 January 2015
Available online 14 January 2015

Keywords:
Nitroso–redox balance
ROS
Exercise
Phospholamban
Phosphatase

ABSTRACT

Excessive oxidative stress in the heart results in contractile dysfunction. While antioxidant therapies have been a disappointment clinically, exercise has shown beneficial results, in part by reducing oxidative stress. We have previously shown that neuronal nitric oxide synthase (nNOS) is essential for cardioprotective adaptations caused by exercise. We hypothesize that part of the cardioprotective role of nNOS is via the augmentation of the antioxidant defense with exercise by positively shifting the nitroso–redox balance. Our results show that nNOS is indispensable for the augmented anti-oxidant defense with exercise. Furthermore, exercise training of nNOS knockout mice resulted in a negative shift in the nitroso–redox balance resulting in contractile dysfunction. Remarkably, overexpressing nNOS (conditional cardiac-specific nNOS overexpression) was able to mimic exercise by increasing VO_{2max}. This study demonstrates that exercise results in a positive shift in the nitroso–redox balance that is nNOS-dependent. Thus, targeting nNOS signaling may mimic the beneficial effects of exercise by combating oxidative stress and may be a viable treatment strategy for heart disease.

© 2015 Elsevier Ltd. All rights reserved.

1. Introduction

Oxidative stress (i.e., an increase in reactive oxygen species — ROS) within ventricular myocytes can be detrimental to the heart. In fact, much of the contractile dysfunction and adverse remodeling that occurs in numerous cardiomyopathies (e.g., heart failure) involves oxidative stress [1]. Unfortunately, clinical trials investigating the effects of antioxidants on heart failure have been disappointing [2,3]. It would appear that simply lowering ROS is not enough to alleviate disease symptoms. It has been proposed that antioxidants were not clinically effective because they failed to restore the normal nitroso–redox balance [4], which is the functional coupling between ROS, specifically the superoxide anion radical ($O_2^{\bullet -}$) and nitric oxide (NO) [5]. Our hypothesis is that to effectively combat oxidative stress, one must not only decrease ROS but also simultaneously increase NO. The present study explored the idea that exercise positively shifts the nitroso–redox balance (i.e., decreasing ROS and increasing NO) which may contribute to its beneficial effects on heart failure [6].

Exercise is utilized as a therapeutic intervention for cardiovascular disease and is also beneficial to overall cardiovascular health by reducing the risk of many cardiovascular maladies [7]. The bases of the beneficial effects of exercise are still not well known, but are likely to involve activation of cellular anti-oxidant pathways and increased NO [8,9]. Exercise is able to reduce oxidative stress in diseases and improve cardiac function, and we suggest that it may include a positive shift in the nitroso–redox balance. Our and others' work has shown that the major regulator of the nitroso–redox balance within ventricular myocytes is the neuronal nitric oxide synthase (nNOS) isoform [4,10]. Notably, we have also found that nNOS is necessary for many of the beneficial adaptations of exercise on the heart [11]. The purpose of this study is to determine if nNOS is responsible for the antioxidant effects of exercise on the heart. We hypothesize that nNOS plays a central role for the augmentation of the antioxidant defense with exercise. Our study demonstrated that exercise activates nNOS signaling and this prevents elevated ROS levels and shifts the nitroso–redox balance to reduce oxidative stress. Genetic deletion of nNOS caused a negative shift in the nitroso–redox balance (i.e., increasing ROS and decreasing NO) after exercise training leading to reduced contractility (cell shortening and Ca^{2+} transient) via activation of protein phosphatases that decrease phospholamban (PLB) Serine16 phosphorylation. On the other hand, myocyte specific overexpression of nNOS (nNOSOE) mimicked the effects of exercise. Collectively, these studies suggest that

* Corresponding author at: Department of Physiology & Cell Biology, The Ohio State University, 304 Hamilton Hall, 1645 Neil Avenue, Columbus, OH 43210, USA. Tel.: +1 614 688 7905; fax: +1 614 688 7999.
E-mail address: ziolo.1@osu.edu (M.T. Ziolo).

http://dx.doi.org/10.1016/j.yjmcc.2015.01.003
0022-2828/© 2015 Elsevier Ltd. All rights reserved.

exercise improves the heart's response to oxidative stress by increasing nNOS activity to positively shift the nitroso–redox balance.

2. Methods

2.1. Murine exercise protocol

A high intensity aerobic interval treadmill training protocol was used as previously described [11]. C57Bl/6 and nNOSKO mice (Jackson Laboratories, Bar Harbor, Maine), 5 months of age at sacrifice, underwent treadmill (Columbus Instruments, Columbus, OH) training 5 days a week for 8 weeks starting at 30 min/day and increased to 80 min. Mice were challenged at a high intensity fast pace for 4 min followed by 1 min of low intensity recovery pace. This interval set was repeated until the designated time (30–80 min) was reached. An adequate warm-up of 10 min and cool down of 4 min were instituted. Exercise effects are maintained for 2 weeks [12]. Thus to avoid detraining, we isolated ventricular myocytes from Sed and Ex mice within 1 week of completing our 8 week training protocol.

2.2. nNOSOE model

Conditional, cardiac myocyte specific nNOS overexpression mice were generated as previously described [13]. The human (α-isoform) nNOS coding sequence was used.

2.3. VO_{2max} testing protocol

A metabolic chamber (Columbus Instruments) and Oxymax analyzer (Columbus Instruments) were used for measurements. A protocol was adopted to measure maximal O_2 consumption (VO_{2max}) [14]. Briefly, mice underwent a brief warm-up with 2 min at 10 m/min at 0° incline, 2 min at 12 m/min at 10° incline, and 2 min at 15 m/min at 20° incline. Then, the treadmill speed increased 1 m/min at 20° incline every 30 s. VO_{2max} was defined as the absolute maximal VO_2 with a respiratory exchange ratio (RER) above 1 and exhaustion was reached (mice unwilling to run and neglecting shock).

2.4. Echocardiography

Echocardiography was performed on WT and nNOSKO mice to measure the in vivo function of the heart using the Vevo 2100 and MS-400 transducer (Visualsonics, Toronto, Ontario, Canada).

2.5. Cardiomyocyte isolation

Ventricular myocytes were isolated from Ex and age-matched Sed mice along with nNOSOE and non-induced littermates [15]. Briefly, the heart was cannulated and hung on a Langendorff apparatus. It was then perfused with Ca^{2+} free tyrode solution (see Solutions and drugs below) for 4 min. The solution was then switched to a tyrode solution containing Liberase Blendzyme II (0.077 mg/ml) (Roche Applied Science, Indianapolis, IN). After 3–5 min, the heart was taken down, the ventricles were minced, and myocytes were dissociated by trituration. Subsequently the myocytes were filtered, centrifuged, and resuspended in tyrode solution containing 200 μmol/l Ca^{2+}. Myocytes were used within 4 h of isolation.

2.6. Measurement of myocyte Ca^{2+} transients and shortening

Ca^{2+} transient and shortening measurements were performed at room temperature, as previously described [15]. Briefly, myocytes were loaded at room temperature with Fluo-4 AM (10 μmol/l, Molecular Probes, Eugene, OR) for 30 min. An additional 30 min was allowed for intracellular de-esterification. The instrumentation used for cell fluorescence measurements was a Cairn Research Limited (Faversham, UK) epifluorescence system. $[Ca^{2+}]_i$ was measured by Fluo-4 epifluorescence with excitation at 480 ± 20 nm and emission at 535 ± 25 nm. The illumination field was restricted to collect the emission of a single cell. Data are expressed as $\Delta F/F_0$, where F is the fluorescence intensity and F_0 is the intensity at rest. Data for cell shortening were collected using a video edge detection system (Crescent Electronics). Myocytes were stimulated at 1 Hz via platinum electrodes connected to a Grass Telefactor S48 stimulator (West Warwick, RI).

2.7. Measurement of ROS levels with CM-H2DCFDA fluorescence

As previously described [16], isolated myocytes were loaded at room temperature with CM-H2DCFDA (ROS-sensitive fluorescent dye — 10 μM) for 20 min and allowed to de-esterify for an additional 20 min. Fluorescence was observed on an Olympus Fluoview 1000 laser scanning confocal microscope by exciting at 488 nm line of an argon laser and emission was collected at 500–560 nm. Data was normalized to F_0 and background subtracted. Myocytes were stimulated at 1 Hz.

2.8. Western blot analysis

Homogenized ventricles were used to measure specific phospholamban phosphorylation at Serine16 (Badrilla, Leeds, UK) with phospho-specific antibodies, phospholamban (custom — Zymed, Invitrogen; Carlsbad, CA) and SERCA total (custom — Zymed, Invitrogen; Carlsbad, CA) and normalized to GAPDH, as previously described [11].

2.9. Solutions and drugs

Normal tyrode (NT) solution consisted of (in mmol/l): 140 NaCl, 4 KCl, 1 $MgCl_2$, 1 $CaCl_2$, 10 glucose, and 5 HEPES, pH 7.4 adjusted with NaOH or HCl. Myocytes were superfused with MENO (5 μM), allopurinol (100 μM), apocynin (100 μM), or rotenone (0.3 μM). Myocytes were incubated with EMEPO (1 mmol/l), synthesized as previously described [4], or S-methyl-L-thiocitrulline (SMLT, 10 μM, Calbiochem, La Jolla, CA) for 30 min. All chemicals were from Sigma (St. Louis, MO) except where indicated.

2.10. Statistical analysis

Data were presented as mean ± SEM. Differences between 2 groups were evaluated for statistical significance ($P < 0.05$) by paired or unpaired Student's t tests. To test statistical difference between multiple groups, a one-way ANOVA was used.

3. Results

3.1. Increased antioxidant effect of exercise on the heart is nNOS dependent

Acute periods of aerobic exercise increase ROS production within cardiac myocytes [17–23]. Therefore, the heart has to increase its anti-oxidant defenses to prevent oxidative injury. We have previously shown that increased NO production specifically via nNOS is involved in the beneficial effects (contraction/relaxation and hypertrophy) of exercise on the heart [11]. In the present experiments we determined if nNOS signaling is necessary for the increased antioxidant defense induced by exercise. We measured ROS levels in ventricular myocytes isolated from sedentary (Sed) and exercise-trained (Ex) wildtype (WT) and nNOS knockout (nNOSKO) mice. As shown in Fig. 1A, ventricular myocytes isolated from Ex-WT mice had decreased ROS levels compared to corresponding Sed-WT myocytes. Interestingly, we observed the opposite effect when we trained nNOSKO mice. That is, Ex-nNOSKO myocytes had exacerbated

Download English Version:

https://daneshyari.com/en/article/2190498

Download Persian Version:

https://daneshyari.com/article/2190498

Daneshyari.com