Effect of exercise training on Ca\(^{2+}\) release units of left ventricular myocytes of spontaneously hypertensive rats

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Abstract

In cardiomyocytes, calcium (Ca\(^{2+}\)) release units comprise clusters of intracellular Ca\(^{2+}\) release channels located on the sarcoplasmic reticulum, and hypertension is well established as a cause of defects in calcium release unit function. Our objective was to determine whether endurance exercise training could attenuate the deleterious effects of hypertension on calcium release unit components and Ca\(^{2+}\) sparks in left ventricular myocytes of spontaneously hypertensive rats. Male Wistar and spontaneously hypertensive rats (4 months of age) were divided into 4 groups: normotensive (NC) and hypertensive control (HC), and normotensive (NT) and hypertensive trained (HT) animals (7 rats per group). NC and HC rats were submitted to a low-intensity treadmill running protocol (5 days/week, 1 h/day, 0% grade, and 50-60% of maximal running speed) for 8 weeks. Gene expression of the ryanodine receptor type 2 (RyR2) and FK506 binding protein (FKBP12.6) increased (270%) and decreased (88%), respectively, in HC compared to NC rats. Endurance exercise training reversed these changes by reducing RyR2 (230%) and normalizing FKBP12.6 gene expression (112%). Hypertension also increased the frequency of Ca\(^{2+}\) sparks (HC = 7.61 ± 0.26 vs NC = 4.79 ± 0.19 per 100 μm/s) and decreased its amplitude (HC = 0.260 ± 0.08 vs NC = 0.324 ± 0.10 ΔF/μs), full width at half-maximum amplitude (HC = 1.05 ± 0.08 vs NC = 1.26 ± 0.01 μm), total duration (HC = 11.51 ± 0.12 vs NC = 14.97 ± 0.24 ms), time to peak (HC = 4.84 ± 0.06 vs NC = 6.31 ± 0.14 ms), and time constant of decay (HC = 8.68 ± 0.12 vs NC = 10.21 ± 0.22 ms). These changes were partially reversed in HT rats (frequency of Ca\(^{2+}\) sparks = 6.26 ± 0.19 μm/s, amplitude = 0.282 ± 0.10 ΔF/μs, full width at half-maximum amplitude = 1.14 ± 0.01 μm, total duration = 13.34 ± 0.17 ms, time to peak = 5.43 ± 0.08 ms, and time constant of decay = 9.43 ± 0.15 ms). Endurance exercise training attenuated the deleterious effects of hypertension on calcium release units of left ventricular myocytes.

Key words: Physical activity; Hypertension; Cardiomyocyte; Calcium handling; Sarcoplasmic reticulum

Introduction

In cardiomyocytes, calcium release units (CRUs) refer to clusters of intracellular Ca\(^{2+}\) channels located on the sarcoplasmic membrane (SR) (1). Intracellular Ca\(^{2+}\) release channels are known as RyR2 and are largely arranged in supramolecular arrays (10-300 RyR2) separated from the sarcolemma membrane by a dyadic subspace of ~15 nm. Local Ca\(^{2+}\) elevations resulting from the synchronized opening of RyR2 are defined as calcium sparks. The normal functioning of CRUs is essential to local control of Ca\(^{2+}\) release during excitation-contraction coupling (ECC) (2) and is influenced by several factors including tight junction protein-protein

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interactions. One of these, the RyR2-FKBP12.6 interaction is important (3) because it occurs in around 45% of the available sites in the rat RyR2 (4). FKBP12.6 is anchored in the corner of the RyR2 homotetramer, forming a structural complex that stabilizes and regulates the closed state of these channels, preventing intracellular Ca²⁺ leak and arrhythmogenesis (5).

The spontaneously hypertensive rat (SHR) is a widely used model of human essential hypertension. This rat strain may develop the compensated state of hypertension as early as 3 months of age and heart failure (HF) between 18-24 months of age (6). Previous studies have shown that hypertension and HF promote serious dysfunctions that may affect the CRUs, including orphaned RyR2, loss of the RyR2-FKBP12.6 interaction (7) and dyssynchronous Ca²⁺ sparks (8).

Pharmacological approaches that have been proposed for the treatment of heart disease include those that target CRU components like the RyR2, and the restoration of RyR2-FKBP12.6 interactions (9). Endurance exercise training (EET) has also been proposed as a heart-disease therapy. A previous study showed that EET reduced the frequency and increased the amplitude of spontaneous Ca²⁺ sparks in cardiomyocytes of diabetic rats, attenuating cardiac RyR2 dysregulation (10). Recently, our group showed that EET had beneficial effects on the molecular (11), mechanical (12), and electrical properties (13) of left ventricular myocytes of SHRs. However, whether the beneficial antihypertensive effects of EET can be extended to CRU components and Ca²⁺ sparks needs to be elucidated. Thus, the present study evaluated whether EET could attenuate the deleterious effects of hypertension on CRU components and Ca²⁺ sparks in left ventricular myocytes of SHRs.

Material and Methods

Animals

Four-month-old male SHRs and normotensive Wistar rats were divided into 4 experimental groups of 7 animals each: normotensive and hypertensive controls (NC and HC), and normotensive and hypertensive trained (NT and HT). The body weight (BW) of all rats was measured every week; systolic arterial pressure (SAP) and resting heart rate (RHR) were recorded at the beginning and 48 h after the experimental period using tail cuffs (11). All experiments were approved by the Ethics Committee in Animal Use at the Universidade Federal de Viçosa (#66/2011).

EET protocol

The EET protocol was carried out on a motor-driven treadmill (Insight Equipamentos Científicos, Brazil) 5 days/week, 60 min/day, for 8 weeks. Before the beginning of the EET program, the animals were placed on the treadmill for adaptation (10 min/day, 0% grade, 0.3 km/h) for 5 days. After adaptation, all animals performed a maximal running speed (MRS) test at the beginning of the study, at the end of fourth week in order to update the training intensity, and 48 h after the last training session, to measure the total exercise time-to-fatigue (TTF). Throughout the EET period, intensity was monitored by the progressive increase of time and running speed, which reached 1 h/day, 0% grade, at 50-60% of MRS during the exercise protocol (11).

Cardiomyocyte isolation

Two days after the last MRS test, rats were weighed and killed by cervical dislocation under resting conditions. The hearts were quickly removed, and left ventricular myocytes were enzymatically isolated (11). Briefly, the hearts were mounted on a customized Langendorff system (37°C) and perfused sequentially with a modified HEPES-Tyrode solution for 5 min, a calcium-free solution with EGTA (0.1 mM) for 6 min, and a solution containing 1 mg/mL collagenase type II (Worthington, USA) for 15-20 min. The ventricles were then removed and weighed. The left ventricles were separated and weighed to calculate the ventricular hypertrophy index and they were then cut into small pieces. After centrifugation (2500 g for 30 s), the resulting left ventricular cells were suspended in modified HEPES-Tyrode solution.

RyR2 and FKB12.6 gene expression

The relative gene expressions of RyR2 and FKB12.6 in the left ventricle were analyzed by quantitative real-time polymerase chain reaction (qRT-PCR) (11). The expression of RyR2 and FKB12.6 mRNA was assessed by oligonucleotide primers: RyR2: 5’-GAC ATG AAG TGT GAC GAC ATG CT-3’ and 5’-CAC GGA CGC CCA CAT ACA-3’; FKB12.6: 5’-CCC CTG ATG TGG CAT ATG G -3’ and 5’-TGG CAT TGG GAG GGA GGA TGA-3’. The expression of GAPDH (5’-GCT GAT GCC CCC ATG TTT G-3’ and 5’-ACC AGT GGA TGC AGT G G-3’) was measured as an internal control for sample variation in the reverse transcriptase reaction.

Spontaneous Ca²⁺ sparks

Cells were loaded with 5 μM of fluo-4 AM (Molecular Probes, USA) and scanned with a confocal microscope (Meta LSM 510, Carl Zeiss GmbH, Germany). Prior to registration of images, cardiomyocytes were stimulated at 1 Hz, and then 10 images per cell were captured and subsequently analyzed using the SparkMaster program (14). Spontaneous Ca²⁺ sparks were measured as ΔF/F₀, where F₀ was the resting Ca²⁺ fluorescence. The sparks results are shown as amplitude, frequency, full width at half-maximum amplitude (FWHM), total duration (FullDur), time to peak (Tpeak), and time constant of decay (τ).

Statistical analysis

Two-way ANOVA followed by the post hoc Tukey test was used to compare the effects of training and
hypertension on measured parameters. Differences in the Ca^{2+} sparks distributions were analyzed for significance by the two-sample Kolmogorov-Smirnov test. Results are reported as means±SEM. A statistical significance level of 5% was adopted.

Results

General characteristics and physical capacity

It is important to note that SHR presented with increased (P<0.05) ventricular hypertrophy index, i.e., elevated ventricular weight to body weight ratio (VW/BW, HC=4.68±0.12 vs NC=4.03±0.25 mg/g) and left ventricular weight to body weight ratio (LVW/BW, HC=3.60±0.16 vs NC=3.18±0.19 mg/g). EET increased (P<0.05) these parameters in trained rats (VW/BW, NC=4.03±0.25 vs NT=4.74±0.12; HC=4.68±0.12 vs HT=5.42±0.10 mg/g; LVW/BW, NC=3.18±0.19 vs NT=3.59±0.18; HC=3.60±0.16 vs HT=3.99±0.15 mg/g). EET reduced (P<0.05) SAP in hypertensive rats (HC=176.2±3.3 vs HT=160.8±3.3 mmHg) and RHR in trained rats (NT_initial=342±9 vs NT_final=319±12; HT_initial=373±8 vs HT_final=343±10 bpm). Moreover, EET increased (P<0.05) the TTF of trained rats (NC=9.95±1.52 vs NT=19.24±1.43; HC=11.53±1.46 vs HT=21.95±1.43 min).

RyR2 and FKBP12.6 gene expression

As shown in Figure 1A and B, hypertension resulted in a significant 270% increase in RyR2 gene expression in the HC group compared with the NC group. Similarly, EET significantly increased RyR2 expression by 280% in the NT group. In the HT group, EET restored RyR2 expression to levels similar to those in the NC group. Regarding FKBP12.6, there was significantly lower expression in the HC group (88%) than in the NC group. Despite the fact that EET did not modify FKBP12.6 response in the NT group, EET normalized FKBP12.6 gene expression in the HT group.

Spontaneous Ca^{2+} sparks

Representative images of spontaneous Ca^{2+} sparks are illustrated in Figure 2 and quantified in Table 1. Cells from the HC group had a higher frequency of spontaneous Ca^{2+} sparks than cells from the NC group. On the other hand, the amplitude, FWHM, FullDur, T_peak, and τ were lower in HC than in NC cardiomyocytes. EET reduced the frequency and increased the amplitude, FWHM, FullDur, T_peak, and τ in the NT compared with the NC group. Importantly, all alterations observed in HC group Ca^{2+} sparks were partially restored by the EET program.

Discussion

It is established that hypertension imposes early intracellular calcium cycling defects in intact hearts (15).
reduced those events. Similar results have previously been reported in diabetic animals, and were shown to be related to improvement of RyR2 function (10). Moreover, EET reduced the frequency of spontaneous Ca\textsuperscript{2+} waves in the left ventricular myocytes in rats with myocardial infarcts, although the mechanisms have not been elucidated (17). We also observed that EET increased the amplitude, width and duration of the spontaneous Ca\textsuperscript{2+} sparks in the left ventricular myocytes of SHR, and to our knowledge, this is the first study that showed the effects of EET on these parameters in hypertensive rats.

Heart diseases may alter the properties of spontaneous Ca\textsuperscript{2+} sparks as a function of changes in signaling mechanisms that control Ca\textsuperscript{2+} handling. One of the main problems is modification of the structure and activity of RyR2, which can promote changes in the spatial organization of RyR2 clusters, in the proteins associated with RyR2 (e.g., FKBP12.6), alterations in the sensitivity of RyR2 to Ca\textsuperscript{2+}, increased phosphorylation of RyR2 by protein kinase A (PKA) at serine 2808, and by kinase II pathway-dependent Ca\textsuperscript{2+}/calmodulin (CaMKII) at serine 2814. These modifications can lead to an increase in the spontaneous release of Ca\textsuperscript{2+} from SR and could generate Ca\textsuperscript{2+} waves, thus producing cardiac abnormal

![Figure 2. Representative images (Ai to Aiv) of spontaneous Ca\textsuperscript{2+} sparks recorded from left ventricular isolated cardiomyocytes. NC: normotensive control; HC: hypertensive control; NT: normotensive trained; HT: hypertensive trained.](image)

| Table 1. Characteristics of spontaneous Ca\textsuperscript{2+} sparks in left ventricular isolated myocytes. |
|-----------------------------------------------|
|                                              |
| **NC** | **HC** | **NT** | **HT** |
| Frequency (100 \(\mu\)m/s) | 4.79 ± 0.19 | 7.61 ± 0.26* | 3.71 ± 0.11* | 6.26 ± 0.19* |
| Amplitude (\(\Delta F/F_0\)) | 0.324 ± 0.10 | 0.260 ± 0.08* | 0.331 ± 0.14* | 0.282 ± 0.10* |
| FWHM (\(\mu\)m) | 1.26 ± 0.01 | 1.05 ± 0.08* | 1.69 ± 0.02* | 1.14 ± 0.01* |
| FullDur (ms) | 14.97 ± 0.24 | 11.51 ± 0.12* | 21.87 ± 0.46* | 13.34 ± 0.12* |
| T\(_{\text{peak}}\) (ms) | 6.31 ± 0.14 | 4.84 ± 0.06* | 7.62 ± 0.19* | 5.43 ± 0.08* |
| \(\tau\) (ms) | 10.21 ± 0.22 | 8.68 ± 0.12* | 14.98 ± 0.39* | 9.43 ± 0.15* |

Data are reported as means±SE of 60-70 cells in each group. NC: normotensive control; HC: hypertensive control; NT: normotensive trained; HT: hypertensive trained; FWHM: full width at half-maximum amplitude; FullDur: total duration; T\(_{\text{peak}}\): time to peak; \(\tau\): time constant of decay. *\(P<0.05\), compared to NC; **\(P<0.05\), compared to NT; ***\(P<0.05\), compared to HC (two-sample Kolmogorov-Smirnov test).
electrical activity and extrasystoles (5, 18).

Our results showed that hypertension promoted an increase in the left ventricular gene expression of RyR2 (270%) and a decrease in FKBP12.6 expression (88%). In the early stages of hypertension, there is an increased Ca\(^{2+}\) release from SR and cell contractility to compensate pressure overload and to maintain cardiac output. This process probably occurs due to the increased activity and expression of RyR2 (19). The RyR2 associates with some proteins to form a complex macromolecular structure that regulates the activity of these Ca\(^{2+}\) channels. Among these proteins are FKBP12.6, PKA, phosphatase 1 (PP1) and 2A (PP2A) (2, 3, 18). The linkage between the RyR2 channels is regulated by FKBP12.6, which binds four monomers of RyR2 and coordinates Ca\(^{2+}\) release activity from the SR during systole and diastole, forming a group of channels that open and close adequately and simultaneously (3, 18).

In the present study, we observed that hypertension promoted a noteworthy reduction in the expression of FKBP12.6 and an increase in the expression of RyR2, causing an imbalance between these two genes in the left ventricle of SHR. Simultaneously, we also observed an increase in frequency and decrease in amplitude of spontaneous Ca\(^{2+}\) sparks. The increase in the expression and activity of RyR2 promoted by the hypertensive process can promote dysfunction in these channels and consequently in the associated proteins (2, 16, 19). Dissociation of FKBP12.6 is able to significantly alter the biophysical properties of RyR2, increasing sensitivity and the spontaneous release of Ca\(^{2+}\) from these channels when they are at rest (9, 18). Nevertheless, we did not measure the content of these proteins, and others have shown that there is no direct involvement of such a mechanism, suggesting that FKBP12.6 might not be vital to RyR2 stabilization in the rat (4). However, such associations were not addressed in the present study and need to be elucidated.

It has been shown that the inhibition of FKBP12.6 increased the likelihood and the duration of RyR2 opening in single cardiomyocytes. FKBP12.6 may afford an intrinsic mechanism to terminate RyR2 opening and it may exert a negative feedback on CICR in cardiac cells (20). These data could likely help to understand the results of our study regarding the morphology of spontaneous Ca\(^{2+}\) sparks in the left ventricular myocytes of hypertensive rats, inasmuch as we observed increased frequency, and consequently, reductions in amplitude, width, and duration of spontaneous Ca\(^{2+}\) sparks.

In conclusion, we found that exercise training attenuated the deleterious effects of hypertension on CRUs of left ventricular myocytes of SHR.

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