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Skeletal muscle electrical stimulation improves baroreflex sensitivity and heart rate variability in heart failure rats

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ABSTRACT

The goal of the current study was to evaluate the effects of electrical stimulation (ES) on the arterial baroreflex sensitivity (BRS) and cardiovascular autonomic control in rats with chronic heart failure (CHF). Male Wistar rats were designated to one of four groups: placebo sham (P-Sham, n = 9), ES sham (ES-Sham, n = 9), placebo CHF (P-CHF, n = 9) or ES CHF (ES-CHF, n = 9). The ES was adjusted at a low frequency (30 Hz), duration of 250 μs, with hold and rest time of 8 s (4 weeks, 30 min/day, 5 times/week). It was applied on the gastrocnemius muscle with intensity to produce a visible muscle contraction. The rats assigned to the placebo groups performed the same procedures with the equipment turned off. The two-way ANOVA and the post hoc Student–Newman–Keuls tests ($P < 0.05$) were used to data comparison. The BRS was higher in ES-Sham group compared to the P-Sham group and the ES-CHF group compared to the P-CHF group. ES was able to decrease heart rate sympatho-vagal modulation and peripheral sympathetic modulation in ES-CHF compared to P-CHF group. Interestingly, heart rate sympatho-vagal modulation was similar between ES-CHF and P-Sham groups. Thus, ES enhances heart rate parasympathetic modulation on heart failure (ES-CHF) compared to placebo (P-CHF), with consequent decrease of sympatho-vagal balance in the ES-CHF group compared to the P-CHF. The results show that a 4 week ES protocol in CHF rats enhances arterial BRS and cardiovascular autonomic control.

1. Introduction

Chronic heart failure (CHF) is considered a clinical syndrome involving multiple organs that is developed by any functional or structural impairment of heart blood ejection or ventricular filling (Yancy et al., 2013). The CHF shows abnormalities of other systems, beyond the heart (Ventura-Clapier et al., 2002). In this syndrome, the sympathetic hyperactivity and the parasympathetic hypoactivity are characteristics of the neurohumoral excitation (Floras, 2009), which are connected with the baroreflex sensitivity (BRS) attenuation (La Rovere et al., 1998). Considering these circumstances, heart-rate variability (HRV) reduction and an impairment of short-term control of arterial pressure (AP) (La Rovere et al., 1998) have been related with an augmented risk of sudden death from cardiac cause (Schwartz and La Rovere, 1998).

Skeletal muscles are involved in the CHF syndrome. Skeletal muscle myopathy (Mancini et al., 1992), muscle fiber atrophy (Drexler et al., 1992), reduction in muscle strength and in the cross-sectional area of skeletal striated muscle fibers (Buller et al., 1991) are predictors of exercise tolerance decrease in CHF patients. One of the recommendations as part of the treatment for CHF patients is the physical training (Working Group on Cardiac R, Exercice P and Working Group on Heart Failure of the European Society of C, 2001). However, some individuals do not adapt to the physical training and others are not able to support including low levels of exercise.

Several studies show that exercise and muscle afferent activation can interfere in the arterial baroreflex in humans (Gademan et al., 2011; Scherrer et al., 1990) and animals (Hammond et al., 2000; Kim et al., 2005; Lima et al., 2015). The autonomic regulations are produced by both voluntary muscle activation and the central stimulation (Scherrer et al., 1990). At the same time, muscle metaboreflex is activated by the CHF syndrome during strenuous exercise (Hammond et al.,

Abbreviations: ES, Electrical stimulation; CHF, Chronic heart failure; BRS, Baroreflex sensitivity; MI, Myocardial infarction; MAP, Mean arterial pressure; HRV, Heart rate variability; BPV, Blood pressure variability; LF/HF, Sympatho-vagal balance; LV, Left ventricle; RV, Right ventricle; LVSP, LV systolic pressure; $+dP/dt_{max}$, LV maximum change in pressure over time; $-dP/dt_{max}$, LV minimum change in pressure over time; LVEDP, LV end-diastolic pressure; HW, Heart weight; BW, Body weight; SBP, Systolic arterial blood pressure; PI, Pulse interval.

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2000) and consequently, sympathetic hyperactivity occurs. Transcutaneous electrical nerve stimulation (TENS) that is a muscle afferent stimulus was applied in CHF patients, an increase in BRS was found (Gademan et al., 2011) and this modulates sympathetic activity. The use of electro-acupuncture has shown in CHF rats, similar results (Lima et al., 2015). Functional electrical stimulation (FES) a modality of ES that causes muscle contraction has shown promising beneficial effects in CHF patients, such as increase in oxidative enzyme levels, skeletal striated muscle mass (type I fibers), prevention of muscle atrophy (Nuhr et al., 2004), peak VO_2 (Dobsak et al., 2006a), endothelial function and emotional status improvement (Karavidas et al., 2013). Both therapies suggest to be alternative treatments for the CHF subjects who cannot adapt to the conventional exercise training programs (Gademan et al., 2011; Smarta et al., 2013). Nevertheless, the effect of ES on neurohumoral control of the cardiovascular system in CHF rats has not been evaluated.

Therefore, this study was conducted to test the hypothesis that a 4-week protocol of electrical stimulation could be associated with improvement in baroreflex sensitivity and heart rate variability in rats with CHF.

2. Material and methods

2.1. Animals

Thirty-six male Wistar rats (230 to 280 g) from the Animal Breeding Unit of the Universidade Federal de Ciências da Saúde de Porto Alegre (UFCSPA) were used for the experiments. The animals received food and water ad libitum and they were housed two per cage in an animal room kept at 22 °C under a 12:12-h light–dark cycle. The experiment followed the ethical procedures established by the Guide for Care and Use of Experimental Animals, number 85-23, revised in 1996, published by the National Institutes of Health. All procedures from this study were approved by the Ethics and Research Committee from UFCSPA (protocol 006/10).

2.2. Surgery to induce myocardial infarction (MI)

To induce MI, rats were intubated and artificially ventilated after being anesthetized with xylazine (12 mg/kg ip) and ketamine (90 mg/kg ip). As previously described (Pfeffer et al., 1979), the left coronary artery ligation and the sham surgeries were carried out. To prevent pain and infection after operation, a single dose of monofenew (0.05 ml/100 g) and gentamicin (0.05 ml/100 g) were administered.

2.3. Experimental design

The time for the CHF animals to recover and to develop the CHF state following MI surgery was 6 weeks (Pfeffer et al., 1979). The sham groups had the same time period to recover. Five weeks after the operation, the sham rats were randomly designated into one of the following groups: placebo sham rats (P-Sham, n = 9) or ES sham rats (ES-Sham, n = 9); and the CHF animals were randomly assigned into one of the groups: placebo CHF rats (P-CHF, n = 9) or ES CHF rats (ES-CHF, n = 9).

2.4. Electrical stimulation protocol

The adaption protocol to the electrical stimulation started on the 5th week after the surgery and included 5 min on first day and accrued 5 min per day until completing 5 days. The ES protocol began in the 6th week. It comprised 30 min/day, 5 days/week for 4 weeks. The right leg of each rat was shaved and they laid on a platform with its right knee extended. During the ES, the animals were kept awake in a device built in our laboratory that mimics a burrow (Lima et al., 2015) and allows a comfortable positioning. The electrical stimulator (FES VIF 995, Quark, Piracicaba, Brazil) was applied on the gastrocnemius muscle of the right leg with a surface electrode (7.5 mm × 7.5 mm). The current was symmetric biphasic adjusted at a low frequency (30 Hz), duration of 250 μs, with hold and rest time of 8 s, with necessary intensity to produce a visible muscle contraction without causing any apparent discomfort (de Leon et al., 2011). The rats from the placebo groups performed the same procedures with the equipment turned off.

2.5. Awake measurements of cardiovascular parameters

Under general anesthesia as above described, in the week after the ES protocol, two catheters filled with saline (0.06 ml) and heparin (0.01 ml) were implanted into the abdominal aorta and inferior vena cava, which were used to measure mean arterial pressure (MAP) and drugs administration, respectively. Conscious rats were studied on the subsequent day after the catheter placement (Jaenisch et al., 2011; Quagliotto et al., 2008). The arterial catheter was connected to a 40 cm tube attached to a strain-gauge pressure transducer (Miniature Pulse Transducer RP-155, Houston, USA), coupled to a pressure amplifier (General Purpose Amplifier 4 — model 2, Stemtech Inc., Houston, USA), and blood-pressure signals were recorded over a 15-min period, 1 kHz sampling frequency (Windaq — AT/CODAS, Dataq Instruments Inc., Akron, USA). On a beat-to-beat basis the measured data were analyzed to quantify the variables of interest (Jaenisch et al., 2011; Quagliotto et al., 2008).

2.6. Baroreflex sensitivity

On the subsequent day following the catheter placement, MAP and HR were registered for 15 min as baseline control. HR changes to test BRS were recorded during peak changes (augment or reduction) in MAP due to a single dose of venous injection of phenylephrine (8 μg/ml; Sigma Chemical, St. Louis, USA) or sodium nitroprusside (100 μg/ml; Sigma Chemical), respectively (Jaenisch et al., 2011; Quagliotto et al., 2008). The alterations in MAP were within the 10 to 30 mm Hg range and the BRS determination was made by fitting the MAP and HR alterations to a sigmoidal logistic equation (Head and McCarty, 1987).

2.7. Cardiovascular autonomic control

Spectral analysis of systolic arterial blood pressure (SBP) and pulse interval (PI) to evaluate the sympathetic and parasympathetic cardiovascular modulation was performed by an autoregressive method. From the original recordings, samples were exported to create a database for the analysis, according to the HRV guidelines (Guidelines, 1996). Succinctly, continuous series of PI (tachogram) were supplied by a derivative-threshold algorithm. The systogram was created through the beat-to-beat SBP derived from BP signals. The "low frequency" (LF, 0.2–0.75 Hz) and "high frequency" (HF, 0.75–3.0 Hz) spectral components of PI and SBP were explicit in absolute values (ms^2 and mm Hg, respectively) and in normalized units (n.u.). The n.u. were obtained after calculate the relation between the power of either LF or HF components divided by the total power subtracted of the power of the very low frequency component (frequencies ≤ 0.2 Hz). The result is then multiplied by 100.

2.8. Cardiac hemodynamic evaluation

In the subsequent day (24 h) following the autonomic evaluation, the animals were anesthetized, as above described, for cardiac hemodynamic evaluation. For that, a catheter (PE-50) was introduced into the right carotid artery and during a 5-min period the AP was registered. Later, the catheter was placed inside the left ventricle (LV) and the standard graphic recordings of ventricular pressure were used to monitor the pulse wave and registered for 5 min, following the protocol