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Serca2a and Na^+/Ca^{2+} exchanger are involved in left ventricular function following cardiac remodelling of female rats treated with anabolic androgenic steroid

Andrews Marques do Nascimento [a], Ewelyne Miranda de Lima [a], Girlandia Alexandre Brasil [a], Izabela Facco Caliman [a], Josiane Fernandes da Silva [c], Virgínia Soares Lemos [c], Tadeu Uggere de Andrade [b], Nazaré Souza Bissoli [a,*]

[a] Department of Physiological Sciences, Federal University of Espirito Santo, Vitória, Espirito Santo, Brazil
[b] Department of Pharmacy, University Vila Velha, Vila Velha, Espirito Santo, Brazil
[c] Department of Physiology and Biophysic, Federal University of Minas Gerais, Minas Gerais, Brazil

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ABSTRACT

Anabolic-androgenic steroids are misused, including by women, but little is known about the cardiovascular effects of these drugs on women. Aim: To evaluated the effects of nandrolone decanoate (ND) and resistive physical exercise on cardiac contractility in young female rats. Main methods: Female Wistar rats were separated into 4 groups: C (untrained animals); E (animals were submitted to resistance exercise by jumping in water 5 times per week); ND (animals were treated with ND, 20 mg/kg/week for 4 weeks); and NDE (trained and treated). The haemodynamic parameters ($+dP/dt_{max}$, $-dP/dt_{min}$ and Tau) were assessed in the left ventricle. The heart was collected for histological analyses and collagen deposition. The gastrocnemius muscle was weighed, and hypertrophy was assessed by the ratio of their weights to gastrocnemius/tibia length. The expression of calcium handling proteins was measured by western blot analysis. Results: ND treatment and physical exercise increased cardiac contractility and relaxation. In addition, ND promoted increases in phospholamban phosphorylated (p-PLB) and isoforms of sarcoplasmic/endoplasmic reticulum calcium ATPase 2 (SERCA2a) expression, while resistance exercise increased the phosphorylation of PLB and expression of Na^+/Ca^{2+} exchangers (NCX). Cardiac hypertrophy and collagen deposition were observed after ND treatment. Conclusion: Regulatory components of cytosolic calcium, such as SERCA2a and p-PLB, play important roles in modulating the contractility and relaxation effects of ND in females.

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1. Introduction

The use of anabolic-androgenic steroids (AAS) has grown considerably in recent years by both men and women. It is estimated that the broad use of AAS by adults, young athletes and non-athletes has increased threefold in recently (Bojsen-Møller and Christiansen, 2010; Evans, 2004). A study by Miller et al. (2005) conducted in the United States showed that approximately 4.1% of boys and 2% of girls have already used AAS at least once in their lives, indicating that abuse of AAS is no longer restricted to men.

The scientific literature reported about several adverse cardiovascular effects after AAS, e.g. cardiac hypertrophy, hypertension, congestive heart failure, cardiomyopathy, arrhythmias and sudden death (Fineschi et al., 2007; Liu et al., 2003). Cardiac hypertrophy caused by AAS is demonstrated by pathological hypertrophy, in which changes occur in the myofibrils, as well as mitochondrial swelling and stretching of the myocardium indicating early stages of heart failure (Beutel et al., 2005; Melchert and Welder, 1995; Pereira-Junior et al., 2006).

Research from our group showed the influence of high doses of AAS (nandrolone decanoate) on the cardiovascular system. These studies demonstrated that the chronic use of nandrolone in sedentary male rats impaired the cardiopulmonary Bezold-Jarisch reflex, induced cardiac hypertrophy and increased blood pressure in animals, with the participation of the renin angiotensin system (RAS) in these processes (Andrade et al., 2008; Franquni et al., 2013). We also observed that cardiac hypertrophy was associated with increased collagen deposition in the extracellular matrix and with an imbalance in cardiac cytokines, with the prevalence of pro-inflammatory cytokines (Franquni et al., 2013).

* Corresponding author at: Departamento de Ciências Fisiológicas, Universidade Federal do Espírito Santo – UFES, Avenida Marechal Campos, 1468 Maruípe, Vitória, ES 29043-900, Brazil.
E-mail address: nazarebissoli@gmail.com (N.S. Bissoli).

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Stimuli such as exercise also promote cardiac hypertrophy; however, in the case of exercise, this process is beneficial and adaptive in response to an increase in mechanical load (Lorell and Carabello, 2000). The use of AAS is usually associated with exercise; however, the beneficial effect of exercise seems to be lost, with the occurrence of unfavourable cardiovascular adaptations (Melchert and Welder, 1995) and impaired ventricular function, especially diastolic function, myocardial fibrosis and cardiomyocyte disarrangement (Yamamoto et al., 2002).

Hypertrophy is associated with increased efficiency of muscle contraction processes, due to increases in contractile proteins (Kraemer et al., 2002). For the cardiac contraction process, Ca^{2+} is a key regulatory component acting as an electric signal regulator that determines heart rate excitatory coupling and contraction. Reduction of intracellular Ca^{2+} levels and impairment of calcium pump activity are changes found in human heart failure (Adachi, 2010; Dash et al., 2001). Furthermore, cytosolic calcium regulation contributes not only to cardiac contractility but also to relaxation, and this adjustment occurs place through Ca^{2+} channels, ATPase pumps, heart exchangers, carriers and the binding of proteins to Ca^{2+} (Kho et al., 2012; Opie, 2004). The Na^{+}/Ca^{2+} exchangers (NCXs) and the isoform of the sarcoplasmic/endoplasmic reticulum calcium ATPase 2 (SERCA2a) are responsible for the extrusion and reception of calcium, respectively.

There is a clear concern with the use of male hormones by women (Braunstein, 2007; Sirianni et al., 2012), but there have been no studies about the influence of these substances on cardiac contractility in women. In addition, there have been no studies evaluating the relationships of the deleterious effects of AAS on the cardiovascular level in rats submitted to exercise. Therefore, the aim of this study was to evaluate the influence of AAS on arterial and left ventricular pressure, left ventricular function, cardiac morphometry and the expression of calcium handling proteins in female rats submitted to physical resistance training.

2. Methods

2.1. Animals

Adult female Wistar rats weighing 180–200 g were obtained from the breeding unit at the Federal University of Espirito Santo. The rats were housed in room with controlled temperature (22 °C), humidity (50%) and light cycle (12-h light/dark), and the rats were given water and standard rat chow (Purina Labina, SP-Brazil) ad libitum. The procedures were conducted in compliance with the guidelines for the ethical use of animals in scientific research as stated by the National Council for Animal Experiments Control and were approved by the Ethics Committee for Animal Use at the Federal University of Espírito Santo (031/2012).

Four groups of female rats were studied: sedentary controls animals (C): treated weekly with the vehicle (peanut oil with benzyl alcohol, 90:10, v/v); animals submitted to exercise (CE): 5 days per week and treated weekly with the vehicle; sedentary animals treated with nandrolone (ND) (nandrolone decanoate: 20 mg/kg/week (Franquni et al., 2013), divided in two administrations; Deca Durabolin®, Organon Inc., São Paulo, SP, Brazil); and animals doing exercise and treated with nandrolone (NDE). All drugs were administered for 4 weeks by intramuscular injection and were performed for 4 weeks. The treatment was made by intramuscular injection, and the vehicle used was peanut oil with benzyl alcohol, 90:10, v/v. C and CE received only vehicle as drug treatment. The oestrous cycle of the animals was evaluated daily. Vaginal smears were obtained from each female rat as previously described (Becker et al., 2005). The experimental protocols were performed when the animals in the C group were in pro-oestrous, i.e. the stage of the cycle that is when the estrogen production are higher. The animals treated with nandrolone remained in dioestrous II. The reason for the experiments being carried out at different stages of the oestrous cycle of rats between groups was the influence of high levels of androgens. It is known that high levels of androgenic hormones, through negative feedback, reduce the production of steroid hormones such as estrogen and progesterone (Bordbar et al., 2014; Gerez et al., 2005), which in turn influences the oestrous cycle (Kaeoket et al., 2001). Gerez et al. (2005) found that females treated with ND remain in dioestrous and exhibit reduced reproductive capacity.

2.2. Resistance training (RT)

The RT protocol was designed as described by Khananshvili, (2013) with slight modifications. One week before the tests, the animals were adapted to water (31 ± 1 °C) for 60 min every day, without exercise training adaptation.

The RT protocol was conducted as depicted below. First, the animals performed a jump series in the water (31 ± 1 °C; the water depth corresponded to three times the length of the animal) for 6 min (30 s of exercise and 30 s of rest) with a load of 50% of body weight. After a period of 9 min, the animals performed 12 min of exercise (60 s followed by 30 s of rest), with a load of 30% of body weight. After the last series, 25 μL blood samples were obtained from a cut at the tip of the tail to evaluate capillary blood lactate levels (BM-Lactate® strips and Accutrend® Lactate monitor; Roche, São Paulo, Brazil) to confirm that the exercise was performed under anaerobic conditions (indicated by blood lactate values >7.0 mmoL/L; (Voltarelli et al., 2002). These protocols were performed 5 days per week for 4 weeks (Cunha et al., 2005).

2.3. Measurement of arterial blood pressure (BP) and heart rate (HR)

Forty-eight hours after the experimental protocol, mean arterial pressure (MAP) and heart rate (HR) were determined by direct measurement in each group at the end of treatment (Bissoli et al., 2009). For this procedure, on the day before the measurement, a catheter filled with saline (PE-50) was inserted into the left femoral artery under anaesthesia (ketamine 100 mg/kg, xylazine 10 mg/kg). The free end of the catheter was exteriorized at the cervical dorsal area. For BP measurement, the arterial catheter was attached to a 40-cm polyethylene catheter during the 40-min recording period. BP measurements were obtained from quiet, conscious rats that had complete freedom to move throughout the cage. The BP was recorded using a pressure transducer coupled to a MP-100 System Guide (model MP100-CE; Biopac Systems, Santa Barbara, CA, USA). HR was calculated instantaneously from the intervals of pressure pulses.

2.4. Haemodynamic evaluation

After measuring BP and HR, the animals were anesthetized with ketamine (100 $mg \cdot kg^{-1}$, i.p., Agener União, Brazil) and xylazine (10 $mg \cdot kg^{-1}$, i.p., Bayer, Brazil). The Left Ventricular (LV) function of the animals was assessed as previously described (Wang et al., 2003). Briefly, the right common carotid artery was separated from connective tissue and was catheterized with a fluid-filled polyethylene catheter (P50). The catheter was connected to a pressure transducer, and LV recorded using a pressure transducer coupled to a MP-100 System Guide (model MP100-CE; Biopac Systems, Santa Barbara, CA, USA). After 15 min of stabilization, the arterial systolic and diastolic blood pressures were recorded. The catheter was then advanced to the left ventricle. For additional 15 min of stabilization, functional parameters were measured as LV $+dP/dt_{max}$, which is the maximum rate of ventricular pressure increase or the peak positive value of the first derivative of the left ventricular pressure, as well as the rate of pressure decay ($-dP/dt_{min}$) and the time constant of isovolumic relaxation of LV (Tau). The signal was expressed in mm Hg/s. Following this procedure, the catheter was withdrawn from the LV, and the arterial

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