Life Sciences 145 (2016) 9–18

Contents lists available at ScienceDirect

Life Sciences

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

Full length article

Erectile dysfunction in heart failure rats is associated with increased neurogenic contractions in cavernous tissue and internal pudendal artery

Fernanda Luciano Rodrigues [a], Rhéure Alves M. Lopes [a], Rafael Sobrano Fais [a], Lucas de Oliveira [a], Cibele M. Prado [b], Rita C. Tostes [a], Fernando S. Carneiro [a,*]

[a] Department of Pharmacology, Ribeirao Preto Medical School, University of Sao Paulo, Ribeirao Preto, SP, Brazil
[b] Department of Pathology and Legal Medicine, Ribeirao Preto Medical School, University of Sao Paulo, Ribeirao Preto, SP, Brazil

ARTICLE INFO

Article history:
Received 2 July 2015
Received in revised form 24 October 2015
Accepted 2 December 2015
Available online 9 December 2015

Keywords:
Heart failure
Erectile dysfunction
Corpora cavernosa
RhoA/Rho kinase pathway

ABSTRACT

Aims: The rates of erectile dysfunction (ED) in heart failure (HF) are extremely high. This study tested the hypothesis that rats with HF display ED and that HF leads to increased sympathetic-mediated contractile tone of the cavernous tissue and/or internal pudendal arteries (IPA) as potential mechanisms contributing to ED.
Main methods: HF was induced in Wistar rats by ligation of the left anterior descending coronary artery. Changes in the ratio of intracavernosal pressure/mean arterial pressure (ICP/MAP) after electrical stimulation of major pelvic ganglion were determined *in vivo*. Cavernosal and IPA contractions were induced by electric field stimulation (EFS) and phenylephrine. RhoA, Rho kinase 2 (ROCK 2) and myosin phosphatase target protein 1 (MYPT-1) protein expression and phosphorylation levels were also determined.
Key findings: HF rats display impaired erectile function represented by decreased ICP/MAP responses. EFS-mediated contractions were increased by HF in cavernous tissue and IPA. Contractions induced by phenylephrine were increased in cavernous tissue of HF rats, but decreased in IPA rings. Moreover, HF decreased RhoA protein expression, but increased ROCK 2 and MYPT-1 phosphorylation levels in cavernous tissue. In conclusion, rats with HF induced by myocardial infarction display ED *in vivo* and increased sympathetic-mediated contractile responses in cavernous tissue and IPA. Increased sympathetic-mediated contractile responses were associated with increased ROCK 2 and MYPT-1 phosphorylation in cavernosal tissue, suggesting the involvement of ROCK signaling pathway in ED genesis.
Significance: Our findings suggest new mechanisms linking HF to ED, providing potential therapeutic targets for treating ED associated to HF.

1. Introduction

Erectile dysfunction (ED) is defined as the inability to achieve or maintain a penile erection for satisfactory sexual intercourse [1]. The multifactorial nature of ED is evident and population studies show that cardiovascular and metabolic diseases such as arterial hypertension, dyslipidemia, obesity, diabetes and insulin resistance are major risk factors for the development of vasculogenic ED [2]. Clinical studies showed that in high-risk cardiovascular patients, ED is highly predictive of all-cause of deaths and the composite of cardiovascular deaths, myocardial infarction, stroke and heart failure (HF) [3]. ED and HF share many risk factors, which is not surprising since ED is considered a consequence of vascular disease [4]. HF occurs mainly as a consequence of cardiovascular diseases, such as acute myocardial infarction. While HF alone poses many dramatic effects on a patient's lifestyle, ED represents one of these effects and greatly contributes to poor life quality and depression in patients with HF [5]. Moreover, HF elicits profound negative effects on many aspects of patients' sexual function such as decreased libido, decreased intercourse frequency, negative changes in sexual performance and general dissatisfaction related to sexual function [6]. Although the association between ED and HF is well established, there is not enough information on the mechanisms linking both conditions.

Several HF-specific complications can either cause or further exacerbate sexual dysfunction. The decrease in exercise tolerance is considered a major limitation to sexual performance in patients with HF. Patients with HF have increased vasoconstriction and decreased vasodilatation in response to exercise [7]. Although the reduction in exercise tolerance may contribute to ED and sexual dissatisfaction in HF, there is no evidence that exercise limitation is the sole causal agent of ED in patients with HF.

Since HF is largely an outcome of coronary atherosclerosis, it is suggested that ED is the result of atherosclerotic load in patients with HF [8]. However, some studies have shown that 56% of patients with HF

* Corresponding author at: Department of Pharmacology, Ribeirao Preto Medical School, – University of Sao Paulo, Av Bandeirantes, 3900, 14049-900 Ribeirao Preto, SP, Brazil.
E-mail address: fcarneirousp@yahoo.com.br (F.S. Carneiro).

http://dx.doi.org/10.1016/j.lfs.2015.12.005
0024-3205/

without coronary diseases have ED [9], suggesting that other mechanisms besides atherosclerotic load are involved in the pathogenesis of ED.

The penile erection involves both cavernosal smooth muscle relaxation and vasodilatation of penile arterial blood vessels to maintain an adequate blood inflow into the cavernous tissue [10–12]. Inadequate penile arterial blood flow is one of the major causes of ED [13,14]. Hypertension, hyperlipidemia, cigarette smoking, aging and diabetes are common risk factors associated with arterial insufficiency and ED [15,16]. Blood is carried to the penis by the internal pudendal arteries (IPA), from where it is distributed to the corpus cavernosum, corpus spongiosum and skin by deep and superficial penile arteries [17]. Since erection is primarily a neurovascular phenomenon, common vascular abnormalities in HF patients may directly affect erectile function. Therefore, considering the crucial role of IPA to deliver blood to the penis, functional changes in IPA may contribute to the vasculogenic component of ED associated to HF.

Although the rates of ED in patients with HF are alarming and the association between the two diseases is clearly established, no experimental or human study to date has evaluated the effects of HF on the cavernous tissue and/or IPA function. We hypothesize that HF induces direct functional and molecular changes in the corpus cavernosum and/or IPA that contribute to the genesis of ED. Additionally, taking into account that sympathetic overactivity is a hallmark of HF, we tested the hypothesis that HF leads to increased sympathetic-mediated contractile tone of the cavernous tissue and/or IPA of rats, thus contributing to the development of ED.

2. Methods

All experiments were carried out in male Wistar rats (200–250 g) housed with free access to water and standard chow and maintained on a 12 h light–dark cycle. All experimental procedures were carried out in accordance with the *Guide for the Care and Use of Laboratory Animals* [Dept. of Health, Education and Welfare, Publication No. (NIH) 85-23, Revised 1985; Office of Science and Health Reports, DRR/NIH, Bethesda, MD]. The experimental protocols were reviewed and approved by the Ethics Committee in Animal Research of the Ribeirao Preto Medical School, University of Sao Paulo (protocol no 119/2011).

2.1. Heart failure induction

All the animals were anesthetized with a mixture of ketamine (50 μg/g, sc União Química Farmacêutica Nacional S/A, Embu Guaçu, SP, Brazil) and xylazine (10 μg/g, sc Hertape Calier Saúde Animal S/A, Juatuba, MG, Brazil). Rats were endotracheally intubated and mechanically ventilated with a respirator (Advanced Safety Ventilator, Harvard Apparatus, MA1 55-7059, Holliston, MA, USA). Heart failure was induced by coronary artery ligation as previously described by Pfeffer *et al.* [18]. Briefly, a left thoracotomy was performed through the fifth intercostal space, the pericardium was opened and the heart was externalized. The left anterior descending coronary artery was subsequently ligated with polyester suture (4-0: Ethicon, São José dos Campos, SP, Brazil). The heart was brought back to the thoracic cavity, and the incision was sutured closed. Sham-operated control rats underwent a similar surgical procedure without coronary ligation. At the end of the surgery, the animals received analgesic (flunixin meglumine, 2.5 mg/kg, sc) and were maintained in the animal care facility for six to seven weeks until data collection. Six weeks after HF induction, the animals appeared healthy, eating normally and moving freely.

2.2. Cardiac function analysis

Under light anesthesia with a mixture of isoflurane 2% and oxygen (O_2), the rat thoraxes were shaved 24 h before the echocardiography. Transthoracic echocardiography (Vevo 2100 system; Visual Sonics, Toronto, Ontario, Canada) was performed four weeks after coronary artery ligation or sham surgery under anesthesia with a mixture of isoflurane 3% and O_2. The left ventricle internal diameter and the left ventricle posterior and anterior walls diameters were obtained throughout the cardiac cycle from the short-axis, motion-imaging mode. Ejection fraction and fractional shortening were automatically calculated using the measured parameters.

2.3. In vivo measurements of intracavernosal pressure/mean arterial pressure (ICP/MAP)

Intracavernosal pressure (ICP) in response to electrical stimulation of the major pelvic ganglion was assessed in sham and HF rats as previously described [19]. The animals were anesthetized with a mixture of isoflurane 4% and O_2. The left femoral artery and the right crura were cannulated with polyethylene catheters (Clay-Adams, Northridge, CA, USA) and fitted to a heparinized saline-filled pressure transducer to monitor and calculate mean arterial pressure (MAP) and ICP, respectively. The baseline MAP was calculated before the electrical stimulation. The major pelvic ganglion was assessed via a midline incision and stimulated with a bipolar silver electrode. ICP changes were then monitored in response to frequency curves (0.2–20 Hz; 1 ms pulses at 6 V). The stimulation at each frequency lasted for 45 s and each frequency curve was repeated after 5 min intervals. The erectile response was calculated using the maximum ICP response normalized to MAP at the time of maximum ICP. The ratio between the area under the curve (AUC) for ICP and MAP was also recorded with stimulation at 12 Hz for 40 s period. Three measurements were analyzed for each animal.

2.4. Ex vivo cardiac morphological analysis

At the end of *in vivo* ICP/MAP experiments, the rat thoracic cavity was opened, exposing the heart still beating. The hearts were removed, washed in cold potassium chloride (KCl) solution (150 mM) and weighed to calculate the heart weight index. Heart samples were fixed in phosphate-buffered 4% paraformaldehyde solution (24 h), cryoprotected in phosphate-buffered 30% saccharose solution (24 h) and subsequently frozen in Tissue-Tek OCT Compound (Sakura Finetek USA, Inc.; Torrance, CA, 90501 USA). Each block was cut into 10-μm serial sections in cryostat, beginning at the midventricular surface. The sections were stained with picrosirius red solution for the analysis of the infarct size. The infarct size was calculated by the ratio between the length of infarcted area and the total circumference of the left ventricle and was expressed as a percentage. It was considered in the study only animals with infarcted area greater than 40%. The right lung was also removed and weighed to calculate the lung weight index. The infarct size was measured using the public domain software NIH Image J (developed by U.S. National Institutes of Health and available on the site http://rsb.info.nih.gov/nih-image).

2.5. Functional studies in cavernosal strips

Cavernosal strips preparations were obtained from the corpora cavernosa as previously described [20]. Cavernosal strips were mounted in 5 mL myograph chambers (Danish Myo Technology, Aarhus, Denmark) containing physiological salt solution at 37 °C and continuously bubbled with a mixture of 95% oxygen (O_2) and 5% carbon dioxide (CO_2). The tissues were stretched to a resting tension of 5.0 mN and changes in isometric force were recorded using the acquisition data system PowerLab/8SP (LabChart software, version 7.2; ADInstruments, Colorado Springs, CO). To verify the contractile ability of the preparations, a KCl solution (120 mM) was added to the organ baths at the end of the stabilization period. Cumulative concentration-response curves to phenylephrine (10^{-9} M to 3×10^{-5} M), in the presence or absence of Nω-nitro-L-arginine methyl ester (L-NAME) (10^{-4} M) plus atropine (10^{-6} M) were performed with strips under basal tone.