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Impact of myocardial infarction on cardiac autonomic function in diabetic rats ☆

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ABSTRACT

Aims: We evaluated autonomic and hemodynamic parameters and maximal oxygen consumption (VO_2max) as possible determinants of mortality in streptozotocin (STZ) diabetic rats after myocardial infarction (MI).
Method: Male Wistar rats were divided into (n=8 of each): control sham (CS), diabetes sham (DS), MI (I), and diabetes+MI (DI). MI was induced 15 days after STZ induction. VO_2max was measured at 3 (basal), 30, 60, and 91 days after MI. Hemodynamic and autonomic parameters were evaluated 92 days after MI.
Results: MI area was similar in infarcted groups (~44%). Mortality rate increased in the DI (70%) compared with I (53%) group. Cardiopulmonary baroreflex, sympathetic (48%) and vagal (33%) tonus, low frequency (LF) band (57%), and LF/high frequency (HF) band ratio (53%) were reduced in DI compared with I animals. Furthermore, cardiac output (CO), peripheral vascular resistance (PVR) impairment, and VO_2max reductions were observed in the DI compared with the I group.
Conclusions: Our data suggest that the CO and PVR changes as well as VO_2max reduction were probably associated with additional cardiac autonomic control impairment, and, consequently, increased mortality rate in diabetic rats after a chronic myocardial infarction.

1. Introduction

The prevalence of diabetes in developed countries is approaching epidemic proportions and has been associated with an increased risk of cardiovascular abnormalities and microvascular complications (Haffner, Lehto, Ronnemaa, Pyorala, & Laakso, 1998). One of the most serious complications of diabetes is cardiovascular autonomic neuropathy, which results in abnormalities in heart rate control and vascular dynamics (Maser, Mitchell, Vinik, & Freeman, 2003) and has been related to the subsequent incidence of a fatal or nonfatal cardiovascular event (Liao, Carnethon, Evans, Cascio, & Heiss, 2002).

Diabetic patients have a two-fold higher risk of short-term mortality after MI, even after adjustment for the extent of coronary heart disease (Woodfield et al., 1996). In the thrombolytic era, many diabetic patients have survived cardiac events; however, how diabetes affects the long-term prognosis of these early MI survivors is less certain. Gustafsson et al. (2000) evaluating pre-admission history, presentation, initial treatment, and long-term mortality in patients with MI and diabetes concluded that diabetic compared with nondiabetic patients treated with oral hypoglycemic agents or insulin, but not those treated with diet alone, have a significantly increased mortality following acute MI.

In post-MI diabetic patients, autonomic function can be affected by diabetes sequelae, MI sequelae, or both, and has been associated with increased risk of morbidity and total mortality (Barthel et al., 2011). In the experimental setting, our group has previously evidenced autonomic dysfunction in streptozotocin diabetic rats (De Angelis et al., 2000; Schaan et al., 2004; Farah et al., 2007; De Angelis, Irigoyen, & Morris, 2009; Mostarda et al., 2009) and MI animals (Lacerda et al., 2007; Flores et al., 2010; Mostarda et al., 2010; Jorge et al., 2011), being normalized by aerobic exercise training. However, the autonomic function when associated with these experimental conditions has been poorly investigated.

Exercise intolerance is a common characteristic observed in diabetic (Vinik & Ziegler, 2007) and chronic heart failure patients (Negrao & Middlekauff, 2008) and has been considered an important determinant of mortality in these populations. Autonomic dysfunction may impair exercise tolerance and has been shown to reduce heart rate, blood pressure, and cardiac output responses to exercise in persons with diabetes (Vinik & Ziegler, 2007). Furthermore, it has been proposed that impaired cardiac reserve and peripheral abnormalities, rather than resting cardiac function, may be more directly correlated with impaired exercise ability in heart failure patients (Negrao & Middlekauff, 2008). In this sense, it is

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possible that the set of abnormalities intrinsic to diabetes or MI, or both together, may play a critical role in the abnormal exercise performance in these individuals.

We hypothesized that the association between diabetes and chronic MI could be related to additional worsening of cardiac autonomic dysfunction, resulting in hemodynamic and exercise capacity impairments, with consequently increase in mortality rate. Thus, the purpose of this study was to conduct a screening of cardiac autonomic function and hemodynamic measurements, and to evaluate the maximal oxygen consumption (VO_2max), and the possible repercussion of these clinical parameters on the mortality rate of diabetic rats after chronic MI.

2. Materials and methods

2.1. Experimental design

Experiments were performed using adult male Wistar rats (230–260 g) obtained from the animal facility at the University of São Paulo (São Paulo, Brazil). The rats were fed standard laboratory chow and water ad libitum. They were housed in collective polycarbonate cages in a temperature-controlled room (22 °C) with a 12-h dark–light cycle (light 07:00–19:00 h). The Institutional Animal Care and Use Committee of the Medical School of the University of São Paulo approved this protocol; this investigation was conducted in accordance with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1985). The rats were randomly assigned to 4 groups: control sham (CS, n = 10), diabetic sham (DS, n = 20), control infarcted (I, n = 30), and diabetes + myocardial infarction (DI, n = 30). Experimental diabetes was induced by an intravenous injection of 50-mg/kg streptozotocin (STZ) (Sigma Chemical Co., St. Louis, MO) dissolved in citrate buffer (pH 4.2). The rats were fasted overnight before the STZ injection. The CS and I rats were injected with buffer only (10 mM citrate buffer, pH 4.5). Forty-eight hours after the STZ injection, diabetes was confirmed by the measurement of blood glucose levels above 200 mg/dL in fasted (6 h) rats.

2.2. Myocardial infarction

Fifteen days after the STZ injection or administration of the citrate buffer, the rats were anesthetized (80 mg/kg Ketamine and 12 mg/kg Xylazine, administered i.p.) and underwent MI induction by surgical occlusion of the left coronary artery, as described elsewhere (Rodrigues, Figueroa, et al., 2011; Rodrigues, Rosa, et al., 2011; Jorge et al., 2011). Briefly, after intubation, animals were positive-pressure ventilated with room air at 2.5 mL, 65 strokes/min with a pressure cycled rodent ventilator (HARVARD Apparatus, Model 683, Holliston, MA). For induction of MI, a 2-cm left lateral thoracotomy was performed in the third intercostal space, and the left anterior descending coronary artery was occluded with a nylon (6.0) suture at approximately 1 mm from its origin below the tip of the left atrium. The chest was closed with a silk suture. The animals were maintained under ventilation until recovery. The sham rats (CS and DS) underwent the same procedures except that myocardial ischemia was not induced.

The mortality rate was investigated during the 90 days of the protocol (n = 90) beginning after the MI and/or Sham surgery (to exclude the influence of anesthesia or stress from the surgical procedure). Eight animals from each group were evaluated 90 days after myocardial infarction induction (105 days after diabetes induction) to perform the following experimental protocols.

2.3. Myocardial infarction area determinations

The MI area was evaluated by echocardiography 2 days (initial evaluation) and 90 days (final evaluation) after MI surgery and was delimited leading to analysis of the movement of the LV walls. Regions with systolic shortening classified as absent were considered infarcted (Rodrigues, Figueroa, et al., 2011; Rodrigues, Rosa, et al., 2011; Jorge et al., 2011). To confirm the echocardiographic measurement, the MI area was also evaluated after all evaluations by dissecting the fibrous scar from the remaining LV muscle. Initially, the MI area was confirmed by gross observation of the LV scar. After dissection of the scar area, the outlines of fragments were drawn on graph paper, and their areas were measured by the cross-point method (Flores et al., 2010; De Angelis, Leirner, Irigoyen, & Cestari, 2001; Lindpaintner et al., 1993). The interventricular septum was considered part of the left ventricle.

2.4. Maximal oxygen consumption (VO_2max)

VO_2max was measured at basal (3 days), 30, 60, and 91 days after MI or Sham surgery. The VO_2 max was determined by analyzing expired gas during a progressive exercise ramp protocol, with 3 m/min increments every 3 min and no grade until exhaustion. The experimental animals only started the exercise test when the levels of resting VO_2 were near to 30 ± 5 mL/kg/min. Gas analysis was performed using an oxygen (S-3A/I) analyzer (Ametek, Pittsburgh, PA, USA). The VO_2 was calculated using the measured flow through the metabolic chamber, the expired fraction of effluent oxygen, and the fraction of oxygen in room air (Rodrigues et al., 2007; Jorge et al., 2011).

2.5. Hemodynamic measurements

One day after the last maximal oxygen consumption evaluation, 2 catheters filled with 0.06 mL of saline were implanted into the femoral artery and femoral vein of the anesthetized rats (80 mg/kg Ketamine and 12 mg/kg Xylazine, i.p.). Twenty-four hours later, the arterial cannula was connected to a strain-gauge transducer (Blood Pressure XDCR; Kent Scientific, Torrington, CT, USA), and arterial pressure (AP) signals were recorded over a 30-min period in conscious animals by a microcomputer equipped with an analog-to-digital converter board (WinDaq, 2 kHz, DATAQ, Springfield, OH, USA). The recorded data were analyzed on a beat-to-beat basis to quantify changes in mean AP (MAP) and heart rate (HR). Pulse interval variability (PIV) in time domain was determined by using the standard deviation of the basal HR recording period for 30 min (Rodrigues, Rosa, et al., 2011; Jorge et al., 2011).

Responses to stimulation of cardiopulmonary baroreflex sensitivity (CBS) (Bezold–Jarish reflex) were determined in conscious animals. After recording 30 min of resting AP and HR, sequential bolus injections (0.1 mL) of increasing doses of serotonin (2, 4, and 8 μg/kg; Sigma Chemical Co. Perth, West Australia) were given to the animals while AP and HR were recorded. For data analysis, maximum reductions in HR and AP for each given dose were used to calculate the CBS. Injections were not repeated until the recorded parameters had returned to basal levels (Lacerda et al., 2007; Flores et al., 2010).

After CBS assessment, AP and HR were continuously recorded during the basal state and after methylatropine (3 mg/kg, IV) injection (0.2 mL). Because the HR response to the drug reaches its peak within 3 to 5 min, this time interval was allowed to elapse before HR measurement. Atenolol (8 mg/kg, IV) was injected (0.2 mL) 10 min after methylatropine, and again the response was evaluated after simultaneous blockade with atenolol and methylatropine. The next day, the sequence of injections was inverted (first atenolol and then methylatropine). The intrinsic heart rate (IHR) was evaluated after simultaneous blockade with atenolol and methylatropine. Sympathetic tonus was determined as the difference between maximum HR after methylatropine injection and IHR. Vagal tonus was obtained by