Autonomic Neuroscience: Basic and Clinical 177 (2013) 231–236

Contents lists available at ScienceDirect

Autonomic Neuroscience: Basic and Clinical

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

Carvedilol recovers normal blood pressure variability in rats with myocardial infarction

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ARTICLE INFO

Article history:
Received 4 November 2012
Received in revised form 27 April 2013
Accepted 9 May 2013

Keywords:
Arterial blood pressure
Autonomic nervous system
Power spectral analysis
Beta-blocker therapy

ABSTRACT

Background: The aim of this study was to investigate the effects of chronic treatment with carvedilol in blood pressure (BPV) and heart rate (HRV) variability of rats with myocardial infarction (MI).
Methods: MI was produced in male rats by ligature of anterior interventricular branch of left coronary artery. Control rats were submitted to a sham surgery (SO). MI and SO rats were randomized to receive for 30 days placebo (Plac 0.5% metilcelulose) or carvedilol (Carv, 2 mg/Kg body weight/day, drinking water): SO-Plac ($N = 10$), SO-Carv ($N = 10$), MI-Plac ($N = 12$), MI-Carv ($N = 13$). Blood pressure (BP) was directly recorded in the awake animals and BPV was determined, in time (variance, $mmhg^2$) and frequency domains by the autoregressive method. Statistical significance was set in $P < 0.05$. Data are median and interquartile range.
Results: No significant changes in HRV was observed in MI rats, while BPV showed significant decreasing of blood pressure variance (SO-Plac = 42.08 (39.21) $mmHg^2$ vs. MI-Plac = 21.67 (12.58) $mmHg^2$, $P < 0.05$), reversed by the Carv treatment (MI-Plac = 21.67 (12.58) vs. MI-Carv = 38.64 (29.25), $P < 0.05$). In the frequency domain analyses, MI reduced absolute and normalized LF component (LF ($mmHg^2$): SO-Plac = 8.98 (14.84) vs. MI-Plac = 2.08 (4.84), $P < 0.05$; LF(nu): SO-Plac = 79.48 (45.03) nu vs. MI-Plac = 24.25 (40.67) nu, $P < 0.05$) and increased the normalized HF component of the BPV (SO-Plac = 20.51 (39.18) vs. MI-Plac = 60.51 (39.73). Carv treatment significantly attenuated the LF component fall.
Conclusion: Chronic treatment with carvedilol restored the variance of BPV altered by the MI.

1. Introduction

Cardiovascular diseases represent today the major cause of death and disability in the world (World Health Organization, 2008; Gaziano et al., 2010; Mittal and Singh, 2010). Ischemic heart diseases have accounted for about 7.2 millions of deaths in 2004 (World Health Organization, 2008). Blood pressure (BP) is one of the most important parameters to maintain cardiovascular homeostasis and its regulation depends on a complex beat-to-beat and long-term integration of efferent signals in the central nervous system to produce fine adjustments of the autonomic balance to the heart and blood vessels, thus modulating heart rate, cardiac output and peripheral vascular resistance. In pathological conditions, either afferent as well as efferent signaling may change, and thus affect BP regulation. For the better understand the hemodynamic deregulation occurring in several cardiovascular diseases, studies of patterns of blood pressure variability (BPV) have received increasing attention because it may provide sign of risk of cardiovascular death (Akselrod et al., 1985; Mancia et al., 1994; Martinka et al., 2005) and valuable information on the mechanisms of cardiovascular deregulation (Pagani et al., 1988).

The impact of myocardial infarction (MI) on BPV has received little attention until now, mainly in experimental animals in which, unlike the studies in humans, a more detailed exploration of variables interfering in blood pressure regulation is possible (Mostarda et al., 2010). Long-term use of beta-blockers has been strongly recommended to prevent cardiac arrhythmias and cardiac death in patients with MI (Gunnar et al., 1990). Since adrenergic blockers interfere on the efferent sympathetic control of heart rate and vascular resistance, studies on BPV under such drugs influence are clinically relevant. Carvedilol is a non-selective beta-blocker also exhibiting alpha-1 blockade properties with increasing use in patients with MI and heart failure (Packer et al., 1996a, 1996b; Dargie, 2001). However, the long-term

Abbreviations: BPV, blood pressure variability; MI, myocardial infarct; HRV, heart rate variability; VLF, very low frequency; LF, low frequency; HF, high frequency; SO, sham operated; Plac, placebo; Carv, carvedilol.

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http://dx.doi.org/10.1016/j.autneu.2013.05.007

effects of carvedilol on BPV after MI remain unknown until now. Therefore, we carried out this study to investigate the interference of chronic use of carvedilol on the spectral and temporal parameters of BPV of rats submitted to MI.

2. Materials and methods

2.1. Sample and myocardial infarct procedure

Fifty-one male Wistar rats of our department colony were used in the experiments. The study was approved by our institutional ethical committee (Ethics Committee in Use of Animals, protocol no. 056/11) and the experiments were conducted in accordance with the Guide for Care and Use of Laboratory Animals published by the US National Institute of Health (NIH Publication #85-23, revised 1996). The animals (8 to 10 weeks old) were anesthetized (35 mg/kg Ketamine + 10 mg/kg Xylazine, im), the thorax was opened in the fourth left intercostal space, the heart was rapidly eviscerated and a 6–0 mononylon thread was used to produce a permanent ligature of the anterior interventricular branch of left coronary artery ($N = 31$), as before described (Mill et al., 1992). Another group of rats was subjected to a sham operation (SO, $N = 20$), in which the coronary ligature around the coronary vessels was not tied. Twenty-four hours later, the rats were re-anesthetized with the same drugs to record a standard electrocardiogram (ECG). Presence of MI was confirmed by the presence of wide Q wave in D1 lead (Pimentel et al., 2012). Six rats were excluded from the study because Q wave was absent, suggesting an unsuccessful coronary ligature. The MI ($N = 25$) and SO groups were then randomized to receive a daily treatment with carvedilol (Carv, 2 mg/Kg) or placebo (Plac, 0.5% methylcellulose vehicle) in the drinking water. The volume of water supplied was determined by the average weekly consumption in a pilot study. The rats were kept in a room with controlled temperature (20–22 °C) with 12:12-h light–dark-cycle and food was provided ad libitum.

2.2. Hemodynamic evaluation of blood pressure and ECG acquisition

The treatment was discontinued one month later. The rats were then weighted, anesthetized (90 mg/Kg Ketamine + 13 mg/Kg Xylazine, ip) to insert a PE50 catheter into the left femoral artery to direct BP recording and to implant metallic electrodes under the skin to ECG recording. After 24 h, the arterial cannula was washed with saline heparinized solution (1:500 UI) and attached to a pressure transducer (TRA021, LSI, Letica, Scientific Instruments) to blood pressure monitoring (sampling rate 2 KHz). Simultaneously the metallic electrodes were connected to the input of a bioelectric amplifier (ML 132 Bio Amp) to record ECG (sampling rate 2 KHz). The pulsatile blood pressure and the ECG signals were simultaneously processed in a data acquisition system (Powerlab 4 sp, sp 4922, Adinstruments; Bridge Amp, ML 110) and analyzed in a dedicated computer with Chart 5.5.1 software. After adaption to the recording system, blood pressure and ECG were continuously recorded for 30 min. Systolic, diastolic and mean blood pressure as well as heart rate were averaged during the 30 min recording period to give basal values of these variables in the awake and unrestrained animals.

2.3. Blood pressure and heart rate variability

Time and frequency domains analyses of systolic BPV and HRV were obtained from the 30 min recording period. An algorithm was used to detect cycle-to-cycle peak systolic pressure and R waves, thus generating the beat-to-beat series of systolic pressure and R–R intervals. The series were visually inspected and artifacts and ectopic beats were manually removed (Task Force, 1996). Time domain analysis included variance of systolic BP and variance of R–R intervals in the entire record. Frequency domain analysis was performed by autoregressive modeling with model order set in 16 (Dantas et al., 2012). Oscillatory components were calculated by the Yule–Walker method with the Levison Durbin recursion (Souza et al., 2008). The oscillatory components were divided in following bands: very low frequency (VLF, 0.01–0.20 Hz), low frequency (LF, 0.20–0.75 Hz) and high frequency (HF, 0.75–2.50 Hz) (Malliani et al., 1991). Low and high frequency components were expressed also in normalized units. Normalization consisted in dividing the power of a given spectral component by the total power minus the power of VLF and multiplying the ratio by 100. LF/HF ratio was obtained by division of the components of low by high frequency, in normalized units (Malliani et al., 1991).

2.4. Evaluation of cardiac weight and structure

After hemodynamic recording, the rats were anesthetized (90 mg/Kg Ketamine + 13 mg/Kg Xylazine, ip) and euthanatized with an intravenous injection of 3 M KCl. The thorax was opened, the heart quickly removed, washed in saline solution, dried on paper-filter, weighed (atria and ventricles), wrapped in plastic, and stored for 30 min at −12 °C. The atria were then removed and the ventricles were cut transversely into four equal segments, from the apex to the base. The sections were immersed in a 10% tripheniltetrazolium chloride solution at 37 °C for 10 min, and finally immersed in 10% buffered formalin solution for 20 min. This procedure allowed increasing the contrast between the intact myocardial and the infarct scar. Images of sections were scanned (Scanjet 2400) and analyzed by planimetry with a software (Image J 1.40 g). Infarct size, expressed as percentage, was calculated by dividing the sum of infarct areas from all sections by the sum of left ventricle areas from all sections (including those without infarct scar) and multiplying by 100 (Takagawa et al., 2007). Relative heart weight was calculated dividing heart weight by body weight.

2.5. Statistical analyses

Normality of data was evaluated by Shapiro–Wilk test and variance homogeneity by Levene test. MI size was compared with two-tail unpaired *t*-test. Body weight, relative cardiac weight, heart rate, BPV and HRV variables, were ranked before comparisons. These variables, and the hemodynamic parameters (systolic, diastolic, and mean blood pressures), body weight and cardiac weight were compared by two-way ANOVA followed by the Bonferroni post hoc test for multiple comparisons. Only the following comparisons between groups were tested in the post-hoc test: SO-Plac vs. SO-Carv (to test the effect of the Carv), SO-Plac vs. MI-Plac (to test the effect of the infarct) and MI-Plac vs. MI-Carv (to test the effect of Carv in presence of MI). Statistical significance was set in $P < 0.05$ and SPSS 15.0 was used to perform all analyses. Unless otherwise stated, data are expressed as mean ± standard deviation.

3. Results

Body weight before surgery was similar in the four groups (SO-Plac = 245 ± 12 g; SO-Carv = 250 ± 7 g; MI-Plac = 238 ± 9 g; MI-Carv = 223 ± 5 g; $P > 0.05$; two-way ANOVA). The body weight gain during the treatment period was similar in the four groups, so that the final body weight was also similar in the four groups at the end of the study (SO-Plac = 376 ± 19 g; SO-Carv = 384 ± 7 g; MI-Plac = 362 ± 11 g; MI-Carv = 328 ± 7 g; $P > 0.05$; two-way ANOVA).

3.1. Cardiac structure

No visible scar was observed in rats submitted to sham surgery, while a remarkable transmural scar was observed in all animals