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Circadian changes in autonomic function in conscious rats with heart failure: Effects of amiodarone on sympathetic surge

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ABSTRACT

Cardiovascular events are characterized by circadian periodicity with a peak prevalence during the awakening period, which suggests a morning surge in sympathetic activity. We developed an experimental system to determine circadian changes in heart rate (HR), blood pressure (BP), locomotor activity (Loc), respiratory rate and autonomic function in conscious, unrestrained rats. The effects of amiodarone on circadian variation of these variables were determined in rats with myocardial infarction and subsequent congestive heart failure (CHF). We continuously recorded BP, HR and Loc for 24 h in rats with CHF ($n=16$) or after a sham operation (Sham; $n=7$). To determine circadian changes in sympathovagal balance, digitized BP and HR data throughout 24 h were analyzed based on maximum entropy. The study was repeated after 3 weeks of oral amiodarone (50 mg/kg/day) or saline administration. Baseline HR, mean BP, and Loc were higher in the dark period than in the light period (all $p<0.05$) in both CHF and Sham rats, which is consistent with the circadian periodicity of nocturnal animals. Low-frequency components of diastolic BP variability (LFdp), an index of sympathetic tone, were significantly higher during the awakening period (16:00–20:00) than during the sleeping period (08:00–14:00), a finding analogous to the sympathetic morning surge in men. Amiodarone suppressed this transient increase in LFdp power during the awakening period. Our experimental system could detect sympathetic surge in conscious rats. Amiodarone suppressed the sympathetic surge, which could explain, at least in part, beneficial effects of amiodarone in patients with CHF.

1. Introduction

Sudden death accounts for about 30 to 50% of the total mortality of patients with congestive heart failure (CHF), and it occurs more frequently among patients with less severe CHF (New York Heart Association functional class II) (MERIT-HF study group, 1999). However, the likelihood of death from worsening heart failure increases with an increase in the severity of heart failure (MERIT-HF study group, 1999). Sudden death generally peaks during the period of awakening (Willich et al., 1987), and in patients with CHF, in the morning (Moser et al., 1994) or evening (Carson et al., 2000). The prevention of sudden death might therefore be important for decreasing mortality in patients with CHF.

Activation of the sympathetic nervous system influences both disease progression and survival in patients with CHF (Floras, 2009; Triposkiadis et al., 2009). Sleep disturbances have recently become an area of interest with respect to the pathogenesis of enhanced sympathetic activity (Somers et al., 2008), which is potentially carried over into the daytime. Although pathophysiological changes in CHF could be attributable, at least in part, to an abnormal circadian variation in autonomic nervous activity, continuous evaluation of 24-h diurnal changes in autonomic function is difficult in conscious, unrestrained rats. Thus, we developed an experimental system that enables continuous 24-h sampling of blood pressure (BP) and heart rate (HR) in conscious, unrestrained rats to determine circadian variations in BP, HR and autonomic nervous function. We also studied the effects of amiodarone in rats with CHF induced by myocardial infarction (MI) using this system, because amiodarone is effective as prophylaxis against cardiac events in patients with CHF (Amiodarone trial meta-analysis investigators, 1997; Doval et al., 1994).

2. Methods

2.1. Preparation of animals

The Animal Care and Use Committee of the University of Toyama approved all of the study protocols, which conformed to the guiding principles of the Physiological Society of Japan. Male Wistar rats weighing between 300 and 350 g were anesthetized with 3% isoflurane, intubated, placed on a rodent respirator, and maintained on 2%

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isoflurane in oxygen. The CHF model was produced by coronary artery ligation as described elsewhere (Pfeffer et al., 1979). We induced MI after thoracotomy by ligating the left coronary artery about 2 mm distal to the origin using a 6-0 Ethilon suture. The Sham group underwent thoracotomy but not coronary artery ligation. The abdominal cavity of surviving rats was implanted with an abdominal aortic catheter attached to a TA11PA-C40 radio-telemetry transmitter (Data Sciences International, St. Paul, MN, USA) under isoflurane anesthesia at 4 weeks after MI ($n=16$) or Sham operation ($n=7$) to measure BP and HR. The rats recovered for 1 week under a 12-h light–dark cycle and received food and water *ad libitum*.

2.2. Data acquisition and analysis

An acrylic chamber containing rats was placed on a signal-receiving board (RMC-1, Data Science International) to receive telemetric BP signals that were digitized at 1 kHz by an analog-digital converter (DT9804-USB, Data Translation Inc., Marlborough, MA, USA) and continuously stored on a personal computer (Dimension 8200, Dell, Round Rock, TX, USA) for 24 h (Fig. 1). These beat-by-beat BP time series were interpolated to generate a new time series for BP that was sampled at an equidistant sampling interval of 0.01 s (sampling rate, 100 Hz) using the MATLAB 6 program (The MathWorks, Inc., Natick, MA, USA). Mean BP was calculated using the formula,

$$\text{mean}\,BP = \text{diastolic}\,BP + (\text{systolic}\,BP - \text{diastolic}\,BP)/3.$$

Heart rate was calculated from pulse intervals between the successive maximum points of dBP/dt. Respiratory rate was determined by respiratory curve extracted from BP fluctuations by applying the Butterworth filter (Butterworth, 1930) with a passband of 0.8–2 Hz. Locomotor activity (Loc) in unrestrained, freely moving rats was determined using an infrared beam system (LCM-20, Melquest, Toyama, Japan; Fig. 1).

Throughout the entire 24 h, power spectral analysis with a maximum entropy method was applied to 1-min segments of re-sampled time-series data. BP and HR variability was determined every 10 s by shifting sequential 1-min segments. We determined the low-frequency components of diastolic BP (LFdp, 0.15–0.79 Hz) as an index of sympathetic tone and the high-frequency components of HR (HFhr, 0.8–3 Hz) as an index of vagal tone (Brown et al., 1994; Cerutti et al., 1991; Goso et al., 1999; Henze et al., 2008; Japundzic et al., 1990; Persson et al., 1992; Rubini et al., 1993; Waki et al., 2006).

To determine circadian variations in these variables, the 24-h period was separated into light (07:00–19:00; asleep) and dark (19:00–07:00; awake) periods. In particular, the period of 16:00–20:00 was designated as the period of awakening.

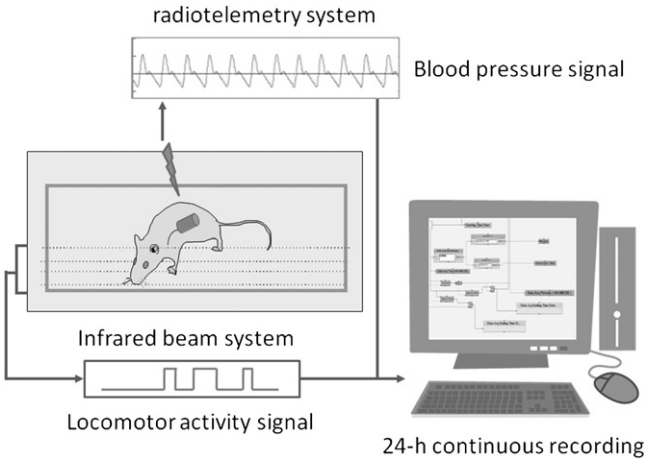

Fig. 1. Our system of simultaneously recording blood pressure (BP) and locomotor activity (Loc). Continuous 24-h BP and Loc data were obtained from conscious, unrestrained rats. Heart rate was determined from BP traces.

2.3. Experimental protocol

The CHF rats ($n=16$) were randomized into two groups after 24 h of pre-treatment data acquisition. One group received amiodarone (50 mg/kg daily; $n=8$), and the other received normal saline ($n=8$) for 3 weeks under the same housing conditions, with food and water *ad libitum*. The dosage of amiodarone was selected following a previous experimental study (Tachikawa et al., 2005). Each treatment was given by gastric gavage once a day. The study was repeated over a 24-h period following 3 weeks of either amiodarone or saline administration.

On the last day of the experiment, a 2F micromanometer-tipped catheter (SPR 320, Millar Instruments, Houston, TX, USA) was inserted into the right carotid artery and advanced into the left ventricle (LV) to determine LV pressure under light isoflurane anesthesia. After the measurements, the rats were euthanized with an overdose of pentobarbital sodium. The rats were weighed and then the heart and lungs were removed, the right ventricle (RV) and LV plus septum were separately weighed, and the LV was placed in 10% buffered formalin for subsequent determination of infarct size.

2.4. Determination of LV infarct size

After formalin fixation for at least 24 h, the LV plus septum was cut into four transverse slices. Sections (7 μm thick) were cut and stained with Masson's trichrome to maximally discriminate between the fibrous area of the infarct and viable muscle. Histological images were digitized through a frame grabber and analyzed (BZ Analyzer, Keyence, Osaka, Japan). Infarct size was calculated from the 4 slices by dividing the sum of the endocardial lengths of infarcted regions by the sum of the total endocardial circumferences (Pfeffer et al., 1979).

2.5. Statistical analyses

Data are presented as means ± SEM. Differences among continuous variables were determined by the paired *t*-test, the unpaired *t*-test or the analysis of variance (ANOVA) for repeated measures followed by the post hoc Bonferroni test (SPSS 14.0; SPSS, Chicago, IL, USA) if appropriate. A p value of <0.05 was considered statistically significant.

3. Results

The entire group given amiodarone survived but two rats in the saline group died before the treatment period was completed. One rat died suddenly two days, and the other, 8 days after starting saline administration. The latter might be due to congestive heart failure because this rat showed rapid and shallow respiration, and autopsy showed pleural effusion and ascites. Therefore, the remaining 6 rats comprised the saline group in the following analyses. The structural and hemodynamic characteristics of the three groups are summarized in Table 1. Morphological analysis of the hearts revealed MI with LV dilation in the CHF rats, indicating compensatory remodeling. MI involved 34 ± 2% of the LV, and hypertrophy of the viable LV wall was evident in the CHF group. The rats of this group were regarded as being in a chronic stage of compensated CHF because of increased RV weight (Table 1).

3.1. Baseline circadian variation

Representative raw data of BP, HR, respiratory rate and Loc throughout 24 h in a rat with CHF are shown in Fig. 2. Respiratory rate, HR, and Loc increased during the dark period, a consistent finding with the circadian rhythms of a nocturnal animal. The baseline data of