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Spectral analysis of cooling induced hemodynamic perturbations indicates involvement of sympathetic activation and nitric oxide production in rats

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ABSTRACT

Aims: Oscillations in arterial pressure and heart period in response to cold stress are poorly understood. We used a telemetric device with spectral and cross-spectral analyses to assess variabilities in the heart rate (HRV) and blood pressure (BPV) of conscious rats receiving a cooling stimulus (CS) (rapid immersion of palms and soles into 4 °C water) at ambient thermoneutral conditions (TC), in a cold room (CC), and when under pentobarbital anesthesia (UA).
Main methods: Power spectra of very low, low, and high frequencies (VLF: 0.02 to 0.2 Hz, LF: 0.2 to 0.6 Hz, and HF: 0.6 to 3.0 Hz), dicrotic notch (Dn) and plasma nitric oxide (NO) levels were measured for statistical comparisons.
Key findings: When compared to resting rats (PreCS), CS evoked in rats the following hemodynamic perturbations: 1) pressor and tachycardia; 2) increases in VLF_{BPV}, LF_{BPV}, HF_{BPV}, and TP_{BPV} but decreases in VLF_{HRV}, LF_{HRV}, and TP_{HRV}; 3) a positive correlation between LF_{BPV} and VLF_{BPV} but an inverse correlation between VLF_{HRV} and VLF_{BPV} and LF_{HRV} and LF_{BPV}; 4) high coherence value at frequency region of LF between BPV and HRV; and 5) increase of NO production and disappearance of Dn. Additionally, CS of CC and UA rats compared with TC rats evoked different patterns of hemodynamic perturbations; CC rats were activated but UA rats were inactivated.
Significance: Our findings indicate that changes in VLF_{BPV} are related to the myogenic vascular responsiveness to CS. Power spectra changes to CS are highly relevant to sympathetic activation and NO production.

1. Introduction

A cold pressor test is a novel maneuver commonly used in clinical practice to evaluate autonomic functions in cardiovascular regulation. Stressful cooling, such as rapidly immersing a hand in cold water, will evoke pressor and tachycardia and increase sympathetic neurotransmissions [19]. The mechanisms underlying this stress response are not clearly understood. In general, cold stress evokes hemodynamic perturbations characterized by instability of the cardiovascular system. The stress response includes unstable oscillations in blood pressure (BP), heart rate (HR), and vascular resistance, and an initial vasoconstriction followed by vasodilatation and a secondary progressive vasoconstriction [2,3,8] that allows greater blood flow and tissue perfusion to the cooled areas to avoid damage [11].

The interplay between initial vasoconstriction and subsequent vasodilatation through prolonged cooling is complex and requires intact sympathetic activity and sensory functions together with baroreflex compensation and the release of humoral substances [2,3,8,13,14]. The evoked vasodilatation, a myogenic vascular response, is suggestive of a reduction in α-adrenergic vasoconstriction and an increases in nitric oxide (NO) production [6,8,13,24,26].

Spectral analyses of BP variability (BPV) and HR variability (HRV) using a frequency domain approach have been widely used for investigations into baroreflex control of cardiovascular homeostasis — a dynamic interplay between the ongoing BP oscillations and compensatory hemodynamic responses [4,7,9,16]. The standard frequency bands commonly used are the following: 1) high frequency (HF) of BPV (HF_{BPV}) to characterize the oscillatory cardiac output secondary to the mechanical respiratory sinus arrhythmia and HF of HRV (HF_{HRV}) to characterize the oscillatory respiration and vagal modulation of HR; 2) low frequency (LF) of BPV (LF_{BPV}) and HRV (LF_{HRV}) to characterize the rhythmic activity of sympathetic modulation in respective BP and HR analyses; 3) LF/HF ratio of HRV (LF/HF_{HRV}) to characterize the overall balance between sympathetic and vagal modulation in HR regulation; 4) total power (TP) of BPV (TP_{BPV}) and HRV (TP_{HRV}) to characterize the overall variance in rhythmic activity of autonomic modulations in respective vasculature and heart; and 5) very-low frequency (VLF) of BPV (VLF_{BPV}) and HRV (VLF_{HRV}) to characterize the heterogeneous frequency bands

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of their physiological background. However, such VLF bands have been ascribed to thermoregulatory vasomotor modulation, activity of hormonal systems, and the autonomic nervous system itself [5,10,17,22].

The aim of our present study is to determine the underlying mechanisms of cold pressor tests. A rapid and noxious cooling stimulus (CS) was given to the glabrous palms and soles of conscious rats under three different circumstances to highlight the hemodynamic perturbations of the vascular wall and heart when under thermoneutral temperatures. We postulate that both sympathetic activation and production of NO will contribute to the underlying mechanism and corresponding spectral changes that occur to vascular wall and heart during cooling challenge.

2. Materials and methods

2.1. Animals

Adult male Sprague–Dawley rats (BioLASCO, Taiwan (ROC)) arrived at the animal center of the National Defense Medical Center (NDMC, Taipei, Taiwan) weighing between 300 and 350 g one week before experiments commenced. The experiments were performed according to a protocol approved by the animal care committee of NDMC. All efforts were made to keep the number of animals used as low as possible and to minimize animal suffering during the experiments. All rats were housed in a temperature and humidity controlled holding facility with a 12-h light/dark cycle (light on from 07:00 to 19:00) maintained by manual light control switches as required by the experiment. Rats in the same experimental group were housed together. All rats received food and water ad libitum. The experiments were performed between 08:30 and 17:30 with all rats being tested at the same time every day when possible.

2.2. Experimental protocols and cooling procedure

Rats were randomly divided into three groups for a stressful cooling procedure under three different experimental conditions including ambient thermoneutral conditions (TC, 23 °C: n = 8), 30-min in a cold room (CC, 4 °C: n = 8) before CS, and under pentobarbital anesthesia (UA: n = 7). Prior to the experiments, all of the rats were adapted to their environment for approximately 30 min. Following a complete hemodynamic and temperature stabilization, the individual rats were quickly placed in a Plexiglas cage filled with ice-water (depth = 2 cm; temperature = 4 °C) to immerse their glabrous palms and soles for a period of 10 min. After this cooling treatment, the rats were removed from the cage and dried with a cloth in a similar cage for 30 min to facilitate recovery. The beat to beat BP signals were continuously monitored via a telemetric device (TL11M2-M2-C50-PXT, DSI, USA) throughout three equally spaced 10-min during the entire 1-h experimental course: 10 min before (PreCS), 20 min after (PostCS), and during cooling (CS). Afterward, successive signals from a 5-min period (3 to 8 min) at each stage were taken for spectral analyses due to the stability of the mean and variance of VLF_{BPV} signals and the fluctuations of systolic blood pressure (SBP) during this period were found to be stationary. Dicrotic notch (Dn) and counts were manually assessed.

2.3. Surgical intervention

The telemetry transmitter was implanted intra-abdominally into each rat while it was under anesthesia (sodium pentobarbital, 50 mg/kg). A laparotomy was performed under aseptic procedure and the catheter of the transmitter inserted into the abdominal aorta, distal to the kidneys, and fixed. Experiments were started after the rat had fully recovered from surgery (7 days).

2.3.1. Spectrum signal acquisition and processing

The pulse signals were obtained after magnetic activation of the transmitter at least 1 h before starting the experiments to generate a calibrated analog signal (UA10; DSI, St. Paul, MN) with a range of ±5 V and a 12-bit resolution. Individual rats from each group were then placed on top of the receiver (PhysioTel® RPC-1) for telemetric signal acquisition. Five receivers were connected to a PC desktop computer via a matrix (Dataquest ART Data Exchange Matrix) and the received signals were recorded with Dataquest Acquisition software (Dataquest ART 4.33). A series of successive SBP and inter-beat interval (IBI) signals collected throughout the experiment were then digitized at a 500 Hz sampling rate and processed off-line using Matlab software (Terasoft Co.).

Beat-by-beat oscillations in SBP and IBI series were analyzed to quantify their frequency and power in BPV and HRV using autoregressive spectral decomposition. The BPV calculation was based on software kindly written for us by Prof. P.L. Lee, National Central University, Taiwan. Briefly, the acquired SBP signals were preprocessed by applying a band-pass filter (0.1–18 Hz, zero-phase 4th-order) to remove DC components. After finding all the maximum SBP peaks between two zero cross points, the extracted beat-by-beat SBP time series were detrended, interpolated and re-sampled at 0.05 s to generate a new time series of evenly spaced SBP samplings that allowed for a direct spectral analysis of each distribution using a Fast Fourier Transform (FFT) algorithm. The HRV calculation was based on Chart software developed by PowerLab, ADInstruments, USA. Spectral indexes of BPV and HRV were independently computed to obtain the total power (0.00 to 3.0 Hz, TP) and three major spectral bands: very-low frequency (0.02 to 0.2 Hz, VLF), low frequency (0.20 to 0.60 Hz, LF), and high frequency (0.60 to 3.0 Hz, HF). The normalized LF and HF were also calculated as nLF or nHF = LF or HF / TP − VLF × 100%. The modulus of the BP or HR spectrum (ordinates) had units of mm Hg^2 and ms^2, respectively. In addition, to examine the strength of the linear link between oscillations across a given frequency region between BPV and HRV signals, further computation was performed on the data by cross-spectrum analysis. As our estimate has approximately 7 degrees of freedom, an estimated value ($K^2_{IBI/SBP}$) > 0.585 was used to signify that two variability signals covary significantly at different frequency regions.

2.3.2. NO assay

Tail vein NO levels in the TC rats (n = 6) was assessed before and after 10 min of a CS treatment. The plasma NO level was determined indirectly by the content of nitrite and nitrate using enzyme-linked immunosorbent assay (ELISA) kits (CAT No. 780,001) supplied by Cayman Chemical Company (Ann Arbor, MI, USA). The results were expressed in μM.

2.3.3. Statistics

Statistical analyses were performed using the SPSS software version 18.0. The homogeneity of variance was first confirmed using the Kolmogorov–Smirnov test and afterward, was performed by a repeated measurement two-way ANOVA followed by a post-hoc Scheffe's test. Student's t-test was used to detect differences between two groups. Univariate correlations were calculated using Pearson's correlation analysis to estimate associations between selected frequency bands. The results are expressed as the mean ± SEM. A P-value < 0.05 is considered statistically significant.

3. Results

A representative example of BP and IBI tracings for rats challenged with CS under various experimental conditions is shown in Fig. 1. Averaged data are shown in Table 1 and Figs. 2–5.