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Effects of autonomic ganglion blockade on fractal and spectral components of blood pressure and heart rate variability in free-moving rats

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ABSTRACT

Fractal analysis is a promising tool for assessing autonomic influences on heart rate (HR) and blood pressure (BP) variability. The temporal spectrum of scale coefficients, $\alpha(t)$, was recently proposed to describe the cardiovascular fractal dynamics. Aim of our work is to evaluate sympathetic influences on cardiovascular variability analyzing $\alpha(t)$ and spectral powers of HR and BP after ganglionic blockade.

BP was recorded in 11 rats before and after autonomic blockade by hexamethonium infusion (HEX). Systolic and diastolic BP, pulse pressure and pulse interval were derived beat-by-beat. Segments longer than 5 min were selected at baseline and HEX to estimate power spectra and $\alpha(t)$. Comparisons were made by paired t-test.

HEX reduced all spectral components of systolic and diastolic BP, the reduction being particularly significant around the frequency of Mayer waves; it induced a reduction on $\alpha(t)$ coefficients at $t<2$ s and an increase on coefficients at $t>8$ s. HEX reduced only slower components of pulse interval power spectrum, but decreased significantly faster scale coefficients ($t<8$ s). HEX only marginally affected pulse pressure variability.

Results indicate that the sympathetic outflow contributes to BP fractal dynamics with fractional Gaussian noise ($\alpha<1$) at longer scales and fractional Brownian motion ($\alpha>1$) at shorter scales. Ganglionic blockade also removes a fractional Brownian motion component at shorter scales from HR dynamics. Results may be explained by the characteristic time constants between sympathetic efferent activity and cardiovascular effectors. Therefore fractal analysis may complete spectral analysis with information on the correlation structure of the data.

1. Introduction

The analysis of heart rate (HR) and blood pressure (BP) spontaneous variability is a powerful tool to derive information on the cardiovascular autonomic regulation (Parati et al., 1995; Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology, 1996). Most past studies focused on spectral analysis and described how experimental set-ups or clinical conditions influencing the autonomic tone modify the amplitude of HR and BP fluctuations at specific frequencies. However, approaches based on nonlinear analysis or on "complexity" analysis have been also increasingly used (Aubert et al., 2009; Huikuri et al., 2009). These approaches describe structural aspects of the cardiovascular dynamics, rather than the amplitude of HR or BP fluctuations.

Among complexity-based methods, the evaluation of HR or BP "self-similarity" appears promising. A time series is "self-similar" if any small data segment extracted from it looks like the original series, at least in a statistical sense, when plotted at higher resolution properly rescaling the amplitude (Eke et al., 2002). This is a "fractal" property of the time series. In fact also when a geometric fractal is broken down, its parts can have all the complexity of the original object, whatever small they are. For "monofractal" series, a single "stretching factor" may rescale the vertical axis assuring self-similarity at all the temporal scales. This stretching factor is often assessed by detrended fluctuation analysis (DFA) and is termed "scale coefficient" α (Eke et al., 2002). The α coefficient distinguishes two families of fractal processes with self-similar behavior: the fractional Gaussian noises, stationary processes with $\alpha<1$, and the fractional Brownian motions, nonstationary processes with $\alpha\geq 1$. Since real HR series are not properly described by monofractal processes (Tan et al., 2009), most studies estimate short-term ($\alpha 1$) and long-term ($\alpha 2$) scale coefficients by DFA (Peng et al., 1993). More recently a whole temporal spectrum of DFA coefficients, $\alpha(t)$, has been introduced to get a richer picture of the autonomic

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influences on HR and BP multi-fractal dynamics (Castiglioni et al., 2009, 2011b).

Different clinical conditions induce changes in sympathetic activity which substantially modify the HR dynamics (Malliani et al., 1991; Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology, 1996). It may be important therefore to identify autonomic changes by applying spectral and fractal analysis to spontaneous HR and BP variability. Influences of the sympathetic outflow on $\alpha(t)$ of HR and BP were previously studied in human volunteers by making use of pharmacological interventions (Castiglioni et al., 2011a). Cardiac parasympathetic influences were investigated by vagal blockade with atropine, cardiac sympathetic influences by sympathetic blockade with propranolol. The effects of a central reduction of the overall sympathetic activity were studied, instead, by administering clonidine. This provided evidence that sympathetic outflows influence the cardiac and vascular temporal spectrum of DFA coefficients, but with two limitations. First, the effects of cardiac sympathetic reduction could have been underestimated in volunteers studied while resting supine, a condition with an already low basal sympathetic tone. Second, the effects of a complete (cardiac and vascular) sympathetic blockade were not assessed.

Therefore aim of the present study is to better understand the effects of sympathetic influences on the fractal structure of cardiovascular signals by evaluating how a combined cardiac and vascular sympathetic blockade modifies the fractal dynamics, with particular attention to the temporal spectrum of scale coefficients $\alpha(t)$. This will be done by inducing a pharmacological ganglionic blockade in conscious rats by means of hexamethonium, and by correlating changes in the fractal structure with changes in spectral powers.

2. Methods

2.1. Data collection and pre-processing

The study was conducted on arterial BP recordings obtained in 11 Wistar-Kyoto rats (Charles River Italia, Calco, Italy), aged between 11 and 12 weeks. These recordings were selected from a larger study, aimed at investigating very-low frequency oscillations in sympathectomized rats (Radaelli et al., 2006). Selection criterion was the presence of an artifact-free stable segment of BP recording of at least 5-minute duration. Experimental procedures were conformed with Italian Government directives concerning the protection of animals used for scientific purposes, and were approved by the Ethical Committee of the University of Milano–Bicocca. In each rat, polyethylene catheters were implanted in the femoral artery for arterial BP recording and in two femoral veins for drug injections. Stress associated with surgery may increase the sympathetic nervous activity, and telemetry studies in rabbits actually showed higher sympathetic activity up to 4–5 days after surgery (Barrett et al., 2001). On the other hand waiting too long increases the probability that catheters occlude because of endovascular thrombosis, or that the animals start biting or tearing the catheters. As a compromise between these opposite requirements, the animal was examined 24 hours after surgery and BP recorded if there were no signs of sufferance or altered behavior. Otherwise, the experiment was postponed to the following day. Performing recordings at least 24 hours after surgery also allowed the animals to get used to the experimental environment, i.e., a wide cage where they were free to move, eat, and drink. Recordings were obtained before (baseline) and after induction of ganglionic blockade by hexamethonium bolus (30 mg/kg) followed by continuous hexamethonium infusion at 1.5 $mg \times kg^{-1} \times min^{-1}$ (HEX) to maintain the blockade over time. Hexamethonium is a nicotinic receptor antagonist that acts on autonomic receptors at pre-ganglionic sites. It may block outflows of both sympathetic and parasympathetic nervous systems. In rats, however, it has been shown that hexamethonium reduces the cardiac parasympathetic outflow only weakly even at doses that substantially decrease BP (Abdel-Rahman, 1989).

Beat-by-beat values of systolic BP, diastolic BP, pulse pressure (difference between systolic BP and diastolic BP of the preceding beat) and pulse interval (time interval between consecutive systolic BP peaks) were derived from each recording. In each animal, stable segments not shorter than 5 min were selected in baseline and HEX for DFA and spectral analysis.

2.2. Temporal spectrum of scale exponents

The DFA spectrum of scale exponents, $\alpha(t)$, was estimated for systolic and diastolic BP, for pulse pressure and for pulse interval, as described in (Castiglioni et al., 2011a). Briefly, given the beat-by-beat time series $x(t_k)$ of length N beats, the integrated series $y(t_k)$ is obtained after subtraction of the mean:

$$y(t_k) = \sum_{i=1}^{k} (x(t_i) - \overline{x}) \quad k = 1, \ldots, N \tag{1}$$

Then, $y(t_k)$ is segmented into blocks of size n beats and fit in each block to a regression line $y_n(t_k)$. The mean squared residual $F(n)$ is calculated over all the blocks of size n:

$$F(n) = \sqrt{\frac{1}{N}\sum_{k=1}^{N} (y(t_k) - y_n(t_k))^2} \tag{2}$$

For monofractal processes, $F(n)$ increases proportionally to n^{α} and α is estimated as slope of the regression line between $\log[F(n)]$ and $\log[n]$. In order to take into account possible deviations of real physiological data from the monofractal model, a whole spectrum of exponents $\alpha'(n)$ is calculated as the derivative of $\log[F(n)]$ vs. $\log[n]$:

$$\alpha'\left(n_p\right) = \frac{\log\left[F\left(n_{p+1}\right)\right] - \log\left[F\left(n_{p-1}\right)\right]}{\log\left[n_{p+1}\right] - \log\left[n_{p-1}\right]} \quad 1 < p < M \tag{3}$$

where n_p with $1 \le p \le M$ indicates the size of each of the M blocks. The temporal spectrum $\alpha(t)$ is obtained by mapping $\alpha'(n_p)$ from the samples domain, n, to the time-domain t, as $\alpha(t_p) = \alpha'(n_p)$ with $t_p = n_p/f_{HR}$ and f_{HR} the mean heart rate, in Hz; and by linearly interpolating $\alpha(t_p)$ over time to obtain a continuous function $\alpha(t)$. The larger temporal scale where $\alpha(t)$ can be calculated without substantial estimation bias depends on the length of the recording. Considering 5-minute recordings of heart rate, the estimation bias is lower than the 5% tolerance limit up to time scales of 50 s (Castiglioni et al., 2011b). Moreover, analyses on synthesized monofractal Gaussian noises and Brownian motions showed that in a physiological range of self-similarity values ($0.5 \le \alpha \le 1.1$) bias is negligible also for block sizes as short as $n = 4$ (Hu et al., 2001). To remain within these limits, $\alpha(t)$ was calculated for temporal scales t between 0.8 and 45 s.

2.3. Power spectrum

Power spectral densities were estimated by fast Fourier transform (FFT) on the same data segments selected for DFA. Beat-by-beat times series were resampled evenly at 10 Hz after linear interpolation. The periodogram was then calculated over 90% overlapped data windows of 120-s length, after mean removal, linear detrending and Hann windowing, with broadband smoothing procedure (Di Rienzo et al., 1996). The root mean square around the mean, RMS, was taken as measure of overall variability. Since the overall variability may increase with the length of the recording for nonstationary self-similar signals, like fractional Brownian motions (Eke et al., 2002), RMS was calculated over the 120-s running windows used for spectral analysis.