Nonlinear Dynamics of Heart Rate Variability after Superoxide Dismutase Inhibition in Rats

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Abstract

Superoxide dismutase (SOD) is a crucial enzyme for endogenous oxidative balance. We tested how diethylthiocarbamate (DETC), a nonspecific SOD inhibitor and oxidant, influence heart rate variability (HRV).

Fourteen Wistar rats (divided into two groups) with implanted subcutaneous ECG electrodes, maintained unrestrained in experimental setup. Time-series of 1024 RR intervals (RRi) were extracted from continuous ECG recording for HRV analysis. SOD activity was measured by dedicated assay kit. Intraperitoneal injection of DECT resulted in SOD inhibition by 30%. HRV analysis confirms a role of ROS in the regulation of sinoatrial rhythm. Increased HR after DETC injection was followed by a reduction in overall HRV including LF/HF. A significant reduction of the recurrence quantification analysis (RQA) parameters: %REC, %DET, DFAα1 and DFAα2 is indicative of some loss of complexity and self-similarity within the tested RRi time-series. The reduction of intrinsic harmonic correlations can be interpreted as a simplification of the regulatory feedback loops with more random heart rhythm poorly controlled by autonomic nervous system. Nonlinear dynamics methods such as RQA have a potential to increase the reliability of data in experimental studies.

1. Introduction

Reactive oxygen species (ROS) are involved in a wide array of cardiovascular regulatory mechanisms. Several lines of experimental data indicate a role for ROS in autonomic regulation of heart including a chemoreflex response and heart rate variability (HRV) [1,2]. Extensive generation or insufficient removal of ROS, especially superoxide anion ($O_2^{\bullet-}$) have been implicated in pathogenesis of multiple cardiovascular diseases, including atherosclerosis, hypertension and heart failure. Superoxide dismutase (SOD), which catalyzes dismutation of superoxide radical ($O_2^{\bullet-}$) into molecular oxygen (O_2) and hydrogen peroxide (H_2O_2) is the crucial enzyme for oxidative balance.

Our experimental study was designed to investigate the effect of SOD inhibition by diethylthiocarbamate (DETC) on HRV in unrestrained rats. DETC acts as a nonspecific SOD inhibitor to evoke oxidative stress with increased level of $O_2^{\bullet-}$and reduction of H_2O_2. An increased exposure to $O_2^{\bullet-}$ is supposed to trigger chemoreflex response and to dysregulate autonomic control of the heart rhythm [3].

2. Materials and methods

The study was approved by and carried out under the supervision of the Local Ethics Committee.

2.1. Experimental protocol

Fourteen male Wistar rats (350 g) were used in this study. Animals was randomly divided into control group (N=7) and experimental (DETC) one (N=7). Three silver ECG electrodes were implanted subcutaneously and exteriorized on occipital area. Following 48 hours of recovery the experiments were performed on conscious animals, previously thoroughly habituated to the experimental environment which were transparent chambers with enough space for unrestrained body movements.

DETC or vehicle (control group) were injected intraperitoneally at a dose of 250 mg/kg. ECG signals were continuously recorded for 1 hour with the use of LabChart 7 Pro software (ADInstruments Sydney, Australia) with sampling rate 4 kHz. The tachogram was calculated using automatic R-peak detection of the ECG signal (LabChart 7 Pro software, ADInstruments, Sydney, Australia; Microsoft Excel 2013, USA). All QRS complexes were thoroughly checked to avoid false positive detections and missed beats. Time series of 1024 consecutive RRi were derived between the 35th and 55th minute of each ECG recording when the rats were found to exhibit unrestrained behavioral patterns.

2.2. Chemical analysis protocol

SOD activity was measured by using Cayman Assay Kit (no: 706002; Cayman Chemical, MI, USA) in erythrocyte lysate. The blood was collected on heparin just after finishing the experimental procedure from animals of both groups. Blood was centrifuged at 1000 × g for 10 minutes at 4°C and plasma was pipetted off. Next, erythrocytes were lysed in ice-cold HPLC-grade water and centrifuged at 10000 × g at 4°C. Supernatant was frozen at -80°C until all blood samples was collected. Thereafter, SOD activity was measured following Cayman's detailed instruction for SOD assays.

2.3. HRV analysis

HRV parameters in time-domain: HR (heart rate), SDNN (the standard deviation of all normal RR intervals) and rMSSD (the root mean square of successive heartbeat interval differences), spectral power (FFT method [4]): power spectral density (PSD) within low frequency (LF; 0.2 – 0.75 Hz) and high frequency (HF; 0.75 – 2.5 Hz) bands as well as total spectral power (TSP) [5] and nonlinear: recurrency (%REC), determinism (%DET), DFAα1 (short-term fractal exponent of Detrended Fluctuation Analysis that correspond to a period of 4 – 16 RRi) and DFAα2 (long-term fractal exponent of DFA that correspond to a period of 16 – 64 RRi), Sample Entropy (SampEn) and Approximate Entropy (ApEn) were assessed using KubiosPro2.0 software (Kuopio, Finland). Statistical analysis was performed by the Statistica 12 software (StatSoft, Tulsa, USA).

3. Results

We started with checking whether DETC effectively inhibited SOD. SOD activity was reduced by 30% in comparison with control as detecting analysing blood samples collected following the experiments. (Figure 1).

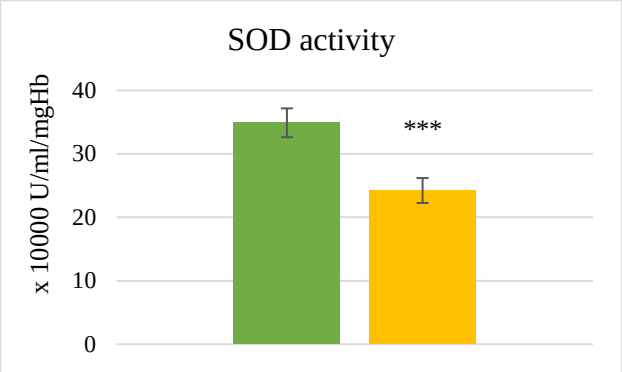

Figure 1. SOD activity in erythrocyte lysate after the experiment. Green bar – control group, yellow – SOD activity after inhibition by DETC. Data shown as mean ± SD; *** p<0.001.

DETC-driven SOD inhibition resulted in a significant increase in HR (p=0.002) from 319±36 to 429±44 bpm by shortening RR interval from 190±21 to 141±15 ms which in turn was followed by a significant suppression of the overall HRV as shown by reduced SDNN, TSP, and LF (Table 1). In contrast, HRV indexes of vagal drive: rMSSD and HF exhibit insignificant decreasing tendency (Table 2; p = 0.09, and p = 0.07; respectively), whereas LF/HF ratio, known as an index of the sympathovagal balance, decreased significantly (Table 1).

As for nonlinear HRV parameters, SampEn and ApEn remained unaffected by inhibited SOD activity, while %REC, %DET and both DFAα1 and DFAα2 were significantly reduced (Table 3).

Table 1. Time- and spectral-domain parameters of overall HRV after SOD inhibition by DETC. Data shown as mean ± SD.

Parameter	Control	DETC	p
HR (bpm)	319±36	429±44	0.002
RR (ms)	190±21	141±15	0.003
SDNN (ms)	3.68±0.98	1.53±1.08	0.023
TSP FFT (ms^2)	13.08±6.32	2.21±2.10	0.011
LF FFT (ms^2)	2.31±1.21	0.13±0.08	0.004
LF/HF FFT	0.85±0.34	0.12±0.03	0.001

Table 2. Time- and spectral-domain parameters of vagal activity of HRV after SOD inhibition by DETC. Data shown as mean ± SD. NS – non-significant.

Parameter	Control	DETC	p
rMSSD (ms)	3.13±0.97	1.74±0.95	NS
HF FFT (ms^2)	2.62±1.30	1.31±1.14	NS

Table 3. Nonlinear parameters of HRV after SOD inhibition by DETC. Data shown as mean ± SD. NS – non-significant.

Parameter	Control	DETC	p
SampEn	1.38±0.19	1.56±0.41	NS
ApEn	1.33±0.11	1.33±0.12	NS
%REC	46.33±4.40	32.93±10.09	0.021
%DET	99.37±0.27	97.97±1.26	0.035
DFAα1	1.08±0.15	0.53±0.17	0.001
DFAα2	1.25±0.12	1.01±0.27	0.034

4. Discussion

The major finding of this study is that oxidative stress which follows SOD inhibition seems to destabilise autonomic control of the heart as shown by reduced HRV and sympatovagal imbalance. Our data confirm the hypothesis of permanent involvement of ROS in cardiovascular regulatory control.

The detailed profile of changes observed in time- and frequency-domain HRV analysis seems ambiguous. Increased HR following DETC administration suggests sympathetic dominance, whereas significantly reduced TSP, LF, and LF/HF ratio indicate sympathetic suppression. Although high LF/HF ratio is commonly believed to reflect an increased risk of sudden arrhythmic events related to sympathetic overdrive [6], such simple interpretation of LF/HF index has been recently disputed in light of nonlinear, in part non-reciprocal interactions among sympathetic, vagal, and respiratory regulators. As suggested by Billman, Cohen and Taylor, Carnevali at al. and Taylor et al. [5,7-9] in case of low baseline vagal activity even small suppression of vagal input may result in an increase of HR.

Unlike in humans, cardiac vagal activity is much lower in rats [10,11] therefore sympathetic activity is not overridden by parasympathetic drive. The tendency towards a decrease of rMSSD and HF indicating vagal inhibition during oxidative challenge may promote sympathetic override. The overall suppression of autonomic control as shown by significant reduction of variability (TSP, SDNN) allows intrinsic pacemaker to take more control or even to override heart rhythm. As for resting tachycardia commonly reported in denervated hearts following heart transplantation [12,13] the increased HR observed after DETC administration may mostly reflect the intrinsic pacemaker properties. In such case, routine time-domain or spectral HRV measures would not be representative for autonomic regulatory control. Supposed predominant role of intrinsic pacemaker and related higher irregularity was not confirmed by expected [14] increase of entropy parameters (SampEn, ApEn). However, a significant reduction of the tested parameters of recurrence quantification analysis (RQA; %REC, %DET, DFAα1 and DFAα2) evoked by oxidative challenge indicate partial loss of complexity and self-similarity of the RR-time-series. Moreover, a significant decrease of DFAα1 from 1.08±0.15 to the level near of the Gaussian white noise (0.53±0.17; Table 3) [15] indicates almost complete loss of short term harmonic correlations. Such reduction of DFAα1 may reflect highly impaired vagal input, which was confirmed by HRV parameters assessed from linear method analysis: rMSSD and HF. Lack of intrinsic harmonic correlations may be interpreted as simplification of regulatory feedbacks. Heart rhythm is more random, poorly controlled and vulnerable to uncontrolled arrhythmias.

Even in baseline conditions heart rhythm is influenced by unpredictable environmental factors, artifacts and systemic noise. As opposed to traditional approach, nonlinear dynamics methods such as RQA have a potential to increase the robustness of data interpretation in experimental studies.

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