Temporal Analysis of the Spontaneous Baroreceptor Reflex during Acute and Chronic Shaker Stress in Freely Moving Rats

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Abstract

Radiotelemetred male Wistar conscious rats were exposed to acute and chronic shaker stress and the spontaneous baroreceptor reflex (sBRR) functioning was evaluated using the method of sequences. Baroreflex sensitivity (BRS) is calculated using traditional, local and global approach. Contour coverage area (CCA, area embedding the sequence points in SBP-PI plain) with coordinates of baricentre (mass center of CCA) were proposed to evaluate the range and the set point of sBRR. Acute shaker stress increased vigilance and HR, induced no changes in BRS and decreased the range and the set point of sBRR, which was displaced towards lower PI and higher SBP values. Chronic shaker stress reduced only sBRR range. In the post-stress period of acute and chronic shaker stress shortening of baroreflex sequences and SBP ramps, SBP and PI increments and swings were noted, indicating that the BRR functioning is shifted toward faster BP changes.

1. Introduction

Baroreflex (BRR) presents the major negative feedback regulator of arterial blood pressure (BP). Under basal physiological conditions, an increase of arterial pressure induces a reflex lengthening of the pulse interval (PI), while under the stress increase of arterial BP is accompanied by concomitant shortening of PI. This phenomenon has raised the question as to how the arterial baroreflex is altered by stress. Furthermore, chronic psychological stress is associated with cardiovascular morbidity, arterial BP deregulation and primary hypertension [1]. Therefore elucidating the effects of emotional stress on the functioning of the BRR is of great interest.

Recent development of computer based techniques for the analysis of the spontaneous baroreceptor reflex activity both in time and frequency domain [2] allowed analysis of the BRR without use of vasoactive drugs. Consequently we propose to use novel, computer based techniques, in time domain, for the evaluation of the spontaneous BRR (sBRR) activity in rats exposed to acute and chronic shaker stress.

2. Methods

2.1. Materials and experiment

Animals: male Wistar outbred rats weighting 250-300g were used. Rats were housed individually in controlled environment (12h/12h light/darkness cycle, temperature 21°C ± 2 and humidity 65%± 9) with access to food and tap water *ad libitum*.

Surgery: radiotelemetric probes (TA11-PA C40, Data Science International, Transoma Medical) were implanted in abdominal aorta under combined ketamine and xylazine anesthesia (0.4 ml 10 % ketamine i.p. plus 0.1 ml 2 % xylazine i.p. per animal). Three days before and after surgery rats were treated with gentamicin (25mg/kg i.m.). In addition, at the end of surgery, rats received one injection of metamizol (200mg/kg i.m.) for pain relief and were left to recover fully for 8 days.

Experimental protocol: animals were kept under standard laboratory conditions and experiments started around 10 A.M. every day. Based on previously published protocols [3,4], shaker stress was performed for 3 days. BP of rats was recorded 20 minutes before stress (BASELINE), 10 minutes during exposure to shaking platform at 200 cycles/min (AS - ACUTE STRESS) and 30 min after stress (PAS - POST ACUTE STRESS). Chronic exposure to 5 minutes-long shaking period was performed 18 times per day and BP was recorded during the last exposure, on day 3 (CS-CHRONIC STRES), as well as 30 minutes after exposure to chronic stress (PCS-POST CHRONIC STRESS).

2.2. Cardiovascular signal analysis

The arterial blood pressure signal (BP) was digitized at 1000 Hz and relayed to a PC equipped with Dataquest A.R.T. 4.0 software, DSI for acquisition and analysis of cardiovascular signals. Systolic blood pressure (SBP) and pulse interval (PI) were derived from the arterial BP as maxima in the pulse wave signal and interval between them, respectively. After careful visual examination and removal of artifacts, SBP and PI series were analyzed in the time domain.

The sequence method: Spontaneous baroreflex (sBRR) analysis is technique developed for dynamic study of the arterial baroreflex control of the sinus node [5]. This traditional method evaluates BRR from SBP and PI by detecting spontaneously occurring ramps of increase/decrease of consecutive SBP values followed by unidirectional changes of PI. According to [6,7], to apply the sequence method to SBP and PI time series of rats, the respiration induced variability has to be filtered-out by moving average over 10 cardiac cycles.

SBP series was examined for the chain of at least three consecutive samples that are either increasing or decreasing (i.e. "ramp"). To find a matching sequence in PI series a delay of three, four and five beats was applied, based on the estimated baroreflex time delay from a change in BP (point of baroreflex receptor) to a reflex response in PI (point of sinoatrial node) in rats [7]**.** A chain of *SBP-PI* pairs was considered a *BRR* sequence (BS) if it consisted of minimum three beats. Differences between the successive SBP and PI samples were reduced due to the moving average filter, so no minimal difference value ("threshold") was set. To fully describe the functioning of the *BRR* following parameters were introduced:

- ***N*** –number of sequences/minute;
- N_R –number of *SBP* ramps/minute;
- ***BEI*** – ratio of number of sequences *N* vs. number of *SBP* ramps, N_R [8];
- S_{SBP}, (S_{PI}) – *SBP* (*PI*) swing, a mean difference in [mmHg] or [ms] between the highest and the lowest *SBP* or *PI* value in one sequence
- N_B – mean number of *SBP-PI* pairs per sequence [beats].
- N_{BR} – mean number of SBP values per ramp [beats].
- Δ_{PI} and Δ_{SBP} – absolute *PI* and *SBP* increment in [ms] and [mmHg] respectively – the mean absolute difference between the successive *PI* (*SBP*) values in a sequence;
- ***Contour plots*** - the contour plots embed all of the sequence points in the SBP-PI plane prior to mean removal, or the regions where the number of sequence points exceeds specified limit, to emphases the area where the points are clustered.
- ***CCA - Contour Coverage Area*** is the area covered with all of the sequence points; it is expressed as the percentage of the SBP-PI plotting area for all the experimental protocols. The axis values for plotting area were obtained as minimum and maximum values of SBP and PI in all of the experimental protocols.

Since the density of the sequence points is not uniform, the coordinate of the barycentre of the CCA were calculated to indicate the change in position of the coverage area (PI_B, SBP_B). Contour plot for the baseline protocol for one of the rats is presented in Fig.1a The contour labeled with 1 embeds the areas with more than one sequence points, whereas the contour with label 15 embeds the areas with more than a 15 sequence points. The barycentre position is indicated with the black point

sBRS estimation was done using local (traditional), global and total approach [9]. The local approach is based on estimating the regression slope for each one of BS. The mean was removed from each BS and the slope was estimated from detrended values (x_{PI}, x_{SBP}) using least squares method (LS). BRS based on local approach, $\mathbf{BRS_L}$, is calculated as a mean of the local slopes.

The global approach proposes that all of the x_{PI} and x_{SBP}, values from all of the BSs found are used to estimate the slope of linear regression using LS method resulting in global BRS estimate $\boldsymbol{BRS_G}$.

In contrast to the dispersion diagram of the sequence points in Fig 1a, Fig. 1b reveals the linear relationship between SBP and PI in sequence points after mean removal; the blue broken line has a slope BRS_L and red solid line has a slope BRS_G.

The total approach uses more robust global method based on outlier rejection rule and slope estimation using total least square (TLS) minimization that takes into account errors of both variables, x_{PI} and x_{SBP}. Before TLS slope estimation, outlier segments were excluded from the analysis, based on their influence, f^k, upon the TLS slope. The influence of the k^{th} segment is evaluated as: f^k =(TLS slope when the segment is excluded from analysis)/(TLS slope when all data are used). BSs were considered as outliers if their influence differed from median of influences more than two median absolute deviations (MAD) divided by 0.6745 [9].

After the outlier removal, the TLS slope $\boldsymbol{\alpha}$ was estimated from remaining pairs of values divided by corresponding MAD values to compensate for inherent dependence of TLS on scale changes. Finally, total BRS estimator, $\boldsymbol{BRS_T}$ equals to:

$$BRS_T = \alpha * MAD(x_{PI})/MAD(x_{SBP}) \quad (1)$$

In the Fig 1c sequence points from remained BS are shown, as well as the line with slope that equals to BRS_T.

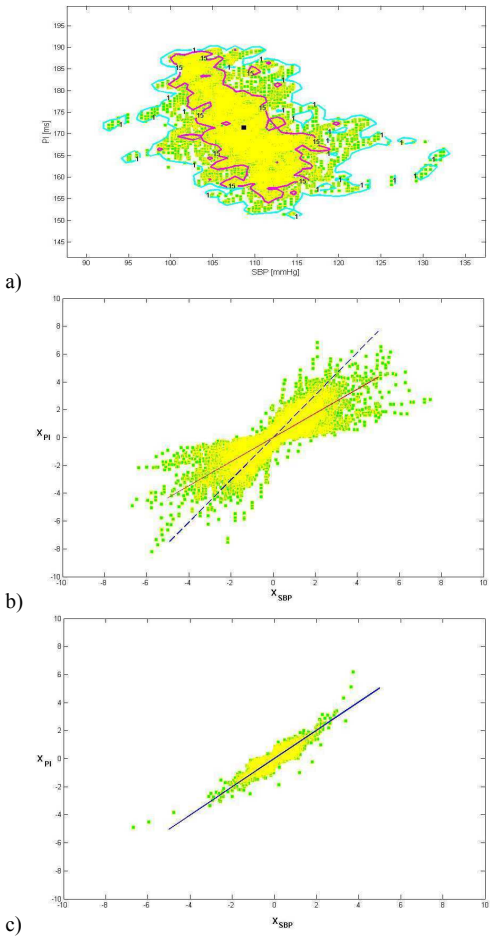

Figure 1. The dispersion diagram of sequence points a) contour plots prior to mean removal with black point indicating baricentre position. b) after mean removal with indication of BRS_L (slope of dotted line) and BRS_G (slope of solid line); c) after outlier removal with line whose slope equals BRS_T

3. Results

Shaker stress produced passive coping behavior and increased vigilance in rats. The cardiovascular response to acute and chronic exposure to shaker stress, followed by post-stress periods, is shown in Fig.2.

Rats exposed to acute shaker stress exhibited increase in HR, without significant changes in SBP, *BEI*, N_R, *N*, N_{BR}, N_B, SBP or PI increments and swings (Table 1). There is a decrease in mean value of BRS, for all three approaches, but with no significance. However, the *CCA* was significantly decreased, while the barycentre was significantly displaced towards lower PI and higher SBP values, depicting reduced sBRR operating range and resetting. In the post stress period *(PAS)*, all the parameters returned to basal value. (Table 1).

Chronic shaker stress evoked the reduction of the *CCA*, without the changes of other parameters, including the baricentre coordinates, suggesting that the sBRR range is reduced without resetting. Parameters evaluated during the post-stress period (PCS) show no difference in comparison to baseline values. (Table 1).

Contour plots for one rat, during the time-course of experiment, are shown in Fig. 3. The contours embed areas with more than 30 sequence points. In this way, the change in sBRR operating range and eventual resetting (change in the barycentre, i.e. set point position) is visible.

4. Discussion and conclusions

The sBRS estimates calculated using local, total and global approach, provide almost the same estimate, without the significant changes in respect to baseline conditions. However, they preserve the same mutual relationship $BRS_G<BRS_T<BRS_L$ as found in humans [9], with the global approach showing the least variability.

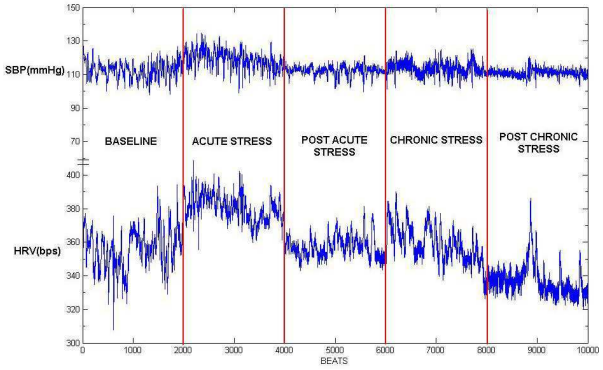

Figure 2. SBP and PI response to acute and chronic shaker stress followed by post stress periods.

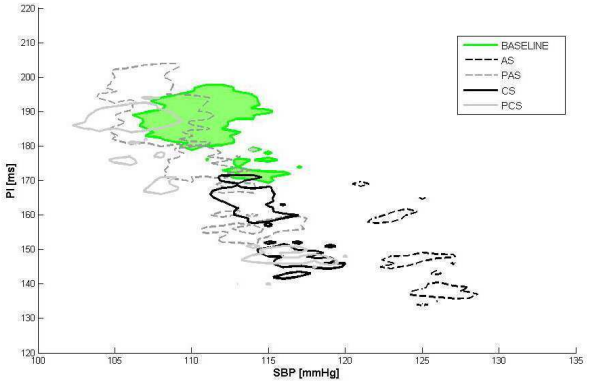

Figure 3. The contour plots of one rat embedding more than 30 sequence points. Filled areas indicates baseline BRR operating range, dashed lines refer to AS (black) and PAS (gray), while solid ones to CS (black) and PCS (gray)

Table 1 Parameters of sequence technique and contour plots

	BASELINE	AS	PAS	CS	PCS
SBP(mmHg)	114.3±3.2	125.5±4.7	114.8±5.2	117.6±1.2	111.0±2.8
HR(bpm)	353.5±7.2	381.5±9.2*	354.7±8.3	367.0±7.1	335.0±9.3
BRS_L	1.26±0.19	1.07±0.19	1.36±0.20	1.08±0.11	1.41±0.11
BRS_G	0.76±0.13	0.61±0.13	0.75±0.13	0.64±0.06	0.88±0.08
BRS_T	0.86±0.17	0.71±0.17	0.88±0.17	0.73±0.07	1.03±0.07
BEI	0.82±0.04	0.83±0.02	0.76±0.02	0.73±0.04	0.71±0.017
N	34.95±1.22	39.07±1.76	37.47±1.59	35.11±2.13	35.62±1.32
N_R	43.16±2.91	47.01±1.76	49.09±2.78	48.42±2.96	50.30±1.56
N_B	6.42±0.21	6.01±0.29	5.67±0.17	5.76±0.26	5.35±0.264
N_{BR}	8.99±0.74	8.41±0.16	7.84±0.25	7.83±0.48	7.13±0.04
Δ_{PI}	0.26±0.03	0.24±0.05	0.24±0.05	0.22±0.03	0.19±0.02
S_{PI}	2.07±0.18	1.81±0.49	1.71±0.36	1.53±0.27	1.36±0.22
Δ_{SBP}	0.33±0.03	0.32± 0.02	0.27±0.03	0.28±0.02	0.21±0.017
S_{SBP}	2.61±0.35	2.34±0.19	1.93±0.25	2.01±0.23	1.35±0.15
CCA	5.05±0.31	2.85±0.48 **	5.51±1.04	2.88±0.33**	5.54±0.35
PI_B	172.5±3.6	157.6±4.3 *	171.6±4.1	166.0±2.7	183.4±5.2
SBP_B	113.5±3.2	125.4±4.8*	114.3±5.1	116.7±1.1	109.9±2.6

Values are represented as MEAN±SEM. Statistical analysis using repeated measures one-way ANOVA followed by a *post hoc* Bonferroni test. Significance level * $p<0.05$, ** $p<0.01$, *** $p<0.005$.

In this paper we suggest, for the first time, the contour plots, contour coverage area and baricentre as new non-invasive measures of the sBRR set point and sBRR operating range, thus expanding the method of sequence.

BRS estimates calculated using local, total and global approach show that shaker stress does not alter the barroreflex sensitivity, neither after acute nor after chronic exposure. Nevertheless, introduction of new parameters such as contour plots, contour coverage area and baricentre, uncover that shaker stress modifies the functioning of sBRR by reducing its operating range and by resetting it towards higher HR and SBP values.

Acknowledgements

This work is supported by research grant OI145062, Ministry of Science, Republic of Serbia.

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