Time-Domain and Spectral Analysis of Heart Rate Variability in Rats Challenged with Hypoxia

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Abstract

Different approaches based on spectral and time-domain analysis of heart rate variability (HRV) were used to evaluate cardiac rhythm regulatory response to hypoxia. Nineteen male Wistar rats with previously implanted subcutaneous ECG electrodes, were maintained unrestrained in originally designed experimental setup, during normoxia and hypobaric hypoxia (-400 mmHg).

RR intervals were extracted from continuous ECG recording. Time- and frequency-domain HRV estimates were computed from time-series of 1024 consecutive RRi. Power spectrum was obtained using both, fast Fourier transformation and autoregressive (AR) modeling. Compared to normoxia, hypoxia resulted in a significant increase of mean RRi, SDNN, rMSSD. AR and FFT analyses provided concordant power spectra across all tested bands. TP, including VLF, HF and predominantly LF, was increased in hypoxia, whereas LF/HF index of the sympatho-vagal balance remained unchanged. Changes in spectral power (TP, VLF, LF, HF) evoked by hypoxia correlated positively with simultaneous changes in SDNN and rMSSD. Interestingly, correlation coefficients derived from AR and FFT did not vary

1. Introduction

Experimental studies on human cardiovascular function are obviously limited for safety reasons. This is why laboratory animals have been used in research where noninvasive methods were being increasingly implemented in cardiac autonomic regulation including heart rate variability analysis (HRV). Measuring stationary time series of RR-intervals (RRi) is prerequisite for reliable HRV assessment with use of parametric (e.g. autoregressive model) and especially non-parametric methods (e.g. fast Fourier transform [FFT] in most studies) in time- and frequency-domain. The key issue limiting widespread HRV studies in rats is inadequate reproducibility of data related to difficulties in maintaining stationary conditions matched with and high behavioral vulnerability to environmental changes. Furthermore, Silva et al. have demonstrated that HRV parameters obtained with use of FFT or AR modeling in rats that underwent surgical vascular intervention are discordant and difficult for physiological interpretation [1]. Intriguing point is whether it is possible to provide experimental conditions of sympathovagal activation in rat in which assumption of stationarity would be at least roughly met. To date, there are no comparative reports on AR and FFT data processing of HRV during controlled hypoxic challenge. In this study we investigated the HRV-response to hypoxia in possibly unrestrained rats thoroughly habituated to the laboratory conditions. We developed an experimental model of controlled mild hypobaric hypoxia to assess temporary steady-state stationary response. Time- and frequency-domain HRV parameters obtained with use of FFT or AR modeling were analyzed simultaneously and were derived from equinumerous RRi-time-series.

2. Materials and methods

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

Nineteen male Wistar rats (350g) were used in this study. Three silver ECG electrodes were implanted subcutaneously and exteriorized. Following 48h recovery the experiments were performed on conscious animals, previously thoroughly habituated to the experimental environment - transparent hypobaric chambers, which provided enough space for unrestrained body movements. The procedure took two hours and consisted of two consecutive phases: normobaric normoxia (1h) and controlled hypobaric hypoxia (1h). During the latter the chamber pressure was reduced by 400 mmHg within initial 10 min. ECG signals were continuously recorded with the use of LabChart 7 Pro (ADInstruments Sydney, Australia) with sampling rate 4 kHz. The tachogram was calculated using automatic R-peak detection of the ECG signal (LabChart 7 Pro, ADInstruments Sydney, Australia; Microsoft Excel 2013, USA). All QRS complexes were thoroughly checked to avoid false positive detections and missed beats.

For each experimental phase, the time series of 1024 consecutive RRi was always derived from the 20 minutes between the 35th and 55th minute of an hour when the experiment took place. This timing was to ensure possibly unrestrained rat behavior. These RRi series have been further used for all analyses shown in this publication.

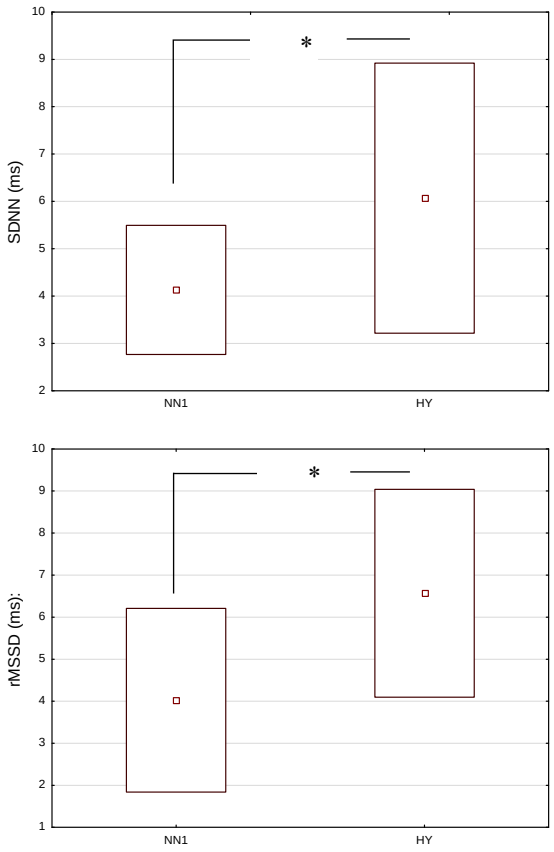

Figure 1. Effect of hypoxia on time-domain HRV parameters. NN1 – normoxia, HY – hypoxia. Data shown as mean ± SD; ** p<0,01; *** p<0,001.

3. Analysis

HRV parameters in time-domain: rMMSD, SDNN and spectral powers: total (TP), VLF (0,02-0,2 Hz), LF (0,2 – 0,75 Hz), HF (0,75 – 2,5 Hz) [2] and LF/HF ratio - used as an indicator of sympathovagal balance - were assessed from both FFT and AR using KubiosPro2.0 software (Kuopio, Finland). AR order was set at 40. All calculations were carried out on entire 1024 RRi segments, without overlapping. Statistical analysis, including linear regression analysis and Student t-test, was performed by the Statistica 10 software (StatSoft, Tulsa, USA).

4. Results

Hypobaric hypoxia significantly affected HR (p<0,05) – with the decrease from 321 to 301 bpm by prolonged RR intervals (from 188±17 to 203±28 ms), that was accompanied by increased overall HRV including all analyzed frequency segments.

In addition we observed a tendency towards higher sympathetic impact upon the heart as shown by increased LF/HF ratio.

Table 1. Mean ± SD of the values of the frequency-domain parameters. Power shown in ms^2 units. NS – not significant; * p<0,05.

	Normoxia	Hypoxia
TP AR	16,8 ± 12,1	45,0 ± 46,5 *
AR VLF	7,0 ± 3,4	17,4 ± 19,1 *
AR LF	4,2 ± 3,4	15,9 ± 24,1 *
AR HF	5,6 ± 7,4	11,6 ± 8,4 *
AR LF/HF	1,0 ± 0,4	1,2 ± 1,4 NS
TP FFT	17,4 ± 12,6	55,5 ± 65,9 *
FFT VLF	7,2 ± 4,2	21,3 ± 27,5 *
FFT LF	4,4 ± 3,7	22,0 ± 35,1 *
FFT HF	5,9 ± 8,1	12,1 ± 9,7 *
FFT LF/HF	1,0 ± 0,5	1,5 ± 2,0 NS

All data was gathered using time-domain parameters such as rMSSD, SDNN (Figure 1) and spectral measures obtained with either FFT or AR method (Table 1). Time-domain HRV measures (rMSSD, SDNN) correlated with spectral power (total power or VLF, LF, and HF) in both circumstances - control conditions (normoxia) and hypoxia (Table 2).

Table 2. Correlations coefficients (r) between time-domain and frequency-domain parameters during normoxia and hypoxia. a: p<0,001; b: p<0,01; c: p<0,05.

		SDNN Normoxia	SDNN Hypoxia	rMSSD Normoxia	rMSSD Hypoxia
TP	AR	0,980[a]	0,934[a]	0,935[a]	0,624[b]
	FFT	0,965[a]	0,908[a]	0,912[a]	0,608[b]
VLF	AR	0,668[b]	0,918[a]	0,330	0,583[b]
	FFT	0,455[c]	0,829[a]	0,118	0,512[c]
LF	AR	0,923[a]	0,808[a]	0,874[a]	0,417
	FFT	0,893[a]	0,846[a]	0,831[a]	0,485[c]
HF	AR	0,876[a]	0,753[a]	0,979[a]	0,929[a]
	FFT	0,855[a]	0,746[a]	0,975[a]	0,920[a]
LF/	AR	-0,146	0,702[a]	-0,426	0,209
HF	*FFT*	-0,036	0,733[a]	-0,327	0,244

Furthermore, hypoxia-induced relative changes in time-domain parameters, seemed to be related to spectral indices (Table 3).

Table 3. Correlation coefficients (r) between hypoxia-dependent changes in time- and frequency-domain HRV parameters. a – p<0,001; b – p<0,01; c – p<0,05.

Hypoxia - Normoxia		SDNN	rMSSD
TP	AR	0,943[a]	0,702[a]
	FFT	0,925[a]	0,698[a]
VLF	AR	0,928[a]	0,622[b]
	FFT	0,843[a]	0,561[c]
LF	AR	0,821[a]	0,495[c]
	FFT	0,866[a]	0,577[b]
HF	AR	0,725[a]	0,936[a]
	FFT	0,727[a]	0,947[a]
LF/HF	AR	0,699[a]	0,246
	FFT	0,765[a]	0,331

Correlation coefficients calculated with use of FFT corresponded with those obtained from AR modeling. Predictably, correlation values from two pairs: SDNN/Total Power and rMSSD/HF - were close to 1 (see [3]) in both conditions (normoxia and hypoxia) as well as in normoxia – hypoxia Δ (delta) as shown in Figure 2.

5. Discussion

In rats, sympathetic efferents are dominant regulatory components which control heart rate [4]. Their HRV resembles its human counterpart, consisting of two principal components: the LF and HF [5,6]. The primary type of adaptive response (reduction of oxygen consumption and dominant component of sympathetic inhibition) was reported in rats challenged with prolonged hypoxia.

In contrast to confounding reports describing spectral indices using AR or FFT processing [1,9-12] we hereby demonstrate data which indicates comparability and compatibility of both methods.

Rat-handling approach established in our laboratory ensured unrestrained and thus almost stationary conditions were provided even during hypoxic challenge. Previously published results, showing that AR and FFT analyses were inconsistent with each other, seemed to have been obtained in evidently unstable physiological conditions. The RRi-time-series were acquired during postural tilting tests [9,10], post-surgical recovery [1], dynamic exercise [11] and acute restrained stress [12]. In majority of those reports the FFT analysis was based on equidistant tachograms consisting of different RRi number. Such approach might have resulted in variable spectral power outputs and misleading interpretations.

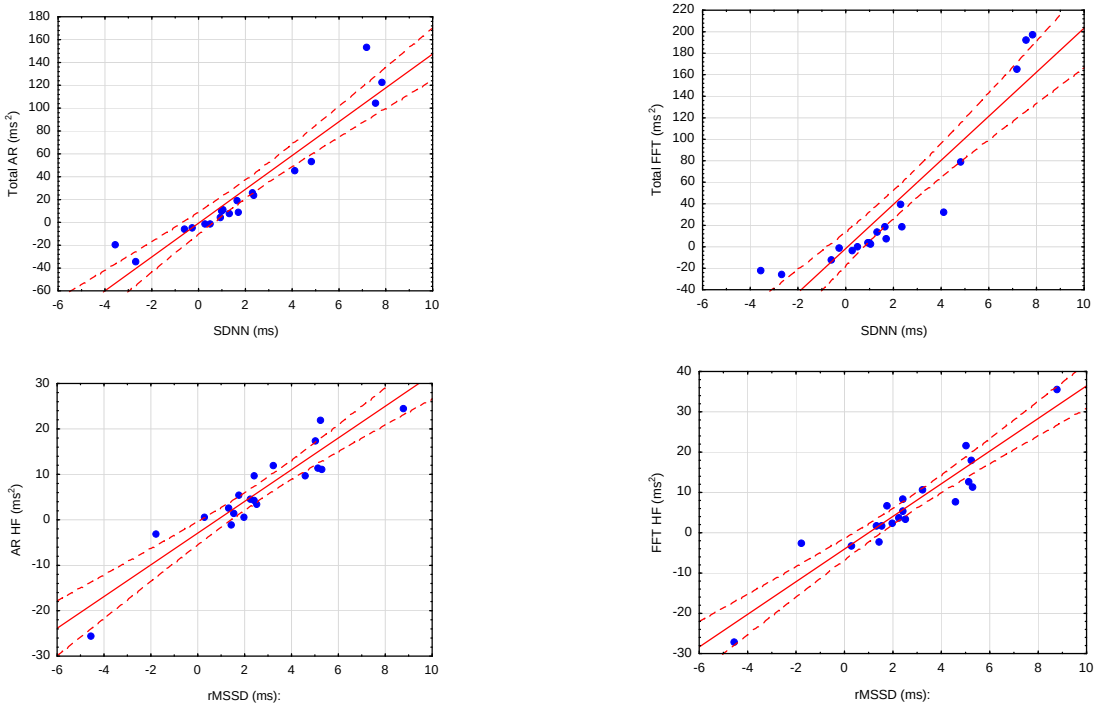

Figure 2. Correlations between hypoxia-dependent changes in time- and frequency-domain HRV parameters obtained using both AR and FFT methods.

Instead to compare data segments of identical lengths we employed time-series composed of the same RRi number (1024 RRi). Moreover our long-standing experience in rat studies permitted us to use a relatively high AR order (40).

Our data shows the following. A decrease in HR, generally considered to result from a shift of sympathovagal balance towards vagus, was associated with a modest increase in LF/HF which on the contrary suggests sympathetic gain. Possible explanation is that in case of simultaneous activation of sympathetic and vagal efferents, which our HRV data suggest, the physiological effect of vagus is dominant. Low baseline vagal activity that was previously reported in rats [7,8] might need only a small increase to cause vagal functional domination at a primary sinus pacemaker node.

Tight links seem to exist between time- and frequency-domain HRV measures, as suggested by high correlation coefficients between rMSSD and HF- spectral power or SDNN and TP (Table 3, Figure 2), indicating consistency of obtained data.

6. Conclusion

We found FFT and AR methods of spectral HRV analysis in rats in both baseline, steady-state conditions and during controlled hypoxia - concordant and reproducible. Stationary conditions were successfully provided in time long enough for sufficient RRi-time-series recording despite hypoxia-driven destabilization of cardiovascular homeostasis.

Acknowledgments

The study was supported by National Science Center (DEC-2011/01/N/NZ4/01132).

References

[1] Silva GJJ, Ushizima MR, Lessa PS, Cardoso L, Drager LF, Atala MM. Critical analysis of autoregressive and fast Fourier transform markers of cardiovascular variability in rats and humans. Braz J Med Biol Res 2009; 42:386–96

[2] Carnevali L, Trombini M, Porta A, Montano N, de Boer SF, Sgoifo A. Vagal withdrawal and susceptibility to cardiac arrhythmias in rats with high trait aggressiveness. PLoS One 2013; 8(7):e68316

[3] Sztajzel J. Heart rate variability: a noninvasive electro-cardiographic method to measure the autonomic nervous system. Swiss Med Wkly 2004;4;134:514–22

[4] Mansier P, Clairambault J, Charlotte N, Médigue C, Vermeiren C, LePape G, et al. Linear and non-linear analyses of heart rate variability: a mini review. Cardiovasc Res 1996;31:371–9

[5] Altimaras J. Understanding autonomic sympathovagal balance from short-term heart rate variations. Are we analyzing noise? Comp Biochem Physiol A Mol Integr Physiol 1999;124:447–60.

[6] Japundzic N, Grichois M-L, Zitoun P, Laude D, Elghozi J L. Spectral analysis of blood pressure and heart rate in conscious rats: effects of autonomic blockers. J Auton Nerv Syst 1990;30(2):91–100.

[7] Hayward LF, Johnson a K, Felder RB. Arterial chemoreflex in conscious normotensive and hypertensive adult rats. Am J Physiol 1999;276(4 Pt 2):H1215–22.

[8] Przybylski J, Trzebski A, Przybyszewski A. Circulatory responses to acute hypoxia in spontaneously hypertensive and normotensive rats. Acta Physiol Pol 1980;31:463–8

[9] Badilini F, Maison-Blanche P, Coumel P. Heart rate variability in passive tilt test: comparative evaluation of autoregressive and FFT spectral analyses. Pacing Clin Electrophysiol 1998;21:1122–32.

[10] Pichon A, Roulaud M, Antoine-Jonville S, de Bisschop C, Denjean A. Spectral analysis of heart rate variability: interchangeability between autoregressive analysis and fast Fourier transform. J Electrocardiol 2006;39:31–7.

[11] Mendonca GV, Fernhall B, Heffernan KS, Pereira FD. Spectral methods of heart rate variability analysis during dynamic exercise. Clin Auton Res 2009;19:237–45.

[12] Ramaekers D, Beckers F, Demeulemeester H, Aubert AE. Cardiovascular autonomic function in conscious rats: a novel approach to facilitate stationary conditions. Ann Noninvasive Electrocardiol 2002;7:307-18.

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