Power Spectrum Analysis of Heart-Rate Variability in the Young Zucker Rat

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Abstract

Cardiac autonomic control was studied in a group of five young hyperinsulinaemic, insulin resistant, Zucker fatty rats (ZFR) compared with a control group of five young Zucker lean rats (ZLR). Power spectrum analysis (PSA) of heart rate variability (HRV) was performed with autoregressive (AR) models. All rats were anaesthetised with sodium pentobarbital. Mean insulinaemia and glycaemia levels were 185±27 μU/ml and 159±38 mg/dl, respectively, in the ZFR, and reduced to 13±13 μU/ml ($p<0.05$) and 101±15 mg/dl ($p<0.05$), respectively, in the ZLR. No significant difference in basal blood pressure was found between the two groups. Analysis of power spectrum density, normalized by the total area, showed a marked reduction ($p<0.05$) in the peak amplitude of the high frequency (HF) component in the ZFR group. No significant difference was observed in the peak amplitude of low frequency (LF) component between the two strains. LF/HF ratio was higher ($p<0.05$) in the ZFR group. These results indicate that in the young ZFR, hyperinulinemia associates with altered sympathovagal balance in the autonomic cardiovascular control, before the onset of significant changes in both sympathetic nervous activity and blood pressure.

1. Introduction

Several studies, including our own [5-8, 13], have reported an association between hyperinsulinaemia, insulin resistance, arterial hypertension and cardiovascular diseases. The notion that hypertension is a metabolic as well as a vascular disease has opened a new paradigm for the treatment of this disorder, thus advocating for further studies that may take advantage from the assessment of adequate animal models. In a previous study [12], we investigated the homozygote, Zucker fatty rat (ZFR), as a possible, suitable experimental model. Minimal model analysis of intravenous glucose tolerance test data showed a significant insulin sensitivity reduction in a group of young (7-wk-old) ZFRs, compared with a control group of heterozygote, Zucker lean rats (ZLR). Analysis of mean arterial pressure (MAP) and heart rate (HR) response to electrical stimulation of sciatic nerve showed an enhanced somathosympathetic reactivity in the ZFR group with no sign of hypertension [12].

In the present study, power spectral analysis (PSA) of heart-rate variability (HRV) was performed in a group of young ZFRs, compared with an age-matched group of ZLRs, to provide new information about the cardiac autonomic control and the balance between sympathetic and parasympathetic components in this model of type 2 diabetes.

2. Methods

Animals and data acquisition. Experiments were performed in five young (8.2±1.6 weeks) homozygote (fa/fa; ZFR) Zucker rats, and five age-matched young (8.8±1.9 weeks) heterozygote (fa/-; ZLR) Zucker rats, obtained from the Charles River Breeding Farms, NY. They were housed in controlled conditions of temperature (21±1 °C), humidity (60±10%) and lighting (8-20h) and received standard raw chow containing 0.3% sodium, with tap water and libitum. All rats were anaesthetised with sodium pentobarbital (40 mg/kg body wt, i.p.). The experiments were performed in accordance with the Italian national guidelines on animal experimentation (Decreto Legislativo 27/1/1992, no. 116, Attuazione della Direttiva no. 86/609/CEE in materia di protezione degli animali utilizzati a fini sperimentali o ad altri fini scientifici). This study was approved by the Ethical Committee of the University of Genoa and by the Italian Ministry of Health. Rectal temperature was controlled and maintained at 37.5±0.5 °C by a heating pad.

The trachea and the right femoral artery and vein were cannulated. The venous cannula was used for drugs injection. Two basal blood samples (200 μl) were taken from the arterial catheter to measure plasma insulin and glucose concentrations. The arterial cannula, connected to a pressure transducer (Spectramed Statham P23XL, Viggo-Spectramed, Oxnard, California, USA) provided a recording of arterial pressure (AP) through a Grass

preamplifier, model 7P14A (Grass Instruments, Quincy, Massachusetts, USA). HR was monitored using a Grass tachograph (model 7P4), triggered by lead II of the electrocardiogram (ECG). AP, ECG and HR were digitized by an A/D converter (CED Power1401, Cambridge Electronic Design, Cambridge, UK), stored on a PC and analysed by laboratory software (Spike2, CED).

Data processing. From the ECG signal, QRS complexes were detected via a classical derivative-threshold algorithm (Spike 2 software). An array containing the absolute values of the R-R intervals was saved. For each R-R sequence, ectopic and missing beats were automatically detected and refilled using methods reported by others [9]. From the R-R series, about 15 epochs (steady traces) of 300 samples, overlapping by a half, were extracted. This window length was selected as the best compromise between the need for a large time series, in order to achieve a greater accuracy in the computation, and the need of a stationary series, easier to obtain from short-time periods. Each epoch underwent spectral analysis by means of autoregressive models of order *m* (see section 2.3). To reduce variability due to different orders of the AR model, we first estimated the optimum order *m* that fulfilled the Akaike information criterion (section 2.3) for each epoch of each animal. The mean *m,* averaged over a group, was used as the optimal value for that group. All the spectra relative to the same animal were averaged to reduce the amount of noise.

Spectral analysis. Epoch by epoch, the n^{th} R-R value was considered as the output, *y(n)*, of an AR model of order *m*, driven by a white noise, *w(n)*, with zero mean and variance λ^2:

$$y(n) = \sum_{k=1}^{m} a(k) \cdot y(n-k) + w(n) \qquad (1)$$

This linear prediction equation is characterized by *m* unknown parameters, a(k). Within each epoch, the series was assumed to be stationary. The *a(k)* parameters and λ^2 were, then, estimated with the Yule-Walker method [10].

The order of the model was chosen as the one that minimises the Akaike information criterion (AIC) figure of merit [1]:

$$AIC(m) = N \cdot \ln(\hat{\lambda}^2) + 2m \qquad (2)$$

where *N* is the number of data samples, and $\hat{\lambda}^2$ is the estimated white noise variance. Power spectrum density, *PSD(f)*, as a function of frequency, *f*, was computed by the following relation [6, 7]:

$$PSD(f) = \lambda^2 \Big/ \left| 1 + \sum_{k=1}^{m} a(i) \cdot \exp(-j2\pi fk) \right|^2 \qquad (3)$$

In equation 3, the sampling period is unitary and the measure units of *PSD* are sec^2/Hz. Normalized units (*nu*), for easier comparison of spectra, were obtained by dividing individual spectra by the total area.

Statistical analysis. Student's t-test was applied for statistical analysis of differences between results from the ZFR and ZLR groups. Variations were considered statistically significant when $p<0.05$.

3. Results

Mean values of HR, diastolic (*DBP*) and systolic (*SBP*) blood pressure, reported in Table 1, showed no significant differences between ZFR and ZLR groups. Mean insulinaemia and glycaemia basal levels (Table 1) were significantly higher ($p<0.05$) in the ZFR. The weight of the ZFR group (275±56 g) was higher, but not significantly, than that of the ZLR group (234±61 g).

Optimal order, *m*, of AR models for power spectrum analysis was found to be 15 for the ZFR group and 17 for the ZLR group.

Typical examples of normalized *PSD* in a ZFR and a ZLR are shown in Figures 1 and 2. High-frequency (HF) and low-frequency (LF) components are evident in the ZLR. On average, the ZFR group showed a significant reduction in the mean peak amplitude of the high frequency (HF) component compared with the ZLR group (Table 2 and Figure 3). Indeed, the HF component was negligible or absent in the ZFR. By contrast, mean peak amplitude of the LF component showed no significant difference between the two strains. The LF/HF ratio was significantly higher ($p<0.05$) in the ZFR group (Table 3).

Table 1. Haemodynamic and metabolic basal data.

	ZFR	ZLR
HR (bpm)	309±31	310±18
DBP (mmHg)	89±7	90±9
SBP (mmHg)	128±3	127±9
I (μU/ml)	185±27	13±13*
G (mg/dl)	159±38	101±15*

Values are means ±SE. *HR*, heart rate; *DBP*, diastolic blood pressure; *SBP*, systolic blood pressure; *I*, insulinemia; *G,* glycemia; ***** : $p<0.05$, Student's t-test.

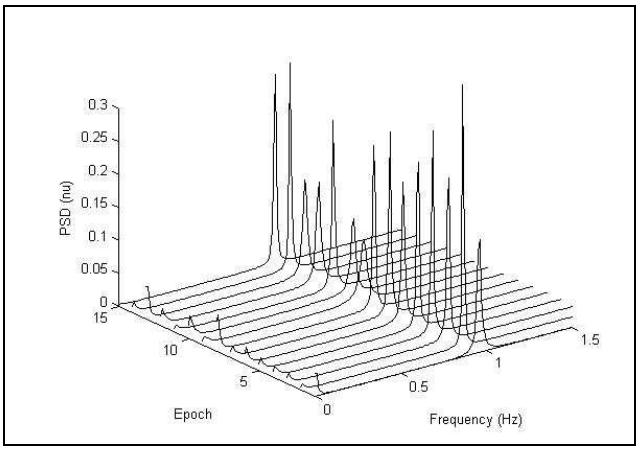

Figure 1. Typical example of *PSD* as a function of frequency, for different epochs, in a ZFR.

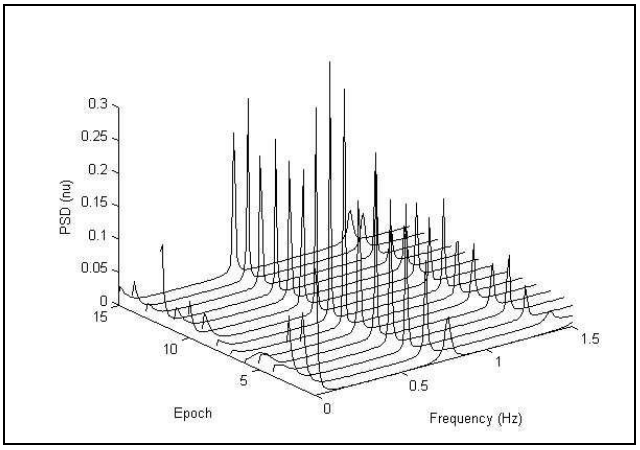

Figure 2. Typical example of *PSD* as a function of frequency, for different epochs, in a ZLR.

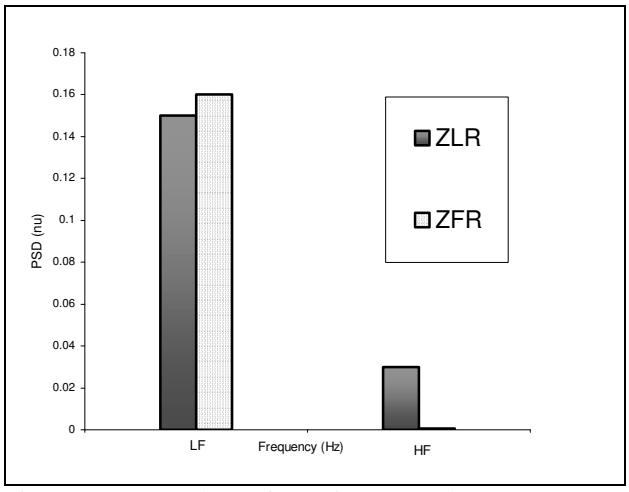

Figure 3. Mean values of *PSD* in ZFR and ZLR groups.

Table 2. Comparison between spectral components in the ZFR and ZLR groups.

	ZFR	ZLR
LF CF (Hz)	1.06 ±0.19	0.76±0.20*
LF A	0.16±0.08	0.15±0.12
HF CF (Hz)	1.40±0.12	1.34±0.04
HF A	$(7.00\pm6.75)10^{-4}$	0.03±0.03*
LF/HF	407±270	101±221*

Values are means ± standard deviation. LF CF, central frequency (CF) of low frequency (LF) components; LF A: peak amplitude (A) of LF components; HF CF, central frequency (CF) of high frequency components (HF); HF A: peak amplitude (A) of HF components; LF/HF, ratio of LF to HF; notation * indicates $p<0.05$ of Student's t-test.

4. Discussion and conclusions

This study provides new information about the autonomic system status and the balance between its sympathetic and parasympathetic components associated with alterations of glucose metabolism. Our HRV analysis with AR models identified two major components in the power spectra density of young ZFR and ZLR groups. The first component, at low frequency (LF), was centred at 1.06 Hz in the ZLR and at 0.76 Hz in the ZLR. The second component, at high frequency (HF), was centred at 1.40 Hz in the ZFR and 1.34 in the ZLR. A relatively small component at very low frequency (VLF), close to 0 Hz, was also identified. However, this component was disregarded in our analysis since its meaning is uncertain [11].

The power density of LF component is generally considered to represent mainly sympathetic activity and, to a much lesser extent, parasympathetic activity. The HF component is closely associated with parasympathetic activity, such that the ratio (LF/HF) between the PSD components at LF and HF indicates the extent of temporal predominance between the parasympathetic and sympathetic pathways [2-4, 11, 14]. Our results showed a significant reduction of HF mean normalized PSD amplitude in the ZFR group compared with the ZLR group, whereas no significant differences were found between the two groups in the LF components. Reduction of HF amplitude suggests a substantial decrease of parasympathetic activity in the ZFR, associated with hyperinsulinaemia and hyperglycaemia (Table 1). As a whole, a relative predominance of the sympathetic activity in the autonomic control of the heart occurs in the

ZFR, as a consequence of the significant increase of LF/HF ratio in this strain, before the onset of significant changes in both sympathetic nervous activity and blood pressure. These results are in agreement with previous reports in humans [3] and evoke for further studies in the homozygote Zucker rat as a suitable experimental model to investigate the link between metabolic alterations and cardiovascular diseases.

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