Characteristics of Autonomic Nervous Function in Zucker-Fatty Rats: Investigation by Power Spectral Analysis of Heart Rate Variability

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Abstract: We investigated the characteristics of autonomic nervous function in Zucker-fatty and Zucker-lean rats. For this purpose, a long-term electrocardiogram (ECG) was recorded from conscious and unrestrained rats using a telemetry system, and the autonomic nervous function was investigated by power spectral analysis of heart rate variability (HRV). Although heart rate (HR) in Zucker-fatty rats was lower than that in Zucker-lean rats throughout 24 h, apparent diurnal variation in HR was observed in both strains and HR during the dark period was significantly higher than that in light period. Diurnal variation in locomotor activity (LA) in Zucker-fatty rats was also observed, but LA was lower than that in Zucker lean rats, especially during the dark period. There were no significant differences, however, in high-frequency (HF) power, low-frequency (LF) power, and the LF/HF ratio between Zucker-fatty and Zucker-lean rats. The circadian rhythm of these parameters was mostly preserved in both strains of rats. Moreover, the effect of autonomic blockades on HRV was nearly the same in Zucker-fatty and Zucker-lean rats. These results suggest that the autonomic nervous function of insulin-resistant Zucker-fatty rats remain normal, from the aspect of power spectral analysis of HRV.

Key words: autonomic nervous function, circadian rhythm, heart rate variability, radiotelemetry, Zucker fatty rats

Introduction

Changes in autonomic nervous activity have been hypothesized to be one of underlying causes of many cardiovascular diseases and diabetic peripheral neuropathy [4, 8, 9, 14]. Moreover, the necessity of the study of the circadian variation of the autonomic nervous function has greatly increased. Previously, we have investigated the characteristics of diurnal variation of autonomic nervous activity using power spectral analysis of heart rate variability (HRV) in some animal species [1, 12, 16]. Because power spectral analysis of HRV
has also been introduced to detect autonomic dysfunction in diabetic patients, this analysis can serve as a clinical test of autonomic function [23, 24]. Insulin resistance is considered to be a risk factor in diabetes and other diseases such as hyperlipidemia, hypertension and arteriosclerosis. However, to our knowledge, effects of insulin resistance on autonomic nervous system function, including diurnal variations, have not been studied.

The Zucker-fatty rat showing hyperphagia due to mutation of the leptin receptor gene is a well-established model of insulin resistance [5, 21]. Plasma glucose level and blood pressure in Zucker-fatty rats are relatively similar to those in Zucker-lean rats [14, 20]. These characteristics show that the Zucker-fatty rat may be suitable for research on effects of insulin resistance on autonomic nervous function. Therefore, we conducted this study to clarify autonomic nervous function in Zucker-fatty and Zucker-lean rats using power spectral analysis of HRV.

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### Materials and Methods

This study was performed following ethical guidelines, which meet the generally accepted international criteria, for animal care, handling, and euthanasia in our institution.

#### Animals

Five male Zucker-fatty (*fafa*) rats and 5 Zucker-lean (*fa/+*) rats (9–10 weeks of age) were purchased from Charles River Japan Inc. (Yokohama, Japan). They were housed in individual cages within a light-proof chamber (MIR-553 or MIR-252, Sanyo, Tokyo, Japan). In the chamber, a light-dark cycle (LD 12:12, lights on at 08:00 h) was maintained under constant temperature (24 ± 1°C). Standard diet (MF, Oriental Yeast, Co., Ltd., Tokyo, Japan) and water were supplied *ad libitum*.

#### Surgical procedure

Animals were anesthetized with an intraperitoneal injection of sodium pentobarbital (50 mg/kg), and a telemetric ECG radio-transmitter (TA10EA-F20, Data Sciences International, St. Paul, MN, USA) was implanted in a dorsal subcutaneous pouch. The paired wire electrodes for the precordial bipolar lead (apex-base lead) were placed at the cervical subcutaneous region over the trapezius, and the skin was closed by suture. After the recovery period (7–10 days), the animals were used in each experiment.

#### Data recording

Each rat cage was placed on a signal receiving board (RLA1020 or CTR86, Data Sciences International, St. Paul, MN, USA) in the chamber. The ECG signal was continuously recorded by an ECG processor analyzing system (Softron, Tokyo, Japan). The recorded signals were sampled at 1 msec intervals and stored on a Magneto Optical (MO) disk using the ECG processor. The signal of locomotor activity (LA) from the telemetric transmitter was sampled every 5 min by a Data Quest analyzing system (Data Sciences International), and the average value of 1-h length segments was calculated.

#### Data analysis

Power spectral analysis of HRV was made on an ECG processor analyzing system (Softron, Tokyo, Japan), using ECG data recorded on a MO disk. Twenty-four-hour data was divided into 1-h lengths, and 30 min of stable data were extracted from each period. The computer program first detected R waves and calculated the RR interval tachogram as the raw HR variability in sequence order. The coefficient of variation (CV) of all normal RR intervals was also calculated. From this tachogram, data sets of 512 points were re-sampled at 80 msec. The Hanning window and the fast Fourier transform were applied to each set of data to obtain the power spectrum of the fluctuation. Squared magnitudes and the products of the computed discrete Fourier transforms were averaged to obtain spectral estimates. We used frequency bands of low frequency (LF) and high frequency (HF) according to a previous study [15]: LF 0.04–1.0 Hz, HF 1.0–3.0 Hz. The ratio of LF and HF power (LF/HF) was also calculated. The average values for light, dark, and for the entire day period and diurnal variability (mean value for every hour) were also determined for the above-mentioned parameters.

#### Effects of autonomic nervous blockade

To block autonomic nervous activity, atropine sulfate (2 mg/kg, Sigma-aldrich Co., St Louis, Missouri, USA), propranolol hydrochloride (4 mg/kg, Sigma-aldrich Co.) and a simultaneous dose of both drugs were injected intraperitoneally to the animals at 24 h
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intervals. Saline was injected as a control. The ECG was recorded with the ECG processor analyzing system. The ECG data for 10 min during the period 20 to 30 min after the administration was analyzed to generate spectral power parameters.

Statistical analysis

Comparison of data from spectral power parameters and LA between Zucker fatty and lean rats was analyzed by the unpaired Student’s t-test for all 24 h. To examine the differences between dark and light periods, the average value of each parameter in the respective periods was compared with the paired Student’s t-test. In the experiments using autonomic blockers, the percentage changes for the drugs against saline treatment was compared between Zucker fatty and lean rats with unpaired Student’s t-test. A statistically significant difference was defined as P<0.05.

Results

Clinical findings

The changes in body weights of Zucker-fatty and Zucker-lean rats are shown in Fig. 1. Zucker-fatty rats were obviously obese in appearance and heavier in weight than Zucker-lean rats.

Power spectral analysis and LA

The typical diurnal rhythm, obtained from the average of 2 days, of spectral power parameters and LA in Zucker-fatty and Zucker-lean rats (13–14 wk of age) is shown in Fig. 2 and the results of statistical analysis are shown in Table 1. Apparent diurnal variation in HR was observed in Zucker-fatty rats as well as Zucker-lean rats. In both strains, HR in the dark period was significantly higher than that in the light period (Figs. 2a and 3a). Meanwhile, HR in Zucker-fatty rats was lower than that in Zucker-lean rats throughout 24 h, especially from midnight to noon. Furthermore the difference in HR between the dark and light period in Zucker-fatty rats was somewhat smaller than that in Zucker-lean rats (35 and 54 bpm, respectively). The CV value in Zucker-fatty rats was significantly lower than that in Zucker-lean rats (Fig. 2b, Table 1). Moreover, a significant difference in CV value between the dark and light period was found in Zucker-lean rats (Fig. 3b). No distinct differences in diurnal patterns of LF, HF and LF/HF were observed between Zucker-fatty and lean rats (Fig. 2c, d, e). LF and HF powers during the light period were significantly higher than those during the dark period in Zucker-lean rats (Fig. 3c, d).

Nocturnal patterns of LA were observed in both Zucker-fatty and Zucker-lean rats (Fig. 2f). Although there were significant differences of LA between the dark and light period in both strains, LA in Zucker-fatty rats was significantly lower than that in Zucker-lean rats (Fig. 3f, Table 1).

Table 1. Results of statistical analysis of the spectral power parameters and LA in Zucker-fatty and Zucker-lean rats

<table>
<thead>
<tr>
<th>Item</th>
<th>Zucker-fatty vs Zucker-lean</th>
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<tbody>
<tr>
<td>HR</td>
<td>**</td>
</tr>
<tr>
<td>CV</td>
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<td>LF</td>
<td>NS</td>
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<tr>
<td>HF</td>
<td>NS</td>
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<tr>
<td>LF/HF</td>
<td>NS</td>
</tr>
<tr>
<td>LA</td>
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*: p<0.05, **: p<0.01, NS: no significant differences.
Effects of autonomic nervous blockade on Spectral Power Parameters

The basal value of HR and spectral power parameters from saline treated animals are shown in Table 2. These values were almost the same as the average of the 24 h value as shown in Fig. 3. Atropine increased HR and decreased LF and HF powers. Propranolol decreased HR and increased HF power in both Zucker-fatty and Zucker-lean rats (Fig. 4). Simultaneous dosage of both blockades decreased all the parameters examined in both Zucker-fatty and Zucker-lean rats. No significant differences in these changes were observed between Zucker-fatty and Zucker-lean rats.

Discussion

We investigated the diurnal variation of autonomic nervous functions in Zucker-fatty and Zucker-lean rats using power spectral analysis of HRV. HR clearly showed a nocturnal pattern in both strains of rats. The diurnal patterns of HF, LF, and LF/HF in Zucker-fatty rats were almost the same as those in Zucker-lean rats. There was no difference in the changes of spectral power parameters between Zucker-fatty and Zucker-lean rats after the administration of autonomic blockers. These results suggest that the autonomic nervous function of Zucker-fatty rats mostly remains normal from...
Table 2. Base value of HR and spectral power parameters in Zucker-fatty and Zucker-lean rats treated with intraperitoneal injection of saline

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>HR (bpm)</th>
<th>LF (msec²)</th>
<th>HF (msec²)</th>
<th>LF/HF</th>
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<tbody>
<tr>
<td>Zucker-fatty rats</td>
<td>5</td>
<td>374 ± 40</td>
<td>2.746 ± 1.231</td>
<td>0.982 ± 0.882</td>
<td>3.840 ± 2.491</td>
</tr>
<tr>
<td>Zucker-lean rats</td>
<td>5</td>
<td>410 ± 30</td>
<td>4.547 ± 1.622</td>
<td>0.805 ± 0.445</td>
<td>6.544 ± 2.854</td>
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</table>

Values represent mean ± SD.

Fig. 3. Spectral power parameters and LA of averaged 24 h, dark and light period in Zucker-fatty and Zucker-lean rats. Each column represents the mean value for 4 (fatty) or 5 (lean) rats with SD bar. a) HR, b) CV, c) LF, d) HF, e) LF/HF, f) LA. Light: 8:00–20:00, Dark: 20:00–8:00, *: p<0.05, **: p<0.01, dark period vs light period.
the aspect of power spectral analysis of HRV.

We have previously reported that the sympathetic nervous activity has nocturnal rhythms in Wistar rats [12]. Both Zucker-fatty and Zucker-lean rats did not show clear nocturnal rhythm in the sympathetic nervous activity, because LF/HF in the light phase was slightly higher than that in the dark phase. However, parasympathetic nervous activity became predominant in the light phase as shown by HF changes. A decrease in HF and LF powers are often seen in diabetic patients with autonomic nerve dysfunction or animals such as BB rats, especially accompanying severe neuropathy [22, 23, 28]. In streptozotocin induced diabetic rats, a decrease in HF power was also observed [7, 10, 18]. Although the diurnal pattern of autonomic nervous activity was somewhat altered in both species of rats, HF power was almost equal in Zucker-fatty rats compared with Zucker-lean rats. Therefore, it seems that the autonomic nervous function in Zucker-fatty rats was almost preserved as normal.

Diabetes, i.e. insulin resistance, is considered to be a factor relating to cardiac failure or sudden death [6]. Furthermore, one of the causes of cardiac failure and sudden death is thought to be sympathetic hyperactivity and parasympathetic hypoactivity, scince the plasma level of insulin, but not plasma glucose level, was correlated with QTc dispersion, which was closely related to sudden death in human diabetes [27]. Although there is no information about the QT interval in Zucker-fatty rats, we clearly showed that Zucker-fatty rats with insulin resistance have no characteristics such as sympathetic overactivity. These results suggest that the autonomic neuropathy might develop in association with insulin resistance and other risk factors. Further
examination will be needed to clarify the relationship between insulin resistance and autonomic nervous function, however, this animal model may provide useful information on risk factors of cardiovascular disease under the insulin resistance condition.

A significant reduction in HR was observed in Zucker-fatty rats compared with Zucker-lean rats. These results agree with earlier reports using telemetric monitoring in Zucker-fatty rats [2, 3, 19]. This tendency was also observed in WBN/Kob diabetic rats and streptozotocin-diabetic rats [11, 13]. Although autonomic neuropathy is a well-documented complication in diabetic patients, Van Buren et al. [25] reported that the reduced HR in diabetic rats is not derived from autonomic nervous dysfunction. HR in Zucker-fatty rats was lower than that in Zucker-lean rats after simultaneous administration of two autonomic blockers, atropine and propranolol, suggesting that the lowered HR in Zucker-fatty rats may be dependent on a lower level of intrinsic HR rather than altered autonomic nervous function.

Sympathetic overactivity during sleep duration is often seen in diabetic patients with insulin resistance [14]. Insulin can enhance the sympathetic activity mediated by the central nervous system [17]. The plasma level of insulin in Zucker-fatty rats at 12 weeks of age was about 10 times as much as in Zucker-lean rats of the same age [26]. However, Zucker-fatty rats with hyperinsulinemia showed no sympathetic overactivity during the resting (light) period. Therefore, insulin resistance in the central nervous system appeared to be the well balanced with hyperinsulinemia in Zucker-fatty rats at the age examined in this study. This remains to be proved through further experiments observing the autonomic changes during the process of development of insulin resistance, or on the functional changes of the autonomic nervous system when drugs are used to improve insulin resistance.

In conclusion, the autonomic nervous function of Zucker-fatty rats remains almost normal from the aspect of power spectral analysis of HRV. This result suggests that the development of autonomic neuropathy in diabetes may be associated with insulin resistance and other risk factors.

References

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