Power Spectral Analysis of Heart Rate Variability for Assessment of Diurnal Variation of Autonomic Nervous Activity in Guinea Pigs

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Abstract: We established characteristics of power spectral analysis of heart rate variability, and assessed the diurnal variations of autonomic nervous function in guinea pigs. For this purpose, an electrocardiogram (ECG) was recorded for 24 hr from conscious and unrestrained guinea pigs using a telemetry system. There were two major spectral components, at low frequency (LF) and high frequency (HF) bands, in the power spectrum of HR variability. On the basis of these data, we defined two frequency bands of interest: LF (0.07–0.7 Hz) and HF (0.7–3.0 Hz). The power of LF was higher than that of HF in the normal guinea pigs. Atropine significantly reduced power at HF. Propranolol also significantly reduced power at LF. Furthermore, the decrease in the parasympathetic mechanism produced by atropine was reflected in a slight increase in the LF/HF ratio. The LF/HF ratio appeared to follow the reductions of sympathetic activity produced by propranolol. Autonomic blockade studies indicated that the HF component reflected parasympathetic activity and the LF/HF ratio seemed to be a convenient index of autonomic balance. Nocturnal patterns, in which the values of heart rate in the dark phase (20:00–06:00) were higher than those in the light phase (06:00–20:00), were observed. However, the HF, LF and the LF/HF ratio showed no daily pattern. These results suggest that the autonomic nervous function in guinea pigs has no clear circadian rhythmicity. Therefore, this information may be useful for future studies concerning the autonomic nervous function in this species.

Key words: autonomic nervous system, circadian rhythm, heart rate variability, power spectral analysis, radiotelemetry

Introduction

Many physiological functions in rats and mice have periodic fluctuations such as the well-known circadian rhythms [3, 11]. Although some investigators have observed daily variations in cortisol and melatonin levels, respiratory measures, and blood histamine response in guinea pigs [4, 15–17], there is considerable controversy in the literature regarding the 24 hr distribution of sleep and waking and the presence of a circadian rhythm [18–20].

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rest-activity or sleep-wake rhythm in guinea pigs [2, 7, 8, 13, 18]. Moreover, we have shown that characteristics of rhythmicity of heart rate, body temperature, and locomotor activity in guinea pigs are different from other rodents such as rats and mice [1]. The autonomic nervous control of the cardiovascular system has been generally thought to have a diurnal variation in its activity. Recently, we clearly demonstrated the diurnal variation of autonomic nervous activity in rats and miniature pigs using power spectral analysis of heart rate (HR) variability [5, 9]. However, some guinea pigs showed circadian rhythms in heart rate, but others did not show significant daily patterns. We subsequently hypothesized that guinea pigs may have individual characteristics of rhythmicity in autonomic nervous activity. Moreover, it is conceivable that the power spectral analysis of heart rate variability might provide specific information concerning the diurnal variation of autonomic nervous activity in guinea pigs. Therefore, the purposes of this study were to establish the characteristics of power spectral analysis of HR variability and to assess the diurnal variation of autonomic nervous activity in guinea pigs.

Materials and Methods

Animals and housing

Seven male Hartley guinea pigs (Saitama Experimental Animals Supply Co., Ltd., Saitama, Japan) weighing 500 to 800 g were used. They were housed in individual cages floor with wood shavings within a light-proof chamber (Sanyo, MIR-553 or MIR-252, Tokyo, Japan). In the chamber, a light-dark cycle (LD 14:10; lights-on at 6:00) was maintained under constant temperature (24 ± 1 C). Standard guinea pig pellets (RC4; Oriental Yeast Co., Tokyo, Japan) and water were supplied ad libitum.

Implanting the transmitter

A telemetric transmitter for ECG (TA10ETA-F20 or TA10EA-F20, Data Sciences, St Paul, MN, USA) was implanted under pentobarbital sodium anesthesia (40 mg/kg, i.p.). Paired wire electrodes in a precordial bipolar lead (Apex-Base lead) were placed at the cervical subcutaneous region over the trapezius and the skin was closed by suture. All animals were studied 1 week after surgery.

Data recording

The cage housing the guinea pig was placed on a signal receiving board (CTR-86, Data Sciences, St Paul, MN, USA) in the chamber. ECG waveform was continuously recorded by an ECG processor (Softron, Tokyo, Japan). The recorded signals were sampled at 1 ms intervals and stored on a Magneto Optical disk using the ECG processor.

Power spectral analysis

Off-line analysis was performed using the ECG processor analyzing system (Softron, Tokyo, Japan) for power spectral analysis. The computer program first detected R waves and calculated the RR interval tachogram as the raw HR variability in sequence order and obtained the following time domain parameters: the mean of all normal RR intervals (mean RR); the SD of all normal RR intervals (SDRR); and the coefficient of the variation of all normal RR intervals (CV). From this tachogram, data sets of 512 points were resampled at 100 ms. This length of the tachogram was selected as the best compromise between the need for a large time series to achieve greater accuracy in the computation, and easiest for short periods [14]. Any RR intervals before and after artifacts were excluded from analysis. We then applied each set of data to the Hamming window and the fast Fourier transform 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 obtained the low frequency (LF) power, the high frequency (HF) power, and the LF to HF (LF/HF) ratio from the recordings. For assessment of diurnal variations, each hour’s spectral value was prepared from the average of 60 spectra computed about 1 min periods. The LF and HF components of the power spectrum were evaluated in absolute values (LF and HF) and normalized units (LFnu and HFnu) [12, 14].

Autonomic nervous blockade study

Autonomic nervous blockade study was carried out after 30 min of control recordings. Atropine (2 mg/kg), propranolol (4 mg/kg), and atropine (2 mg/kg) and propranolol (4 mg/kg) were injected intraperitoneally in that order at 24 hr intervals to block autonomic nervous activity. The doses of the drugs were selected according to preliminary experiments based on the HR changes.
Moreover, almost the same doses were used for rats [10]. Parameters were continuously recorded from before injection of the drugs to 30 min after injection.

Statistical analysis
All values are expressed as mean ± SEM. Statistical analysis based on Student’s paired t-test was performed for comparisons.

Results

Spectral analysis
All guinea pigs shared a characteristic pattern in their power spectrum analysis. A representative power spectrum of HR variability in guinea pigs is shown in Fig. 1A. There were two major spectral components of LF and HF spectra for HR variability. The HF peaks appeared between 1.0 and 2.0 Hz. The LF peaks appeared below 0.6 Hz. On the basis of these data, two frequency bands of interest were decided: LF (0.07–0.7 Hz) and HF (0.7–3.0 Hz).

Effects of autonomic nervous blockade on HR variability
Atropine reduced the LF area and abolished the HF area (Fig. 1B). Propranolol reduced the LF area, but the HF area was still present (Fig. 1C). A double blockade also reduced both the HF and LF areas similar to when atropine was given alone (Fig. 1D). Moreover, a double blockade reduced the LF area. The changes of HR, mean RR, SDRR, CV, LF, HF and LF/HF ratio by autonomic blockade are summarized in Table 1. HR was significantly increased by the administration of atropine, and decreased by propranolol. The simultaneous administration of atropine and propranolol increased HR. CV was reduced significantly by propranolol, while atropine increased it. The LF was significantly reduced by the administration of propranolol and a double blockade. The HF was significantly reduced by atropine and a double blockade. The LF/HF ratio was significantly reduced by a double blockade and tended to be reduced by propranolol.
Table 1. Effects of autonomic nervous blockade on heart rate variability in guinea pigs

<table>
<thead>
<tr>
<th>Measure</th>
<th>Saline</th>
<th>Atropine</th>
<th>Propranolol</th>
<th>Atropine + Propranolol</th>
</tr>
</thead>
<tbody>
<tr>
<td>HR (bpm)</td>
<td>223.2</td>
<td>272.6</td>
<td>215.0</td>
<td>249.8</td>
</tr>
<tr>
<td>Mean RRR (msec)</td>
<td>269.3</td>
<td>220.8 (8.3)*</td>
<td>278.8 (5.0)</td>
<td>240.6 (8.3)*</td>
</tr>
<tr>
<td>SDRR (msec)</td>
<td>1.4 (1.4)</td>
<td>12.9 (8.3)</td>
<td>7.2 (0.6)*</td>
<td>10.3 (0.7)</td>
</tr>
<tr>
<td>CV (%)</td>
<td>4.2 (0.4)</td>
<td>5.9 (1.2)</td>
<td>2.6 (0.2)*</td>
<td>4.3 (0.3)</td>
</tr>
<tr>
<td>LF (msec²)</td>
<td>6.2 (0.9)</td>
<td>4.1 (1.4)</td>
<td>2.4 (0.5)*</td>
<td>1.2 (0.6)*</td>
</tr>
<tr>
<td>HF (msec²)</td>
<td>1.7 (0.5)</td>
<td>0.9 (0.3)*</td>
<td>1.1 (0.3)</td>
<td>0.5 (0.2)*</td>
</tr>
<tr>
<td>LFHF (%)</td>
<td>79 (5)</td>
<td>82 (4)</td>
<td>67 (8)*</td>
<td>58 (9)*</td>
</tr>
<tr>
<td>HFHF (%)</td>
<td>21 (5)</td>
<td>18 (4)</td>
<td>33 (8)*</td>
<td>42 (9)*</td>
</tr>
<tr>
<td>LF/HF</td>
<td>4.7 (1.1)</td>
<td>5.4 (1.1)</td>
<td>3.3 (1.3)</td>
<td>1.8 (0.5)*</td>
</tr>
</tbody>
</table>

Mean (SEM) obtained from 7 guinea pigs. *Significantly (p<0.05) different from values for saline treatment.

Diurnal variations in autonomic nervous activity

The changes in the 24 hr plot of the hourly HR, mean RR, SDRR, CV, LF, HF, and LF/HF ratio are shown in Figs. 2 and 3. A nocturnal pattern, in which the values of HR in the dark phase were higher than those in the light phase, was observed. There was a clear peak after lights off in HR. The SDRR and CV showed the same tendency from that of the mean RR. However, the HF, LF, and the LF/HF ratio showed no daily pattern. There were no significant differences between light and dark phases in any of the parameters (Table 2).

Discussion

The results of this study document the characteristics of power spectral analysis of HR variability and indicate the absence of a diurnal rhythm in autonomic nervous activity in guinea pigs. There were two major spectral components at LF and HF in the power spectrum. The HF peaks appeared between 1.0 and 3.0 Hz. The LF peaks appeared below 0.6 Hz. On the basis of these data, we defined two frequency bands of interest: LF (0.07–0.7 Hz) and HF (0.7–3.0 Hz). HR in the dark phase tended to be higher than those in the light phase. There were no clear diurnal variations in LF and HF powers, and the LF/HF ratio. These results suggest that there is no diurnal variation in the autonomic nervous activity of guinea pigs.

Power spectral analysis of HR variability is a noninvasive tool which can quantify the relative amount of sympathetic and vagal activity distributed to the heart. The power spectrum of HR variability in guinea pigs resembled the power spectra derived from humans and other animal species. Two major spectral components were observed. The LF peaks appeared below 0.7Hz and HF peaks appeared between 1.0 and 2.0 Hz. The possibility that power spectral analysis can provide an index of autonomic nervous activity in rats, dogs, and humans is well established, especially for parasympathetic activity. Therefore, we tested the effects of autonomic nervous blockade on power spectral analysis of HR variability for assessment of autonomic nervous activity in guinea pigs. Atropine significantly increased HR and reduced HF and LF power. Proprao-
nolol decreased HR and LF power. These results indicate that the parasympathetic nervous system mediated HR variability in the HF and LF components, whereas the sympathetic nervous system mediated only the LF component. Accordingly, the ranges of LF and HF defined in this study were appropriate for assessing the autonomic nervous function in the guinea pigs. This study in guinea pig confirmed earlier observations in conscious rats, dogs, and humans [6, 10, 14].

It has been suggested that spectral analysis of HR variability might reflect a balance between the activities of the parasympathetic and sympathetic nervous systems [6, 14]. The decrease in the parasympathetic nervous function induced by atropine was reflected in an increase of the LF/HF ratio. Moreover, the LF/HF ratio was decreased by the reductions of sympathetic
Table 2. Light- and dark-phase heart rate variability in guinea pigs

<table>
<thead>
<tr>
<th>Measure</th>
<th>Light</th>
<th>Dark</th>
<th>Overall</th>
</tr>
</thead>
<tbody>
<tr>
<td>HR (bpm)</td>
<td>243.1</td>
<td>245.0</td>
<td>243.9</td>
</tr>
<tr>
<td>Mean RR (msec)</td>
<td>246.8</td>
<td>245.1</td>
<td>246.1</td>
</tr>
<tr>
<td>SDRR (msec)</td>
<td>10.9 (0.5)</td>
<td>10.9 (0.9)</td>
<td>10.9 (0.6)</td>
</tr>
<tr>
<td>CV (%)</td>
<td>4.4 (0.2)</td>
<td>4.5 (0.4)</td>
<td>4.4 (0.3)</td>
</tr>
<tr>
<td>LF (msec²)</td>
<td>5.7 (1.2)</td>
<td>6.5 (1.5)</td>
<td>6.0 (1.3)</td>
</tr>
<tr>
<td>HF (msec²)</td>
<td>1.6 (0.2)</td>
<td>1.8 (0.3)</td>
<td>1.7 (0.3)</td>
</tr>
<tr>
<td>LF/HF (%)</td>
<td>77 (2)</td>
<td>77 (3)</td>
<td>77 (3)</td>
</tr>
<tr>
<td>HF/HF (%)</td>
<td>23 (2)</td>
<td>23 (3)</td>
<td>23 (3)</td>
</tr>
<tr>
<td>LF/HF</td>
<td>4.0 (0.5)</td>
<td>4.0 (0.5)</td>
<td>4.0 (0.5)</td>
</tr>
</tbody>
</table>

Mean (SEM) obtained from 7 guinea pigs.

nervous activity produced by propranolol. Therefore, the LF/HF ratio seems to be a convenient index of parasympathetic and sympathetic balance in guinea pigs.

Representation of LF/HF and HF/HF emphasizes the controlled and balanced behavior of the two branches of the autonomic nervous system [12, 14]. Moreover, normalization tends to minimize the effect of the changes in total power on the values of LF and HF components. Therefore, we also calculated LF/Fr and HF/Fr. Absolute LF and HF power were decreased by atropine, whereas that of LF/HF was increased. Thus, normalization leads to predominant LF and smaller HF powers, which express the alteration of spectral power attributable to the administration of atropine. Furthermore, the effects of propranolol and double blockade on the spectral power in normalized units also clearly expressed the balanced behavior of the two branches of the autonomic nervous system. These results suggest that the normalization procedure may provide useful information concerning the underlying autonomic nervous activity in guinea pigs.

In humans, power spectral analysis of HR variability, even when applied to ambulatory subjects, could successfully evaluate the circadian rhythm of sympathovagal balance. Moreover, we clearly showed the diurnal variation of autonomic nervous activity in rats and miniature pigs using power spectral analysis of heart rate variability [5, 9]. However, the present results obtained from guinea pigs confirm that the LF and HF (both absolute and normalized units) powers and the LF/HF ratio have no diurnal variations. Therefore, these results suggest that autonomic nervous functions do not have a diurnal rhythm in guinea pigs.

Recently, we showed that characteristics of rhythmicity of heart rate, body temperature, and locomotor activity in guinea pigs are different from other rodents such as rats and mice [1]. Moreover, the HR fluctuation in guinea pigs is largely dependent on the locomotor activity. Therefore, it seems that the regulation of HR in guinea pigs may be affected by the locomotor activity rather than the autonomic nervous system, although the autonomic nervous control of the cardiovascular system has been generally thought to have a diurnal variation in its activity.

In conclusion, we established characteristics of power spectral analysis of heart rate variability, and assessed the diurnal variations of autonomic nervous function in guinea pigs. Nocturnal patterns were observed in the HR. However, the HF and LF powers, and the LF/HF ratio showed no daily pattern. These results suggest that the autonomic nervous function in guinea pigs has no clear circadian rhythmicity. This information may be useful for future studies concerning the autonomic nervous function in this species.

References


