Spectral analysis of circadian rhythms in heart rate variability of dogs

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Objective—To determine characteristics of power spectral analysis of heart rate variability (HRV) during a 24-hour period in dogs and to evaluate the effects of vagal and sympathetic tone on HRV.

Animals—16 healthy adult Beagles.

Procedure—Power spectral analysis of HRV was conducted, using 24-hour ambulatory ECG recordings. Circadian rhythms were evaluated in terms of absolute units of low-frequency (LF) and high-frequency (HF) powers, their ratio (LF:HF), and their adjusted (normalized) units (LF[norm] and HF[norm]). Three or 4 dogs were used for simultaneous measurement of heart rate and respiratory waveform as well as to evaluate treatment (propranolol, atropine, or both) administered to cause blockade of the autonomic nervous system.

Results—Values for LF and HF powers, LF:HF, LF[norm], and HF[norm] had obvious rhythmicity in clinically normal dogs. The HF power of HRV in dogs was extremely high, compared with that of other species, and HF peaks corresponded to peaks obtained from respiratory waveforms. Blockade of the autonomic nervous system documented that HRV in dogs was mostly attributable to vagal activity.

Conclusion and Clinical Relevance—We determined characteristics of power spectral analysis of HRV in dogs, including circadian rhythm of the autonomic nervous system. Power spectral analysis of HRV may provide a useful noninvasive technique for assessing the effects of drugs on activity of the autonomic nervous system in dogs. (Am J Vet Res 2001;62:37-42)

Heart rate variability (HRV) has been used as a research tool to evaluate function of the autonomic nervous system.1-4 Sympathetic and parasympathetic influences on ventricular arrhythmias have been of interest in cardiovascular research. Many investigators have reported that activity of the sympathetic nervous system promotes cardiac arrhythmias, especially malignant ventricular arrhythmias such as ventricular tachycardia and ventricular fibrillation. The parasympathetic nervous system generally plays a protective role in the onset of cardiac arrhythmias.5-10 but it is not always cardioprotective.11 There are many techniques available for evaluating HRV, which are classified into time- and frequency-domain analyses.11,12 Frequency-domain analysis of HRV is most commonly used for mechanistic studies, because it resolves more precisely the sympathetic and parasympathetic influences than does time-domain analysis.13,14 In fact, many investigators have used frequency-domain analysis to assess function of the autonomic nervous system in humans and other animals,15-21 but most analyses have been done on short-term recordings of 2 to 5 minutes. Recently, it was reported that the risk of sudden death in humans may be predicted by spectral analysis of circadian variation in HRV.22,23 Although there are some reports on the relationship between activity of the autonomic nervous system and sudden death in dogs24-26 or assessment of autonomic nerve function in other animals,27,28 there are few reports in the literature regarding assessment of function of the autonomic nervous system by use of power spectral analysis of 24-hour HRV in clinically normal dogs. We are not aware of any reports on spectral analysis of HRV that could be used for assessment of safety and pharmacologic effects of drugs in Beagles, which rank high among the choices of animals that can be used for research on the circulatory system. Therefore, the purpose of the study reported here was to clarify the characteristics of power spectral analysis of HRV and to evaluate the effects of autonomic blocking compounds on function of the autonomic nervous system in Beagles. To achieve this, we performed spectral analysis of HRV for day and night periods, using 24-hour ECG recordings.

Materials and Methods

Animals—Sixteen Beagles of either sex (9.1 to 13.3 kg, 11 to 19 months old)24 were used for evaluating the circadian rhythm of HRV. Dogs were housed separately in stainless-steel cages (80 x 90 x 90 cm) in a climate-controlled room (23 ± 2°C; relative humidity, 55 ± 15%; 12-h light:12-h dark). They were fed 300 g of a certified solid diet27/d, tap water was available ad libitum. Care and use of the dogs was in accordance with guidelines established by the Mochida Pharmaceutical Company.

Simultaneous measurement of HRV and respiration variability—Three of the 16 dogs were acclimated to being placed in a sling and being restrained in the hanging position. Respiration (CO2 monitor)28 and ECG (standard limb leads)29 were recorded simultaneously to enable us to compare the power spectrum of HRV with that of respiration variability. Both recordings were digitized by use of an ECG processor30, and spectral analyses of HRV and respiration variability were performed for 5-minute segments, using the ECG processor. Then, interaction between HRV and respiration variability was assessed by the coherence spectra for each dog.

Recording and analysis of ambulatory electrocardiography—Using a Holter recorder,31 we attempted to obtain 24-hour ambulatory electrocardiographic recordings in 8 Beagles. Self-adhesive electrodes32 were attached to the skin of the manubrium sterni in the region of the second thoracic
Effects of pharmacologic blockade of the autonomic nervous system on HRV—Four dogs (2 of the dogs used for simultaneous measurement of HRV and respiration variability and 2 of the remaining 13 dogs) were treated with propranolol hydrochloride (1 mg/kg of body weight, IV), atropine sulfate (0.05 mg/kg, IV), or a combination of both drugs on separate days to assess the autonomic nervous system. Dosages of the drugs were based on results reported by Billman and Hogsins. The dogs were continuously administered physiologic saline (0.9% NaCl) solution intravenously by drip infusion (2 ml/min) before and during the experiment, and atropine, propranolol, or both was added to the infusion tube to avoid the influence of venipuncture on autonomic nervous tone. An ECG (standard limb leads) was recorded for 10 minutes before and 10 minutes after each treatment, and spectral analysis of HRV was performed on stable data for a 5-minute period for each point.

Statistical analysis—Comparison of mean values for HRV during the day with those during the night in clinically normal dogs was used to assess circadian rhythm of HRV, using a paired t-test. Differences in heart rate, absolute units of LF and HF powers, LF:HF, and LF and HF normalized units (LF[norm] and HF[norm]) between values before and after blockade of the autonomic nervous system also were assessed by use of paired t-tests. A significant difference was defined as $P < 0.05$.

Results

Simultaneous measurement of HRV and respiration variability—The HF peak of HRV appeared at the same frequency as the peak of respiration variability for each dog (Fig 1). Moreover, interaction between HRV and respiration variability was assessed by use of the
coherence spectra for each dog. A high coherence between HRV and respiration variability was detected at 0.5 to 0.6 Hz (data not shown). Maximum coherence and frequency at which maximum coherence was evident for HRV and respiration variability in 3 dogs were (mean ± SD) 0.967 ± 0.021 and 0.482 ± 0.100 Hz, respectively (data not shown). This result revealed a relationship that is similar to the ensemble spectra.

Circadian rhythm of HRV in clinically normal dogs—Heart rate had an obvious circadian rhythm; heart rate was high in the morning and low during the night (Fig 2). Maximum heart rate was 115 ± 21 beats/min (bpm), which was observed at 9 AM, and the minimum was 67 ± 11 bpm, which was recorded at 4 AM and again at 5 AM. The LF:HF had a similar but more distinct trend, compared with changes in heart rate. The HF values in dogs were extremely high and variable. The HF power increased continuously from afternoon to early morning, but it rapidly declined after the lights were turned on in the morning. The LF power was high during the night and low during the day (ie, the same

Table 1—Results of power spectral analysis to detect circadian rhythms in 16 clinically normal Beagles

<table>
<thead>
<tr>
<th>Variable</th>
<th>Day</th>
<th>Night</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart rate (bpm)</td>
<td>98 ± 14</td>
<td>76 ± 8*</td>
</tr>
<tr>
<td>HF (ms²)</td>
<td>5,868 ± 1,714</td>
<td>16,650 ± 5,243*</td>
</tr>
<tr>
<td>LF (ms²)</td>
<td>810 ± 388</td>
<td>1,103 ± 352</td>
</tr>
<tr>
<td>LF:HF</td>
<td>0.187 ± 0.073</td>
<td>0.080 ± 0.040*</td>
</tr>
<tr>
<td>Adjusted values</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HF (norm)</td>
<td>67.2 ± 10.0</td>
<td>78.4 ± 5.4*</td>
</tr>
<tr>
<td>LF (norm)</td>
<td>12.0 ± 4.3</td>
<td>6.2 ± 2.6*</td>
</tr>
</tbody>
</table>

Values reported are mean ± SD.

*Value differs significantly (P < 0.001) from value for day. †Value differs significantly (P < 0.05) from value for day. bpm = Beats per minute. HF = High frequency power. LF = Low frequency power. HF (norm) = Adjusted (normalized) value for HF. LF (norm) = Adjusted (normalized) value for LF.
trend as seen for HF power). Results for adjusted LF and HF powers revealed that LF(norm) was high during the day and low during the night; however, values for HF(norm) were the reverse of the LF(norm) values (Fig 3). Overall, heart rate, LF:HF, and LF(norm) decreased significantly during the night, compared with values for those variables during the day. Conversely, HF and LF powers as well as HF(norm) increased significantly (Table 1). Therefore, heart rate, LF, and HF powers, LF:HF, LF(norm), and HF(norm) had an obvious rhythmicity in clinically normal dogs.

The peaks of HF bands were distributed between 0.1 and 0.6 Hz. Most peaks were detected between 0.1 and 0.4 Hz (Fig 4).

Effects of pharmacologic blockade of the autonomic nervous system on HRV—Propranolol induced a significant decrease in heart rate and significant increase in HF power, but it did not affect LF power, resulting in a significant reduction in LF:HF. When expressed in normalized units, propranolol did not cause a change in HF(norm) but did cause a significant decrease in LF(norm). Atropine alone or in combination with propranolol induced a significant increase in heart rate and a severe, significant reduction in LF and HF. On the other hand, when the data were expressed in normalized units, atropine alone or in combination with propranolol decreased HF(norm) values and caused a significant increase in LF(norm) values (Table 2). Values for LF:HF after treatment with atropine alone or in combination with propranolol were not subjected to analysis, because LF and HF powers were too low to obtain reliable ratios.


discussion

Although power spectral analysis of HRV during a 24-hour period is more labor-intensive and time-consuming than time-domain analysis, it provides more information regarding hourly fluctuations in HRV. Therefore, we conducted power spectral analysis of HRV in conscious Beagles, using ECG recordings during a 24-hour period, and evaluated the effects of vagal and sympathetic tone on HRV. Consequently, we documented that function of the autonomic nervous system clearly had circadian rhythms and that the responses to pharmacologic blockade of the autonomic nervous system in dogs were entirely or partially similar to those in other animals.

Circadian rhythms clearly were evident in heart rate, absolute units of HF and LF powers, LF:HF, and LF(norm) and HF(norm) in the dogs reported here. Rhythmicity of heart rate resembled that of LF, HF, and LF(norm), with high values in the morning and low values at night, whereas the absolute HF and LF powers and HF(norm) represented opposite circadian rhythms. Although there are few reports on circadian rhythm of HRV in animals, the rhythm we found in our dogs coincided with that reported in humans.31,32 The HF power in these dogs was extremely high, which resulted in a low LF:HF, compared with that in rats,15,35,36 cats,39 horses,38 and humans.31,32,33 This high power of HF is probably attributable to spontaneous respiratory sinus arrhythmia in dogs, because it is known that variations in the R-R interval reflect vagal activity caused by spontaneous respiration,39-41 and HF power is considered to reflect vagal activity. Peak for the HF bands was detected at 0.15 to 0.25 Hz (Fig 4), which may have been a result of the lower respiratory rate (9 to 15 breaths/min) during sleep or rest.

Analysis of results of pharmacologic blockade of the autonomic nervous system also suggested that HRV in dogs was mostly attributable to vagal activity, because β-adrenergic blockade with propranolol induced an increase in HF power and decreases in LF:HF and heart rate. Furthermore, vagal blockade with atropine almost abolished HRV, resulting in low values of HF and LF powers. Atropine also induced greater changes in HF(norm) and LF(norm) values, compared with values after administration of propranolol, although significant differences were not detected because of large deviations in the values. Although it is known that the effects of pharmacologic blockade on HRV differ among species, these responses to atropine or propranolol in dogs were entirely or partially similar to those in other animals.15,32,34 The intrinsic heart rate after administration of sympathetic and parasympathetic blockers was higher than the typical heart rate, and the effect of atropine on HRV was similar to that of the atropine-propranolol combination. This result also may suggest the predominance of parasympathetic nervous activity in dogs. Simultaneous measurement of HRV and respiration variability proved that the HF peaks we observed originated from fluctuations in respiration. The characteristic of

<table>
<thead>
<tr>
<th>Variable</th>
<th>Before treatment</th>
<th>Propranolol*</th>
<th>Atropine*</th>
<th>Propranolol and atropine*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart rate (bpm)</td>
<td>114 ± 8</td>
<td>98 ± 111</td>
<td>207 ± 291</td>
<td>173 ± 311</td>
</tr>
<tr>
<td>Absolute values</td>
<td>HF (ms²)</td>
<td>1,993 ± 754</td>
<td>3,810 ± 1,085</td>
<td>4 ± 76</td>
</tr>
<tr>
<td>LF (ms²)</td>
<td>330 ± 150</td>
<td>385 ± 189</td>
<td>3 ± 26</td>
<td>5 ± 58</td>
</tr>
<tr>
<td>LF:HF</td>
<td>0.164 ± 0.029</td>
<td>0.105 ± 0.0461</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Adjusted values</td>
<td>HF(norm)</td>
<td>65.5 ± 6.6</td>
<td>71.9 ± 5.5</td>
<td>32.6 ± 29.0</td>
</tr>
<tr>
<td>LF(norm)</td>
<td>11.7 ± 2.5</td>
<td>7.4 ± 3.01</td>
<td>59.1 ± 28.1</td>
<td>38.4 ± 9.7</td>
</tr>
</tbody>
</table>

Values reported are mean ± SD. *Propranolol (1 mg/kg of body weight) and atropine (0.05 mg/kg) were administered IV. †Value differs significantly (P < 0.05) from value obtained before treatment. §Value differs significantly (P < 0.01) from value obtained before treatment. ‡Value differs significantly (P < 0.001) from value obtained before treatment. ND = Not determined.
HF distribution is considered to reflect intrinsically variable cardiopulmonary functions in dogs.

Hajigholi et al. reported that LF:HF was of little use in detecting decreases in sympathetic activity in settings in which the sympathetic tone is already low, because LF fluctuations are caused by the combined action of sympathetic and vagal nerves, and HF fluctuations are caused only by vagal activity. Hoshikawa and Yamamoto also reported that LF:HF was of little use in assessing autonomic balance in settings with high sympathetic tone but without specific enhancements of LF fluctuations in sympathetic nerves. Therefore, we evaluated our data by analyzing normalized units of LF and HF in addition to absolute powers and LF:HF to minimize dependence on heart rate or total power. Consequently, the study reported here precisely assessed the effects of pharmacologic blockade of the autonomic nervous system, especially when normalized units were used.

In the study reported here, we documented the characteristics of power spectral analysis of HRV in dogs, using 24-hour ambulatory electrocardiographic recordings in laboratory-housed Beagles. However, these dogs may not be representative of other breeds of dogs or of dogs maintained in a domestic environment. Furthermore, it is scarcely possible to say that we excluded the influence of human interaction on the circadian rhythm of HRV in our dogs. The number of dogs used was not always sufficient for simultaneous measurement of HRV and respiration variability (3 dogs) or for evaluation of autonomic pharmacologic blockade with propranolol or atropine (4 dogs). Propranolol, an adrenergic antagonist, did not cause a change in HF(norm) values but caused a decrease in LF(norm) values. On the other hand, a pure parasympatholytic agent, (ie, atropine) would have been expected to possibly decrease HF(norm) values and cause no change or an increase in LF(norm) values. However, atropine did not cause significant changes in LF(norm) or HF(norm) values. These results may have been attributable to an insufficient number of dogs and large deviations of the values. Therefore, further investigation may be necessary regarding this point.

In the study reported here, we clarified the characteristics of power spectral analysis of HRV, including circadian rhythm of the autonomic nervous system. We believe that power spectral analysis of HRV provides a useful noninvasive technique for assessing the effect of drugs on activity of the autonomic nervous system in dogs.

References


