Entropy measures of heart rate variation in conscious dogs

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Palazzolo, James A., Fawzy G. Estafanous, and Paul A. Murray. Entropy measures of heart rate variation in conscious dogs. Am. J. Physiol. 274 (Heart Circ. Physiol. 43): H1099–H1105, 1998.—Our goal was to determine the contributions of sympathetic and parasympathetic activity to entropy measures of heart rate variability (HRV). We compared our results with two commonly used methods to analyze HRV: standard deviation (SDNN) and power spectral analysis (HF norm). Beat-by-beat analysis of R-R intervals was performed in conscious dogs. The R-R intervals were analyzed with approximate entropy (ApEn) and entropy of symbolic dynamics (SymDyn) to assess the effects of reducing system complexity. This was achieved by pharmacologically inhibiting sympathetic, parasympathetic, and total autonomic nervous system regulation of heart rate. Three conditions were examined: rest, standing, and systemic hypotension. At rest or standing, sympathetic inhibition (propranolol) had no effect on ApEn or SymDyn, whereas parasympathetic (atropine) and combined (propranolol + atropine) inhibition reduced both entropy measures to near zero. Systemic hypotension reduced both entropy measures in intact dogs. When hypotension was induced after sympathetic inhibition, ApEn was increased compared with hypotension alone, whereas parasympathetic inhibition with hypotension resulted in near-zero ApEn. Changes in the entropy measures of HRV were directionally similar to changes in SDNN and HF norm. These results indicate that the entropy of R-R intervals reflects parasympathetic modulation of heart rate.

approximate entropy; symbolic dynamics; autonomic nervous system; propranolol; atropine

BEAT-BY-BEAT MEASUREMENT of heart rate and its related analysis is commonly referred to as heart rate variability (HRV). HRV is a broad-spectrum signal of colored noise that contains one harmonic at the frequency of respiration. Typically, HRV is analyzed using the standard deviation (SDNN) of all the R-R intervals or using the power spectrum of a heart rate or R-R interval signal (17). Mathematical models of R-R intervals can be classified as either deterministic or stochastic. There is evidence that R-R intervals are generated by a composite deterministic system with some stochastic or “noise” input (14). Additionally, the model can be either linear or nonlinear. A linear system is the special condition of a nonlinear system where the properties of superposition and homogeneity hold for inputs. In the case of R-R intervals, the preponderance of interpretations of SDNN and spectral analysis has been based on underlying assumptions of linear models.

The concept of entropy, as it applies to signals like R-R intervals, is to quantify the repetition of patterns in that signal. Larger values of entropy correspond to greater apparent randomness or irregularity, whereas smaller values correspond to more instances of recognizable patterns in the data. One algorithm for evaluating the entropy that has been applied to R-R intervals is approximate entropy (ApEn) (13). A second method involves calculating the entropy of a simplified dynamic system based on a sequence of symbols (SymDyn) (6). The entropy calculations, here applied to beat-to-beat heart rate, can be shown to obliquely provide a positively correlated barometer of the extent of complication of an underlying network model in many diverse systems, with larger values implying a more complex feedback or feedforward system (10, 11).

One general hypothesis is that system complexity is decreased by certain pathophysiological conditions (11, 14). ApEn has been shown to decrease in infants that have had episodes of aborted sudden-infant-death syndrome (12). Distressed fetuses with acidosis also have lower ApEn (15). In the adult cardiac surgical population, a correlation has been found between a decreased ApEn late in surgery and the occurrence of ventricular dysfunction (5). SymDyn has been tested in patients 1–3 wk after a myocardial infarction (19). When used in combination with linear statistics like SDNN, SymDyn was found to be useful in detecting altered HRV in patients with high risk for sudden cardiac death (19).

Entropy measures of HRV have no previously described well-established physiological correlate (14). The overall objective of the present study was to determine the contribution of sympathetic and parasympathetic activity to entropy measures of HRV. Specifically, we used pharmacological antagonists to inhibit sympathetic, parasympathetic, and total autonomic nervous system control of heart rate (8). Experiments were performed in conscious dogs at rest, in the standing position, and during systemic hypotension. We hypothesized that ApEn and SymDyn would be decreased by the autonomic nervous system antagonists, by upright posture, and by systemic hypotension. We compared our results with two well-accepted methods to measure parasympathetic regulation of HRV, i.e., SDNN and the normalized high-frequency (HF norm) component of the power spectrum of HRV.

METHODS

All surgical procedures and experimental protocols were approved by the Institutional Animal Care Committee.

Chronic Instrumentation

Eight mixed-breed dogs (30 ± 2 kg) were chronically instrumented for this study. All dogs were premedicated with morphine sulfate (10 mg im) and anesthetized with pentobar-
bital sodium (20 mg/kg iv) and fentanyl citrate (15 µg/kg iv). After the dogs were endotracheally intubated, the lungs were mechanically ventilated at 15 ml/kg tidal volume and 15 breaths/min. Anesthesia was maintained with halothane (~1% end tidal). A left thoracotomy was performed through the fifth intercostal space using sterile technique. Tygon catheters (1.02-mm ID, Norton) were inserted into the descending thoracic aorta and right atrium to measure systemic arterial pressure and to administer the pharmacological antagonists, respectively. A hydraulic occluder (22-mm ID, In Vivo Metric) was positioned around the inferior vena cava. The free ends of the catheters and occluder were threaded through the chest wall and tunneled subcutaneously to exit between the scapulae. A chest tube was placed in the left thorax and removed on the first postoperative day. Morphine sulfate (10 mg im) was administered postoperatively for pain as required. Ampicillin (1 g iv), cefazolin (1 g iv), and gentamicin (80 mg iv) were administered for 10 postoperative days. The dogs were allowed to recover for at least 2 wk before experimentation. At the time of experimentation, systemic arterial pressure, blood gases, rectal temperature, heart rate, respiratory rate, and body weight were all within normal limits.

HRV Measurements

For each experiment, a three-lead electrocardiogram (ECG) and three-lead respiratory activity monitor (Hewlett-Packard) were applied using pregelled silver/silver chloride electrodes. The computer digitized the ECG at 1,000 Hz. The R waves in the ECG were detected with an amplitude and derivative threshold and with a minimum refractory period between R waves. The R-R intervals were then measured in the original ECG, with a maximum error of 2 ms. The R-R intervals were manually inspected for noise. Any experiment with an arrhythmia, ectopic beat, or loss of ECG quality was discarded, and the experiment was repeated. Any experiment in which mean heart rate changed because the dog slept, barked, or became excited was also repeated (4). The first 2,048 beats were used as the R-R interval vs. beat number to be analyzed for HRV.

Power Spectrum

Both moment statistics and spectral analysis were calculated for comparison with entropy measures. The SDNN of the R-R intervals was calculated. The R-R intervals were interpolated to heart rate vs. time using the method of Berger et al. (1). The mean was subtracted from the heart rate signal, and a Hamming window was applied. It was then recreated into the frequency domain using the fast Fourier transform. The spectrum was corrected for coherent gain and the Berger interpolation. The power spectrum was integrated using the trapezoid rule between the frequency bounds: low frequency (LF; 0.04–0.15 Hz) and high frequency (HF; 0.15–0.4 Hz). The spectrum was corrected for coherent gain and the Berger interpolation. The power spectrum was integrated using the trapezoid rule between the frequency bounds: low frequency (LF; 0.04–0.15 Hz) and high frequency (HF; 0.15–0.4 Hz). Each power range was then represented as the fraction of the total spectral power (LF norm, HF norm).

ApEn

ApEn was calculated using the algorithm by Pincus and co-workers (10, 13). The ApEn calculation has three parameters (N, m, r). In the ApEn calculation, the number of points (N = 2,048) and the embedding dimension (m = 2) were fixed. The vector comparison length (r) for ApEn was calculated as 15% of the standard deviation of the R-R intervals. The phase space was created with a delay of 1, and the correlation integral was calculated as the number of vectors with a maximum distance less than or equal to r to the template vector. The natural logarithm of the correlation integral was averaged over the 2,048 beats. This process was repeated for m = 3, and ApEn was the difference between the values at m = 2 and m = 3.

StatAv was used to determine the stationarity of the R-R intervals (12). The R-R intervals were divided into 20 groups, the means of which were calculated. The ratio of the standard deviation of the 20 means to the overall standard deviation is referred to as StatAv. A StatAv near zero indicates stationarity, whereas large values of StatAv indicate greater nonstationarity.

SymDyn

SymDyn is based on the concept of coarse graining the dynamics of a complex system (6). The system dynamics are recreated in phase space using successive differences in R-R intervals with a fixed delay (τ = 1) (3). An example of this calculation with hypothetical data is shown in Fig. 1. The phase space is divided into either four or six sections defined by ±2SDNN and ±1SDNN or ±1SDNN only. Each region of the divided phase space is given a symbol, creating four (0–3) or six (0–5) possible symbols. The order that the regions are visited by the evolving dynamics creates a symbol sequence. The symbol sequence is then divided into word sequences with a length of two or three symbols. The frequency of occurrence for each word is counted, and a histogram is generated.
generated. The Shannon entropy is then calculated from this histogram. If a word occurs with a probability of <0.001, it is considered a “forbidden word.” The number of forbidden words is counted for a word sequence. The SymDyn are analyzed with the following parameter set: length two words and four symbols (V-2); length two words and six symbols (P-2); length three words with four symbols (V-3); and length three words and six symbols (P-3).

Experimental Protocols

Resting condition. During the recovery from surgery, the dogs were trained to lie on a padded table. Experiments were initiated at the same time each day to eliminate effects of circadian cycles. Experiments were performed with the dogs lying on their right sides in a quiet laboratory environment. Systemic arterial pressure was measured via a strain gauge manometer (Gould Statham P23 ID), which was referenced to atmospheric pressure and positioned at the level of the spine. Baseline experimental recordings were made for 30 min (intact condition). Sympathetic β-adrenoreceptor inhibition was then achieved with propranolol hydrochloride (1.0 mg/kg iv). After a 5-min equilibration period, experimental measurements were recorded continuously for 30 min. At the end of the experiment, the efficacy of sympathetic β-adrenoreceptor inhibition was tested with isoproterenol hydrochloride (1.0 µg/kg). If isoproterenol increased heart rate by >10%, the experiment was repeated.

On a subsequent day, baseline experimental recordings were again made for 30 min, followed by muscarinic receptor inhibition with atropine methyl bromide (0.1 mg/kg iv). After equilibration and an additional 30-min recording period, the efficacy of muscarinic receptor inhibition was tested with acetylcholine chloride (40 µg/kg iv). If acetylcholine decreased heart rate by >10%, the experiment was repeated.

On the third experimental day, measurements were made in the intact condition for 30 min, followed by a 30-min recording period after combined sympathetic and parasympathetic inhibition with propranolol and atropine, respectively.

Standing condition. The dogs were trained to stand in a Pavlov-style sling, which held their torsos stationary but did not support body weight. The pressure transducer was positioned at the level of the heart. Experiments were initiated when the dog could stand quietly for 60 min. Experiments were performed on separate days in the intact condition and after pretreatment with propranolol, atropine, or combined inhibition.

Systemic hypotension. Experiments were performed with the dogs lying on their right sides. The pressure transducer was positioned at the level of the spine. Systemic hypotension was induced by slowly inflating the hydraulic occluder implanted around the inferior vena cava over a 10- to 15-min period until mean systemic arterial pressure was reduced to ~60 mmHg. Experiments were performed on separate days in the intact condition or after pretreatment with propranolol, atropine, or combined inhibition.

Data Analysis

Group data are presented as means ± SE. Differences between group means were tested with the Student’s t-test. Differences in group variance were assessed with the F-test. Differences were considered significant when P < 0.05.

RESULTS

Resting Condition

Intact values for SDNN, HF norm, ApEn, SymDyn, and LF norm in the resting, standing, and hypotension conditions are summarized in Table 1. Compared with the intact condition, propranolol had no effect on heart rate or the mean R-R interval (Table 2) nor did it alter any of the four measures of HRV (Fig. 2). In contrast, atropine and combined inhibition increased heart rate and decreased the mean R-R interval (Table 2). Atropine and combined inhibition also markedly decreased P-3 by 10.2 ± 0.3 on October 1, 2017 http://ajpheart.physiology.org/ Downloaded from
Respiratory rate was unchanged by any intervention (Table 2).

Standing Condition

Compared with the intact resting condition, LF norm and heart rate were increased, and the mean R-R interval was decreased with standing (Tables 1 and 2). Intact values of SDNN, HF norm, and SymDyn were decreased in the standing compared with the resting condition (Table 1). ApEn was also decreased in the standing condition, but the change did not achieve statistical significance ($P = 0.10$). Propranolol failed to alter any measure of HRV in the standing condition, whereas all values were decreased ($P < 0.05$) by atropine and combined inhibition (Fig. 3). StatAv values indicate that the R-R intervals were moderately stationary in the intact condition and after propranolol but nonstationary after atropine and combined inhibition (Table 2).

Hypotension Condition

Inflating the hydraulic occluder implanted around the inferior vena cava gradually (10–15 min) reduced systemic arterial pressure from $108 \pm 4$ to $60 \pm 1$ mmHg. Compared with the intact resting condition, LF norm and heart rate were increased, and the mean R-R interval was decreased with hypotension (Tables 1 and 2). Intact values of SDNN, HF norm, ApEn, and SymDyn were all decreased during hypotension compared with the resting condition (Table 1). In general, there was a greater degree of variability in all measures of HRV during hypotension. Propranolol increased ($P < 0.05$) HF norm and ApEn but not SDNN or SymDyn (Fig. 4). Atropine and combined inhibition decreased ($P < 0.05$) all four measures of HRV (Fig. 4), although the decreases in SDNN and SymDyn were smaller than the changes observed in either the resting or standing conditions. StatAv values indicate that the R-R intervals were nonstationary during hypotension (Table 2).

Forbidden Words

Another method for quantifying alterations in system complexity is by counting the number of forbidden words from SymDyn. The number of forbidden words increases as system complexity decreases, and vice
versa. The calculation for the number of forbidden words is shown in Fig. 5 for an individual conscious dog. The intact condition shows a phase portrait (Fig. 5A) that has dynamics in each of the defined regions. The histogram (Fig. 5B) for that phase portrait shows that most of the six-symbol length three words occur. After atropine, the phase portrait (Fig. 5C) shows considerably less variation. The maximum range of variation (320–360 ms), as well as the distance of variation from the diagonal, is minimal. The symbol regions beyond those immediately adjacent to the diagonal contain none of the R-R interval dynamics. This is reflected in the histogram (Fig. 5D), which shows that most words are forbidden. The only words that occur are 000, 003, 030, 033, 300, 303, 330, and 333.

Intact values for the number of forbidden words were 131 ± 6 at rest, 155 ± 5 while standing, and 189 ± 13 during systemic hypotension, i.e., the number of forbidden words was increased (P < 0.05) by standing and hypotension when compared with the resting condition. Changes in the number of forbidden words with autonomic inhibition are summarized in Fig. 6 for each condition. Propranolol had no effect on the number of forbidden words in any condition. Atropine and combined inhibition increased (P < 0.05) the number of forbidden words for each condition, indicating a decrease in system complexity.

DISCUSSION

The overall objective of this study was to characterize the contribution of sympathetic and parasympathetic activity to entropy measures of HRV, i.e., ApEn and SymDyn. The simplest measure of HRV is SDNN, which has been used as an index of cardiac parasympathetic activity (7). In power spectral analysis, the high-frequency (HF norm) component is also thought to reflect parasympathetic activity (2, 9, 16). In this study, we used SDNN and HF norm as benchmarks to assess the effects of pharmacological denervation on entropy measures of HRV.

It is important to note that in contrast to SDNN and the power spectrum, the entropy measures used in this study are independent of any type of theoretical model. Entropy measures were used to quantify the regularity in the R-R intervals. Variability measures like SDNN reflect the magnitude of variation. Although SDNN and
entropy represent two distinct methods of analysis, they are correlated, e.g., in a "fixed r" version of ApEn, not implemented here. To decorrelate these two methods, ApEn was normalized by selecting r as a percentage of SDNN. The resultant versions of ApEn and SymDyn employed herein are shift and scale invariant and thus provide orthogonal and complementary perspective to analyses provided by moment statistics, such as mean R-R interval and SDNN. In a similar fashion, the phase space regions of SymDyn were selected as multiples of SDNN. As a result, a decrease in entropy reflects a decrease in complexity, not a decrease in variability.

Resting Condition

Our results demonstrate that the strongest contribution to the entropy of HRV is parasympathetic control of heart rate. Inhibiting sympathetic activity with propranolol had no effect on ApEn or SymDyn. As expected, SDNN and HF norm were also unchanged by propranolol. When parasympathetic activity was inhibited with atropine, both ApEn and SymDyn were markedly decreased. These changes paralleled the decreases in SDNN and HF norm in response to atropine.

When evaluating SymDyn, four different parameter sets were analyzed: P-2, V-2, P-3, and V-3. The findings for each parameter set were both qualitatively and quantitatively similar, so only the P-3 results were presented. The phase space representation of the R-R intervals in the intact condition has a distinctive "V" shape (Fig. 5). This was quantified using Shannon entropy. This phase space description (i.e., Shannon entropy) was unchanged when sympathetic activity was inhibited with propranolol. In contrast, when atropine was administered, most of the variation around the phase space diagonal was eliminated, resulting in a more correlated and less dynamic phase space.

In the intact condition, the R-R intervals were generally stationary (StatAv = 0.34 ± 0.02). Inhibiting sympathetic control of heart rate did not alter stationarity, whereas parasympathetic inhibition increased StatAv to very large values, quantifying the associated nonstationarities. These results are likely because of the concomitant decrease in SDNN induced by atropine. However, it is unlikely that the changes in ApEn and SymDyn were primarily the result of changes in stationarity, because the absolute magnitude of the trend changes in the R-R interval was small (5–20 ms). The increase in nonstationarity caused by inhibition with atropine may affect the power spectrum, which is sensitive to nonstationarity and outliers (18). This may, in turn, introduce some error into LF norm and HF norm calculated from the power spectrum.

Standing Condition

At rest, heart rate regulation in the dog is predominantly parasympathetic and minimally sympathetic. To moderately increase sympathetic activity, we repeated experiments in the standing condition. Heart rate was increased and the mean R-R interval was decreased in the standing position, and these effects were inhibited by propranolol. Compared with rest, SDNN, HF norm, and SymDyn, but not ApEn, were decreased in the standing condition. These effects are likely because of withdrawal of vagal activity, because propranolol had no effect on these measures of HRV. Atropine markedly reduced SDNN and HF norm, and these results were paralleled by changes in ApEn and SymDyn.

The intact standing R-R intervals were partially nonstationary (StatAv = 0.50 ± 0.02). This likely reflects an increased level of alertness and freedom of movement in the standing compared with the resting condition. Just as we observed in the resting condition, propranolol had no effect on StatAv, whereas atropine increased StatAv to very large values, quantifying the associated nonstationarities.

Hypotension Condition

To assess the effects of pharmacological denervation on measures of HRV in the setting of high sympathetic activity, systemic hypotension (60 ± 1 mmHg) was induced by gradually inflating the hydraulic occluder implanted around the inferior vena cava. Systemic hypotension markedly decreased SDNN, HF norm, ApEn, and SymDyn. Although speculative, these changes may reflect the combined influence of an increase in sympathetic activity and a concomitant decrease in parasympathetic activity. These simultaneous changes would reduce system complexity to a greater extent than the standing condition. In support of this concept, propranolol increased both HF norm and ApEn. During hypotension, there was a greater degree of variability in all measures of HRV. This could be because of homeostatic adaptations in blood pressure regulation during the 30-min period of hypotension. During the recording period, we noted very long-term trending patterns in the R-R interval, which were reflected in increased values of StatAv (0.82 ± 0.08). During hypotension, administration of atropine, alone or in combination with propranolol, reduced HRV measures to a lesser extent than during the resting or standing conditions. Thus other unknown factors may contribute to system complexity during hypotension.

Forbidden Words

Forbidden words were independently calculated from SymDyn and possibly provide a separate measure of system complexity. An increase in the number of forbidden words is thought to represent a decrease in system complexity. Compared with the resting condition, the number of forbidden words increased in the standing condition and increased even further during hypotension. Similar to all measures of HRV, propranolol had no effect on the number of forbidden words in the resting and standing conditions. However, in contrast to HF norm and ApEn, propranolol failed to have an effect on the number of forbidden words during hypotension. Either this method is not sensitive to a sympa-
thetic contribution to system complexity or changes in the HRV measures under these circumstances do not reflect a sympathetic component.

**Low-Frequency Component of the Power Spectrum**

The low-frequency component of the power spectrum is generally thought to primarily reflect sympathetic, and to a lesser extent parasympathetic, activity (17). This is particularly the case when low frequency is normalized to the total power (LF norm). Consistent with this concept, LF norm increased in the standing condition and increased further during systemic hypotension. In contrast, SDNN, HF norm, ApEn, and SymDyn all decreased during the standing and hypotension conditions.

**Summary**

We investigated the contributions of sympathetic and parasympathetic activities to entropy measures (ApEn and SymDyn) of HRV in chronically instrumented, conscious dogs. Our results suggest that under varying physiological conditions and in response to pharmacological denervation, the entropy of R-R intervals reflects parasympathetic modulation of heart rate.

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