Dynamic Analysis of Renal Nerve Activity Responses to Baroreceptor Denervation in Hypertensive Rats

Gerald F. DiBona, Susan Y. Jones

Abstract—Sinoaortic and cardiac baroreflexes exert important control over renal sympathetic nerve activity. Alterations in these reflex mechanisms contribute to renal sympathoexcitation in hypertension. Nonlinear dynamic analysis was used to examine the chaotic behavior of renal sympathetic nerve activity in normotensive Sprague-Dawley and Wistar-Kyoto rats and spontaneously hypertensive rats before and after complete baroreceptor denervation (sinoaortic and cardiac baroreceptor denervation). The peak interval sequence of synchronized renal sympathetic nerve discharge was extracted and used for analysis. In all rat strains, this yielded systems whose correlation dimensions converged to similar low values over the embedding dimension range of 10 to 15 and whose greatest Lyapunov exponents were positive. In Sprague-Dawley and Wistar-Kyoto rats, complete baroreceptor denervation was associated with decreases in the correlation dimensions (Sprague-Dawley: 2.42±0.04 to 2.16±0.04; Wistar-Kyoto: 2.44±0.04 to 2.34±0.04) and in the greatest Lyapunov exponents (Sprague-Dawley: 0.199±0.004 to 0.130±0.015; Wistar-Kyoto: 0.196±0.002 to 0.136±0.010). Spontaneously hypertensive rats had a similar correlation dimension, which was unaffected by complete baroreceptor denervation (2.42±0.02 versus 2.42±0.03), and a lower value for the greatest Lyapunov exponent, which decreased to a lesser extent after complete baroreceptor denervation (0.183±0.006 versus 0.158±0.006). These results indicate that removal of sinoaortic and cardiac baroreceptor regulation of renal sympathetic nerve activity is associated with a greater decrease in the chaotic behavior of renal sympathetic nerve activity in normotensive compared with hypertensive rats. This suggests that the central neural mechanisms that regulate renal sympathetic nerve activity in response to alterations in cardiovascular reflex inputs are different in spontaneously hypertensive rats from those in Sprague-Dawley and Wistar-Kyoto rats. (Hypertension. 2001;37:1153-1163.)

Key Words: nonlinear dynamics ■ denervation ■ baroreceptors ■ renal nerves

The sinoaortic and cardiac baroreceptor reflexes exert important control over sympathetic nerve activity. The details of these control mechanisms have been studied by observing steady state changes in mean arterial pressure (MAP), heart rate (HR), and sympathetic nerve activity at various times after complete disruption of these reflexes.

After sinoaortic baroreceptor denervation, MAP, HR, and renal sympathetic nerve activity (RSNA) were increased on day 1 but returned to control levels on day 14.1 On day 1, variability of MAP was increased, while that of HR and RSNA was decreased. On day 14, variability of MAP remained increased, while that of HR and RSNA returned to control levels. MAP and RSNA were strongly (≈90%) negatively correlated before sinoaortic baroreceptor denervation but only 30% negatively correlated on days 1 and 14 and 25% positively correlated on days 1 and 14. These results indicate that low MAP variability results from sinoaortic baroreflex–mediated fluctuations in HR and RSNA that are inversely related and that high MAP variability after sinoaortic baroreceptor denervation is infrequently positively corre-

lating with RSNA. Because MAP variability can be reduced by interventions that block the sympathetic nervous system,2,3 it appears that MAP variability associated with sinoaortic baroreceptor denervation is mediated largely by a permissive role of peripheral sympathetic nervous system activity. This is especially prominent in the conscious state, in which MAP, HR, and RSNA responses to environmental alerting stimuli are exaggerated.

Time series of normal heartbeat (ie, R-R intervals), arterial pressure, and peak intervals of synchronized RSNA display complex nonlinear dynamics, including deterministic chaos. In normal animals subjected to sinoaortic and cardiac baroreceptor denervation, the regulation of arterial pressure4–6 (dogs) and RSNA7 (rats) became more simple, with significant reduction in 2 indices of chaotic behavior, the correlation dimension and greatest Lyapunov exponent. Similarly, the heartbeat8 of patients and the RSNA9 of rats with congestive heart failure showed marked reduction in chaotic behavior compared with the normal state. This is of interest because human subjects and animals with congestive heart failure...
have impaired sinoaortic and cardiac baroreflex regulation of HR, arterial pressure, and RSNA.9 Since removal of sinoaortic and cardiac baroreceptor regulation of RSNA, occurring either physiologically or pathophysiologically, is associated with a reduction in both the transmission of afferent inhibitory information to the central nervous system and the chaotic behavior of RSNA, this suggests that the continued presence of tonic afferent inhibitory neurotransmission to the central nervous system contributes to sustaining the normal chaotic behavior of RSNA.

Compared with the genetically normotensive control Wistar-Kyoto rat (WKY), the genetically spontaneously hypertensive rat (SHR) has altered sinoaortic10 and cardiac11 baroreflex regulation of RSNA. Consequences of these alterations are that for a given level of arterial or left-sided cardiac filling pressure, afferent inhibitory input to the nucleus tractus solitarius is less and RSNA is greater in SHR than in WKY. Basal values for MAP, HR, and RSNA are greater in SHR and WKY. In comparison to Wistar rats, the RSNA of stroke-prone SHR (SHRSP) appeared to represent a simpler system with a significantly lower correlation dimension but a similar greatest Lyapunov exponent.12 These several studies suggested that removal of sinoaortic and cardiac baroreceptor regulation of RSNA might have different effects on the chaotic behavior of RSNA in SHR compared with WKY.

This purpose of the present study was to examine the dynamic and chaotic behavior of RSNA in SHR and WKY before and after complete baroreceptor (sinoaortic and cardiac baroreceptor) denervation.

Methods

SHR, WKY, and Sprague-Dawley rats, aged 14 to 16 weeks, allowed free access to normal sodium rat pellet diet (Teklad) and tap water, were used for all experiments. All animal procedures were performed in compliance with the University of Iowa Policies and Guidelines Concerning the Use of Animals in Research and Teaching and the US Public Health Service Guide for the Care and Use of Laboratory Animals.

Anesthesia

Rats were anesthetized with pentobarbital 50 mg/kg IP with supplemental doses of 5 to 10 mg/kg IV at regular intervals.

Sinoaortic and Cardiac Baroreceptor Denervation

Sinoaortic and cardiac baroreceptor denervation was performed by methods previously used and validated in this laboratory.5,13 Sinoaortic baroreceptor denervation was verified by noting the absence of decreases in RSNA after the administration of 3 μg/kg IV phenylephrine. Cardiac (vagotomy) baroreceptor denervation was verified by noting the absence of decreases in RSNA after the administration of 50 μg/kg IV 2-methyl-serotonin.14

Procedures

Catheterization

Catheters were placed in the right carotid artery and jugular vein for the measurement of pulsatile arterial pressure (PAP), MAP, and HR and infusion of solutions (0.9% NaCl at 0.05 mL/min) or drugs, respectively.

RSNA Electrode

The left kidney was exposed through a left flank incision via a retroperitoneal approach. With the use of a dissecting microscope, a renal nerve branch from the aortocaval ganglion was isolated and carefully dissected free. The renal nerve branch was then placed on a recording electrode. RSNA was amplified (×20 000 to 50 000) and filtered (low, 30 Hz; high, 3000 Hz) via a Grass HIP511 high-impedance probe, which led to a Grass P511 bandpass amplifier. The amplified and filtered neurogram signal was channeled to a Tektronix 5113 oscilloscope and Grass model 7D polygraph for visual evaluation and to an audio amplifier/loudspeaker (Grass model AM 8) for aural evaluation. The quality of the RSNA signal was assessed by its pulse synchronous rhythmicity; signal-to-noise ratio ranged between 3:1 and 5:1. An additional assessment was made during an injection of phenylephrine 3 μg/kg IV; as MAP increased, RSNA decreased. When an optimal RSNA signal was observed, the recording electrode was fixed to the nerve preparation with a silicone cement (Wacker Sil-Gel). The electrode cable was sutured to the back muscles and tunneled to the back of the neck, where it was exteriorized. The left flank incision was closed in layers.

Experimental Protocols

Conscious Rats

Marked suppressive effects of pentobarbital anesthesia on basal values of MAP, HR, and RSNA and, more importantly, on PAP, HR, and RSNA spectral power have been demonstrated in Sprague-Dawley rats.15 To examine whether a similar effect occurs in WKY and SHR, additional WKY and SHR were anesthetized, and surgical insertion of the arterial and venous catheters and implantation of the RSNA recording electrode were performed by the aforementioned methods. Then the rats were allowed to recover from the effects of anesthesia and surgery in their home cages overnight (∼15 hours) before experimental use. At that time, the arterial and venous catheters and the RSNA recording electrode were connected to the appropriate measuring device, and after an additional 30-minute equilibration period, a 30-minute recording period was made in the conscious state. Thereafter, sinoaortic and cardiac baroreceptor function was tested. Then the rat was killed, and postmortem RSNA was recorded for 30 minutes; this value was subtracted from all experimental values of RSNA.

Sinoaortic and Cardiac Baroreceptor Denervation in Anesthetized Rats

SHR, WKY, and Sprague-Dawley rats were anesthetized, and surgical insertion of the arterial and venous catheters and implantation of the RSNA recording electrode were performed by methods described above. At this point, intact sinoaortic and cardiac baroreceptor function was verified by the methods described above. Then isolation and identification of the nerves (looped ligatures) required for sinoaortic and cardiac baroreceptor denervation were done to allow subsequent complete baroreceptor denervation in as little time as possible. Sinoaortic and cardiac baroreceptor function was again tested to verify that the isolation and identification procedure had not disrupted it. Thereafter, 1 hour was allowed to elapse for stabilization. Then, during continuous measurement of MAP, HR, and RSNA, a 30-minute control period was made. Thereafter, sinoaortic and cardiac baroreceptor denervation was performed as rapidly as possible (generally within 30 seconds). Continuous measurement of MAP, HR, and RSNA was continued through this rapid denervation procedure and for an additional 30-minute experimental period. Then sinoaortic and cardiac baroreceptor function was again tested. The rat was killed, and postmortem RSNA was recorded for 30 minutes; this value was subtracted from all experimental values of RSNA.

Analytical Protocols

The amplified and filtered renal neurogram was full wave rectified and integrated (Grass 7P3 Resistance-Capacitance Integrator, 20 ms time constant) and stored as RSNA on videotape (Vetter 4000A PCM) along with the neurogram, PAP, and HR (Grass 7P4 Tachograph) signals for later offline analysis, as described below.

Sympathetic Peak Detection Program

The steady state RSNA displayed positive deflections that were proportional to the frequency discharge in the original neurogram.
and generally occurred with each cardiac cycle. Individual nerve bursts, still observable in the RSNA record, were smoothed by subsequent filtering at 35 Hz. This smoothed RSNA was used for analysis of synchronized renal sympathetic nerve discharge characteristics. With the use of an analog-to-digital converter (Laboratory-PC+) and standard data acquisition software (LabVIEW), the steady state RSNA was sampled at 200 Hz over the identical 30-minute periods as used above. The characteristics of RSNA were determined with a statistically based computerized algorithm, the Sympathetic Peak Detection Program.16–19 The Sympathetic Peak Detection Program allows the simultaneous determination of the amplitude (height), duration, and periodicity (peak interval) of synchronized sympathetic discharges or peaks. The minimum acceptable peak height was set at >25% of the maximum peak height in the data series. Since peak height depends on the number of active fibers, this choice indicates that a sufficient number of fibers is active so as to generate a peak whose height is >25% of the peak generated by the maximum number of fibers active in the data series, ie, the maximum peak height. After the synchronized peaks had been identified for each data series, data on interpeak interval (ms), individual peak height (mV), MAP, and HR were extracted.

**Power Spectral Analysis and Transfer Function**

Tape-recorded signals of PAP and RSNA were sampled continuously at 50 Hz with an analog-to-digital converter in a Pentium IBM compatible computer (30-minute record=90 000 samples). All signals were corrected by subtraction of the death signals. The dynamic fluctuations in PAP and RSNA were investigated in the frequency domain with spectral analysis techniques.20 The time series was divided into half-overlapping sequential blocks of 1024 data points (20.5 seconds). Each block was subjected to linear trend removal and cosine tapering of the first and last 60 data points before calculation of spectral density power. For each parameter, spectral density power was calculated as the average over the sequential blocks for each period in each rat. The analysis of the influence of fluctuations in RSNA on the fluctuations in PAP, the transfer function between RSNA (input signal) and PAP (output signal) was calculated as the quotient of the cross spectrum and the input spectrum. The value of the transfer function represents the degree to which fluctuations in the RSNA signal are transferred into the PAP signal, with lower values (closer to 0) reflecting less and higher values (closer to 1) reflecting more transfer; values ≥1 suggest that the RSNA fluctuations are possibly either amplified or generated within the cardiovascular system. The phase of the transfer function reflects the temporal relationship between the input and output signals in the frequency domain, ie, that oscillations in the one signal may induce a similar frequency oscillation in the other signal delayed in time. The phase angle enables the determination of whether one rhythm is preceding or following the other rhythm. A phase angle of 0° (0 radians) indicates synchrony between the 2 signals, and a phase angle of 180° (π radians) indicates a reciprocal relationship between the 2 signals. The phase spectra were converted into time delay spectra by dividing by the product of 2π and the respective frequency. The coherence function yields a value that varies between 0 and 1 and is a frequency domain estimate of a linear regression coefficient, indicating the degree to which variance in one signal can be explained by a linear operation on the variance in the other signal. For each period in each rat, results from these analyses were averaged over the sequential data blocks to reduce variance.

**Nonlinear Dynamic Analysis**

**Data**

Each original data set consisted of approximately 11 000 to 13 000 interpeak intervals. The data sets were continuous without artifacts. For assessment of stationarity, the original data set was divided into 2 equal portions. The values of correlation dimension and greatest Lyapunov exponent determined in each of these portions and in the original data set were compared. The determinations of correlation dimension and greatest Lyapunov exponent were made in subsets (1024 peak intervals each) of the original data set as well as the original data set. For the chaos detection algorithm, the original data set was divided into 10 subsets of 1000 peak intervals each. The original data set size of ~12 000 agrees well with estimates of the minimal number of data points necessary to identify nonlinear structures.21–24 $10^{5}$ or $10^{4}$, where d is the dimension of the structure under study.

**Correlation Dimension**

The Grassberger-Procaccia algorithm was used to determine the correlation dimension, defined as a dimension with noninteger values.25,26 The correlation dimension is an estimate of the least number of independent variables that characterize the system (given sufficient fine-scale resolution). With each pass through the data, a new data point is taken, and a hyperdimension sphere of embedding dimension D and radius r is centered on that point. The fraction of subsequent data points in the record within that sphere [C(r)] is then calculated for various values of r (length scale), and a plot is made of the log C(r) versus the log r for a range of embedding dimensions. The slope of this relationship is the correlation dimension. These slopes were plotted against r to identify values of the correlation dimension that were independent of both r and the embedding dimension. The correlation dimension was calculated over a wide range of embedding dimensions (1–15) to enable the detection of a plateau of the values of the calculated correlation dimension with increasing values of the embedding dimension. The time delay was determined from the first zero of the autocorrelation function27–29 or from the minimum of the time delayed mutual information function,27–29 which were in close agreement for all data sets. The minimal sufficient embedding dimension was determined by the false nearest neighbor method.27–29 The length scale and its upper and lower limits were kept constant for the analysis of control and experimental original data sets as well as the matched surrogate data sets.

**Lyapunov Exponent**

The Lyapunov exponent is a measure of the exponential rate at which nearby trajectories in phase space diverge (given sufficient fine-scale resolution). The Lyapunov exponent, λ, is directly related to the magnitude of chaos in the system. Periodic processes have λ = 0, wherein trajectories eventually converge, while uncorrelated random data (ie, noise) have λ = 0. Chaotic systems have 0 < λ < 0, indicating that the trajectories diverge; ie, insignificant differences in the initial conditions become significant over time, which is a defining feature of chaos. The greatest Lyapunov exponent was estimated by the fixed evolution computer program of Wolf et al30 and the algorithm of Kantz27–29 (lyap_k program27) over the ranges of embedding dimensions similar to those used for determination of correlation dimension. The length scale and its upper and lower limits were kept constant for the analysis of control and experimental original data sets as well as the matched surrogate data sets.

**Chaos Detection Algorithm**

This algorithm detects nonlinear determinism in a time series by iteratively generating a family of polynomial autoregressive models. The null hypothesis (ie, that the time series is stochastic with linear dynamics) is rejected if there is at least one nonlinear model that provides a significantly better fit to the data in a parsimonious manner than linear autoregressive models of all dynamic orders. The statistical test is highly robust and sensitive, in that it is resistant to noise contamination and is applicable to short time series (<1000 data points). The technique provides a highly specific test for deterministic chaos in that the null hypothesis is not readily rejected in the presence of random noise unless the underlying system is chaotic. The level of noise corruption that can be tolerated is directly related to the magnitude of the greatest positive Lyapunov exponent, a measure of the degree of chaos in the underlying noise-free data. The best linear and nonlinear models are obtained for both the original and the surrogate data series. This is defined as the model that minimizes the cost function, $C(p)=log(p)+p/N$, where $e(p)$ is the residual error, p is the number of polynomial terms, and N is the length of the time series. Chaos is established when the best nonlinear model from the original data series is significantly more predictive than both the best linear model from the original data.
series and the best linear and nonlinear models obtained from the surrogate data series. This is determined by statistical comparison of the residual errors for the models using the F-ratio test at the 1% significance level. The algorithm was applied to each of the 1000 data point subsets of the original data set, and the frequency of linear and nonlinear model selection was tabulated.

**Surrogate Data**
Iterative fast Fourier transform surrogate data sets\(^{27-32}\) were generated\(^{27-29,31,32}\) (surrogates program\(^{27}\)) with the use of a subsequence of the original data set with negligible end point mismatch and minimal loss of data (end-to-end program\(^{27}\)). The surrogate data sets have the same Fourier amplitudes and distribution of values. The linear properties (ie, mean, SD, power spectra, autocorrelation function) of the surrogate data sets are identical to those of the original data set. The null hypothesis being tested is that the original data set arises from a stationary, possibly rescaled, linear gaussian random (stochastic) process. As a measure of nonlinearity, a nonlinear prediction error statistic (predict program\(^{27}\)) was used with similar parameters of embedding dimension, time delay, and radius for both the original and surrogate data sets. For a 1-sided test to detect a significantly smaller error with a residual probability \(\alpha\) of a false rejection, corresponding to a level of significance of 100% \((1-\alpha)\), then \(1/1-\alpha\) surrogate data sets are required; for \(\alpha=0.99\), 100 surrogate data sets were constructed. The assessment of nonlinearity is important because, while deterministic chaos implies nonlinearity, the reverse is not true; thus, not all nonlinear systems are chaotic.

**Computer Software**
Computer software programs were obtained from the following sources: FFT,\(^{20}\) U. Wittmann, University of Heidelberg (Germany); Chaos Data Analyzer (professional version),\(^{33}\) American Institute of Physics, Physics Academic Software, North Carolina State University, Raleigh; FET\(^{20}\) (a program that quantifies chaos in a time series); Chaos Detection Algorithm\(^{34}\) (algorithm for detection of nonlinear dynamics in short, noisy time series); and Time Series Analysis (TISEAN).\(^{27-29,32}\)

**Statistical Analysis**
Statistical analyses were conducted with ANOVA and Scheffé’s test for pairwise comparisons among means and \(t\) test for comparison between groups\(^{35}\); statistical significance was taken at a value of \(P<0.05\). Statistical comparison of the residual errors for the models in the Chaos Detection Algorithm was performed with the F-ratio test, with statistical significance taken at a value of \(P<0.01\). Data in text, tables, and figures are mean±SE.

**Results**

**Conscious WKY and SHR**
Compared with conscious WKY \((n=6)\), conscious SHR \((n=6)\) had higher MAP \((153±4\) versus \(111±5\) mm Hg), HR \((400±10\) versus \(365±9\) bpm), and RSNA \((45.3±4.9\) versus \(30.1±3.1\) mV/s · s) \((all P<0.05)\). As seen in Figure 1, PAP (top) and RSNA spectral power (middle) were greater in SHR than in WKY. In SHR, both PAP and RSNA spectral power showed distinct peaks near 0.4 Hz, while PAP and RSNA spectral power in WKY at the same frequency were nearly 10-fold lower. Near 0.4 Hz frequency, coherence (Figure 1, bottom) was 0.932±0.031 in SHR \((0.34\) Hz) and 0.859±0.021 in WKY \((0.39\) Hz). Thus, as previously identified in conscious Sprague-Dawley rat,\(^{15}\) there is also a close coupling between RSNA and PAP at 0.4 Hz.

**Tests of Sinoaortic and Cardiac Baroreflex Function**
The results of serial testing of sinoaortic and cardiac baroreflex function (Table 1) showed that procedures used for isolation and identification of the nerves required for sinoaortic and cardiac baroreceptor denervation did not affect the renal sympatoinhibitory responses to either phenylephrine (sinoaortic) or 2-methyl-serotonin (cardiac), indicating preserved sinoaortic and cardiac baroreceptor function. However, when sinoaortic and cardiac baroreceptors were denervated, the renal sympatoinhibitory responses to either phenylephrine (sinoaortic) or 2-methyl-serotonin (cardiac) were abolished. These results indicated effective sinoaortic and cardiac baroreceptor denervation.

**Steady State Data**
In the control period (Table 2; Figure 2A through 2D, top), basal values for MAP, HR, RSNA, peak frequency, and peak amplitude were similar in Sprague-Dawley rats and WKY but, except for peak frequency, were lower than those in SHR. In all 3 strains, sinoaortic and cardiac baroreceptor denervation increased MAP, HR, RSNA, and peak amplitude, while peak frequency was unaffected. In the sinoaortic and cardiac baroreceptor denervation period, values for MAP, HR, RSNA, and peak amplitude were greater in SHR than in Sprague-Dawley rats and WKY, which were similar.

When we used the absolute value of the standard deviation \((SD_{\text{abs}})\) as a measure of variability (Figure 2A through 2D, bottom), in all 3 strains sinoaortic and cardiac baroreceptor denervation increased the variability of MAP and decreased the variability of HR, RSNA, and peak amplitude, while the variability of peak frequency was unaffected (not shown).
Power Spectral Analysis and Transfer Function

**Sprague-Dawley Rats**
In the control period (Figure 3, top), RSNA spectral power was greater than PAP spectral power. The PAP spectra showed low frequency power at <0.1 Hz and a broad respiratory oscillation near 1.0 Hz. The RSNA spectra showed multiple oscillations at frequencies <1.0 Hz and a distinct respiratory oscillation near 1.0 Hz. Strong oscillations in the vicinity of 0.4 Hz were not seen. When we used RSNA as the input signal and PAP as the output signal (Figure 4, right), coherence was 0.5 to 0.8 over the entire frequency range, except for lower values at 0.7 Hz and >1.1 Hz; the largest value was at the respiratory oscillation frequency near 1.0 Hz. Transfer gain showed higher values at 0.2, 0.7, and 0.9 Hz (clearly less than the respiratory oscillation frequency near 1.0 Hz). The phase angle was variably positive and negative, with prominent negative values at 0.15, 0.6 Hz, and >1.0 Hz and prominent positive values at 0.8, 1.0 Hz (the respiratory oscillation frequency), and 1.35 Hz. At frequencies <0.6 Hz, time delays were small and variable. At the respiratory oscillation frequency near 1.0 Hz, the change in PAP preceded the change in RSNA by 220 ms.

After sinoaortic and cardiac baroreceptor denervation (Figure 3, bottom), spectral power increased more in RSNA than in PAP. The PAP spectra showed low frequency power at <0.1 Hz, a small oscillation near 0.4 Hz, and a more narrow respiratory oscillation near 1.15 Hz. The RSNA spectra again showed multiple oscillations, with a more prominent oscillation near 0.4 Hz (same frequency as the oscillation in the PAP spectra) that had a coherence of 0.7. Transfer gain was slightly less than during control. Phase angle was positive over the entire frequency range, indicating that changes in PAP preceded changes in RSNA, with the maximum time delay of 140 ms occurring at 1.15 Hz.

**Wistar-Kyoto Rats**
In all respects, the data on WKY were not significantly different from those on Sprague-Dawley rats (not shown).

**Spontaneously Hypertensive Rats**
In the control period (Figure 5, top), RSNA power was greater than PAP power except at frequencies <0.1 Hz. The PAP spectra showed low frequency power at <0.1 Hz and a broad respiratory oscillation near 1.2 Hz. The RSNA spectra showed multiple oscillations at frequencies <1.0 Hz and a distinct respiratory oscillation near 1.2 Hz. Strong oscillations in the vicinity of 0.4 Hz were not seen. With the use of RSNA as the input signal and PAP as the output signal (Figure 4, right), coherence was 0.5 to 0.8 over the entire frequency range, except for lower values at 0.7 Hz and 0.9 Hz; the largest value was at the respiratory oscillation frequency near 1.2 Hz. Transfer gain showed higher values at 0.34, 0.44, and near 1.2 Hz (respiratory oscillation frequency). The phase angle was variably negative and positive. At frequencies >0.4 Hz, time delays were small and variable.

After sinoaortic and cardiac baroreceptor denervation (Figure 5, bottom), power increased more in RSNA than in PAP. The PAP spectra showed low frequency power at <0.1 Hz and a respiratory oscillation near 1.15 Hz. The RSNA spectra again showed multiple oscillations, with a respiratory oscillation near 1.15 Hz. Strong oscillations in the vicinity of 0.4 Hz were not seen. Coherence was 0.5 to 0.8 over the entire frequency range, with the highest value at the respiratory oscillation frequency near 1.15 Hz. Transfer gain was slightly less than during control, with a larger value near the respiratory oscillation frequency. Phase angle was variably positive and negative over the entire frequency range. At frequencies >0.4 Hz, time delays were small and variable.

**Nonlinear Dynamic Analysis**
As previously reported, the values for the greatest Lyapunov exponent determined by the algorithm of Wolf (FET), the algorithm of Kantz, and Chaos Data Analyzer were within 8% of each other with the use of 2 benchmark data series. In addition, the Chaos Detection Algorithm also showed a

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### TABLE 1. Tests of Sinoaortie and Cardiac Baroreceptor Function

<table>
<thead>
<tr>
<th>Variable</th>
<th>Sprague-Dawley Rats</th>
<th>WKY</th>
<th>SHR</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Isolation</td>
<td>Denervation</td>
</tr>
<tr>
<td>PHE, Δ% RSNA</td>
<td>-96±2</td>
<td>-94±4</td>
<td>-3±2*</td>
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<td>2-MS, Δ% RSNA</td>
<td>-71±4</td>
<td>-69±3</td>
<td>-2±4*</td>
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PHE indicates phenylephrine 3 μg/kg IV; 2-MS, 2-methyl-serotonin 50 μg/kg IV.

### TABLE 2. Effect of Sinoaortie and Cardiac Baroreceptor Denervation

<table>
<thead>
<tr>
<th>Variable</th>
<th>Sprague-Dawley Rats</th>
<th>WKY</th>
<th>SHR</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>SADVX</td>
<td>Control</td>
</tr>
<tr>
<td>MAP, mm Hg</td>
<td>109±6</td>
<td>137±5*</td>
<td>110±3</td>
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<tr>
<td>HR, bpm</td>
<td>384±9</td>
<td>418±9*</td>
<td>376±8</td>
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<tr>
<td>RSNA, mV·s</td>
<td>23.8±2.0</td>
<td>31.6±2.1*</td>
<td>24.8±2.0</td>
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<tr>
<td>Peak frequency, Hz</td>
<td>6.4±0.3</td>
<td>6.1±0.2</td>
<td>6.5±0.2</td>
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<tr>
<td>Peak amplitude, mV</td>
<td>36.7±2.3</td>
<td>44.8±3.7*</td>
<td>34.1±2.1</td>
</tr>
</tbody>
</table>

SADVX indicates sinoaortic and cardiac baroreceptor denervation.

*P<0.05, SADVX vs control within rat strain.
†P<0.05, SHR vs both Sprague-Dawley rat and WKY within period.
significant nonlinear component in both benchmark data series.

The values of correlation dimension and greatest Lyapunov exponent calculated for each half of the original record agreed with each other and with the values calculated from the entire original record to within 5%. This was the case for each rat strain before and after sinoaortic and cardiac baroreceptor denervation.

Figure 6 (top) shows the relation between the logarithm of the correlation sums, C(r), and the logarithm of the radius, r, for a Sprague-Dawley rat after sinoaortic and cardiac baroreceptor denervation. Each curve signifies a different embedding dimension (from 6 to 15) whereby the correlation dimension, \( D(r) \), is given by the slope of the linear segment in these curves:

\[
D(r) = \frac{d \log C(r)}{d \log(r)}
\]

It can be seen that the curves contain a linear segment in which the slope converges to a constant value as the embedding dimension increases. Figure 6 (bottom) shows the relation between the correlation dimension, \( D(r) \), and \( r \) for the same range of embedding dimensions. A plateau (ie, scaling range) is identified around a value of \( r = 10 \), where the correlation dimension, \( D(r) \), is independent of changes in either the embedding dimension or \( r \). For values of \( r \) greater or less than this scaling range, the correlation dimension,
D(r), shows dependence on both the embedding dimension and r. In the vicinity of r = e^{1.1}, D(r) = 2.09 ± 0.07 (averaged over the 10 embedding dimensions). The nonlinear prediction error for the original data set was 1.9104, while those for the 100 surrogate data sets ranged from 2.0943 to 2.1339, so that the null hypothesis that the original data set arises from a stationary, possibly rescaled, linear gaussian random process was rejected at the 99% level of significance. The correlation dimension for the original data set, 2.09, was outside the range of correlation dimensions for the 100 surrogate data sets, 3.21 to 5.68. The greatest Lyapunov exponent for the original data set, 0.065, was outside the range of greatest Lyapunov exponents for the 100 surrogate data sets, 0.074 to 0.099.

Figure 6. Top, Estimate of correlation dimension by the method of Grassberger and Procaccia. The slope of the linear segment in a plot of log correlation sum C(r) vs log radius (r) is taken as the correlation dimension D(r). Each curve refers to a different value of the embedding dimension, with the uppermost curve being 6 and the lowermost curve being 15 in steps of 1. The curves contain a linear segment whose slope converges to a constant value as embedding dimension is increased. Bottom, Relation between the correlation dimension, D(r), and r for the same range of embedding dimensions. A plateau (ie, scaling range) is identified around an intermediate value of e^{-1.1}, where the correlation dimension, D(r), is relatively independent of changes in either the embedding dimension or r.

hypothesis that the original data set arises from a stationary, possibly rescaled, linear gaussian random process was rejected at the 99% level of significance. The correlation dimension for the original data set, 2.09, was outside the range of correlation dimensions for the 100 surrogate data sets, 3.21 to 5.68. The greatest Lyapunov exponent for the original data set, 0.065, was outside the range of greatest Lyapunov exponents for the 100 surrogate data sets, 0.074 to 0.099.
Table 3: Correlation Dimensions and Greatest Lyapunov Exponents

<table>
<thead>
<tr>
<th>Group</th>
<th>Correlation Dimension</th>
<th>Greatest Lyapunov Exponent</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>SADVX</td>
</tr>
<tr>
<td>Sprague-Dawley</td>
<td>2.42±0.04</td>
<td>2.16±0.04*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.199±0.004</td>
</tr>
<tr>
<td>WKY</td>
<td>2.44±0.04</td>
<td>2.34±0.04*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.196±0.002</td>
</tr>
<tr>
<td>SHR</td>
<td>2.42±0.02</td>
<td>2.42±0.03</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.183±0.006†</td>
</tr>
</tbody>
</table>

Abbreviations are as defined in Table 2.
*P<0.05, control vs SADVX within each measurement.
†P<0.05, SHR control vs SD control and WKY control.

Discussion

Conscious Versus Anesthetized Rats During Control State

The design of these experiments permitted an analysis of the immediate responses of MAP, HR, and RSNA to sinoaortic and cardiac baroreceptor denervation, with each rat serving as its own control. This design minimized the variability between rats, which is generally larger than the variability within each rat. This is of special importance for RSNA, wherein it is not possible to keep the number of renal sympathetic nerve fibers on the recording electrode and their contact with the recording electrode constant between rats. This design required the use of anesthesia, which may have multiple effects on cardiovascular reflex behavior. However, given the time limit for reliable RSNA recordings in conscious rats, avoiding the effects of anesthesia by using conscious rats resulted in the comparison of 2 different groups of rats: intact versus sinoaortic and cardiac baroreceptor denervated. In this case, there was a greater degree of between-rat variability, albeit without the potentially confounding aspects of anesthesia.

Because an aim of the study was to examine the immediate response to sinoaortic and cardiac baroreceptor denervation, the experiments were conducted under anesthesia to permit a rapid denervation procedure with continuous recording. From previous studies in Sprague-Dawley rats, it was known that pentobarbital anesthesia (as used herein) had substantial but different effects on basal values of MAP, HR, and RSNA and the spectral power of PAP, HR, and RSNA. Anesthesia, while not affecting MAP, decreased HR by 8% and RSNA by 15%. However, anesthesia markedly decreased maximum spectral power of PAP (−90%), HR (−96%), and RSNA (−66%), while not affecting the frequency at which the maximum

Table 4: Percentage of Linear and Nonlinear Model Detection by Chaos Detection Algorithm in Sprague-Dawley Rats, WKY, and SHR during Control and Sinoaortic and Cardiac Baroreceptor Denervation Periods

<table>
<thead>
<tr>
<th>Group</th>
<th>Control Group</th>
<th>SADVX</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Linear</td>
<td>Nonlinear</td>
</tr>
<tr>
<td>Sprague-Dawley</td>
<td>4 ± 1</td>
<td>96 ± 3</td>
</tr>
<tr>
<td></td>
<td>51 ± 4</td>
<td>49 ± 3</td>
</tr>
<tr>
<td>WKY</td>
<td>3 ± 1</td>
<td>97 ± 4</td>
</tr>
<tr>
<td></td>
<td>49 ± 3</td>
<td>51 ± 3</td>
</tr>
<tr>
<td>SHR</td>
<td>12 ± 2*</td>
<td>88 ± 3*</td>
</tr>
<tr>
<td></td>
<td>62 ± 3*</td>
<td>38 ± 3*</td>
</tr>
</tbody>
</table>

Abbreviations are as defined in Table 2.
*P<0.05, SHR vs Sprague-Dawley rat or WKY within experimental period.
spectral power occurred. Anesthesia did not affect the maximum coherence between PAP and RSNA or the frequency at which this maximum coherence occurred. In these conscious SD, the maximum peak spectral power for PAP was at 0.08 Hz, while that for RSNA was at 0.4 Hz. However, there was an ≈70% smaller peak in spectral power for PAP that was also located at 0.4 Hz. The maximum coherence between PAP and RSNA of 0.9 occurred at 0.4 Hz.

To examine whether the effects of anesthesia on basal values of MAP, HR, and RSNA and on the spectral power of PAP, HR, and RSNA in WKY and SHR were different from those previously reported in Sprague-Dawley rats,15 groups of conscious WKY and SHR were studied so that their results could also be compared with those from anesthetized WKY and SHR. Conscious WKY and SHR had similar basal MAP and HR but higher basal RSNA than anesthetized WKY and SHR. Power spectral analysis in conscious WKY and SHR showed spectral power in both PAP and RSNA at 0.4 Hz that was stronger in SHR than in WKY; there was strong coherence between PAP and RSNA spectral power at 0.4 Hz in both WKY and SHR. These results are similar to those in conscious Sprague-Dawley rats15 and indicate a coupling between RSNA and PAP at 0.4 Hz.

However, during the control period in anesthetized SHR, both PAP and RSNA spectral power were markedly decreased compared with that in conscious SHR; this was the case for WKY as well (data not shown). Anesthetized Sprague-Dawley rats and WKY did not display prominent PAP or RSNA spectral power at 0.4 Hz, while SHR showed RSNA but not PAP spectral power at 0.4 Hz. Coherence values were relatively low at 0.4 Hz (0.617±0.031 in Sprague-Dawley rats, 0.512±0.029 in WKY, and 0.306±0.021 in SHR). Thus, the suppressive effect of anesthesia on PAP and RSNA spectral power in WKY and SHR was similar to that previously reported for Sprague-Dawley rats.15

**Immediate Effect of Sinoaortic and Cardiac Baroreceptor Denervation**

**Absolute Values and Variability**

Immediately after sinoaortic and cardiac baroreceptor denervation, MAP, HR, RSNA, and the peak amplitude of synchronized RSNA were increased in Sprague-Dawley rats, WKY, and SHR; the peak frequency of synchronized RSNA was unchanged. In synchronized RSNA, peak amplitude reflects the number of active renal nerve fibers, and peak frequency reflects the activity of central rhythm oscillators, which may be influenced by afferent input from peripheral reflex mechanisms. Thus, these results indicate that the immediate response of synchronized RSNA to sinoaortic and cardiac baroreceptor denervation involves an increase in the number of active renal nerve fibers rather than an increase in the frequency of central rhythm oscillators. Since the number of fibers on the recording electrode is unchanged, this indicates that there is recruitment of previously silent fibers to begin firing. Since the renal nerves are a heterogeneous population with respect to effects on various renal functions,36–40 this makes possible the engagement of functionally specific renal sympathetic nerve fibers after sinoaortic and cardiac baroreceptor denervation. Variability, as reflected by SD_{xx}, was increased in MAP and HR but decreased in RSNA and peak amplitude.

In prior studies comparing groups of conscious Sprague-Dawley rats,1 those studied 1 day after sinoaortic baroreceptor denervation showed increases in MAP, HR, and RSNA. As a measure of variability, sinoaortic baroreceptor denervation increased SD_{MAP} but decreased SD_{HR} and SD_{RSNA}. In the group studied 14 days after sinoaortic baroreceptor denervation, MAP, HR, and RSNA had returned to normal, as had SD_{HR} and SD_{RSNA}, while the SD_{MAP} remained increased. At both 1 and 14 days after sinoaortic baroreceptor denervation, the correlations between MAP and RSNA were negative (ie, RSNA inversely related to MAP) in 30% to 40% of the cardiac cycles (compared with 90% in control rats) and were positive (ie, RSNA directly related to MAP) in 30% of the cardiac cycles (compared with 0% in control rats).

**Frequency Domain Analysis**

In prior studies comparing groups of conscious Sprague-Dawley rats, the magnitude of PAP spectral power in the 0.3- to 0.5-Hz frequency range was reduced when examined at both 7 ±1 (≈−50%) and 14 ±2 (≈−75%) days after sinoaortic baroreceptor denervation. The effect of sinoaortic baroreceptor denervation on RSNA spectral power was not reported in those studies.

Herein, under anesthesia, before sinoaortic and baroreceptor denervation, prominent PAP spectral power at 0.4 Hz was not identified in Sprague-Dawley rats, WKY, or SHR, while RSNA spectral power at 0.4 Hz was greater in SHR than either Sprague-Dawley rats or WKY. With the large increase in RSNA after sinoaortic and cardiac baroreceptor denervation in Sprague-Dawley rats, WKY, and SHR, prominent RSNA spectral power was identified at 0.4 to 0.5 Hz, which showed high coherence (≈0.7) with smaller oscillations in PAP spectral power at the same frequency.

It appears that the absence of prominent PAP or RSNA spectral power at 0.4 Hz during the control period likely reflects the marked suppressive effect of pentobarbital anesthesia on both PAP and RSNA spectral power. However, with the increase in MAP and RSNA after sinoaortic and cardiac baroreceptor denervation, these suppressive effects of pentobarbital anesthesia were partially offset by the increases in RSNA spectral power. Under these conditions, prominent RSNA spectral power and residual PAP spectral power were seen at 0.4 to 0.5 Hz (with increased [compared with control period] coherence values of 0.71±0.02 in Sprague-Dawley rats, 0.70±0.03 in WKY, and 0.71±0.02 in SHR).

When the results from conscious and anesthetized studies ranging from immediate (seconds) to longer-duration (1 to 2 weeks) responses to sinoaortic and/or cardiac baroreceptor denervation in normotensive and hypertensive rats are synthesized, it appears that there are immediate increases in MAP, HR, and RSNA that are accompanied by increased variability in MAP but decreased variability in HR and RSNA. These effects are sustained for at least 1 day, but by 14 days the only residual alteration is an increase in MAP variability. Despite different baseline levels of MAP, HR, and RSNA between Sprague-Dawley rats, WKY, and SHR, the
immediate responses to sinoaortic and cardiac baroreceptor denervation are qualitatively similar. Power spectral analysis shows a strong coupling between RSNA and PAP near 0.4 Hz, which is qualitatively similar between normotensive (Sprague-Dawley rats and WKY) and hypertensive rats; it is readily detected in the conscious state but is substantially depressed by anesthesia and appears to be related to sinoaortic (and possibly cardiac) baroreceptor-dependent mechanisms.

**Chaos Analysis**

 Interruption of afferent input from the sinoaortic and cardiac baroreceptors resulted in a decrease in the correlation dimension and the greatest Lyapunov exponent of the nonlinear dynamic characteristics of synchronized RSNA that was different among Sprague-Dawley rats, WKY, and SHR. In Sprague-Dawley rats, the immediate responses to sinoaortic and cardiac baroreceptor denervation were qualitatively similar to those seen in previous studies in which 60 minutes was allowed to elapse between the completion of the denervation procedure and the collection of the experimental data. However, there are important quantitative differences in that the decrease in the correlation dimension was less in the present study (immediate, 2.42 versus 2.16, −11%) than in the previous study (60 minutes, 2.65 versus 1.64, −38%), while the situation was opposite for the greatest Lyapunov exponent (immediate, 0.199 versus 0.130, −35%; 60 minutes, 0.201 versus 0.177, −12%). With respect to central nervous system control mechanisms, it seems likely that multiple adaptive and compensatory adjustments occur in the first 60 minutes after sinoaortic and cardiac baroreceptor denervation.

Compared with Sprague-Dawley rats, the immediate effect of sinoaortic and cardiac baroreceptor denervation in normotensive WKY was a lesser decrease in the correlation dimension with a similar decrease in the greatest Lyapunov exponent, whereas SHR showed no change in the correlation dimension and a lesser decrease in the greatest Lyapunov exponent. The chaotic behavior of synchronized RSNA discharge in normotensive Wistar rats and SHRSP has been examined with the use of another reflex stimulus, brachial nerve stimulation (simulate somatic afferent stimulation). Somatic afferent stimulation decreased both correlation dimension and greatest Lyapunov exponent in Wistar rats but did not affect these measurements in SHRSP. These studies concur in the view that, in the SHR genetic model of hypertension, the correlation dimension and the greatest Lyapunov exponent are unaffected by 2 different maneuvers that alter afferent neural input to the brain. Thus, the agreement of these studies reinforces the notion that the normal control mechanisms within the brain that determine the pattern of RSNA are different in SHR (and SHRSP) from those in WKY (and Wistar rats). It is likely that reflex alterations in central neural input have limited effects on the chaotic behavior of synchronized RSNA in SHR. Because somatic afferent stimulation involves an increase in central input of both excitatory and inhibitory signals, while sinoaortic and cardiac baroreceptor denervation involves a decrease in central input of predominantly inhibitory signals, it is speculated that the central neural mechanisms governing the chaotic behavior of synchronized RSNA in SHR might be somewhat rigid and inflexible, ie, uninfluenced by bidirectional afferent inputs from a variety of different peripheral receptor stations. This may be of potential significance in terms of the generation and maintenance of the increased level of single fiber renal sympathetic nerve activity known to be present in SHR.

In summary, conscious WKY and SHR, like conscious Sprague-Dawley rats, exhibit a strong coupling between arterial pressure and RSNA at 0.4 Hz. Under anesthesia, the immediate response of Sprague-Dawley rats, WKY, and SHR to sinoaortic and cardiac baroreceptor denervation involves increases in MAP, HR, and RSNA, with the increase in RSNA derived from an increase in peak height (but not peak frequency) of synchronized RSNA discharge. There is increased variability in MAP but decreased variability in HR, RSNA, and peak height. Before sinoaortic and cardiac baroreceptor denervation, the suppressive effect of anesthesia on PAP and RNSA spectral power obscures the 0.4-Hz coupling that is readily observed in the conscious state. After sinoaortic and cardiac baroreceptor denervation, the overall increases in MAP and RSNA, and especially RSNA spectral power, enable the 0.4-Hz coupling to be observed even under anesthesia. In Sprague-Dawley rats and WKY, sinoaortic and cardiac baroreceptor denervations decrease 2 indices of chaotic behavior of synchronized RSNA discharge, the correlation dimension and the greatest Lyapunov. In contrast, in SHR these indices were unchanged (correlation dimension) or decreased to a lesser extent (greatest Lyapunov exponent), indicating that the central neural mechanisms that regulate RSNA in response to alterations in cardiovascular reflex inputs are different in SHR from those in Sprague-Dawley rats and WKY.

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**References**

DiBona and Jones Renal Nerve Activity Dynamic Analysis


Dynamic Analysis of Renal Nerve Activity Responses to Baroreceptor Denervation in Hypertensive Rats
Gerald F. DiBona and Susan Y. Jones

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