Potentiated Sympathetic Nervous and Renin-Angiotensin Systems Reduce Nonlinear Correlation Between Sympathetic Activity and Blood Pressure in Conscious Spontaneously Hypertensive Rats

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Background—Patients with a reduced nonlinear component of heart rate regulation have a poorer outcome.

Methods and Results—We investigated whether a nonlinear correlation between renal sympathetic nerve activity (RSNA) and blood pressure or renal blood flow is reduced in conscious, spontaneously hypertensive rats (SHR) by comparing them with normotensive Wistar-Kyoto rats (WKY). We also determined the linearity and nonlinearity of the correlation in SHR who were given an angiotensin II receptor blocker, candesartan, orally for 2 weeks. The RSNA value was higher in SHR than in WKY, and coherence peaks of transfer function were found at 0.05 and 0.80 Hz (ie, below respiratory- and cardiac-related fluctuations). The coherence (linearity) of the transfer function was significantly higher and gain was smaller in SHR than in WKY. Because mutual information values (linear and nonlinear correlation) were similar in both strains, we found the nonlinear correlation to be lower in SHR than in WKY. Time delay values calculated by the mutual information method demonstrated that RSNA preceded blood pressure and renal blood flow by 0.5 to 1.0 s. In SHR given candesartan, the RSNA value was lower, and the linearity was lower and nonlinearity higher than SHR given vehicle.

Conclusions—Linear correlation between RSNA and blood pressure or renal blood flow was higher in SHR than in WKY, whereas the nonlinear correlation was lower. Oral treatment with candesartan increased the nonlinearity and reduced the linearity in SHR. Increased RSNA and the renin-angiotensin system may be responsible for the lower nonlinearity and higher linearity in hypertension. (Circulation. 2002;106:620-625.)

Key Words: hypertension • nervous system, sympathetic • angiotensin

The cardiovascular system is regulated by nonlinear dynamics.1-3 Huikuri et al4 recently found a reduction in the nonlinear correlation of heart rate (HR) intervals was a good predictor of mortality in patients who had suffered acute myocardial infarction. Several diseases, such as congestive heart failure and arrhythmia, are characterized by a reduction in nonlinearity and loss of complexity, whereas nonlinearity is high in the healthy state.5 Although Skinner et al6 and Osaka et al7 showed that a reduction in the nonlinear correlation of HR intervals preceded ventricular fibrillation or tachycardia in humans, the link that connects reduced nonlinearity with poor prognosis remains unknown.

Although hypertension is an independent risk factor for cardiovascular events and mortality,8 the linear and nonlinear correlations among blood pressure (BP), renal sympathetic nerve activity (RSNA), and renal blood flow (RBF) have not been determined in hypertensive humans or animals. Also, few methods have accurately assessed which parameter precedes others in intact animals.

In the present study, we hypothesized that the activated sympathetic nervous system (SNS) and renin-angiotensin system (RAS) would be responsible for the high linearity and low nonlinearity in spontaneously hypertensive rats (SHR). We used a new measurement technique, the mutual information method, to quantify the nonlinear and linear correlation of 2 variables and to detect any time delay between them.9,10 First, we used a combination of transfer function and mutual information to test whether the nonlinear correlations among BP, RBF, and RSNA are reduced in SHR by comparing them with those in normotensive Wistar-Kyoto rats (WKY). BP, HR, RSNA, and RBF on the same side were simultaneously recorded in conscious rats, a technique that has not been reported to be successful. The reasons that we chose SHR were that the SNS and RAS are more activated than in WKY.
and the SHR has been approved as an appropriate model of human essential hypertension. Second, to elucidate mechanisms of higher linearity, we infused conscious SHR with the nitric oxide donor L-arginine for 60 minutes, because we previously demonstrated that the L-arginine infusion decreased RSNA in rats. Third, we determined linearity and nonlinearity in SHR given either the angiotensin II receptor blocker candesartan or vehicle orally for 2 weeks. We chose candesartan for 2 reasons. First, this drug blocks the RAS and SNS effectively. We previously demonstrated that candesartan reduced the activity of bulbospinal neurons in the rostral ventrolateral medulla (RVLM; sympathetic nervous center) of SHR using the whole-cell patch-clamp (T. Matsuura, MD, et al, unpublished data, July 2001). Second, Candesartan can decrease the electrophysiological properties of RVLM neurons situating behind the blood-brain barrier even when administered orally. On the basis of these experiments, we attempted to find the missing link that connects the lower nonlinearity with disease state/poor outcome of hypertension.

**Methods**

**Recording of BP, ECG, HR, RSNA, and RBF**

Male 15-week-old WKY (n = 7) and age-matched SHR (n = 22) were purchased from Oriental Kobo, Tokyo, Japan. All experimental procedures were performed in accordance with institutional animal care guidelines. Before surgical preparation, the rats were deeply anesthetized with pentobarbital sodium, 30 mg/kg IV initially and supplemented with 10 mg/kg as needed.

We used a telemetry system to record ECG and HR monitoring of conscious and unrestrained rats. The transmitter (TA10EA-F20; Data Science, USA) was implanted in the peritoneal cavity 1 to 2 days before the experiment, and the receiver (RPC1; Data Science) was placed underneath the rat cage. The raw analog data from the ECG were digitized with an A/D converter (Power Laboratory, ADI) and converted to HR data. Multifiber recordings of RSNA were performed as described previously. Polytetrafluoroethylene-coated multistrand stainless wire electrodes (A-M System) were placed on the left renal nerve fascicle. Subsequently, a pulsed Doppler flow probe (inner diameter, 1.0 mm; 20 MHz, CBI Co) was placed on the left renal artery. The nerve fascicle and electrodes and the artery and Doppler probe were covered with Sil-Gel 604A and B (Wacker-Chemie).

**Data Acquisition and Data Analysis**

At least 24 hours later, a conscious and unrestrained rat was placed in a rat cage. Neural recording electrodes were connected to a high-impedance probe (JB101J, Nihon Kohden Co), which was connected to a differential amplifier with a band-pass filter of 50 to 1000 Hz. The filtered neurogram was integrated by a resistance-capacitance circuit (time constant, 20 ms). The pulsed Doppler probe was connected to a Doppler blood flow velocimeter (PDV-20, Crystal Biotech). BP, ECG, RSNA, and ipsilateral RBF were simultaneously recorded in the conscious state for >60 s and a half minutes. The signals were sampled at 2000 Hz.

A smooth, instantaneous HR time series was constructed from R waves of the ECG by using the algorithm proposed by Berger et al. The time series of BP, HR, RSNA, and RBF was splined and sampled at 64 Hz so that values of the entire constructed series were made to occur simultaneously.

**Transfer Function**

To quantify the linear dependence, the transfer function between RSNA (as input) and BP or RBF (as output) and between BP (as input) and RBF (as output) were calculated. After dividing the data into multiple epochs, we optimized auto- and cross-spectra estimates by ensemble averaging 9 to 13 data epochs of 4096 points (64 s) by Welch’s method. To reduce the loss of stability, we sectioned the original data with a 50% overlap. Before applying the fast Fourier algorithm, linear trends were removed from the data, and the data were tapered with a Hanning window. Coherence is a measure of the linear statistical link between 2 variability series at any given frequency. We considered coherency values >0.5 to be significant. The gain from the input signal to the output signal was computed by dividing the ensemble-averaged cross-spectrum by the ensemble power spectrum of the input. We normalized its magnitude by the ratio of the steady-state values of the output signal and input signal.

**Mutual Information Method**

To define the nonlinear and linear relationship between RSNA and BP or RBF, which were passed through a low-pass filter <0.1 Hz, we calculated the mutual information values according to an algorithm of Fraser and Swinney that was described in our previous report. For each pair of time series, S = [s(t)] and Q = [q(t)], mutual information in the system I(S, Q) was defined as the answer to the question, “Given a measurement of s, how many bits on average can be predicted for q?” The mutual information value depends on data length, and the values were normalized so that they would fall between 0 and 1. On the basis of our previous study, a mutual information value >0.047 indicates a significant linear and nonlinear correlation. We calculated the mutual information I(T) of (S, Q), where S is the time series of RSNA(t) and Q is another time series with a time delay of T, eg, S = RSNA(t) and Q = BP(t + T). Data length was 214 points (ie, 256 seconds). T was between −5 s and 5 s in 0.25-s steps. Iml(S, Q) denotes the maximum value of I(T) between S and Q, when T lies between −5 s and 5 s. We consider the time delay Tml(S, Q), at which the maximum value of I(T) of (S, Q) is obtained, to be the physiological delay between these parameters. When Tml(S, Q) is positive, S precedes Q.

**Protocols**

**Protocol 1: Linear and Nonlinear Correlations in WKY and SHR**

We examined the coherence of the transfer function (linear correlations among BP, RSNA, and RBF) and the mutual information value (linear and nonlinear correlation) in conscious WKY and SHR. We also determined the time delay among the parameters.

**Protocol 2: L-Arginine Infusion**

Nitric oxide has been reported to inhibit the SNS. To examine whether the higher linearity is due to an impaired nitric oxide system, we infused conscious SHR (n = 8) with L-arginine hydrochloride (50 μmol/kg per minute) for 60 minutes.

**Protocol 3: Oral Treatment With Candesartan**

To test the hypothesis that increased SNS and RAS account for the impaired linearity/nonlinearity, we compared the linearity and nonlinearity in SHR given candesartan (1 mg/kg per day; n = 8) or vehicle (n = 7) orally for 2 weeks.

All values are given as mean±SEM. Differences were considered significant at P<0.05. Intergroup differences were analyzed using Student’s unpaired t test. The differences in the parameters within the groups were analyzed using the paired t test.

**Results**

**Spectral Analysis**

The mean value of RSNA in conscious SHR was significantly higher than in WKY (15.4±6.1 versus 7.3±2.4 arbitrary units [AU]; P = 0.002), despite their higher BP (151±16 versus 114±6 mm Hg, P = 0.0002), but mean HR and RBF did not differ between SHR and WKY.

In power spectral components of BP, HR, RSNA, and RBF at 0 to 10 Hz, RSNA peaks were found at frequencies of 0.05, 0.80 to 0.90, 1 to 2, and 6 to 8 Hz in both strains, and RSNA...
powers at these frequencies were larger in SHR than in WKY. Because RSNA frequencies at 1 to 2 Hz are known to be respiratory-related and frequencies at 6 to 8 Hz are cardiac-related, we paid attention to the frequency band below 1.0 Hz. The power spectra at 0 to 1.0 Hz are shown in Figure 1. RSNA peaked at 0.05 and 0.80 to 0.90 Hz in both strains, and the result of these oscillations in RSNA was that BP, HR, and RBF showed a tightly coupled rhythmicity at the same frequencies. Therefore, assuming that RSNA components <1.0 Hz play an important role in regulating BP and RBF, we assessed the linear correlation by calculating the transfer function.

**Transfer Function**
One example of transfer function from RSNA (as input) to BP (as output) at 0 to 1.0 Hz in a WKY and an SHR is shown in Figure 2a. Coherence peaked at 0.05 and 0.80 Hz in both strains. In SHR, a more significant ($P>0.5$) and higher coherence was found at the broader frequency band than in WKY. Coherence (linearity) was significantly higher in SHR than in WKY, but the gain was smaller (Figure 2b). Similar results were obtained for the transfer function from RSNA (as input) to RBF (as output). Coherence was significantly higher in SHR than in WKY ($0.75\pm0.08$ versus $0.49\pm0.04$; $P<0.05$), whereas the gain was significantly smaller in SHR. In contrast, the coherence of the transfer function from BP (input) to RBF (output) was very high ($0.92\pm0.06$) and did not differ between WKY and SHR. Our data regarding the gain show that the relation between RSNA and BP or RBF functioned as a low-pass filter, whereas the relation between BP and RBF functioned as a high-pass filter, as Wittmann et al.$^{17}$ reported.

**L-Arginine Infusion**
After a 60-min intravenous infusion of L-arginine in SHR, RSNA decreased significantly (from $13.6\pm2.3$ to $11.9\pm1.8$ AU; $P<0.05$) and RBF increased (from $6.2\pm0.9$ to $6.6\pm0.9$ mL/min; $P<0.05$), but BP and HR remained unchanged. The coherence between RSNA and BP and between RSNA and RBF was significantly reduced by L-arginine (Figure 3), but the gain was unchanged. The high coherence between BP and RBF was not reduced by L-arginine (from $0.94\pm0.04$ to $0.88\pm0.05$; $P=0.20$). The latter data are compatible with a study performed in normotensive dogs.$^{18}$

**Mutual Information Method**
The maximum mutual information ($I_{\text{max}}$) values between RSNA and BP at 0 to 0.1 Hz indicate that RSNA and BP have

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**Figure 1.** Power spectrum of each parameter at 0 to 1.0 Hz. RSNA power peaks were found at 0.05 and 0.80 to 0.90 Hz. Similar peaks were found in BP, HR, and RBF.

**Figure 2.** Transfer function from RSNA (as input) to BP (as output) (a, one example; b, mean values in 7 rats of each strain). a, Coherence peaks were found at 0.05 and 0.80 Hz in both strains. A significant ($>0.5$) and higher coherence was found at the broader frequency band in SHR than in WKY. b, We divided the 0 to 1 Hz frequency band into 2 different bands: 0 to 0.5 Hz and 0.5 to 1.0 Hz. The coherence (linearity) in SHR was significantly higher than in WKY.
a strong general (both linear and nonlinear) correlation, but the values do not differ between WKY and SHR (Figure 4a). Similarly, the significance of $I_{\text{max}}$ values between RSNA and RBF suggests a strong correlation (Figure 4b). We calculated the time delay to determine the order of the parameters in intact animals. Mutual information analysis at 0 to 0.1 Hz showed that RSNA preceded BP and RBF in both strains (Figures 4c and 4d). The time delay between RSNA and RBF in SHR was significantly longer than in WKY. Simultaneous recordings that are low-pass filtered at $<0.1$ Hz (Figure 5) indicate the reliability of the time delay values.

### Long-Term Oral Treatment With Candesartan

The RSNA value in SHR given candesartan for 2 weeks was significantly lower (9.8±1.6 versus 15.2±3.2 AU; $P<0.01$) than in SHR given vehicle, despite the lower BP (131±4 versus 152±7 mm Hg; $P<0.05$). Coherence between RSNA and BP (Figure 6) or RBF (data not shown) was smaller in SHR given candesartan than in SHR given vehicle. The coherence in SHR given candesartan was as low as in WKY ($P=0.13$).

By contrast, the $I_{\text{max}}$ (linear and nonlinear correlation) between RSNA and BP or RBF was larger in SHR given candesartan than in SHR given vehicle (Figure 7). Thus, the nonlinearity was higher in SHR given candesartan. The time delay value between RSNA and BP or RBF was significantly smaller in SHR given candesartan than in SHR given vehicle.

### Discussion

**Linear and Nonlinear Correlations**

The coherence (linearity) between RSNA and both BP and RBF in SHR was significantly higher than in WKY. The high coherence between RSNA and BP around 0.4 Hz was consistent with the data of Brown et al.\(^1\) Our results are incompatible with an earlier study showing that the low-frequency component of splanchnic SNA was unrelated to BP.\(^2\) The reason for the difference may be that renal SNA regulates BP more directly than splanchnic SNA. Mutual information values demonstrated low-frequency components of RSNA to have strong linear and nonlinear correlations with BP and RBF. The linear and nonlinear correlations were similar in WKY and SHR (Figure 4), but the linear correlation was greater in SHR. Therefore, the nonlinear correlation was weaker in SHR than in WKY.

Reduced nonlinearity of HR variability is a predictor of high mortality in patients who have had myocardial infarction and is an indicator of sudden onset of ventricular fibrillation.\(^4\),\(^6\),\(^7\) However, the factors that connect the reduced nonlinearity with the poor outcome have not been identified. We demonstrated that 2 weeks of oral treatment with candesartan reduced the higher linearity and increased the lower nonlinearity in SHR. As far as we know, few reports have shown that angiotensin II receptor blockers improve the low nonlinearity and high linearity in hypertension. The RSNA value was higher in SHR than in WKY, and it was lower in SHR given candesartan than in SHR given vehicle. We had shown that candesartan suppresses the electrophysiological activity of RVLM neurons of SHR by the whole-cell patch-clamp method (T. Matsuura, MD, et al, unpublished data, July 2001). Therefore, our results suggest that the potentiated SNS and RAS are responsible for the higher linearity and lower nonlinearity in SHR. However, we recognize that this does not necessarily mean this is the only exact mechanism underlying the altered cardiovascular control in SHR. An impaired endogenous nitric oxide system is another factor

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**Figure 3.** Coherence in SHR before and after a 1-hour infusion of L-arginine. Coherence between RSNA and BP or RBF was significantly reduced by L-arginine, but the gain remained unchanged. High coherence between BP and RBF was not significantly reduced.

**Figure 4.** Maximum mutual information values ($I_{\text{max}}$) between RSNA and BP (a) and between RSNA and RBF (b) at 0 to 0.1 Hz. The $I_{\text{max}}$ values did not differ between WKY and SHR. Time delay values showed that RSNA precedes BP (c) and RBF (d). The time delay between RSNA and RBF was significantly longer in SHR.
that increases the linearity in SHR, because L-arginine infusion decreased RSNA and reduced the linearity of the RSNA-BP and RSNA-RBF relations. Because the nonlinearity decreased and the SNS was activated in baroreceptor-denervated normotensive dogs,3 the baroreflex system contributes to normal nonlinearity and complexity. The baroreflex system is reported to be impaired in SHR.21 Therefore, the higher linearity and lower nonlinearity in SHR can be explained by strong dependence of the cardiovascular regulation on 1 or 2 predominant neurohumoral systems (the SNS and RAS) and/or by a decrease in the number of neurohumoral systems (nitric oxide and baroreflex). By contrast, the higher nonlinearity and complexity in normotensive WKY may imply that regulation of BP and RBF depends on various neurohumoral systems and not solely on the SNS.

By directly recording RSNA, we were able to show that the increased RSNA plays a key role in reducing the nonlinearity and increasing the linearity of SHR. The potentiated SNS in SHR may be the missing link connecting reduced nonlinearity and poor prognosis of hypertension, because the potentiated SNS is an independent risk factor for cardiovascular diseases.22

**Time Delay Value Determined by the Mutual Information Method**

To test whether BP precedes RSNA via the baroreflex, we calculated the time delay among BP, RSNA, and RBF by assuming that the time delay, at which the maximum mutual information value between the signals occurs, represents a physiological delay. We demonstrated RSNA to precede BP and RBF at 0 to 0.10 Hz in conscious rats. Because it is difficult to determine which parameter precedes the other in intact animals, Guil et al23 assessed the time difference by electrically stimulating the renal nerves in anesthetized rabbits. However, because electrical stimulation cannot mimic the selective recruitment of individual nerves that occurs in the ongoing of sympathetic activity,23 we calculated the time delay between the naturally occurring RSNA and RBF or BP in conscious rats. The time delay value between RSNA and RBF in WKY is consistent with their result that RBF is reduced 670 ms after nerve stimulation.23 Our time delay values are also compatible with neuroeffector delays.24 Earlier studies used baroreceptor denervation as a method to perturb and examine cardiovascular control in conscious animals.3 Our method of calculating time delay under intact conditions is free from pharmacological interventions.
closed-loop conditions without cutting any part of the neurocirculatory system can obtain important data similar to those obtained by baroreceptor denervation. If BP precedes RSNA, RSNA would be suppressed by BP elevation via the baroreflex. However, because the time delay values (Figures 4c and 4d) and Figure 5 demonstrate that the RSNA increase precedes BP elevation, this correlation in the low-frequency band (<0.1 Hz) is baroreflex-independent. Wagner et al.3 showed that although the nonlinearity of BP regulation was attenuated by baroreceptor denervation, some of the nonlinear component remained. That study3 strongly supports our results. Although most investigators have reported the relations between BP, HR, and RSNA are regulated exclusively by baroreflex, Taylor and Eckberg25 have demonstrated that the low-frequency component (0.1 Hz) of transfer function between BP and HR is baroreflex-independent in normotensive humans. Therefore, we think that the distinct slow oscillations of sympathetic activity arise separately from the central nervous system, such as RVM neurons, and do not necessarily reflect either the resonance or lag time of the baroreflex.26

In summary, we have demonstrated the following new method and findings. (1) BP, HR, RSNA, and RBF on the same side were simultaneously recorded in conscious rats to examine the linear and nonlinear correlations among the parameters. (2) Distinct coherence between RSNA and BP or RBF was found at 0.05 and 0.80 Hz. (3) Oral treatment with candesartan reduced the higher linearity and increased the lower nonlinearity in SHR.

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