Effects of autonomic blockers on linear and nonlinear indexes of blood pressure and heart rate in SHR

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MESTIVIER, Denis, Hubert Dabire, and Nguyen Phong Chau. Effects of autonomic blockers on linear and nonlinear indexes of blood pressure and heart rate in SHR. Am J Physiol Heart Circ Physiol 281: H1113–H1121, 2001.—Recent results in normotensive Wistar-Kyoto (WKY) rats show that nonlinear method may be more specific to quantify sympathetic and parasympathetic activities than the low (LF) and high frequencies (HF) spectral powers of blood pressure (BP) and R-R interval (RR). The present study extends this conclusion to spontaneously hypertensive rats (SHR). Blood pressure was recorded for 30 min before and after intravenous injection of saline, hexamethonium, atropine, atenolol, or prazosin. Mean level, standard deviation (SD), spectral LF and HF components, and three nonlinear indexes (percentage of recurrence, percentage of determinism, and length index of the recurrence plot method) were used to analyze the BP and RR signals. In conscious SHR, sympathetic but not parasympathetic blockade reduced BP level and LF-BP, and increased nonlinear indexes of BP. RR increased after β-sympathetic and ganglionic blockade, decreased after parasympathetic blockade, and remained unchanged after α1-sympathetic blockade. SD-RR decreased after ganglionic and α1 blockade, whereas HF-RR increased after β-sympathetic blockade. The effects on nonlinear indexes of RR are clear and consistent: only α1-blockade increased the indexes. Our nonlinear indexes may be useful to investigate cardiovascular functions in normotension and hypertension.

METHODS

The experiments were performed in conscious, male SHR (Charles River France; St. Aubin-les-Elbeuf, France). The rats were received in the laboratory at 12 wk of age and were housed three per cage during 1 wk at 22–24°C, with lights on from 0600 to 1800 and pellets and water ad libitum. The rats were randomized into five groups of six or seven rats each: control group (NaCl 0.9%, 100 μl/kg iv, n = 7); atropine group (atropine, 0.5 mg/kg iv, n = 6); β-blocking group (atenolol, 1 mg/kg iv, n = 6); α1-blocking group (prazosin, 1 mg/kg iv, n = 6); and ganglion-blocked group (hexamethonium, 20 mg/kg iv, n = 6). The drugs were dissolved in saline; doses refer to the salt.

In recent studies, it has been argued that the mechanisms regulating heart rate and blood pressure are most probably nonlinear (17, 34) and several authors (5, 24, 28, 33, 38–40) have used nonlinear techniques to analyze heart rate and blood pressure series in healthy condition and in various pathological states and have obtained reliable results. Dabire et al. (10) showed that in normotensive Wistar-Kyoto (WKY) rats, ganglionic blockade by hexamethonium, and α1-sympathetic blockade by prazosin increased some “nonlinear indexes” of blood pressure. In contrast, parasympathetic blockade by atropine increased some nonlinear indexes of R-R interval (RR). These results indicate that nonlinear methods might be useful to explore the sympathetic and parasympathetic systems in the normotensive rats. Nonlinear indexes were more specific markers of sympathetic and parasympathetic tones than spectral indexes (10). The aim of the present study was to investigate the relationships between the same nonlinear indexes and autonomic system in spontaneously hypertensive rats (SHR). We used the same protocol as in Ref. 10, which addressed to normotensive rats. In this study, the sympathetic and/or parasympathetic systems were blocked by infusion of atropine, hexamethonium, atenolol, or prazosin. Intra-arterial blood pressure and heart rate were measured. Nonlinear indexes defined by the recurrence plot (see METHODS for details) and spectral indexes were analyzed in rats under drugs compared with rats under neutral saline infusion.

Since the study by Akselrod et al. (4) was published in 1981, it has been suggested that the high-frequency (HF) component of heart rate spectral power may be a marker of parasympathetic tone, whereas the low-frequency (LF) component of blood pressure spectral power may be a good marker of the sympathetic activity. In contrast, the LF component of heart rate spectral power may reflect both sympathetic and parasympathetic activities (2, 25, 29). However, the use of these indexes as sympathetic and parasympathetic markers is still under debate (11, 15, 16, 18, 26, 31).

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Nonlinear indexes and sympathetic tone in SHR

The experimental procedure, animal surgery, blood pressure recording, and analysis have been described by Dabiré et al. (10). In brief, 2 days before blood pressure recording, the rats were anesthetized with pentobarbital sodium (60 mg/kg ip). Two polyethylene (PE) catheters [a PE-10 (ID 0.28 mm, OD 0.61 mm; Clay Adams, Parsippany, NJ) fused to a PE-50 (ID 0.58 mm, OD 0.96 mm; Guerbet; Louvres, France)] filled with heparinized 0.9% NaCl (50 U/ml) were inserted into the lower abdominal aorta via the left femoral artery and the left femoral vein for blood pressure recording and intravenous drug injection, respectively. The two catheters were tunnelled subcutaneously under the skin of the back to exit between the scapulae and were plugged with a short piece of stainless steel wire. The rats were then allowed to recover from anesthesia for 48 h in individual cages. The two catheters were flushed twice daily with a solution of heparinized NaCl.

Recording of arterial pressure and intravenous injection of drugs were performed in unrestrained rats after the 2 days of recovery. The venous catheter was connected to a syringe for saline or drug injections. The arterial catheter was connected to a pressure processor via a pressure transducer (Statham model P23 ID, Gould Instruments; Longjumeau, France). After 60 min of stabilization, arterial blood pressure was recorded on an eight-channel digital audiotape recorder (model DTR-1800, Biologic; Claiex, France). A series of two recordings (30 min each) was performed, the first one immediately after the 1-h period of stabilization. After that, saline or a drug was injected and, 20 min later, a second series (30 min) of recording was performed. Each rat received a single injection of saline or a drug. Injections were flushed with 30 μl of saline. At the end of the second series of 30-min recording the rat was euthanized.

The two 30-min blood pressure signal periods were sampled at 1 kHz through the digital audio tape recorder by a MacLab system (ADInstruments; London, UK). From this blood pressure wave, local maxima [systolic blood pressure (SBP)], local minima [diastolic blood pressure (DBP)], and time intervals from systolic-to-systolic blood pressure (RR) were computed. Each 30-min record afforded a series of 8,000–12,000 beat-to-beat SBP and DBP. SBP and DBP outside the range of 60–300 and 30–250 mmHg, respectively, were considered artifacts; <1% of values were in that group. To handle artifacts, a moving window of 200 values was screened along the series. In each window, we computed the mean value and SD of SBP and DBP. Whenever an artificial SBP or DBP was encountered, the values of SBP or DBP were replaced by the mean of the windows.

We used a package of personal programs based on the formulas of Anderson (7) for Fourier analysis of blood pressure and RR. We calculated the total area under the Fourier spectrum and the percentage of this area in the LF band (0.25–0.75 Hz) and HF band (0.75–2.56 Hz) (8).

Nonlinear indexes were defined by the recurrence plot method (24, 38, 39). To explain the recurrence plot method, let us consider an example. Given a series of length N, the recurrence plot method looks for recurrent values in the series and records these recurrences in an N × N square plot. Two values in this series, \( X_i \) and \( X_j \) (where \( i \) and \( j \) run from 1 to \( N \)), are recurrent if their mutual distance is less than a threshold \( r \), \( X_i - X_j < r \). If \( X_i \) and \( X_j \) are recurrent, we draw a black pixel at the location \([i, j]\). The plot is symmetrical to the diagonal line; therefore, only one-half of the figure is plotted with the diagonal excluded. Of particular interest are the diagonal segments of length \( k \) in the plot: when a recurrence is found at \( X_i \) and \( X_j \), the trajectories issued from \( X_i \) and \( X_j \) remain parallel for \( k \) subsequent beats.

Figure 1A, as an example, shows a series of 10 SBP \((\times 1, \times 2, \times 3, \ldots , \times 10)\). Take \( r = 2 \) to define the distance threshold. We see that \( \times 7 \) is recurrent to \( \times 1 \). Furthermore, the subsequent points, \( \times 8, \times 9, \) and \( \times 10 \), are recurrent to \( \times 2, \times 3, \) and \( \times 4 \), respectively. To mark these recurrences, we plotted...
in Fig. 1B the points [1,7]-[2,8]-[3,9]-[4,10]. These points form a diagonal line. If several long diagonal lines are observed, this means that sequences of data issued from similar levels remain long time parallel. We say that the dynamic is highly deterministic. From the plot, we computed three indexes: 1) the percentage of recurrence (%rec), which counts the percentage of black pixels in the plot; 2) the percentage of determinism (%det), which counts the percentage of black pixels that are in diagonal segment of length >2; 3) $L_{\text{max}}$, the length of the longest diagonal segment; $L_{\text{max}}$ is inversely related to the highest “Lyapunov exponent,” a measure of divergence of trajectories (39). In Fig. 1A, we have %rec = $(4 + 2)/(9 \times 10/2) = 0.12$, %det = $(3)/(4 + 2) = 0.50$, and $L_{\text{max}} = 4$. Figure 1, C and E, show the SBP series from a SHR treated with solvent or prazosin, respectively. Figure 1, D and F, show the recurrence plot for a series of 250 data.

In fact, the effective calculations are more complex: as in our previous work (10), we embedded the series in a $p$-dimensional Euclidian space, using the time-delay reconstruction of Takens. The recurrence threshold $r$ was set as $r = \sqrt{p} \cdot SD$, where SD is the average of all standard deviation of the series. Values used were $r = 7$ mmHg for SBP, 6 mmHg for DBP, and 5 ms for RR. The embedding dimension was set to $p = 10$.

The results are expressed as means ± SE. Student’s $t$-test for paired comparisons was used to assess the drug effects. One-way analysis of variance, followed by a Bonferroni test for multiple comparisons was used to compare baselines values in SHR (22). Comparisons between WKY and SHR were performed with the use of two-way analysis of variance, followed by a Bonferroni correction for multiple comparisons, yielding strain effect, treatment effect, and interaction. $P < 0.05$ was considered statistically significant.

RESULTS

Baseline values of DBP, SBP, and RR, and their linear and nonlinear indexes did not differ in the five groups of SHR used. Intravenous administration of the solvent did not change any of these parameters.

Effects of autonomic blockade on linear indexes. Figure 2 shows the effects of the treatments on blood pressure, RR, and their SDs. Although both hexamethonium and prazosin significantly ($P < 0.001$ for both) reduced DBP, only prazosin significantly ($P < 0.01$) decreased SD-DBP. Atenolol and atropine did not change DBP and its SD. Similar results were observed...
on SBP and SD-SBP. Atenolol significantly \((P < 0.001)\) increased RR but did not change its SD. In contrast, hexamethonium significantly \((P < 0.05)\) increased RR but reduced SD-RR \((P < 0.01)\). Atropine reduced RR \((P < 0.05)\) but did not change its SD. In contrast, prazosin did not change RR but decreased SD-RR \((P < 0.01)\).

Figure 3 shows the effects of the treatments on blood pressure and RR power spectra. Hexamethonium and prazosin significantly reduced LF-DBP \((P < 0.01\) and \(P < 0.001\), respectively). LF-DBP remained unchanged under atropine and atenolol. Similar results were observed on LF-SBP. None of the treatments changed the HF component of DBP or SBP. Likewise, LF-RR remained unchanged and only atenolol significantly increased HF-RR \((P < 0.01)\).

Effects of autonomic blockade on nonlinear indexes. Figure 4 shows the effects of the treatments on the three nonlinear indexes of blood pressure and RR. Hexamethonium and prazosin significantly increased \%rec, \%det, and \(L_{\text{max}}\) of DBP \((P < 0.01\) for all treatments on all indexes, except \(P < 0.05\) for hexamethonium on \%det). Similar results were observed on the nonlinear indexes of SBP. Atenolol and atropine did not change nonlinear indexes of blood pressure. On RR, only prazosin significantly increased its nonlinear indexes \((P < 0.05\) for \%rec, \(P < 0.01\) for \%det, and \(L_{\text{max}}\)). The other treatments did not change the nonlinear indexes of RR.

Comparisons between WKY rats and SHR. Figure 5 shows the differences in baseline values between WKY rats and SHR. Data on WKY rats are from Dabiré et al.
(10). SHR have higher DBP, SBP and their SD ($P < 0.001$ for both) than WKY rats. Although RR was also higher in SHR than in WKY rats ($P < 0.001$), no difference was observed in SD-RR between the two strains. The LF components of DBP, SBP, and RR were significantly lower in SHR than in WKY rats ($P < 0.01$, $P < 0.01$, and $P < 0.05$, respectively). In contrast, only the HF component of SBP was lower ($P < 0.001$) in SHR than in WKY rats. The nonlinear indexes ($%\text{rec}$, $%\text{det}$, and $L_{\text{max}}$) of DBP and SBP were significantly lower ($P < 0.001$) in SHR than in WKY rats. In contrast, only $%\text{rec}$-RR was lower ($P < 0.05$) in SHR than in WKY rats.

**DISCUSSION**

Table 1 summarizes the results of the present study. In conscious SHR, sympathetic but not parasympa-
Fig. 5. Comparison between normotensive Wistar-Kyoto (WKY) rats and SHR. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$. ns, Not significant, using two-way analysis of variance, followed by a Bonferroni test for multiple comparisons.
and remained unchanged after blockade, it decreased after parasympathetic blockade, sympathetic tone, and more specifically, the LF of blood pressure, suggesting the participation of a particular property remains controversial (23).

Central effects of effect of prazosin on RR may be ascribed to the putative mediated heart rate in conscious rats (1). The lack of hexamethonium does not adequately block the vagally in heart rate. On the other hand, the effects of hexamethonium could be related to its inadequate component of blood pressure power spectra and increased nonlinear indexes of blood pressure. Although RR increased after β-sympathetic and ganglionic blockade, it decreased after parasympathetic blockade, and remained unchanged after α₁-sympathetic blockade; SD of RR decreased after ganglionic and α₁-blockade, whereas HF-RR increased after β-sympathetic blockade. The effects on nonlinear indexes of RR are more homogenous because only α₁-blockade increased them.

Our results show that in conscious SHR, sympathetic and parasympathetic blockade induced the well-known effects on blood pressure and RR levels. Indeed, ganglionic and α₁-sympathetic, but not β-sympathetic, blockades significantly reduced blood pressure. Antonnacio et al. (8) have shown that β-adrenoceptor blockade reduced blood pressure in conscious SHR provided that sufficient time is allowed for this observation to occur, this effect being unrelated to the acute changes in heart rate. On the other hand, the effects of hexamethonium on heart rate are species dependent and hexamethonium does not adequately block the vagally mediated heart rate in conscious rats (1). The lack of effect of prazosin on RR may be ascribed to the putative central effects of α₁-blockers (20), although this property remains controversial (23).

Ganglionic blockade by hexamethonium and in particular α₁-blockade by prazosin significantly reduced LF of blood pressure, suggesting the participation of sympathetic tone, and more specifically, the α-adrenergic component, in the LF component of the blood pressure power spectrum. These results agree with those suggesting the LF component of the blood pressure power spectrum as a marker of sympathetic tone (9, 21, 30).

The participation of the parasympathetic system in the HF component of RR is less clear cut, because in our experiments, atropine did not change the HF component of the blood pressure power spectra. These results strengthened the conclusion that the indexes derived from spectral analysis could not be considered as specific markers of parasympathetic tone (11, 15, 16, 18, 19, 26, 31, 32).

Results obtained with nonlinear indexes are more clear cut than those observed in the time and frequency domains. Parasympathetic blockade by atropine and β-sympathetic blockade by atenolol did not change %rec, %det, and Lmax of blood pressure or RR. These results ruled out the participation of the parasympathetic system and that of the β-sympathetic component of the sympathetic system in the modification of nonlinear indexes of blood pressure and RR. In contrast, sympathetic blockade by hexamethonium and in particular α₁-sympathetic blockade by prazosin, significantly increased both %rec, %det, and Lmax of blood pressure, indicating the participation of the sympathetic tone and, more specifically α₁-sympathetic component, in the changes of the three nonlinear indexes of blood pressure. Therefore, our results suggest that nonlinear indexes of blood pressure may be used as good markers of sympathetic tone.

The results obtained with nonlinear indexes of RR are more difficult to discuss because only prazosin increased nonlinear indexes of RR. The lack of effect of hexamethonium could be related to its inadequate blockade of the vagal component at the ganglionic level (1). On the other hand, our results are in concordance with those studies (17, 35, 41) showing that in rabbits and humans, β-adrenoceptor blockade did not change the highest Lyapunov exponent of heart rate.

**Table 1. Effects of autonomic blockade on linear and nonlinear indexes of blood pressure and R-R interval in SHR**

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SHR, spontaneously hypertensive rats; $L_{\text{max}}$, length index; SD, standard deviation; LF, low frequency; HF, high frequency; %rec, percentage of recurrence; %det, percentage of determinism; ↓, reduction; ↑, increase; →, no change. 1 Arrow, $P < 0.005$; 2 arrows, $P < 0.01$; 3 arrows, $P < 0.001$, using Student’s $t$-test for paired comparisons.
Taken together our results clearly indicate the participation of sympathetic nervous system, in particular $\alpha$-sympathetic system, in the modifications of nonlinear indexes of blood pressure. Thus, in contrast to the linear indexes derived from the spectral analysis, nonlinear indexes derived from recurrence plot method better reflect sympathetic tone.

Realized under the same experimental conditions as those already published (10) in WKY rats, the present results obtained in SHR allow us to perform comparisons between the two strains in term of baseline values and drugs effects. Baseline values of blood pressure and RR were higher and LF component of blood pressure and RR lower in SHR than in WKY rats. If we assume a dysbalance in the autonomic regulation of blood pressure in SHR, an increased sympathetic drive and/or decreased parasympathetic tone should be expected, with an increased blood pressure and heart rate as a result. Indeed, an increased sympathetic tone is commonly accepted in hypertension (6, 12, 13, 16). Therefore, sympathetic markers may increase in hypertension. However, although an increased level of blood pressure is reported (3, 27, 31) in SHR compared with WKY rats, no difference in heart rate is frequently observed between the two strains. Moreover, conflicting results have been reported on the linear markers of sympathetic activity, namely the LF of blood pressure and heart rate. Whereas Akselrod et al. (3) reported significantly lower low- and midfrequency fluctuations in blood pressure in SHR compared with WKY rats, others have shown no difference in the midfrequency power of blood pressure and heart rate (27, 31). The results of our experiments are thus in agreement with the precedent ones.

In a pharmacological point of view, results obtained in both SHR and WKY rats suggest the implication of the sympathetic tone, and more specifically, the $\alpha$-sympathetic component in the modifications of nonlinear indexes of blood pressure. On the other hand, although not directly related to the variance, the $%$rec may be considered as a measure of the variability of the series, the higher the $%$rec, the lower the variability of the series. The $%$det may be considered as an index of the predictability or regularity of the dynamic. The higher the $%$det, the higher the dynamic is regular or can be predicted. The length index $L_{\text{max}}$ is inversely related to the highest Lyapunov exponent, allowing measurement of divergence of trajectories. A high-Lyapunov exponent, i.e., a short $L_{\text{max}}$, expresses a "chaotic" dynamic. The present results show that the nonlinear indexes of blood pressure were lower in SHR than in WKY rats suggesting that the dynamic of blood pressure in SHR is more chaotic than in WKY rats. In other words, the dynamic of blood pressure is more variable (lower $%$rec), less predictable (lower $%$det), and less sensitive to initial conditions (lower $L_{\text{max}}$) in SHR than in WKY rats. However, this seems at variance with the increased periodicity associated with increased regularity or predictability observed in some pathologic conditions, such as severe congestive heart failure (14). However, it might be relevant to note that most of the results summarized in this review have been obtained in human and on heart rate series. To our knowledge, little data are available on blood pressure in animal. Moreover, Yip et al. (36, 37) observed almost periodic oscillations of tubular pressure in the kidneys of normal rats. The oscillations had more aperiodic character after arterial clipping of one kidney and in SHRs. The results of our present study were in the same direction as those of Yip et al. (37). Therefore, it seems that the question whether physiological dynamics are more or less chaotic in healthy status compared with some disease status cannot be stated in a general manner. The answer depends on the parameter analyzed, the type of pathology, the species, or both.

In conclusion, the present results indicate that compared with linear indexes, nonlinear indexes derived from the recurrence plot method may be useful quantitative tools to investigate cardiovascular functions in normotension as well as hypertension.

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