Quantification of sympathetic and parasympathetic tones by nonlinear indexes in normotensive rats

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Quantification of sympathetic and parasympathetic tones by nonlinear indexes in normotensive rats. Am. J. Physiol. 275 (Heart Circ. Physiol. 44): H1290–H1297, 1998.—Because the use of spectral powers of blood pressure (BP) and R-R interval (RR) in the low (LF) and high frequencies (HF) to quantify sympathetic and parasympathetic activities is still under debate, we questioned whether nonlinear methods may give better results. The BP signal was recorded for 30 min before and after intravenous injection of hexamethonium (20 mg/kg), atropine (0.5 mg/kg), atenolol (1 mg/kg), and prazosin (1 mg/kg) in conscious, normotensive Wistar-Kyoto rats. Three nonlinear indexes [percentage of recurrence, percentage of determinism, and length index (Lmax)] extracted from the recurrence plot method were used to analyze the BP signal. Sympathetic but not parasympathetic blockade reduced BP level and its LF component. RR increased and decreased after β- and α-blockades, respectively. Hexamethonium increased HF, and atropine reduced LF, of RR. Sympathetic blockade and, in particular, α-sympathetic blockade increased nonlinear indexes of BP. In contrast, parasympathetic blockade by atropine increased nonlinear indexes of RR. These results suggest that, compared with spectral indexes, nonlinear indexes may be more specific markers of sympathetic and parasympathetic tones.

autonomic nervous system; cardiovascular control; spectral analysis; recurrence plot

IT HAS BEEN SUGGESTED that spectral analysis of blood pressure and heart rate may provide information on the function of the autonomic nervous system in cardiovascular control. Results obtained in both humans and animals suggest 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 better marker of sympathetic activity. In contrast, the LF component of heart rate spectral power reflects both sympathetic and parasympathetic activities (2, 6, 13, 19, 22).

However, recent clinical and experimental results showed that the HF component of the heart rate power spectrum does not always reliably measure parasympathetic tone and that no blood pressure or pulse interval power in the midfrequency and LF ranges can be regarded as a specific marker of sympathetic activity (8, 10, 12, 20, 25).

In recent years, it was argued that the mechanisms regulating heart rate and blood pressure are most probably nonlinear (11, 14, 24, 28, 29, 32). Indeed, several authors used nonlinear techniques to analyze heart rate and blood pressure time series in healthy conditions and in various pathological states and obtained reliable results (3, 17, 21, 27, 33, 34, 36).

However, most of these studies were focused on the calculation of “nonlinear indexes” of cardiovascular time series, but little attention was devoted to the physiological and/or pharmacological significance of these indexes. Therefore, the present study was designed to investigate specifically the latter issues. We studied in normotensive Wistar-Kyoto (WKY) rats the modifications of nonlinear indexes after different pharmacological interventions in the autonomic nervous system: vagal blockade by atropine, ganglionic blockade by hexamethonium, β-sympathetic blockade by atenolol, and α-sympathetic blockade by prazosin. The blood pressure and heart rate signals were analyzed in the time domain (standard deviation), the frequency domain (Fourier transform), and by the recurrence plot method. This last method has been used to explore nonlinear regulations in physiological time series (9, 33).

METHODS

Blood Pressure Recording

The experiments were performed in conscious, normotensive male WKY rats (Charles River France, St. Aubin-lès-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.

Two days before blood pressure recording, the rats were anesthetized with pentobarbital sodium (60 mg/kg ip). Two polyethylene 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 tunneled 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 Gould 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 audio tape recorder (DTR-3800, Biologic, Claix, 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 killed.

The rats were randomized into five groups of six to seven rats each: control group (NaCl 0.9%, 100 µl/kg iv, n = 6); atropine group (atropine, 0.5 mg/kg iv, n = 6); β-blocking group (atenolol, 1 mg/kg iv, n = 7); α₁-blocking group (prazosin, 1 mg/kg iv, n = 6), and ganglion-blocked group (hexamethonium, 20 mg/kg iv, n = 7). The drugs were dissolved in saline; doses refer to the salt.

Blood Pressure Analysis

Blood pressure levels. The three 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 [R-R intervals (RR)] were computed. Each 30-min record afforded a time 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 time series. In each window, we computed the mean value and SD of SBP and DBP. Whenever an artifactual SBP or DBP was encountered, the values of SBP or DBP were replaced by the mean of the windows.

Linear methods: Spectral analysis. We used a package of personal programs based on the formulas of Anderson (5) 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 (0.25–0.75 Hz) and HF (0.75–2.56 Hz) bands (6).

Nonlinear methods: Recurrence plot method. One characteristic of nonlinear dynamics that we consider most important is the “Lyapunov exponent.” The meaning of this index is simple to explain. Some dynamics have the property of “sensitivity to initial conditions.” Starting from two similar values, the systems may generate two sequences that quickly (exponentially) diverge one from the other. The exponent of this divergence is called the Lyapunov exponent.

The direct calculation of the Lyapunov exponent from an experimental time series is complicated and requires very long and stationary series (31). To obtain an approximation of the exponent, we used an index called Lmax derived from the recurrence plot method (9, 35). The recurrence plot method is described in the Appendix. The method defines two other indexes, the percentage of recurrence (%rec) and the percentage of determinism (%det) (30). The recurrence plot method has been applied to various biological data in previous reports and has given interesting results (17, 30, 33–35).

Statistical Analysis

The results are expressed as means ± SE. A Student’s t-test for paired comparisons was used to assess the significance of the results. Comparison of baselines values was performed by an analysis of variance followed by a Bonferroni test for multiple comparisons (16). 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 WKY rats used. Intravenous administration of the solvent did not change any of these parameters.

Effects of Autonomic Blockade on Linear Indexes

Figure 1 shows the effects of autonomic nervous system blockade on blood pressure and RR. Hexamethonium, atenolol, and prazosin significantly reduced the DBP level (P < 0.001, P < 0.01, and P < 0.05, respectively). In contrast, atropine did not change the DBP level. Hexamethonium and prazosin significantly (P < 0.05 and P < 0.01, respectively) reduced the SD of DBP in parallel with the decrease in DBP level. In contrast, although the DBP level was reduced by atenolol, its SD remained unchanged. Like the DBP level, the SD of DBP remained unchanged by atropine. Similar effects were observed for the SBP level. Only prazosin and atenolol significantly reduced (P < 0.05) the SD of SBP in parallel to the decrease in the SBP level.

Hexamethonium and atropine did not change RR, whereas atenolol significantly increased (P < 0.001) and prazosin significantly reduced (P < 0.05) RR. However, all four treatments significantly decreased (P < 0.05 for both) the SD of RR.

Figure 2 shows the effects of autonomic nervous system blockade on blood pressure and RR power spectra. Hexamethonium and prazosin significantly reduced the LF of DBP (P < 0.01 for both). In contrast, atropine and atenolol did not change the LF. Similar effects were observed on the LF of SBP. The HF of DBP significantly decreased with atropine (P < 0.05), increased with prazosin (P < 0.05), and remained unchanged by hexamethonium and atenolol. Only atenolol significantly increased (P < 0.01) the HF component of the SBP power spectrum.

Atropine slightly decreased (P < 0.05) the LF component and hexamethimation increased (P < 0.001) the HF component of RR. The other treatments did not change the RR power spectral components.

Effects of Autonomic Blockade on Nonlinear Indexes

Figure 3 shows the effects of autonomic nervous system blockade on the nonlinear indexes of blood pressure and RR. Hexamethonium and prazosin significantly increased %rec (P < 0.01 and P < 0.001, respectively), %det (P < 0.001 for both), and Lmax (P < 0.01 for both) of DBP. In contrast, atropine and atenolol did not change %rec, %det, or Lmax of DBP. Similar effects were observed on nonlinear indexes of SBP.

Results obtained for %rec, %det, and Lmax of RR were less clear-cut than those for blood pressure. Hexamethonium, atropine, and atenolol significantly increased
%rec of RR (P < 0.01, P < 0.05, and P < 0.05, respectively). However, only hexamethonium and atropine significantly increased %det (P < 0.05 and P < 0.001, respectively) and L_max (P < 0.05 for both) of RR. Atenolol and prazosin did not change the other nonlinear indexes of RR.

**DISCUSSION**

The results of the present study are summarized in Table 1. In conscious, normotensive WKY rats, sympathetic but not parasympathetic blockade reduced blood pressure level and its linear indexes and increased nonlinear indexes of blood pressure. Although RR increased after β-blockade and decreased after α-blockade, all four treatments reduced the SD of RR. Sympathetic blockade increased the HF component, whereas parasympathetic blockade reduced the LF component, of the RR power spectrum. On nonlinear indexes of RR, hexamethonium and atropine significantly increased %rec, %det, and L_max of RR, atenolol increased only %rec of RR, and prazosin did not change the nonlinear indexes of RR.

Linear Indexes of Blood Pressure and RR

Sympathetic and parasympathetic blockade induced the well-known effects on blood pressure level, i.e., no change after atropine and a significant reduction of blood pressure level by hexamethonium, atenolol, and prazosin. The hypotension was accompanied with reflex tachycardia (reduction of RR) in prazosin-treated rats only. Although hexamethonium reduces blood pressure in animals and humans, its effects on heart rate are species dependent. In our experiments, hexamethonium did not change RR. Similar results have been reported in conscious, normotensive rats (13) and may be ascribed to the fact that hexamethonium does not adequately block the vagally mediated heart rate response in conscious rats (1).

Ganglionic blockade by hexamethonium and α1-blockade by prazosin significantly reduced the LF
component of blood pressure, suggesting the participation of sympathetic tone and, more specifically, the \(\alpha\)-adrenergic component in the LF component of the blood pressure power spectrum. These results agree with those suggesting the LF component of blood pressure power spectrum as a marker of sympathetic tone (6, 13, 23). However, the modifications of the SD and the LF component of blood pressure spectral power did not always parallel those of the blood pressure level, indicating dissociation between changes in blood pressure level and changes in blood pressure variability assessed in the time and frequency domains (4, 7).

The participation of the parasympathetic system in the HF component of RR is less clear-cut in the present results. According to Japundzic et al. (13), in normotensive rats parasympathetic blockade with atropine almost suppressed the HF component of the heart rate power spectrum, whereas hexamethonium, prazosin, and atenolol did not change it. In our experiments, parasympathetic blockade by atropine almost suppressed the HF component of the power spectrum but significantly reduced the LF component. By contrast, ganglionic blockade increased the HF component of the RR power spectrum. This apparent contradiction between our results and those of Japundzic et al. (13) may result from the methods of quantification and the subdivision of the spectrum (20). However, our finding did support the participation of both sympathetic and parasympathetic systems in the different components of the RR power spectrum (2, 13, 19, 22), but it remained difficult to draw a clear-cut conclusion from the present results. Taken together, our results agree with the conclusion that the LF component of the blood pressure power spectrum and the HF component of RR cannot be considered as specific markers of sympathetic and parasympathetic tone, respectively (8, 10, 12, 20, 25).

Nonlinear Indexes of Blood Pressure and RR

In contrast to the results obtained in the time and frequency domains, those observed with nonlinear indexes are clear-cut. Parasympathetic blockade by atropine and \(\beta\)-sympathetic blockade by atenolol did not
change %rec, %det, or $L_{\text{max}}$ of blood pressure. In contrast, sympathetic blockade by hexamethonium and in particular $\alpha_1$-sympathetic blockade by prazosin significantly increased %rec, %det, and $L_{\text{max}}$ of blood pressure. It is interesting to note that nonlinear indexes of DBP seem more sensitive than nonlinear indexes of SBP to autonomic blockade. These results clearly indicate the participation of the sympathetic tone and, more specifically, the $\alpha_1$-sympathetic component, in the changes of the three nonlinear indexes of blood pressure and suggest that these indexes may be used as good markers of sympathetic tone.
Although both linear and nonlinear indexes of blood pressure are changed by ganglionic blockade and $\alpha_1$-adrenoceptor blockade, the former decreased whereas the latter increased under these treatments. Thus modifications in linear indexes are positively correlated to those of blood pressure level, whereas modifications in nonlinear indexes are negatively correlated to those of blood pressure level. This observation confirmed another observation we recently made. In spontaneously hypertensive rats with a higher blood pressure level than normotensive rats, the nonlinear index $L_{\text{max}}$ of blood pressure is significantly lower than in normotensive WKY rats. Treatment of spontaneously hypertensive rats with clonidine, a drug known to inhibit sympathetic tone, significantly reduced blood pressure level and increased $L_{\text{max}}$ of blood pressure (18). It is interesting to note that these opposite modifications in linear and nonlinear indexes and blood pressure level were also reported in dogs. Wagner et al. (27) showed that in conscious dogs, baroreceptor denervation increased blood pressure level and its SD but reduced the highest Lyapunov exponent, indicating that the degree of "chaos" does not correspond to the amount of variability. Because $L_{\text{max}}$ is the reverse of the Lyapunov exponent, $L_{\text{max}}$ may be reduced with increased blood pressure level.

The results obtained with nonlinear indexes of RR mirrored those obtained with nonlinear indexes of blood pressure: parasympathetic blockade did not change nonlinear indexes of blood pressure but increased those of RR, and sympathetic blockade increased nonlinear indexes of blood pressure but did not change those of RR. These results indicate that the modifications in nonlinear indexes of RR are related to the parasympathetic system and suggest that these indexes may be used as markers of parasympathetic tone. The changes in nonlinear indexes of RR after ganglionic blockade could be explained by the fact that hexamethonium blocks the neurotransmission of both sympathetic and parasympathetic systems at the ganglionic level. On the other hand, the increase in %rec induced by atenolol may be ascribed to its selectivity for cardiac $\beta_1$-adrenoceptors. In both rabbits and humans, $\beta$-adrenoceptor blockade did not change the highest Lyapunov exponent of heart rate (11, 32, 37).

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

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<thead>
<tr>
<th>Linear Indexes</th>
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<td>SD LF HF %rec</td>
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SD, standard deviation; LF, low frequency; HF, high frequency; %rec, percentage of recurrence; %det, percentage of determinism. See APPENDIX for calculation of length index ($L_{\text{max}}$). $\downarrow$, reduction; $\uparrow$, increase; $\rightarrow$, no change. 1 Arrow, $P < 0.05$; 2 arrows, $P < 0.01$; 3 arrows, $P < 0.001$ using Student's $t$-test for paired comparisons.

Fig. 4. Recurrence plot method. A: example of blood pressure time series, $x_1, x_2,..., x_{\text{BP}}$. B: recurrence plot. A 20 × 20 square is drawn. If BP $x_i$ differs from $x_j$ by less than a given value (here 2 mmHg), then we plot the point ($i, j$). Diagonal lines, for example [1,7]–[2,8]–[3,9], represent parallel trajectories. Lengths of diagonal lines account for dependence of dynamics on initial values. C: histogram of lengths of diagonal lines. Longest length is $L_{\text{max}} = 4$. D: histogram of lengths of diagonal lines corresponding to data in C. E: histogram of lengths of diagonal lines corresponding to data in D.
APPENDIX

To explain the recurrence plot method, let us consider an example. Figure 4A shows a series of 20 SBP values (x, where \(i = 1, 2, ..., 20\); in mmHg). The recurrence plot looks for repeated sequences in the data. We consider that two SBP values are the same if their difference is less than a small number (r), say 2 mmHg. Starting from \(x_i\), we are interested to see whether the same value \((\pm 2\) mmHg) occurs later in the series (i.e., for some \(j\), with \(j > i\)). In Fig. 4A, the same values are found at \(i = 4, 7, 10, 13,\) and 19. To mark these recurrences, we plot on a 20 × 20 square the points \([1, 4, 1, 7, 1, 10, 1, 13,]\) and \([1, 19]\) (Fig. 4B). These points start from \(x_1\) and plot the recurrent points \([2, 8]\) and \([2, 16]\). Figure 4B shows the recurrent points of the total series. The figure is symmetrical to the diagonal line. Therefore, only one-half of the figure was plotted with the diagonal excluded. Of particular interest are the diagonals in the figure. One example is the line \([1, 7, 2, 8, 3, 9]\). This line means that when a recurrence is found, the two sequences starting from these recurrent points remain close together for several subsequent beats: the trajectory \(x_1\rightarrow x_2\rightarrow x_3\) is parallel to the trajectory \(x_7\rightarrow x_8\rightarrow x_9\). Figure 4C describes the histograms of the diagonal lengths (in semilog scale). We observe a decreasing line. The length where this line cuts the abscissa is taken as the \(L_{\text{max}}\).

We recall that the Lyapunov exponent of a dynamic is a measure of the divergence of two trajectories starting from two points close to each other. Therefore, the Lyapunov exponent is inversely related to the \(L_{\text{max}}\) index (9). A high Lyapunov exponent, i.e., a short \(L_{\text{max}}\), expresses a “chaotic” dynamic. A series of 1,000 real data points is shown in Fig. 4D, and the corresponding histogram of diagonal length is shown in Fig. 4E. In this example, \(L_{\text{max}} = 122\).

Let \(n\) be the total number of observed series; the total number of points in the half of the \(n \times n\) grid (diagonal excluded) is then \(m = n(n - 1)/2\). Let \(k\) be the number of the recurrence points. The percentage of recurrences is then defined as \(\%\text{rec} = 100k/nm\). To get a first estimate of the fact that trajectories issued at similar levels remained parallel, we counted the number \(q\) of recurrent points in diagonal lines of length 2 or more. The percentage of determinism was defined as \(\%\text{det} = 100q/k\).

To analyze blood pressure data, we embedded the blood pressure data in a p-dimensional Euclidean space, using the time-delay reconstruction of Takens (26, 30). The delay of reconstruction was 1 beat. Using the false-neighbors method of Kennel et al. (15), the embedding dimension was taken as \(p = 10\). The recurrence plot method was applied in dimension 10, using the Euclidean norm. Let the cut-off value of the recurrence plot method in dimension 1 be \(r_1\). In dimension \(p\), we used \(r = r_1\sqrt{p}\). This formula gives the length of a hypercube of side \(r_1\). We used \(r_1 = SD\), where SD is the standard deviation of the data. The SD of blood pressure was \(\sim 4-5\) mmHg. We finally selected \(r = 5\) or \(10\) mmHg for SBP and \(r = 4, 10\) mmHg for DBP. Each blood pressure series included \(~10,000\) values. We calculated \(L_{\text{max}}\), \(\%\text{rec}\), and \(\%\text{det}\) for nonoverlapping segments of 1,000 consecutive readings and took the mean values of these indexes obtained in the different segments. As suggested by Eckmann et al. (9), we also plotted the histograms of the density of the recurrences in the diagonal lines to see whether some drift might exist in these segments.

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