Use of nonlinear methods to assess effects of clonidine on blood pressure in spontaneously hypertensive rats

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Mestivier, Denis, Hubert Dabiré, Michel Safar, and Nguyen Phong Chau. Use of nonlinear methods to assess effects of clonidine on blood pressure in spontaneously hypertensive rats. J. Appl. Physiol. 84(5): 1795–1800, 1998.—In spontaneously hypertensive rats (SHR), chronic infusion of clonidine failed to decrease blood pressure and blood pressure variability. We used nonlinear methods to get a deeper insight on the effects of clonidine on blood pressure dynamics. For 24 h and 4 wk, clonidine (0.1 mg·kg⁻¹·day⁻¹·sc) was infused by minipumps in the conscious SHRs, and, for comparison, a vehicle was infused in SHRs and in Wistar-Kyoto rats. Blood pressure was recorded for 30 min before and after treatments. We used the Lyapunov exponent, approximated by the inverse of the Iₘₐₓ index derived from the recurrence plot method, to characterize nonlinear dynamics. Before treatment, Iₘₐₓ index of blood pressure was lower (P < 0.01) in the SHRs than in the Wistar-Kyoto rats. Clonidine significantly increased Iₘₐₓ (P < 0.01) to the level observed in normotensive rats, at 24 h and up to 4 wk after infusion. We conclude that clonidine has a significant chronic effect on blood pressure dynamics, as evidenced by nonlinear methods. Our study also suggests that the mechanisms governing blood pressure variations are nonlinear.

recurrence plot; Iₘₐₓ index; Lyapunov exponent; cardiovascular dynamics

IN HUMANS AND IN ANIMALS, clonidine, a centrally acting antihypertensive agent (19), decreases both blood pressure level and blood pressure variation assessed by spectral analysis (2, 5, 8, 18). In contrast, the effects of clonidine on the blood pressure variability, assessed by the standard deviation (SD), are inconsistent. Although clonidine reduced the SD of blood pressure in normotensive rats and hypertensive patients (5, 8), it did not change SD of blood pressure in hypertensive rats or normotensive volunteers (2, 9, 18). Moreover, chronic treatment with clonidine failed to decrease blood pressure level and its SD in conscious spontaneously hypertensive rats (SHRs) (2, 4). The fact that clonidine had no chronic effect on blood pressure level and on blood pressure variability was surprising. We suggest that the use of linear indexes, such as mean value, SD, and spectral method was insufficient to analyze the effect of clonidine on blood pressure dynamics.

In recent years, it has been argued that the mechanisms regulating blood pressure are most probably nonlinear (1, 20, 21). Therefore, nonlinear methods should also be used to analyze blood pressure time series. Several authors have used nonlinear techniques to analyze heart rate and blood pressure in healthy conditions and in various pathologies and have obtained useful results (see, for example, Refs. 1, 14, 15, 20, 26–28).

One marker of nonlinear dynamics that we consider as the 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 system may generate two sequences that quickly (exponentially) diverge one from the other. The exponent of this divergence is called the Lyapunov exponent. In this work, we used a simple and intuitive method to estimate this exponent. Our aim was to explore whether, in chronic dosing, clonidine has modified the Lyapunov exponent of the blood pressure dynamics.

METHODS

Male SHRs (n = 24), aged 13 wk, and age-matched normotensive Wistar-Kyoto (WKY) rats (n = 16) (Charles River France, St. Aubin-les-EElbeuf, France) were used according to the Guide for the Care and Use of Laboratory Animals published by the US National Institute of Health (NIH Publication No. 85–23, revised 1985). The SHRs were randomized into four groups of six rats each. Groups 1 and 2 (SHRs) were treated with a vehicle (0.9% NaCl) and 0.1 mg·kg⁻¹·day⁻¹·sc clonidine, respectively, for 24 h. Groups 3 and 4 (SHRs) were infused with the vehicle and with 0.1 mg·kg⁻¹·day⁻¹·sc clonidine, respectively, for 4 wk. Groups 5 and 6 (WKY rats, n = 8 in each group) were infused with the vehicle for 24 h and 4 wk, respectively. Vehicle and clonidine infusions were performed via osmotic minipumps implanted subcutaneously, while animals were under ether anesthesia, in the ventral region of the abdomen (Alzet models 2ML1 and 2ML4, total volume 2 ml each, nominal rate 10 and 2.5 µl/h, respectively; Charles River France, St. Aubin-les-EElbeuf, France).

Blood Pressure Recording

Rats were anesthetized with pentobarbital sodium (60 mg/kg ip). A catheter (PE-50 fused to PE-10; Clay Adams, Parsippany, NJ), filled with heparinized 0.9% NaCl (50 U/ml) was introduced into the lower abdominal aorta via the femoral artery and was tunnelled subcutaneously under the skin of the back to exit between the scapulae. Animals were then allowed to recover from anesthesia, in individual cages, for 24 h. The arterial catheter was connected to a Gould pressure processor via a pressure transducer (Statham model P23 ID; Gould Instruments, Claudioeu, France), and arterial blood pressure was recorded on an eight-channel digital audiotape recorder (DTR-1800; Biologic, Claix, France) for 30 min, after 30 min of stabilization.

Acute treatments. At 24 h before the beginning of infusion of vehicle or clonidine, the arterial catheter was implanted as described above, and baseline values of arterial pressure were recorded in conscious, freely moving rats 24 h after surgery. After blood pressure was recorded, the minipumps (Alzet...
model 2ML4) were implanted while animals were under anesthesia. Another recording of blood pressure was performed in the same rats 24 h later. Because of the life span of extra-arterial catheters, baseline values were recorded only in the sample of rats used in acute treatments.

Chronic treatments. In these experiments, the minipumps (Alzet model 2ML4) were implanted at day 0, and the rats were housed in individual cages for 28 days. At day 27, they were prepared for recording of blood pressure as described above. Blood pressure was recorded 24 h after implantation of extra-arterial catheters.

Beat-to-Beat Blood Pressure

Blood pressure signal was sampled through the digital audio tape recorder by a MacLab system (AD Instruments, London, England). From this blood pressure wave, systolic and diastolic blood pressures were computed. Each 30-min record afforded a times series of 8,000–12,000 beat-to-beat systolic blood pressure and diastolic blood pressure. Blood pressure readings outside the range of 100–300 mmHg systolic and 50–250 mmHg diastolic were considered as artifacts, but <1% of values were in that range. To handle artifacts, a moving window of 200 values was screened along the time series. In each window, we computed the mean-value, m, and SD of systolic blood pressure and diastolic blood pressure. Whenever an artifactual systolic or diastolic blood pressure was encountered, the values of systolic and diastolic blood pressures were replaced by the mean value in the windows.

Lyapunov Exponent: The $l_{max}$ Index of the Recurrence Plot

The direct calculation of the Lyapunov exponent from an experimental time series is complicated and requires very long and stationary series (23). To obtain an approximation of the exponent, we used an index called $l_{max}$ derived from the method of recurrence plot (3). The method has been applied to several biological data in previous reports and has given interesting results (14, 22, 26, 27).

To illustrate the method, Fig. 1A shows a series of 20 systolic blood pressure values, $x_i$ (in mmHg), $i = 1, 2, ..., 20$. The recurrence plot looks for repeated sequences in the data. We consider that two blood pressure values are the same if their difference is less than a small number ($r$), say 2 mmHg. Starting from $x_1$, we are interested to see whether the same value ($±2$ mmHg) occurs later on in the series (i.e., for some $j$ with $j > 1$). In Fig. 1A, the same values are found at $j = 4, 7, 10, 14, 18$. To mark these recurrences, we plot on a $20 \times 20$ square the points $(1, 4), (1, 7), (1, 10), (1, 14), (1, 18)$ (Fig. 1B). Then we start from $x_2$ and plot the recurrent points $(2, 8), (2, 11), (2, 16)$. Figure 1B shows the recurrent points of the total series. 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 remained close together for several subsequent beats: the trajectory $x_1-x_2-x_3$ was parallel to the trajectory $x_2-x_5-x_6$. We recall that the Lyapunov exponent of a dynamic is a measure of the divergence of two trajectories that start from two points close to each other. Therefore, the Lyapunov exponent is inversely related to the $l_{max}$ index (3). A high Lyapunov exponent, i.e., a short $l_{max}$, expresses a chaotic dynamic.

To analyze blood pressure data, we embedded the one-dimensional blood pressure data in a $p$-dimensional Euclidean space by using the time-delay reconstruction of Takens (16; see also Ref. 22). The delay of reconstruction was one beat. With use of the false neighbors method of Kennel et al. (10), the embedding dimension was taken as $P = 10$. The recurrence plot method was applied in dimension 10 by using the Euclidean norm. The cutoff value in the recurrence plot method in dimension 1, $r_1$, was generally taken as $r_1 = f \cdot \text{SD}$ where $f$ is a fraction of one unit or less. In dimension $p$, we used $r = r_1 \sqrt{p}$; this formula gives the diagonal length of a hypercube of side $r_1$. The SD of blood pressure was $\sim 4–5$ mmHg. Here we used $f = 1$. Because systolic blood pressure was somewhat more dispersed than diastolic blood pressure,
we selected finally, \( r = 5 \sqrt{10} \text{mmHg} \) for systolic blood pressure and \( r = 4 \sqrt{10} \text{mmHg} \) for diastolic blood pressure. Each blood pressure series included ~10,000 values. We calculated the \( I_{\text{max}} \) for nonoverlapping segments of 1,000 consecutive readings and took the mean values of the \( I_{\text{max}} \) obtained in the different segments. As suggested by Eckmann et al. (3), we also plotted the histograms of the density of the recurrences in the diagonal lines to see if some drift might exist in these segments.

Statistical Analysis

The results are expressed as means ± SD. Comparisons between groups were performed by the nonparametric Mann-Whitney test (11).

RESULTS

The effects of clonidine were similar on systolic and diastolic blood pressures. In the following, the term blood pressure means either of the two pressures.

Effects on Blood Pressure Level and Blood Pressure Variability

As expected before treatment, systolic blood pressure and diastolic blood pressure mean values and their SD (in mmHg) were significantly greater (\( P < 0.01 \)) in the SHR group (mean systolic: 204 ± 4, mean diastolic: 150 ± 5, SD systolic: 8.6 ± 12, SD diastolic: 6.9 ± 0.5) than in the WKY group (mean systolic: 136 ± 9, mean diastolic: 103 ± 7, SD systolic: 5.7 ± 0.9, SD diastolic: 5.0 ± 0.8). All these variables were the same in the vehicle-treated SHRs and in the clonidine-treated SHRs.

After 24 h of treatment, mean value and SD of blood pressure in the clonidine-treated SHRs were significantly decreased compared with the vehicle-treated SHRs (\( P < 0.05 \) and \( P < 0.01 \), respectively). However, the level of blood pressure in the clonidine-treated SHRs was still significantly higher (\( P < 0.01 \)) than in the vehicle-treated WKY rats, whereas the SD of blood pressure in the clonidine-treated SHRs was reduced to the level observed in the vehicle-treated WKY rats (Fig. 2).

After 4 wk of infusion, blood pressure in the clonidine-treated SHRs unexpectedly rose and recovered to the level observed in the vehicle-treated SHRs. Similarly, the SD of blood pressure were the same in the clonidine-treated SHRs and in the vehicle-treated SHRs. Thus, in contrast to its acute effects, clonidine in chronic treatments did not change the level and the SD of blood pressure (Fig. 2).

Effects on the \( I_{\text{max}} \) Index of the Blood Pressure Dynamics

In the histograms of the density of the recurrences along the diagonal lines, we observed no apparent drift in the observed series of data. Before treatment, the \( I_{\text{max}} \) index of blood pressure was significantly lower (\( P < 0.01 \)) in the SHR group (systolic \( I_{\text{max}} \): 46 ± 7, diastolic \( I_{\text{max}} \): 45 ± 7) than in the WKY group (systolic \( I_{\text{max}} \):...
97 ± 22, diastolic \( l_{\text{max}} \): 84 ± 17). This index was the same in the vehicle-treated and in the clonidine-treated SHR.

After 24 h of treatment, the \( l_{\text{max}} \) of blood pressure was significantly increased (\( P < 0.01 \)) in the clonidine-treated SHR compared with the vehicle-treated SHR. The index in the clonidine-treated SHR had joined the level observed in the vehicle-treated WKY rats (Fig. 3).

After 4 wk, the \( l_{\text{max}} \) index of blood pressure in the clonidine-treated SHR was still significantly increased (\( P < 0.01 \)) compared with the vehicle-treated SHR. This index remained at the same level as in the vehicle-treated WKY rats (Fig. 3).

**DISCUSSION**

After 24 h of treatment with clonidine in conscious SHR, blood pressure level and its SD significantly decreased. These effects are consistent with previously published results (5, 8, 9, 18) and may be interpreted as the consequence of the centrally mediated inhibition of sympathetic tone induced by the drug (19).

In contrast to acute treatments, after 4 wk of infusion, clonidine no longer changed blood pressure level and its SD. The lack of effectiveness of clonidine on blood pressure level may be ascribed to the development of tolerance rather than to a malfunctioning of the micropumps. Indeed, after 4 wk of infusion, flesinoxan, another centrally acting antihypertensive agent (13, 24), significantly reduced blood pressure level in SHR (2). On the other hand, tolerance also develops to the antihypertensive effects of rilmenidine when it is infused in SHR (4). Observations of results on the SD of blood pressure were also inconsistent. Whereas clonidine reduced the SD of blood pressure in normotensive rats and hypertensive patients (5, 8), it did not change the SD in hypertensive rats and in normotensive volunteers (2, 9, 18).

We suggest that the absence of chronic effect of, and/or inconsistent results with, clonidine might be caused by the method of analysis. The use of blood pressure level and SD or spectral method might not be instructive enough, and the method was unable to explore the deep impact of clonidine on the blood pressure dynamics. It is clear that if we randomly perturb the initial order of the blood pressure series, then we obtain a new series with the same mean value and the same SD as the initial series. Therefore, mean value and SD cannot account for the temporal structure of the initial series. On the other hand, in the Fourier method, one decomposes the blood pressure signal into a sum of many cosine functions with different amplitudes and phases. However, the spectrum of the series uses only the amplitudes of the cosines and cannot account for a change in the phases of the different cosines. More subtle is the reason why the Fourier method cannot account for a possible sequential nonlinear relationship in the initial series. Theiler et al. (17) have shown that one can random modify a time series without modifying its linear sequential relationship (i.e., its linear autocorrelation coefficients). In the new series, the Fourier spectra are the same as in the initial series. For these reasons, nonlinear methods are needed to explore a possible nonlinear time-dependent relationship in the original data.

In this work, we have used a nonlinear \( l_{\text{max}} \) index, obtained by the recurrence plot method, to characterize the dynamic of blood pressure variation before and after treatment with clonidine. The meaning of \( l_{\text{max}} \) is simple (see details in the **METHODS** section). This index is approximately proportional to the inverse of the well-known Lyapunov exponent, which characterizes the dependency of the dynamic on initial conditions.

We have calculated the \( l_{\text{max}} \) in dimension 10. This dimension was suggested by the application of the false-neighbors method of Kennel et al. (10) to our observations. This result is in agreement with previous reports analyzing physiological data. Weber and Zbilut (22) used the same dimension to study electromyogram recording. Layne et al. (12), estimating fractal dimension of electroencephalogram data, showed that

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![Fig. 3](image-url)  
**Fig. 3.** \( l_{\text{max}} \) index of SBP \( l_{\text{max}}(\text{SBP}) \); A and of DBP \( l_{\text{max}}(\text{DBP}) \); B at baseline, after 24-h infusion (acute), and after 28 days of infusion (chronic) in SHR and WKY rats treated with vehicle or clonidine. Symbols same as in Fig. 2.
the fractal dimension was stabilized for an embedding dimension larger than \( \sim 10 \).

Nonlinear methods, in particular the recurrence plot method, have been used in previous reports. It was shown that the new methods could discover some important properties in the time series that were unrevealed by linear methods (14, 22).

It is interesting to notice that, before treatment, the \( l_{\text{max}} \) index is shorter in the SHRs than in the WKY rats. This means that the blood pressure dynamic is more sensitive to initial conditions in the hypertensive rats than in the normotensive rats. It has been suggested that physiological dynamics show a large variability in the healthy condition whereas a loss of variability is a sign of some pathology (7). Wagner et al. (20) showed that blood pressure regulation in the intact dog has a positive Lyapunov exponent and a decreased exponent was seen after baroreceptor denervation. Similarly, when analyzing electroencephalogram data of patients suffering from multiple sclerosis, Ganz et al. (6) also observed a decrease of Lyapunov exponent in pathological conditions. We also observed a longer \( l_{\text{max}} \) of heart rate variation in patients with diabetes mellitus and dysautonomy compared with control patients without dysautonomy (14). However, in contrast to the above results, Yip et al. (25) observed almost periodic oscillations of tubular pressure in the kidneys of normal rats. The oscillations had more an aperiodic character after arterial clipping of one kidney and in SHRs. Results of the present study were in the same direction as those of Yip et al. (25). Therefore, it seems that the question of 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.

We have shown that 24 h after dosing, clonidine significantly increased the \( l_{\text{max}} \) index of systolic and diastolic blood pressures in the direction of normality, and this effect was maintained until the end of the period of treatment (28 days). One interesting point is the fact that the index, at the 24th h after treatment, and on day 28, reached the level observed in the normotensive rats.

In contrast to \( l_{\text{max}} \), blood pressure level and its SD returned to the pretreatment level 28 days after clonidine infusion. It might be surprising that blood pressure dynamics in the SHRs before and after 4 wk of clonidine may have different regulations, as witnessed by two different \( l_{\text{max}} \) coefficients, and yet have the same mean level and the same SD. An explanation may be that many factors might act on mean level, SD, and the sensitivity of blood pressure to initial conditions. We give an example to illustrate this point. Fig. 4 shows two series generated by the recurrence equation \( x_{n+1} = ax_n - bx_n^2 \), with two coefficients of regulation, \( a \) and \( b \). In this model, coefficient \( a \) acts on the sensitivity to initial conditions and mean values of the time series, while coefficient \( b \) acts solely on the mean level. We

![Fig. 4. Left, A and B: 2 time series generated by recurrence equation \( x_{n+1} = ax_n - bx_n^2 \), with \( a = 3.95, b = 4.45 \) in series A and \( a = 3.86, b = 4.36 \) in series B. The 2 series have almost the same mean value (0.51 and 0.52, respectively) and SD (0.28 and 0.27, respectively). Right, A and B: histograms of lengths of diagonal lines (see Lyapunov Exponent: The \( l_{\text{max}} \) Index of the Recurrence Plot). Series A and B have quite different \( l_{\text{max}} \) indexes: \( l_{\text{max}} = 18 \) in series A; \( l_{\text{max}} = 37 \) in series B. L, length of diagonal lines.](http://jap.physiology.org/pdfs/1.2003.5.1799.f4)}
chose $a = 3.95$, $b = 4.45$ in series A and $a = 3.86$, $b = 4.36$ in series B. The two series have almost the same mean values (0.51 and 0.52, respectively) and SD (0.28 and 0.27, respectively), but they have quite different $l_{max}$ indexes: $l_{max}$ is equal to 18 in series A, and $l_{max}$ is equal to 37 in series B.

In conclusion, the present study demonstrates that clonidine has a significant chronic effect on blood pressure dynamics, but this effect can be evidenced only by nonlinear methods. Our study also suggests that the mechanisms governing blood pressure variations are nonlinear, and nonlinear methods should be used in complement to linear methods (mean values, SD, and spectral power). One important point is the relationship between the $l_{max}$ index and the known physiological mechanisms that govern blood pressure and heart rate variations. More data are needed to document this issue.

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