Relationship between noradrenaline and nonlinear indexes of blood pressure dynamics in normotensive and spontaneously hypertensive rats

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ABSTRACT
Recent studies in normotensive Wistar–Kyoto (WKY) and spontaneously hypertensive (SHR) rats show that some nonlinear indexes derived from the recurrence plot method may be better markers of sympathetic activity than the spectral powers of blood pressure (BP). We herein investigated the relationships between nonlinear indexes and plasma noradrenaline concentration in conscious WKY rats and SHRs. Blood pressure was recorded for 30 min after intravenous injection of saline (0.9% NaCl, 100 µL/kg), hexamethonium (20 mg/kg), atropine (0.5 mg/kg), atenolol (1 mg/kg) or prazosin (1 mg/kg). Spectral power in the low-frequency (LF) band and the nonlinear index ($I_{max}$), calculated on diastolic (DBP) and systolic blood pressures (SBP), were used to analyse the BP signal. Noradrenaline concentration was determined by radioenzymatic technique. A robust stepwise regression analysis – using noradrenaline concentration as dependent variable, and LF, $I_{max}$ and treatment, as independent variables – shows that treatment is the main variable explaining the variance of noradrenaline level in WKY rats, excluding the use of the pooled data to explore the relationship between noradrenaline concentration and LF or $I_{max}$. In contrast, in SHRs, treatment has no effect on the variance of noradrenaline concentration and the pooled data were then used. In this group, no correlation was observed between noradrenaline concentration and LF. In contrast, very high positive correlation was observed between noradrenaline level and $I_{max}$-DBP ($r = 0.59; P = 0.0005$) or $I_{max}$-SBP ($r = 0.53; P < 0.002$). The results strengthen our previous suggestion that nonlinear indexes may be better tools than spectral powers to investigate the sympathetic nervous system.

INTRODUCTION
Many techniques have been proposed to evaluate the activity of the sympathetic nervous system. Among these are direct recording of sympathetic nerve activity in both human beings and animals and, measurement of plasma noradrenaline spillover [1]. Since the report of Akselrod et al. [2] using spectral analysis of blood pressure (BP) and heart rate (HR), it has been suggested that the high-frequency (HF) component of HR spectral power may be a marker of parasympathetic tone, whereas the low-frequency (LF) component of BP spectral power may be a marker of the sympathetic activity [3,4]. However, the use of these indexes as sympathetic and parasympathetic markers is still under debate [5–8]. For instance, no consistent significant correlation between these indexes and noradrenaline and adrenaline has been found [1, 9–11]. Likewise, a lack of correlation between sympathetic nerve activity and the LF-BP has been reported in healthy human beings [12].

In recent reports, we have used nonlinear methods to analyse HR and BP time-series in Wistar–Kyoto (WKY)
rats and in spontaneously hypertensive rats (SHR), before and after treatment with different autonomic blockers. We used the so-called ‘recurrence plot method’, and defined some ‘nonlinear indexes’ from BP and HR time-series. We showed that, compared with spectral techniques, the nonlinear indexes are better markers of sympathetic and parasympathetic activities [13,14]. However, the relationships between noradrenaline and nonlinear indexes have never been studied. Therefore, the aim of the present study was to investigate the putative relationships between our nonlinear indexes derived from the recurrence plot method and plasma noradrenaline concentrations in WKY rats and in SHRs.

MATERIALS AND METHODS

The experiments were performed in conscious, normotensive male WKY rats and age-matched SHRs (Charles River France, St. Aubin-les-Elbeuf, France). The rats, received in the laboratory at 12 weeks of age, were housed three per cage for a duration of 1 week at 22–24 °C, with lights on from 06:00 to 18:00 hours and pellets and water provided ad libitum. All procedures were conducted in accordance with the INSERM Animal Ethic Committee and conform with the Guide for Care and Use of Laboratory Animals published by the US National Institutes of Health.

The rats of each strain were randomized into five groups of six to seven rats each: control groups (NaCl 0.9%, 100 µL/kg i.v.; WKY n = 6; SHR n = 7); atropine groups (atropine, 0.5 mg/kg i.v., n = 6 for each strain); β-blocking groups (atenolol, 1 mg/kg i.v.; WKY n = 7; SHR n = 6); α1-blocking groups (prazosin, 1 mg/kg i.v., n = 6 for each strain) and ganglion-blocked groups (hexamethonium, 20 mg/kg i.v.; WKY n = 7; SHR n = 6).

Animal surgery and BP recording and analysis (linear and nonlinear methods) have been previously described in details [13,14]. A brief summary is given below. After surgery, the rats were allowed to recover from anaesthesia for 48 h in individual cages. The two catheters were flushed twice daily with a solution of heparinized NaCl 0.9% (50 units/mL). Recording of arterial pressure was performed in unrestrained rats after the 2 days of recovery. At the end of the recording, 2 mL of blood was drawn, immediately centrifuged (2000 g for 15 min at 4 °C) and plasma was kept at −30 °C until measurement of catecholamines. Catecholamines were measured according to a radioenzymatic technique [15]. The sensitivity of the assay was between 0.5 and 1 pg for noradrenaline and adrenaline. The interassay coefficient of variation was 11.8% for noradrenaline and 10.2% for adrenaline (n = 36).

After sampling the BP signal at 1 kHz, we used a package of personal programs based on the formulas of Anderson [16] for Fourier analysis of BP and RR-interval (RR). Beat-to-beat diastolic (DBP) and systolic blood pressure (SBP) were submitted to Fourier analysis and recurrence plot method. We calculated the total area under the Fourier spectrum and the percentage of this area in the low-frequency (LF) band (0.25–0.75 Hz) to define LF-DBP and LF-SBP [17].

The recurrence plot method identifies points that return to the same level in a suitably embedding space. Existence of long sequences of recurrent points indicates that the dynamic is ‘deterministic’, i.e. is faithful to initial values. Let M be the number of recurrent points, M2 the number of sequences of recurrent points longer than 2 and Mmax be the length of the longest recurrent sequence. Mmax is inversely related to the largest Lyapunov exponent of the dynamic. In previous works [18–20], the two indexes, 100 * (M2/M), named ‘the percent of determinism’ and Mmax have been used to characterize the dynamic. In correlation analyses, the two gave comparable results. Therefore, in the present analysis, only the index Mmax has been used.

The results are expressed as mean ± SEM. An ANOVA followed by a Fisher’s protected least significant difference (PLSD) test for multiple comparisons was used to assess the significance of the results. To analyse if our variables can predict plasma noradrenaline concentration, we used a robust stepwise regression analysis, with noradrenaline as dependent variable and treatment, strain (when SHRs and WKY rats are pooled together), LF-DBP, LF-SBP, Mmax-DBP and Mmax-SBP, as independent variables. When treatment has no effect on the noradrenaline level, then the data can be used to analyse the relationships between noradrenaline and linear and nonlinear indexes of BP. P < 0.05 was considered statistically significant.

RESULTS

Effects of autonomic blockade on plasma noradrenaline and adrenaline

Table I shows the effects of the different treatments on plasma noradrenaline and adrenaline concentrations in WKY rats and SHRs. In vehicle-treated rats, noradrenaline but not adrenaline, was slightly lower (P < 0.05) in SHRs compared with WKY rats. Prazosin significantly increased noradrenaline concentration in both WKY rats and SHRs (P < 0.001) and adrenaline concentration
only in SHRs ($P < 0.001$). Atropine significantly increased ($P < 0.01$) adrenaline concentration only in WKY rats.

### Correlations between linear and nonlinear indexes and plasma noradrenaline

Table II summarizes the results of the multiple stepwise robust regression analysis. In WKY rats, treatment explained up to 22% ($P < 0.001$) of the variance of noradrenaline concentration. Therefore, in this strain, the variability of noradrenaline is mainly related to the effect of treatment. In contrast, in SHRs, treatment did not play any role in the variance of noradrenaline concentration. Similar results were observed when the results on both SHRs and WKY rats were pooled together. In SHRs, $L_{\text{max}}$-SBP was the most important independent variable, explaining up to 19% ($P < 0.001$) of the variance of noradrenaline concentration. Once $L_{\text{max}}$-SBP and $L_{\text{max}}$-DBP have been introduced in the regression equation, the linear index LF-SBP contributed only of 2% to the variance of noradrenaline concentration.

In WKY rats, although the variability of noradrenaline is mainly explained by the treatments (see above), we have shown the scatter plots between noradrenaline and LF-DBP and LF-SBP (Figure 1, left panels) and between noradrenaline and $L_{\text{max}}$-DBP and $L_{\text{max}}$-SBP (Figure 2, left panels). No correlation was observed in both cases. In SHRs, no correlation existed between noradrenaline concentration and LF-DBP or LF-SBP (Figure 1, right panels). In contrast, a high positive correlation was observed between noradrenaline and $L_{\text{max}}$-DBP and $L_{\text{max}}$-SBP (Figure 2, right panels).

### DISCUSSION

In previous studies [13,14], using the recurrence plot method to analyse BP and RR time series before and after autonomic nervous system blockade, we have shown, in both WKY rats and SHRs, that nonlinear indexes derived from this method might be better markers of sympathetic nervous system activity than linear indexes derived from the spectral analysis. We have herein investigated the putative correlations between our nonlinear indexes and the biochemical marker of the sympathetic nervous system activity, namely, the plasma noradrenaline concentration. The results obtained show that in SHRs, no correlation was observed between noradrenaline concentration and LF-BP whereas very high positive correlation was displayed between noradrenaline and $L_{\text{max}}$-SBP and $L_{\text{max}}$-DBP (Figure 2, right panels).
Figure 1 Correlations between noradrenaline (NA) concentration and low frequency (LF) power spectrum of diastolic (DBP) and systolic (SBP) blood pressure in normotensive Wistar–Kyoto rats (WKY; left panels) and in spontaneously hypertensive rats (SHR; right panels).

r = -0.12; P = 0.518

Figure 2 Correlations between noradrenaline (NA) concentration and $L_{\text{max}}$ of diastolic (DBP) and systolic (SBP) blood pressure in normotensive Wistar–Kyoto rats (WKY; left panels) and in spontaneously hypertensive rats (SHR; right panels).

r = -0.22; P = 0.248
in the variance of noradrenaline concentration. In this strain, no correlation was observed between noradrenaline concentration and LF-DBP or LF-SBP while a high correlation was observed between noradrenaline concentration and \( I_{\text{max}} \)-DBP and \( I_{\text{max}} \)-SBP. These results are in agreement with observations in human beings. Indeed, in normal subjects, no significant correlation has been found between LF and HF of heart rate and plasma noradrenaline and adrenaline concentrations in baseline conditions and during psychological stress which increased the sympathetic activity, suggesting little relationship between neurohumoral and spectral estimates of cardiac sympathetic activity [11,23,24]. Similar results have been also reported in hypertensive patients [25]. In addition, a lack of correlation between sympathetic nerve activity and the LF of mean arterial pressure has been reported in healthy human beings [12].

The limitation of the present study may be the use of plasma noradrenaline concentration as a marker of sympathetic activity. Indeed, more reliable markers such as noradrenaline spillover or sympathetic nerve activity could be used [1]. However, it is noteworthy that both noradrenaline spillover and microneurography measure local sympathetic nervous system activity. In the present experiments, the noradrenaline concentration observed in solvent-treated SHRs was slightly although significantly lower than in solvent-treated WKY rats. This observation might be surprising. However, it seems that plasma noradrenaline concentration does not invariably reflect changes in sympathetic activity [1]. In contrast, although plasma adrenaline concentration in vehicle-treated SHRs was about 50% higher than in vehicle-treated WKY, it was not statistically significant. However, an activation of the adrenergic system is likely to be present as a large and highly significant increase of plasma adrenaline concentration was observed in prazosin-treated SHR while it remained unchanged in prazosin-treated WKY rats. This is in agreement with the concept suggesting an alteration of the adrenergic system as the first dysregulation of the sympathetic system in essential hypertension [21,22].

In conclusion, we suggest that linear indexes derived from the spectral analysis do not reliably measure sympathetic activity. In contrast, the nonlinear index \( I_{\text{max}} \) is highly associated with plasma noradrenaline concentration, and therefore may be a useful quantitative tool to investigate the sympathetic nervous system.

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