Steady-State and Dynamic Responses of Renal Sympathetic Nerve Activity to Air-Jet Stress in Sinoaortic Denervated Rats

Christian Barrès, Yong Cheng, Claude Julien

Abstract—This study examined the role of arterial baroreceptors in mediating the relationship between changes in the mean level and the amplitude of slow oscillations of renal sympathetic nerve activity (RSNA) during environmental stress. In 7 sham-operated (control) and 7 chronically (2 weeks before study) sinoaortic baroreceptor denervated (SAD) conscious rats, arterial pressure (AP) and RSNA were simultaneously recorded during two 15-minute periods, before and during the application of a mild environmental stressor (jet of air). Air-jet stress induced a similar degree of sympathoexcitation in both groups of rats. During stress in control rats, AP and RSNA spectral power in the mid-frequency (MF) range (0.27 to 0.74 Hz) increased, mainly as a consequence of an amplification of strongly coherent oscillations of \( \approx 0.4 \text{ Hz} \). In SAD rats, MF fluctuations of AP and RSNA were reduced but not abolished before stress, tended to increase during stress, and were linearly related under both experimental conditions. However, in the MF range, there was no well-defined oscillation at any specific frequency. At the peak coherence frequency (\( \approx 0.4 \text{ Hz} \)), the gain of the transfer function from RSNA to AP did not change during stress in control rats and was similar to that measured in SAD rats, indicating that it mainly reflected the properties of the feedforward effect of RSNA on AP (ie, vascular reactivity). In summary, the parallelism between stress-induced changes in the mean level of RSNA and the amplitude of slow RSNA oscillations requires the functional integrity of the baroreceptor reflex, which is consistent with the hypothesis that slow AP and RSNA rhythms are resonant oscillations within the baroreceptor reflex loop. (Hypertension. 2004;43:629-635.)

Key Words: arterial pressure ■ Mayer waves ■ spectral analysis ■ sympathetic nervous system ■ transfer function

Sympathetic overactivity has been implicated in the initiation and progression of several cardiovascular diseases, including essential hypertension.1 Methods currently recommended for assessing the activity and reactivity of the sympathetic nervous system, ie, microneurographic recordings and catecholamine spillover measurements,2 are invasive and technically difficult to realize. The search for a noninvasive measure of sympathetic nerve activity (SNA) has been one of the main goals of the studies on cardiovascular oscillations. It was shown soon after the introduction of computers in cardiovascular research that some of these oscillations might be related to SNA, because they were almost invariably enhanced by sympathoexcitatory stimuli.3 In particular, the so-called Mayer waves, usually referred to as low- or mid-frequency arterial pressure (AP) oscillations, were shown to grow in amplitude during tilt,4,5 active standing,6 mental stress,6–8 or drug-induced baroreceptor unloading.9 However, the mechanism of this association has not been clarified until now. More specifically, two hypotheses have been proposed to explain the genesis of coherent oscillations of AP and SNA at Mayer wave’s frequency. One hypothesis assumes that the SNA rhythm derives from the pacemaker activity of autonomous oscillators located within central nervous and/or spinal structures,10,11 which in turn results in a cycle of vasoconstriction and vasodilation. The second hypothesis implicates a positive feedback within the arterial baroreceptor reflex loop, especially because of the presence of fixed time delays in the sympathetic limb of the loop, which results in self-sustained oscillations of AP and SNA at a particular frequency called the resonance frequency.12–14 Experimental support to the latter hypothesis came from the observation that in conscious cats and rats, spectral power in the frequency band containing Mayer waves was decreased after arterial baroreceptor denervation, ie, after opening the baroreceptor reflex loop at the level of afferent pathways.15–17 However, Mayer waves are barely visible or even absent under conditions of low sympathetic activation.9,18 During reflexly induced sympathetic activation, SNA oscillations at Mayer wave’s frequency are amplified when the baroreceptor reflex is normally functioning.9,19 After acute administration of the \( \alpha \)-adrenoceptor blockade, phentolamine, the baroreceptor reflex loop is opened at the vascular neuroeffector junction, and the reflex sympathetic activation occurring in response to the decrease in AP is not accompanied by a parallel increase in the amplitude of SNA oscillations at Mayer wave’s frequency. On the contrary,
these oscillations are strongly attenuated or even abolished, which further favors a major role of the baroreceptor reflex in their production. During centrally induced sympathetic activation, it is not known whether the amplification of SNA oscillations at Mayer wave’s frequency requires the functional integrity of the baroreceptor reflex. The aim of the present study was therefore to examine the effect of stress-induced sympathetic activation on SNA rhythms at Mayer wave’s frequency in rats with and without intact arterial baroreceptors. The sinoaortic baroreceptor denervation is a unique tool to study the role of feedback pathways in generating SNA rhythms. In addition, as feedforward pathways are left intact after denervation, transfer function from SNA to MAP can be used to assess vascular reactivity under various conditions.

Methods

Animals

All experiments were performed in accordance with the guidelines of the French Ministry of Agriculture for animal experimentation. Fourteen male Sprague-Dawley rats (Charles River Laboratories, L’Arbresle, France) aged 10 to 12 weeks were used. Surgical procedures have been previously described in detail. Two weeks before the study, under anesthesia with a mixture of acepromazine maleate (12 mg/kg intraperitoneally) and ketamine hydrochloride (120 mg/kg intraperitoneally), animals were submitted to bilateral denervation of aortic and carotid sinus baroreceptors (SAD group) (n=7) or sham surgery (control group, n=7). One day before the study, rats were anesthetized with pentobarbital sodium (630 mg/kg), and femoral arterial and venous polyethylene catheters were inserted for AP measurement and drug administration, respectively. Then, a major branch of the left renal nerve was placed on a bipolar electrode and insulated with a silicone gel for renal SNA (RSNA) measurement. The renal nerve was left intact so that efferent and afferent renal nerve activities were recorded. However, in our experimental conditions, afferent nerve activity appears negligible because the activity recorded after acute ganglionic blockade is similar to postmortem activity (unpublished observation). Catheters and electrode were led subcutaneously to exit at the back of the neck. After each surgical intervention, rats received a single intraperitoneal injection of penicillin G (40 000 IU).

Experimental Protocol

On the day of the experiment, in conscious and unrestrained rats, the sensitivity of the baroreceptor reflex system was evaluated by means of intravenous injections of phenylephrine hydrochloride (1.5 g/kg). Then, AP and RSNA were simultaneously and continuously recorded during two 20-minute periods, before (baseline) and during the application of an acute environmental stress elicited by means of a jet of air blown into the cage. To avoid the potential interference of heart rate fluctuations in the genesis of Mayer waves, recordings were performed while the rats were under cardiac autonomic blockade (atropine methyl nitrate and atenolol, 2 mg/kg intravenous, each). After recovery from the stress application, the background noise level of RSNA was estimated as the residual electrical activity obtained after administration of the short-acting ganglionic blocker, trimethaphan camysylate (10 mg/kg intravenous). At the end of the experiments, the rats were euthanized with an intravenous overdose of pentobarbital sodium.

Data Collection and Analysis

AP was measured by connecting the arterial catheter to a precali- brated pressure transducer (Statham P23 ID; Gould, Cleveland, Ohio) coupled to an amplifier (model 13-4615-52; Gould). RSNA was amplified (×50 000), band-pass filtered (300 to 3000 Hz; Model P-511J; Grass, Quincy, Mass), and rectified (analog homemade rectifier including a low-pass filter with a cutoff frequency of 5Hz). Using a computer equipped with an analog-to-digital converter (model AT-MIO-16; National Instruments, Austin, Tex) and a LabVIEW 5.0 software (National Instruments), the AP and RSNA data were sampled at 500 Hz and stored on CD-ROM.

Offline processing of data was performed on a workstation (Sparc1; Sun Microsystems, Mountain View, Calif). Mean AP (MAP) (mm Hg) was computed on a beat-to-beat basis and resampled at 10 Hz after linear interpolation. MAP has been reported to be suitable for studying Mayer waves. Values of RSNA (μV) were averaged over consecutive 100-ms periods, the background noise was subtracted, and all values were normalized by the mean RSNA value calculated over the baseline period (normalized unit [μV]). From each 20-minute period, a 15-minute period was selected to exclude from calculations transient effects at the onset of the stress trial. Actual periods started 2.9±0.3 minutes and 3.7±0.3 minutes after the beginning of the stress in control and SAD rats, respectively. Then, 34 data sets of 512 points (51.2 seconds) overlapping by half were processed. For each data set, after removal of the linear trend and application of a Hanning window, power spectral density was computed using a fast Fourier transform algorithm. The spectra obtained for the different data sets were averaged. The frequency resolution was 0.0195 Hz and the highest frequency studied was 5 Hz. Three frequency bands were defined: a low-frequency (LF) band from 0.0195 to 0.254 Hz; a mid-frequency (MF) band from 0.273 to 0.742 Hz; and a high-frequency (HF) band from 0.762 to 5 Hz. In each band, spectral powers were calculated by integration.

Cross-spectral techniques were used to calculate the magnitude squared coherence function γ2(τ) between RSNA and MAP. Coherence is the equivalent in the frequency domain of the coefficient of determination (r2) of a linear regression. It quantifies the amount of linear coupling between two variables as a function of frequency. However, coherence cannot be used without applying a statistical test to determine the threshold (γ2min) above which it exceeds zero with a significance level α. When using the periodogram method (Welch method), γ2min depends on the number of segments used for computing spectra and a factor determined by the shape of the window used to taper data. According to Koopmans: where F is the Fisher distribution with 2 and 2(n–1) degrees of freedom.

For N samples of evenly spaced data divided into segments of length L and multiplied by a window:

In the case of the Hanning window, wi=0.5(1−cos(2πi/L)), so that k=0.75 and n=(2.67N/L)2.

| TABLE 1. Effect of Air-Jet Stress on Mean Levels of Cardiovascular Variables in Control and SAD Rats Under Cardiac Autonomic Blockade |
|---------------------------------|-------------|-------------|-------------|-------------|
| Variable                        | Baseline    | Stress      | Baseline    | Stress      |
| MAP (mm Hg)                     | 103±3       | 113±2*      | 104±4       | 129±8†      |
| RSNA (μV)                       | 0.92±0.22   | 1.95±0.25*  | 1.07±0.17   | 2.17±0.31*  |
| HR (bpm)                        | 337±7       | 352±5*      | 326±9       | 338±10      |

Values are mean±SEM; MAP indicates mean arterial pressure; RSNA, renal sympathetic nerve activity; HR, heart rate.

*P<0.05 vs baseline. †P<0.05 vs control.
In the present study, $N=8704$ and $L=512$, thus $n=22.695$ and $2(n-1)=43.39$. For $2(n-1)=43$ and $a=0.01$, $F_{n}(0.01)=5.1356$, which yields a significance threshold for coherence $\gamma_{mn}=0.193$.

Cross-spectral analysis was also used to calculate the gain of the transfer function from RSNA to MAP.

Baroreceptor reflex sensitivity was estimated as the ratio of the peak change in heart rate (bpm) or RSNA (percentage change) to the peak change in MAP (mm Hg) after phenylephrine and nitroprusside administrations.

**Statistics**

All data are presented as mean±SEM. Statistical comparisons between control and SAD rats were made by the non-parametric Mann-Whitney $U$ test for unpaired data. Within each group of rats, paired comparisons (stress versus baseline) were made by the Wilcoxon signed rank test (SigmaStat 2.03; SPSS, Chicago, Ill). Statistical significance was taken at a value of $P<0.05$.

**Results**

On the day of the experiment, control and SAD rats had similar body weights (334±6 and 315±6 g, respectively). The SAD procedure markedly reduced cardiac and sympathetic baroreflex sensitivities measured after either phenylephrine ($-0.81±0.25$ versus $-1.88±0.19$ bpm/mm Hg, $P<0.02$ and $-1.02±0.09$ versus $-1.94±0.11$/mm Hg, $P<0.001$) or nitroprusside ($-0.58±0.08$ versus $-3.54±0.32$/mm Hg, $P<0.001$) administrations.
versus $2.81 \pm 0.26$ bpm/mm Hg, $P<0.001$ and $1.47 \pm 0.11$ versus $-7.23 \pm 0.71\%$/mm Hg, $P<0.001$ administrations.

**Baseline Conditions**

Mean levels of MAP, RSNA, and heart rate were comparable in control and SAD rats (Table 1). As expected, SAD rats had enhanced total and LF powers of MAP, together with a decreased power in the MF band containing the Mayer waves (Figure 1). In contrast, spectral powers for RSNA did not differ between control and SAD rats. However, examination of the RSNA spectra (Figure 2 middle panels) indicated that the RSNA periodicity observed at $0.4$ Hz in control rats was absent in SAD rats.

**Effect of Air-Jet Stress on Mean Levels and Spectral Powers**

The air-jet stress induced a comparable 2-fold RSNA increase in both groups of rats, which was associated with an increase in MAP that was significantly larger in SAD than in control rats (Table 1). In control rats, during stress exposure, all spectral indices of RSNA variability were significantly enhanced while only MF power of MAP showed a significant increase (Figure 1). In SAD rats, the stress-induced increases in RSNA spectral powers were significant only in the HF band, although there was a trend in the MF band ($P=0.078$). Considering MAP, the MF and HF components of its variability were significantly increased during stress (Figure 1). Differences between control and SAD rats were not altered during air-jet stress, with the exception of the reduction of MF power of RSNA in SAD rats that became significant.

**Effect of Air-Jet Stress on 0.4 Hz Oscillations of MAP and RSNA**

In control rats, the stress-induced increases in the MF power of MAP and RSNA were largely a consequence of an amplification of the peaks that were already present in the band under baseline conditions (Figure 2). This amplification of the MAP and RSNA rhythmicity during stress is illustrated in Figure 3. On the contrary, in SAD rats, increased MF power during stress was not attributable to the appearance of any obvious periodicity, considering either MAP or RSNA (Figure 2).

**Transfer Function From RSNA to MAP Under Baseline and Stressful Conditions**

In both groups of rats and under both experimental conditions, the coherence function clearly reached a maximum in the MF band (Figure 2 and Table 2). The gain of the transfer function relating RSNA and MAP was calculated from $0.02$ to $1$ Hz (Figure 4). In both groups of rats, it was not markedly altered during air-jet stress. In particular, the gain measured at the peak coherence frequency remained unchanged during stress in control rats and was only marginally ($P=0.078$) enhanced in SAD rats (Table 2). Finally, gain at peak coherence frequency tended ($P=0.053$) to be lower in SAD
than in control rats under baseline but not under stressful conditions.

Discussion

The results of the present study support the conclusion that in rats, the arterial baroreceptor reflex largely mediates the stress-induced increases in Mayer waves and associated RSNA oscillations. In rats with chronic SAD, stress enhances fluctuations of MAP and RSNA in the frequency band containing Mayer waves but does not unmask any predominant rhythmicity at Mayer wave’s frequency.

In baroreceptor-intact and SAD rats, a continuous jet of air blown into the cage increased RSNA and MAP, which is characteristic of the defense reaction in rats. In SAD rats, although the stress-induced increase in RSNA was similar to that observed in control rats, the increase in MAP was larger. The latter finding accords with a previous report. These differential effects of stress on MAP were not related to cardiac factors because the autonomic innervation of the heart had been blocked. Stress did not markedly alter the gain of the transfer function between RSNA and MAP in SAD rats, which suggests that the feedforward link between RSNA and MAP, ie, vascular reactivity, was essentially unaltered. Therefore, it can be proposed that the more pronounced pressor effect observed during stress in SAD rats was related to a stronger sympathoexcitation in vascular beds other than the kidneys. We have previously shown that during air-jet stress in SAD rats, the mesenteric vasoconstriction is enhanced whereas the hindquarters vasodilation is attenuated.

Two aspects of MAP and RSNA variabilities estimated by means of spectral analysis were considered in this study. The first one concerned the absolute value of the spectral power calculated in the MF band containing Mayer waves, independently of the presence of peaks in the spectra. The second aspect concerned the presence of authentic oscillations demonstrated by the presence of peaks in the spectra. In SAD rats, the sympathoexcitation accompanying air-jet stress was associated with an increase in MAP and RSNA spectral powers calculated in the MF band. However, under resting and stressful conditions, no well-defined peaks were observed in the MF band of RSNA and MAP spectra. The absence of Mayer waves in resting SAD rats has been reported first in cats, then in rats. More recently, the absence of RSNA oscillations at Mayer wave’s frequency has been reported in conscious SAD rats. However, significant residual power was present in the MF band of RSNA and MAP spectra. The absence of Mayer waves in resting SAD rats has been reported first in cats, then in rats. More recently, the absence of RSNA oscillations at Mayer wave’s frequency has been reported in conscious SAD rats. The functional significance of this residual RSNA power was demonstrated by the almost complete abolition of MF power of MAP after ganglionic blockade in resting SAD rats. Interestingly, the presence of slow AP fluctuations in the frequency band containing Mayer waves (0.04 to 0.15 Hz) was reported in patients with multiple system atrophy. In these patients, sympathetic activity is chronically elevated and is uncontrolled by the baroreceptor reflex. It was demonstrated that these slow AP fluctuations were of sympathetic origin as they were attenuated during progressive ganglionic blockade. However, these observations are difficult to evaluate in the context of the present study, because the presence or absence of peaks in the MAP spectra of the patients was not reported.

During air-jet stress in SAD rats, the mean RSNA level increased together with RSNA spectral power in all frequency bands, although this enhancement was especially marked in the HF band, which made the main contribution to total power. In the MF band, increased spectral power of RSNA was not accompanied by any shaping of power at a preferred frequency. Under stressful conditions, coherence analysis indicated that fluctuations of RSNA and MAP were

<table>
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<th>Variable</th>
<th>Control (n=7)</th>
<th>SAD (n=7)</th>
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<tr>
<td>Peak coherence frequency (Hz)</td>
<td>0.40±0.02</td>
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<td>Peak coherence</td>
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<td>0.60±0.08†</td>
<td>0.63±0.06†</td>
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<tr>
<td>Gain at peak coherence frequency (mm Hg/nu)</td>
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<td>0.027±0.004</td>
</tr>
<tr>
<td></td>
<td>0.017±0.003</td>
<td>0.030±0.006</td>
</tr>
</tbody>
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Values are mean±SEM; nu indicates normalized units. †P<0.05 vs control.

Figure 4. Group-average spectra of the transfer function gain from RSNA to MAP computed before (Baseline) and during air-jet stress in 7 control and 7 SAD rats. Standard error lines have been omitted for legibility.
still linearly correlated. It was also observed that the gain function did not change markedly, so that enhanced RSNA fluctuations were translated into enhanced MAP fluctuations in the MF band.

In baroreceptor intact rats, air-jet stress increased spectral power of MAP in the MF band. This confirms previous observations in normotensive rats of the Lyon strain and in Fischer rats. This increase in MF power of MAP was paralleled by an increase in the MF power of RSNA. Both effects were mainly attributable to an amplification of coherent oscillations centered ~0.4 Hz that were already present before stress application. Therefore, by combining the observations made in baroreceptor intact (closed-loop conditions) and SAD (open-loop conditions) rats, it emerges that during stress-induced sympathoexcitation, the integrity of the baroreceptor reflex is required for the amplification of MAP and RSNA oscillations in the MF band. Under resting and stressful conditions in SAD rats, the absence of peaks in the MF band of MAP and RSNA spectra does not support the hypothesis that SNA and AP oscillations at Mayer wave’s frequency result from the operation of a central oscillator, whatever the level of sympathetic activation. By contrast, the key role played by the arterial baroreceptor reflex in the genesis of Mayer waves in resting rats is confirmed in the present study, and this role is extended to a situation of centrally induced sympathoexcitation. This important contribution of 0.4-Hz oscillations to the MF spectral power of MAP and RSNA has already been reported by Brown et al in rats under conditions of normal (conscious) and low (light pentobarbital anesthesia) sympathetic activation. Within the frame of the baroreflex hypothesis for the genesis of 0.4-Hz RSNA and MAP oscillations, the maintenance of these oscillations during stress suggests that the functionality of the baroreceptor reflex was maintained during stress. However, because air-jet stress increased RSNA and MAP, the preservation of a functional baroreflex would require some degree of resetting toward higher MAP values. Such a resetting has indeed been demonstrated for the cardiac component of the reflex in rats during air jet stress.

Perspectives

The major role played by the baroreceptor reflex in the production of coherent AP and SNA oscillations at Mayer wave’s frequency has been demonstrated in resting subjects, i.e., under conditions of low SNA, and during reflexly induced increases in SNA. The present study allows extending this conclusion to a situation of centrally induced sympathetic activation accompanying exposure to a mild environmental stressor, which is particularly relevant to daily life situations. A quantitative approach of the various determinants of Mayer wave’s amplitude, especially mean SNA level versus baroreflex sensitivity, is now required. For example, it would be interesting to evaluate the effects of partial baroreceptor denervation on Mayer wave’s amplitude, because such a procedure is supposed to diminish, but not abolish, baroreflex sensitivity.

References


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