Altered frequency characteristic of central vasomotor control in SHR

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Kuo, Terry B. J., and Cheryl C. H. Yang. Altered frequency characteristic of central vasomotor control in SHR. Am. J. Physiol. Heart Circ. Physiol. 278: H201–H207, 2000.—Previous work from our laboratory has demonstrated that the very low-frequency (VLF: 0–0.25 Hz) and low-frequency (LF: 0.25–0.8 Hz) power of arterial pressure variability (APV) are related to vasomotor reactivity in response to control signals from the rostral ventrolateral medulla (RVLM) through the sympathetic system in the rat. The present study evaluated the differences in the dynamic property of central vasomotor control between spontaneously hypertensive rats (SHR) and normotensive Wistar-Kyoto rats (WKY). Experiments were conducted in 10- to 12-wk-old rats that were anesthetized with continuous infusion of pentobarbital sodium, paralyzed with pancuronium, and maintained on mechanical ventilation. We found that SHR exhibited significantly higher arterial pressure (AP), heart rate (HR), and VLF, LF, and high-frequency (0.8–2.4 Hz) power of APV than WKY under resting state. Broad-band electrical stimulation of the RVLM elicited parallel APV in the VLF and LF ranges in both rat strains. The evoked APV and transfer magnitude of the APV to stimulus spike rate variability (RVLM-AP magnitude) were significantly higher in SHR, especially in the LF range. The response frequency of central vasomotor control, represented by the high-cut frequency of RVLM-AP magnitude, was also extended in SHR. The disparity in RVLM-AP transfer magnitude between SHR and WKY became virtually absent after combined α- and β-adrenoceptor blockade by phentolamine and propranolol. These results suggest that the dynamic control of RVLM on AP reactivity is enhanced in SHR, in which the adrenergic system may play a major role.

arterial pressure variability; rostral ventrolateral medulla; broad-band stimulation; coherence; transfer function

A restricted area in the rostral ventrolateral medulla (RVLM) functions as “premotor sympathetic neurons” in the regulation of arterial pressure (AP) by providing tonic excitation to preganglionic sympathetic neurons of the spinal cord (11, 27). Electrical or chemical excitation of the RVLM elicits a pressor response that is antagonized by adrenoceptor blockade (27). Although the magnitude characteristic of RVLM control of AP has been extensively studied, the temporal characteristic, which is crucial to understanding the response rate of the cardiovascular system to central command, is less discussed. This is caused in part by the limitations of traditional static methods that must operate under a steady state. Our laboratory recently developed (16) a method to evaluate the dynamic property of central vasomotor control that uses transfer-function analysis of the broad-band electrical stimulation-response relationship in the frequency domain. This technique may quantify both the magnitude and temporal characteristics of RVLM control of AP. We showed (16) that broad-band stimulation of RVLM may evoke parallel AP variability (APV) in the frequency range <0.8 Hz via the sympathetic system in the rat. Such a demonstration offers a close linkage between the vasomotor center and vasomotor-related APV.

Enhanced basal and environmentally evoked sympathetic neural activity has been implicated in the development and maintenance of the hypertension observed in experimental animal models such as the spontaneously hypertensive rat (SHR). In support of this hypothesis, increased plasma catecholamine levels (24), increased sympathetic nerve activity (12), and sympathetic hyperreactivity to stressful stimuli (18) have been noted in the SHR compared with its normotensive control, the Wistar-Kyoto rat (WKY). Several investigators showed that cardiovascular responses to RVLM stimulation are enhanced in SHR (6, 21, 31). These data support the hypothesis that the sympathetic hyperfunction resulting from the deterioration of modulatory systems in the central nervous system may be considered one of the major causes of essential hypertension. In this study, we proposed to further characterize potential differences in the dynamic property of central vasomotor control in SHR and WKY strains using transfer-function analysis. In adopting this method, we hypothesized that, in addition to an elevation in magnitude characteristic, the central vasomotor control of SHR may also have a wider frequency range of operation. Furthermore, such physical characteristics may be reflected by alterations in vasomotor-related APV as well as a transfer function between RVLM stimulation and AP response.

MATERIALS AND METHODS

Preparation of animals. Experiments were carried out in male SHR and WKY (National Laboratory Animal Breeding and Research Center, Taipei, Taiwan) that were 10–12 wk old. As described previously (16, 30), animals were anesthetized with an induction dose of pentobarbital sodium (50...
mg/kg ip). Anesthetic maintenance was provided by intravenous infusion of the same anesthetic at 20 mg·kg$^{-1}$·h$^{-1}$. This anesthetic management offered maintained anesthesia while preserving the capacity of cardiovascular regulation (16, 30). The trachea was intubated for connection to an animal respirator (Harvard 683). During the experiment, animals were paralyzed with pancuronium bromide (2 mg·kg$^{-1}$·h$^{-1}$ iv) and were mechanically ventilated (1.4–1.6 Hz, 2.4 ml/stroke). Animals were placed supine on a heating pad throughout the experiment, and the rectal temperature was maintained at 37 ± 0.5°C.

Broadband electrical stimulation of RVLM. After the completion of surgery, the head of the rat was placed in a stereotaxic head holder (Kopf 900). Electrical stimulation of the RVLM was delivered by a stainless steel bipolar concentric electrode (diameters: 250 µm shaft, 100 µm tip; Rhodes Medical SNE-100). Activation of the RVLM (average coordinates: 3.2–3.8 mm posterior to parietooccipital suture, 1.7 mm lateral to midline, and 8.7–9.2 mm ventral from cerebellar surface with incisor bar set at −3.5 mm) (11, 16) was effected with cathodal rectangular current pulses (50 µA, 1 ms), delivered via a constant current isolation unit.

Similar to our previous study (16), the stimulus spike rate was controlled by an IBM-PC-compatible computer. A specially designed computer program developed by our laboratory controlled the pattern of the stimulus pulses. Following the terminology commonly used in the literature for the display of frequency responses (7, 16), we designated the independent variable as the modulation frequency (in Hz) and the number of stimulus pulses per second (pps) as the spike rate. To excite the RVLM with broadband electrical stimulation, the stimulus spike rate was changed at a variance generated in proportion to Gaussian-distributed random numbers (2, 16) about a mean rate. We set the mean spike rate at 15 pps, with a root mean square of 15 pps. The stimulus was broadband modulated between 0 and 3 Hz, and each stimulation lasted for 3 min.

Recording of AP and electrocardiogram signals. The left femoral artery was cannulated with a PE-50 catheter filled with heparinized saline (200 U/ml). The arterial catheter was connected to a pressure transducer (Statham P23 ID, frequency range DC to 200 Hz) and in turn to a universal amplifier (Gould G-20–4615–58) by means of which the AP signals were amplified and filtered (frequency range DC to 200 Hz). Lead II electrocardiogram was also amplified and filtered (3–300 Hz) by a universal amplifier (Gould G-20–4615–58) and was synchronously acquired with the AP signals.

Data acquisition and storage. Both RVLM stimulus and AP signals were acquired by a 12-bit analog-digital converter (Advantech PCL1800) at a sampling rate of 2,048 Hz, in conjunction with another computer. The computer we used was a general-purpose personal computer equipped with an Intel Pentium microprocessor and 16 MB of random access memory. The acquired data were analyzed on-line but were a general-purpose personal computer equipped with an Intel Pentium microprocessor and 16 MB of random access memory. The acquired data were analyzed on-line but were synchronously acquired with the AP signals.

Power spectral analysis was accomplished by fast Fourier transform, and average periodograms were generated by averaging the autospectra from 16 time segments. Cross-spectral analysis was carried out on the same 16 sets of data on RVLM stimulus spike rate and AP signals, which led to the computation of the two functions. First, a squared coherence function

\[ k^2(f) = \frac{SS_{AB}(f)^2}{SS_A(f) \times SS_B(f)} \]  

where \( SS_{AB}(f) \) and \( SS_A(f) \) are the respective power spectra for spike rate of RVLM electrical stimulation and AP signals, and \( SS_B(f) \) is their cross spectrum. \( k^2(f) \) ranges from 0 to 1, which provides an assessment of the linear relationship at each frequency and of the statistical reliability of transfer function. A value \( \leq 0.5 \) was taken to be statistically significant (8, 16, 19). Second, a transfer function

\[ H(f) = \frac{SS_{AB}(f)}{SS_A(f)} \]  

with a magnitude defined as

\[ |H(f)|^2 = [H_r(f)]^2 + [H_i(f)]^2 \]  

where \( H_r(f) \) and \( H_i(f) \) are the real and imaginary parts of the complex \( H(f) \) values and are expressed as millimeters of mercury per pulse per second.

Statistical analysis. The AP power was quantified by integration of its average periodogram in the very low-frequency (VLF, 0.0–0.25 Hz), low-frequency (LF, 0.25–0.8 Hz), and high-frequency (HF, 0.8–2.4 Hz) ranges (4, 16, 30). The relationship between stimulation spike rate and evoked APV was assessed by coherence and transfer magnitude in each frequency range. We also quantified the frequency characteristic of RVLM-APV transfer function by the high-cut frequency (16), which was defined as the high end of frequency at which the magnitude of transfer function decayed to one-half of maximum. Values are presented as means ± SE. Data between groups were compared by Student’s t-test or two-way ANOVA as appropriate. Difference was considered statistically significant at \( P < 0.05 \).

RESULTS

Autospectral analysis of APV in SHR and WKY. Figure 1 provides illustrative examples of AP, heart rate (HR), and APV in SHR (Fig. 1B) and WKY (Fig. 1A). Continuous autospectral analysis, together with average periodogram analysis of APV, revealed a significantly different picture between these two strains. In the SHR, the autospectrum of APV exhibited a continuous display of prominent power especially in the LF and HF ranges (Fig. 1B, top). These spectral manifestations were clearly demonstrated by the corresponding average periodogram of APV with an enhanced resolution (Fig. 1B, bottom). As summarized in Fig. 2, AP, HR, and APV in the VLF, LF, and HF ranges of SHR were significantly higher than those in WKY. However, the most significant disparity was noted in the LF power of APV, which in SHR was ~10 times that in WKY.

Cross-spectral analysis of RVLM stimulation and AP response. Figure 3 is a time- and frequency-domain illustration of the relationship between RVLM electrical stimulation and AP response in SHR and WKY. Activation of the RVLM with broad-band-modulated
current pulses evoked an irregular APV in both rats. Concurrent frequency-domain analysis further revealed a low-pass characteristic of the stimulation-response relationship. As expected, the autospectrum of the stimulus spike rate manifested a broad distribution of power spectral density. Intriguingly, the corresponding autospectrum of APV exhibited prevailing power density only in the lower frequency range (<0.8 Hz) in both rats. This range covered the VLF (0–0.25 Hz) and LF (0.25–0.8 Hz) components in the APV spectrum. The coherence function demonstrated significant linearity between stimulation and response in the VLF and LF ranges in both rats. However, the power density of the evoked APV as well as the magnitude of the RVLM-AP transfer function was generally higher in SHR. It is interesting to note that the frequency bandwidth of the transfer function was also higher in SHR, as revealed by rightward expansion of transfer magnitude toward higher frequencies.

As summarized in Fig. 4, the broad-band electrical stimulation of the RVLM evoked significant APV in both rat strains. Compared with that shown in Fig. 2, the evoked APV was noted to be ~20 times the spontaneous APV, especially in the LF range. On the basis of the similar stimulation spike rate variability (SRV) of electrical stimulation, it was noted that the evoked APV and the transfer magnitude of APV to SRV were significantly higher in SHR. As for spontaneous APV, the most significant disparity of evoked APV between SHR and WKY was noted in the LF range, at which the power value in SHR was around nine times that in WKY. In addition, the transfer magnitude in the LF range in SHR was noted to be around three times that in WKY.

Figure 5 provides a more detailed description of the frequency characteristic of the central vasomotor control in SHR and WKY. Two-way ANOVA detected significant effects (P < 0.05) of frequency and strain on
RVLM-AP transfer magnitude. The greatest disparity within these two strains occurred at 0.44 Hz, where the transfer magnitude of SHR was 3.1 times that of WKY. The least disparity occurred at 0.06 Hz, where the SHR-to-WKY transfer magnitude ratio was only 1.5. It is interesting to note that the differences between SHR and WKY became virtually absent (P > 0.05) subsequent to combined α- and β-adrenoceptor blockade by phentolamine (2.5 mg/kg iv) and propranolol (1 mg/kg iv) (15).

The difference in the frequency characteristic of central vasomotor control between SHR and WKY can

Fig. 3. Illustrative example of time- and frequency-domain analysis of effect of electrical stimulation of rostral ventrolateral medulla in SHR and WKY with computer-generated current pulses (50 μA, 1 ms) at a spike rate of 15 ± 15 pulse per second (pps) and broad-band modulation frequencies between 0 and 3 Hz. A: stimulus spike rate (SR) and AP during stimulation. B: average periodograms of spike rate variability (SRV) or APV and cross-spectrograms showing coherence (Coher) and transfer magnitude between SRV and APV during stimulation. Note that significant responses are denoted by coherence > 0.5 or by heavy line in magnitude of transfer function.

Fig. 4. SRV of stimulus current pulses on rostral ventrolateral medulla (A), evoked APV (B), and corresponding coherence (C) and transfer magnitude (D) between SRV and APV in VLF (0–0.25 Hz), LF (0.25–0.8 Hz), and HF (0.8–2.4 Hz) ranges in SHR and WKY. Data are expressed as means ± SE (n = 10). *P < 0.05 vs. WKY group by Student's t-test.

Fig. 5. Changes in transfer magnitude between SRV and APV of SHR and WKY as a function of frequency before and after combined α- and β-adrenoceptor blockade by phentolamine (2.5 mg/kg iv) and propranolol (1 mg/kg iv). Data are expressed as means ± SE (n = 10). *P < 0.05 vs. WKY group; †P < 0.05 vs. adrenoceptor blockade.
be further demonstrated by the high-cut frequency of RVLM-AP transfer magnitude. We found, in addition to the grossly enhanced transfer magnitude, that SHR were also characterized by an increased high-cut frequency in comparison with WKY (0.38 ± 0.03 vs. 0.24 ± 0.02 Hz, P < 0.05). This indicates that SHR have a wider frequency range of central vasomotor control.

**DISCUSSION**

Recent advances in the analysis technique have extended our knowledge of APV. APV has a very specific frequency in comparison with WKY (0.38 vs. 0.02 Hz, 6). This indicates that SHR have a higher spontaneous APV. With the technique of frequency-domain analysis, we found that APV between 0.1 and 0.8 Hz coheres well with RVLM excitation via the sympathetic system. This finding also provides a close linkage between RVLM and the LF component. The HF component of APV has been attributed to vagal and respiratory control in the spontaneously breathing dog (1). In the positive-pressure-ventilated rat, however, it has been shown that cardiac sympathetic regulation plays an important role in respiratory-related APV (15, 29), and thus a similar animal preparation was used in the present study. The VLF variability is presumably related to thermoregulation or the angiotensin system (19).

The present study explored the differences in the frequency characteristic of central vasomotor control between SHR and WKY. We used 10- to 12-wk-old SHR, which are at the developmental stage of hypertension (10). In a stable, anesthetized state, the SHR were noted to exhibit a higher spontaneous APV. With the technique of frequency-domain analysis, we found that the LF power of APV was ~10 times more pronounced in SHR compared with WKY. Thus our data support the hypothesis that vasomotor reactivity is higher in SHR. From this observation, it is of interest to ask where the site of action is. We specifically focused on the RVLM-AP functional pathway. To evaluate the dynamic property, we applied the broad-band-modulated spikes of electrical current on RVLM, which evoked a prominent APV and quantified the stimulation-response relationship in the frequency domain. Spectral analysis revealed a LF power of APV in SHR nine times that in WKY during broad-band RVLM stimulation. Although the RVLM-AP transfer magnitude of both rat strains had properties similar to a low-pass filter, the gain of SHR was around three times that of WKY, particularly in the LF range. Interestingly, the elevation of LF power in SHR, either spontaneous or evoked, can be roughly matched by the square of the corresponding transfer magnitude. This indicates that the enhanced APV observed in the SHR may result from its potentiated RVLM-AP functional pathway. It should be noted that the increase of APV or RVLM-AP transfer magnitude cannot be completely explained by the high AP of SHR, which was only ~1.5 times that of WKY. Another interesting finding is that the disparity in RVLM-AP transfer magnitude between SHR and WKY became virtually absent subsequent to combined α- and β-adrenoceptor blockade.

Our data provide the first demonstration that the dynamic control of RVLM on AP reactivity is significantly enhanced in SHR, in which the adrenergic system may play a major role. This finding also offers an explanation, at least in part, for the higher spontaneous LF variability in SHR. The enhanced potency of a RVLM-AP functional pathway may arise in any relay between RVLM and AP. For example, an elevation in excitability of preganglionic sympathetic neurons (22, 28), sympathetic innervation of target organs (3, 20), or sensitivity of vascular smooth muscle (9) may be responsible for the altered RVLM-AP transfer function in SHR. The exact mechanism warrants further exploration. The response frequency is an important consideration for the temporal property of an input-output system. A high response frequency usually coincides with a rapid response rate. For example, the vagal regulation of heart rate is characterized by a higher response frequency and a higher response rate compared with sympathetic regulation (2). For the purpose of quantitative analysis, we have defined the high-cut frequency of RVLM-AP functional pathway from the shape of its magnitude spectrum (16). This parameter describes the upper limit of the response frequency. An increase in the high-cut frequency means a higher extension of the response frequency. Therefore, it can be more convenient to study the temporal property of central vasomotor control.

When electrical stimulation is applied to RVLM, the pressor response may be attributable to excitation of sympathetic vasomotor fibers, release of catecholamine from the adrenal medulla, and release of vasopressin, presumably from the posterior pituitary (27). Because each functional pathway has its response frequency, it is possible to separate them with the application of dynamic analysis. For example, although both pressor responses due to sympathetic vasomotor fibers and adrenal catecholamine may have similar magnitude, the former is characterized by a more rapid phase of increase (27), which is equivalent to a higher response frequency. Because the net response between RVLM stimulation and AP is jointly achieved by all functional pathways, the balance among them will determine the gross response frequency. Our results show that, in addition to a larger response magnitude, the RVLM-AP transfer function of SHR is characterized by a higher extension of response frequency, which can be quantitatively analyzed by the increase of the high-cut frequency. The increase in the transfer magnitude may be caused by the increase in the net potencies of all functional pathways, but the increase in the high-cut frequency represents a change in their balance. Therefore, we suggest that a dominant elevation in the potency of sympathetic vasomotor fibers may be responsible for the altered frequency characteristics of central vasomotor control in SHR.
Controversial results have been noted regarding the spontaneous APV in essential hypertension. Although the experimental results from this study and from previous studies by Murphy et al. (23) and Lucini et al. (17) support an enhanced LF power of APV in spontaneous hypertension, other studies have shown similar APV in hypertension and normotension (25, 26). It should be noted that the present work first demonstrated the coexistence of enhanced spontaneous APV and altered RVLM-AP transfer function in SHR. Because electrical stimulation was applied for transfer-function analysis and the animals were anesthetized throughout the experiments, the influence of pentobarbital anesthesia must be considered. For this reason, we systematically evaluated the effect of pentobarbital on cardiovascular variabilities in a previous study (30) and found the optimal dose for rat experiments to be a continuous intravenous infusion of 20 mg·kg⁻¹·h⁻¹. Such a dose may achieve stable anesthesia while preserving autonomic regulation of cardiovascular variabilities (30). This anesthetic regimen was also applied in the present experiment from which our conclusion is drawn. Although the dose of the anesthetic has been controlled, the possible difference in anesthetic sensitivity between SHR and WKY cannot be completely ruled out.

The elevation in spontaneous vasomotor-related APV found in SHR is compatible with the enhanced magnitude and temporal properties of the RVLM-AP functional pathway. Although essential hypertension may be caused by multiple factors, this study quantitatively demonstrated the coexistence of enhanced spontaneous APV and altered RVLM-AP transfer function in SHR. Because electrical stimulation was applied for transfer-function analysis and the animals were anesthetized throughout the experiments, the influence of pentobarbital anesthesia must be considered. For this reason, we systematically evaluated the effect of pentobarbital on cardiovascular variabilities in a previous study (30) and found the optimal dose for rat experiments to be a continuous intravenous infusion of 20 mg·kg⁻¹·h⁻¹. Such a dose may achieve stable anesthesia while preserving autonomic regulation of cardiovascular variabilities (30). This anesthetic regimen was also applied in the present experiment from which our conclusion is drawn. Although the dose of the anesthetic has been controlled, the possible difference in anesthetic sensitivity between SHR and WKY cannot be completely ruled out.

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REFERENCES


