Differential dynamic baroreflex regulation of cardiac and renal sympathetic nerve activities

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Regional difference in sympathetic efferent nerve activity has been a great concern of many investigators. Ninomiya et al. (25) reported differential arterial baroreflex control of sympathetic nerve activity among neural districts directed to the spleen, kidney, and heart in anesthetized rabbits. We isolated carotid sinuses and randomly perturbed intracarotid sinus pressure (CSP) while simultaneously recording cardiac sympathetic nerve activity (CSNA) and renal sympathetic nerve activities (RSNA). The neural arc transfer function from CSP to CSNA and that from CSP to RSNA revealed high-pass characteristics. The increasing slope of the transfer gain in the frequencies between 0.03 and 0.3 Hz was significantly greater for CSNA than for RSNA (2.96 ± 0.72 vs. 1.64 ± 0.73 dB/octave, \( P < 0.01 \), \( n = 9 \)). The difference was hardly explained by the difference in static nonlinear characteristics of CSP-CSNA and CSP-RSNA relationships or by the difference in conduction velocities in the multifiber recording. These results indicate that the central processing in the brain stem differs between CSNA and RSNA. The neural arc of the baroreflex may exert differential effects on the heart and kidney in response to dynamic baroreflex activation.

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nothing in particular except for inverting the sign of signal and adding some lag time. The discrepancy between their and our data lead us to hypothesize that the dynamic baroreflex regulation differs between CSNA and RSNA.

Although Harada et al. (7) demonstrated that arterial baroreflex control of CSNA and RSNA is uniform in the frequency domain in anesthetized cats, we think the study is incomplete with respect to the following aspects. First, they applied a conventional open-loop transfer function analysis despite the fact that the arterial baroreflex loop was partially closed in their experimental setting (17). Second, the input power spectra were not quite white in the frequency range examined (0.01–0.7 Hz). Therefore, there is room for argument over whether the neural arc transfer functions they estimated were precise enough for uncovering the difference in dynamic baroreflex regulation between CSNA and RSNA. Third, the dynamic responses of CSNA and RSNA were not directly compared in individual animals, which might have made the differentiation between the CSNA and RSNA responses difficult. To circumvent these problems, we performed a baroreflex open-loop experiment in anesthetized rabbits while simultaneously recording CSNA and RSNA. We isolated bilateral carotid sinuses from the systemic circulation so as to accurately impose desired pressure perturbation (8, 11–14, 22, 23, 27, 29). The results indicated that the high-pass characteristics of the neural arc was significantly more enhanced in CSNA than in RSNA.

MATERIALS AND METHODS

Surgical Preparations

Animals were cared for in strict accordance with the Guiding Principles for the Care and Use of Animals in the Field of Physiological Sciences approved by the Physiological Society of Japan. Nine Japanese White rabbits weighing 2.4–3.8 kg were anesthetized via intravenous injection (2 ml/kg) of a mixture of urethane (250 mg/ml) and α-chloralose (40 mg/ml) and mechanically ventilated with oxygen-enriched room air. Supplemental anesthetics were injected as necessary (0.5 ml/kg) to maintain an appropriate level of anesthesia. Aortic pressure (AoP) was measured using a high-fidelity pressure transducer (Millar Instruments; Houston, TX) inserted via the right femoral artery. We isolated the bilateral carotid sinuses vascularity from the systemic circulation by ligating the internal and external carotid arteries and other small branches originating from the carotid sinus regions. The isolated carotid sinuses were filled with warmed physiological saline through catheters inserted via the common carotid arteries. The intracarotid sinus pressure (CSP) was controlled by a servo-controlled piston pump (model ET-126A, Labworks; Costa Mesa, CA). Bilateral vagal and aortic depressor nerves were sectioned at the middle of the neck to eliminate baroreflexes from the cardiopulmonary region and the aortic arch. We exposed the left renal sympathetic nerve retroperitoneally and attached a pair of stainless steel wire electrodes (Bioflex wire AS633, Cooner Wire) to record RSNA. The nerve fibers peripheral to the electrodes were tightly ligated and crushed to eliminate afferent signals from the kidney. We also recorded CSNA from the left cardiac sympathetic nerve through a midline thoracotomy. The nerve fibers peripheral to the electrodes were sectioned to eliminate afferent signals from the heart. To insulate and fix the electrodes, the nerve and electrodes were covered with a mixture of silicone gel (Semicosil 932A/B, Wacker Silicones) and white petrolatum (Vaseline). The preamplified nerve signal was band-pass filtered at 150–1,000 Hz. It was then full-wave rectified and low-pass filtered with a cutoff frequency of 30 Hz to quantize the nerve activity. We exchanged the recording systems for CSNA and RSNA occasionally and confirmed that the recording systems had identical characteristics. Pancuronium bromide (0.3 mg/kg) was administered to prevent contamination of muscular activity in the CSNA and RSNA recordings. Body temperature was maintained at ~38°C with a heating pad.

Protocols

Dynamic protocol. After the surgical preparation was completed, CSP was equilibrated with mean AoP to obtain the operating pressure (OP). To estimate the dynamic characteristics of the baroreflex in regulating CSNA and RSNA, we randomly assigned CSP to either high (OP + 20 mmHg) or low (OP – 20 mmHg) pressure every 500 ms according to a binary white noise sequence (12–14, 19, 28, 29). The input power spectra of CSP were relatively flat up to 1 Hz (Fig. 1). We recorded CSP, CSNA, RSNA, and AoP for 10 min at a sampling rate of 200 Hz using a 12-bit analog-to-digital converter. The data were stored on the hard disk drive of a dedicated laboratory computer system for later analysis.

Static protocol. Because static nonlinear characteristics of the neural arc are thought to affect dynamic baroreflex regulation of sympathetic nerve activity (11, 30), we estimated the static characteristics of the neural arc in six of nine animals. CSP was first decreased to 40 mmHg. After CSNA and RSNA reached steady state, CSP was changed stepwise from 40 to 180 mmHg with an increment of 20 mmHg. Each pressure step was maintained for 60 s. When CSNA and RSNA were completely suppressed and AoP decreased below 50 mmHg at the CSP level of 160 mmHg, the CSP level of 180

Fig. 1. Power spectra of the intracarotid sinus pressure (CSP) during the dynamic protocol. The power was relatively flat up to 1 Hz. Solid and dashed lines represent the mean and mean + SD values, respectively, calculated from all animals.
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Data Analysis

In the dynamic protocol, to estimate the neural arc transfer function of the carotid sinus baroreflex, we treated CSP as the input and CSNA or RSNA as the output of the system. We resampled input-output data pairs at 10 Hz and segmented them into eight sets of 50% overlapping bins of 1,024 data points each. For each segment, a linear trend was subtracted, and a Hanning window was applied. We then performed a fast Fourier transform to obtain frequency spectra of the input and output. We ensemble averaged the input power $S_x(f)$, output power $S_y(f)$, and cross-power between the input and output $S_{XY}(f)$ over the eight segments, where $f$ represents frequency. Finally, we calculated the transfer function $[H(f)]$ from the input to output with the use of the following equation (19)

$$H(f) = \frac{S_{xy}(f)}{S_{xx}(f)}$$

The modulus [or dynamic gain, $|H(f)|$] and phase [$\theta(f)$] of the transfer function were derived from its real $[H_{\text{real}}(f)]$ and imaginary parts $[H_{\text{imag}}(f)]$ with the use of the following equations

$$|H(f)| = \sqrt{H_{\text{real}}(f)^2 + H_{\text{imag}}(f)^2}$$

$$\theta(f) = \tan^{-1}\left(\frac{H_{\text{imag}}(f)}{H_{\text{real}}(f)}\right)$$

To quantify the linear dependence between the input and output signals in the frequency domain, we calculated a magnitude-squared coherence function $[\text{Coh}(f)]$ with the use of the following equation (19)

$$\text{Coh}(f) = \frac{|S_{xy}(f)|^2}{S_{xx}(f)S_{yy}(f)}$$

The coherence value ranges from zero to unity. A unity coherence indicates a perfect linear dependence between the input and output signals, whereas zero coherence indicates total independence between the two signals.

Hereafter in the present paper, $H_{\text{CSNA}}$ and $H_{\text{RSNA}}$ denote the transfer function from CSP to CSNA and from CSP to RSNA, respectively. The system impulse response was derived from the inverse Fourier transform of $H(f)$. To facilitate intuitive understanding of the transfer characteristics, we calculated the system step response from a time integral of the system impulse response up to 10 s. To compare the dynamic responses of CSNA and RSNA directly during CSP perturbation, we also calculated the transfer function from RSNA to CSNA. In this context, the transfer function represented the amplitude ratio and phase difference between RSNA and CSNA rather than the input-output relationship between the two signals.

In the static protocol, we calculated mean sympathetic nerve activity during the last 10 s of each CSP level and performed a regression analysis for the four-parameter logistic curve as follows (11, 15, 28)

$$y = \frac{P_1}{1 + \exp\left(P_2\text{CSP} - P_3\right)} + P_4$$

where $y$ indicates mean CSNA or RSNA, $P_1$ is the response range (i.e., the difference between the maximum and minimum values of $y$), $P_2$ is the coefficient of gain, $P_3$ is the midpoint of operation in the CSP axis, and $P_4$ is the minimum value of $y$.

Statistical Analysis

All data are presented as means ± SD. In all of the following statistics, difference was considered significant when $P < 0.05$. Because the magnitude of sympathetic nerve activity varied depending on such recording conditions as the physical contact between the nerve and the electrodes, CSNA and RSNA were presented in arbitrary units. In the dynamic protocol, we normalized CSNA and RSNA by the gain values of $H_{\text{CSNA}}$ and $H_{\text{RSNA}}$ below 0.03 Hz, respectively. To examine the difference between $H_{\text{CSNA}}$ and $H_{\text{RSNA}}$, we obtained the gain and phase values at 0.01, 0.1, 0.5, and 1 Hz in each animal. Because the baroreceptors were exposed to a broad frequency bandwidth perturbation in the range from 0.01 and 1 Hz, we arbitrarily chose the frequencies for the statistical analysis within this frequency range. To minimize the variance associated with the estimation of the transfer function, values between 0.45 and 0.5 Hz were averaged to represent the value around 0.5 Hz. Similarly, values between 0.9 and 1 Hz were averaged to represent the value around 1 Hz. After the gain and phase values at each frequency in each animal were obtained, group differences in these parameters between $H_{\text{CSNA}}$ and $H_{\text{RSNA}}$ were examined by paired $t$-test (6). We also calculated a slope of the transfer gain in the frequencies between 0.03 and 0.3 Hz in each animal and examined its group difference between $H_{\text{CSNA}}$ and $H_{\text{RSNA}}$ by paired $t$-test.

The coherence values at 0.01, 0.1, 0.5 (averaged from 0.45 to 0.5 Hz), and 1 Hz (averaged from 0.9 to 1 Hz) were obtained in each animal. The group difference in the coherence value at each frequency was then examined by the Wilcoxon signed-ranks test, because the normal distribution was not assumed for the coherence values (6).

The initial response (i.e., maximum negative response) and steady-state response of the step response (averaged between 9 and 10 s) were also calculated in each animal. Group differences in the initial and steady-state responses between the CSNA and RSNA step responses were then examined by paired $t$-test (6). In the static protocol, because mean sympathetic nerve activity was represented in arbitrary units, the absolute $P_1$ and $P_3$ values had no particular biological meanings. Therefore, we compared only the $P_2$ and $P_3$ values between the CSP-CSNA and CSP-RSNA relationships by paired $t$-test (6). Note that the $P_2$ value has a reciprocal relationship with the operating range in the CSP axis independent of the other parameters of the logistic function (see Appendix).

Results

Figure 2 presents typical time series obtained from the dynamic protocol. CSP, CSNA, RSNA, and AoP are shown. CSP was perturbed according to a binary white noise sequence. When CSP was increased, CSNA and RSNA decreased. AoP then decreased with some delay. When CSP was decreased, the opposite responses were observed. Because the carotid sinuses were isolated, changes in AoP did not affect CSP, thereby validating a conventional open-loop transfer function analysis under this experimental condition (12, 14, 19). Although the shape of each burst differs between CSNA and RSNA, global characteristics of dynamic changes are similar between the two activities. A simple inspec-
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CSP to RSNA (H)

H1584

Dashed lines represent the mean and mean 1 frequency in both the gain value approximated unity at the lowest frequencies (H) are shown. As a result of normalization, bottom middle, and coherence functions (bottom) are shown. Figure 3A shows the neural arc transfer functions estimated using CSNA (left) and RSNA (right). Gain plots (top), phase plots (middle), and coherence (Coh) functions (bottom) are shown. As a result of normalization, the gain value approximated unity at the lowest frequency in both HCSNA and HRSNA. The gain values increased as the input frequency of CSP perturbation increased between 0.03 and 0.3 Hz, indicating high-pass characteristics of the neural arc. The increasing slope of the transfer gain was steeper in HCSNA than HRSNA. The gain values were relatively constant in the frequencies between 0.4 and 0.8 Hz and gradually decreased as the frequency approached 1 Hz in both HCSNA and HRSNA. The phase plots indicated an out-of-phase relationship between CSP and sympathetic nerve activity in the lowest frequencies in both HCSNA and HRSNA, reflecting negative feedback in the neural arc. The coherence values were between 0.6 and 0.9 in the frequency range between 0.01 and 0.4 Hz in both HCSNA and HRSNA. Figure 3B shows the step responses corresponding to HCSNA and HRSNA. The initial decrease of the step response was significantly more negative in CSNA than in RSNA, whereas the steady-state values did not differ between the two.

Table 1 summarizes the parameters of the transfer function and step response shown in Fig. 3. The gain value at 0.01 Hz approximated unity due to the normalization of sympathetic nerve activity. Although the gain value at 0.1 Hz did not differ between HCSNA and HRSNA, the gain values at 0.5 and 1 Hz were significantly greater in HCSNA than in HRSNA. The phase value at 0.01 Hz did not differ between HCSNA and HRSNA. The phase values at 0.1, 0.5, and 1 Hz were significantly less negative in HCSNA than in HRSNA. The initial decrease in the step response was significantly more negative in CSNA than in RSNA, reflecting the differential high-

![Figure 2](http://ahpheart.physiology.org.org) Representative recordings of CSP, cardiac sympathetic nerve activity (CSNA), renal sympathetic nerve activity (RSNA), and aortic pressure (AoP) during the dynamic protocol. CSP was changed according to a binary white noise signal. au, Arbitrary units.

![Figure 3](http://ahpheart.physiology.org.org) A: transfer function from CSP to CSNA (HCSNA) and from CSP to RSNA (HRSNA). The gain plots (top), phase plots (middle), and coherence (Coh) functions (bottom) are shown. The increasing slope of the transfer gain was greater in HCSNA than in HRSNA. B: step responses (Step Res) corresponding to HCSNA and HRSNA. Solid and dashed lines represent the mean and mean + SD values, respectively.

Table 1. Parameters of the neural arc transfer function and step response

<table>
<thead>
<tr>
<th>Parameter</th>
<th>HCSNA</th>
<th>HRSNA</th>
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<tbody>
<tr>
<td><strong>Gain, U/mmHg</strong></td>
<td></td>
<td></td>
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<tr>
<td>0.01 Hz</td>
<td>1.02 ± 0.06</td>
<td>0.97 ± 0.04</td>
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<tr>
<td>0.1 Hz</td>
<td>1.78 ± 0.40</td>
<td>1.57 ± 0.27</td>
</tr>
<tr>
<td>0.5 Hz</td>
<td>3.75 ± 1.65*</td>
<td>2.33 ± 0.84</td>
</tr>
<tr>
<td>1 Hz</td>
<td>2.88 ± 1.11*</td>
<td>1.99 ± 0.65</td>
</tr>
<tr>
<td><strong>Phase, rad</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.01 Hz</td>
<td>−3.00 ± 0.18</td>
<td>−2.92 ± 0.22</td>
</tr>
<tr>
<td>0.1 Hz</td>
<td>−2.75 ± 0.25*</td>
<td>−3.00 ± 0.16</td>
</tr>
<tr>
<td>0.5 Hz</td>
<td>−3.99 ± 0.25*</td>
<td>−4.13 ± 0.16</td>
</tr>
<tr>
<td>1 Hz</td>
<td>−5.02 ± 0.36*</td>
<td>−5.43 ± 0.49</td>
</tr>
<tr>
<td><strong>Coherence</strong></td>
<td></td>
<td></td>
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<tr>
<td>0.01 Hz</td>
<td>0.66 ± 0.15</td>
<td>0.63 ± 0.19</td>
</tr>
<tr>
<td>0.1 Hz</td>
<td>0.79 ± 0.09</td>
<td>0.86 ± 0.06</td>
</tr>
<tr>
<td>0.5 Hz</td>
<td>0.66 ± 0.10†</td>
<td>0.74 ± 0.11</td>
</tr>
<tr>
<td>1 Hz</td>
<td>0.47 ± 0.12†</td>
<td>0.64 ± 0.05</td>
</tr>
<tr>
<td><strong>Slope, dB/octave</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.03–0.3 Hz</td>
<td>2.96 ± 0.72*</td>
<td>1.64 ± 0.73</td>
</tr>
<tr>
<td><strong>Initial response, units</strong></td>
<td>−3.08 ± 0.88*</td>
<td>−2.21 ± 0.61</td>
</tr>
<tr>
<td><strong>Steady-state response, units</strong></td>
<td>−1.01 ± 0.14</td>
<td>−0.98 ± 0.11</td>
</tr>
</tbody>
</table>

Data are means ± SD. HCSNA, neural arc transfer function estimated using cardiac sympathetic nerve activity; HRSNA, neural arc transfer function estimated using renal sympathetic nerve activity. *P < 0.01 by paired t-test and †P < 0.01 (P = 0.0078) by the Wilcoxon signed-rank test.
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The steady-state value of the step response did not differ between CSNA and RSNA.

Figure 4 shows the amplitude ratio and phase difference between CSNA and RSNA. Although the amplitude ratio of CSNA to RSNA approximated unity in the frequencies below 0.1 Hz, it became greater than unity in the higher frequencies up to 1 Hz. Despite the frequency-dependent difference in the amplitude ratio of CSNA to RSNA, the phase approximated 0 rad over the frequency range under study. If we take a close look at Fig. 4, however, the phase difference is slightly positive in the higher frequencies, indicating that CSNA preceded RSNA. The phase difference at 1 Hz approximated $\pi/10$ rad, which corresponded to a lag time of $\sim 50$ ms. Given that the recording positions for CSNA and RSNA were separated by $\sim 20$–$25$ cm, the average conduction velocity between CSNA and RSNA was 4–5 m/s, which fell among the conduction velocities of preganglionic sympathetic neurons (3–15 m/s), postganglionic sympathetic neurons (0.7–2.3 m/s) (26), and spinal sympathetic conduction (1.6–8 m/s) (31). The coherence function between RSNA and CSNA ranged from 0.7 to 0.9.

Figure 5 presents typical results obtained from the static protocol. Figure 5A is the time series of CSP, CSNA, and RSNA. Mean CSNA and RSNA were decreased in response to a pressure increment in CSP. Figure 5B illustrates the relationship between CSP and mean CSNA and between CSP and mean RSNA calculated from data shown in Fig. 5A. The fitted logistic functions were almost superimposable by the arbitrary scaling of the ordinate. The group-averaged parameters of $P_2$ and $P_3$ did not differ between the CSP-CSNA and CSP-RSNA curves ($P_2$: $0.110 \pm 0.028$ vs. $0.106 \pm 0.031$ mmHg$^{-1}$; $P_3$: $114.6 \pm 11.9$ vs. $112.1 \pm 14.2$ mmHg, $n = 6$).

DISCUSSION

The present study demonstrated that the neural arc transfer function of the carotid sinus baroreflex showed high-pass characteristics regardless of whether CSNA or RSNA was used as the output of the neural arc (Fig. 3). However, the extent of the high-pass filter or the increasing slope of the transfer gain was significantly greater in $H_{CSNA}$ than $H_{RSNA}$ (Table 1). To our knowledge, this is the first demonstration of regional difference in the dynamic baroreflex regulation between CSNA and RSNA.

Differential High-Pass Characteristics of Neural Arc Transfer Function

Although high-pass characteristics were common for $H_{CSNA}$ and $H_{RSNA}$, the extent of the high-pass filter or increasing slope of transfer gain was significantly greater in $H_{CSNA}$ than $H_{RSNA}$ (Fig. 3 and Table 1). The differential CSNA and RSNA responses to CSP perturbation were also supported by the apparent transfer function between RSNA and CSNA where the ampli-
tude ratio of CSNA to RSNA increased greater than unity in the frequencies above 0.1 Hz (Fig. 4). Because arterial pressure regulation via changes in cardiac output is much faster than via changes in urine excretion (24), the CSNA response would be more sensitive to rapid changes in baroreceptor pressure input than would the RSNA response. The neural arc transfer function of the carotid sinus baroreflex is a cascade of the transfer function from pressure input to carotid sinus afferent nerve activity and from carotid sinus afferent nerve activity to sympathetic efferent nerve activity. According to our previous studies (28, 30), the amplitude of aortic depressor nerve activity increased by about two times when the frequency of baroreceptor pressure input increased from 0.01 to 0.5 Hz. If we assume that these dynamic characteristics are also applicable to the carotid sinus baroreceptors, a major part of the high-pass characteristics of $H_{RSNA}$ would be attributable to the dynamic transduction properties at baroreceptors, being consistent with the interpretation proposed by Kubo et al. (17). On the other hand, the high-pass characteristics were more exaggerated in $H_{CSNA}$ than in $H_{RSNA}$. The amplitude of the CSNA response increased by about four times when the frequency of CSP perturbation increased from 0.01 to 0.5 Hz (Table 1). Therefore, the dynamic transduction properties of the baroreceptors alone cannot account for the high-pass characteristics of $H_{CSNA}$. The central processing in the brain stem would play an important role in enhancing the high-pass characteristics of $H_{CSNA}$ compared with $H_{RSNA}$.

The coherence values associated with the neural arc transfer function ranged from 0.6 to 0.9 in the frequency range below 0.4 Hz, suggesting that there existed a significant linear dependence between CSP and CSNA (or RSNA) (Fig. 3). At the same time, however, coherence values less than unity suggest the existence of CSNA and RSNA that could not be described by linear dynamics with baroreceptor pressure input. The reduction of coherence values is attributable to several factors: a nonlinear system response, a central command component of sympathetic nerve activity uncoupled with baroreceptor pressure input, and physical noise in the nerve activity recording procedure. Among these possibilities, the central command component would be the most significant factor. For instance, the sympathetic preganglionic nuclei are known to receive inputs from sources other than the rostral ventral medulla (4). Such inputs generate physiological signals in nerve activity independent of the arterial baroreflex, which are then treated as the inherent noise of the system in terms of linear system analysis.

**Effect of Static Nonlinear Characteristics of Neural Arc on Dynamic Baroreflex Regulation**

We examined whether mechanisms other than the central processing could account for the observed differential high-pass characteristics between $H_{CSNA}$ and $H_{RSNA}$. The neural arc of the carotid sinus baroreflex has a nonlinear sigmoidal relationship between CSP and steady-state sympathetic nerve activity (9, 24, 27). Because of the sigmoidal nonlinearity, the response of sympathetic nerve activity is saturated when the input amplitude increases. Note that the high-pass characteristics of baroreceptor transduction properties augment the amplitude of baroreflex afferent signal in the higher frequencies, even when the amplitude of input pressure remains constant (28, 30). If the central processing in the brain stem also has a sigmoidal non-linearity between baroreflex afferent signal and sympathetic efferent nerve activity, the saturation effect on sympathetic nerve activity will become pronounced in the higher frequencies of CSP perturbation. Therefore, if the operating range of the CSP-RSNA curve is narrower than that of the CSP-CSNA curve, it will result in the saturation of dynamic RSNA response relative to dynamic CSNA response in the higher frequencies. However, the coherence values associated with $H_{RSNA}$ were slightly but significantly greater than those associated with $H_{CSNA}$ at 0.5 and 1 Hz (Table 1), suggesting that the linearity between CSP and RSNA during the dynamic protocol was not less than that between CSP and CSNA. These results were against the presumption that the operating range of the CSP-RSNA curve was narrower than that of the CSP-CSNA curve.

We also directly examined whether the operating range differed between the CSP-CSNA and CSP-RSNA curves in the static protocol (Fig. 5). There were no significant differences in $P_2$ and $P_3$ between the CSP-CSNA and CSP-RSNA curves. In other words, the operating range did not differ between the CSP-CSNA and CSP-RSNA curves (see Appendix). Therefore, the differential high-pass characteristics between $H_{CSNA}$ and $H_{RSNA}$ cannot be attributed to the difference in the static nonlinear characteristics between the CSP-CSNA and CSP-RSNA curves. The fact that the differential high-pass characteristics between $H_{CSNA}$ and $H_{RSNA}$ were persistently observed when the peak-to-peak amplitude of CSP perturbation was reduced to 20 mmHg in the dynamic protocol (data not shown) also supports the interpretation that the differential high-pass characteristics are independent of the saturation effect via the static nonlinearity.

**Effect of Multifiber Recording on High-Pass Characteristics**

The amplitude of the compound action potential decreases and the duration increases as the conduction distance is prolonged in the multifiber recording of nerve activity (3, 5, 26). This is due to the difference in conduction velocities among the nerve fibers that make up the nerve bundle. Because the conduction distance between the brain stem and nerve is longer for the RSNA than for the CSNA recordings, we examined, with the use of a simulation, whether the difference in conduction distance and the nature of multifiber recording could affect the dynamic baroreflex regulation of sympathetic nerve activity. According to previous studies (8, 14, 22), we modeled the neural arc transfer...
function as a first-order high-pass filter with a lag time as follows

\[ H(f) = -K \left(1 + \frac{f}{f_c} \right) \exp(-2\pi f j L) \]  \hspace{1cm} (6)

where \( K, f_c, \) and \( L \) are steady-state gain, corner frequency (in Hz), and lag time (in s), respectively. \( f \) and \( j \) indicate the frequency (in Hz) and the imaginary unit, respectively. To mimic the observed transfer characteristics, we set \( K = 1 \) and \( f_c = 0.12 \) Hz. We then averaged \( H(f) \) from 5,000 nerve fibers with the following equation

\[ \overline{H(f)} = \frac{1}{5,000} \sum_{n=1}^{5,000} \left[ -K \left(1 + \frac{f}{f_c} \right) \exp(-2\pi f j L_n) \right] \]  \hspace{1cm} (7)

where \( n \) and \( L_n \) represent an index number of each fiber and corresponding lag time, respectively. \( L_n \) was randomly assigned according to a Gaussian distribution, so that \( L_n \) had a mean value of 500 ms for \( H_{CSNA} \) and 550 ms for \( H_{RSNA} \). The difference in the mean lag time represents the difference in conduction distance between CSNA and RSNA recordings. The SD of the lag time (SD\(_{lag}\)) was set at 0, 15, and 30% of the mean value and represents the difference in conduction velocity among nerve fibers. Although the distribution of the conduction velocities of nerve fibers in the present settings was unknown, according to the study on the human ulnar nerve by Barker et al. (3), the 80% spread of the distribution (i.e., the difference between the two velocities below which 10 and 90% of the total number of fibers lie) is \( \approx 30\% \) of the distribution mean velocity. Our simulation covered the reported distribution of the conduction velocities. Figure 6 presents the simulation results calculated from a total number of 5,000 nerve fibers. Figure 6A shows the histograms of lag time. When SD\(_{lag}\) is zero, all 5,000 fibers show lag times of 500 and 550 ms for cardiac and renal sympathetic nerves, respectively. When SD\(_{lag}\) is set at 15 or 30%, the histograms of lag time show the corresponding normal distributions around 500 and 550 ms for cardiac and renal sympathetic nerves, respectively. Figure 6B presents the gain and phase plots of the simulated neural arc transfer functions averaged from 5,000 nerve fibers (the solid line and dashed line indicate the simulated \( H_{CSNA} \) and \( H_{RSNA} \), respectively). When SD\(_{lag}\) is zero, the gain plots of \( H_{CSNA} \) and \( H_{RSNA} \) are identical. The gain increases as the frequency increases above 0.1 Hz. The high-pass characteristics continue beyond 1 Hz. The phase plot reveals a phase difference between \( H_{CSNA} \) and \( H_{RSNA} \) corresponding to the difference in the lag time (50 ms). Although increasing SD\(_{lag}\) attenuated the transfer gain in frequencies above 0.8 Hz, the gain plots below the frequency were indistinguishable between \( H_{CSNA} \) and \( H_{RSNA} \). Therefore, despite the potential low-pass effect on the transfer gain in the higher frequencies, the distribution of the conduction velocities among nerve fibers may not account for the differential high-pass characteristics observed in the frequencies between 0.03 and 0.3 Hz.
Open-Loop Versus Closed-Loop Experiments

The differential high-pass characteristics between CSNA and RSNA responses to CSP perturbation are inconsistent with the results by Harada et al. (7) in anesthetized cats. The difference may be explained as follows. First, as mentioned in the introduction, the arterial baroreflex was partially closed in their study. In other words, changes in AoP by aortic balloon inflations and deflations altered sympathetic nerve activity, whereas changes in sympathetic nerve activity, in turn, affected the AoP. Under these conditions, a closed-loop system identification method rather than a conventional open-loop transfer function analysis should be employed to avoid any biased estimation (12, 14). In the present study, the carotid sinus baroreflex loop was opened so that the conventional open-loop analysis was applied. Second, the power spectra of AoP perturbation were not quite white in the frequencies between 0.01 and 0.7 Hz in their study, possibly due to a mechanical damping by the arterial compliance. The lack of input power might have affected the precise assessment of high-pass characteristics of the CSNA and RSNA responses. In contrast, because we isolated the carotid sinuses, we were able to impose CSP perturbation of relatively flat power spectra up to 1 Hz (Fig. 1). Third, they normalized CSNA and RSNA by the respective values under control conditions, whereas we normalized CSNA and RSNA by the gain values below 0.03 Hz in the respective neural arc transfer functions. Because sympathetic nerve activity under control conditions consisted not only of phasic activity (dynamic component) but also of tonic activity (direct current component) and because the transfer function dealt with the dynamic component alone, their normalization procedure might have masked differential high-pass characteristics between \( H_{\text{CSNA}} \) and \( H_{\text{RSNA}} \). Finally, the species difference between cats and rabbits should also be taken into account.

Although we focused on the frequency range from 0.01 to 1 Hz on the basis of the frequency bandwidth of the total baroreflex function (8), the baroreceptors are normally exposed to higher frequency input mainly associated with the heart rate component (3–4 Hz in rabbits) and its harmonics. Frequency-dependent depression of synaptic transmission has been reported at the first synapse of the baroreflex in the nucleus tractus solitarius mainly in the frequencies above 1 Hz (2, 18). The apparent contradiction between our high-pass filter characteristics and the phenomenon of frequency-dependent depression may be explained as follows. First, the frequency range tested is different. Second, because we defined the input frequency as the frequency of baroreceptor pressure perturbation, it corresponds to a modulation frequency of stimulation rather than stimulation frequency itself of baroreflex afferent fibers. Further open-loop experiments are clearly needed where input frequencies of CSP perturbation are expanded beyond 1 Hz to integrate the different aspects of dynamic characteristics of signal transduction in the neural arc.

Limitations

There are several limitations to this study. First, we investigated the carotid sinus baroreflex in anesthetized rabbits. Although we chose an anesthetic agent that is less suppressive to cardiovascular regulation, the absolute gain values of the carotid sinus baroreflex might have been affected to some degree. However, because we recorded CSNA and RSNA simultaneously, we believe that the differential high-pass characteristics would exist even in the absence of anesthesia.

Second, whether it is CSNA or RSNA that is a better representative of dynamic systemic sympathetic nerve activity remains unclear. Despite a slight but significant difference in the neural arc transfer function, the coherence values were sufficiently high for both \( H_{\text{CSNA}} \) and \( H_{\text{RSNA}} \) to accurately describe the linear input-output relationship between CSP perturbation and sympathetic nerve activity (Fig. 3). Therefore, we will be able to use both CSNA and RSNA to describe dynamic baroreflex regulation as long as we recognize the potential difference in the extent of high-pass characteristics. The difference between the CSNA and RSNA responses to CSP perturbation implies that the neural arc transfer function would be differently estimated when other neural districts are examined. Regional difference in the neural arc transfer characteristics among other neural districts awaits further investigation.

Third, we could not identify the mechanism for the differential high-pass characteristics between \( H_{\text{CSNA}} \) and \( H_{\text{RSNA}} \). Although a difference in the static nonlinear characteristics of the neural arc could theoretically affect the high-pass characteristics of the neural arc, there were no significant differences between the CSP-CSNA and CSP-RSNA curves. Furthermore, the simulation study indicated that the difference in conduction velocities among the nerve fibers could not account for the differential high-pass characteristics between \( H_{\text{CSNA}} \) and \( H_{\text{RSNA}} \) in the frequencies between 0.03 and 0.3 Hz. Further studies focusing on central processing in the brain stem are required to identify the mechanism for the differential high-pass characteristics.

Finally, we filled the isolated carotid sinuses with warm physiological saline. Because the ionic content affects the sensitivity of the baroreceptors (1), the absolute gain values of the carotid sinus baroreflex might be different from normal physiological values. The extent of chemoreceptor activation might also affect CSNA and RSNA or interfere with the carotid sinus baroreflex. However, because we changed neither intravascular ion content nor oxygen content in the isolated carotid sinuses during CSP perturbation, dynamic changes in CSNA and RSNA should be mainly attributable to baroreceptor pressure input. The baroreflex sensitivity to pressure input was relatively unchanged even in a prolonged protocol under this experimental setting (27). In addition, CSNA and RSNA were simultaneously recorded. We think, therefore, that the comparison of the neural arc transfer...
functions between the CSNA and RSNA responses is a fair one.

In conclusion, we found differential high-pass characteristics between the CSNA and RSNA responses to dynamic CSP perturbation. The differential high-pass characteristics of the neural arc imply a differential central processing in the brain stem. Although we did not confirm any physiological significance of the differential high-pass characteristics between \( H_{CSNA} \) and \( H_{RSNA} \) in regulating arterial pressure, we speculate that the neural arc of the baroreflex exerts differential effects on the heart and kidney in response to dynamic baroreflex activation.

**APPENDIX**

**Relationship between coefficient of gain of logistic function and operating range of the system.**

In the logistic function, the threshold pressure \( (P_{th}) \) and saturation pressure \( (P_{sat}) \) can be described as:

\[
P_{th} = P_3 - k/P_2 \\
P_{sat} = P_3 + k/P_2
\]

where \( k \) is an arbitrarily defined constant, \( P_3 \) is the midpoint of operation of the CSP axis, and \( P_2 \) is the coefficient of gain. For instance, Kent et al. (15) adopted the solution of the third derivative of the logistic function at its minimum \( (k = 1.317) \).

The width of the operating range of the system is calculated irrespective of the value of \( k \) as follows:

\[
P_2 = \frac{2k}{P_{sat} - P_{th}}
\]

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