Summation of dynamic transfer characteristics of left and right carotid sinus baroreflexes in rabbits

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Kawada, Toru, Takayuki Sato, Toshiaki Shishido, Masashi Inagaki, Teiji Tatemaki, Yusuke Yanagiya, Masaru Sugimachi, and Kenji Sunagawa. Summation of dynamic transfer characteristics of left and right carotid sinus baroreflexes in rabbits. Am. J. Physiol. 277 (Heart Circ. Physiol. 46): H857–H865, 1999.—Although interactions among parallel negative-feedback baroreflex systems have been extensively investigated with respect to their steady-state responses, the dynamic interactions remain unknown. In anesthetized, vagotomized, and aortic-denervated rabbits, we perturbed isolated intracarotid sinus pressure (CSP) unilaterally or bilaterally around the physiological operating pressure according to binary white noise. The neural arc transfer function from CSP to cardiac sympathetic nerve activity (SNA) and the peripheral arc transfer function from SNA to aortic pressure were estimated. The gain values of the neural arc at 0.01 Hz estimated by the left (L) and right (R) CSP perturbations were 0.94 ± 0.31 and 0.96 ± 0.25, respectively. The gain value increased to 2.17 ± 0.97 during the bilateral identical CSP perturbation and was not significantly different from L + R. The phase values of the neural arc did not differ among protocols. No significant differences were observed in the peripheral arc transfer functions among protocols. We conclude that summation of the dynamic transfer characteristics of the bilateral carotid sinus baroreflexes around the physiological operating pressure approximates simple addition.

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THE CAROTID SINUS BAROREFLEX is one of the important negative-feedback systems that stabilize systemic arterial pressure against pressure perturbation. Because baroreceptor input and such final physiological quantities as arterial pressure and heart rate (12, 24). Despite the dramatically different observations reported, the steady-state response to simultaneous activation of bilateral carotid sinus baroreflexes around the physiological operating pressure approximates simple addition, at least in some experimental conditions (18, 19).

Because the baroreflex operates dynamically under the routine circumstances of daily activity, analysis of the baroreflex system should include not only its steady-state response but also its transient, dynamic response (9, 11, 14, 15). However, previous studies concerning the interactions between the baroreflexes focused mainly on steady-state responses. The purpose of the present study was to examine the dynamic interactions between the left and right carotid sinus baroreflexes. We used a framework of separating the carotid sinus baroreflex system into its two constituent principal arcs: a neural arc representing the characteristics from the baroreceptor input to the efferent sympathetic nerve activity (SNA) and a peripheral arc representing the characteristics from the efferent SNA to aortic pressure (AoP) (9, 11, 14, 15). The results indicate that summation of the left and right carotid sinus baroreflexes around the physiological operating pressure approximated simple addition in relation to their dynamic transfer characteristics.

MATERIALS AND METHODS

Surgical preparations. Animal care was in accordance with the guiding principles of the Physiological Society of Japan. Six Japanese White rabbits weighing 2.4–3.0 kg were anesthetized via intravenous injection (2 ml/kg) of a mixture of urethan (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. AoP was recorded using a high-fidelity pressure transducer (Millar Instruments, Houston, TX) inserted from the right femoral artery. We isolated bilateral carotid sinus nerves from the systemic circulation by ligating the internal and external carotid arteries and other small branches originating from the carotid sinus regions (9, 11, 14, 15). Each of the isolated carotid nerves was flushed and filled with warmed physiological saline through a catheter inserted from the common carotid artery. The left and right intracarotid sinus pressures (CSP) were controlled separately using two independent servo-controlled piston pumps (model ET-126A, Labworks, Costa Mesa, CA). Bilateral vagal nerves and aortic depressor nerves were sectioned at the middle of the neck to eliminate baroreflexes from the cardiopulmonary region and the aortic arch. We sectioned one of the left cardiac sympathetic nerves.
and recorded the efferent SNA using a pair of platinum electrodes. To prevent desiccation and to provide insulation, the electrodes and the nerve were soaked in a mixture of white petrolatum (Vaseline) and paraffin. Pancuronium bromide (0.3 mg/kg) was given to prevent contamination of muscular activity in the nerve recording. 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 cut-off frequency of 30 Hz to quantify the nerve activity. The body temperature was maintained at ∼38°C with a heating pad.

Protocols. The physiological operating pressure was obtained in advance of the carotid sinus isolation, vagotomy, and aortic denervation. The study consisted of the following three protocols. In protocol 1, which was done to assess the dynamic characteristics of unilateral carotid sinus baroreflex, we perturbed either the left or right CSP around the physiological operating pressure according to a binary white noise sequence (11, 16, 21) with a peak-to-peak amplitude of 40 mmHg. The contralateral CSP was kept constant at the physiological operating pressure. The minimum switching interval of high- and low-pressure values of the binary white noise signal was set at 700 ms to provide relatively flat input power spectra up to 0.7 Hz. In protocol 2, which was done to assess the dynamic characteristics of bilateral carotid sinus baroreflexes, we perturbed bilateral CSP simultaneously and identically according to the binary white noise sequence. In protocol 3, which was done to examine the independence of dynamic characteristics between the left and right carotid sinus baroreflexes, we perturbed bilateral CSP simultaneously and independently according to a different series of binary white noise sequences. The order of the protocols was randomized among animals to reduce the likelihood of bias or systematic error in our identification approach. We recorded CSP, SNA, and AoP at a sampling rate of 200 Hz, using a 12-bit analog-to-digital converter. The data were stored on the hard disk of a dedicated laboratory computer system for later analysis.

Data analysis. The data were analyzed beginning at 2 min after the onset of pressure perturbation. To estimate the neural arc transfer function of the carotid sinus baroreflex, we treated CSP as the input and SNA as the output of the system. In protocol 1, we calculated the neural arc transfer functions during the left and right CSP perturbations, respectively, by means of an analysis for one-input, one-output systems (see APPENDIX). In protocol 2, we calculated the neural arc transfer function during the bilateral identical CSP perturbation by means of the same analysis for one-input, one-output systems. In protocol 3, we calculated the neural arc transfer functions related to the left and right CSP perturbations by means of an analysis for two-input, one-output systems (see APPENDIX).

To estimate the peripheral arc transfer function of the carotid sinus baroreflex, we treated SNA as the input and AoP as the output and applied the analysis for one-input, one-output systems. In protocol 1, we estimated the peripheral arc transfer functions during the left and right CSP perturbations, respectively. In protocol 2, we estimated the peripheral arc transfer function during the bilateral identical CSP perturbation. In protocol 3, we did not present the estimated peripheral arc transfer function because the meaning of protocol 3 was less clear with respect to the peripheral arc transfer function because of the fact that SNA was a single quantity despite the bilateral independent CSP perturbation. In protocols 1 and 2, we also estimated the transfer function of the total baroreflex loop by treating CSP as the input and AoP as the output of the system to demonstrate how the neural arc and peripheral arc transfer functions combine into the overall function.

Statistical analysis. All data are presented as means ± SD. Because the magnitude of SNA varied among animals depending on recording conditions such as the physical contact between the nerve and the electrodes, SNA was normalized in each animal and described in arbitrary units. We first calculated a normalization factor based on the mean modulus of the neural arc transfer functions <0.03 Hz obtained from protocol 1. We then multiplied the neural arc transfer functions obtained from all the protocols by the normalization factor. Finally, we multiplied the peripheral arc transfer functions obtained from all the protocols by an inverse of the normalization factor. Thus the gain of the total baroreflex loop was kept unchanged by the normalization procedure. Hereafter, we refer to the modulus as the gain of the transfer function.

To quantitatively examine the differences among the transfer functions, we used the gain and phase values at 0.01, 0.1 and 0.5 Hz (averaged between 0.45 and 0.55 Hz). The selected frequencies were arbitrary and had no particular biological significance. We compared the gain and phase values among the neural arc transfer functions (or the peripheral arc transfer functions) estimated in protocols 1 and 2 by the Student-Newman-Keuls test for nonparametric simultaneous multiple comparisons (5). To examine the differences of gain and phase values of the neural arc transfer function related to left (or right) CSP perturbation between protocol 1 and 3, we used a paired t-test (5). Differences were considered statistically significant when P < 0.05.

RESULTS

Figure 1 presents typical recordings obtained from protocol 1 showing left CSP, right CSP, SNA, and AoP. In the recording shown in Fig. 1A, we perturbed the left CSP alone according to a binary white noise sequence. We fixed the right CSP at the physiological operating pressure throughout the experimental run. When the left CSP was raised, SNA decreased and AoP increased after some delay. When the left CSP was decreased, the opposite responses were observed. In the recording shown in Fig. 1B, we perturbed the right CSP alone while fixing the left CSP. The responses of SNA and AoP to the right CSP perturbation were similar to those observed during the left CSP perturbation.

Figure 2 presents typical recordings obtained from protocol 2, showing left CSP, right CSP, SNA, and AoP. We perturbed the bilateral CSP simultaneously and identically according to a binary white noise sequence. When CSP was raised, SNA ceased and AoP decreased after a small delay. When CSP was decreased, the opposite responses were observed. The cessation of SNA in response to CSP increase was more evident in Fig. 2 than in Fig. 1. Accordingly, changes in AoP were observed to be greater in Fig. 2 than in Fig. 1.

Figure 3 shows the neural arc transfer functions estimated by left (Fig. 3A), right (Fig. 3B), and bilateral identical (Fig. 3C) CSP perturbations. Gain plots, phase plots, and coherence functions are shown. As a result of the normalization of SNA, gain values of the neural arc transfer functions estimated by the left and right CSP perturbations approximated unity at the lowest frequencies. The gain values increased as the frequency increased, indicating high-pass filter characteristics of the neural arc. The gain plot obtained from
the bilateral identical CSP perturbation showed an approximately twofold parallel upward shift compared with that obtained from the left or right CSP perturbation. The phase plots indicated an out-of-phase relationship between CSP and SNA in the frequency range under study. The coherence values in the frequency range of 0.01 to 0.5 Hz were between 0.4 and 0.7 during the left and right CSP perturbations. Although the coherence values were between 0.5 and 0.8 during the bilateral identical CSP perturbation and were slightly greater than those during the unilateral CSP perturbation, there were no statistically significant differences among the protocols.

Table 1 summarizes the gain and phase values of the neural arc transfer functions shown in Fig. 3. The gain values of the neural arc transfer function estimated by the bilateral identical CSP perturbation were significantly greater than those estimated by the left or right CSP perturbation at 0.01, 0.1, and 0.5 Hz. The phase values did not differ significantly among the neural arc transfer functions.

Figure 4 shows the peripheral arc transfer functions estimated by left (Fig. 4A), right (Fig. 4B), and bilateral identical (Fig. 4C) CSP perturbations. Gain plots, phase plots, and coherence functions are shown. The gain values decreased as the frequency increased in all of the peripheral arc transfer functions, indicating low-pass filter characteristics of the peripheral arc. A peak in the gain plot at 0.6 Hz corresponded to the artificial respiration frequency. The phase values approached 0 rad at the lowest frequencies, indicating an in-phase relationship between SNA and the steady-state AoP response. The phase values delayed as the frequency increased. The coherence values in the frequency range of 0.01 to 0.5 Hz were between 0.4 and 0.7 and between 0.5 and 0.8 during the left and right CSP perturbations, respectively. The coherence values during the bilateral identical CSP perturbation were between 0.5 and 0.8. The differences in coherence values among the protocols were not statistically significant.

Table 2 summarizes the gain and phase values of the peripheral arc transfer functions shown in Fig. 4. No statistically significant differences were observed in gain and phase values at 0.01, 0.1, or 0.5 Hz among the peripheral arc transfer functions.

Figure 5 shows the transfer functions of the total baroreflex loop estimated by left (Fig. 5A), right (Fig. 5B), and bilateral identical (Fig. 5C) CSP perturbation.
tions. Gain plots, phase plots, and coherence functions are shown. The gain values decreased as the frequency increased, indicating low-pass filter characteristics of the total baroreflex. The extent of the low-pass filter (i.e., the decreasing slope of the gain values in the higher frequencies) was attenuated compared with that of the corresponding peripheral arc transfer function (Fig. 4). The phase values approached $-\pi$ rad at the lowest frequencies, indicating the negative-feedback characteristics of the total baroreflex loop. The coherence values were between 0.4 and 0.7 during the left and right CSP perturbations, respectively. The coherence values during the bilateral identical CSP perturbation were between 0.5 and 0.8.

Figure 6 presents typical recordings obtained from protocol 3, showing left CSP, right CSP, SNA, and AoP. We imposed a statistically independent perturbation on left and right CSP according to a different series of binary white noise sequences. As a result of the complexity of input signals, no apparent relationship between CSP and SNA could be readily seen by simply inspecting the time-series data. AoP showed dynamic changes in response to changes in SNA.

Table 1. Gain and phase values of neural arc transfer functions estimated by left, right, and bilateral identical carotid sinus pressure perturbations

<table>
<thead>
<tr>
<th>Frequency (Hz)</th>
<th>Left (Gain, U/mmHg)</th>
<th>Right (Gain, U/mmHg)</th>
<th>Bilateral (Gain, U/mmHg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.01 Hz</td>
<td>0.94 ± 0.31</td>
<td>0.96 ± 0.25</td>
<td>2.17 ± 0.97**</td>
</tr>
<tr>
<td>0.1 Hz</td>
<td>1.78 ± 0.41</td>
<td>1.91 ± 0.73</td>
<td>4.30 ± 1.57**</td>
</tr>
<tr>
<td>0.5 Hz</td>
<td>4.29 ± 1.24</td>
<td>4.01 ± 1.74</td>
<td>9.02 ± 3.36**</td>
</tr>
<tr>
<td>Phase, rad</td>
<td>-3.08 ± 0.84</td>
<td>-2.78 ± 0.68</td>
<td>-2.80 ± 0.84</td>
</tr>
<tr>
<td>0.1 Hz</td>
<td>-2.61 ± 0.45</td>
<td>-2.50 ± 0.47</td>
<td>-2.75 ± 0.23</td>
</tr>
<tr>
<td>0.5 Hz</td>
<td>-3.41 ± 0.57</td>
<td>-3.77 ± 0.41</td>
<td>-3.90 ± 0.39</td>
</tr>
</tbody>
</table>

Data are means ± SD. *P < 0.05 from left value; †P < 0.05 from right value.

Figure 7 shows the neural arc transfer functions related to the left (Fig. 7A) and right (Fig. 7B) CSP perturbations obtained from protocol 3. Gain plots, phase plots, and partial coherence functions are shown. The gain plots indicated high-pass filter characteristics. The phase plots indicated an out-of-phase relationship between CSP and SNA. No statistically significant differences were observed in gain and phase values at 0.01, 0.1, and 0.5 Hz between the neural arc transfer functions estimated in protocols 1 and 3 (Fig. 3A vs. Fig. 7A and Fig. 3B vs. Fig. 7B). The partial coherence is a frequency-domain representation of the linear dependence of SNA on either one of the left and right CSP values during simultaneous independent CSP perturbations. The partial coherence values associated with the left and right CSP perturbations were between 0.3 and 0.6 in the frequency range of 0.01 to 0.5 Hz. The multiple coherence (Fig. 7C) is a frequency-domain measure of the extent to which SNA is explained by a linear combination of left and right CSP values during simultaneous independent CSP perturbations. The multiple coherence values were between 0.5 and 0.8.

DISCUSSION

The present study demonstrated the following characteristics of the carotid sinus baroreflex system when tested around the physiological operating pressure: 1) the left and right carotid sinus baroreflexes showed similar dynamic transfer characteristics; 2) the summation of the dynamic transfer characteristics of the left and right carotid sinus baroreflexes approximated simple addition; and 3) no apparent dynamic interactions existed between the left and right carotid sinus baroreflexes.

Dynamic characteristics of left and right carotid sinus baroreflexes. The neural arc transfer function showed high-pass filter characteristics (Fig. 3), whereas the peripheral arc transfer function showed low-pass...
filter characteristics (Fig. 4). Therefore, we can interpret the fast neural arc of the baroreflex as compensating for the slow AoP response to SNA. As shown in Fig. 5, the decreasing slope in the gain plot was less in the transfer function of the total baroreflex loop than in the corresponding peripheral arc transfer function. Our previous study using computer simulation indicated that this compensation of the neural arc for the peripheral arc baroreflex was almost optimal to attain quick and stable regulation of arterial blood pressure (9).

Although we recorded SNA from the left cardiac sympathetic nerve, similar neural arc transfer functions were estimated by the left and right CSP perturbations (Fig. 3, A and B; Table 1). Because we normalized SNA by a single value (normalization factor) in each animal, the laterality in the carotid sinus baroreflexes, if any, persisted even after the normalization procedure. Thus afferent signals from the left and right carotid sinus baroreceptors almost completely converged at the level of the sympathetic efferent pathway. A similar result was reported in anesthetized cats with respect to the static characteristics of the carotid sinus baroreflexes, which did not show a difference in the left inferior cardiac SNA either by contralateral or ipsilateral changes in CSP (23). Thus there appears to be no apparent functional laterality in the neural arc of the carotid sinus baroreflexes. This is also reflected in the finding that the peripheral arc transfer functions during the left and right CSP perturbations did not differ significantly each other (Fig. 4, A and B; Table 2). These results suggest that the neural arc of the carotid sinus baroreflex cannot afford to fully make use of the laterality in the sympathetic regulation of the cardiovascular system such as that observed in heart rate and ventricular contractility responses to sympathetic stimulation (16).

According to anatomic and histochemical studies, the ipsilateral projection of the carotid sinus nerve fibers to the nucleus tractus solitarii (NTS) (3, 17) predominates over the contralateral projection. The rostral ventrolateral medulla (RVLM) has been well established to play a crucial role in the baroreflex regulation of the SNA (4). Spinally projecting cells of the RVLM terminate specifically in sympathetic preganglionic nuclei. The afferent signals from the left and right carotid sinus baroreceptors might converge within the central pathway from the NTS to the RVLM, which may include the caudal ventrolateral medulla.

**Table 2. Gain and phase values of peripheral arc transfer functions estimated by left, right, and bilateral identical carotid sinus pressure perturbations**

<table>
<thead>
<tr>
<th>Frequency (Hz)</th>
<th>Left</th>
<th>Right</th>
<th>Bilateral</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.01</td>
<td>0.39 ± 0.13</td>
<td>0.35 ± 0.14</td>
<td>0.38 ± 0.14</td>
</tr>
<tr>
<td>0.1</td>
<td>0.13 ± 0.05</td>
<td>0.14 ± 0.05</td>
<td>0.14 ± 0.08</td>
</tr>
<tr>
<td>0.5</td>
<td>0.009 ± 0.001</td>
<td>0.008 ± 0.003</td>
<td>0.007 ± 0.002</td>
</tr>
<tr>
<td>Phase, rad</td>
<td>–0.60 ± 0.16</td>
<td>–1.08 ± 0.36</td>
<td>–1.06 ± 0.37</td>
</tr>
<tr>
<td>0.1</td>
<td>–2.59 ± 0.33</td>
<td>–2.70 ± 0.10</td>
<td>–2.64 ± 0.17</td>
</tr>
<tr>
<td>0.5</td>
<td>–5.59 ± 0.38</td>
<td>–5.66 ± 0.44</td>
<td>–5.77 ± 0.27</td>
</tr>
</tbody>
</table>

Data are means ± SD.
To confirm whether the summation of the dynamic transfer characteristics of the left and right carotid sinus baroreflexes approximate simple addition in a more intuitive fashion, we calculated the step response of SNA by integrating the impulse response obtained from the neural arc transfer function via the inverse Fourier transformation. Figure 8 shows the step responses of SNA estimated by left (Fig. 8A), right (Fig. 8B), and bilateral identical (Fig. 8C) CSP perturbations. The simple addition of an average step response related to the left and right CSP perturbations is depicted by a thin line in Fig. 8C, which conforms to the average step response related to the bilateral identical CSP perturbation. Thus the summation of the left and right carotid sinus baroreflexes approximated simple addition with respect to their dynamic characteristics when the system operated around the physiological operating pressure.

As shown in Fig. 4, the peripheral arc transfer function during the bilateral identical CSP perturbation did not differ significantly from that during the left or right CSP perturbation. Thus, in the present study, the peripheral arc of the carotid sinus baroreflex was not saturated during the bilateral identical CSP perturbation. As a consequence, the gain of the total baroreflex loop was approximately double during the bilateral identical CSP perturbation compared with that during the left or right CSP perturbation (Fig. 5). Our recent study in rats also indicated that the static relationship between SNA and AoP had a wider operating range compared with that between CSP and SNA (20). It is important to note that doubling the total gain cannot be achieved by simply doubling the peak-to-peak amplitude of unilateral CSP perturbation to 80 mmHg, because the transfer gain from CSP to SNA would decrease as a result of saturation phenomena with such a large amplitude of input (20, 21, 24). Thus parallel baroreceptors provide a mechanism that bypasses the saturation phenomenon at the baroreceptor transduction and increases the gain of the total baroreflex loop.

The coherence values associated with the neural arc transfer function were slightly greater during the bilateral identical CSP perturbation than during the unilateral CSP perturbation (Fig. 3), although the difference did not reach statistical significance. The coherence values reflect both the degree of system linearity and the signal-to-noise ratio in the output signal (13). In the present study, we can expect higher
coherence values in protocol 2 than in protocol 1 because of a higher signal-to-noise ratio. The sympathetic preganglionic nuclei are known to receive inputs from sources other than the RVLM (4). Such inputs generate physiological signals in SNA independent of the baroreflex, which are then treated as the inherent noise of the baroreflex system in terms of the linear systems analysis. During the bilateral identical CSP perturbation, baroreflex-related SNA increased and thus the signal-to-noise ratio was increased.

Dynamic interactions of left and right carotid sinus baroreflexes. Although Fig. 8 clearly demonstrates that the summation of the dynamic transfer characteristics in the neural arc of the left and right carotid sinus baroreflexes was a type of simple addition, we performed a more comprehensive analysis of the system. As shown in Fig. 7, the neural arc transfer functions related to the left and right CSP perturbations could be estimated separately when the left and right CSP were perturbed simultaneously and independently. No significant differences were observed in gain and phase values between the neural arc transfer functions estimated in protocols 1 and 3 (Fig. 3A vs. Fig. 7A and Fig. 3B vs. Fig. 7B). The multiple coherence values (Fig. 7C) were as high as the coherence values during the bilateral identical CSP perturbation (Fig. 3C). Although the analysis for the two-input, one-output systems cannot on its own exclude the possibility of dynamic interactions between the left and right carotid sinus baroreflexes (2), it appears that the carotid sinus baroreflexes around the physiological operating pressure essentially obey the linear system dynamics.

Greene et al. (6) reportedly observed no contralateral effects of acute resetting of the baroreflex in anesthetized dogs. On the basis of a dynamic system analysis, we can interpret the acute resetting as one of several manifestations of the dynamic transfer characteristics of the baroreflex (22). Thus the present results showing no apparent dynamic interactions between the left and right carotid sinus baroreflexes are consistent with the results of Greene et al.

Limitations. There are several limitations to this study. The first limitation is related to the fact that we investigated the carotid sinus baroreflexes in anesthetized rabbits. Although we chose an anesthetic agent that is minimally suppressive to circulatory regulation, the absolute gain values of the carotid sinus baroreflex might have been affected to some degree. However, we compared the transfer functions to different pressure inputs under the same anesthetic procedure and randomized the order of the protocols. Therefore, we believe that the summation of the dynamic transfer characteristics of the bilateral carotid sinus baroreflexes would be found to approximate simple addition even in the absence of anesthesia.

The second limitation is related to the fact that we used the cardiac SNA as the output of the carotid sinus baroreflex. Because there are thought to be regional
differences in SNA, the use of the SNA associated with other neural districts such as the renal sympathetic nerve might affect the estimation of the neural arc and peripheral arc transfer functions. However, the relatively high coherence values observed during bilateral identical CSP perturbation (Figs. 3C, 4C, and 5C) support the relevance of having the cardiac SNA represent the systemic SNA. In addition, simultaneous recordings of cardiac and renal SNA in anesthetized cats indicated that the dynamic transfer characteristics of the arterial baroreflex control of these two nerves were indistinguishable (7).

The third limitation is related to the fact that we filled the isolated carotid sinuses with warmed physiological saline. Because ion content affects the sensitivity of baroreceptors (1), the absolute gain values of the carotid sinus baroreflexes might have been different had we used a different solution such as Ringer solution. However, we did not change the ion content of the isolated carotid sinuses among protocols. Thus the effects of ion content on the current conclusion would be minimal.

Finally, we sectioned bilateral vagi to simplify our system identification approach. Therefore, the current study did not assess any parasympathetic contribution to overall arterial baroreflex. Because the heart rate control by the vagal system is quicker than that by the sympathetic system (10), inclusion of the vagal system would alter the overall transfer characteristics from CSP to arterial blood pressure. Further studies are clearly required to assess the parasympathetic contribution to the arterial baroreflex.

In conclusion, summation of the dynamic transfer characteristics of the left and right carotid sinus baroreflexes approximates simple addition when the system operates around the physiological operating pressure. Given such characteristics, parallel baroreceptors were shown to bypass the saturation phenomenon at the baroreceptor transduction and increase the gain of the total baroreflex loop without changing the dynamic characteristics of the baroreflex.

APPENDIX

Transfer function of one-input, one-output 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 (8). For each segment, the linear trend was removed and a Hanning window was applied. We then performed fast Fourier transform and obtained the frequency spectra of the input [S_x(f)] and the output [S_y(f)].

We ensemble averaged the input power [S_{xx}(f)], the output power [S_{yy}(f)], and the cross power between the input and the output [S_{xy}(f)] over the eight segments. Finally, we estimated the transfer function [H(f)] from the input to the output using the following equation (13)

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

We also calculated the magnitude-squared coherence function. The coherence function [\text{Coh}(f)] is a measure of linear dependence between the input and the output in the frequency domain. It was calculated using the following equation (13)

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

Transfer functions of two-input, one-output system. For a two-input, one-output system, we obtained a frequency spectr for the inputs [S_x(f) and S_U(f)] and the output [S_y(f)]. We ensemble averaged the input powers [S_{xx}(f) and S_{UU}(f)], the output power [S_{yy}(f)], and the cross powers for combinations of these [S_{xy}(f), S_{XY}(f), and S_{UX}(f)] over the eight segments. We calculated the transfer function relating to the input X [H_x(f)] using the following equations (2)

\[ S_{XY}(f) = S_{xy}(f) - \frac{|S_{UX}(f)|^2}{S_{UU}(f)} \]
\[ S_{XX}(f) = S_{xx}(f) - \frac{|S_{UX}(f)|^2}{S_{UU}(f)} \]
\[ H_x(f) = \frac{S_{XY}(f)}{S_{xx}(f)} \]

The transfer function relating to the input U can be calculated by exchanging X and U in the above equations.

We also calculated the magnitude-squared partial coherence function relating to the input X [\text{Coh}_X(f)], which is a measure of linear dependence between the input X and the output Y in the frequency domain. \text{Coh}_X(f) is estimated as follows (2)

\[ S_{YY}(f) = S_{yy}(f) - \frac{|S_{UY}(f)|^2}{S_{UU}(f)} \]
\[ \text{Coh}_X(f) = \frac{|S_{XY}(f)|^2}{S_{XX}(f) \cdot S_{YY}(f)} \]

The partial coherence function between the input U and the output Y can be calculated by exchanging X and U in the above equations. We also calculated the multiple-coherence function [\text{Coh}_M(f)] between the inputs (X and U) and the output Y, which is a measure of the extent to which the output is linearly explained by a combination of the inputs. \text{Coh}_M(f) is calculated from the following equation (2)

\[ \text{Coh}_M(f) = \frac{H_x(f) \cdot S_{XY}(f) + H_u(f) \cdot S_{UY}(f)}{S_{yy}(f)} \]

where S_{XY}(f) and S_{UY}(f) are the complex conjugates of S_{XY}(f) and S_{UY}(f), respectively.

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