Input-size dependence of the baroreflex neural arc transfer characteristics

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Submitted 10 April 2002; accepted in final form 17 September 2002

Kawada, Toru, Yusuke Yanagiya, Kazunori Uemura, Tadayoshi Miyamoto, Can Zheng, Meihua Li, Masaru Sugimachi, and Kenji Sunagawa. Input-size dependence of the baroreflex neural arc transfer characteristics. Am J Physiol Heart Circ Physiol 284: H404–H415, 2003. First published September 26, 2002; 10.1152/ajpheart.00319.2002.—Static characteristics of the baroreflex neural arc from pressure input to sympathetic nerve activity (SNA) show sigmoidal nonlinearity, whereas its dynamic characteristics approximate a derivative filter where the magnitude of SNA response becomes greater as the input frequency increases. To reconcile the static nonlinear and dynamic linear components, we examined the effects of input amplitude on the apparent linear transfer function of the neural arc. In nine anesthetized rabbits, we perturbed isolated carotid sinus pressure by using binary white noise while varying the input amplitude among 5, 10, 20, and 40 mmHg. With increasing input amplitude, the transfer gain at 0.01 Hz decreased from 1.21 ± 0.27 to 0.49 ± 0.28 arbitrary units/mmHg (P < 0.01). Moreover, the slope of the transfer gain between 0.03 and 0.3 Hz decreased from 14.3 ± 3.7 to 6.5 ± 2.5 dB/decade (P < 0.01). We conclude that the model consisting of a sigmoidal component following rather than preceding a derivative component explains the observed results and thus can be used as a first approximation of the overall neural arc transfer characteristics.

The arterial baroreflex system plays an important role in stabilizing arterial pressure (AP) against exogenous pressure perturbation such as that induced by changes in posture. The sympathetic limb of the arterial baroreflex may be divided into the neural and peripheral arc subsystems (9, 18, 21). The neural arc represents the relationship between carotid sinus pressure input and efferent sympathetic nerve activity (SNA), whereas the peripheral arc represents the relationship between SNA and AP. Knowledge of the static and dynamic characteristics of the two arcs is essential for the systematic understanding of how the baroreflex system regulates AP. The static characteristics provide information on the operating point of the baroreflex system (13, 18, 21), whereas the dynamic characteristics determine the stability and quickness of the baroreflex system (9, 14). Combining the static-nonlinear and dynamic-linear characteristics is essential to better understand the overall behavior of the arterial baroreflex such as self-sustained oscillations in AP (20, 25). With respect to the neural arc of the carotid sinus baroreflex, the static characteristics can be identified from the input-output relationship between carotid sinus pressure (CSP) and SNA in the steady state. The static characteristics of the baroreflex neural arc approximate a sigmoidal curve with threshold and saturation nonlinearity (13, 18, 21). The dynamic characteristics of the baroreflex neural arc can be identified by using a transfer function analysis. The transfer function from CSP to SNA approximates a derivative filter where the magnitude of SNA response becomes greater as the input frequency of CSP perturbation increases (9, 12, 14).

A sigmoidal nonlinearity causes input-size dependence of the system response around the operating point (26). Although such static nonlinearity can theoretically affect the dynamic response of the system, to the best of our knowledge, no studies have focused on the effects of input size on the apparent linear transfer function of the baroreflex neural arc, assuming a cascade connection of the dynamic linear and static nonlinear components. The dynamic linear and static nonlinear components represent the derivative characteristics and sigmoidal nonlinearity of the baroreflex neural arc, respectively. Because the two components lumped the characteristics of the baroreflex neural arc, no specific counterparts are assumed in terms of anatomy. Two major schemes may be put forward to most simply reconcile the static nonlinear and dynamic linear components in the neural arc. The first scheme consists of a sigmoidal component followed by a derivative component (Fig. 1A). In this model, an increase in the input amplitude of binary white noise results in a decrease in dynamic gain of the apparent linear transfer function in a frequency independent manner (Fig. 1B) (see APPENDIX A for details). In other words, the

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degree of derivative characteristics in the neural arc remains unchanged irrespective of the input amplitude of binary white noise. The second scheme consists of a derivative component followed by a sigmoidal component (Fig. 1C). In this model, an increase in the input amplitude of binary white noise attenuates dynamic gain more in the higher frequencies than in the lower frequencies, blunting the derivative characteristics of the neural arc (Fig. 1D) (see Appendix A for details). With these considerations in mind, we designed the present study to determine which of the two models was more suitable to represent the overall transfer characteristics of the neural arc. The results indicate that the model consisting of the derivative component followed by the sigmoidal component can serve as a first approximation to the overall transfer characteristics of the neural arc.

MATERIALS AND METHODS

Surgical preparations. Animals were cared for strictly in 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 2.6 to 3.7 kg were anesthetized by intravenous injection (2 ml/kg) of a mixture of urethane (250 mg/ml) and α-chloralose (40 mg/ml), and they were mechanically ventilated with oxygen-enriched room air. Supplemental anesthetics were injected as necessary (0.5 ml/kg) to maintain an appropriate level of anesthesia. AP was measured using a high-fidelity pressure transducer (Millar Instruments; Houston, TX) inserted via the right femoral artery. We isolated the bilateral carotid sinuses 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. CSP was controlled by a servo-controlled piston pump. 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 exposed the left cardiac sympathetic nerve through a midline thoracotomy and attached a pair of stainless steel wire electrodes (Bioflex wire AS633; Cooner Wire) to record SNA. The nerve fibers peripheral to the electrodes were sectioned to eliminate afferent signals from the heart. The nerve and electrodes were covered with a mixture of silicone gel (Semicosil 932A/B, Wacker Silicones) and white petrolatum (Vaseline) for insulation and fixation. The preamplified nerve signal was bandpass filtered at 150–1,000 Hz and was then full-wave rectified and low-pass filtered at 30 Hz to quantify the nerve activity. Pancuronium bromide (0.3 mg/kg) was administered to prevent artifacts of muscular activity from appearing in the SNA recording. Body temperature was maintained at around 38°C with a heating pad.

Protocols. After surgical preparations were completed, CSP was adjusted to AP via a servo controller until the steady state was reached. The operating pressure was determined from mean CSP at the steady state. CSP was then assigned either high- or low-pressure values around the operating pressure according to a binary white noise sequence. The input amplitude was varied among 5, 10, 20, and 40 mmHg in random order. Each amplitude of CSP perturbation was measured for 10 min. Hereafter, we denote the protocols as P5, P10, P20, and P40, respectively. Downward arrows indicate a frequency-independent decrease in dynamic gain associated with an increase in the input amplitude (see Appendix A for details).
amplitude affect the identification of the transfer function. The input-output data pairs were resampled at 10 Hz and segmented into eight sets of 50%-overlapping bins of 1,024 points each. For each segment, a linear trend was subtracted and a Hanning window was applied. A fast Fourier transform was performed to obtain the frequency spectra of the input and output (2). The ensemble averages of input power $S_{XX}(f)$, output power $S_{YY}(f)$, and crosspower between the input and output $S_{XY}(f)$ were obtained over the eight segments. Finally, the linear transfer function $[H(f)]$ from the input to output was calculated as (16)

$$H(f) = \frac{S_{XY}(f)}{S_{XX}(f)}$$

To quantify the linear dependence between the input and output signals in the frequency domain, a magnitude-squared coherence function $[\text{Coh}(f)]$ was calculated as (16)

$$\text{Coh}(f) = \frac{|S_{XY}(f)|^2}{S_{XX}(f)S_{YY}(f)}$$

To facilitate intuitive understanding of the transfer function, the step response corresponding to the transfer function was also calculated as follows. The system impulse response was derived from the real part of the inverse Fourier transform of $H(f)$. The system step response was obtained from the time integral of the impulse response.

Statistical analysis. All data are presented as means ± SD values across the nine animals. Because the amplitude of SNA varied depending on such recording conditions as the physical contact between the nerve and electrodes, SNA was presented in arbitrary units (au). The neural and peripheral arc transfer functions were normalized in each animal so that the average gain values below 0.03 Hz in the $P_5$ protocol became unity. The transfer function of the total baroreflex loop was presented without normalization. To compare the neural arc transfer functions across all protocols, a transfer gain value at 0.01 Hz ($G_{0.01}$) and an average slope of the transfer gain between 0.03 and 0.3 Hz ($\text{Slope}_{0.03-0.3}$) were calculated. To compare the peripheral arc or total loop transfer functions across all protocols, $G_{0.01}$ and an average slope of the transfer gain between 0.1 and 0.5 Hz ($\text{Slope}_{0.1-0.5}$) were calculated. To examine the difference in the power spectral densities of SNA and AP among protocols, power values at 0.01 and 0.5 Hz (averaged from 0.45 to 0.5 Hz) were used. The frequencies of the parameters were chosen arbitrarily so that these parameters could properly represent changes in the transfer functions and power spectral densities. In the step response of the neural arc, the steady-state step response at 50 s ($S_{50}$), the peak negative value ($S_{\text{peak}}$), and the time to the negative peak ($T_{\text{peak}}$) were calculated. In the step response of the peripheral arc or the total baroreflex loop, $S_{50}$ and the 50% rise time ($T_{50}$) were calculated. $T_{50}$ indicates the time at which the 50% of $S_{50}$ was attained in the step response. These parameters were tested for all four protocols by using a repeated-measures analysis of variance followed by a Dunnett’s multiple-comparison procedure (5). Differences with respect to the $P_5$ protocol were considered significant when $P < 0.05$.

RESULTS

Typical time series of CSP, SNA, and AP are shown in Fig. 2. CSP was perturbed using a binary white noise sequence. The same binary sequence at a different amplitude was applied to each animal. Although the panels were ordered according to the input amplitude, the protocols were performed in random order. Whereas the mean CSP was kept unchanged, mean SNA and AP were decreased as the input amplitude increased. The amplitude of AP response did not change between 0.03 and 0.3 Hz ($\text{Slope}_{0.03-0.3}$) were calculated. To compare the peripheral arc or total loop transfer functions across all protocols, $G_{0.01}$ and an average slope of the transfer gain between 0.1 and 0.5 Hz ($\text{Slope}_{0.1-0.5}$) were calculated. To examine the difference in the power spectral densities of SNA and AP among protocols, power values at 0.01 and 0.5 Hz (averaged from 0.45 to 0.5 Hz) were used. The frequencies of the parameters were chosen arbitrarily so that these parameters could properly represent changes in the transfer functions and power spectral densities. In the step response of the neural arc, the steady-state step response at 50 s ($S_{50}$), the peak negative value ($S_{\text{peak}}$), and the time to the negative peak ($T_{\text{peak}}$) were calculated. In the step response of the peripheral arc or the total baroreflex loop, $S_{50}$ and the 50% rise time ($T_{50}$) were calculated. $T_{50}$ indicates the time at which the 50% of $S_{50}$ was attained in the step response. These parameters were tested for all four protocols by using a repeated-measures analysis of variance followed by a Dunnett’s multiple-comparison procedure (5). Differences with respect to the $P_5$ protocol were considered significant when $P < 0.05$.

Table 1. Mean levels of CSP, SNA, and AP

<table>
<thead>
<tr>
<th></th>
<th>$P_5$</th>
<th>$P_{10}$</th>
<th>$P_{20}$</th>
<th>$P_{40}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean CSP, mmHg</td>
<td>91.4 ± 4.7</td>
<td>91.5 ± 4.8</td>
<td>91.5 ± 4.5</td>
<td>91.5 ± 4.3</td>
</tr>
<tr>
<td>Mean SNA, au</td>
<td>85.2 ± 34.6</td>
<td>78.0 ± 34.4</td>
<td>67.0 ± 29.5</td>
<td>63.6 ± 27.4</td>
</tr>
<tr>
<td>Mean AP, mmHg</td>
<td>103.0 ± 18.5</td>
<td>96.8 ± 19.6</td>
<td>85.2 ± 21.1</td>
<td>74.6 ± 26.4</td>
</tr>
</tbody>
</table>

Values are means ± SD. CSP, carotid sinus pressure; SNA, sympathetic nerve activity; AP, arterial pressure; $P_5$, $P_{10}$, $P_{20}$, $P_{40}$, protocols at 5, 10, 20, and 40 mmHg, respectively; au, arbitrary units. *$P < 0.05$ and †$P < 0.01$ against $P_5$ by Dunnett’s multiple-comparison procedure.
increase proportionally to the increase in the input amplitude of CSP, suggesting a saturation phenomenon in the AP response to CSP perturbation.

Mean levels of CSP, SNA, and AP obtained from all animals are summarized in Table 1. CSP was kept unchanged across protocols. The mean levels of SNA and AP were significantly lower in the P20 and P40 protocols than in the P5 protocol.

Power spectral densities of CSP, SNA, and AP averaged from all animals are shown in Fig. 3. The CSP power was fairly flat up to 1 Hz in each protocol. The twofold increase in the input amplitude corresponds to a fourfold increase in the CSP power. The SNA power showed higher values in the frequencies between 0.3 and 1 Hz. AP power showed decreasing values as the frequency increased. Solid and dashed lines indicate means and means ± SD values, respectively. Mean and SD values were calculated after common logarithmic transformation of the power values.

Table 2 summarizes the parameters of the power spectral densities averaged from all animals. The SNA power at 0.01 Hz was significantly greater in the P40 than in the P5 protocol. The SNA power at 0.5 Hz did not differ across all protocols. The AP powers at 0.01 and 0.5 Hz did not differ for all four protocols.

Figure 4A shows the neural arc transfer functions averaged from all animals. Gain plots (Fig. 4A, top), phase plots (Fig. 4A, middle) and coherence functions (Fig. 4A, bottom) are presented. The transfer gain increased as the input frequency increased in each protocol, indicating the derivative characteristics of the neural arc. The gain value at the lowest frequency became smaller as the input amplitude increased. Moreover, the slope of the increasing gain became shallower as the input amplitude increased. The phase approach −π radians at the lowest frequency in each protocol, reflecting the negative feedback character of the baroreflex neural arc. The phase plot did not differ among the protocols. The coherence values were lower than 0.2 at frequencies below 0.06 Hz and increased to 0.5 at frequencies above 0.1 Hz in the P5 protocol. Coherence values increased as the input amplitude was increased. Figure 4B illustrates the step responses of SNA corresponding to the transfer functions shown in Fig. 4A. The initial drop of the SNA response as well

**Table 2. Spectral power densities of SNA and AP**

<table>
<thead>
<tr>
<th></th>
<th>P5</th>
<th>P10</th>
<th>P20</th>
<th>P40</th>
</tr>
</thead>
<tbody>
<tr>
<td>SNA0.01, au²</td>
<td>1.24 ± 0.83</td>
<td>1.31 ± 0.63</td>
<td>2.19 ± 1.32</td>
<td>4.07 ± 3.54†</td>
</tr>
<tr>
<td>SNA0.5, au²</td>
<td>18.8 ± 12.4</td>
<td>24.8 ± 17.7</td>
<td>25.1 ± 19.0</td>
<td>23.1 ± 13.9</td>
</tr>
<tr>
<td>AP0.01, mmHg²</td>
<td>2.43 ± 1.82</td>
<td>2.85 ± 1.88</td>
<td>2.82 ± 1.63</td>
<td>3.04 ± 1.47</td>
</tr>
<tr>
<td>AP0.5, × 10⁻³ mmHg²</td>
<td>6.90 ± 6.74</td>
<td>8.17 ± 9.19</td>
<td>7.38 ± 6.20</td>
<td>4.24 ± 4.12</td>
</tr>
</tbody>
</table>

Values are means ± SD. SNA0.01 and SNA0.5, SNA powers at 0.01 and 0.5 Hz, respectively; AP0.01 and AP0.5, AP powers at 0.01 and 0.5 Hz, respectively. †P < 0.01 against P5 by Dunnett’s multiple-comparison procedure.
The phase lagged with increasing frequency up to 1 Hz. Coherence values at frequencies below 0.1 Hz appeared to increase as the input amplitude was increased. Figure 5A illustrates the step responses of AP corresponding to the transfer functions shown in Fig. 5A. The AP response gradually increased to reach the steady state. The step response did not differ among the protocols (Table 3). Figure 6A depicts the total loop transfer function averaged from all animals. The transfer gain decreased as the input frequency increased in each protocol, indicating the low-pass characteristics of the total baroreflex loop. The slope of decreasing gain was slower than that in the corresponding peripheral arc transfer function. The gain value at the lowest frequency became smaller as the input amplitude in-

**Table 3. Parameters of transfer functions and corresponding step responses**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>P5</th>
<th>P10</th>
<th>P20</th>
<th>P40</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neural arc</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G0.01, au/mmHg</td>
<td>1.21</td>
<td>0.75</td>
<td>0.68</td>
<td>0.49</td>
</tr>
<tr>
<td>Slope0.03–0.3, dB/decade</td>
<td>14.3</td>
<td>13.3</td>
<td>9.0</td>
<td>6.5</td>
</tr>
<tr>
<td>S50, au</td>
<td>-1.09</td>
<td>-0.54</td>
<td>-0.67</td>
<td>-0.54</td>
</tr>
<tr>
<td>Speak, au</td>
<td>-5.23</td>
<td>-3.75</td>
<td>-2.18</td>
<td>-1.30</td>
</tr>
<tr>
<td>Tpeak, s</td>
<td>0.38</td>
<td>0.48</td>
<td>0.66</td>
<td>0.71</td>
</tr>
<tr>
<td>Peripheral arc</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G0.01, mmHg/au</td>
<td>1.09</td>
<td>1.02</td>
<td>1.11</td>
<td>1.02</td>
</tr>
<tr>
<td>Slope0.1–0.5, dB/decade</td>
<td>-35.0</td>
<td>-37.5</td>
<td>-36.6</td>
<td>-36.6</td>
</tr>
<tr>
<td>S50, mmHg</td>
<td>0.98</td>
<td>0.96</td>
<td>1.14</td>
<td>1.00</td>
</tr>
<tr>
<td>T50, s</td>
<td>7.8</td>
<td>6.6</td>
<td>6.8</td>
<td>6.5</td>
</tr>
<tr>
<td>Total baroreflex loop</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G0.01</td>
<td>2.28</td>
<td>1.91</td>
<td>1.06</td>
<td>0.58</td>
</tr>
<tr>
<td>Slope0.1–0.5, dB/decade</td>
<td>-21.4</td>
<td>-27.2</td>
<td>-29.9</td>
<td>-34.2</td>
</tr>
<tr>
<td>S50</td>
<td>-1.97</td>
<td>-1.39</td>
<td>-0.99</td>
<td>-0.58</td>
</tr>
<tr>
<td>T50, s</td>
<td>4.5</td>
<td>3.4</td>
<td>4.1</td>
<td>4.9</td>
</tr>
</tbody>
</table>

Values are means ± SD. G0.01, transfer gain at 0.01 Hz; Slope0.03–0.3 and Slope0.1–0.5, averaged slope of dynamic gain between 0.03 and 0.3 Hz and between 0.1 and 0.5 Hz, respectively; S50, step response at 50 s; Speak, negative peak response; T50, 50% rise time of the step response. *P < 0.05 and †P < 0.01 against P5 by Dunnett’s multiple-comparison procedure.
creased. The phase approached $-\pi$ radians at lowest frequencies, reflecting the negative feedback attained by the total baroreflex loop. The coherence values were lower than 0.3 at frequencies below 0.04 Hz and increased to 0.5 at frequencies above 0.05 Hz in the P5 protocol. Coherence values increased as the input amplitude was increased. Figure 6B illustrates the step responses of AP corresponding to the transfer functions shown in Fig. 6A. The step response in the P5 protocol was more variable than that in the other protocols, possibly due to a low signal-to-noise ratio in the system identification process. The maximum negative step response was significantly attenuated as the input amplitude increased (Table 3).

Parameters of the transfer functions and step responses are summarized in Table 3. In the neural arc, $G_{0.01}$ was significantly smaller in the P10, P20, and P40 protocols than in the P5 protocol. Slope 0.03–0.3 was significantly smaller in the P20 and P40 protocols than in the P5 protocol. $S_{\text{peak}}$ as well as $S_{50}$ was significantly smaller in the P10, P20, and P40 protocols than in the P5 protocol. $T_{\text{peak}}$ was significantly longer in the P20 and

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**Fig. 5.** A: peripheral arc transfer functions obtained from 4 protocols. Gain plots, phase plots, and Coh are shown. Transfer function did not differ among protocols. B: Step Res corresponding to transfer functions showing AP response to the unit change in the SNA. Solid and dashed lines represent means and means ± SD values, respectively.

**Fig. 6.** A: transfer functions of total baroreflex loop obtained from 4 protocols. Gain plots, phase plots, and Coh are shown. Transfer gain decreased as the input amplitude was increased. B: Step Res corresponding to transfer functions. AP responses to the unit changes in input pressure to the carotid sinuses are shown. Solid and dashed lines represent means and means ± SD values, respectively.
P_{40} protocols than in the P_{5} protocol. In the peripheral arc, G_{0.01} was unchanged among the protocols. Slope0_{1–0.5} did not differ significantly among the protocols. Neither S_{50} nor T_{50} varied among the protocols. In the baroreflex total loop, G_{0.01} was significantly smaller in the P_{20} and P_{40} protocols than in the P_{5} protocol. Slope0_{1–0.5} was significantly more negative in the P_{10}, P_{20}, and P_{40} protocols than in the P_{5} protocol. Whereas T_{50} did not differ among the protocols, S_{50} was significantly smaller in the P_{20} and P_{40} protocols than in the P_{5} protocol.

**DISCUSSION**

We have demonstrated that the dynamic gain and the slope of increasing gain in the baroreflex neural arc decreased as the input amplitude of the binary white noise was increased (Fig. 4). In contrast, the peripheral arc transfer function remained unchanged irrespective of the input amplitude of CSP (Fig. 5). As a consequence, the total baroreflex gain decreased as the input amplitude was increased (Fig. 6).

**Input-size dependence of the neural arc transfer function.** As mentioned in the introduction, the input-size dependence of the apparent linear transfer function in the baroreflex neural arc provides a clue to creating a model for the neural arc using the static sigmoidal and dynamic derivative components. The degree of the derivative characteristics or the slope of transfer gain decreased as the input amplitude of the binary white noise increased (Fig. 4A, Table 3). This phenomenon is consistent with what is predicted by the derivative-sigmoidal (Fig. 1, C and D) rather than sigmoidal-derivative cascade model (Fig. 1, A and B). Previous studies indicate that the transfer function from AP to aortic diameter does not possess derivative characteristics (10), whereas the pressure-diameter relationship of the baroreceptor region reveals threshold and saturation nonlinearity (7, 19). Therefore, the nonlinearity in the pressure-diameter relationship could be a sigmoidal component preceding the derivative component in the neural arc. However, the present results indicate that the nonlinearity in the pressure-diameter relationship plays little role in determining the overall neural arc transfer characteristics around the normal physiological operating pressure. If the dynamic range of the pressure-diameter relationship were narrowest among the neural arc cascade components, the neural arc transfer function would have revealed a frequency-independent decrease in dynamic gain with increasing input amplitude of the binary white noise as shown in Fig. 1B.

In a previous study, Sugimachi et al. (23) demonstrated that a dynamic linear-static nonlinear model is useful for explaining the unique aspects of baroreceptor transduction properties such as adaptation and resetting. The present results indicate that the same type of dynamic linear-static nonlinear model can be used as a first approximation of the overall neural arc transfer characteristics. This does not mean, however, that we attributed the overall neural arc transfer characteristics to the baroreceptor transduction properties alone. The neural arc transfer characteristics include not only baroreceptor transduction but also afferent signal transduction, central processing, and efferent signal transduction. Therefore, the parameters of the dynamic linear and static nonlinear components for the overall neural arc would be determined differently from those fitted to the baroreceptor transduction properties. For instance, the derivative characteristics are more pronounced in the overall neural arc transfer function than in the baroreceptor transduction properties alone (12, 22).

**Deviation of operating point.** The mean level of SNA was significantly lower in the P_{20} and P_{40} protocols than in the P_{5} protocol, although mean CSP was kept constant for all protocols (Table 1). The decrease in mean SNA indicates an increased baroreceptor activity in response to the increased input amplitude. Chapleau et al. (3) demonstrated that pulsatile pressure input causes an increased baroreceptor activity compared with static pressure input when the mean input pressure is lower than the midpoint of the sigmoid curve representing the pressure-baroreceptor activity relationship. Because we determined the operating pressure by imposing native pulsatile pressure on CSP, the operating pressure might be influenced not only by the CSP-SNA relationship but also by the SNA-AP relationship. When we directly estimated the midpoint of the sigmoid curve in the static CSP-SNA relationship using experimental settings similar to the present study, the midpoint pressure was 115 mmHg (12). Thus the operating pressure of 91 mmHg was indeed lower than the midpoint pressure (Table 1), probably causing input-size dependent decrease in the mean level of SNA.

The deviation of operating pressure from the midpoint of the sigmoid curve requires some modification of the simulations in Fig. 1. However, when we simulated the effects of input amplitude on the apparent linear transfer function using mean input pressure displaced from the midpoint of the sigmoid nonlinearity, the simulation results were qualitatively similar to those illustrated in Fig. 1, B and D, with regard to the following points. The sigmoidal-derivative cascade model cannot affect the degree of derivative characteristics, whereas the derivative-sigmoidal cascade model blunts the derivative characteristics with an increase in the input amplitude of the binary white noise. Thus the derivative-sigmoidal cascade model is likely to be a better representation of the overall neural arc transfer characteristics even when the effects of a different operating point are taken into account.

The mean level of SNA as well as the mean level of AP were both significantly lower in the P_{20} and P_{40} protocols than in the P_{5} protocol (Table 1), indicating that the peripheral arc transfer functions were estimated under different operating points among protocols. Furthermore, the SNA power (the input power to the peripheral arc) at 0.01 Hz was significantly greater in the P_{40} than in the P_{5} protocol (Table 2). Regardless of these differences in the operating point and input
power, the peripheral arc transfer functions were unchanged among the protocols (Fig. 5, Table 3). Because the neural arc showed significant saturation in response to the large input amplitude, changes in the SNA power among protocols were much smaller than those in the CSP power (Fig. 3). Furthermore, judging from the static input-output characteristics, the SNA-AP relationship is much more linear than the CSP-SNA relationship (21). Thus the differences in the operating point and input power would not have been large enough to yield a nonlinear system response in the present study. The modeling study by Ringwood et al. (20) also suggested an insignificant change in Fig. 8. The total baroreflex gain changed from unity to 5 in each panel. The input amplitude of the stepwise pressure perturbation was varied among 5, 10, and 20 mmHg. In the linear model (Fig. 8A), increasing G above 2 resulted in an oscillatory AP response, suggesting an instability in the linear system. The magnitude of the oscillatory AP response increased proportionally to the input amplitude. On the other hand, the AP responses in the G range above 2 were more stable in both the nonlinear-linear (Fig. 8B) and linear-nonlinear (Fig. 8C) cascade models. The oscillatory AP response disappeared in response to an input amplitude of 20 mmHg. This was because the operating point was displaced from the midpoint of the sigmoid curve at steady state, making the effective gain too small to cause an oscillatory AP response. Although the stability of the AP response was attained at the expense of the quickness of the AP response, AP still reached a steady state within ~10 s. Thus the nonlinearity in the baroreflex neural arc plays an important role in achieving a stable transient AP response with a minimal sacrifice of the quickness of AP response.

Both the nonlinear-linear and linear-nonlinear cascade models were effective in providing a stable transient AP response against a large input amplitude (Fig. 8, B and C). Although the linear-nonlinear cascade model showed less oscillatory AP responses for pressure perturbations of 5 and 10 mmHg compared with the nonlinear-linear model, the physiological advantage of the linear-nonlinear model relative to the non-linear-linear model is unclear from the simulation results shown in Fig. 8. However, the physiological significance of the linear-nonlinear model may be analyzed from a somewhat different viewpoint as described by the following. In a previous study, we demonstrated that the high-cut characteristics of the neural arc transfer function in the frequency range above 1 Hz are essential to preserve total baroreflex gain in the face of pulsatile pressure input (14). The conclusion was based on the presumption that a derivative component is followed by a nonlinear limiter component in the neural arc. The present results confirm that the linear-nonlinear cascade model can represent the overall neural arc transfer characteristics.

Nonlinear system identification. The models shown in Fig. 1 have their generic names: Hammerstein (Fig. 1A) and Wiener (Fig. 1C) systems (8). The impulse response of a given Wiener system is proportional to the impulse response of the linear component of the system when the input is Gaussian white noise. Hence, the nonlinear component can be directly estimated by comparing the linear prediction versus the actual output of the system (4, 6). However, because the Gauss-

**Fig. 7. Simulators of the baroreflex system.** A: linear model; B: nonlinear-linear model; C: linear-nonlinear model. $H_N$, neural arc transfer function; $H_P$, peripheral arc transfer function; $G$, total baroreflex gain. A stepwise perturbation was applied to the baroreflex negative feedback system (see APPENDIX B for details).
ian white noise input also yields the impulse response proportional to that of the linear component of a given Hammerstein system, it does not allow us to determine which of the Wiener and Hammerstein models is more suitable to represent the overall neural arc transfer function without calculating higher-order kernels. Several factors confound nonlinear system identification based on the higher-order kernels: the existence of a significant noise component in SNA unrelated to the baroreflex and may not have Gaussian distribution; the sigmoidal input-output relationship that cannot be described by a low-order polynomial function of the input; and the limited data length relative to the frequency bandwidth of the system. In contrast, the binary white noise input yields different transfer functions between the Wiener and Hammerstein systems as shown in Fig. 1. Thus the present approach of examining the amplitude dependence of system response to the binary white noise would be more practical when determining the sequence of subsystems in biological systems.

The coherence function is a frequency-domain measure of the linear dependence between input and output signals. Unity coherence indicates perfect linear dependence between the input and output signals, whereas zero coherence indicates total independence between the two signals. With respect to the neural arc transfer function, coherence values were $<0.2$ at frequencies below 0.06 Hz in the $P_5$ protocol (Fig. 4A). Several factors can reduce the coherence value from unity: a nonlinear system response, a central command component in SNA, and physical noise associated with the SNA recording procedure. In the present study, the nonlinearity of the SNA response would have increased as the input amplitude increased, because of the saturation of signal transduction in the neural arc. However, the coherence values at frequencies $<0.06$ Hz became greater as the input amplitude increased (Fig. 4A). The apparent contradiction may be explained as follows. Because not only nonlinear but also linear SNA response components increased as the input amplitude increased, the linear response component might have been increased relative to the baroreflex-uncoupled component in SNA. As a result, the signal-to-noise ratio in terms of a linear system analysis was increased as the input amplitude increased, causing increased coherence values. Thus the degree of system
nonlinearity cannot be assessed from the deviation of coherence values from unity alone in the baroreflex neural arc.

The reduction of $G_{0.01}$ from the $P_5$ to $P_{40}$ protocols was $\sim 40\%$ in the neural arc transfer function (Table 3). $G_{0.01}$ did not differ sizably between the $P_5$ and $P_{40}$ protocols in the peripheral arc transfer function. Accordingly, the reduction of $G_{0.01}$ from the $P_5$ to $P_{40}$ protocols should have been $\sim 40\%$ in the total loop transfer function if the total loop transfer function had been the product of the neural and peripheral arc transfer functions. However, the reduction of $G_{0.01}$ from the $P_5$ to $P_{40}$ protocols was $\sim 25\%$ in the total loop transfer function, suggesting that the total loop transfer function was not the linear product of the neural and peripheral arc transfer functions. We examined whether the product of the neural and peripheral arcs without normalization approximated the total loop transfer function in each animal (data not shown). The results indicated that the product tended to underestimate the total loop transfer function in the $P_5$ protocol. If the system nonlinearity had been the major source of the discrepancy between the product and the total loop transfer function, the discrepancy should have been larger in the $P_{40}$ protocol. We speculate that the estimation of linear transfer function was inaccurate due to the lower signal-to-noise ratio during the small input amplitude, resulting in the discrepancy between the product and the total loop transfer function in the $P_5$ protocol.

Limitations. There are several limitations in this study. First, we investigated the carotid sinus baroreflex in anesthetized rabbits. Because anesthesia affects SNA (24), the results might have differed had the experiment been performed in conscious animals. For instance, the baroreflex-uncoupled central command component in SNA could be expected to increase in conscious animals, reducing the accuracy of the estimation of baroreflex transfer functions.

Second, we represented the SNA responsible for the AP regulation by cardiac SNA. Because there could be regional differences in SNA in response to pressure perturbation, utilizing the SNA associated with other neural districts such as the renal or muscle nerve activity might affect the estimation of the neural and peripheral arcs of the carotid sinus baroreflex. However, differences in cardiac and renal SNA values were significant but small in dynamic characteristics in our previous study (12). In addition, high coherence values between cardiac SNA and AP (Fig. 5) suggest that AP tightly related to cardiac SNA (9). Cardiac SNA might convey common information to regulate AP as well as specific information to regulate the heart. Thus representing systemic SNA by cardiac SNA would be relevant in this study.

Third, we filled isolated carotid sinuses with warm physiological saline. Because the ionic content affects the sensitivity of the baroreceptors (1), it might also affect the dynamic characteristics of the neural arc. However, because we did not change the intravascular ionic content in the isolated carotid sinuses and performed the protocols in random order, changes in the apparent linear transfer function in the neural arc were most likely associated with changes in the input amplitude.

Finally, we sectioned vagi to remove the influences of low-pressure baroreflexes on the carotid sinus baroreflex neural arc. Accordingly, the transfer function of the total baroreflex loop shown in Fig. 6 disregarded the vagal limb of the baroreflex. The vagal efferent system affects AP via the direct control on heart rate (11) and the indirect control on ventricular contractility through the vago-sympathetic interactions (17). Although we focused on the sympathetic limb of the carotid sinus baroreflex in the present study, further studies are clearly required to identify the neural and peripheral arcs of the vagal system in regulating AP.

In conclusion, dynamic gain and the slope of increasing gain in the apparent linear transfer function of the baroreflex neural arc decreased as the input amplitude was increased. The phenomenon matched the prediction of a model consisting of a linear derivative component followed by a nonlinear sigmoidal component. Although the model is simplistic, as long as we keep the limitations in mind, the derivative-sigmoidal cascade model should prove to be useful in simulating baroreflex behavior and to improve our understanding of how the baroreflex system regulates AP against exogenous pressure perturbations.

APPENDIX A

Effects of Input Amplitude on the Apparent Linear Transfer Functions

We used Matlab Simulink toolbox (Math Works; Natick, MA) to simulate the effects of input amplitude on the apparent linear transfer functions associated with the models in Fig. 1, A and C. We modeled a sigmoidal nonlinearity in the baroreflex neural arc by a four-parameter logistic function using Eq. A1

$$y = \frac{p_1}{1 + \exp[p_2(x - p_3)]} + p_4 \quad (A1)$$

where $x$ and $y$ are input (in mmHg) and output (in au) values of the sigmoid curve. $p_1$ is the response range (in au), $p_2$ is the coefficient of gain, $p_3$ is the midpoint of input range (in mmHg), and $p_4$ is the minimum output value (in au). We set $p_1 = 40$, $p_2 = 0.1$, $p_3 = 0$, and $p_4 = -20$. These settings yielded the maximum negative gain of $-1$ at $x = 0$. The $p_2$ value was determined based on the static CSP-SNA relationship obtained from a previous study (12). The saturation point in the input axis reciprocally relates to the $p_2$ value. If we adopt a constant of 1.317 according to a model by Kent et al. (15), the saturation point in the input axis becomes $\pm 13.17$ mmHg. Because we set $p_3 = 0$, the simulation results showed changes in AP ($\Delta AP$) rather than absolute AP values.

The derivative characteristics of the neural arc were modeled using Eq. A2 according to a previous study (14)

$$H(f) = \frac{1 + \frac{f}{f_{c1}}}{(1 + \frac{f}{f_{c3}})^{\frac{2}{3}}} \exp(-2\pi f|L|) \quad (A2)$$
where \( f \) and \( j \) represent frequency (in Hz) and imaginary units, respectively. \( f_{c1} \) and \( f_{c2} \) (\( f_{c1} < f_{c2} \)) are the corner frequencies (in Hz) for derivative and high-cut characteristics of the neural arc, respectively, and \( L \) is the dead time (in s). We set \( f_{c1}, f_{c2}, \) and \( L \) at 0.1 Hz, 0.8 Hz, and 0.2 s, respectively (14). Note that a negative sign for the negative feedback was omitted as the sigmoid curve to be connected (Eq. AI) inverted the signal.

We simulated the SNA response to the CSP perturbation according to a binary white noise sequence with a switching interval of 500 ms. The input amplitude was varied from 5 to 50 mmHg in 5-mmHg increments. The transfer functions were calculated using the time series obtained from the simulations.

**APPENDIX B**

**Physiological Significance of the Sigmoidal Nonlinearity**

To simulate the closed-loop AP response to stepwise pressure perturbations (Figs. 7 and 8), we used the following models. The linear transfer function of the neural arc, \( H_{N} \), was simulated using Eq. A2 with the same parameter settings described in APPENDIX A. The linear transfer function of the peripheral arc, \( H_{P} \), was simulated by a second-order low-pass filter with the dead time as follows

\[
H_{P} = \frac{1}{1 + 2f_{N} \zeta j - \left( \frac{f_{N}}{L} \right)^{2}} \exp(-2\pi f j L)  \quad (B1)
\]

where \( f_{N} \) and \( \zeta \) are the natural frequency (in Hz) and damping ratio, respectively. We set \( f_{N} \) and \( \zeta \) at 0.07 Hz and 1.37, respectively, according to a previous study (9). In the linear model (Fig. 7A), a linear inverter was used to simulate negative feedback in the neural arc. In the nonlinear models (Fig. 7, B and C), the sigmoid curve of Eq. A1 was used with the same parameter settings described in APPENDIX A. To modify the total baroreflex gain, a linear gain component, \( G \), was inserted in a series connection in all the models.

The input amplitude of the stepwise pressure perturbation was varied among 5, 10, and 20 mmHg. The total baroreflex gain, \( G \), was varied among 5, 10, and 20 mmHg. The total baroreflex gain, \( G \), was varied among 5, 10, and 20 mmHg. The transfer function of the neural arc, \( f_{N} \) and \( j \) are the natural frequency (in Hz) and imaginary unit, respectively. The linear transfer function of the neural arc, \( A \), was calculated using Eq. B1 with the same parameter settings described in APPENDIX A. The transfer function of the peripheral arc, \( B \), was simulated by a second-order low-pass filter with the dead time as follows

\[
H_{P} = \frac{1}{1 + 2f_{N} \zeta j - \left( \frac{f_{N}}{L} \right)^{2}} \exp(-2\pi f j L)  \quad (B1)
\]

where \( f_{N} \) and \( \zeta \) are the natural frequency (in Hz) and damping ratio, respectively. We set \( f_{N} \) and \( \zeta \) at 0.07 Hz and 1.37, respectively, according to a previous study (9). In the linear model (Fig. 7A), a linear inverter was used to simulate negative feedback in the neural arc. In the nonlinear models (Fig. 7, B and C), the sigmoid curve of Eq. A1 was used with the same parameter settings described in APPENDIX A. To modify the total baroreflex gain, a linear gain component, \( G \), was inserted in a series connection in all the models.

The input amplitude of the stepwise pressure perturbation was varied among 5, 10, and 20 mmHg. The total baroreflex gain, \( G \), was varied among 5, 10, and 20 mmHg. The closed-loop AP response was simulated up to 30 s (Fig. 8).

This study was supported by the following: Research Grants for Cardiovascular Diseases (9C-1, 11C-3, and 11C-7) from the Ministry of Health and Welfare of Japan; a Health Sciences Research Grant for Advanced Medical Technology from the Ministry of Health and Welfare of Japan; a Grant-in-Aid for Scientific Research (B-11694337, C-11680862, and C-11670730) and a Grant-in-Aid for the Encouragement of Young Scientists (13770378) from the Ministry of Education, Science, Sports and Culture of Japan; Research and Development for Applying Advanced Computational Science and Technology from the Japan Science and Technology; and the Program for Promotion of Fundamental Studies in Health Science from the Organization for Pharmaceutical Safety and Research.

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