Dynamics of sympathetic baroreflex control of arterial pressure in rats

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Sato, Takayuki, Toru Kawada, Masashi Inagaki, Toshiaki Shishido, Masaru Sugimachi, and Kenji Sunagawa. Dynamics of sympathetic baroreflex control of arterial pressure in rats. Am J Physiol Regul Integr Comp Physiol 285: R262–R270, 2003; 10.1152/ajpregu.00692.2001.—By a white noise approach, we characterized the dynamics of the sympathetic baroreflex system in 11 halothane-anesthetized rats. We measured sympathetic nerve activity (SNA) and systemic arterial pressure (SAP), while carotid sinus baroreceptor pressure (BRP) was altered randomly. We estimated the transfer functions from BRP to SNA (mechanoneural arc), from SNA to SAP (neuromechanical arc), and from BRP to SAP (total arc). The gain of the mechanoneural arc gradually increased about threefold as the frequency of BRP change increased from 0.01 to 0.8 Hz. In contrast, the gain of the neuromechanical arc rapidly decreased to 0.4% of the steady-state gain as the frequency increased from 0.01 to 1 Hz. Although the total arc also had low-pass characteristics, the rate of attenuation in its gain was significantly slower than that of the neuromechanical arc, reflecting the compensatory effect of the mechanoneural arc for the sluggish response of the neuromechanical arc. We conclude that the quantitative estimation of the baroreflex dynamics is vital for an integrative understanding of baroreflex function in rats.

baroreflex sensitivity; dynamic system; feedback system; mechanoneural arc; neuromechanical arc

THE MOST UNIQUE FUNCTION of arterial baroreflex is to quickly and sufficiently attenuate the effect of an external disturbance on arterial pressure (19). For example, the change in arterial pressure induced by a postural change from lying to standing is sensed by arterial baroreceptors located within the walls of the aortic arch and internal carotid arteries. This fall in pressure is neurally encoded and relayed viaafferent pathways to a brain stem vasomotor center and then immediately causes an appropriate degree of compensatory vasoconstriction through activation of sympathetic efferent pathways. Without such quick compensation, the simple act of standing would cause a fall in arterial pressure responsible for perfusing the brain, resulting potentially in loss of consciousness. Therefore, the characterization of dynamic, as well as static, properties of baroreflex function is vital for an understanding of its function.

A useful animal model for a variety of clinical diseases ranging from hypertension to heart failure, the rat is very important to cardiovascular research (4, 8, 24, 25, 28). Its usefulness in molecular-based investigations is also an important advantage (28). For these reasons, the physiology and pathophysiology of arterial baroreflex function have been frequently examined in rats under diseased as well as normal conditions. These studies, however, have been performed under closed-loop conditions due to inherent difficulties in the rat in isolating the baroreceptors and in opening the feedback loop. In such investigations, the pressure changes imposed on the baroreceptors were produced by the injections of vasoactive agents (4, 8, 12, 13, 16, 26) or blood (6). It is important to recognize that analyses of dynamic physiological systems under closed-loop conditions could lead to erroneous conclusions (14, 21). Specifically, the experimental preparations lacking strict controllability of the rate of pressure change could result in an erroneous estimation of baroreflex function. To overcome these limitations, we developed a new simple method for isolation of baroreceptor regions of the rat (20) and demonstrated the static characteristics of the baroreflex control of sympathetic efferent activity and arterial pressure (19). However, another important feature of baroreflex function of the rat, i.e., baroreflex dynamics, was not yet clarified. In rabbits, our previous study suggested a significance of derivative responses of sympathetic nerve activity (SNA) to change in baroreceptor pressure (BRP) in stabilization of arterial pressure (9). Bertram et al. (2) studied the frequency response of arterial pressure to electrical stimulation of the aortic depressor nerve of the rat, suggesting the significance of dynamic properties of the arterial baroreflex control of arterial pressure.

The purpose of this investigation was to characterize the dynamics of the baroreflex control of SNA and arterial pressure by a system identification method based on a white noise approach. The results provided the first and quantitative data on the dynamic charac-

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teristics of the arterial baroreflex in rats and also suggested some limitations of the routine methods in estimation of so-called baroreflex sensitivity, i.e., the maximum gain of the baroreflex control of sympathetic nerve activity.

METHODS

Theoretical Consideration

We opened the feedback loop of the arterial baroreflex system and divided the system into its controlling element, mechanoneural arc, and its controlled element, neuromechanical arc. In the mechanoneural arc, the input is arterial BRP and the output is SNA. In the neuromechanical arc, the input is SNA, and the output is systemic arterial pressure (SAP). Inasmuch as the variables characterizing the functions of the two arcs are common, the operating point of the feedback system should be given as an intersection between them on an equilibrium diagram. Our previous study (19) showed that the two functional curves of the two arcs intersect each other at the point where the steady-state gain of each arc attained its maximum. Because each arc curve is significantly sigmoid shaped and the steady-state gain depends on the operating point, much attention should be paid to the design of the mean and range of BRP perturbation for estimation of baroreflex dynamics. Therefore, in the present study, to estimate the dynamics of each arc around the operating point, we first measured the operating point of the system and then designated the perturbation pressure imposed on the baroreceptors.

Animals and Surgical Procedures

The care of animals was in strict accordance with the “Guiding Principles for Research Involving Animals and Human Beings” of American Physiological Society (1). A total of 11 male Sprague-Dawley rats weighing 280–350 g were used. The rat was first placed in a glass jar where it inspired a mixture of 2% halothane (Fluothane; Takeda Pharmaceuticals, Tokyo, Japan) in oxygen-enriched air for 5–10 min. After the induction of anesthesia, an endotracheal tube was introduced orally, and the rat was ventilated artificially via a volume-controlled rodent respirator (model 683; Harvard Apparatus, South Natick, MA). The respiratory rate was controlled at 1.5 Hz. In accordance with Ono et al. (16), anesthesia was maintained through the use of 1.2% halothane during surgical procedures and 0.6% halothane during data recording. Polyethylene tubings (PE-10; Becton Dickinson, Parsippany, NJ) were inserted into the right femoral vein and the left common carotid artery. Pancuronium bromide (0.8 mg·kg⁻¹·h⁻¹ iv) was administered to eliminate spontaneous muscle activity. Arterial blood gases were monitored with a blood gas analyzer (IL-13064; Instrumentation Laboratory, Lexington, MA). For the prevention of dehydration during experiments, physiological saline was continuously infused at a rate of 5 ml·kg⁻¹·h⁻¹ with a syringe pump (CPV-3200; Nihon Kohden, Tokyo, Japan). For measurement of SAP, a 2-Fr catheter-tip micromanometer (SPC-320; Millar Instruments, Houston, TX) was placed in the aortic arch through the right femoral artery.

To open the feedback loop of the arterial baroreflex system, we cut the vagi and the aortic depressor nerves and isolated the carotid sinus baroreceptor regions by the embolization method (15, 20). In our previous study (20), we extensively described the surgical procedures for isolation of carotid sinus baroreceptors with ball bearings. Briefly, the external carotid artery was ligated at its root of the bifurcation of the common carotid artery, and then the internal carotid and pterygopalatine arteries were embolized with two ball bearings of 0.8 mm diameter. Two short polyethylene tubings (PE-50) were placed into both carotid sinuses and connected to a fluid-filled transducer (DX-200; Viggio-Spectramed, Singapore) and to a custom-made servo-controlled pump system (22, 23) based on an electromagnetic shaker and power amplifier (ARB-126; AR Brown, Osaka, Japan). We used the servo-controlled pump to impose various pressures on carotid sinus baroreceptor regions. To record SNA, we identified the left renal nerve branch from the aortorenal ganglion via a retroperitoneal approach through a left flank incision. The nerve branch was isolated, carefully dissected free, and cut. The central end of the nerve branch was placed on a pair of Teflon-coated platinum wires (7720; A-M Systems, Everett, WA). The implantation site of the wires was embedded in silicone rubber (Sil-Gel 604; Wacker, Munich, Germany). Finally, the flank incision was closed in layers. The nerve activity was amplified and band-pass filtered in the frequency range between 150 and 3,000 Hz by 8573 (ARJ and AB-610J, Nihon-Kohden). The enveloped waveform of SNA was generated through a custom-made circuit with a cut-off frequency of 100 Hz (–3 dB).

Data Recording

Protocol 1: measurement of operating points under closed-loop conditions. To measure the operating points under the closed-loop conditions of the arterial baroreflex system, we closed the feedback loop of the system with our servo-controlled pump system. A dedicated laboratory computer (PC-9801RA21; NEC, Tokyo, Japan) in real time commanded the power amplifier to make carotid sinus BRP identical with SAP by means of a digital-to-analog converter (DA12-4-98; Contec, Osaka, Japan), while digitizing SAP at a rate of 2 kHz through a 12-bit analog-to-digital converter (AD12-16D-98H; Contec). Using this technique, we were able to impose the same pressure waveform as SAP on the carotid sinus baroreceptors in the frequency range up to 10 Hz. BRP, SNA, and SAP were recorded for 3 min under the closed-loop conditions.

Protocol 2: white noise system identification of mechanoneural and neuromechanical arcs. To estimate the transfer functions characterizing dynamic characteristics of the mechanoneural and neuromechanical arcs and the total arc, we used a white noise system identification method. We randomly altered BRP between the measured operating pressure of ±10 mmHg every 0.4 s. The electrical signals of BRP, SNA, and SAP were low-pass filtered with antialiasing filters having a cutoff frequency of 100 Hz (–3 dB) and an attenuation slope of ~80 dB/decade (ASIP-0260L; Canopus, Kobe, Japan) and were then digitized at 200 Hz by means of the analog-to-digital converter for 1 h. To calibrate the level of SNA for each rat, we measured SNA at 80 and 160 mmHg of BRP and then recorded the background noise after the renal nerve was crushed between the aortorenal ganglion and the recording site.

Data Analysis

Because the absolute voltage value of SNA depends on various physical recording conditions such as the size and positioning of the electrodes, we expressed the level of SNA in an arbitrary unit (au). For each rat, SNA was normalized by the values at 80 and 160 mmHg of BRP after the average background noise level was subtracted.
To characterize the dynamic properties of the mechanoneural, neuromechanical, and total arcs, we estimated the transfer functions from BRP to SNA, from SNA to SAP, and from BRP to SAP. The transfer function $H_{x\rightarrow y}$ from input $x$ to output $y$ was computed with a fast Fourier transform algorithm (14, 21–23). The digitized data of $x$ and $y$ were resampled at 20 Hz after a moving average. The time series of each data point was divided into 50 segments of 2,048 points each, with 1,024 points of overlap between segments. The length of each segment was 102.4 s in duration. To suppress spectral leakage, we applied a Hann window to each segment and then computed the raw autospectra of $x$ and $y$ and the raw cross-spectrum between the two. To reduce an error in estimating the spectrum, we calculated the ensemble average of 50 raw spectra. Finally, we computed the transfer function over the frequency range of 0.0098–1.25 Hz with a resolution band width of 0.0098 Hz as follows

$$H_{x\rightarrow y} = \frac{S_{xy}}{S_{xx}}$$

where $S_{xx}$ is the ensemble autospectrum of $x$, and $S_{yy}$ is the ensemble cross-spectrum of $x$ and $y$. $H_{x\rightarrow y}$ is, in general, a complex quantity and is therefore expressible in polar form as

$$H_{x\rightarrow y} = |H_{x\rightarrow y}| \exp(j\phi_{x\rightarrow y})$$

where $j^2 = -1$ and $|H_{x\rightarrow y}|$ and $\phi_{x\rightarrow y}$ are the gain and phase of the transfer function, respectively. The gain spectrum was normalized by its value at the lowest frequency. The squared coherence function, a measure of linear dependence between normalized by its value at the lowest frequency. The gain spectrum was estimated by evaluating the following equation

$$\text{coh} = \frac{|S_{xy}|^2}{S_{xx} \times S_{yy}}$$

where $S_{yy}$ is the ensemble autospectrum of $y$.

The step response that represents a transient change in $y$ in response to a sudden step increase in $x$ was estimated by integration of the impulse response function computed from an inverse Fourier transform of the transfer function (14). The estimated step response had a resolution time interval of 0.4 s. Values were expressed as means ± SD.

**RESULTS**

The arterial pressure at the operating point of the closed-loop system was $118 \pm 5$ mmHg for 11 rats. Shown in Fig. 1A is a representative example of original tracings of BRP, SNA, and SAP during random pressure perturbation to carotid sinus baroreceptors. An increase in BRP seems to rapidly suppress SNA and gradually lower SAP and vice versa. The power of change in BRP was fairly constant up to 1.25 Hz (Fig. 1B). Considering that the pressure perturbation with a bandwidth of interest (≤1.25 Hz) was prerequisite for the accurate and reliable estimation of the transfer functions from BRP to SNA and BRP to SAP, our perturbation seems to be sufficient and valid for estimation.

Shown in Fig. 2 are the averaged transfer functions and step responses from BRP to SNA, from SNA to SAP, and from BRP to SAP for 11 rats. The gain from input to output is normalized by the value at the lowest frequency, i.e., steady-state gain, showing the relative change in the gain in response to the change in the input frequency. The steady-state gain from BRP to SNA was $0.021 \pm 0.003$ au/mmHg, that from SNA to SAP was $98 \pm 13$ mmHg/au, and that from BRP to SAP was $2.5 \pm 0.3$. The gain of the mechanoneural arc gradually increased about threefold up to 0.8 Hz and then decreased with input frequencies (Fig. 2A). Whereas the phase spectrum shows that the input-output relationship was out of phase at steady state, a small but significant phase lead was found below 0.5 Hz. On the basis of these characteristics, the mechanoneural arc is considered as a filter with both derivative and high-cut characteristics. The normalized gain had a peak at 0.8 Hz and a phase change of $\pi/2$ over the frequency range. These properties of the mechanoneural arc are clearly indicated by the step response estimated from the transfer function. The
SNA response to a step-wise increment in BRP exhibits an initial overshoot inhibition with the dead time of 0.12 ± 0.01 s (see Appendix for calculation of dead time). The response peaked at 0.4 s to 281 ± 13% of the steady-state value and then rapidly reached the steady-state value within 10 s. The gain of the neuro-mechanical arc rapidly decreased to 0.4% of the steady-state value as the frequency increased from 0.01 to 1 Hz (Fig. 2B). The phase shift increased with frequencies. These low-pass characteristics are clearly indicated by the step response estimated from the transfer function. The SAP exhibits a sluggish pressor response to a step-wise increment in SNA. The dead time was 0.68 ± 0.19 s, and the response reached 90% of the steady-state value at 25 ± 3 s. Similar to the neuro-mechanical arc, the total arc had low-pass characteristics (Fig. 2C). However, the rate of the gain attenuation of the total arc was significantly slower than that of the neuromechanical arc, reflecting the compensatory effect of the mechanoneural arc for the sluggish response of the neuromechanical arc. Such a difference was also clearly found in the step responses. Compared with the step response of the neuromechanical arc, that of the total arc was quick to attain the steady-state value; the elapsed time to reach 90% of the steady-state response was 7.2 ± 2 s (Fig. 2C, bottom).

The dead time was 0.79 ± 0.21 s. It should be noted that the squared coherence value was close to unity up to 0.7 Hz. High coherence values suggested that the input-output relationships of the mechanoneural, neuromechanical, and total arcs were predominantly governed by linear dynamics and that the transfer functions and step responses well described the dynamic properties of the respective arcs of arterial baroreflex.

**DISCUSSION**

Using the previously developed methods for isolation of the baroreceptor region (20) and a white noise technique (14, 21), we systematically identified the dynamic properties of the baroreflex control of arterial pressure. To our knowledge, this is the first study showing the quantitative results of baroreflex dynamics in rats, which provide many important animal models for cardiovascular pathophysiology.

**Open-Loop Approach**

Bertram et al. (2) studied the frequency response of arterial pressure to electrical stimulation of the aortic depressor nerve of the rat, suggesting the significance of dynamic properties of the arterial baroreflex control of arterial pressure. They showed the transfer function...
from the electrical stimulation of the left aortic depressor nerve to SAP under the intact conditions of both carotid sinus nerves and the right aortic depressor nerve. They also reported that denervation of both carotid sinus nerves and the right aortic depressor nerve had no significant effect on the characteristics of the transfer function. However, our previous work (10) tested the validity of the estimation of the transfer function using the random electrical stimulation of the aortic depressor nerve in rabbits, concluding that the unbiased or accurate transfer function could not be estimated if carotid sinus and/or aortic baroreceptor reflexes were functioning.

As shown in our previous study (19), baroreceptor transduction properties in rats exhibit a significant transient response. Therefore, our pressure loading method is more suitable for characterization of the arterial baroreflex control of SAP than the nerve stimulation method. In terms of systems physiology, for characterization of the feedback system, the input should be a feedback variable, i.e., BRP, and the output should be a controlled variable, i.e., SAP. According to the open-loop approach, we can characterize the most important function of the arterial baroreflex, i.e., total-loop dynamics.

Our experimental preparations for alternating between open- and closed-loop conditions of the baroreflex system even after isolation of baroreceptor regions and for servocontrolling BRP at any level allowed us to quantitatively evaluate the baroreflex dynamics in each animal. Our previous study (19) revealed that the effectiveness of baroreflex in attenuation of the effect of external disturbance depended on the operating point of the baroreflex system before the disturbance. Interestingly, the static open-loop gain from BRP to SAP was well optimized to be 2–3 at the operating point of the closed-loop system before the external perturbation, i.e., 110–130 mmHg. Such a relationship was also observed in the mechanoelectrical transduction properties of rat baroreceptors (20). Thus to clarify the physiological baroreflex dynamics, we should pay much attention to the design of BRP. In the present study, we carefully selected the level of BRP for each rat, so that the level was matched to the operating point of the closed-loop system at the baseline conditions.

System Identification by White Noise Approach

For characterization of the dynamic properties of the baroreflex control of SNA and SAP accurately and quantitatively, a white noise approach for system identification (14, 21) is more suitable than traditional methods with step and sine-wave stimuli. Because imposition of truly step-wise, i.e., with an infinitesimal rise and/or fall time, waveforms of pressure on baroreceptors is impossible (3), the accurate step response cannot be measured. Because of the limited input frequencies of sinusoidal waveforms of pressure, it would be difficult to accurately estimate the gain and phase characterizing the input-output relationship in the frequency domain (14, 21). In the white noise approach, the system is, on the other hand, tested with every possible stimulus and thus would be well characterized in the frequency domain. Moreover, the identification of the physiological system through the white noise technique is largely unaffected by the types of contaminating noise usually present in such a system (14).

The white noise method has another important advantage (14). If the transfer function of a system is accurately identified, we can estimate the impulse- and step-response functions. Thus we can quantitatively, in the time domain, visualize a transient response of the system and simulate the response to any waveform input in the frequency range of interest (21). These data help us understand the dynamics of the system.

Interestingly, arterial pressure of conscious rats (13, 15) changes within the BRP range used in the present study and the high coherence values are found in the input-output relationships of the mechanoneural and neuromechanical arcs and the total arc in this pressure range. Therefore, the arterial baroreflex system would be considered to be governed by linear dynamics under the physiological conditions.

Baroreflex Dynamics in Rat

Persson et al. (17) and Di Rienzo et al. (5) examined the influence of the baroreflex on the different spectral components of SAP variability through the broad-band spectral analysis of SAP fluctuations in dogs and cats before and after sinoaortic denervation, suggesting that the arterial baroreflex system has a limited control on SAP at higher frequencies. This suggestion was consistent with the present result that the gain of the total arc shown in Fig. 2C decreased progressively at higher frequencies. Petiot et al. (18) showed the transfer function from the electrical stimulation of the aortic depressor nerve to SNA in rats, indicating that the gain increases threefold at 0.8–1 Hz and the phase leads up to 0.5–0.6 Hz. Such characteristics seem to be similar to the transfer function from BRP to SNA shown in the present study. Therefore, the dynamic properties of the rat arterial baroreceptors are not apparent in this frequency range, which would accord with the findings by Brown et al. (3).

In our previous study (9), we examined the dynamic properties of the baroreflex control of SNA and SAP in rabbits. The derivative characteristics of the mechanoneural arc compensated the low-pass characteristics of the neuromechanical arc, and thus the closed-loop baroreflex control of SAP was optimally accelerated. The derivative characteristics of the mechanoneural arc shown in Fig. 2 would be important in optimization of the baroreflex control of SAP in rats as well as in rabbits.

To clarify the effect of the high-pass characteristics of the mechanoneural arc, we evaluated the difference in the transient responses of SAP to an external disturbance in pressure between the closed-loop systems with and without the derivative characteristics. Shown in Fig. 3A is a block diagram of the baroreflex feedback system. Under the closed-loop conditions, the effect of the external disturbance in pressure, $\Delta P_d$, is attenu-
Fig. 3. A: block diagram of the sympathetic baroreflex feedback system. Under the closed-loop conditions, a change in BRP, \( \Delta \text{BRP} \), is the same as a change in SAP, \( \Delta \text{SAP} \), and thus the effect of an external disturbance in pressure, \( \Delta P_a \), is attenuated. \( H_{\text{MN}}(f) \) and \( H_{\text{NM}}(f) \) represent transfer functions of the mechanoneural and neuromechanical arcs, \( \Delta \text{SNA} \), change in SNA. B: graph showing the simulated results of \( \Delta \text{SAP} \) in response to a step-wise \( \Delta P_a \). \( \Delta P_a \) with a magnitude of 1 mmHg is imposed at 0 s. Under the open-loop conditions (dashed line), the effect of \( \Delta P_a \) is not attenuated at all. Assuming that \( H_{\text{MN}}(f) \) and \( H_{\text{NM}}(f) \) are the averaged transfer functions of the respective arcs shown in Fig. 2, B and C, and that the steady-state gain of the total arc is 2, the effect of \( \Delta P_a \) is attenuated to one-third at the steady state. The time for the peak attenuation is 3.6 s (solid line); the transient response converges within the range of \( \pm 10\% \) of the steady-state response (shadow zone) in 4.4 s (Fig. 3B, solid line). However, if only \( H_{\text{MN}}(f) \) was replaced by an all-pass filter with flat gain and phase characteristics, the underdamped oscillatory response became clearer (Fig. 3B, dotted line). The time for the peak attenuation increased to 4.8 s; the time for the convergence within the range of \( \pm 10\% \) of the steady-state response was prolonged to 11.6 s. These results, therefore, suggest that the derivative characteristics of the mechanoneural arc significantly contribute to the quick and stable attenuation of the effect of the external disturbance on SAP.

In our previous study on the rabbit baroreflex system (9), the simulated response of the closed loop to step-wise pressure perturbation showed no oscillation of SAP, which is different from the present result. This inconsistency would result from the difference in animal species between these two studies. Another possibility is the difference in the value of the total arc gain (19). The gain estimated in the previous study was less than one-half of the value obtained in the present study. The difference in the estimated value for the baroreflex gain could be ascribed to the difference in the level of BRP used for transfer function analysis. In the previous study (9), the mean level of BRP was quite different from that of SAP (75 vs. 128 mmHg). Thus the level of BRP was not matched to the operating point of the closed-loop system. As discussed in Open-Loop Approach, this mismatching could produce the underestimation of the total arc gain. Therefore, to clarify how dynamically the baroreflex system attenuates the effect of an external disturbance under the closed-loop conditions, we should carefully design BRP.

**Implications for Baroreflex Studies in Nonisolated Preparations**

Many studies concerning baroreflex function mediated by the arterial baroreceptors have been conducted under closed-loop conditions in rats. Ramp changes in arterial pressure were routinely produced by the injections of vasoactive agents (4, 8, 12, 13, 16, 26) and blood (6). The rate of BRP change could not be strictly controlled under such conditions, and thus the results would be inconsistent and imprecise. The rates of BRP change induced by the conventional methods were considerably different (0.28–10 mmHg/s) among these studies; a large difference in the rate was found even within individual studies. The effect of the rate of BRP change, however, has never been evaluated quantitatively.

To clarify the effect of the rate of a ramp BRP change on a widespread index for baroreflex function, baroreflex sensitivity (BRS), we performed a simulation study. Using a convolution algorithm (14, 21) and the representative impulse-response function \( h(t) \) computed by an inverse Fourier transform of the transfer function estimated in the present study, we simulated the responses of SNA \( y(t) \) to ramp BRP changes \( x(t) \) with different rates (0.5–10 mmHg/s, every 0.5 mmHg/s).
\[ y(t) = \int_{0}^{t} h(\tau) x(t - \tau) d\tau \]

The examples of simulated results of time-series data of SNA responses to ramp BRP rises and falls at the rates of 0.5 and 10 mmHg/s are presented in Fig. 4, A and B. For these cases, we plotted the instantaneous responses of SNA to changes in BRP (Fig. 4C). The BRS value, i.e., the maximum slope estimated from the instantaneous SNA response curve, obviously depends on the rate of BRP change. Figure 4D indicates the relationship between the estimates for the BRS and the rates of BRP change, suggesting that the BRS value is inevitably overstimulated during the faster ramp change in BRP. Therefore, the BRS should be recognized as a function of the rate of BRP change and should be redefined as the maximum gain at a specific rate of BRP change. From these results, we conclude that the strict control of the rate of BRP change is vital for the accurate and reliable evaluation of baroreflex function, because of baroreflex dynamics.

**Limitations**

As shown in Fig. 2, the gain values from SNA to SAP and from BRP to SAP were pretty small at the higher frequencies over 0.7 Hz. We used a 12-bit analog-to-digital converter for data acquisition of electrical signals of ±5 V. The signal of arterial pressure was amplified to be 0 V for 0 mmHg and 5 V for 250 mmHg. Therefore, an error in quantization, i.e., a signal resolution, was 0.12 mmHg. These limitations affected coherence values. For example, if we imposed changes in BRP with an amplitude of 20 mmHg, and if the absolute gain values of the total arc at 0.01 and 1 Hz were 2 and 0.01, respectively, the measured changes in SAP at 0.01 and 1 Hz should be 40 and 0.2 mmHg, respectively. It is difficult to accurately measure a change of 0.2 mmHg using our system. Therefore, lower coherence values at the higher frequencies in the transfer functions of the neuromechanical and total arcs would result from the lower signal-to-noise ratio.

The purpose of this study was to characterize the arterial baroreceptor reflex with carotid sinus baroreceptor afferent and the sympathetic efferent limbs. Therefore, for the sake of simplicity, we bilaterally cut the vagal nerves to exclude the effect of cardiopulmonary receptors (7, 17) and the vagal control of heart rate. Because of difficulty in the control of aortic baroreceptor pressure, we also cut the aortic depressor nerves to open the arterial baroreflex loop. Therefore, in the present study, we could characterize the dynamic properties of the arterial baroreceptor reflex with carotid sinus baroreceptor afferent and the sympathetic efferent limbs. Thus the transfer function of the mechanoneural arc did not include the effect of the baroreceptors of the aortic depressor nerve. It is noted that the transfer function of the neuromechanical arc reflected the dynamic properties of the sympathetic

![Fig. 4. Graphs showing the simulated responses of SNA to ramp rises (A) and falls (B) in BRP at the rates of 0.5 (solid line) and 10 (dotted line) mmHg/s, and the instantaneous relationship between changes in BRP and SNA (C). D: graph showing the relationship between the baroreflex sensitivity (BRS) estimated from the instantaneous response curve and the rate of BRP change. au, Arbitrary units. See text for details.](image-url)
modulation of not only vascular but also cardiac characteristics such as heart rate and cardiac contractility.

Anesthetic agents used in the present study could also affect the dynamic properties of arterial baroreflex. The vasomotor center of the arterial baroreflex is affected by higher-order centers such as the limbic-hypothalamic systems and also receives various afferents from the periphery such as sympathetic afferent cardiac and splanchnic fibers. In the present study, we ignored these components. Thus further investigation concerning the effects of these components is needed for clarifying the dynamics of arterial baroreflex.

In conclusion, we characterized the dynamic properties of the mechanoneural, neuromechanical, and total arcs of the arterial baroreflex of the rat by the white noise system identification method. The derivative nature of the mechanoneural arc compensated for the sluggish response of the neuromechanical arc, and thus the total arc response was significantly accelerated. The simulated result with the transfer function of the mechanoneural arc suggested that the apparent maximum gain estimated from the instantaneous relationship between BRP and SNA, i.e., baroreflex sensitivity, depended on the rate of change in BRP because of the dynamic nature of the mechanoneural arc. We conclude that the quantitative estimation of the dynamic characteristics of arterial baroreflex is vital for an understanding of baroreflex function and that the routine methods lacking strict controllability of the rate of BRP change could result in erroneous conclusions.

APPENDIX

As described previously (11), we could model the transfer function of the mechanoneural arc as follows

\[ H_{MN}(f) = \frac{1 + \left(\frac{f}{f_{MN1}}\right)^j}{1 + \left(\frac{f}{f_{MN2}}\right)^j} \exp(-2\pi j f L_{MN}) \]

where \( j \) and \( f \) represent the frequency (in Hz) and imaginary units, respectively; \( f_{MN1} \) and \( f_{MN2} \) (\( f_{MN1} < f_{MN2} \)) are corner frequencies for derivative and high-cut characteristics, respectively; and \( L_{MN} \) is a pure delay or dead time (in s). When \( f_{MN2} \) is set greater than \( f_{MN1} \), dynamic gain increases in the frequency range from \( f_{MN1} \) to \( f_{MN2} \) and decreases above \( f_{MN2} \). When \( f_{MN2} \) is far greater than the frequency range of interest, \( H_{MN}(f) \) approximates a derivative filter. The phase shift due to derivative and high-cut filters and the pure delay yield the overall phase characteristics. Introduction of the pure dead time \( L_{MN} \) would be reasonable because the baroreceptor transduction, nerve conduction, and synaptic transmission should be assumed in the transfer function from BRP and SNA.

Similarly, we could model the transfer function of the neuromechanical arc with a second-order low-pass filter as follows

\[ H_{NM}(f) = \frac{1}{1 + 2\zeta \left(\frac{f}{f_{NM}}\right)^j + \left(\frac{f}{f_{NM}}\right)^j} \exp(-2\pi j f L_{NM}) \]

where \( f_{NM} \) and \( \zeta \) indicate a natural frequency and a damping ratio, respectively. The pure delay \( L_{NM} \) would be due to the electromechanical transduction from SNA to SAP.

The transfer function of the total arc could be modeled as the product of \( H_{MN}(f) \) and \( H_{NM}(f) \). Finally, the dead time of the total arc was calculated from the sum of \( L_{MN} \) and \( L_{NM} \).

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