Dynamic transduction properties of in situ baroreceptors of rabbit aortic depressor nerve

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Animal Preparations

The care of animals was in strict accordance with the guiding principles of the Physiological Society of Japan. Twelve Japanese White rabbits weighing 2–3 kg were anesthetized with urethan (1.5 g/kg iv) followed by a maintenance dosage of urethan (0.1 g·kg⁻¹·h⁻¹·iv). The rabbits were intubated and ventilated artificially with room air. $P_{\text{aco}}$ and $P_{\text{ao}}$ were maintained in the ranges of 30–40 mmHg and 90–100 mmHg, respectively. Pancuronium bromide (0.5 mg/kg iv) was administered to eliminate spontaneous muscle activity. After midline incision in the neck and median thoraectomy, the aortic arch, brachiocephalic trunk, innominate artery, right subclavian artery, and bilateral common carotid arteries were identified. Although some variations in the branches of the aortic arch and in nomenclature for the vasculature have been reported in rabbits, we used the same nomenclature reported by Sawin and Edmonds (16). They described the brachiocephalic trunk as arising from the aortic arch and giving origin in turn to the innominate artery and...
the left common carotid artery. If the left common carotid artery arose from the aortic arch directly, the brachiocephalic trunk was considered to be absent. For the measurement of aortic pressure, 18-gauge polyethylene tubing (PE-160, Becton Dickinson, Parsippany, NJ) was cannulated through the left common carotid artery and advanced into the aortic arch. A cuff occluder was placed around the descending aorta. The right subclavian artery was tied proximal to the roots of the vertebral and internal thoracic arteries. The 10-cm-long polyethylene tubing was cannulated into the right common carotid artery and connected to a servo-controlled piston pump (model ET-126A, Labworks, Costa Mesa, CA). The rise time of the servo pump in controlling intracarotid pressure was <50 ms. Right common carotid pressure and aortic arch pressure were recorded via fluid-filled transducers (DX-200, Viggo-Spectramed, Singapore) connected to the cannulated tubing. After the innominate artery was tied at its root, water sealing of the right common carotid artery was checked. Occasionally leakage from a few small branches from the innominate artery such as Neubauer’s artery (arteria thyreoidea ima) was found, but ligation of the branches ensured a completely watertight chamber (Fig. 1). The right vagus was cut at the level of the right subclavian artery. The right superior and middle cervical sympathetic ganglia were removed.

Aortic Depressor Nerve Recording

The right aortic depressor nerve was identified, separated free, and cut at the junction with the superior laryngeal nerve and nodose ganglion under a dissecting microscope. The distal end of the nerve was desheathed and placed on a pair of platinum-iridium wire electrodes. The multifiber neural activity was amplified and band-pass filtered between 0.15 and 3.0 kHz. The envelope of nerve activity was generated by full-wave rectification and low-pass filtering of the raw nerve signal with a cutoff frequency of 100 Hz (−3 dB).

Experimental Protocols

Protocol 1. To examine how uniquely aortic depressor nerve activity represents intracarotid pressure without contamination with other regions, we kept the intracarotid pressure at 20 mmHg with the servo-controlled piston pump. We then occluded the aorta with the cuff occluder to alter aortic pressure while measuring aortic depressor nerve activity.

Protocol 2. To examine the linearity of the baroreceptor transduction in the physiological pressure range, we changed intracarotid pressure stepwise from 80 to 85, from 80 to 90, from 80 to 95, and from 80 to 100 mmHg with the servo-controlled piston pump. If the transduction was linear, the step response should be linearly scaled for the amplitude of input. Each pressure step was maintained for 50 s. Before the step pressure was given, intracarotid pressure was kept at the baseline pressure of 80 mmHg for 5 min.

Protocol 3. To examine dynamic transduction properties of the baroreceptors, we altered intracarotid pressure randomly according to a binary quasi-white noise. The perturbation pressure distribution between 80 and 100 mmHg with a frequency bandwidth of 10 Hz. While the perturbation was given, we recorded the intracarotid pressure and the envelope of aortic depressor nerve activity through an analog-to-digital converter (AD12–16D98H, Contec, Osaka, Japan) at 200 Hz for 40 min.

Protocol 4. To examine the static, nonlinear nature of the baroreceptor transduction, we changed intracarotid pressure stepwise from 20 to 180 mmHg in 20-mmHg steps. Each pressure step was maintained for 50 s. This protocol was performed last, because the exposure of baroreceptors to sustained high pressure could damage them irreversibly.

Data Analysis

The digitized data were resampled at 10 Hz after a 20-point moving average to avoid aliasing. The 40-min data of protocol 3 were analyzed in the frequency domain (13). The time-series data of intracarotid pressure and aortic depressor nerve activity were segmented into 1,024-point (102.4 s) data. To reduce spectral leakage, we applied a Hanning window w(n) to each segment

\[ w(n) = 0.5 - 0.5 \cos \left( \frac{2\pi n}{N} \right) \]

where \( n \) is the point number and \( N \) is the number of data points. We computed the raw autospectra of intracarotid pressure and aortic depressor nerve activity and the raw cross-spectrum between the two signals with an algorithm based on a fast Fourier transform. To reduce error in estimating the spectrum, we calculated the ensemble average of 50 raw spectra. The equivalent bandwidth of the spectra was ~0.01 Hz. The transfer function \( H(f) \) from intracarotid pressure to aortic depressor nerve activity was estimated from the raw autospectra of intracarotid pressure \( S_{pp}(f) \) and the ensemble cross-spectrum \( S_{pn}(f) \)

\[ H(f) = \frac{S_{pn}(f)}{S_{pp}(f)} \]

\( H(f) \) was, in general, a complex quantity and was therefore expressible in polar form as

\[ H(f) = |H(f)| \exp \{ j \phi(f) \} \]

where \( |j| = -1 \), and \( H(f) \) and \( \phi(f) \) were the gain and phase of the transfer function, respectively. The squared coherence function \( \text{coh}(f) \), a measure of linear dependence between intracarotid pressure and aortic depressor nerve activity, was estimated with the following equation

\[ \text{coh}(f) = \frac{|S_{pn}(f)|^2}{S_{pp}(f) \times S_{nn}(f)} \]
where $S_{nn}(f)$ is the ensembled autospectrum of aortic depressor nerve activity.

The step response that represents a transient change in nerve activity in response to a sudden increase in intracarotid pressure was estimated by integration of the impulse response function computed from an inverse Fourier transform of the transfer function (13). To examine the linearity of the baroreceptor transduction, we compared the estimated step response with the actual step response obtained in protocol 2.

The data from protocol 4 were analyzed with a four-parameter logistic regression analysis (11)

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

where $y$ is nerve activity and $x$ is pressure. The four parameters were defined as follows: $p_1$, the range of change in $y$ (i.e., maximum − minimum values of $y$); $p_2$, the coefficient for calculation of gain; $p_3$, the value of $x$ corresponding to the midpoint over the range of $y$; and $p_4$, the minimum value of $y$. The maximum gain was $-p_1p_2/4$.

RESULTS

Figure 2 shows an example of original tracings of aortic arch pressure, intracarotid pressure, and aortic depressor nerve activity during transient occlusion of the descending aorta. The right aortic depressor nerve did not respond to aortic arch pressure while intracarotid pressure was kept constant. Similar results were obtained from all of the rabbits used in the present study.

Shown in Fig. 3A is an example of the transient responses of aortic depressor nerve activity against sudden changes in intracarotid pressure with different magnitudes. An increase in amplitude of step pressure produced a linear increase in aortic depressor nerve activity at the peak and steady state in the pressure range of 80–100 mmHg. Figure 3B demonstrates the relationship between the peak and steady-state responses and the amplitude of step pressure in 12 rabbits. For each animal, aortic depressor nerve activity was normalized by the values at the baseline and at

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the steady-state response to a 20-mmHg step (Fig. 3B). Linear regression analysis indicated that Pearson’s correlation coefficient for each data set was $>0.98$. The linearity between input and output was, therefore, acceptable in this input range. Both peak and steady-state responses appeared to increase linearly, whereas input pressure amplitudes increased.

Illustrated in Fig. 4 are the original tracing during random pressure perturbation and the autospectra of pressure perturbation and aortic depressor nerve activity. Input power into the baroreceptors was flat up to $\sim 5$ Hz (Fig. 4B). Output power increased with frequency up to 1 Hz (Fig. 4C), suggesting the derivative nature of the baroreceptor transduction.

Shown in Fig. 5 is the transfer function of signal transduction from pressure to afferent nerve activity. The gain increased about threefold as the frequency increased from 0.01 to 2 Hz, and then the gain decreased at higher frequencies. A small phase lead was found at low frequencies. The coherence spectrum was close to unity. Thus aortic depressor nerve activity was linearly attributable to intracarotid pressure in the frequency range between 0.01 and 5 Hz.

Illustrated in Fig. 6 are the step response estimated from the transfer function and the step response actually observed. The estimated step responses are indistinguishable from the measured step response. The standard error of the estimate was only $0.8 \pm 0.3\%$. The fact that we could predict the actual step response with fidelity indicates that the estimated transfer function described the overall and accurate dynamic transduction properties of baroreceptors.

To disclose the steady-state nonlinearity of the baroreceptor transduction, we altered the intracarotid pressure and measured aortic depressor nerve activity. Figure 7A demonstrates an example of the response of
aortic depressor nerve activity to 20-mmHg steps lasting for 50 s from various baseline pressures. For each animal, aortic depressor nerve activity was normalized by the steady-state values at 20 and 180 mmHg. The peak responses were almost saturated at 120 mmHg. This saturation effect was even clearer when we plotted the nerve activity as a function of intracarotid pressure, as shown in Fig. 7B.

DISCUSSION

The main results of the present study were as follows. 1) Barosensitive regions innervated by the right aortic depressor nerve were completely isolated in situ by the simple method described in METHODS. This result suggests that the right aortic depressor nerve does not innervate the aorta functionally. 2) By the white noise technique, the dynamic transduction properties from pressure to afferent nerve activity were estimated well in the frequency ranges of 0.01–5 Hz. The gain of transduction increased two to three times as the frequency of pressure change increased from 0.01 to 2 Hz, and then the gain decreased rapidly at higher frequencies. 3) The estimated step response from the frequency domain analysis corresponded well to the actual step response.

In Situ Isolation of Baroreceptors

After the discovery of the aortic depressor nerve of rabbits by Cyon and Ludwig (5), rabbits have been used widely in experimental research on baroreceptor and baroreflex, because the rabbit aortic depressor nerve runs separately and is purely barosensitive, unlike that of dogs or cats (8, 14, 17). Tello (19) has examined the developmental neuroanatomy of the aortic depressor nerve in rabbits. At the beginning of embryogenesis, a prototype of the aortic depressor nerve comes out from the nodose ganglion and grows into the fourth intersegmental artery at the neck. With the migration of the truncus arteriosus into a thoracic cavity, the right fourth intersegmental artery develops into the root of the right subclavian artery and the distal end of the innominate artery. On the left side, the fourth intersegmental artery develops into the short segment of the aortic arch between the brachiocephalic trunk and the left subclavian artery. Tello (19) concluded that the right aortic depressor nerve did not innervate the aortic arch or the heart. Similar findings have also been reported by Nonidez (15). Based on these neuroanatomic characteristics of the right aortic depressor nerve, we made an attempt to isolate the in situ baroreceptive regions of the aortic depressor nerve.

In the present study, we confirmed the validity of our method for isolating in situ baroreceptive regions. Generally, aortic depressor nerve-mediated baroreflex functions in rabbits were examined under closed-loop conditions because the isolation of baroreceptive area was considered difficult. However, our method provides a new preparation technique for clarifying the aortic depressor nerve activity to 20-mmHg steps lasting for 50 s from various baseline pressures. For each animal, aortic depressor nerve activity was normalized by the steady-state values at 20 and 180 mmHg. The peak responses were almost saturated at 120 mmHg. This saturation effect was even clearer when we plotted the nerve activity as a function of intracarotid pressure, as shown in Fig. 7B.
depressor nerve-mediated baroreflex functions of rabbits under open-loop conditions without complex surgical procedures such as the bypass or cross-circulation technique used in dogs (12). In light of the fact that the aortic depressor nerve is purely barosensitive in rabbits, the in situ isolation preparations of the baroreceptor area of the aortic depressor nerve by our method would be more suitable for studies of baroreflex control than those of carotid sinus employed routinely. Moreover, compared with a method for isolating the carotid sinus, our method for making a watertight chamber is simple.

Angell-James (1) and Brown et al. (3, 4) have developed ex vivo preparations to examine the characteristics of arterial baroreceptors. The aortic arch with the left aortic depressor nerve was excised and adjusted to approximate its in vivo position. However, difficulty in the preservation of the in situ normal configuration of the aortic arch could result in an erroneous estimate of the transduction properties. The pressure threshold of myelinated A fibers for steady-state discharges has been reported to be 104 mmHg in ex vivo preparation (3) and to be 35 mmHg in in vivo preparation (20). This large difference would be ascribed to the difference in the preparation method between these two studies.

White Noise Technique

Little information is available on the dynamic transduction properties of the aortic depressor nerve of rabbits. Angell-James (1) used ex vivo perfused, isolated preparations of the rabbit aortic arch and gave sinusoidal pressure perturbation into the aortic arch. Indicating the number of impulses recorded from only two nerve fibers during 1.8- and 3.5-Hz perturbation, she concluded qualitatively that the frequency of pressure change affected both the maximum and minimum impulse frequencies. Similarly, almost all the studies on the dynamic transduction properties of baroreceptors of other animals (4, 7) have been conducted under particular sets of input such as sinusoidal and step pressure changes and thus demonstrated only limited aspects of the dynamic properties (13).

Compared with the traditional approach of testing dynamic properties of a physiological system with step (7) and sine wave (3, 4) stimuli, the white noise approach has definite advantages as follows (13). First, if a step stimulus is applied, we learn the response of the system to this step and have little notion of the response of the system to any other type of stimulus. If a sinusoidal pulse is applied, then we know the response of the system to such a stimulus and little else. The same applies for any other specific waveform. In the white noise approach, the system is, on the other hand, tested with every possible stimulus. Namely, the white noise stimulus is a very rich stimulus. It should be emphasized that the white noise method is perfectly suited to the analysis of linear systems. Second, 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 (13).

Sugimachi et al. (18) demonstrated that the white noise technique was useful in identifying the overall dynamic transduction properties of the rabbit aortic depressor nerve and estimated the unbiased transfer function in the frequency range up to 0.5 Hz. In light of the fact that the heart rate in conscious, resting rabbits, pressure perturbation of higher frequencies would be needed for a detailed description of the transduction properties. Sugimachi et al. (18) altered aortic pressure by ventricular pacing to impose a pressure perturbation on the baroreceptor area and failed to provide the rapid pressure perturbation. In the present study, the isolation preparations of baroreceptor regions produced an adequately broad and continuous frequency band of pressure perturbation. The perturbation pressure was randomly changed according to a binary quasi-white noise between 80 and 100 mmHg, because the level and amplitude of perturbation pressure were located in linearly operating regions as demonstrated in Figs. 3 and 7. Interestingly, arterial pressure of conscious, resting rabbits changes within this range (2, 20), and thus the information of arterial pressure would be linearly translated into the nerve activity under physiological conditions.

Dynamic Transduction Properties

The present study identified the dynamic transduction properties of the rabbit aortic depressor nerve in a broad frequency range, indicating that a cutoff frequency is 3–4 Hz. The accuracy of our estimated transfer function was confirmed by the reproducibility of the step response with high fidelity. Using an ex vivo rat aortic arch preparation, Brown et al. (4) evaluated the gain of the discharge rate of a myelinated fiber of the aortic depressor nerve to several sinusoidal pressure perturbations. The gain reached ~1.6 times as large as that at baseline frequency, peaked between 5 and 9 Hz, and decreased rapidly at higher frequencies. Brown et al. speculated a possible correlation between the cutoff frequency and the heart rate of rats, although the physiological significance of such a correlation remains unclear. In light of the fact that the heart rate of conscious, resting rabbits (2) is between 2 and 3 Hz, the speculation by Brown et al. could be applied to rabbits. However, the gain reported by Brown et al. (4) was smaller than that shown in this study. The difference could be ascribed to the differences in species and methodology.

In our previous study (10), we examined the open-loop dynamic property of baroreflex control of vasomotor sympathetic nerve activity and arterial pressure and characterized the transfer functions from carotid sinus pressure to sympathetic nerve activity (neural arc) and from sympathetic nerve activity to systemic arterial pressure (peripheral arc) in rabbits. The high-pass characteristics of neural arc up to 1 Hz compensated the low-pass characteristics of peripheral arc, and thus the closed-loop baroreflex control of arterial pressure was optimized. The neural arc consists of the mechanoelectrical transduction from pressure to baroreceptor activity and the central regulation of sympathetic nerve activity corresponding to baroreceptor...
activity. Therefore, the derivative nature of baroreceptors shown in the present study would be important in optimization of baroreflex control of arterial pressure.

Implications for Baroreceptor and Baroreflex Studies in Nonisolated Preparations

Many studies concerning baroreceptor and baroreflex function mediated by the aortic depressor nerve have been conducted under closed-loop conditions. Pressure perturbation was produced routinely by pressor and depressor drugs (2, 6, 9) or caval and aortic occlusions (20). The rate of pressure change could not be strictly controlled under such conditions, and thus the results would be inconsistent and imprecise. The rates of pressure change induced by the conventional methods were considerably different among studies (0.33–2 mmHg/s); a large difference in the rate was found even within individual studies (2, 6, 9, 20). We simulated the aortic depressor nerve activity during ramp pressure changes from 80 to 60 or to 100 mmHg with different rates (0.1–5 mmHg/s, in steps of 0.1 mmHg/s), using a convolution algorithm (13) and the representative impulse response function computed by an inverse Fourier transform of the transfer function estimated in the present study

\[ y(t) = \int_0^t h(\tau)x(t - \tau) \, d\tau \]

Two examples during slow (0.5 mmHg/s) and rapid (3 mmHg/s) ramps are presented in Fig. 8A. The maximum gain values estimated by the logistic function analysis (11) are 1.27 during the slow ramp and 1.95 during the rapid ramp. Figure 8B clearly reveals that the maximum gain depends on the rate of the pressure change. Thus the accurate and reliable evaluation of baroreceptor and baroreflex function would be difficult unless the rate of change in pressure is strictly controlled.

Fig. 8. A: simulated ADN activity during linear pressure changes from 80 to 60 or to 100 mmHg at rapid (3 mmHg/s) and slow (0.5 mmHg/s) rates and relationship between instantaneous pressure change and nerve activity. B: relationship between maximum gain estimated by logistic regression analysis and rate of pressure change on a semilogarithmic scale. See text for details.
Limitations

The following represent possible limitations of the present study. First, multifiber neural activity and its enveloped waveform are used in the present study as a marker of nerve signals instead of single-fiber activity and its spike frequency. Two types of fibers, myelinated A fibers and nonmyelinated C fibers, have been identified in the rabbit aortic depressor nerve and reported to differ from each other functionally (20). Compared with myelinated fibers, nonmyelinated fibers showed high thresholds and incoherent discharge patterns with pressure stimulation. The operational range of C fibers of the rabbit aortic depressor nerve might be presumed to be >140 mmHg. Therefore, the present study would describe the characteristics of myelinated fibers. High coherence values of our results also suggest that the nerve activity recorded in the present study derived predominantly from myelinated fibers.

Second, the isolated baroreceptor regions in the present study were not perfused with temperature-controlled or oxygenated solution. The possibility that the characteristics of arterial wall and baroreceptor were affected, therefore, could not be denied (7).

Third, we did not measure vascular diameters simultaneously. Transduction from pressure to afferent nerve activity is subdivided into two steps, mechanical transduction from pressure to vascular strain and mechano-electrical transduction from strain to nerve activity (4). In this study, we could estimate overall dynamic transduction properties from pressure to nerve activity. Further examination by the in situ simultaneous measurement of pressure, diameter, and nerve activity is needed for characterizing detailed transduction properties.

In conclusion, we developed a method for in situ isolation of baroreceptor area of the right aortic depressor nerve of rabbits and estimated the dynamic transduction properties from pressure to afferent nerve activity. The gain of transduction increased two to three times as the frequency of pressure change increased from 0.01 to 2 Hz, and then the gain decreased rapidly at higher frequencies. The information of arterial pressure would be transduced into the nerve activity under physiological conditions according to linear dynamics.

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