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Consideration on Step Duration to Assess Open-loop Static Characteristics of the Carotid Sinus Baroreflex in Rats

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Abstract—**The carotid sinus baroreflex is one of the most important negative feedback systems to stabilize arterial pressure. Although static characteristics of the carotid sinus baroreflex can be assessed by using a stepwise input protocol under baroreflex open-loop conditions, the step duration has been determined empirically. In the present study, we examined the effects of different time windows (5-10, 15-20, 25-30, 35-40, 45-50, and 55-60 s) on the static characteristics estimated by using a 60-s stepwise input protocol in 10 anesthetized rats. Based on the results, we compared the static characteristics between actual 60-s and 20-s stepwise input protocols. Most of the parameters of the static characteristics did not differ significantly between the 60-s and 20-s stepwise input protocols, suggesting that the open-loop baroreflex static characteristics can be estimated by using a stepwise input with the step duration as short as 20 s in normal rats.**

I. Introduction

The carotid sinus baroreflex system is one of the most important negative feedback systems to stabilize arterial pressure (AP). The carotid sinus baroreflex may be divided into two principal subsystems [1], [2]. One is a neural arc subsystem that acts as a controller for regulating sympathetic nerve activity (SNA) in response to a baroreceptor pressure input. The other is a peripheral arc subsystem that serves as a plant for yielding AP according to SNA through cardiovascular responses. In order to assess the open-loop static characteristics of these two subsystems, a stepwise (staircase-wise) input has been employed. The levels of input pressure are changed stepwise to cover the whole input pressure range of the arterial baroreflex, e.g., between 60 and 180 mmHg in rats. Each input pressure level is sustained for certain duration to make the system response reach steady state at a given input pressure level. Empirically, 60-s step duration seems to be appropriate for estimating the baroreflex static characteristics in rats [3], [4]. Although minimizing the step duration would contribute to shortening the total experimental time, too short duration can violate the assumption of acquiring the steady-state response. In the present study, we examined possible shortest step duration necessary for estimating the baroreflex open-loop static characteristics in rats.

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II. Materials and Methods

A. Animal Preparation

Animals were cared for in strict accordance with the *Guiding Principles for the Care and Use of Animals in the Field of Physiological Sciences*, which has been approved by the Physiological Society of Japan. All experimental protocols were reviewed and approved by the Animal Subjects Committee at National Cerebral and Cardiovascular Center.

The study was conducted using ten male Sprague-Dawley rats. Each rat was anesthetized by an intraperitoneal injection (2 ml/kg) of a mixture of urethane (250 mg/ml) and α-chloralose (40 mg/ml), and mechanically ventilated through a tracheal tube with oxygen-enriched room air. A venous catheter was inserted into the right femoral vein for a maintenance dose of the above anesthetic mixture diluted by 20 fold (2-3 $ml \cdot kg^{-1} \cdot h^{-1}$). An arterial catheter was inserted into the right femoral artery to measure AP, from which heart rate (HR) was detected. Another venous catheter was inserted into the left femoral vein for the infusion of Ringer solution (6 $ml \cdot kg^{-1} \cdot min^{-1}$) to maintain fluid balance.

In order to record SNA, a postganglionic branch from the splanchnic sympathetic nerve was exposed through a left flank incision. A pair of stainless steel wire electrodes (Bioflex wire, AS633, Cooner Wire, CA, USA) was attached to the nerve, and the nerve and electrodes were covered with silicone glue (Kwik-Sil, World Precision Instruments, FL, USA). To quantify the nerve activity, the preamplified signal was band-pass filtered at 150-1000 Hz, and was full-wave rectified and low-pass filtered with a cut-off frequency of 30 Hz. Pancuronium bromide (0.4 $mg \cdot kg^{-1} \cdot h^{-1}$) was administered to prevent muscular activity from contaminating the SNA recording. At the end of the experiment, an intravenous bolus injection of a ganglionic blocker, hexamethonium bromide (60 mg/kg), was given to confirm the disappearance of SNA. The noise level was then recorded and served as zero SNA. Because the absolute magnitude of SNA varied among animals depending on recording conditions, mean SNA value corresponding to the carotid sinus pressure (CSP) of 60 mmHg calculated at the time window of 55-60 s was assigned to be 100 au (arbitrary units).

Bilateral vagal and aortic depressor nerves were sectioned at the neck to avoid reflexes from the cardiopulmonary region and aortic arch. The carotid sinus regions were isolated from the systemic circulation using previously reported procedures [5], [6] with modifications. A 7-0 polypropylene suture with a fine needle (PROLENE, Ethicon, GA, USA) was passed through the tissue between the external and internal carotid arteries, and the external carotid artery was ligated close to the carotid bifurcation. The internal carotid artery was embolized by the injection of two to three steel balls with a diameter of 0.8 mm (Tsubaki Nakashima, Nara, Japan) via the common carotid artery. The isolated carotid sinuses were filled with warmed Ringer solution via the catheter inserted into the common carotid arteries. CSP was controlled using a servo-controlled piston pump. Heparin sodium (100 U/kg) was given intravenously to prevent blood coagulation. Body temperature was maintained at approximately 38ºC with a heating pad and a lamp.

B. Estimation of Baroreflex Open-loop Static Characteristics Using Different Time Windows in a 60-s Stepwise Input

To estimate the open-loop static characteristics of the total baroreflex, neural arc, peripheral arc, and HR control, CSP was first decreased to 60 mmHg for four min, and increased stepwise from 60 to 180 mmHg at increments of 20 mmHg every minute.

Mean values of SNA, AP, and HR were calculated from time windows of 5-10, 15-20, 25-30, 35-40, 45-50, and 55-60 s at each CSP level. In each rat, data from two consecutive 60-s stepwise input cycles were averaged. The static characteristics of the total baroreflex (the CSP-AP relationship), neural arc (the CSP-SNA relationship), and HR control (the CSP-HR relationship) were quantified using a four-parameter logistic function as [7]:

$$y = \frac{P_1}{1+\exp[P_2(x-P_3)]} + P_4$$

where x and y denote the input and output values, respectively; P_1 is the response range; P_2 is the slope coefficient, P_3 is the midpoint input pressure; and P_4 is the minimum value of the output.

The static characteristics of the baroreflex peripheral arc (the SNA-AP relationship) were quantified by a linear regression analysis as:

$$AP = a \times SNA + b$$

where a and b represent the slope and intercept, respectively.

C. Estimation of Baroreflex Open-loop Static Characteristics Using a 20-s Stepwise Input

Based on preliminary results of the open-loop static characteristics using different time windows in a 60-s stepwise input described above, the system response to a 20-s stepwise input was examined. The 20-s stepwise input protocol was conducted before (n = 5) or after (n = 5) the 60-s stepwise input protocol to make the possible time effect be even between the two protocols. Mean values of SNA, AP, and HR were obtained during the last 5 s (15-20 s) at each CSP level. In each rat, data from two consecutive 20-s stepwise input cycles were averaged.

D. Statistical Analysis

All data are expressed as means±SE values. To compare the effects of differing the time windows of analysis (5-10, 15-20, 25-30, 35-40, 45-50, and 55-60 s) on the parameters of the baroreflex static characteristics, repeated-measures analysis of variance (ANOVA) was used [8]. If there was a significant difference, a Dunnett's test was applied to identify the difference against the data calculated from a time window of 55-60 s. To compare the parameters of the baroreflex static characteristics between the 60-s and 20-s stepwise input protocols, a paired-t test was used. Differences were considered to be significant when $P < 0.05$. We used a rule of thumb that the parameters derived from two protocols were considered to be similar when $P > 0.2$.

III. Results and Discussion

Fig. 1 represents typical recordings of CSP, SNA, AP, and HR during 60-s and 20-s stepwise input protocols. A white line in the SNA recording is a 2-s moving averaged signal. An increase in CSP decreased SNA, AP, and HR. The maximum and minimum values of SNA, AP, and HR responses did not differ significantly between the two input protocols.

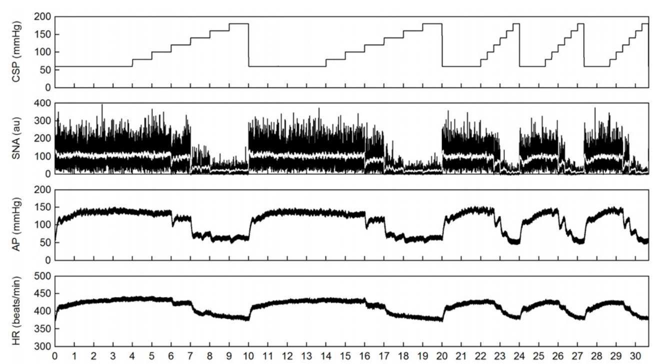

Fig. 1. Typical experimental recordings during 60-s and 20-s stepwise input protocols. CSP: carotid sinus pressure, SNA: sympathetic nerve activity, AP: arterial pressure, HR: heart rate. The white line in the SNA recording represents a 2-s moving averaged signal.

Figure 2 (the last page) summarizes the open-loop static characteristics of the total baroreflex, neural arc, peripheral arc, and HR control, obtained from the 60-s stepwise input protocol, with the analyses using different time windows. The characteristics of the total baroreflex, neural arc, and HR control approximated an inverse sigmoid curve. The characteristics of the peripheral arc approximated a straight line. The data obtained during 55-60 s served as controls. The estimated parameter values, except those estimated during 5-10 s, did not differ significantly from those obtained during 55-60 s (Table 1), suggesting that the open-loop static characteristics of the baroreflex could be obtained using a stepwise input with step duration as short as 20 s.

Figure 3 (the last page) compares the open-loop static characteristics of the total baroreflex, neural arc, peripheral arc, and HR control between actually applied 20-s and 60-s stepwise input protocols. Data were calculated from the last 5 s of each step. The lines of mean data obtained from the two protocols were very close (Fig. 3, right panels, dashed line: 20-s, solid line: 60-s). In the parameters of the total baroreflex, no significant differences were detected between the two protocols (Table 2). In the neural arc, although the slope coefficient was significantly smaller by 0.006 in the 20-s stepwise input protocol, the magnitude of the difference was comparable to the corresponding SE value (0.006) in the 60-s stepwise input protocol. Other parameters of the neural arc did not differ significantly. Parameters of the peripheral arc did not differ significantly between the two protocols. In the HR control, although the response range was significantly smaller by 3.6 beats/min in the 20-s stepwise input protocol, the magnitude of the difference was less than the corresponding SE value (7.5 beats/min) in the 60-s stepwise input protocol. Other parameters did not differ significantly. Although we did not carry out an equivalence test, if we use a rule of thumb that the two parameter values are considered to be similar when $P > 0.2$, the midpoint input pressure (P_3) could be different in all of the total baroreflex, neural arc, and the HR control. The percent difference of P_3 values relative to the value estimated by the 60-s stepwise input protocol was, however, less than 5% on the average. Collectively, although several parameters differed slightly, the 20-s stepwise input protocol provided parameter values similar to those obtained from the 60-s stepwise input protocol. The differences of the parameters between the two protocols could not be detected if we applied an unpaired-t test instead of a paired-t test, suggesting that the detected difference was within the inter-individual variations.

Although too short step duration in a stepwise input protocol will violate the assumption that the system's steady-state response is obtained, too long step duration will also violate the assumption that the system remains stationary. Minimizing the step duration may contribute to shortening the total experimental time and making the assumption for stationarity more feasible in biological experiment. In addition, when examining the effects of certain interventions on the system characteristics, reducing the step duration would increase the time resolution for tracking the effects of interventions on the system characteristics. In other words, by using a 20-s stepwise input protocol, we may be able to increase the time resolution of the systems analysis by 3 fold compared to a 60-s stepwise input protocol.

There is a limitation to the present study. We estimated the baroreflex static characteristics in normal anesthetized rats. In diseased conditions such as chronic heart failure, the cardiovascular responses could be blunted [3]. In such conditions, longer step duration may be required for AP to reach a new steady state at a given input pressure, and thus the 20-s stepwise input protocol may not work well. Apparently, some priori knowledge or preliminary studies are needed to use the 20-s rather than the 60-s stepwise input protocol.

IV. Conclusion

The open-loop static characteristics of the carotid sinus baroreflex in normal rats may be obtained by the stepwise input protocol with step duration as short as 20 s. The shortening of the step duration can reduce the total amount of experimental time. Moreover, it would also make it possible to analyze the time effect of drugs on the baroreflex static characteristics with a better time resolution.

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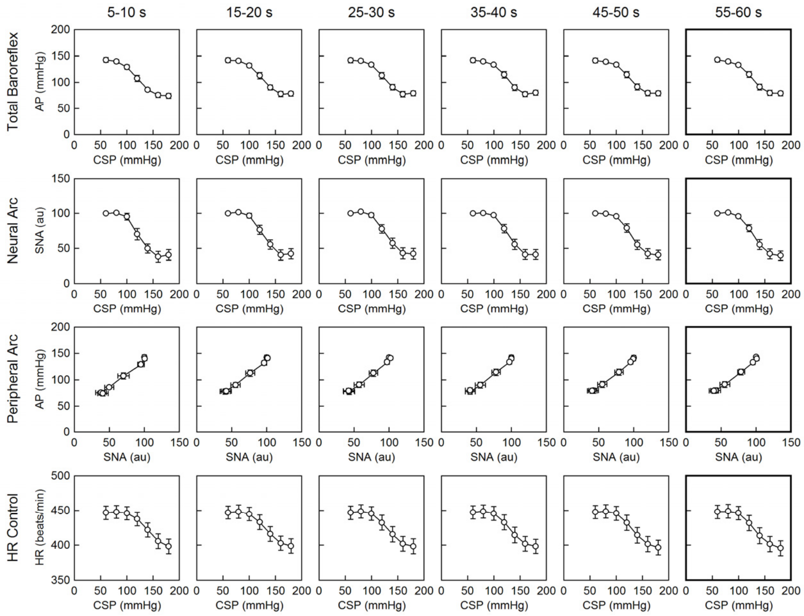

Fig. 2. Open-loop static characteristics of the carotid sinus baroreflex estimated at different time windows of the 60-s stepwise input protocol. CSP: carotid sinus pressure, AP: arterial pressure, SNA: sympathetic nerve activity, HR: heart rate. The rightmost panels serve as controls.

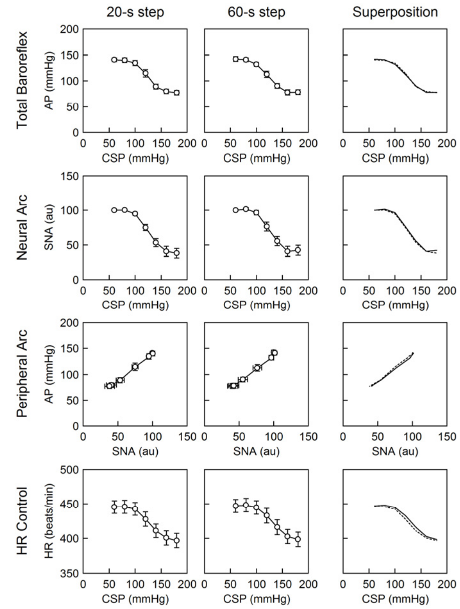

Fig. 3. Open-loop static characteristics of the carotid sinus baroreflex estimated using 20-s and 60-s stepwise input protocols. CSP: carotid sinus pressure, AP: arterial pressure, SNA: sympathetic nerve activity, HR: heart rate.

Table 1. Parameters of open-loop static characteristics of the carotid sinus baroreflex estimated at different time windows in the 60-s stepwise input protocol.

	5-10 s	15-20 s	25-30 s	35-40 s	45-50 s	55-60 s
Total Baroreflex						
P_1, mmHg	72.5±8.6**	68.8±8.0	67.9±7.8	66.4±7.7	65.0±7.8	65.4±7.1
P_2, mmHg^{-1}	0.088±0.011	0.089±0.009	0.095±0.011	0.099±0.009	0.097±0.009	0.091±0.008
P_3, mmHg	118.1±3.6**	122.1±3.5	122.8±3.6	123.6±3.5	124.0±3.6	123.7±3.6
P_4, mmHg	72.9±4.8**	74.9±5.2	75.1±5.2	75.5±5.1	76.2±5.1	76.3±4.8
Neural Arc						
P_1, au	65.5±7.6	63.0±6.7	62.6±8.0	62.8±7.3	61.3±7.1	63.1±6.5
P_2, mmHg^{-1}	0.115±0.014	0.102±0.012	0.102±0.010	0.100±0.009	0.101±0.010	0.088±0.006
P_3, mmHg	120.6±3.6**	125.1±3.7	126.0±3.8	127.8±3.8	127.2±3.6	127.0±3.6
P_4, au	37.6±7.8	39.3±7.4	40.1±8.1	38.4±7.3	39.3±7.1	38.7±6.7
Peripheral Arc						
a, mmHg/au	1.06±0.07	1.06±0.07	1.10±0.07	1.09±0.08	1.07±0.07	1.06±0.07
b, mmHg	31.0±7.4	31.2±6.9	27.0±8.0	29.4±8.1	32.0±6.4	32.1±7.0
HR Control						
P_1, beats/min	52.8±8.2	51.8±8.4	52.3±8.2	51.7±8.0	53.8±8.0	54.8±7.5
P_2, mmHg^{-1}	0.077±0.006	0.082±0.008	0.089±0.010	0.093±0.009	0.087±0.008	0.083±0.007
P_3, mmHg	138.5±2.9**	131.0±3.1	131.0±3.4	130.5±3.3	131.1±3.6	130.8±3.5
P_4, beats/min	395.7±10.8	397.2±10.4	396.8±10.8	397.4±10.3	395.7±10.6	395.2±10.5

Data are means±SE values. **P < 0.01 by Dunnett's test from the value estimated at a time window of 55-60 s.

Table 2. Parameters of open-loop static characteristics of the carotid sinus baroreflex estimated by actual 20-s and 60-s stepwise input protocols.

	20-s step	60-s step	P value	%difference
Total Baroreflex				
P_1, mmHg	65.9±7.5	65.4±7.1	0.779	0.1±3.5
P_2, mmHg^{-1}	0.094±0.009	0.091±0.008	0.691	1.0±8.1
P_3, mmHg	121.9±3.0	123.7±3.6	0.148	1.4±0.9
P_4, mmHg	76.6±4.3	76.3±4.8	0.893	−0.4±2.0
Neural Arc				
P_1, au	65.5±7.6	63.1±6.5	0.330	−1.5±4.5
P_2, mmHg^{-1}	0.082±0.008*	0.088±0.006	0.011	10.8±3.8
P_3, mmHg	121.8±3.3	127.0±3.6	0.075	4.4±2.3
P_4, au	37.4±7.2	38.7±6.7	0.414	9.6±7.5
Peripheral Arc				
a, mmHg/au	1.09±0.08	1.06±0.07	0.351	−1.1±2.5
b, mmHg	31.4±8.3	32.1±7.0	0.780	7.3±11.1
HR Control				
P_1, beats/min	51.2±8.2*	54.8±7.5	0.041	13.7±7.6
P_2, mmHg^{-1}	0.082±0.008	0.083±0.007	0.820	6.6±8.9
P_3, mmHg	126.5±3.3	130.8±3.5	0.126	3.5±2.1
P_4, beats/min	396.4±10.4	395.2±10.5	0.501	−0.3±0.4

Data are means±SE values. *P < 0.05 by a paired-t test.