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Differential Dynamic Control of Cardiac and Splanchnic Sympathetic Nerve Activity by the Arterial Baroreflex

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Abstract— **The arterial baroreflex is the primary negative feedback system involved in stabilizing arterial pressure from external disturbances. Determining the dynamic characteristics of the baroreflex is important for our understanding of the mechanisms involved in rapid restoration of arterial pressure. This study examined the differences in the dynamic baroreflex control of cardiac (CSNA) and splanchnic (SSNA) sympathetic nerve activity. The baroreceptor region of the right aortic depressor nerve was isolated from the systemic circulation to control baroreceptor region pressure (BRP) with a Gaussian white noise signal while simultaneously recording CSNA and SSNA in anesthetized Sprague-Dawley rats. SSNA was recorded from a postganglionic branch of the splanchnic sympathetic nerve and CSNA was recorded from a branch of the left stellate ganglion. Neural arc transfer functions from BRP to SSNA (H_{SSNA}) and BRP to CSNA (H_{CSNA}) displayed derivative characteristics. When dynamic gain below 0.03 Hz was normalized to unity, H_{SSNA} had a higher gain at frequencies 0.1 and 1 Hz and increasing slope from 0.1 to 1 Hz relative to H_{CSNA}. The peak decrease in the step response was higher for SSNA than CSNA. These data indicate differential dynamic baroreflex control of SSNA and CSNA. Rapid changes in baroreceptor pressure input would result in a larger response in SSNA compared with CSNA.**

I. Introduction

The arterial baroreflex is a negative feedback system that regulates arterial pressure by controlling the parasympathetic and sympathetic nervous systems. For example, a reduction in arterial pressure results in a baroreflex mediated increase in sympathetic nerve activity to resistance vessels and heart. The elevated sympathetic nerve activity to resistance vessels increases peripheral resistance while the increase in activity to the heart increases cardiac output; both contribute to the restoration of arterial pressure towards pre-perturbation levels. Rapid arterial pressure restoration by the baroreflex is essential for maintaining appropriate blood supply to vital organs in the face of hypotensive stress such as associated with standing posture. To understand the mechanisms involved in the rapid restoration of arterial pressure it is important to investigate the dynamic characteristics of the baroreflex.

The dynamic characteristics of the baroreflex can be analyzed by dividing the baroreflex system into two principal subsystems: a baroreflex neural arc and a baroreflex peripheral arc [1]. The neural arc represents the pathway from baroreceptor pressure input to efferent sympathetic nerve activity, and can be regarded as a controller subsystem of the baroreflex. The peripheral arc represents the pathway from sympathetic nerve activity to arterial pressure, and acts as a controlled subsystem.

Previous studies have indicated regional (e.g. heart, muscle, and kidney) differences in the baroreflex control of sympathetic nerve activity [2–4]. The baroreflex control of one sympathetic nerve does not necessarily indicate similar control in another. While the neural arc of the baroreflex has been shown to exert differential effects on cardiac (CSNA) and renal (RSNA) sympathetic nerve activity [4] it remains unclear if there is also a difference between the dynamic baroreflex control of CSNA and splanchnic sympathetic nerve activity (SSNA). The splanchnic sympathetic nerves control splanchnic vascular resistance and capacitance, which could play a significant role in rapid restoration of arterial pressure [5]. Furthermore, it has recently been shown that angiotensin II-induced hypertension in rats fed a high-salt diet is associated with a specific elevation in splanchnic sympathetic tone while sympathetic activity to the kidney, hind limb and heart is largely unchanged [6]. Investigation into the possible differences in the baroreflex control of CSNA and SSNA may provide insight into the mechanism involved in elevated sympathetic nerve activity associated with neurogenic hypertension.

The aim of this study was to determine if there is a difference in the dynamic baroreflex control of CSNA and SSNA. To accomplish this we manipulated pressure at the baroreceptor region of the right aortic depressor nerve while simultaneously recording from splanchnic and cardiac sympathetic nerves. To identify the dynamic characteristics of the baroreflex over a wide frequency range (from 0.01 to 1 Hz), we employed an open-loop systems analysis approach using a Gaussian white noise input [7].

II. Materials and Methods

The study was performed on six anesthetized and vagotomized Sprague-Dawley rats. The 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. The experimental protocols were reviewed and approved by the Animal Subjects Committee at National Cerebral and Cardiovascular Center.

A. Animal Preparation

The animals were anesthetized with an intra-peritoneal injection (2 ml/kg) of a mixture of urethane (250 mg/ml) and α-chloralose (40 mg/ml). After the induction of anesthesia, the trachea was intubated and mechanically ventilated with

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oxygen supplied room air. Supplemental anesthetic mixture was continuously administered via a venous catheter inserted in the right femoral vein. An additional venous catheter was inserted in the left femoral vein for later administration of hexamethonium. An arterial catheter was inserted in the right femoral artery to measure arterial pressure. A postganglionic branch of the splanchnic sympathetic nerve was exposed through a left flank incision, and a pair of stainless steel wire electrodes (AS633, Cooner Wire, USA) was attached to record SSNA [3]. The left chest was opened at the third and fourth intercostal spaces and the left stellate ganglion identified. A pair of stainless steel electrodes was attached to a branch of the stellate ganglion to record CSNA. Preamplified nerve signals were band-pass filtered at 150−1,000 Hz, and were then full-wave rectified and low-pass filtered with a cut-off frequency of 30 Hz to quantify the nerve activities. At the end of the experiment, the ganglionic blocker hexamethonium bromide (60 mg/kg) was intravenously administered in bolus, and the noise level of SSNA and CSNA was measured. This procedure confirmed that we were measuring postganglionic sympathetic nerve activities.

Both vagus and carotid sinus nerves, and the left aortic depressor nerve were sectioned to avoid reflexes from carotid sinus baroreceptors, cardiopulmonary regions and aortic arch baroreceptors associated with the left aortic depressor nerve, respectively. The baroreceptor region of the right aortic depressor nerve was isolated from the systemic circulation according to a previously reported procedure [8]. Briefly, under midline thoracotomy, the right brachiocephalic trunk was ligated at its beginning from the aortic arch. The right subclavian artery was ligated before the branching of the right vertebral artery. Finally, the right common carotid artery was ligated to make the baroreceptor region isolated. The isolated baroreceptor region was filled with warmed Ringer's solution through catheters inserted from the right common carotid artery, and the intra-baroreceptor region pressure (BRP) was controlled by a servo-controlled piston pump.

B. Protocol

To identify dynamic characteristics of the aortic baroreceptor reflex, BRP was controlled according to a Gaussian white noise signal with a mean of 160 mmHg and standard deviation of 20 mmHg [7]. The switching interval of the input signal was set to 500 ms so that the input power spectrum was relatively flat up to 1 Hz. SSNA, CSNA and arterial pressure responses were measured for 20 min under Gaussian white noise perturbation.

C. Data Analysis

Data were sampled at 200 Hz using a 16-bit analog-to-digital converter. To estimate the dynamic characteristics of the baroreflex neural arc, BRP was treated as the input and either SSNA or CSNA was treated as the output of the system.

The input-output data pairs were resampled at 10 Hz. The data were then divided into 10 half overlapping bins of 1,024 data points each. In each segment, a linear trend was subtracted and a Hanning window was applied. The input power spectra, $S_{XX}(f)$, output power spectra, $S_{YY}(f)$, and cross spectra, $S_{YX}(f)$, were calculated over the 10 segments, where f denotes frequency. The transfer function from the input to output was calculated as:

$$H(f) = S_{YX}(f)/S_{XX}(f)$$

The magnitude squared coherence function between the input and output was calculated as:

$$Coh(f) = |S_{YX}(f)|^2/S_{XX}(f)/S_{YY}(f)$$

Because the magnitude of SSNA and CSNA differed within and between animals depending on recording conditions, SSNA and CSNA were normalized and expressed in arbitrary units (a.u.). The normalization was such that the average of dynamic gain values below 0.03 Hz became unity in the respective neural arc transfer functions. The transfer functions from BRP to SSNA and BRP to CSNA are referred to as H_{SSNA} and H_{CSNA}, respectively.

D. Modeling of Transfer Functions

In accordance with previous studies [7, 9], the neural arc transfer functions were modeled as:

$$H_N(f) = K_N \frac{1 + \frac{f}{f_{C1}} j}{\left(1 + \frac{f}{f_{C2}} j\right)^2} \exp(-2\pi f L_N j)$$

where j represents the imaginary units. K_N is the steady-state gain of the neural arc (in a.u./mmHg), f_{C1} is the corner

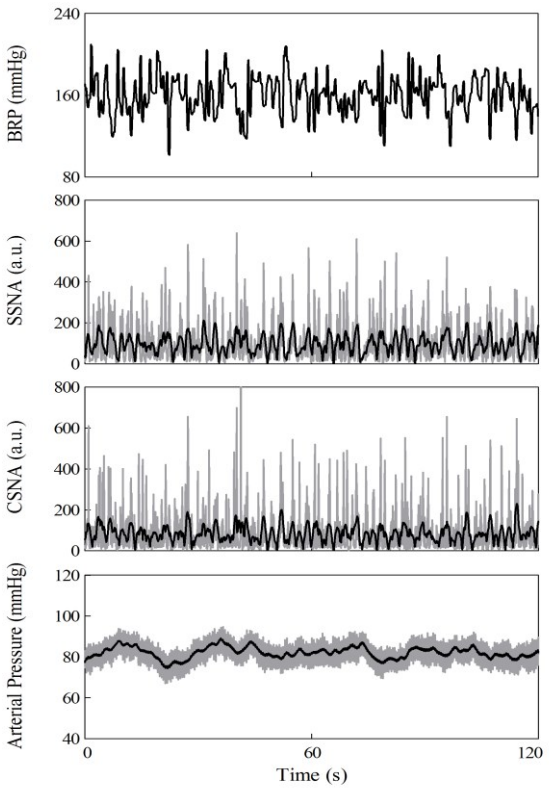

Fig. 1. Typical recordings of baroreceptor region pressure (BRP), splanchnic sympathetic nerve activity (SSNA), cardiac sympathetic nerve activity (CSNA) and arterial pressure (AP). In the SSNA, CSNA, and arterial pressure plots, the gray lines indicate 10-Hz resampled signal, and the black lines indicate 2-s moving average signal. BRP was resampled at 10 Hz.

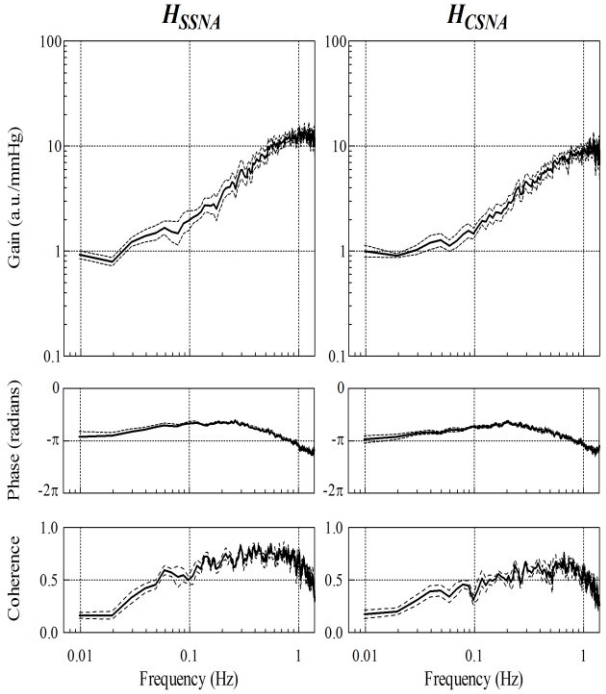

Fig. 2. Neural arc transfer function from baroreceptor region pressure to splanchnic sympathetic nerve activity (H_{SSNA}) and baroreceptor region pressure to cardiac sympathetic nerve activity (H_{CSNA}). Solid and dashed lines are means ± SE, respectively.

frequency determining the derivative characteristics (in Hz), f_{C2} is the corner frequency determining the high-cut characteristics (in Hz), and L_N is the pure dead time (in s).

E. Statistical Analysis

Mean transfer function parameters and step response values were compared with Student's paired-t test. Coherence values were compared with Wilcoxon matched-pairs signed rank test. A statistically significant difference was considered at $P < 0.05$.

III. Results and Discussion

Fig. 1 shows a typical experimental trace taken during a Gaussian white noise perturbation of BRP. SSNA, CSNA, and arterial pressure changed dynamically in response to BRP. Because of the dynamic nature of the Gaussian white noise pressure input it is not easy to identify corresponding baroreflex mediated responses in SSNA and CSNA. However, when BRP was increased, SSNA and CSNA tended to decrease and vice versa.

TABLE I. Neural Arc Parameters

	H_{SSNA}	H_{CSNA}
Gain, a.u./mmHg		
0.01 Hz	0.94 ± 0.10	1.04 ± 0.09
0.1 Hz	2.18 ± 0.43	1.52 ± 0.20*
1 Hz	12.2 ± 0.86	8.95 ± 0.95*
Slope, dB/decade		
0.1 – 1 Hz	16.3 ± 1.64	14.5 ± 1.2*
Coherence		
0.01 Hz	0.17 ± 0.03	0.17 ± 0.04
0.1 Hz	0.44 ± 0.04	0.31 ± 0.05
1 Hz	0.67 ± 0.03	0.54 ± 0.03

Data are means ± SE (n = 6). Gain and slope values were compared using student's paired-t test and coherence values with Wilcoxon matched-pairs signed rank test. *P < 0.05 by paired-t test.

A. Comparison between Neural Arc Transfer Functions

The neural arc transfer functions from BRP to SSNA (H_{SSNA}) and BRP to CSNA (H_{CSNA}) are presented in Fig 2. The dynamic gain of the H_{SSNA} increased as the frequency increased beyond 0.04 Hz, whereas, the dynamic gain of H_{CSNA} increased above 0.07 Hz. While both transfer functions displayed derivative characteristics, the dynamic gain at 0.1 and 1 Hz and the increasing slope between 0.1 to 1 Hz were higher in H_{SSNA} compared with H_{CSNA} (Table I).

The phase was close to $-\pi$ radians at the lowest frequency for both H_{SSNA} and H_{CSNA}. This reflects the negative feedback nature of the neural arc of the baroreflex. The phase deviated towards zero radians until 0.6 Hz and delayed beyond $-\pi$ radians at approximately 1 Hz for both H_{SSNA} and H_{CSNA}. There was no significant difference in the phase plots between H_{SSNA} and H_{CSNA}.

The coherence value was low at 0.01 Hz and increased with increasing frequency for both transfer functions up to 0.8 Hz. There was no significant difference in the coherence values between H_{SSNA} and H_{CSNA} at any measured frequency (Table I).

TABLE II. Model Parameters & Step Response Values

	H_{SSNA}	H_{CSNA}
f_{C1}, Hz	0.045 ± 0.006	0.075 ± 0.005**
f_{C2}, Hz	1.56 ± 0.14	1.63 ± 0.12
L_N, s	0.115 ± 0.004	0.120 ± 0.006
Peak Response, a.u.	-12.9 ± 1.15	-8.3 ± 0.43**
Time to peak , seconds	0.22 ± 0.01	0.23 ± 0.01

Data are means ± SE (n = 6). **P < 0.01 by paired-t test.

B. Modeling of H_{SSNA} and H_{CSNA}

To aid in interpretation of the data the neural arc transfer functions were modelled and time domain step responses calculated as previously described [6]. Mean model parameters are presented in Table II. Fig. 3 illustrates the mathematical models and corresponding step responses for H_{SSNA} and H_{CSNA}. K_N was set to 1 for both H_{SSNA} and H_{CSNA} to reflect our normalization procedure. The corner frequency f_{C1}, was higher in H_{CSNA} compared with H_{SSNA}, while there was no significant difference in f_{C2}.

A small difference in pure dead time was expected between H_{SSNA} and H_{CSNA} because of the longer conduction distance to splanchnic sympathetic nerves from the brainstem compared with cardiac sympathetice nerves. However, we observed no significant difference in L_N.The peak decrease in the step response of H_{SSNA} was significantly more negative than H_{CSNA} (Table II). However, there was no significant difference in the time to peak between the step response of H_{SSNA} and H_{CSNA}. Collectively, these data indicate differential

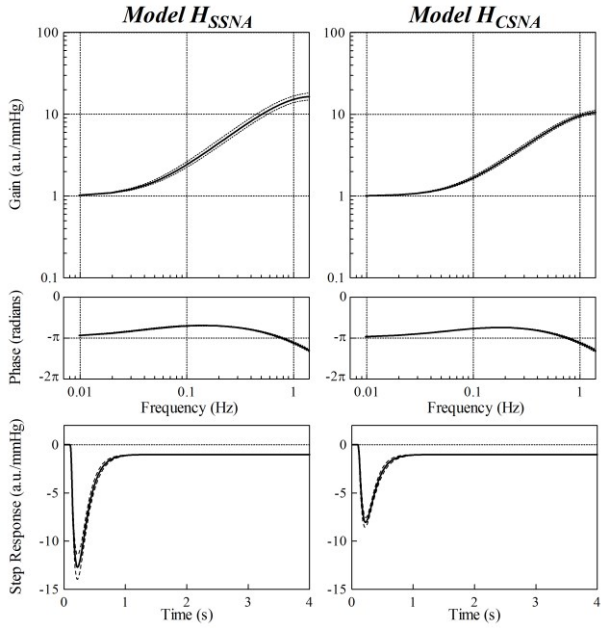

Fig. 3. Mathematical models for neural arc transfer functions. Gain (*top*) and phase (middle) match the estimated transfer functions well. The peak decrease in the step response (*bottom*) was greater for H_{SSNA} than H_{CSNA}. Solid and dashed lines are means ± SE, respectively.

baroreflex control of SSNA and CSNA and that a greater dynamic response is observed in SSNA.This finding was, to a certain extent, surprising because previous studies have indicated that the baroreflex gain for CSNA is higher than the gain for other branches of the sympathetic nervous system in rabbits and cats [3, 4]. In addition, it has been previously suggested that there is minimal baroreflex inhibition of sympathetic nerve activity to the splanchnic vascular bed relative to the baroreflex inhibition of sympathetic nerve activity to the heart and kidney after the infusion of angiotensin II [6, 10], which our findings challenge.

Because we treated BRP as the input and SSNA or CSNA as the output, the transduction property of the baroreceptors was included in the neural arc transfer functions. The transduction property from BRP to aortic depressor nerve activity has a slight derivative characteristics in rabbits [11]. The dynamic gain of the baroreceptor transduction property was increased approximately three fold when the frequency increased from 0.01 to 2 Hz. Therefore, the derivative characteristics of H_{SSNA} and H_{CSNA} may be partly attributable to common baroreceptor transduction property.

C. Study Limitations

While we have observed a difference in how the neural arc (controller subsystem) of the baroreflex controls SSNA and CSNA, the peripheral arc (controlled subsystem) of each should act through different pathways, i.e., vascular resistance and cardiac output, respectively. As such, it is difficult to identify how baroreflex control of SSNA or CSNA will result in regulation of arterial pressure without directly measuring splanchnic vascular resistance and capacitance or cardiac output.

We imposed relatively high mean input pressure (160 mmHg) to baroreceptor regions. Although we tried a mean pressure of 120 mmHg, responses in SSNA and CSNA were not clear compared to those induced by bilateral carotid sinus inputs. An increase in mean input pressure was necessary to improve the signal to noise ratio in the transfer function estimation. Unilateral perturbation and a smaller isolated baroreceptor region for the right aortic depressor nerve may be responsible for the smaller responses in SSNA and CSNA. Nevertheless, because we recorded SSNA and CSNA simultaneously, comparison between SSNA and CSNA may be a fair one.

IV. Conclusion

Rapid perturbations in arterial pressure at baroreceptor regions is likely to result in a greater baroreflex response in SSNA than CSNA. While previous studies have focused on the differences in the baroreflex control to the heart, muscle, and kidney [2–4], this study highlights the importance of splanchnic vascular bed and its ability to shift blood between highly compliant venous and less compliant arterial compartments [5] in the dynamic regulation of arterial pressure. A better understanding of the regional differences in the baroreflex control of the sympathetic nervous system may provide further insight into rapid arterial pressure restoration and regional elevation of sympathetic nerve activity that is associated with some forms of hypertension.

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