

## Supplementary Issue: Heart Failure: An Exploration of Recent Advances in Research and Treatment

### Calibration of Baroreflex Equilibrium Diagram Based on Exogenous Pressor Agents in Chronic Heart Failure Rats

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**ABSTRACT:** A baroreflex equilibrium diagram describes the relation between input pressure and sympathetic nerve activity (SNA) and that between SNA and arterial pressure (AP). To calibrate the SNA axis (abscissa) of the baroreflex equilibrium diagram, the AP response to intravenous bolus injections of phenylephrine (0.2–50 µg/kg) or norepinephrine (NE, 0.02–5 µg/kg) was examined in normal control rats (NC,  $n = 9$ ) and rats with chronic heart failure (CHF,  $n = 6$ ). The maximum slope of the dose–effect curve was significantly smaller in the CHF group than in the NC group ( $57.3 \pm 5.2$  vs  $80.9 \pm 6.3$  mmHg/decade for phenylephrine,  $60.2 \pm 7.8$  vs  $80.4 \pm 5.9$  mmHg/decade for NE;  $P < 0.01$ ). The CHF/NC ratio of the maximum slope was used to calibrate SNA. While the calibrated baroreflex equilibrium diagram showed increased maximum SNA and operating-point SNA in CHF rats compared with NC rats, the magnitude of increase was smaller than that expected from the excess plasma NE concentration in CHF rats. Plasma NE concentration in the CHF group could be disproportionately high relative to SNA.

**KEYWORDS:** carotid sinus baroreflex, open-loop analysis, arterial pressure, phenylephrine, norepinephrine

**SUPPLEMENT:** Heart Failure: An Exploration of Recent Advances in Research and Treatment

**CITATION:** Kawada et al. Calibration of Baroreflex Equilibrium Diagram Based on Exogenous Pressor Agents in Chronic Heart Failure Rats. *Clinical Medicine Insights: Cardiology* 2015:9(S1) 1–9 doi: 10.4137/CMC.S18759.

**RECEIVED:** October 08, 2014. **RESUBMITTED:** December 15, 2014. **ACCEPTED FOR PUBLICATION:** December 26, 2014.

**ACADEMIC EDITOR:** Thomas E. Vanhecke, Editor in Chief

**TYPE:** Original Research

**FUNDING:** This study was partly supported by the Support Program to break the bottlenecks at R&D System for accelerating the practical use of Health Research Outcome from the Japan Science and Technology Agency, and by the Grant-in-Aid for Scientific Research (JSPS KAKENHI grants 26750153, 26461099, 26430103, 13J05473). The authors confirm that the funder had no influence over the study design, content of the article, or selection of this journal.

**COMPETING INTERESTS:** Authors disclose no potential conflicts of interest.

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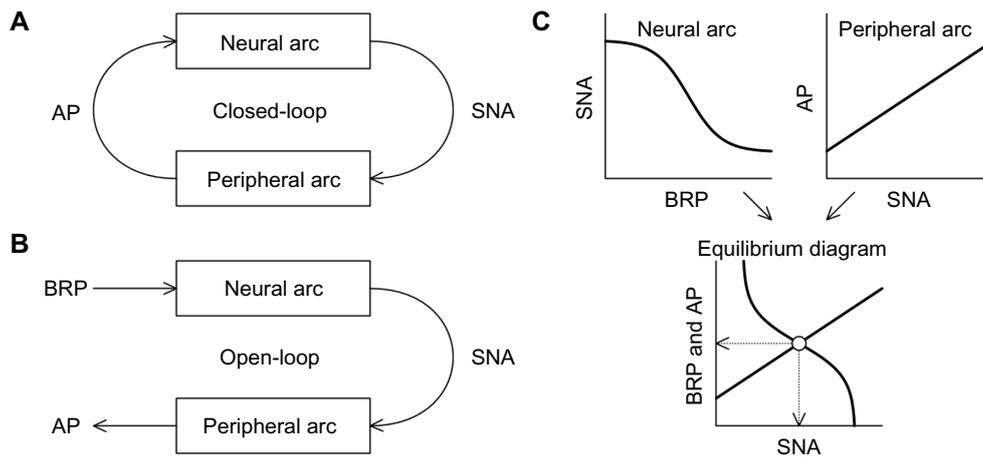
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## Introduction

The arterial baroreflex is a major negative feedback system that stabilizes arterial pressure (AP) during daily activities. From an analytical point of view, this system may be divided into two principal subsystems: a neural arc subsystem that defines the input–output relation between baroreceptor pressure and efferent sympathetic nerve activity (SNA), and a peripheral arc subsystem that defines the input–output relation between efferent SNA and AP.<sup>1,2</sup> Under normal physiological conditions, changes in AP alter SNA via the neural arc, while changes in SNA affect AP via the peripheral arc (Fig. 1A). This closed-loop negative feedback operation

makes it difficult to separately identify the characteristics of the neural and peripheral arcs. For instance, if pharmacological intervention is used to assess the SNA response to changes in AP, it usually becomes impossible to observe the AP response to SNA independently of the drug's effect on AP. To circumvent this problem, we have employed a method where the carotid sinus baroreceptor regions are isolated from the systemic circulation<sup>3,4</sup> so that we can identify the characteristics of the neural and peripheral arcs under open-loop conditions (Fig. 1B).<sup>2,5–7</sup> Once the characteristics of the neural and peripheral arcs are identified, a baroreflex equilibrium diagram can be constructed by drawing the two arcs on a



**Figure 1** (A) Schematic diagram of the closed-loop negative feedback operation between neural and peripheral arcs of the arterial baroreflex.

(B) Schematic diagram of the open-loop analysis of the baroreflex system. (C) Input–output relationships of the neural and peripheral arcs.

**Notes:** The baroreflex equilibrium diagram is obtained by plotting the neural and peripheral arcs on a pressure–SNA plane. The intersection (open circle) indicates the operating point of the baroreflex control. The dotted arrows indicate the operating-point SNA and AP.

**Abbreviations:** AP, arterial pressure; SNA, sympathetic nerve activity; BRP, baroreceptor pressure.

pressure–SNA plane (Fig. 1C). The baroreflex equilibrium diagram provides information on how the operating-point SNA and AP are determined through the interaction of the neural and peripheral arcs.<sup>8–11</sup>

A number of studies have indicated that arterial baroreflex function is depressed in chronic heart failure (CHF).<sup>12–15</sup> We have identified the open-loop static characteristics of the neural and peripheral arcs in rats with CHF following myocardial infarction and compared them with those in normal control (NC) rats.<sup>6,16</sup> These studies indicate that the neural arc characteristics approximate an inverse sigmoid curve, and the response range of the neural arc is reduced in CHF rats compared with NC rats. The inability of the impaired neural arc to suppress SNA could contribute to an increase in SNA and the resultant aggravation of cardiac function typically observed in CHF individuals.<sup>17,18</sup> Peripheral arc characteristics normally approximate a straight line. The slope of the peripheral arc is smaller in CHF rats than in NC rats, in accordance with the notion of a blunted target-organ response in CHF.<sup>14,15</sup> One of the limitations in our open-loop baroreflex studies is that SNA was expressed in percentage units in each animal because the absolute amplitude of multifiber SNA varied largely among animals depending on the recording conditions. As a result, a comparison of the absolute levels of SNA between CHF and NC groups was impossible.<sup>6,16</sup> The response range of the neural arc was smaller in the CHF group than in the NC group, but it could have been underestimated and might have been larger if the SNA was expressed in absolute units. Conversely, the slope of the peripheral arc could have been overestimated in CHF rats, and the AP response to SNA might have been much smaller in CHF rats than in NC rats if the SNA was expressed in absolute units.

If we know the difference in the AP response to adrenergic activation between NC and CHF rats, we may be able to

calibrate the SNA axis (abscissa) of the baroreflex equilibrium diagram because the baroreflex peripheral arc characterizes sympathetic AP control. Feng et al.<sup>19</sup> examined the AP response to electrical preganglionic sympathetic nerve stimulation in pithed CHF rats after myocardial infarction. Their data indicated that the AP response to the sympathetic stimulation was significantly attenuated in CHF rats compared with a sham-operated control group. They also demonstrated that the AP response to pharmacological adrenergic activation was decreased in CHF rats.<sup>19</sup> Although these data may be utilized to calibrate the SNA axis of the baroreflex equilibrium diagram, the severity of CHF could be different between their study<sup>19</sup> and our previous studies.<sup>6,16</sup> In addition, differences in the experimental setting (eg, pithed rats vs rats anesthetized with  $\alpha$ -chloralose and urethane) may affect the AP response to adrenergic activation. Accordingly, the aim of the present study was to compare the AP response to adrenergic activation between NC and CHF rats in our own experimental setting and to calibrate the SNA axis of the baroreflex equilibrium diagram based on the AP response to adrenergic activation.

## Materials and Methods

Animal care was provided in strict accordance with the *Guiding Principles for the Care and Use of Animals in the Field of Physiological Sciences*, approved by the Physiological Society of Japan. All protocols were reviewed and approved by the Animal Subject Committee of the National Cerebral and Cardiovascular Center.

**Surgical preparation.** Myocardial infarction was produced in eight-week-old, male Sprague–Dawley rats according to a previously established procedure.<sup>6,16,20,21</sup> Eight weeks after myocardial infarction, the surviving rats underwent an acute experiment as follows. Rats in the NC ( $n = 9$ ) and the CHF ( $n = 6$ ) groups were anesthetized with an intraperitoneal injection (2 mL/kg) of a



mixture of urethane (250 mg/mL) and  $\alpha$ -chloralose (40 mg/mL) and were mechanically ventilated with oxygen-supplied room air. Anesthesia was maintained by a continuous intravenous infusion of a diluted solution of the above anesthetic mixture from a catheter inserted into the right femoral vein. Another venous catheter was inserted into the left femoral vein for measuring baseline central venous pressure and for administering pharmacological agents. An arterial catheter was inserted into the right femoral artery to measure AP. Body temperature of the animal was maintained at approximately 38 °C using a heating pad and a lamp.

**Protocols.** Hexamethonium bromide (60 mg/kg) was given as an intravenous bolus injection to block sympathetic and parasympathetic ganglionic transmissions. Ten minutes later, 0.5 mL of saline was given as a control injection; thereafter, an  $\alpha_1$ -adrenergic agonist, phenylephrine (PE), was given as intravenous bolus injections (0.2 mL of drug solution followed by 0.3 mL of saline to flush the solution) in an increasing order at 0.2, 0.5, 1, 2, 5, 10, 20, and 50  $\mu$ g/kg. After finishing the PE protocol, a 30-minute recovery period was interposed, and another injection of hexamethonium bromide (60 mg/kg) was added. Ten minutes later, 0.5 mL of saline was given as a control injection; thereafter, norepinephrine (NE) was given as intravenous bolus injections (0.2 mL of drug solution, followed by 0.3 mL of saline to flush the solution) in an increasing order at 0.02, 0.05, 0.1, 0.2, 0.5, 1, 2, and 5  $\mu$ g/kg. An interval of a minimum of three minutes and a maximum of 15 minutes was allowed between successive injections. In three of the NC rats, splanchnic SNA was recorded as previously reported.<sup>5-7</sup> At the end of the PE protocol, a 10-second averaged value of SNA was less than 5% of a 10-second averaged baseline activity measured before the first administration of hexamethonium bromide, indicating a sufficient ganglionic blockade throughout the protocol.

**Data analysis.** Data were continuously recorded at a sampling frequency of 1,000 Hz using a 16-bit analog-to-digital converter. The peak AP response corresponding to each injection was determined from a 10-second moving averaged signal. The relation between the logarithmic dose of PE or NE and the peak AP response was analyzed by linear regression as follows.

$$y = P_a \log_{10}(x) + P_b \quad (1)$$

where  $x$  and  $y$  denote the dose of PE or NE and the peak AP response, respectively.  $P_a$  represents the slope (in mmHg/decade), and  $P_b$  represents the intercept (in mmHg) of the regression line.

The dose–effect curve was also analyzed using a four-parameter logistic function as follows.

$$y = \frac{P_1}{1 + \exp[-P_2(\log_{10}(x) - P_3)]} + P_4 \quad (2)$$

where  $x$  and  $y$  represent the dose of PE or NE and the peak AP response, respectively.  $P_1$  is the response range of AP (in mmHg);  $P_2$  is the slope coefficient;  $P_3$  is the logarithmic dose corresponding to the midpoint of the logistic function; and  $P_4$  is the lower plateau of AP (in mmHg). The parameter values were determined by nonlinear iterative least-square fitting. The maximum slope (in mmHg/decade) was calculated from  $P_1 \times P_2/4$ . The dose corresponding to the midpoint (in  $\mu$ g/kg) was calculated from 10 to the power of  $P_3$ .

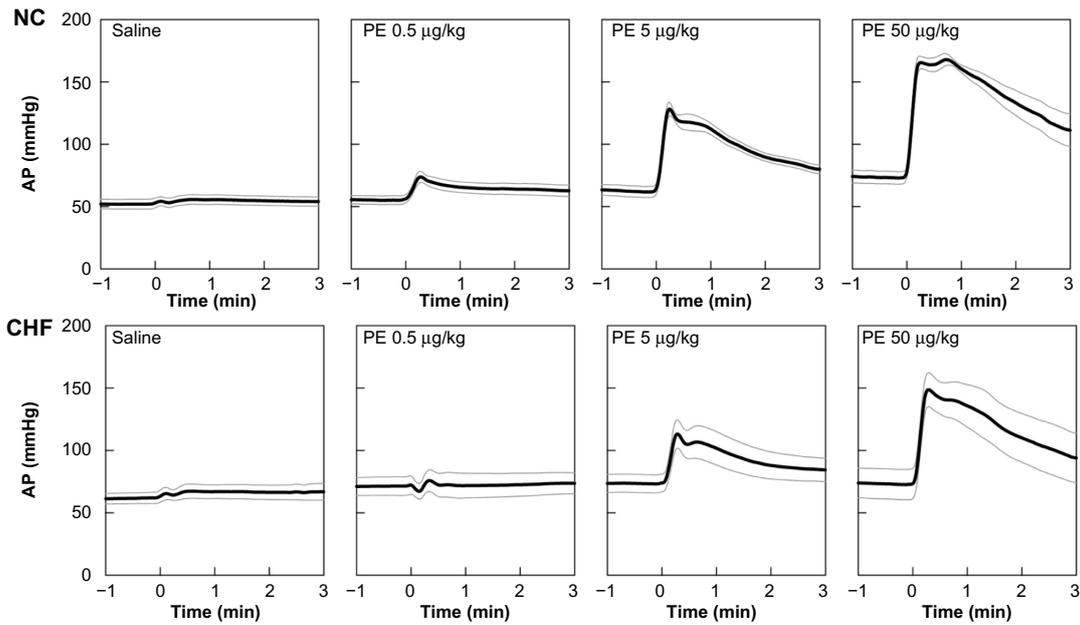
**Statistical analysis.** All data are presented in mean  $\pm$  standard error values. Body weight and baseline hemodynamics were compared between the NC and CHF groups by unpaired  $t$ -test. The slope of the linear regression and the parameters of the fitted logistic function were compared by two-way analysis of variance (ANOVA) in which the first factor was a disease effect (NC vs CHF groups) and the second factor was a drug effect (PE vs NE).<sup>22</sup> The difference was considered significant at  $P < 0.05$ . Because of the logarithmic transformation of the dose of PE or NE, the intercept of the linear regression merely indicates the estimation of an AP value corresponding to a specific dose of 1  $\mu$ g/kg without general meaning. Therefore, the intercept is not reported.

One NC rat died after the PE protocol, possibly due to an acute disappearance of the adrenergic pressor effect, and one CHF rat died in the midst of the NE protocol from a gradual deterioration in hemodynamics. Accordingly, the number of animals in the NE protocol became eight and five for the NC and CHF groups, respectively, and ANOVA was performed as nonrepeated comparisons.

**Table 1.** Body weight, biventricular weight, and baseline hemodynamics.

	NC (N = 9)	CHF (N = 6)	P VALUE
Body weight, g	479 $\pm$ 24	445 $\pm$ 13	0.320
Biventricular weight, g	1.079 $\pm$ 0.056	1.334 $\pm$ 0.053	0.009
Normalized biventricular weight, g/kg	2.099 $\pm$ 0.058	3.013 $\pm$ 0.145	<0.001
Central venous pressure, mmHg	1.72 $\pm$ 0.30	4.20 $\pm$ 1.09	0.029
Mean arterial pressure, mmHg	116.4 $\pm$ 1.8	111.7 $\pm$ 9.0	0.545
Heart rate, beats/min	422.3 $\pm$ 17.0	379.3 $\pm$ 17.9	0.116

**Notes:** Values are means  $\pm$  standard error.  $P$  value was obtained by unpaired  $t$ -test.  
**Abbreviations:** NC, normal control; CHF, chronic heart failure.



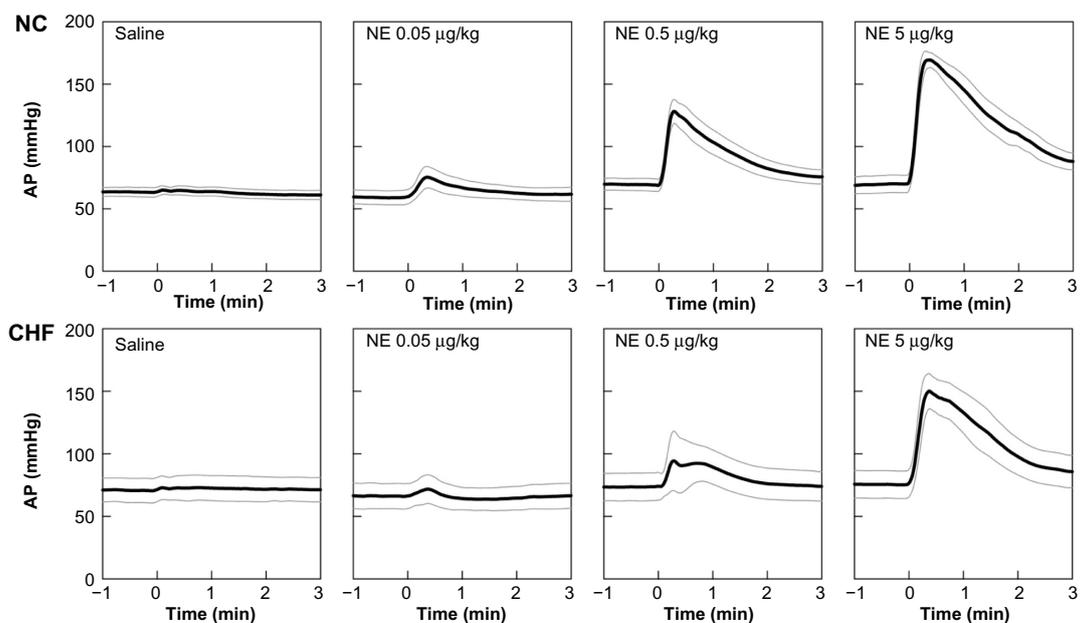
**Figure 2.** Time series of 10-second moving averaged signals of arterial pressure (AP) in response to intravenous injections of saline and 0.5-, 5-, and 50-µg/kg phenylephrine (PE) injections averaged for the normal control group (NC, *n* = 9, top panel) and for the chronic heart failure group (CHF, *n* = 6, bottom panel).

**Note:** Black and gray lines indicate mean values and mean ± standard error values.

## Results

As shown in Table 1, body weight did not differ statistically between the NC and CHF groups, though the mean value was slightly heavier in the NC group. Biventricular weight was significantly heavier in CHF rats than in NC rats, both for the absolute value and for the normalized value relative to

the body weight. Central venous pressure, measured before administering hexamethonium bromide under baseline conditions, was significantly higher in CHF rats than in NC rats. Mean AP under baseline conditions did not differ between the two groups. Although the mean value of heart rate was higher in the NC group than in the CHF group, the heart



**Figure 3.** Time series of 10-second moving averaged signals of arterial pressure (AP) in response to intravenous injections of saline and 0.05-, 0.5-, and 5-µg/kg norepinephrine (NE) injections averaged for the normal control group (NC, *n* = 8, top panel) and for the chronic heart failure group (CHF, *n* = 5, bottom panel).

**Note:** Black and gray lines indicate mean values and mean ± standard error values.

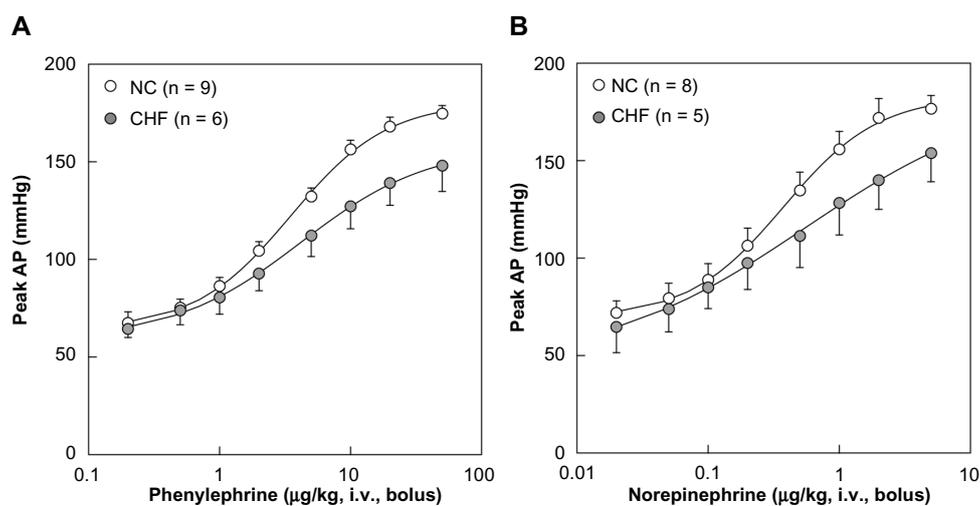
rate showed a large variance among animals and the difference between the two groups was not statistically significant.

Figure 2 depicts group-averaged time series obtained from the PE protocol. Time series only for saline and 0.5-, 5-, and 50- $\mu\text{g}/\text{kg}$  injections of the drug are shown for the sake of simplicity. Black and gray lines indicate mean values and mean  $\pm$  standard error values, respectively. Intravenous saline showed only a slight fluctuation in AP. Intravenous PE elevated AP in a dose-dependent manner in the NC group. Peak AP responses were observed at  $17 \pm 1$ ,  $18 \pm 3$ , and  $37 \pm 8$  seconds in response to 0.5-, 5-, and 50- $\mu\text{g}/\text{kg}$  injections, respectively. Although PE also increased AP dose-dependently in the CHF group, the magnitude of the AP response was smaller compared with that in the NC group. Peak AP responses were observed at  $23 \pm 9$ ,  $21 \pm 5$ , and  $23 \pm 6$  seconds in response to 0.5-, 5-, and 50- $\mu\text{g}/\text{kg}$  injections, respectively.

Figure 3 depicts group-averaged time series obtained from the NE protocol. Time series only for saline and 0.05-, 0.5-, and 5- $\mu\text{g}/\text{kg}$  injections are shown. Intravenous saline did not change AP appreciably; intravenous NE elevated AP in a dose-dependent manner in the NC group. The peak AP responses

were observed at  $28 \pm 7$ ,  $18 \pm 1$ , and  $16 \pm 2$  seconds in response to 0.05-, 0.5-, and 5- $\mu\text{g}/\text{kg}$  injections, respectively. Although NE also increased AP dose-dependently in the CHF group, the magnitude of the AP response was smaller compared with that in the NC group. The peak AP responses were observed at  $16 \pm 4$ ,  $26 \pm 8$ ,  $20 \pm 2$  seconds in response to 0.05-, 0.5-, and 5- $\mu\text{g}/\text{kg}$  injections, respectively.

Figure 4 summarizes the peak AP responses versus the dose of PE (Fig. 4A) and NE (Fig. 4B). Open circles and filled circles represent the NC and CHF data, respectively. As shown in Table 2, the slope of the linear regression was significantly smaller in the CHF group than in the NC group ( $P = 0.002$ ). When a four-parameter logistic function was fitted to the data for each animal, the maximum slope was significantly smaller in CHF rats than in NC rats ( $P = 0.003$ ). The lower plateau of AP was not significantly different ( $P = 0.902$ ), but the response range of AP was significantly narrower in the CHF rats than in the NC rats ( $P = 0.004$ ). The dose corresponding to the midpoint of the logistic function tended to be higher in CHF rats than in NC rats ( $P = 0.077$ ) and was significantly lower for NE than for PE ( $P < 0.001$ ). No interaction effects were observed



**Figure 4.** (A) Relation between the dose of phenylephrine and the peak arterial pressure (AP) response. (B) Relation between the dose of norepinephrine and the peak AP response. Data points represent means and standard errors. Smooth curves represent four-parameter logistic functions derived from the mean data points in each group.

**Abbreviations:** NC, normal control; CHF, chronic heart failure; iv, intravenous administration.

**Table 2.** Relation of peak arterial pressure response to a dose of phenylephrine (PE) or norepinephrine (NE).

	PE		NE		NC VS CHF	PE VS NE	INTERACTION
	NC (N = 9)	CHF (N = 6)	NC (N = 8)	CHF (N = 5)			
Slope of linear regression, mmHg/decade	$54.6 \pm 4.7$	$37.4 \pm 3.9$	$52.5 \pm 3.7$	$36.9 \pm 5.0$	0.002	0.780	0.871
Maximum slope, mmHg/decade	$80.9 \pm 6.3$	$57.3 \pm 5.2$	$80.4 \pm 5.9$	$60.2 \pm 7.8$	0.003	0.851	0.796
Response range of AP, mmHg	$125.1 \pm 8.8$	$89.7 \pm 10.1$	$116.0 \pm 5.7$	$90.9 \pm 14.0$	0.004	0.683	0.590
Lower plateau of AP, mmHg	$61.2 \pm 4.5$	$67.5 \pm 7.8$	$74.3 \pm 9.1$	$69.9 \pm 9.8$	0.902	0.333	0.501
Dose at midpoint, $\mu\text{g}/\text{kg}$	$3.87 \pm 0.59$	$6.15 \pm 1.26$	$0.51 \pm 0.10$	$0.75 \pm 0.24$	0.077	<0.001	0.150

**Notes:** Values are means  $\pm$  standard error.  $P$  value was obtained by two-way analysis of variance.

**Abbreviations:** NC, normal control; CHF, chronic heart failure; AP, arterial pressure.



for any parameters. As an alternative analysis, four-parameter logistic functions were fitted to mean data points in each group (Fig. 4, smooth curves). In the PE protocol, the maximum slopes were estimated to be 78.9 and 50.6 mmHg/decade in the NC and CHF groups, respectively. In the NE protocol, the maximum slopes were estimated to be 78.2 and 44.9 mmHg/decade in the NC and CHF groups, respectively.

## Discussion

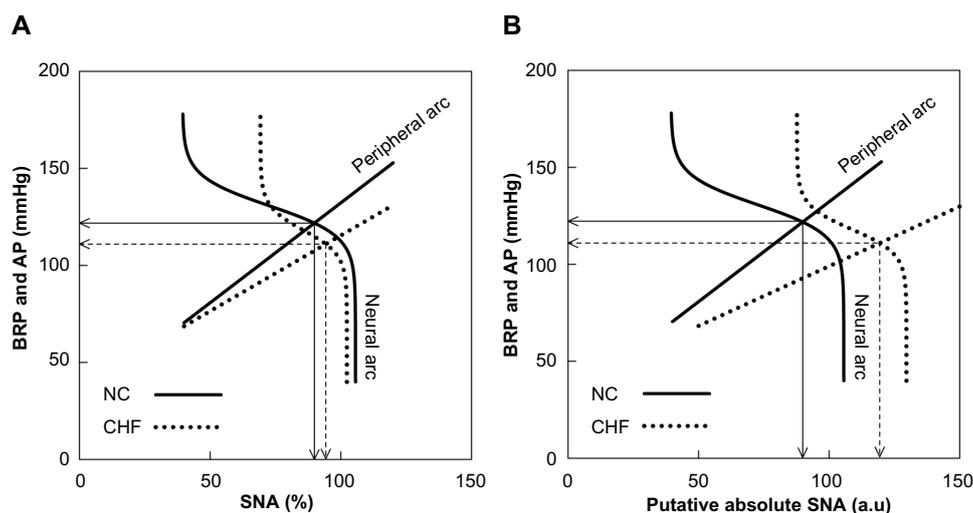
**AP response to pharmacological adrenergic stimulation.** The slope of the linear regression and the maximum slope of the AP response to the intravenous PE were lower in the CHF group compared with the same in the NC group, which is consistent with the results reported by Feng et al.<sup>19</sup> using pithed rats. The decreased AP response to PE in CHF rats is in opposition to the enhanced  $\alpha_1$ -adrenergic receptor-mediated vascular response in CHF rats observed under *in vitro* settings.<sup>23,24</sup> An increased vasoconstrictor response has also been reported in response to renal nerve stimulation in CHF rats.<sup>25</sup> This change accompanies reduced autoregulation of renal blood flow in CHF rats. In contrast, Stassen et al.<sup>26</sup> reported that maximal vasoconstrictor responses to PE in the presence of calcium and the sensitivity to calcium in the presence of PE were reduced in mesenteric arteries isolated from rats five weeks after myocardial infarction. In their study, the contractile responses in the absence of extracellular calcium were not modified, indicating that the agonist-induced calcium influx may be impaired in resistance arteries after myocardial infarction. Adverse milieu such as hypoperfusion, hypoxia,<sup>27</sup> and acidosis<sup>28</sup> might have also restricted the *in vivo* vascular response to PE in CHF rats compared with NC rats. In the study by Feng et al.<sup>19</sup>, the pressor response to electrical preganglionic sympathetic nerve stimulation was decreased in CHF rats compared to that in sham-operated rats. Because percentage changes in cardiac index in response to the preganglionic sympathetic stimulation were not significantly different between the CHF and sham-operated groups, they assumed that the difference in the AP response in the pithed rats mainly represented the difference in the vascular response. However, when cardiac output is reduced in CHF rats, the same amount of changes in the peripheral vascular resistance would yield a smaller AP response compared with the response in NC rats.

PE is a selective  $\alpha_1$ -adrenergic agonist, and therefore any effects on the responsiveness to  $\beta$ -adrenergic activation cannot be assessed in the PE protocol. The sympathetic AP response is mediated by the neurotransmitter NE, which acts on both  $\alpha$ - and  $\beta$ -adrenergic receptors. While high plasma NE levels may not necessarily be the mechanism responsible for the following observation,<sup>29</sup> selective  $\beta_1$ -adrenergic receptor down-regulation occurs in the ventricular myocardium in CHF.<sup>30</sup> To explore whether the AP response is different depending on the type of adrenergic activation, the AP response to exogenous NE was also examined. While the dose necessary to

increase AP was an order of magnitude less for NE than for PE, none of the parameters, such as slope of the regression, maximum slope, lower plateau of AP, and the response range of AP, was significantly different between the PE and NE protocols (Table 2). Therefore, the attenuation of the AP response to adrenergic activation in CHF rats compared with NC rats was observed to a comparable degree regardless of whether the AP response was examined by using PE or NE in the present experimental setting.

**Calibration of baroreflex equilibrium diagram.** The ultimate goal of the arterial baroreflex is to maintain perfusion for vital organs via regulation of AP. While the SNA response to pharmacological pressure perturbation is frequently used to evaluate arterial baroreflex function,<sup>31,32</sup> it does not allow the direct assessment of arterial baroreflex regulation of AP. The AP response to SNA needs to be combined with the SNA response to pressure perturbation to understand the total picture of arterial baroreflex function. The baroreflex equilibrium diagram is useful for describing changes in the baroreflex control of SNA and AP such as those induced by muscle mechanoreflex,<sup>9</sup> electroacupuncture,<sup>11</sup> head-up tilt,<sup>10,33</sup> vagal nerve stimulation,<sup>34,35</sup> and pharmacologic interventions.<sup>5,36</sup> However, when we want to compare the baroreflex equilibrium diagram among different groups of subjects, quantification of SNA becomes problematic. In a previous study, open-loop characteristics of the carotid sinus baroreflex were examined in NC rats and rats with CHF eight weeks postmyocardial infarction; the duration after myocardial infarction is the same as that in the present study.<sup>16</sup> Figure 5A presents the baroreflex equilibrium diagram constructed using mean parameter values of the neural and peripheral arcs obtained from that study.<sup>16</sup> The slope of the peripheral arc in the CHF group was reduced to approximately 0.76 times that in the NC group. The operating-point SNA and AP in the NC group were 89.8% and 121.8 mmHg, respectively (solid arrows). The operating-point SNA and AP in CHF rats were 94.2% and 111.0 mmHg, respectively (dashed arrows). Although the SNA level at the operating point was higher in CHF rats than in NC rats, the difference was very small, casting doubt on the utility of the baroreflex equilibrium diagram for describing sympathetic hyperactivity in CHF rats compared with NC rats when SNA is expressed in percentage units.

In our previous studies regarding baroreflex function in CHF,<sup>6,16</sup> the noise level of SNA measured after ganglionic blockade was assigned to be 0%, which may also be treated as the absolute zero level of SNA. Therefore, using zero as a transform origin, the SNA axis for CHF can be scaled to reflect the difference in the absolute SNA. In our previous study,<sup>6</sup> a putative baroreflex equilibrium diagram was drawn by scaling the SNA axis so that the maximum absolute SNA in the CHF group became two times higher than that in the NC group. The putative baroreflex equilibrium diagram predicted the slope of the peripheral arc in CHF rats to be less than half that in NC rats.<sup>6</sup>



**Figure 5.** (A) Baroreflex equilibrium diagrams of normal control (NC) and chronic heart failure (CHF) constructed using mean parameter values of the neural and peripheral arcs in our previous study.<sup>16</sup> In the neural arc of NC, the response range of SNA is 66.2%, the slope coefficient is 0.13 mmHg<sup>-1</sup>, the midpoint input pressure is 130.8 mmHg, and the lower plateau of SNA is 39.4%. In the peripheral arc of NC, the slope is 1.03 mmHg/% and the intercept is 29.3 mmHg. In the neural arc of CHF, the response range is 33.1%, the slope coefficient is 0.16 mmHg<sup>-1</sup>, the midpoint input pressure is 118.1 mmHg, and the lower plateau of SNA is 69.2%. In the peripheral arc of CHF, the slope is 0.78 mmHg/% and the intercept is 37.5 mmHg. (B) Baroreflex equilibrium diagrams of NC and CHF when the SNA axis for the CHF group was scaled so that the slope of the peripheral arc in CHF relative to that in NC became 0.6. **Notes:** Solid arrows indicate the operating point in NC. Dashed arrows indicate the operating point in CHF.

**Abbreviations:** AP, arterial pressure; au, arbitrary units; BRP, baroreceptor pressure; SNA, sympathetic nerve activity.

In the present study, we aimed to estimate the relative slope of the peripheral arc in CHF rats compared with NC rats, based on the AP response to pharmacological adrenergic activation. The CHF/NC ratio of the maximum slope of the AP response to PE was 0.71 (57.3/80.9) (Table 2). If the four-parameter logistic function was determined using the mean data points of the PE protocol, the ratio was 0.64 (50.6/78.9). The CHF/NC ratio of the maximum slope of the AP response to NE was 0.75 (60.2/80.4) (Table 2). If the four-parameter logistic function was determined using the mean data points of the NE protocol, the ratio was 0.57 (44.9/78.2). These calculations suggest that the CHF/NC ratio of the slope of the peripheral arc may be in the range from 0.6 to 0.7 in our experimental settings. On the other hand, the result of the AP response to PE under pithed conditions indicates that the slope of the AP response in the CHF group relative to the sham-operated group may be as low as 0.5.<sup>19</sup>

When we scale the SNA axis so that the slope of the peripheral arc in the CHF group became 0.6 times that in the NC group, the resulting equilibrium diagram yielded a maximum SNA in CHF rats higher than that in NC rats (Fig. 5B). The operating-point SNA in CHF group was 119.4 au (arbitrary units), while that in the NC group was unchanged at 89.8 au. When we assume the CHF/NC ratio of the slope to be 0.5, the operating-point SNA in the CHF group becomes 143.3 au. Because plasma NE concentration becomes two to three times higher or more in CHF rats compared with NC rats,<sup>37,38</sup> the putative SNA value at the operating point in the CHF group still seems to be lower than that expected from the excess plasma NE concentration in CHF rats. One possible explanation may

be the dissociation of plasma NE concentration from SNA due to impaired NE disposition. In the study by Feng et al.<sup>19</sup>, plasma NE concentration remained 2.4 times higher in CHF rats than in sham-operated rats 40 minutes after pithing, but AP values were not different between the two groups. Impairment of neuronal NE uptake may contribute to the pathophysiology of sympathetic abnormality in CHF.<sup>39</sup> Because plasma NE concentration is significantly increased after the blockade of neuronal NE uptake,<sup>40</sup> the impaired neuronal NE uptake function in CHF would elevate plasma NE concentration disproportionately to SNA. Other mechanisms such as an increased amount of NE release at the same level of SNA may also account for the dissociation between SNA and plasma NE concentration. For instance, presynaptic  $\beta_2$ -adrenergic stimulation by epinephrine facilitates NE release from the sympathetic nerve terminals.<sup>41,42</sup> Peripheral presynaptic inhibition via  $\alpha_2$ -adrenergic receptors is functionally important in inhibiting NE release in healthy humans, but this function is lost in heart failure.<sup>43</sup> It may be worth noting that circulating NE levels do not necessarily correlate with the hemodynamic abnormality in CHF<sup>44</sup> despite its prognostic value of mortality in CHF patients.<sup>45</sup>

**Limitations.** Several limitations in our study need to be addressed. First, the NE protocol was always performed after the PE protocol. Although the response range of AP was not significantly different between the PE and NE protocols, there might have been some aftereffect of  $\alpha_1$ -adrenergic activation induced by high dose of PE on the AP response to NE. Second, AP did not completely return to the baseline levels at higher doses of PE or NE. Accordingly, the



drug effect needs to be interpreted as cumulative to a certain extent. However, because the maximum slope of the dose–effect curve occurred in the midrange concentration (1–10  $\mu\text{g}/\text{kg}$  for PE and 0.1–1  $\mu\text{g}/\text{kg}$  for NE), the cumulative effect on the estimation of the logistic function-derived maximum slope is probably limited. Third, the experiment was performed under anesthetic conditions. Although this was necessary to observe the AP response to adrenergic activation under the same anesthetic conditions as in our previous studies,<sup>6,16</sup> the results may not directly be extrapolated to interpret the baroreflex control under conscious conditions. Finally, systemic administration of pressor agents might have induced vasoconstriction in a relatively homogenous manner throughout the body. In contrast to the pharmacological pressor response, there exist regional differences in sympathetic tone to regulate AP.<sup>46</sup> Further studies are required to elucidate how the regional difference in SNA affects AP regulation in CHF.

## Conclusion

The AP responses to both PE and NE were reduced in CHF rats when compared with the same in NC rats. On the basis of the relative slope of CHF to NC, the SNA axis of the baroreflex equilibrium diagram was calibrated so that the slope of the peripheral arc in CHF rats became 0.6 times that in NC rats. While the calibrated equilibrium diagram explained increased maximum SNA and operating-point SNA in CHF rats compared with NC rats, the magnitude of the difference in SNA between CHF and NC groups seemed smaller than that expected from the difference in plasma NE concentration between CHF and NC rats. Further studies are warranted to solve the discrepancy and to obtain a comprehensive picture of the arterial baroreflex regulation in CHF rats compared with NC rats.

## Author Contributions

Conceived and designed the experiments: TK, YS, MS. Performed the experiments: TK, ML, CZ. Analyzed the data: TK, SS. Wrote the first draft of the manuscript: TK. Contributed to the writing of the manuscript: TK, MJT, SS, MS. Agree with manuscript results and conclusions: TK, ML, YS, CZ, MJT, SS, MS. All authors reviewed and approved of the final manuscript.

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