# Changes in vascular properties, not ventricular properties, predominantly contribute to baroreflex regulation of arterial pressure

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<sup>1</sup>Department of Cardiovascular Medicine, Kyushu University Graduate School of Medical Sciences, Fukuoka, Japan; <sup>2</sup>Iizuka Hospital, Fukuoka, Japan; and <sup>3</sup>Munakata Suikoukai General Hospital, Fukuoka, Japan

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Sakamoto T, Kakino T, Sakamoto K, Tobushi T, Tanaka A, Saku K, Hosokawa K, Onitsuka K, Murayama Y, Tsutsumi T, Ide T, Sunagawa K. Changes in vascular properties, not ventricular properties, predominantly contribute to baroreflex regulation of arterial pressure. Am J Physiol Heart Circ Physiol 308: H49-H58, 2015. First published October 31, 2014; doi:10.1152/ajpheart.00552.2014.-Baroreflex modulates both the ventricular and vascular properties and stabilizes arterial pressure (AP). However, how changes in those mechanical properties quantitatively impact the dynamic AP regulation remains unknown. We developed a framework of circulatory equilibrium, in which both venous return and cardiac output are expressed as functions of left ventricular (LV) end-systolic elastance (Ees), heart rate (HR), systemic vascular resistance (R), and stressed blood volume (V). We investigated the contribution of each mechanical property using the framework of circulatory equilibrium. In six anesthetized dogs, we vascularly isolated carotid sinuses and randomly changed carotid sinus pressure (CSP), while measuring the LV Ees, aortic flow, right and left atrial pressure, and AP for at least 60 min. We estimated transfer functions from CSP to Ees, HR, R, and V in each dog. We then predicted these parameters in response to changes in CSP from the transfer functions using a data set not used for identifying transfer functions and predicted changes in AP using the equilibrium framework. Predicted APs matched reasonably well with those measured ( $r^2$ = 0.85–0.96, P < 0.001). Sensitivity analyses indicated that  $E_{es}$  and HR (ventricular properties) accounted for  $14 \pm 4$  and  $4 \pm 2\%$ , respectively, whereas R and V (vascular properties) accounted for 32  $\pm$  4 and 39  $\pm$  4%, respectively, of baroreflex-induced AP regulation. We concluded that baroreflex-induced dynamic AP changes can be accurately predicted by the transfer functions from CSP to mechanical properties using our framework of circulatory equilibrium. Changes in the vascular properties, not the ventricular properties, predominantly determine baroreflex-induced AP regulation.

baroreflex; hemodynamics; circulatory equilibrium; transfer function

BAROREFLEX IS KNOWN TO BE the fastest negative feedback system to stabilize arterial pressure (3, 8, 11, 18). Baroreceptors sense arterial pressure, and the activated afferent nerves relay the pressure signal to the vasomotor center. The vasomotor center changes the mechanical properties of the ventricle and vascular system to stabilize arterial pressure through modulating the autonomic nervous system (12, 13, 17, 21, 22, 31). The dominant mechanical properties that affect hemodynamics include ventricular contractility and heart rate (HR) for the heart, and arterial resistance, and stressed blood volume for the vascular system. Many inves-

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tigators studied how baroreflex induces changes in some of these mechanical properties. Kubota et al. (12) demonstrated that baroreflex did change both ventricular and arterial properties. However, they did not study the impact of baroreflex on preload or cardiac output (CO). Shoukas and Brunner (21) demonstrated that the baroreflex markedly changed arterial resistance and stressed volume. Greene and Shoukas (7) also evaluated the impact of baroreflex on CO curve and venous return curve. Schmidt et al. (20) showed that CO and peripheral resistance equally contributed to baroreflex regulation of arterial pressure. Liu et al. (13) reported that baroreflex-induced change in HR has almost negligible contribution to arterial pressure. However, both groups did not evaluate the impact of each mechanical property on baroreflex-induced change in arterial pressure. Thus how the baroreflex-induced changes in ventricular and vascular properties quantitatively determine arterial pressure remains poorly understood.

We previously proposed and validated a framework of circulatory equilibrium using ventricular-arterial coupling and a distributed vascular model (28-30). Applying the framework allows us to estimate the circulatory equilibrium from ventricular and vascular properties (15, 24-26, 30). If ventricular properties (i.e., ventricular elastance and HR) and arterial resistance are given, we can derive the integrated CO curve. If left atrial pressure and right atrial pressure are known, we can derive the stressed blood volume for a given CO from the venous return surface (see APPENDIX). Once the integrated CO curve and the venous return surface are derived, the intersection between them defines the circulatory equilibrium point. Thus we hypothesize that if we quantitate the baroreflex-induced changes in ventricular and vascular properties, we can predict the baroreflex-induced changes in arterial pressure.

This study consists of three parts. First, we evaluated the transfer function from carotid sinus pressure (CSP) to each mechanical property by an open loop analysis. Second, we predicted the baroreflex-induced change for each property using the transfer function and predicted the dynamic changes of arterial pressure using the circulatory equilibrium model. Finally, we performed a sensitivity analysis to evaluate the contribution of each mechanical property to baroreflex changes in arterial pressure.

## METHODS

Animal preparation. Experiments and animal care were approved by the Committee on Ethics of Animal Experiment, Kyushu University Graduate School of Medical Sciences and performed in strict accordance with the *Guide for the Care and Use of Laboratory Animals* published by the National Institutes of Health. Six mongrel dogs weighing  $16.5 \pm 1.2$  kg (means  $\pm$  SD) were anesthetized with pentobarbital sodium (15 mg/kg iv). The dogs were intubated and artificially ventilated with room air. A catheter (6 Fr) was placed in the right femoral vein for administration of drugs and fluids. An appropriate level of anesthesia was maintained during experiment via inhalation of isoflurane and intravenous infusion of pentobarbital sodium. We confirmed that the arterial blood pH, Po<sub>2</sub>, Pco<sub>2</sub>, and bicarbonate were within physiological ranges. Body temperature was maintained between 37 and  $38^{\circ}$ C.

The experimental setup is illustrated in Fig. 1. To open the baroreflex feedback loop, we isolated the arterial baroreceptors vascularly according to previously reported methods (20). Briefly, we exposed bilateral carotid arteries and vagosympathetic trunks through a midline cervical incision. After ligations of bilateral internal and external carotid sinuses, both carotid sinuses were cannulated and connected to a servo-controlled piston pump (model ET-126A; Labworks) for perturbation of CSP. The vagosympathetic trunks were cut to eliminate other reflexes. A highfidelity micromanometer (Millar Instruments, Houston, TX) was inserted into the ascending aorta through the left common carotid artery to measure arterial pressure. The chest was opened and the heart was suspended in a pericardial cradle. An ultrasonic flow probe (model 20A594; Transonic, Ithaca, NY) was placed around the ascending aorta to measure CO. Fluid-filled catheters were placed in the left and right atria to measure left atrial pressure  $(P_{LA})$  and right atrial pressure  $(P_{RA})$ , respectively. They were connected to pressure transducers (model DX-360 and MEG-5200; Nihonkohden, Tokyo, Japan). The junction between the inferior vena cava and the right atrium was taken as the reference point for zero pressure. Two pairs of sonomicrometry crystals were implanted in the endocardium of the left ventricle to measure the anterior-to-posterior (short axis, D<sub>SA</sub>) and base-to-apex (long axis, DLA) dimensions. A second micromanometer was inserted into the left ventricle via the apex to measure left ventricular pressure. As shown in Fig. 2, all data were recorded simultaneously during CSP perturbation. We calculated the HR on a beat-to-beat basis by detecting each beat from the aortic flow waveform. We calculated

the left ventricular volume (LVV) by the modified ellipsoid formula: LVV =  $(\pi/6) \times D_{SA} \times D_{SA} \times D_{LA}$  (1, 16). Beat-by-beat left ventricular end-systolic elastance (E<sub>es</sub>) was estimated by dividing instantaneous pressure by volume in excess of V<sub>0</sub> defined by the inferior vena cava occlusion method. Systemic vascular resistance (R) was calculated by dividing arterial pressure in excess of P<sub>RA</sub> by CO. Stressed blood volume (V) was estimated from the venous return surface (28, 29). The venous return surface, CO<sub>v</sub>, is expressed as a function of left atrial pressure, P<sub>LA</sub>, and right atrial pressure, P<sub>RA</sub>, as

$$CO_{V} = \frac{V}{W} - G_{p} \times P_{LV} - G_{s} \times P_{RA}$$
(1)

where W,  $G_{\rm p}$ , and  $G_{\rm s}$  are empirically derived constants (29). Rearranging Eq. 1 yields:

$$\mathbf{V} = W \left( \mathbf{CO}_{\mathbf{V}} + G_{\mathbf{p}} \times \mathbf{P}_{\mathbf{L}\mathbf{V}} + G_{\mathbf{s}} \times \mathbf{P}_{\mathbf{R}\mathbf{A}} \right) \tag{2}$$

Although Eqs. 1 and 2 are based on a steady state, we assumed that these equations are valid under dynamic condition. We measured instantaneous CO (not CO<sub>V</sub>),  $P_{LA}$ , and  $P_{RA}$ . We also assumed that CO equals CO<sub>V</sub>. With these assumptions, once we obtain a data set of CO,  $P_{LA}$ , and  $P_{RA}$ , we can calculate instantaneous V. The absolute values of W,  $G_p$ , and  $G_s$  are 0.129, 3.49, and 19.61, respectively, according the report of Uemura et al. (29).

All analog data signals were digitalized at 200 Hz with a 16-bit analog-to-digital converter (PL3616 PowerLab 16/35; AD Instruments) using a dedicated laboratory computer system and were stored on a hard disk for subsequent analysis.

*Protocol.* To identify open-loop transfer functions from CSP to  $E_{es}$ , HR, R, and V, we used the white-noise method. We perturbed CSP (100 and 160 mmHg) with pseudorandom binary sequences at a shortest interval of 5 s and recorded each variable for ~60 min (12, 23, 27). The power spectral density of CSP was flat from 0.002 Hz until 0.1 Hz. Illustrated in Fig. 3 are representative time series of CSP,  $E_{es}$ , HR, R, and V. Lowering CSP increases  $E_{es}$ , HR, R, and V, indicating that baroreflex dynamically modulates both the ventricular and vascular properties.

*Identification of baroreflex transfer functions.* We resampled the all the 2,816-s time series at 2 Hz and segmented the reduced time series







Fig. 2. A data set of simultaneously measured hemodynamic parameters. ECG, electrocardiogram; LVP, left ventricular pressure; LVV, left ventricular volume.

into 10 segments with 50% overlapping, each consisting of 1,024 points (corresponding to 512 s/segment). We detrended the data and derived ensembled crosspower spectra between the input and output,  $S_{\text{IN-OUT}}(f)$ , and divided it by the ensembled power spectra,  $S_{\text{IN-IN}}(f)$ , of input using the discrete Fourier transform to determine a transfer function, H(f), as:

$$H(f) = \frac{S_{\rm IN-OUT}(f)}{S_{\rm IN-IN}(f)}$$
(3)

We also estimated the magnitude-squared coherence function, which is a frequency domain measure of the linear dependence between the input and output variables, as follows:

$$\operatorname{COH}(f) = \frac{|S_{\mathrm{IN-OUT}}(f)|^2}{S_{\mathrm{IN-IN}}(f) \cdot S_{\mathrm{OUT-OUT}}(f)}$$
(4)

Framework of circulatory equilibrium incorporating ventriculararterial coupling using the pressure-volume relationship of ventricle and arterial system. The framework of circulatory equilibrium consists of a venous return surface representing venous return of the systemic and pulmonary circulations and an integrated CO curve representing the Frank-Starling mechanism of the heart. Applying the concept of ventricular-arterial coupling using the pressure-volume relationship yields CO as follows:

$$CO = \frac{E_{es}}{\frac{E_{es}}{HR} + R} (V_{ed} - V_0)$$
(5)

where R is arterial resistance,  $V_{ed}$  is end-diastolic volume, and  $V_o$  is systolic unstressed volume (24–26, 30).



Fig. 3. Representative time series of changes in mechanical properties during perturbation of carotid sinus pressure.  $E_{es}$ , end-systolic elastance; HR, heart rate; R, systemic vascular resistance; V, stressed blood pressure.

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Fig. 4. Flow diagram of the procedures to predict the changes of arterial pressure in response to CSP perturbation. *H*, transfer function; HR, heart rate; CO, cardiac output; VR, venous return. First using the respective estimated transfer functions, we predicted  $E_{es}$ , HR, R, and V in response to changes in CSP. Then we incorporated these variables into the framework of circulatory equilibrium and estimated the changes of arterial pressure. We compared the predicted arterial pressure with those measured.



Since the end-diastolic pressure-volume relationship is known to approximate a monoexponential curve (4, 6, 32),  $V_{ed}$  can be related to end-diastolic pressure ( $P_{ed}$ ) by

$$P_{ed} = \alpha e^{kVed} + \beta \tag{6}$$

where k,  $\alpha$ , and  $\beta$  are curve-fitting constants. If we approximate  $P_{ed}$  by scaled mean atrial pressure,  $\gamma P_{at}$  ( $\gamma$  is a proportionality constant), substituting  $V_{ed}$  by atrial pressure,  $P_{at}$ , in *Eq. 5* yields:

$$CO = \frac{1}{k} \times \frac{E_{es}}{\frac{E_{es}}{HR} + R} \times \left\{ \ln(P_{at} - F) + H \right\}$$
(7)

where F and H are empirically derived constants (28, 29). The values of F and H used in this study are 2.03 and 0.80, respectively, according to Uemura's report (29).

Once we obtained a set of  $E_{es}$ , R, HR, and V, we simultaneous solved *Eqs. 1* and 7 to yield CO. We then derived arterial pressure by multiplying CO and R (28, 29).

Prediction of baroreflex-induced dynamic changes of arterial pressure. Figure 4 shows the flow diagram of the procedures to predict the changes of arterial pressure in response to CSP perturbation. To examine if the framework of circulatory equilibrium holds under baroreflex-induced dynamic changes in hemodynamic conditions, we used the estimated transfer functions to predicted HR,  $E_{es}$ , R, and V in response CSP changes in using data sets that were not used to estimate the transfer functions. This was done by obtaining the time domain representation of the transfer functions, i.e., the impulse response [for example, the impulse response of HR;  $h_{HR}(t)$ ], by applying the inverse Fourier transformation to H(f). We then predicted the time series of each variable for 512 s by convolving the impulse response with the instantaneous CSP(t) after subtracting the mean value of CSP.

$$HR(t) = \sum_{t=1}^{N} h_{HR}(\tau) \cdot CSP(t-\tau)$$
(8)

where *N* is the total number of impulse responses (N = 1,024),  $\tau$  is the convolution parameter, and *t* is time in increments of 0.5 s. We used the CSP data recorded before t = 0 to estimate the value of HR(0). By repeating the same procedure individually for E<sub>es</sub>, V, and R, we predicted the time series of dynamic changes in these variables in response to CSP changes. Then, we obtained the absolute values of these variables by adding the mean values calculated from the time series data used to estimate the transfer functions. We then substituted the time series of these variables into *Eqs. 1* and 7 and simultaneously solved them every 0.5 s to predict the time series of dynamic changes in arterial pressure induced by baroreflex. To quantify the goodness-of-fit of the predicted arterial pressure, we fitted a straight line to the

measured vs. predicted variables, and calculated the slope and intercept of the best fit line, the correlation coefficient and the standard error of the estimate (SEE) between predicted and measured variables.

### RESULTS

The hemodynamic variables obtained during CSP perturbation are shown in Table 1. Mean arterial pressure, HR,  $E_{es}$ , R, and V were 124  $\pm$  22 mmHg, 168  $\pm$  13 beats/min, 11.3  $\pm$  2.9 mmHg/ml ( $E_{es}$  normalized by body weight: 188  $\pm$  63 mmHg·kg·ml<sup>-1</sup>), 1.37  $\pm$  0.27 mmHg/(ml·min<sup>-1</sup>·kg<sup>-1</sup>), nd 18.8  $\pm$  3.7 ml/kg, respectively. The mean arterial pressure was not significantly different from that of mean CSP (P > 0.05), indicating that the range of perturbed CSP was around the operating pressure under closed baroreflex loop condition.

Characteristics of the baroreflex transfer functions. Figure 5 shows the averaged transfer functions from CSP to  $E_{es}$  ( $H_{CSP\rightarrow Ees}$ ), HR ( $H_{CSP\rightarrow HR}$ ), R ( $H_{CSP\rightarrow R}$ ), and V ( $H_{CSP\rightarrow V}$ ). Solid and dashed lines represent the means  $\pm$  SD, respectively. Note that both the frequency and modulus axes are logarithmically scaled as a Bode plot. In all four transfer functions, the moduli remained relatively constant up to 0.02 Hz and decreased in the high-frequency range as frequency increased. The phase angle was almost out of phase in the low-frequency range. These observations are consistent with the negative feedback mechanism of baroreflex. The magnitude squared coherence functions were close to unity in the frequency range between 0.002 and 0.04 Hz, indicating that  $E_{es}$ , HR, R, and V were highly linearly dependent on CSP in this frequency range.

Table 1. Hemodynamic parameters during perturbation ofcarotid sinus pressure for six dogs

Dog No.	BW, kg	AP, mmHg	HR, beats/min	E <sub>es</sub> , mmHg/ml	Resistance, mmHg/(ml·min <sup>-1</sup> ·kg <sup>-1</sup> )	Volume, ml/kg
1	16.3	104	167	9.8	1.43	15.1
2	16.8	93	157	13.5	1.13	18.9
3	15.1	149	161	9.8	1.60	20.7
4	16.3	127	177	10.2	1.08	24.5
5	15.7	126	158	8.3	1.22	19.4
6	18.6	142	190	16.0	1.76	14.4
Means	16.5	124	168	11.3	1.37	18.8
SD	1.2	22	13	2.9	0.27	3.7

Hemodynamic values are expressed as mean values during perturbation of carotid sinus pressure (CSP). BW, body weight; AP, arterial pressure; HR, heart rate; E<sub>es</sub>, end-systolic elastance; Resistance, systemic vascular resistance; Volume, stressed blood volume.



Fig. 5. Averaged open-loop transfer functions from CSP to end-systolic elastance ( $H_{CSP \rightarrow Ees}$ ), heart rate ( $H_{CSP \rightarrow HR}$ ), systemic vascular resistance ( $H_{CSP \rightarrow RR}$ ), and stressed blood volume ( $H_{CSP \rightarrow V}$ ), with coherence functions from 6 dogs. *Bottom*: step response of each variable calculated from the transfer function. In *top* and *bottom*, the bold and dotted lines indicate mean and means  $\pm$  SD values, respectively.

To facilitate interpretation and understanding of the transfer functions, we present a step response of each variable in the Fig. 5, *bottom*. The step response illustrates how the carotid sinus baroreflex will change each variable in the time domain when the CSP abruptly increases by 1 mmHg. In response to the step change in CSP, the carotid sinus baroreflex decreased  $E_{es}$  by ~0.09 mmHg/ml (normalized by body weight: 1.5 mmHg·kg/ml). All the variables reached steady state in the first 100 s.

*Prediction of baroreflex-induced dynamic change of arterial pressure.* Illustrated in Fig. 6 are the time series of CSP (*top*), predicted arterial pressure (*middle*), and measured arterial pressure (*bottom*) in one animal. Figure 7 shows the relations between measured and predicted arterial pressure obtained from six dogs. The predicted arterial pressure matched reasonably well with those measured. The results of this part of the study are summarized in Table 2, which shows the slope, intercept, correlation coefficient, and SEE for the linear relationship between measured and predicted arterial pressure in each animal. The correlation coefficient ( $r^2$ ) varied between 0.85 and 0.96. The SEE ranged between 3.4 and 7.0 mmHg (2.7–5.6% of mean arterial pressure) suggesting reasonable accuracy of prediction.

*Sensitivity analysis.* Next, we estimated the contribution of the change for each variable to the arterial pressure response regulated by the baroreflex. We nullified the response of a



Fig. 6. Representative time series data set of predicted and measured arterial pressure during perturbation of CSP. The predicted arterial pressure matched reasonably well with those measured.

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Fig. 7. Relations between measured and predicted arterial pressure for 512 s obtained from each of 6 dogs (1,024 points each). Regression analyses reveal that predicted arterial pressure matches reasonably well with measured values.

particular variable and observed the impact on the change in arterial pressure. Figure 8 shows the results of simulation study obtained from all six dogs. As summarized in Fig. 9,  $E_{es}$  and HR (ventricular properties) accounted for  $14 \pm 4$  and  $4 \pm 2\%$ , respectively, whereas R and V (vascular properties) accounted for  $32 \pm 4$  and  $39 \pm 4\%$ , respectively, of baroreflex-induced change in arterial pressure. These data thus suggest that baroreflex-induced changes in the vascular properties, not the ventricular properties, predominantly determine the dynamic change in arterial pressure. The contribution of estimated change in CO to arterial pressure was  $41 \pm 5\%$ .

# DISCUSSION

Contribution of ventricular and vascular properties to baroreflex-induced changes in arterial pressure. This report is the first to systematically describe the contribution of individual ventricular and vascular properties to baroreflex arterial

Table 2. Relation between predicted and measured arterialpressure for six dogs

Dog No.	Slope	Intercept, mmHg	$r^2$	SEE, mmHg
1	0.96	11.2	0.85	7.0
2	1.09	0.7	0.94	3.7
3	0.86	13.3	0.96	3.4
4	1.12	-21.1	0.87	3.9
5	0.87	9.4	0.86	4.7
6	0.87	13.5	0.86	6.5
Means	0.96	4.5	0.89	4.9
SD	0.12	13.4	0.05	1.5

SEE, standard error of the estimate.

pressure regulation. Sensitivity analysis using the circulatory equilibrium model indicates that the major variables that contribute to arterial pressure change are the vascular properties including arterial resistance and stressed blood volume. In contrast, the impact of the changes in ventricular properties on arterial pressure regulation is remarkably small. In other words, the vascular system, not the ventricular system, is the dominant actuator in baroreflex regulation of arterial pressure at least in the setting of normal cardiac function.

Schmidt et al. (20) divided the baroreflex control of arterial pressure into changes in CO and total peripheral resistance and investigated the contribution of each variable using carotid sinus isolation in vagotomized dogs. Their results indicated that both CO and total peripheral resistance contributed equally to arterial pressure regulation in response to CSP changes from 100 to 150 mmHg. In our study, the contribution of total peripheral resistance was 32%. The estimated change in CO in our numerical study was 41%. Since arterial pressure is the product of arterial resistance and CO, this numerical result is not much different from the finding of Schmidt et al. Note that the total percent contribution of ventricular and vascular properties does not add up to 100%, due to the nonlinear and synergistic interactions between different branches of the baroreflex.

Liu et al. (13) investigated the contribution of baroreflexinduced changes in ventricular properties in an open loop rabbit model. They electrically stimulated the aortic depressor nerve before and after administration of beta-blocker and vagotomy to remove the ventricular ability to change



Fig. 8. Results of sensitivity analysis obtained from all six dogs. We estimated the contribution of baroreflex-induced change in each mechanical property to arterial pressure regulation by nullifying the response of a particular variable and observed the impact on change in arterial pressure.

arterial pressure. They concluded that the ventricular contribution to the dynamic regulation of arterial pressure was negligible in rabbits. Our study indicated that the contribution of HR was almost negligible and the contribution of contractility was small. The present results are compatible with those reported by Liu et al.



Fig. 9. Contribution of baroreflex-induced change in each mechanical property to arterial pressure regulation. Data are expressed as means  $\pm$  SD (n = 6).

Some observations in the clinical settings support our results. First, in patients with cardiac transplant, the transplanted hearts are denervated and unable to respond to baroreflex regulation. Despite the absence of baroreflex regulation of the heart, postural changes of blood pressure are negligibly small immediately after heart transplantation (5, 14). These results indicate that the ventricular contribution is not important in the baroreflex control of arterial pressure. Second, patients who sustain cervical cord injury are often affected by serious orthostatic hypotension and symptoms such as fainting and syncope. In these patients, efferent sympathetic preganglionic neurons are disrupted and baroreflex-mediated vasoconstriction cannot take place thereby resulting in serious orthostatic hypotension, even though the heart responds to changes in autonomic nervous activities (2). Both of these clinical observations are consistent with our results.

Baroreflex control of ventricular and vascular properties. We used the white-noise method to obtain open-loop transfer functions from CSP to mechanical properties, because the white-noise method allows estimation of unbiased linear transfer characteristics even in the presence of nonlinear system responses. To minimize the nonlinear effects caused by threshold and saturation of the baroreflex, CSP perturbation was limited to 100 and 160 mmHg. Schmidt et al. (20) used the same CSP range and obtained an almost linear relationship between CSP and arterial pressure. The high magnitude squared coherence obtained supports that we characterized the linear transfer functions reasonably well.

Kubota et al. (12) evaluated the open loop transfer function from CSP to  $E_{es}$  using the white-noise method and autoregressive analysis. The gain and the natural frequency in their report were consistent with our results. Shoukas and Brunner (21) evaluated the relationship between CSP and both arterial resistance and stressed volume. They measured the stressed blood volume directly, while we used the concept of venous return surface to evaluate the stressed blood volume. Regardless of the methodological difference, the gains defined by the ratios of changes in arterial resistance and stressed volume to CSP in the study of Shoukas and Brunner were similar to our results. This indicates that the estimation of stressed volume using the venous return surface is reasonably accurate.

Accuracy of the circulatory equilibrium model. Guyton's classic concept of circulatory equilibrium cannot reflect isolated left heart failure because it does not explicitly incorporate left heart mechanics. To overcome such limitation of the classic Guyton's concept, we opened the circulatory loop at the left atrium as well as the right atrium and incorporated left heart mechanics in the framework (15, 28, 29). Although the modified framework is capable of representing the circulatory equilibrium in the presence of left heart failure, it remained unknown how dynamic neurohormonal regulation affects the circulatory equilibrium. Therefore, in this investigation, we developed a framework to quantitatively predict the impact of baroreflex on dynamic circulatory equilibrium and arterial pressure. To achieve this goal, we applied the framework of ventricular-arterial coupling (end-systolic pressure-volume relationship) to the circulatory equilibrium as reported previously (30). We demonstrated that the framework we developed quantitatively reproduces baroreflex-induced dynamic changes in circulatory equilibrium. To our knowledge, this is the first report of an integrated circulation model capable of quantitatively predicting the impact of baroreflex on dynamic changes in arterial pressure.

*Limitation.* All the experiments of this study were conducted in anesthetized, open-chest dogs. Anesthesia and surgical trauma significantly affect the cardiovascular system. Furthermore, the open-chest condition forcibly equalizes "intrathoracic" pressure to atmospheric pressure, preventing any possible reflex modulation of intrathoracic pressure and the consequent effects on cardiac filling and CO. Abolition of the possible reflex adjustment in this experimental condition could partly explain why baroreflex regulation of the heart was relatively ineffective. Whether the circulatory equilibrium can be applied to conscious, closedchest animals remains to be tested.

We evaluated the pure effect of carotid sinus baroreflex on ventricular vascular function in this study. Since we cut the vagosympathetic trunk to eliminate the buffering effects of the aortic arch baroreflex and the cardiopulmonary baroreflex, care has to be exercised in generalizing the results of this study to all baroreflex mechanisms. If the vagal nerves had been preserved, the gain of  $H_{CSP \to HR}$  and subsequently the contribution to baroreflex control of arterial pressure might be conceivably higher. However, as we already discussed, the impact of baroreflex-induced changes in HR on arterial pressure is limited in rabbits and humans (5, 13, 14). Therefore, we speculate that ignoring the vagal control of HR may not change the result of this study.

We estimated the transfer functions from CSP to left ventricle and systemic vasculature, but not to the right ventricle and pulmonary vasculature, because of the difficulty in measuring right ventricular end systolic elastance. Therefore, we assumed that the slopes of the CO curves for left and right ventricles change in proportional to the baroreflex, according to Sagawa et al. (15). The fact that our prediction of arterial pressure was reasonably accurate suggests that the assumption is valid.

The relationship between CSP and arterial pressure in the open loop condition is known to be sigmoidal (19-21). In this study, we evaluated the CSP between 100 and 160 mmHg only. The reason why we chose that range is to evaluate the baroreflex in the physiological pressure range. The coherence functions of the transfer functions were almost unity, indicating linearity of the relationship between CSP and each mechanical property. However, the results could be different at CSP outside the range used in this study. Further investigation is needed to evaluate in greater detail the baroreflex control of arterial pressure.

We assumed that baroreflex does not affect the slope of the venous return surface. Since we modeled the vasculature using resistance and compliance, baroreflex might potentially affect the slope of the surface. However, Greene and Shoukas (7) reported that the slope of the venous return curve from the systemic vasculature was unaffected by the baroreflex. Based on their report, we assume that baroreflex does not affect the slope of the venous return surface. Further investigation is required to evaluate baroreflexinduced changes in venous return surface.

Although the venous return surface is based on a steady state, we assumed that  $Eq. \ 1$  also applies under dynamic condition. We also assumed that CO equals  $CO_V$ . These assumptions are in principal invalid in a continuous time system. Our results suggest that the dynamic characteristics of baroreflex is quasistatic system because of the relatively low corner frequency and the sluggish response.

Our results were obtained in normal dogs and the findings in disease states such as acute or chronic heart failure and hypertension could be very different. Uemura et al. (28) reported that the framework of circulatory equilibrium used in the present study allowed accurate prediction of hemodynamics after extensive changes in stressed blood volume during heart failure and normal cardiac function. Their results indicate that the framework can be applied to acute heart failure. Left ventricular pressure-volume analysis conceptually could be applied to both normal and pathological conditions. These suggest that the methodology used in this study could be used to clarify the contributions of reflex control to ventricular and vascular properties in common pathological conditions. Further investigation is needed to validate this hypothesis.

*Conclusion.* Baroreflex-induced dynamic arterial pressure changes can be predicted accurately by the transfer functions from CSP to individual ventricular and vascular properties using a circulatory equilibrium model integrated with ventric-



Fig. A1. The vascular system modeled by a distributed system. C(x), P(x), and R(x), compliance, pressure, and cumulative resistance over a distance *x* from the venous port; *L*, distance between the arterial and venous ports.

ular-arterial coupling. In anesthetized normal dogs, changes in vascular properties, not ventricular properties, predominantly determine the baroreflex regulation of arterial pressure.

## APPENDIX

*Concept of integrated venous return.* Sunagawa et al. (26) modeled the vascular system using a distributed model. Suppose that compliance and resistance are distributed in the systemic circulation (Fig. A1). When the compliance distribution [C(x)] and pressure distribution [P(x)] are expressed as a function of distance (*x*) from the venous port, then stressed blood volume (V<sub>s</sub>) in the systemic circulation can be described as:

$$V_{\rm S} = \int_0^L P(x) C(x) dx \qquad (A1)$$

where *L* represents the distance between the arterial and venous ports. If we denote the cumulative resistance over a distance (*x*) from the venous port by R(x), the serial pressure distribution can be described using the venous return,  $CO_V$ , as:

$$P(x) = R(x)CO_{V} + P_{RA}$$
(A2)

Substituting Eq. A2 into Eq. A1 yields:

$$\mathbf{V}_{\mathrm{S}} = \mathrm{CO}_{\mathrm{V}} \int_{0}^{L} \mathrm{C}(x) \mathrm{R}(x) \mathrm{d}x + \mathrm{P}_{\mathrm{RA}} \int_{0}^{L} \mathrm{C}(x) \mathrm{d}x \qquad (A3)$$

Substituting C(x) by  $C_S D_{CS}(x)$ , where  $C_S$  is the total systemic vascular compliance and  $D_{CS}(x)$  is the normalized distribution of compliance as a function of *x* [thus  $\int_0^L D_{CS}(x) dx$  is unity], *Eq. A3* can be rewritten as:

$$\mathbf{V}_{\mathrm{S}} = \mathrm{CO}_{\mathrm{V}} \mathbf{C}_{\mathrm{S}} \int_{0}^{L} \mathbf{D}_{\mathrm{CS}}(x) \mathbf{R}(x) \mathrm{d}x + \mathbf{P}_{\mathrm{RA}} \mathbf{C}_{\mathrm{s}} \int_{0}^{L} \mathbf{D}_{\mathrm{CS}}(x) \mathrm{d}x \quad (A4)$$

The first integral term that sums cumulative resistance weighted by systemic compliance distribution is equivalent to the resistance for systemic venous return,  $R_{VS}$ , of Guyton et al. (9, 10). Because the second integral is unity, *Eq. A4* can be rewritten as:

$$V_{S} = CO_{V}C_{S}R_{VS} + P_{RA}C_{S}$$
(A5)

Stressed blood volume in the pulmonary circulation ( $V_P$ ) can be related to  $CO_V$  and  $P_{LA}$  by the following equation:

$$V_{\rm P} = {\rm CO}_{\rm V} {\rm C}_{\rm P} {\rm R}_{\rm VO} + {\rm P}_{\rm LA} {\rm C}_{\rm P} \tag{A6}$$

where  $C_P$  is the total pulmonary compliance and  $R_{VP}$  is the resistance for pulmonary venous return.

For a given condition, the sum of the stressed blood volume in the systemic circulation and pulmonary circulation, V (i.e.,  $V_S + V_P$ ),

remains constant, irrespective of its distribution. Summing *Eqs. A5* and *A6* and rearranging yields:

$$CO_V = V/W - G_S P_{RA} - G_P P_{LA}$$
(A7)

where W,  $G_S$ , and  $G_P$  are linear parameters and are expressed as

$$W = C_S R_{VS} + C_P R_{VP} \tag{A8}$$

$$G_{\rm S} = C_{\rm S}/W \tag{A9}$$

and

$$G_{\rm P} = C_{\rm P}/W \tag{A10}$$

where the unit for W is minute and that for GS and GP is  $ml \cdot min^{-1} \cdot mmHg^{-1} \cdot kg^{-1}$ .

For a given V,  $CO_V$  can be related to  $P_{RA}$  and  $P_{LA}$  by a surface expressed by *Eq. A7*. When V is kept constant,  $CO_V$  decreases with increases in  $P_{RA}$  and/or  $P_{LA}$ .

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#### DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

#### AUTHOR CONTRIBUTIONS

Author contributions: T.S., T. Tsutsumi, and K. Sunagawa conception and design of research; T.S., T.K., K. Sakamoto, T. Tobushi, A.T., K. Saku, K.H., K.O., Y.M., and T. Tsutsumi performed experiments; T.S. analyzed data; T.S., T.I., and K. Sunagawa interpreted results of experiments; T.S. prepared figures; T.S. drafted manuscript; T.S. and K. Sunagawa edited and revised manuscript; T.S. and K. Sunagawa approved final version of manuscript.

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