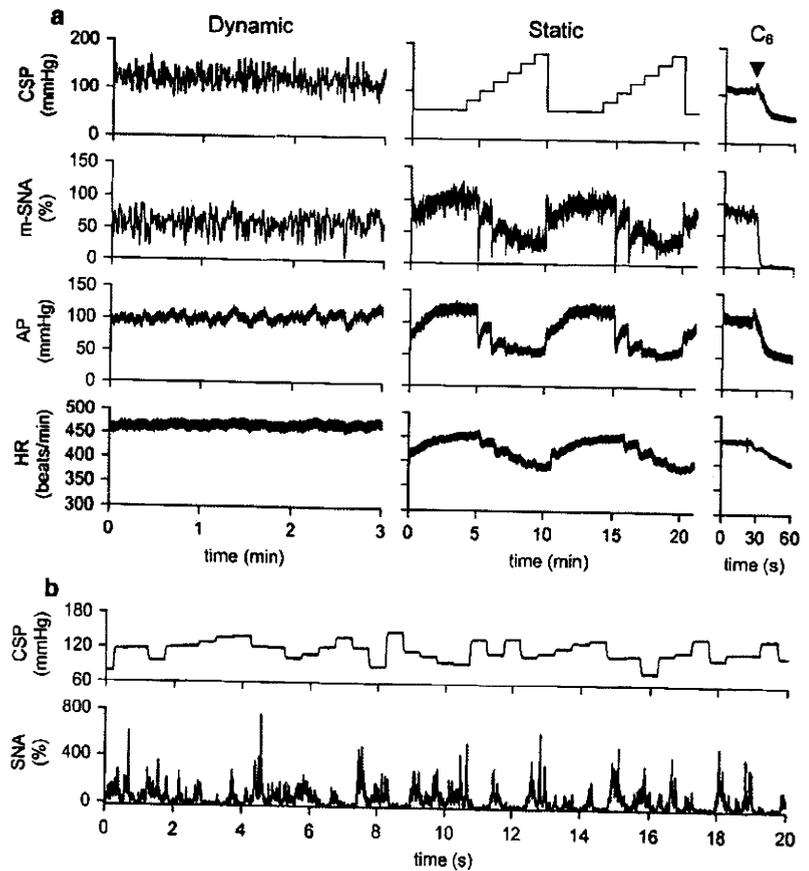


Fig. 1 a Typical recordings (10-Hz decimated data) of carotid sinus pressure (CSP), 2-s moving averaged sympathetic nerve activity (m-SNA), arterial pressure (AP), and heart rate (HR) obtained from a control rat. In the dynamic protocol, CSP was changed according to a Gaussian white noise signal. In the static protocol, CSP was increased from 60 to 180 mmHg. Hexamethonium bromide (C_6) was administered intravenously at the end of the experiment (arrowheads). The noise level of the nerve activity recorded after C_6 administration was assigned 0%, while the m-SNA value averaged for the last 10 s at CSP of 60 mmHg was assigned 100%. **b** CSP and SNA sampled at 200 Hz during the dynamic input protocol



and CHF rats. The neural arc transfer function from CSP to SNA showed derivative characteristics (Fig. 3a). $G_{0.01}$ tended to be lower in the CHF rats, whereas $G_{0.1}$ and G_1 did not differ significantly between the two groups (Table 2). The phase plot showed an out-of-phase relationship in the frequency range from 0.01 to 1 Hz in both groups. The coherence plot with values less than unity suggests that SNA contained an unknown noise signal unrelated to the baroreflex and/or a nonlinear system response to the CSP input. The bottom panel of Fig. 3a represents the step responses of SNA for a unit increase in CSP. Although S_{10} and S_{50} were significantly attenuated in the CHF rats, S_{peak} , T_{peak} , and S_{peak}/S_{50} did not differ significantly between the two groups (Table 2).

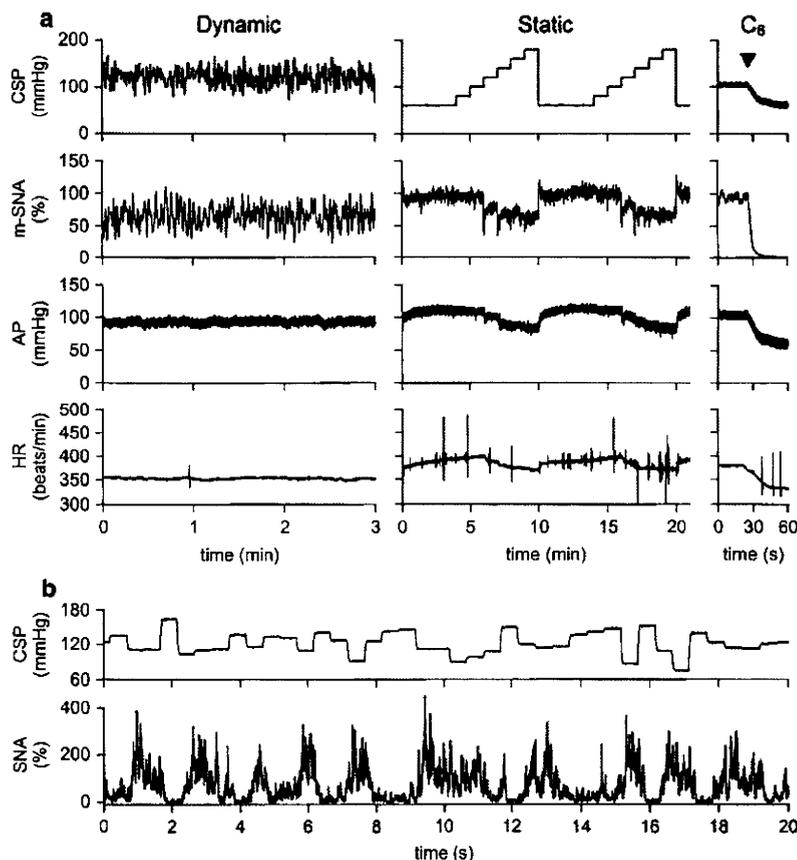
The peripheral arc transfer function from SNA to AP showed low-pass characteristics (Fig. 3b). $G_{0.01}$ and $G_{0.1}$ were significantly smaller in the CHF rats, whereas G_1 did not differ significantly between the two groups (Table 2). The phase was close to zero radians at the lowest frequency and delayed with an increase in frequency in both groups. The coherence plot with values less than unity indicates that a part of AP variation was not explained by the linear dynamics with the SNA variation. In the step response of AP for a unit increase in SNA (Fig. 3b, bottom), S_{50} was

significantly smaller and the initial slope (the dotted line) was significantly gentler in the CHF than in the control rats (Table 2).

The total baroreflex transfer function from CSP to AP showed low-pass characteristics (Fig. 3c). $G_{0.01}$ and $G_{0.1}$ were significantly smaller in the CHF than in the control rats (Table 2), but G_1 did not differ significantly. The phase was close to $-\pi$ radians at the lowest frequency in both groups, reflecting the negative feedback operation attained by the total baroreflex. The phase delayed with an increase in frequency. The coherence values seem lower than those in the peripheral arc transfer function. In the step response of AP for a unit increase in CSP (Fig. 3c, bottom), both S_{50} and the initial slope (the dotted line) were significantly attenuated in the CHF in comparison with the control rats (Table 2).

The transfer function from CSP to HR also displayed low-pass characteristics (Fig. 3d). $G_{0.01}$ and $G_{0.1}$ were significantly smaller in the CHF than in the control rats (Table 2). We did not compare G_1 because the coherence values close to zero and the irregular change in the phase shift above 0.8 Hz suggested poor reliability of the estimated transfer function. In the step response of HR for a unit increase in CSP (Fig. 3d, bottom), both S_{50} and the

Fig. 2 a Typical recordings (10-Hz decimated data) of CSP, m-SNA, AP, and HR obtained from a chronic heart failure rat. In the dynamic protocol, CSP was changed according to a Gaussian white noise signal. In the static protocol, CSP was increased from 60 to 180 mmHg. The noise level of the nerve activity recorded after C_6 administration was assigned 0%, while the m-SNA value averaged for the last 10 s at CSP of 60 mmHg was assigned 100%. b CSP and SNA sampled at 200 Hz during the dynamic input protocol



initial slope (the dotted line) were significantly attenuated in the CHF in comparison with the control rats (Table 2).

Static characteristics of the carotid sinus baroreflex

Figure 4 summarizes the open-loop static characteristics of the carotid sinus baroreflex obtained from the control and CHF rats. The baroreflex neural arc showed a decreasing SNA response with an increase in CSP (Fig. 4a). The response range of SNA was significantly narrower in the CHF rats (Table 3). Consequently, the minimum SNA was significantly higher in the CHF rats. The midpoint pressure on the CSP axis was significantly lower in the CHF rats. Despite the significant attenuation in the response range of SNA, the maximum slope of the neural arc was not reduced in the CHF rats compared with the control rats.

The peripheral arc from SNA to AP approximated a straight line in both the control and CHF rats (Fig. 4b). The slope of the regression line was significantly less steep in the CHF rats, whereas the AP intercept did not differ significantly between the two groups (Table 3).

The total baroreflex function from CSP to AP approximated an inverse sigmoidal curve (Fig. 4c). The response range of AP was significantly narrower in the CHF rats

(Table 3). The slope coefficient, the midpoint pressure on the CSP axis, and the minimum AP did not differ significantly between the two groups. The maximum gain was significantly smaller in the CHF rats compared with the control rats.

The static CSP-HR relationship also approximated an inverse sigmoidal curve (Fig. 4d). The response range of HR was significantly narrower, and the minimum HR was significantly lower in the CHF rats (Table 3). The midpoint pressure on the CSP axis was significantly lower in the CHF rats. The slope coefficient and the maximum slope did not differ significantly between the two groups.

The baroreflex equilibrium diagram is obtained by plotting the neural and peripheral arcs on a pressure-SNA plane (Fig. 4e). The ordinate is either CSP (for the neural arc) or AP (for the peripheral arc). The intersection between the neural and peripheral arcs gives the closed-loop operating point [17, 18]. The operating-point AP (the horizontal arrow) was significantly lower in the CHF rats, whereas the operating-point SNA (the vertical arrow) did not differ significantly between the two groups (Table 3). The total baroreflex gain at the operating point, calculated from the product of the tangential slope of the neural arc ($\Delta SNA/\Delta CSP$) and the slope of the peripheral arc ($\Delta AP/$

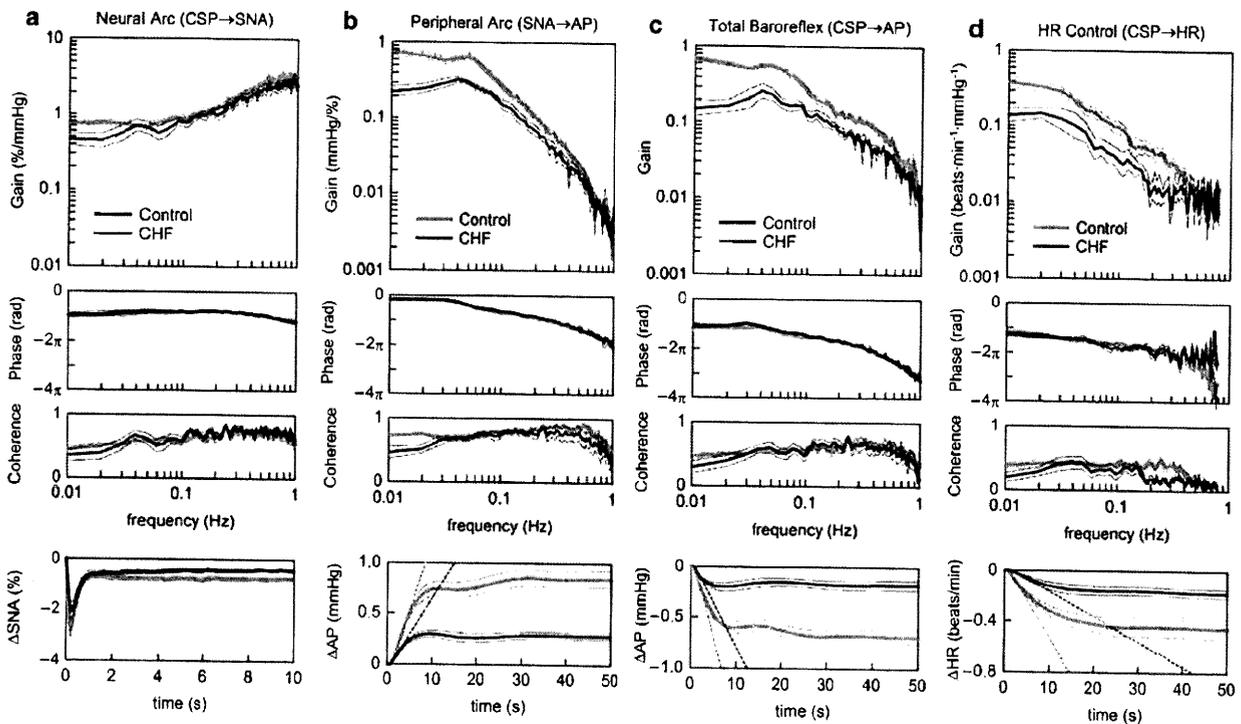


Fig. 3 Dynamic characteristics of the carotid sinus baroreflex averaged for the control rats ($n = 12$) and chronic heart failure (CHF) rats ($n = 7$). **a** Transfer function of the baroreflex neural arc from CSP to SNA. Gain, phase, and coherence plots are shown. The *bottom panel* represents the step response of SNA calculated from the transfer function. There is no significant difference in the negative peak response or the time to peak between control and CHF rats. **b** Transfer function of the baroreflex peripheral arc from SNA to AP. The *bottom panel* represents the step response of AP induced by a unit increase in

SNA. Both initial slope (*dotted lines*) and steady-state response are attenuated in CHF. **c** Transfer function of the total baroreflex from CSP to AP. The *bottom panel* represents the step response of AP induced by a unit increase in CSP. Both initial slope (*dotted lines*) and steady-state response are attenuated in CHF. **d** Transfer function from CSP to HR. The *bottom panel* represents the HR step response calculated from the transfer function. Both initial slope (*dotted lines*) and steady-state response are attenuated in CHF. In each plot, *bold and thin lines* indicate mean and mean \pm SE, respectively

Δ SNA), did not differ significantly between the two groups (Table 3): Δ CSP, Δ SNA, and Δ AP indicate small changes in CSP, SNA, and AP, respectively, at the intersection point. Figure 4f depicts a putative baroreflex equilibrium diagram where the SNA axis is scaled so that the maximum value of SNA in the CHF group becomes two times higher than that in the control group (see Discussion).

Simulation study

A block diagram of the simulation study is shown in Fig. 5a [14–16]. We used impulse responses derived from the group-averaged neural and peripheral arc transfer functions (H_N and H_P) to calculate dynamic responses of the carotid sinus baroreflex. The steady-state gains of H_N and H_P were normalized to unity in order that absolute values of the steady-state gains could be determined by their corresponding static characteristics. The static characteristics of the neural and peripheral arcs were modeled as a logistic function and a regression line, respectively, using group-averaged parameter values (Table 3). A

sinusoidal wave with an amplitude of 15 mmHg (peak-to-peak pressure of 30 mmHg) and a frequency of 5 Hz (corresponding to 300 beats/min) was added to the output from the peripheral arc to mimic pulsatile pressure. The AP signal was fed back into the neural arc to achieve a closed-loop simulation. After the AP signal reached steady state, step disturbances ranging from -10 to -60 mmHg were imposed.

Typical examples of the transient AP response to a step disturbance of -40 mmHg under control and CHF conditions are shown in Fig. 5b. In each panel, the pulsatile pressure is shown in gray, and the mean AP signal is shown as a solid bold line. The horizontal dashed lines represent the mean AP values immediately before and after the onset of the step disturbance. The AP signal decreased abruptly by 40 mmHg at time zero and recovered gradually thereafter. The upward arrow indicates the magnitude of steady-state AP recovery. The recovery was greater in the control than in the CHF simulation.

Figure 5c depicts the percent recovery of AP relative to the size of the step disturbance. The steady-state AP

Table 2 Parameters of the dynamic characteristics of the carotid sinus baroreflex

	Control (<i>n</i> = 12)	CHF (<i>n</i> = 7)	<i>P</i> value
Neural arc			
$G_{0.01}$ (% mmHg ⁻¹)	0.80 ± 0.10	0.50 ± 0.08	0.051
$G_{0.1}$ (% mmHg ⁻¹)	0.99 ± 0.11	0.85 ± 0.14	0.432
G_1 (% mmHg ⁻¹)	3.49 ± 0.34	2.64 ± 0.30	0.093
S_{50} (%)	0.75 ± 0.09	0.39 ± 0.06*	0.012
S_{10} (%)	0.78 ± 0.09	0.42 ± 0.06*	0.010
S_{peak} (%)	2.88 ± 0.25	2.13 ± 0.30	0.076
T_{peak} (s)	0.29 ± 0.01	0.31 ± 0.01	0.327
S_{peak}/S_{50}	5.30 ± 1.60	6.06 ± 1.06	0.743
Peripheral arc			
$G_{0.01}$ (mmHg % ⁻¹)	0.81 ± 0.09	0.24 ± 0.05**	<0.001
$G_{0.1}$ (mmHg % ⁻¹)	0.29 ± 0.04	0.16 ± 0.02*	0.023
G_1 (mmHg % ⁻¹)	0.0032 ± 0.0005	0.0050 ± 0.0015	0.211
S_{50} (mmHg)	0.84 ± 0.08	0.28 ± 0.03**	<0.001
Initial slope (mmHg s ⁻¹)	0.134 ± 0.014	0.071 ± 0.009**	0.007
Total baroreflex			
$G_{0.01}$ (mmHg mmHg ⁻¹)	0.70 ± 0.06	0.17 ± 0.03**	<0.001
$G_{0.1}$ (mmHg mmHg ⁻¹)	0.28 ± 0.03	0.13 ± 0.02**	0.001
G_1 (mmHg mmHg ⁻¹)	0.013 ± 0.002	0.010 ± 0.002	0.324
S_{50} (mmHg)	0.69 ± 0.07	0.18 ± 0.05**	<0.001
Initial slope (mmHg s ⁻¹)	0.166 ± 0.014	0.086 ± 0.009**	<0.001
Heart rate control			
$G_{0.01}$ (beats min ⁻¹ mmHg ⁻¹)	0.43 ± 0.06	0.16 ± 0.03**	0.005
$G_{0.1}$ (beats min ⁻¹ mmHg ⁻¹)	0.11 ± 0.02	0.04 ± 0.01*	0.026
S_{50} (beats min ⁻¹)	0.46 ± 0.08	0.18 ± 0.03*	0.021
Initial slope (beats min ⁻¹ s ⁻¹)	0.059 ± 0.009	0.020 ± 0.004**	0.006

Data are presented as mean ± SE

** *P* < 0.01 and **P* < 0.05 by unpaired *t* test

recovery was approximately 50% in the control and approximately 32% in the CHF simulation for a step disturbance of -10 mmHg, indicating that the feedback AP regulation in the CHF simulation was approximately 64% as effective as that in the control simulation. On the other hand, the AP recovery was approximately 35% in the control and approximately 14% in the CHF simulation for a step disturbance of -60 mmHg, indicating that the efficiency of feedback AP regulation in the CHF simulation reduced to only 40% of that in the control simulation.

Figure 5d shows the initial slope of the AP recovery. The initial slope increased as the size of step disturbance increased in the control simulation. Although the initial slope of the AP recovery in the CHF simulation was comparable to that in the control simulation for a step disturbance of -10 mmHg, it did not increase significantly with an increase in the size of step disturbance.

Discussion

The major findings of the present study are (1) the dynamic characteristics of the baroreflex neural arc were preserved

in CHF, whereas those of the baroreflex peripheral arc were significantly depressed (Fig. 3), and (2) the total baroreflex gain at the closed-loop operating point seemed preserved in CHF, whereas the range of baroreflex operation was significantly narrowed (Fig. 4). Because of these modulations in the baroreflex characteristics, the AP regulation was less robust against exogenous disturbances in CHF (Fig. 5), which may partly explain the incidence of acute decompensation in stable CHF patients caused by noncompliance with salt and water restriction [19].

Dynamic characteristics of the carotid sinus baroreflex in CHF rats

The derivative characteristics of the neural arc are preserved in the CHF rats (Fig. 3a), being consistent with a previous study in heart failure rabbits [20]. Moreover, the present results indicate that the dynamic AP response to SNA is significantly depressed in CHF (Fig. 3b), suggesting impaired end-organ responses to SNA. The total baroreflex function in terms of the AP regulation was more sluggish in the CHF rats (Fig. 3c, bottom) despite the preserved neural arc derivative characteristics.

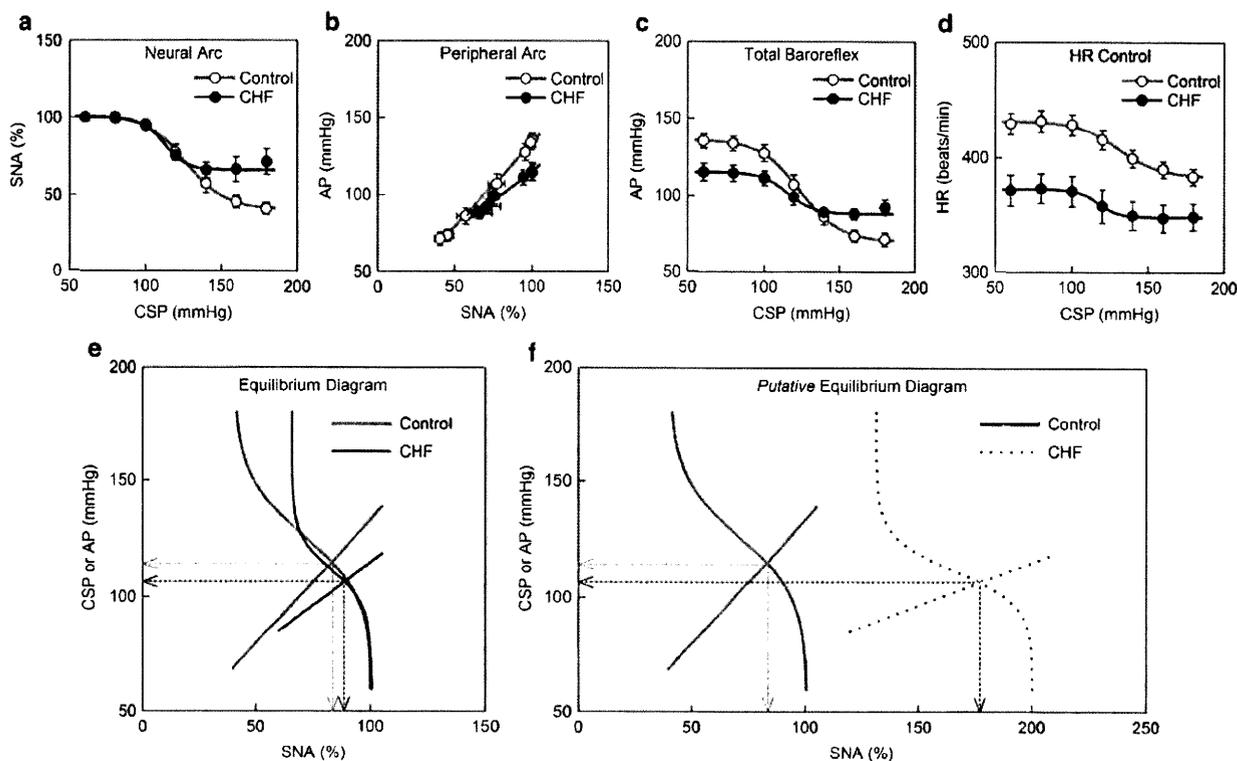


Fig. 4 Static characteristics of the carotid sinus baroreflex averaged for the control ($n = 12$) and chronic heart failure (CHF; $n = 7$) rats. **a** Static characteristics of the baroreflex neural arc. An increase in CSP decreases SNA. The response range in SNA is significantly attenuated in CHF. **b** Static characteristics of the baroreflex peripheral arc. An increase in SNA increases AP in a linear manner. The slope of the regression line is significantly gentler in CHF. **c** Static characteristics of the total baroreflex. CSP and AP show an inverse sigmoidal relationship. The response range of AP and the maximum gain are significantly smaller in CHF. **d** Static characteristics between CSP

and HR. CSP and HR show an inverse sigmoidal relationship. The response range of HR and the minimum HR are significantly smaller in CHF. **e** Baroreflex equilibrium diagram constructed from the fitted logistic function for the neural arc and the regression line for the peripheral arc. **f** Putative baroreflex equilibrium diagram in which the SNA axis is scaled so that the maximum absolute SNA in CHF becomes two times higher than that in control. In panels **e** and **f**, the dotted lines with arrowheads indicate the operating-point AP and SNA in CHF. The dash-dot lines with arrowheads indicate the operating-point AP and SNA in control

In the transfer function from CSP to HR, dynamic gain values were significantly attenuated in the CHF rats (Fig. 3d). Because the dynamic gain of the neural arc transfer function did not reduce significantly ($G_{0.1}$), the attenuation of the dynamic gain of the HR control may be attributable to the reduced HR response to SNA such as that related to the downregulation of β -adrenergic receptors [21].

Static characteristics of the carotid sinus baroreflex in CHF rats

In both the control and CHF rats, the input-output relationship of the peripheral arc approximated a straight line (Fig. 4b), and the sigmoidal nonlinearity is primarily attributed to the neural arc (Fig. 4a). The impairment of the total baroreflex in the CHF rats, characterized by a significant attenuation of the response range of AP and a reduction of the maximum gain (Fig. 4c, Table 3), is in

essence similar to that obtained previously in canine models of heart failure [3, 5, 6].

Wang et al. [4, 5] reported poor end-organ responses and normal central control of renal SNA in the heart failure dogs. The attenuated slope of the peripheral arc in the CHF rats (Fig. 4b) may be consistent with the poor end-organ responses. Although the response range of SNA in the neural arc was narrowed in the CHF rats (Fig. 4a), the maximum slope of the neural arc did not change significantly, which should contribute to the maintained total baroreflex gain within a small range around the operating point (Fig. 4e, Table 3).

The response range of HR was significantly reduced in the CHF rats (Fig. 4d) in agreement with the depressed dynamic HR response to CSP (Fig. 3d). Although 24-h averaged HR was higher in the CHF rats in a previous study [7], the minimum HR was significantly lower in the CHF than in the control rats. Anesthesia and vagotomy might have affected the results, and further studies are

Table 3 Parameters of the static characteristics of the carotid sinus baroreflex

	Control (<i>n</i> = 12)	CHF (<i>n</i> = 7)	<i>P</i> value
Neural arc			
P ₁ , response range (%)	62 ± 4	42 ± 6**	0.008
P ₂ , slope coefficient (mmHg ⁻¹)	0.10 ± 0.01	0.16 ± 0.03*	0.048
P ₃ , midpoint pressure (mmHg)	128 ± 4	115 ± 4*	0.043
P ₄ , minimum SNA (%)	39 ± 4	60 ± 6**	0.008
Maximum slope (% mmHg ⁻¹)	1.62 ± 0.27	1.54 ± 0.27	0.839
Peripheral arc			
<i>a</i> , slope (mmHg % ⁻¹)	1.10 ± 0.08	0.75 ± 0.10*	0.013
<i>b</i> , AP intercept (mmHg)	23.5 ± 7.7	40.7 ± 7.2	0.152
Total baroreflex			
P ₁ , response range (mmHg)	64 ± 4	31 ± 6**	<0.001
P ₂ , slope coefficient (mmHg ⁻¹)	0.10 ± 0.01	0.13 ± 0.01	0.144
P ₃ , midpoint pressure (mmHg)	122 ± 3	117 ± 4	0.326
P ₄ , minimum AP (mmHg)	74 ± 4	85 ± 3	0.065
Maximum gain	1.62 ± 0.22	0.95 ± 0.17*	0.048
Heart rate control			
P ₁ , response range (beats min ⁻¹)	49 ± 5	30 ± 6*	0.033
P ₂ , slope coefficient (mmHg ⁻¹)	0.10 ± 0.01	0.13 ± 0.01	0.099
P ₃ , midpoint pressure (mmHg)	131 ± 4	117 ± 4*	0.046
P ₄ , minimum HR (beats min ⁻¹)	383 ± 7	343 ± 13*	0.011
Maximum slope (beats min ⁻¹ mmHg ⁻¹)	1.28 ± 0.23	0.93 ± 0.20	0.313
Equilibrium diagram			
Operating-point AP (mmHg)	116 ± 3	106 ± 3*	0.042
Operating-point SNA (%)	84 ± 3	90 ± 4	0.227
Operating-point total baroreflex gain	1.23 ± 0.28	0.96 ± 0.39	0.578

Data are presented as mean ± SE

** *P* < 0.01 and **P* < 0.05 by unpaired *t* test

needed to reconcile some of the discrepancies between the present and previous results.

Equilibrium diagram and simulation study

A baroreflex equilibrium diagram provides information on the closed-loop operating point (Fig. 4e) [10, 17, 18, 22–26]. Because absolute SNA is considered to be higher in CHF [7], if we can use the absolute value for SNA, the SNA axis of the equilibrium diagram should be scaled in a manner that renders the peripheral arc much shallower in the CHF than in the control rats (Fig. 4f). The operating-point AP was decreased only by 10 mmHg in the CHF rats and the total baroreflex gain at the operating point did not differ between the control and CHF rats. The baroreflex function may therefore seem preserved in the CHF rats when it is assessed only within a small range around the closed-loop operating point.

We used splanchnic SNA to construct a baroreflex equilibrium diagram. The analysis based on SNA of only one limb of the sympathetic nervous system can be an oversimplification in light of the well-known regional differences in SNAs [27–29]. One rationale for using splanchnic SNA is that the splanchnic region has been

regarded as a major site for the blood flow redistribution [17, 30]. Furthermore, we have continued to observe insignificant differences in the steady-state responses to CSP between left and right cardiac SNAs [31], between cardiac and renal SNAs [32], and among cardiac, renal, and muscle SNAs [33] in anesthetized rabbits. It seems that the carotid sinus baroreflex brings about common activity in addition to regional activity among sympathetic nerves innervating several districts (see Appendix 3). Whether the similarity among SNAs to different districts will hold in other experimental conditions awaits future investigations.

The simulation study is useful and necessary for the integrated understanding of the estimated dynamic and static characteristics of the carotid sinus baroreflex (Fig. 5a). The feedback AP regulation becomes much weaker in CHF as the size of step disturbance increases (Fig. 5c, d), suggesting that a reserve for AP buffering is lost in CHF. Because not only the baroreceptor input but also loading conditions to the heart are changed during orthostatic tilt, the results of the present simulation can not be directly extrapolated to the actual tilting conditions. However, it may be of notice that patients with severe congestive heart failure show a significant drop in systolic AP upon orthostatic tilt regardless of relatively maintained

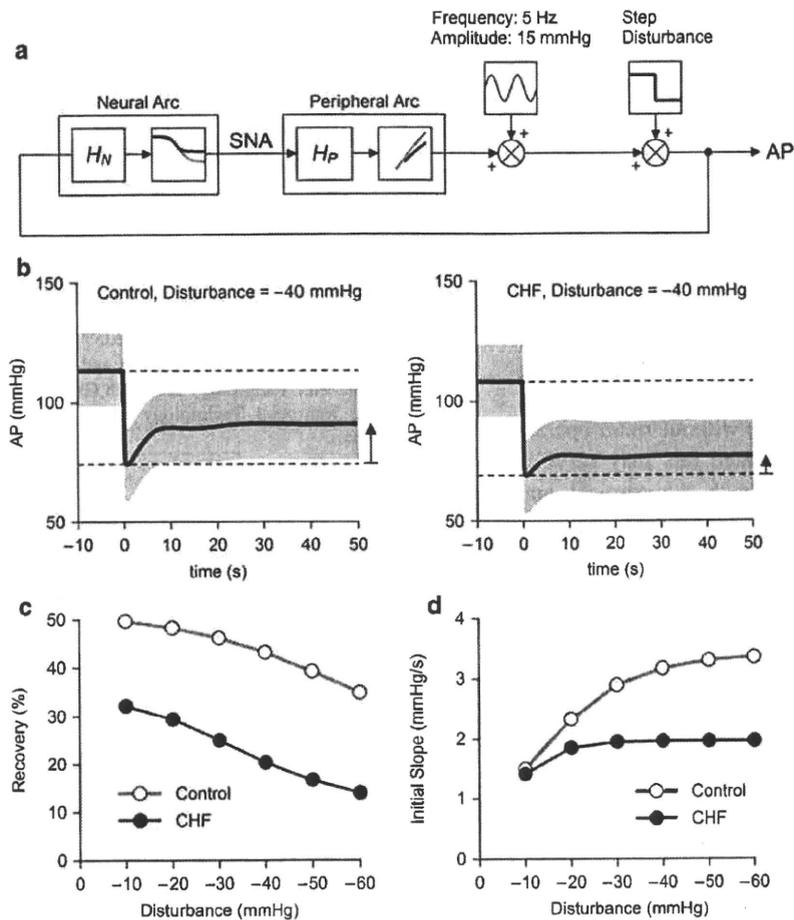


Fig. 5 **a** Block diagram for the simulation of AP regulation. H_N and H_P represent dynamic characteristics of the neural and peripheral arcs, respectively. The static characteristics of the neural arc were modeled as a logistic function. The static characteristics of the peripheral arc were modeled as a straight line. Parameters for the logistic function and the regression line were derived from mean values shown in Table 3. A 5-Hz sine wave with an amplitude of 15 mmHg was added to mimic pulsatile pressure. Exogenous step disturbances ranging from -10 to -60 mmHg were applied, and the closed-loop AP

responses were calculated for control and CHF conditions. **b** Typical simulation results during an exogenous pressure disturbance of -40 mmHg. The pulsatile pressure is shown in gray, and mean AP signal is shown as a bold line. The horizontal dotted lines indicate the mean AP values just before and after the onset of step disturbance. AP recovers less efficiently in the CHF simulation compared to the control simulation. **c** Percent recovery of AP as a function of the size of disturbance. **d** The initial slope of the recovery as a function of the size of disturbance

baseline AP [34]. The exogenous disturbance in the opposite direction may also be hazardous. Even though baseline AP appears normal in stable CHF patients, non-compliance with salt and water restriction may easily collapse the AP regulation and induce acute decompensation [19].

Study perspective

The progression of CHF is closely associated with the autonomic imbalance between sympathetic and vagal nerve activities. Although pharmacological treatments such as β -adrenergic blockers [35], angiotensin converting enzyme inhibitors [36], and angiotensin receptor blockers [37] have been developed, the therapeutic outcome is not always

satisfactory and the mortality rate for CHF remains high. Novel therapeutic strategies for CHF beyond conventional treatments such as electrical vagal nerve stimulation are expected to be developed [7, 38].

Although the mechanisms for the autonomic imbalance in CHF are not completely understood, the loss of baroreflex control of the sympathetic and vagal systems has been considered an important factor [39, 40]. Zucker et al. [41] demonstrated that chronic baroreceptor activation improved the survival of dogs with pacing-induced heart failure. The study seems rudimentary, however, in that the stimulation intensity of the carotid sinus baroreceptors was adjusted intermittently to obtain a prescribed sympathoinhibitory response. The stimulation could be too strong or too weak during the intervals of adjustments. If the

stimulation intensity is feedback-controlled continuously, the therapeutic effect and safety could be enhanced.

Quantification of the dynamic characteristics of a target system is the first step for developing a robust controller [42–44]. Next, the controller should be designed in such a way that the variation of the target system dynamics does not affect the controlling result much [45]. The simulation of the closed-loop AP response in CHF, such as that shown in Fig. 5, will be utilized in designing a robust controller system of the carotid sinus activation.

Limitations

First, we used normal rats without sham operation as the control group. Sham-operated rats may serve as a more appropriate control. Although the surgical operation of thoracotomy itself could have affected the baroreflex function in the CHF group, because over 100 days had elapsed after the surgical operation, the effect of surgical operation might have been limited. Actually, two rats that underwent coronary ligation but did not meet the criteria for CHF retained the maximum gain of the total baroreflex close to the control group (rat A: central venous pressure = 2.28 mmHg, biventricular weight = 1.94 g kg⁻¹, total baroreflex gain = 1.61; rat B: central venous pressure = 1.69 mmHg, biventricular weight = 2.33 g kg⁻¹, total baroreflex gain = 1.67).

Second, the data were obtained under anesthetic conditions. Because the anesthesia affects autonomic nervous activities, the results should be carefully interpreted. Vagotomy and lower perfusion of the brain due to carotid artery occlusion might have also affected the baroreflex function. Nevertheless, the present results would provide a unique clue to integrated understanding of the impaired baroreflex function in CHF.

Third, we did not measure cardiac output or peripheral vascular resistance. Subdividing the peripheral arc into the cardiac and vascular components will be necessary to identify the mechanisms for an approximately linear relationship between SNA and AP in both the control and CHF rats.

Conclusions

The dynamic and static characteristics of the neural arc, peripheral arc, and total baroreflex were analyzed comprehensively in rats with CHF after myocardial infarction. Although the derivative characteristics of the baroreflex neural arc are preserved in the CHF rats, the dynamic AP regulation is depressed in both the magnitude and response speed. The equilibrium diagram indicates that the baroreflex gain may seem preserved in CHF in a small range

around the closed-loop operating point. However, the percent recovery of AP and the speed of recovery are reduced progressively as the size of exogenous disturbance increases in the CHF rats. The reserve for AP buffering may be lost in CHF despite the relatively maintained baseline AP.

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Appendix 1: feature of a Gaussian white noise input

The neural arc of the arterial baroreflex may be roughly modeled by a cascade system, which consists of a dynamic linear subsystem followed by a static nonlinear subsystem [14]. In this type of system, the selection of the mean input pressure and the amplitude of input does not significantly affect the estimation of the system linear dynamics except for a factor of proportionality when the system is tested with a Gaussian white noise (GWN) signal [46]. In order to demonstrate the above notion, we performed a simulation study. In reference to Fig. 6a, the dynamic linear subsystem for the baroreflex neural arc was modeled by the following equation:

$$H_N(f) := \frac{1 + \frac{f}{f_{c1}j}}{\left(1 + \frac{f}{f_{c2}j}\right)^2} \exp(-2\pi f j L) \quad (5)$$

where f_{c1} and f_{c2} are the corner frequencies determining the frequency-dependent changes in the dynamic gain, L denotes the pure dead time, f and j indicate frequency and an imaginary unit, respectively. We set $f_{c1} = 0.1$ Hz, $f_{c2} = 0.9$ Hz, and $L = 0.1$ s to mimic the neural arc transfer function. The static nonlinear subsystem of the neural arc was modeled by the logistic function (Eq. 3) with parameters of $P_1 = 60\%$, $P_2 = 0.1$ mmHg⁻¹, $P_3 = 120$ mmHg, and $P_4 = 40\%$. Using this model, the linear input-output relationship was estimated by a GWN input or a binary white noise (BIN) input. Mean input pressure was changed among 120, 90, and 150 mmHg (P_3 , $P_3 - 30$, and $P_3 + 30$ mmHg).

Figure 6b through 6d shows the estimation results. In each panel, thin smooth curves in the gain and phase plots indicate the transfer function of the dynamic linear subsystem described by Eq. 5. The solid bold curves, dotted thin curves, and dashed thin curves represent the estimation

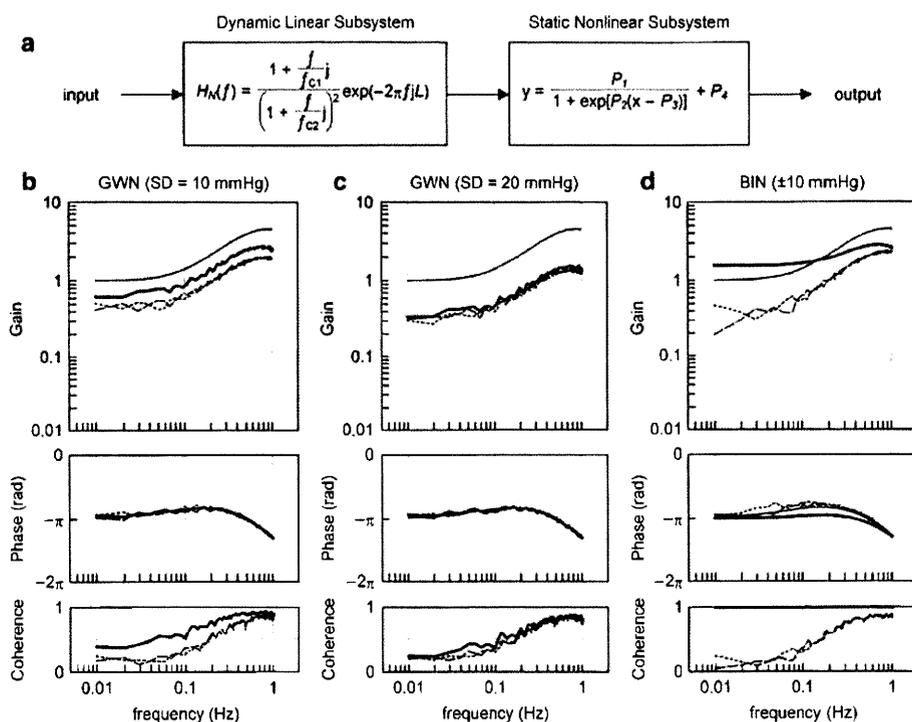


Fig. 6 a A cascade model for the baroreflex neural arc, which consists of a dynamic linear subsystem and a static nonlinear subsystem. The dynamic linear subsystem represents the neural arc transfer function. The static nonlinear subsystem represents the sigmoidal nonlinearity for the neural arc. **b** Estimation results of the system dynamic characteristics using a Gaussian white noise (GWN) input with a standard deviation (SD) of 10 mmHg. **c** Estimation results of the system dynamic characteristics using a GWN input with

an SD of 20 mmHg. **d** Estimation results of the system dynamic characteristics using a binary white noise (BIN) input with an amplitude of 10 mmHg. In panels **b** through **d**, the *thin smooth curve* indicates the transfer function of the given dynamic linear subsystem. The *solid bold curve*, *dotted thin curve*, and *dashed thin curve* represent the estimation results obtained by the input signals with mean input pressures of 120, 90, and 150 mmHg, respectively (see text for explanation)

results obtained by mean input pressures of 120, 90, and 150 mmHg, respectively. Figure 6b and c represents the estimation results obtained by GWNs with standard deviations of 10 and 20 mmHg, respectively. As can be seen, the estimated gain plots are nearly parallel to the gain plot of the given dynamic linear subsystem. The estimated phase plots are superimposable on the phase plot of the given dynamic linear subsystem.

Figure 6d shows the estimation results obtained by BINs with an amplitude of 10 mmHg (i.e., the peak-to-peak pressure of 20 mmHg). None of the estimated gain plots are parallel to the gain plot of the given dynamic linear subsystem. The estimated phase plots also deviated from the phase plot of the given dynamic linear subsystem. When the system was tested by the BIN input with the mean input pressure of 120 mmHg, the coherence value became close to unity, suggesting that the BIN input had caused on-off behavior in the system output.

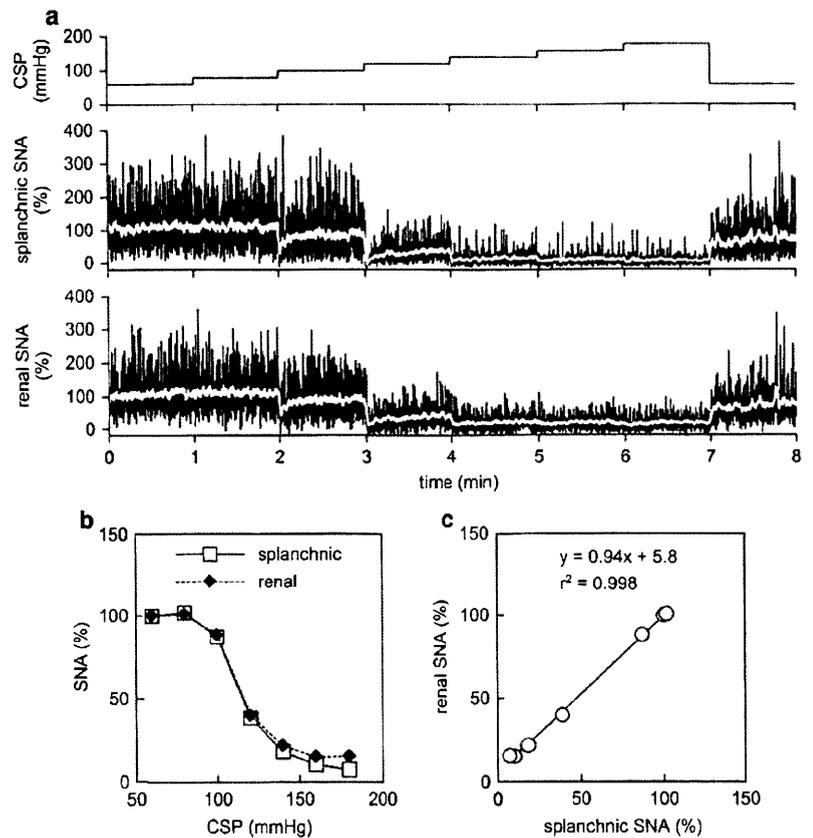
The above simulation results confirm that the selection of the mean input pressure and the amplitude of GWN does

not significantly affect the estimation of the dynamic characteristics of the system except for a factor of proportionality when the mean input pressure and the standard deviation of GWN are not far beyond the system operating range.

Appendix 2: estimation of the initial slope of the step response or recovery response

To characterize the speed of the response, the initial slope of the response was calculated as follows. First, a response threshold is determined as the 5% value of the steady-state step response. In the case of the recovery response, 5% of the maximum recovery is used as the threshold. Next, an initial time point at which the response exceeds the threshold value is determined. Starting from this initial time point, a linear regression analysis giving a slope and an intercept is performed repeatedly while increasing the number of analyzed data points. The steepest slope thus

Fig. 7 a Typical recordings of 10-Hz decimated CSP, splanchnic SNA, and renal SNA obtained from a normal rat in an additional study using the same experimental settings as the main study. The white lines in the SNA recordings represent 2-s moving averaged signals. An increase in CSP decreased splanchnic and renal SNAs. **b** Static characteristics of the baroreflex neural arc drawn based on splanchnic and renal SNAs. **c** Correlation between splanchnic and renal SNAs during the stepwise input protocol. In panels **b** and **c**, splanchnic and renal SNA data were obtained as the averaged values during the last 10 s at each CSP level



obtained is used as the initial slope of the response. By setting the 5% threshold, the linear regression can be performed excluding the initial data points constituting the dead time.

Appendix 3: comparison of splanchnic and renal SNAs under the present experimental conditions

We compared splanchnic and renal SNAs under the present experimental conditions in an additional three rats. A branch of the right renal sympathetic nerve was exposed through a right flank incision, and renal SNA was recorded simultaneously with splanchnic SNA. Figure 7a presents time series of 10-Hz decimated CSP, splanchnic SNA, and renal SNA obtained from one rat. White lines in splanchnic and renal SNAs indicate 2-s moving averaged signals. In each of the splanchnic and renal SNAs, a 10-s averaged value for the last 10 s at CSP of 60 mmHg was defined as 100%, and a 10-s averaged value after the administration of hexamethonium bromide was defined as 0%, in the same manner as the main study. Figure 7b shows the relationships of SNAs versus CSP. Although the renal SNA values were slightly greater than the splanchnic SNA values at CSPs of 160 and 180 mmHg in this rat, the general

sigmoidal relationships were similar between the two SNAs. The regression line for renal SNA (y) versus splanchnic SNA (x) was $y = 0.94x + 5.8$ ($r^2 = 0.998$) in this rat (Fig. 7c). The other two rats showed regression lines of $y = 1.01x + 0.3$ ($r^2 = 0.999$) and $y = 0.99x + 3.7$ ($r^2 = 0.995$), suggesting that both splanchnic and renal SNAs can represent common activity for the AP regulation during the stepwise input protocol under the present experimental conditions.

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Experimental Physiology – Research Paper

Dynamic characteristics of heart rate control by the autonomic nervous system in rats

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We estimated the transfer function of autonomic heart rate (HR) control by using random binary sympathetic or vagal nerve stimulation in anaesthetized rats. The transfer function from sympathetic stimulation to HR response approximated a second-order, low-pass filter with a lag time (gain, 4.29 ± 1.55 beats min^{-1} Hz^{-1} ; natural frequency, 0.07 ± 0.03 Hz; damping coefficient, 1.96 ± 0.64 ; and lag time, 0.73 ± 0.12 s). The transfer function from vagal stimulation to HR response approximated a first-order, low-pass filter with a lag time (gain, 8.84 ± 4.51 beats min^{-1} Hz^{-1} ; corner frequency, 0.12 ± 0.06 Hz; and lag time, 0.12 ± 0.08 s). These results suggest that the dynamic characteristics of HR control by the autonomic nervous system in rats are similar to those of larger mammals.

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Despite extensive use of rats in cardiovascular research, the dynamic characteristics of heart rate (HR) control by the autonomic nervous system in this species remain to be elucidated. To better understand the autonomic control of HR in rats, it is important to quantitatively assess the input–output relationship between autonomic nerve stimulation and HR over a wide range of frequencies that are of physiological interest. By understanding these relationships in the rat, data obtained using this animal model may be more readily extrapolated to larger mammals, including humans.

Heart rate variability is considered to reflect autonomic tone because its components change both physiologically (e.g. standing and ageing) and pathophysiologically (e.g. hypertension and heart failure; Malliani *et al.* 1991). Based on dog and human studies, the very low-frequency (VLF) component (0.02–0.08 Hz) is likely to reflect changes in vasomotor tone in relation to thermoregulation and local adjustment of resistance in individual vascular beds; the low-frequency (LF) component (0.08–0.15 Hz) is considered to be Mayer's wave and a marker of sympathetic activity; and the high-frequency (HF) component (0.15–0.40 Hz) mainly originates from respiratory activity and is considered to be mediated by vagal activity (Pagani *et al.*

1986). Based on the differences in HR spectra between conscious rats and rats in which autonomic blockade was induced pharmacologically, Cerutti *et al.* (1991) determined that the VLF component ranged between 0.017 and 0.26 Hz, the LF component ranged between 0.27 and 0.74 Hz, and the HF component was above 0.75 Hz. Even though these allocations in frequency band in rats corresponded to their considerably higher basal HR (range between 300 and 400 beats min^{-1}) compared with that of larger mammals, such as dogs and humans (range between 60 and 100 beats min^{-1}), there has been no scientific rationale provided for setting these allocations. This is because it is unknown whether the dynamic characteristics of HR control by the autonomic nervous system in rats are significantly different from those in larger mammals, such as rabbits (Kawada *et al.* 1996), cats (Chess & Calaresu, 1971) and dogs (Berger *et al.* 1989).

Given that the release and disposition of neurotransmitters (e.g. noradrenaline and acetylcholine) at autonomic nerve endings may be determined biochemically, regardless of body size, we hypothesized that the dynamic characteristics of HR control by the autonomic nervous system would not differ appreciably among different mammalian species. To test this

hypothesis, we quantified the dynamic characteristics of HR control mediated by sympathetic or vagal nerve stimulation in rats using transfer function analysis. The results provide the first quantitative data on the dynamic characteristics of autonomic HR regulation in rats. Since HR changes dynamically in response to daily activities, quantification of how quickly the HR can respond to sympathetic or vagal nerve stimulation is important. For instance, information on the dynamic HR response is key to understanding the generation of HR variability. The present study aims to expand our knowledge of HR control by the autonomic nervous system.

Methods

Surgical preparation

Animal care was in accordance with the *Guiding Principles for 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 Subjects Committee of the National Cerebral and Cardiovascular Center. Thirteen Sprague–Dawley rats (body weight, 340–670 g) were anaesthetized using a mixture of urethane (250 mg ml⁻¹; Sigma, St. Louis, MO, USA) and α -chloralose (40 mg ml⁻¹; Sigma), initiated with an intraperitoneal bolus injection of 1 ml kg⁻¹. If additional anaesthesia was needed, 0.1 ml kg⁻¹ was given intraperitoneally. The rats were intubated and mechanically ventilated with oxygen-enriched room air. The rats were slightly hyperventilated to suppress chemoreflexes (arterial P_{CO_2} ranged from 30 to 35 mmHg; arterial P_{O_2} > 300 mmHg). Arterial blood pH was within the physiological range. A catheter was placed in the right femoral artery, which was connected to a pressure transducer (DX-200, Nihon Kohden, Tokyo, Japan) to measure arterial pressure (AP). Heart rate was measured using a cardiometer (AT601G, Nihon Kohden, Tokyo, Japan) triggered by the R wave on the electrocardiogram. The HR series were checked by visual inspection. A catheter was introduced into the right femoral vein for drug administration. Sino-aortic denervation was performed bilaterally to minimize changes in the sympathetic efferent nerve activity via arterial baroreflexes. The vagi were sectioned bilaterally at the neck. A pair of bipolar stainless-steel electrodes was attached to the right cervical sympathetic nerve for efferent sympathetic stimulation or the right cervical vagus for efferent vagal stimulation. The stimulation electrodes and nerve were secured with silicon glue (Kwik-Sil, World Precision Instruments, Sarasota, FL, USA). Body temperature was monitored with a thermometer placed in the rectum, and was maintained at 38°C with a heating pad throughout the experiment.

Experimental procedures

The pulse duration was set at 2 ms and the stimulation amplitude was fixed at 10 V for both sympathetic and vagal nerve stimulation. To allow stable haemodynamics, sympathetic and vagal nerve stimulation was started at ~1 h after the end of surgical preparation. Between sympathetic and vagal stimulation protocols, >15 min elapsed to allow AP and HR to return to their respective baseline values.

To estimate the dynamic transfer characteristics from sympathetic stimulation to the HR response, the sectioned end of the right cervical sympathetic nerve was stimulated, employing a frequency-modulated pulse train for 10 min. The stimulation frequency was switched every 1000 ms to either 0 or 5 Hz, according to a binary white-noise signal. The power spectrum of the stimulation signal was reasonably constant up to 0.5 Hz. The transfer function was estimated up to 0.5 Hz because the reliability of estimation decreased owing to the diminution of input power above this frequency. The selected frequency range was determined based on previous results in rabbits (Kawada *et al.* 1996) so that it would sufficiently span the physiological range of interest with respect to the dynamic sympathetic control of HR.

To estimate the dynamic transfer characteristics from vagal stimulation to the HR response, the right vagus was stimulated, employing a frequency-modulated pulse train for 10 min. The stimulation frequency was switched every 500 ms to either 0 or 10 Hz, according to a binary white-noise signal. The power spectrum of the stimulation signal was reasonably constant up to 1 Hz. The transfer function was estimated up to 1 Hz because the reliability of estimation decreased owing to the diminution of input power above this frequency. The selected frequency range was determined based on previous results in rabbits (Kawada *et al.* 1996) so that it would sufficiently span the physiological range of interest with respect to the dynamic vagal control of HR.

The switching intervals differed between sympathetic and vagal stimulation for the following reasons. In theory, the switching interval of binary white noise is inversely related to an upper frequency bound for the input modulation frequency. Prior knowledge in rabbits (Kawada *et al.* 1996) and preliminary results in rats indicated that the upper frequency bound of physiological interest might be lower for the sympathetic transfer function, which rendered the switching interval longer for the sympathetic stimulation. Another reason relates to the stimulation frequency. In the present study, the sympathetic stimulation frequency of 5 Hz and the vagal stimulation frequency of 10 Hz were determined based on the HR response. Once the stimulation frequency is determined, there is a methodological limitation for setting the switching interval. For example, a switching

interval of 500 ms is too close to a pulse interval of 200 ms (i.e. 5 Hz stimulation). In this case, only two pulses can be applied in the shortest interval of 500 ms, which means that the stimulation frequency may be in effect 4 Hz rather than the intended 5 Hz. This problem does not occur with a 1000 ms switching interval in combination with 5 Hz stimulation and 500 ms switching interval in combination with 10 Hz stimulation.

Background sympathetic tone is known to augment vagal HR control (an accentuated antagonism; Levy, 1971; Kawada *et al.* 1996). To eliminate any effect of sympathetic activity, in seven out of the thirteen rats the vagal stimulation protocol was repeated after the administration of the β -adrenergic blocker propranolol (1 mg kg⁻¹ i.v.; Perlini *et al.* 1995).

Data analysis

Data were digitized at 200 Hz using a 12 bit analog-to-digital converter and stored on the hard disk of a dedicated laboratory computer system. The dynamic transfer function from binary white-noise stimulation to the HR response was estimated based on the following procedure. To avoid the possibility that the initial transition from no stimulation to random stimulation biased the transfer function estimation, data were processed only from 2 min after the initiation of random stimulation. Input–output data pairs of the stimulation frequency and HR were resampled at 10 Hz. Subsequently, data pairs were partitioned into eight 50%-overlapping segments consisting of 1024 data points each. For each segment, the linear trend was subtracted and a Hanning window was applied. A fast Fourier transform was then performed to obtain the frequency spectra of nerve stimulation [$N(f)$] and HR [$HR(f)$]. Over the eight segments, the power of the nerve stimulation [$S_{N,N}(f)$], the power of the HR [$S_{HR,HR}(f)$], and the cross-power between these two signals [$S_{N,HR}(f)$] were ensemble averaged. Finally, the transfer function [$H(f)$] from nerve stimulation to the HR response was determined using the following equation (Marmarelis & Marmarelis, 1978):

$$H(f) = \frac{S_{N,HR}(f)}{S_{N,N}(f)} \quad (1)$$

To quantify the linear dependence of the HR response on vagal or sympathetic stimulation, the magnitude-squared coherence function [$Coh(f)$] was estimated by employing the following equation (Marmarelis & Marmarelis, 1978):

$$Coh(f) = \frac{|S_{N,HR}(f)|^2}{S_{N,N}(f) \times S_{HR,HR}(f)} \quad (2)$$

Coherence values range from zero to unity. Unity coherence indicates perfect linear dependence between

the input and output signals; in contrast, zero coherence indicates total independence between the two signals.

As preliminary results suggested that the transfer function from sympathetic stimulation to HR response in rats approximated a second-order low-pass filter with pure delay, as in the case of rabbits (Kawada *et al.* 1996, 2009), we determined the parameters of the sympathetic transfer function using the following equation:

$$H(f) = \frac{K}{1 + 2\zeta\frac{f}{f_N}j + \left(\frac{f}{f_N}j\right)^2} e^{-2\pi f j L} \quad (3)$$

where K is dynamic gain (in beats per minute per herz), f_N is the natural frequency (in herz), ζ is the damping ratio, L is lag time (in seconds), and f and j represent frequency and imaginary units, respectively. The dynamic gain (K , in beats per minute per herz) represents the asymptotic value of transfer gain as the frequency approaches zero, and corresponds to the steady-state response in the step response. The natural frequency (f_N , in herz) determines the frequency limit of the low-pass filter above which the transfer gain reduces as the frequency increases. In the second-order low-pass filter, the maximal negative slope of the gain diminution is 1/100 per 10 Hz. The damping ratio (ζ , unitless) characterizes how the transfer gain varies around the f_N . The lag time (L , in seconds) indicates the latency of signal transmission from nerve stimulation to the initiation of HR response. These parameters were estimated by means of an iterative non-linear least-squares regression.

As preliminary results suggested that the transfer function from vagal stimulation to HR response in rats approximated a first-order low-pass filter with pure delay, as in the case of rabbits (Kawada *et al.* 1996, 2009), we determined the parameters of the vagal transfer function using the following equation:

$$H(f) = \frac{-K}{1 + \frac{f}{f_c}j} e^{-2\pi f j L} \quad (4)$$

where K represents the dynamic gain (in beats per minute per herz), f_c denotes the corner frequency (in herz), L denotes the lag time (in seconds), and f and j represent frequency and imaginary units, respectively. The negative sign in the numerator indicates the negative HR response to vagal stimulation. The dynamic gain (K , in beats per minute per herz) represents the asymptotic value of transfer gain as the frequency approaches zero. The corner frequency (f_c , in herz) represents the frequency at which the transfer gain decreases by 3 dB relative to K . Higher f_c indicates the more rapid HR response to vagal stimulation. In the first-order low-pass filter, the maximal slope of the gain diminution is 1/10 per 10 Hz. The lag time (L , in seconds) indicates the latency of signal transmission from nerve stimulation to the initiation of HR response. These

parameters were estimated by means of an iterative non-linear least-squares regression.

To facilitate the intuitive understanding of the system's dynamic characteristics, we calculated the system step response of HR to 1 Hz nerve stimulation as follows. The system impulse response was derived from the inverse Fourier transform of $H(f)$. The system step response was then obtained from the time integral of the impulse response. The length of the step response was 51.2 s. The 80% rise time for sympathetic step response or the 80% fall time for vagal step response was estimated at the time which the step response reached 80% of the steady-state response, calculated by averaging the last 10 s of data from the step response.

Statistical analysis

All data are represented as means \pm s.d. Student's paired *t* test was used to test differences in haemodynamic parameters. In seven rats, parameters of vagal transfer function were compared before and after propranolol administration using Student's paired *t* test. Values of $P < 0.05$ were considered significant.

Results

Typical recordings

Figure 1 shows typical recordings of sympathetic and vagal stimulation obtained from one rat. While HR varied immediately during random vagal stimulation, it changed only gradually during random sympathetic stimulation. Sympathetic stimulation increased the mean HR from

375.5 ± 33.5 to 444.9 ± 28.4 beats min^{-1} ($P < 0.05$); vagal stimulation decreased the mean HR from 378.2 ± 31.9 to 317.3 ± 47.1 beats min^{-1} ($P < 0.05$). Neither sympathetic nor vagal stimulation altered mean AP (79.1 ± 18.4 versus 75.1 ± 19.4 and 78.2 ± 20.2 versus 72.4 ± 18.3 mmHg, respectively).

Sympathetic transfer function

Figure 2A illustrates the dynamic transfer function characterizing sympathetic HR control averaged from all animals. Gain plots, phase plots and coherence functions are shown. The sympathetic transfer function approximated a second-order, low-pass filter with a lag time (dynamic gain, 4.29 ± 1.55 beats $\text{min}^{-1} \text{Hz}^{-1}$; natural frequency, 0.07 ± 0.03 Hz; damping coefficient, 1.96 ± 0.64 ; and lag time, 0.73 ± 0.12 s). Figure 2B shows the calculated step response of HR to sympathetic stimulation averaged from all animals. As expected, sympathetic stimulation gradually increased HR. The steady-state sympathetic response was 3.86 ± 1.80 beats $\text{min}^{-1} \text{Hz}^{-1}$. The 80% rise time for the sympathetic step response was 13.7 ± 5.1 s.

Vagal transfer function

Figure 2C illustrates the dynamic transfer function characterizing vagal HR control averaged from all animals. The vagal transfer function approximated a first-order, low-pass filter with a lag time (dynamic gain, 8.84 ± 4.51 beats $\text{min}^{-1} \text{Hz}^{-1}$; corner frequency, 0.12 ± 0.06 Hz; and lag time, 0.12 ± 0.08 s). Figure 2D shows the calculated step response of HR to vagal

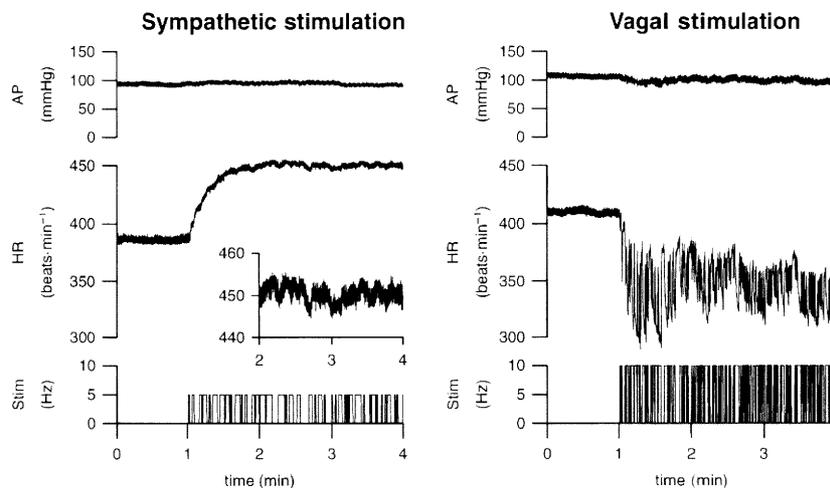


Figure 1. Raw trace of 10 Hz resampled arterial pressure (AP; top) and heart rate (HR; middle) obtained using binary white-noise stimulation (Stim; bottom)

Recordings are shown for sympathetic (left) and vagal nerve stimulation (right). The inset in the panels for sympathetic stimulation indicates the expanded ordinate for the HR response.

stimulation averaged from all animals. As expected, vagal stimulation decreased HR. The vagal HR response appeared more quickly than the sympathetic HR response. The steady-state vagal response was 9.63 ± 5.21 beats min^{-1} Hz^{-1} . The 80% fall time for the vagal step response was 4.13 ± 1.74 s.

β -Adrenergic blockade did not significantly affect any parameters of the vagal transfer function (dynamic gain, 7.96 ± 2.97 versus 7.75 ± 2.49 beats min^{-1} Hz^{-1} ; corner frequency, 0.12 ± 0.05 versus 0.11 ± 0.05 Hz; and lag time, 0.10 ± 0.08 versus 0.17 ± 0.08 s, before and after β -adrenergic blockade, respectively).

Discussion

We have demonstrated the first quantitative data on the dynamic characteristics of HR control by the sympathetic

and vagal nerves in rats. These results suggest that the dynamic characteristics of autonomic HR control in rats are similar to those of larger mammals despite basal HR being higher in these animals.

Sympathetic transfer function

In the present study, we stimulated the preganglionic cardiac sympathetic nerve instead of the postganglionic cardiac sympathetic nerve because of technical reasons. Therefore, the sympathetic transfer function was estimated from the combined dynamic characteristics of ganglionic and postganglionic transmission. Nevertheless, the sympathetic transfer function approximated a second-order delay system (Fig. 2A) and its parameters were similar to those estimated between postganglionic sympathetic stimulation and HR response in rabbits

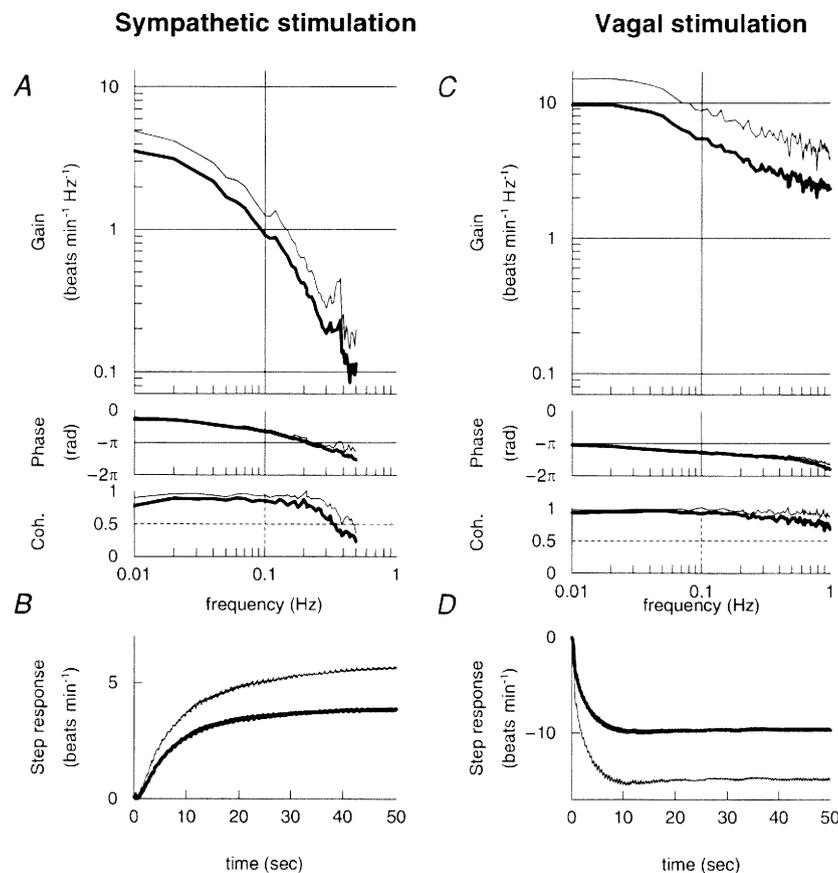


Figure 2. Transfer functions and step responses of HR to sympathetic and vagal stimulation

A, dynamic transfer function relating sympathetic stimulation to the HR response averaged from all animals. Gains (top), phase shifts (middle) and coherence functions (bottom) are presented. B, calculated step response to 1 Hz tonic sympathetic stimulation averaged from all animals. C, dynamic transfer function relating vagal stimulation to the HR response averaged from all animals. Gains (top), phase shifts (middle) and coherence functions (bottom) are presented. D, calculated step response to 1 Hz tonic vagal stimulation averaged from all animals. Thick lines represent the mean, whereas thin lines indicate \pm s.d. values.

(natural frequency, 0.06–0.07 Hz; damping coefficient, 1.34–1.53; and lag time, 0.51–0.65 s; Kawada *et al.* 2009). If we calculate the corner frequency (frequency at which gain decreased by 3 dB from steady-state gain; 0.04 ± 0.01 Hz) instead of natural frequency, our results are comparable to the corner frequency (0.01–0.02 Hz) found by Berger *et al.* (1989) using dogs. Thus, in the present study sympathetic transfer function in rats resembles that of dogs. The 80% rise time for sympathetic step response is also comparable to that of rabbits (14.4–17.2 s) as reported by Kawada *et al.* (2009). Therefore, we contend that the dynamic characteristics in ganglionic transmission contribute little to the determination of the overall low-pass characteristics of sympathetic HR control.

Vagal transfer function

The transfer function of vagal stimulation to HR response can be characterized as a first-order delay system (Fig. 2C), as in previous reports using rabbits (Kawada *et al.* 1996), dogs (Berger *et al.* 1989) and cats (Chess & Calaresu, 1971). The estimated corner frequency of the transfer function from vagal stimulation to HR response in the present study (0.12 ± 0.06 Hz) is different but of the same order as that of rabbits (0.39 Hz; Kawada *et al.* 2009) and cats (0.05 Hz; Chess & Calaresu, 1971). However, if we calculate the corner frequency as the frequency at which gain decreases by 3 dB from steady-state gain, the value is 0.06 ± 0.01 Hz. Furthermore, the 80% fall time for the vagal step response in rats is also greater than that in rabbits (1.3 s; Kawada *et al.* 2009). Hence, the first-order low-pass filter may not be the best approximation of the transfer function from vagal stimulation to HR response in rats, in contrast to the case in rabbits (Kawada *et al.* 1996, 2009). Furthermore, Berger *et al.* (1989) demonstrated that the effective filter characteristics of the system depend on the mean level of stimulatory rates. Additional studies are needed to clarify some of the disparity in results between the present investigation and other reports. Overall, however, the dynamic transfer function characteristics of HR regulation by the vagus were not appreciably different in rats compared with larger mammals.

Although sino-aortic denervation should have minimized the baroreflex-mediated changes in sympathetic nerve activity, this procedure does not necessarily eliminate the background sympathetic tone. It should be noted that tonic sympathetic stimulation may increase the dynamic gain of the vagal transfer function via accentuated antagonism (Kawada *et al.* 1996). The results of propranolol administration indicated that the effects of background sympathetic tone on the dynamic vagal control of HR were insignificant in the present study.

Physiological implications and perspectives

As mentioned in the Introduction, the allocations in frequency band of HR variability in rats (Cerutti *et al.* 1991) are set in a higher frequency band compared with those of larger mammals, such as dogs and humans (Malliani *et al.* 1991). However, parameters of dynamic characteristics in HR control by the autonomic nervous system in rats were not much different from those reported in larger mammals, suggesting the importance of biochemical processes of neurotransmitter release and disposition in determining dynamic HR response. Therefore, the allocations of HR variability in the HF component in rats may not readily be explained in terms of the dynamic characteristics of HR response to sympathetic and vagal nerve stimulation. Nevertheless, the fact that the vagal HR control is much faster than the sympathetic HR control may contribute to the generation of frequency dependence of HR variability.

To simply identify the dynamic characteristics of HR control by the autonomic nervous system in rats, the present study used Sprague–Dawley rats as normal animals. It has been well documented that exercise training results in increased vagal tone (Coote, 2010) and that pathophysiological conditions, such as chronic heart failure and hypertension, result in increased basal sympathetic tone (Billman, 2009) compared with the normal conditions. Further investigations are needed to clarify the impacts of such physiological and pathophysiological conditions on dynamic characteristics of HR control by the autonomic nervous system in rats.

The absolute gain values of the vagal transfer function seem higher than those previously reported in rabbits (Kawada *et al.* 1996, 2009). In those previous studies, the amplitude of vagal stimulation was adjusted in each animal so that HR was decreased by approximately $50 \text{ beats min}^{-1}$ with 5 Hz tonic vagal stimulation. As a result, the stimulation amplitude was below 10 V. In the present study, however, the stimulation amplitude was fixed at 10 V, which would partly account for the larger gain values in the vagal transfer function. The absolute gain values are also affected by the operating HR. When the operating HR is within the saturation range of the HR response, the absolute gain values should be attenuated (Kawada *et al.* 1996). In addition, the magnitude of transfer gain is dependent on a mean stimulation frequency (Berger *et al.* 1989). Accordingly, direct comparison of the absolute gain values among different studies may be difficult based on the currently available data alone.

Limitations

There are several limitations to this study. First, data were obtained from anaesthetized animals. Since anaesthesia affects autonomic tone, the results may not be directly

applicable to conscious animals. Second, we stimulated the autonomic nerve according to binary white noise, which was quite different from the pattern of physiological neuronal discharge. However, the fact that coherence was near unity over the frequency range of interest indicates that, by virtue of their inherent linearity, the system properties would not vary much with differing patterns of stimulation. Finally, we measured AP using a femoral catheter connected to a fluid-filled pressure transducer. Although the computation of the mean pressure might be adequate, a reliable measurement of systolic/diastolic pressure would be difficult in the present study.

Conclusion

In the present study, it was demonstrated for the first time in rats that the transfer function from sympathetic stimulation to HR response approximated a second-order, low-pass filter with a lag time and that from vagal stimulation to HR response approximated a first-order, low-pass filter with a lag time. Despite the large difference in baseline HR, parameters of dynamic characteristics in HR control by the autonomic nervous system in rats are not much different from those reported in larger mammals. As such, the rat can be used as a reliable model for the determination of these parameters.

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Physiological Significance of Pressure-Volume Relationship: a Load-Independent Index and a Determinant of Pump Function

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Kazunori Uemura, and Toshiaki Shishido

Abstract—Pressure-volume relationship permits conceptual integration with time-varying elastance, stress-strain relationship, and pressure-volume area. It has also superior usefulness to other indexes, both as a load-independent index of ventricular contractility and as a determinant of ventricular pump function.

PRESSURE-VOLUME relationship has become a standard framework [1] for discussing the mechanical properties of the ventricles and sometimes atria. It has gained popularity because of its conceptual integration and its superior usefulness, both as a load-independent index and as a determinant of pump function. The concept of pressure-volume relationship agrees with to that of time-varying elastance, that of (time-varying) material properties of myocardium (i.e., stress-strain relationship), and that of pressure-volume area as the major determinant of myocardial oxygen consumption [2].

A. A Load-Independent Index

Pressure-volume relationship (PVR), especially the end-systolic pressure-volume relationship (ESPVR), has been repeatedly shown as one of the least load-sensitive index of ventricular contractility. Although preload-recruitable stroke work (PRSW) has been a rival, it is obvious that PRSW would no longer be load insensitive in extreme cases such as isovolumic beats.

Although detailed examination of ESPVR revealed its load-dependence (such as deactivation and activation associated with ejection) and curvilinearity [3], ESPVR is still the least load-dependent index of ventricular contractility. The apparent linearity of ESPVR seems to be observed just by chance, taking into consideration that ESPVR can be reconstructed from nonlinear (exponential) end-systolic stress-strain relationship of myocardium.

The most important advance what the concept of PVR has

provided are the decoupling of heart from vasculature (preload and afterload), and the fact that actively contracting tissue would change its mechanical properties in cardiac cycles. Decoupling the heart enabled us to separately discuss the changes in the heart and the vasculature, rather than mix them and discuss only the measured hemodynamic variables. The uncovered complex load-dependence and curvilinearity would have not sacrificed the value of decoupling. The concept of changeable material property has simplified the explanation of complex time course of pressure development and ejection.

B. A Determinant of Pump Function

ESPVR has provided a method to precisely predict the stroke volume for given end-diastolic volume, heart rate and afterload resistance. This was accomplished by recoupling ESPVR with effective arterial elastance (mainly determined by heart rate and resistance). This is a major advantage over PRSW. What is more, even the pressure and flow waveform can be reconstructed by recoupling time-varying PVR (for the entire cardiac cycle) and arterial high-resolution impedance [4].

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