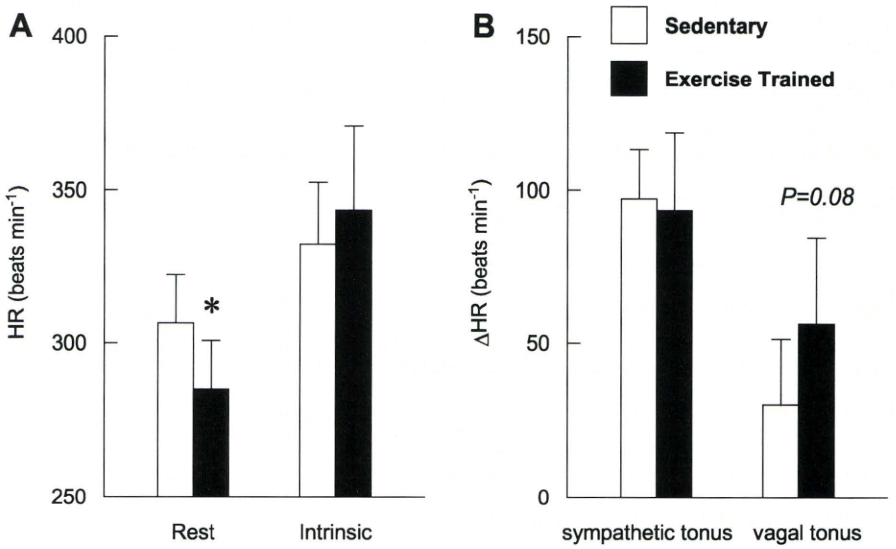

Fig. 1. Heart rate (HR) at rest and intrinsic HR (*A*) and HR sympathetic and vagal tone (*B*) obtained in sedentary and exercised-trained rats. *$P < 0.05$ compared with sedentary group.

DISCUSSION

We have examined the dynamic transfer function of autonomic HR control by using random binary sympathetic and vagal nerve stimulation in sedentary and exercised-trained rats. The major findings in the present study are *1*) that the exercise training did not alter the sympathetic transfer function substantially but augmented the dynamic gain of the vagal transfer function; and *2*) in the frequency domain, exercise training increased the dynamic HR response to vagal stimulation but not sympathetic stimulation, regardless of the frequency band. These findings are the first quantitative data on the effect of exercise training on the dynamic characteristics of peripheral HR control by the sympathetic and vagal systems.

Validity of Exercise Training

The relative ventricular hypertrophy and higher exercise capacity in the exercised-trained compared with the sedentary group suggested that exercise program used in the present study was sufficient to induce physiological adaptations commensurate with an effective training stimulus. As is well known, exercise training induces bradycardia at rest (Fig. 1*A*). Moreover, changes in the spectral parameters for R-R interval (Table 2) and autonomic tone (Fig. 1*B*) induced by the exercise training are consistent with earlier studies in rats (30, 31).

Effect of Exercise Training on Sympathetic and Vagal Transfer Function

Exercise training altered neither dynamic (Fig. 2) nor static sympathetic transfer function (Fig. 5*A*). These results are different than those reported in a previous study in which swim training significantly reduced the HR response to sympathetic nerve stimulation in a double atrial/right stellate ganglion preparation in guinea pigs (22). The discrepancy between investigations may have arisen from differences in the nerves experimentally stimulated (cervical sympathetic nerve vs. stellate ganglion), animal species studied (rats vs. guinea pigs), and/or experimental preparation utilized (in vivo vs. ex vivo). The mechanisms underlying the sympathetically mediated exercise training effect on HR are also controversial. For instance, chronotropic responsiveness to isoproterenol has been reported to be decreased in one study (15) but unchanged in another (22) by exercise training. Furthermore, in response to exercise training, the density and affinity of β-adrenoceptors in the heart have been shown to be reduced in some reports (26, 33), while unchanged in others (3, 34, 35).

Table 3. *Arterial pressure(AP) and heart rate (HR) during dynamic sympathetic stimulation protocol*

	Sedentary		Exercise Trained	
	Prestimulation	During Stimulation	Prestimulation	During Stimulation
AP, mmHg	74 ± 16	68 ± 15†	89 ± 17	84 ± 24
HR, beats/min	377 ± 25	444 ± 23†	381 ± 16	444 ± 26†

Values are means ± SD. †$P < 0.05$ compared with prestimulation.

Exercise training augmented the dynamic gain of the vagal transfer function (Fig. 2). The effect of exercise training was also significant for static vagal transfer function (Fig. 5*B*). These results are in agreement with previous studies showing that exercise training significantly augmented the HR response to vagal nerve stimulation in a double atrial/right vagal nerve preparation using mice (9, 10). In contrast, Negrao et al. (25) demonstrated that the HR response to vagal stimulation was depressed in exercised-trained rats. A possible explanation for this disparate result is that the arterial baroreflexes remained intact in the experimental preparation used in the study (25). In contrast, sinoaortic barodenervation was performed in the present investigation to minimize baroreflex-mediated changes in sympathetic efferent nerve activity. Exercise training has been shown to attenuate the baroreflex-mediated sympathetic nerve response to hypotension (11). Although speculative, in the study by Negrao et al. (25), baroreflex-mediated sympathetic activation in response to vagally-induced hypotension might have been less in exercised-trained compared with sedentary rats. Consequently, the gain of vagal stimulation might have been attenuated in exercised-trained animals relative to sedentary rats. This suggestion is reasonable given that accentuated antagonism is indicative of a diminution in background sym-

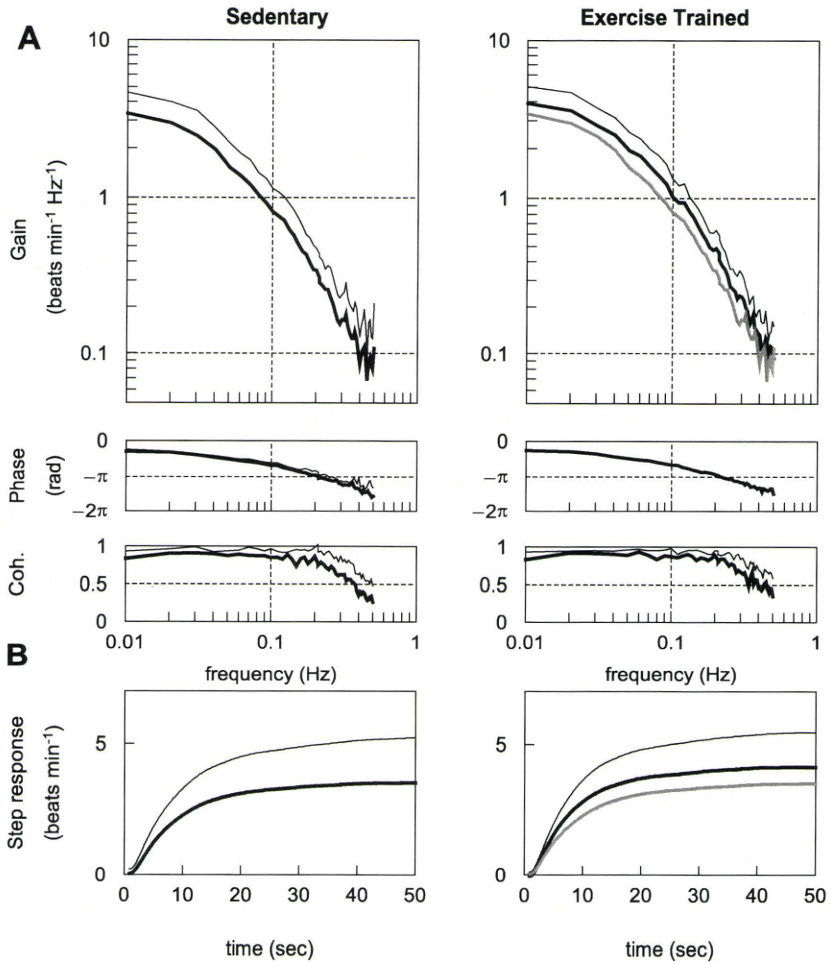

Fig. 2. *A*: transfer function from sympathetic stimulation to the HR response obtained in sedentary and exercised-trained rats. Gains (*top*), phase shifts (*middle*), and coherence (Coh.) functions (*bottom*) are presented. *B*: calculated step response to 1-Hz tonic sympathetic stimulation. Thick lines represent the mean, whereas thin lines indicate ± SD values. The gray solid curves in the gain and step response panels (*right*) duplicates the means (*left*).

pathetic tonus, which can decrease the gain of the vagal transfer function (17).

It has been documented that the intensity of exercise as well as the duration of exercise training are related to the autonomic adaptation to exercise training (28). These factors have been shown to be largely variable among different studies. A well-controlled experimental setup is needed to clarify these issues.

Dynamic Gain Values of Sympathetic and Vagal Transfer Functions Corresponding to HRV Frequency Bands

HRV is considered to reflect autonomic tone (19). The VLF component is likely to reflect changes in vasomotor tone in relation to thermoregulation and local adjustment of resistance in individual vascular beds; the LF component is considered to be a marker of sympathetic activity, although it remains a matter of debate; and the HF component mainly originates from respiratory activity and is considered to be mediated by vagal input (27). In rats, Cerutti et al. (8) determined that the LF component ranged between 0.27 and 0.74 Hz, and the HF component was > 0.75 Hz.

Averaged dynamic gain values of sympathetic transfer function for VLF and LF bands did not differ between the sedentary and exercised-trained groups (Fig. 4*A*). These results suggest that changes in the peripheral sympathetic control of HR likely do not contribute significantly to training-induced alterations in HRV. Therefore, the lower percentage of LF power and LF/HF ratio in the exercised-trained group (Table 2) may indicate reduced activation of sympathetic outflow from autonomic centers (23). In contrast, averaged dynamic gain values of vagal transfer function for VLF, LF, and HF bands (Fig. 4*B*) as

Table 4. *Sympathetic transfer function parameters and step response*

	Sedentary	Exercise Trained
Gain, beats·min^{-1}·Hz^{-1}	4.2 ± 1.5	4.5 ± 1.5
Natural frequency, Hz	0.07 ± 0.01	0.08 ± 0.01
Damping ratio	1.96 ± 0.55	1.69 ± 0.15
Lag time, s	0.71 ± 0.10	0.62 ± 0.11
Steady-state response, beats/min	3.6 ± 1.6	4.2 ± 1.2
80% rise time, s	12.9 ± 2.7	12.1 ± 3.0

Values are means ± SD. See APPENDIX for transfer function parameters.

Table 5. *AP and HR during dynamic vagal stimulation protocol*

	Sedentary		Exercise Trained	
	Prestimulation	During stimulation	Prestimulation	During stimulation
AP, mmHg	72 ± 21	68 ± 15	92 ± 14	80 ± 21
HR, beats/min	373 ± 18	327 ± 38 †	372 ± 14	301 ± 32 †

Values are means ± SD. †$P < 0.05$ compared with prestimulation.

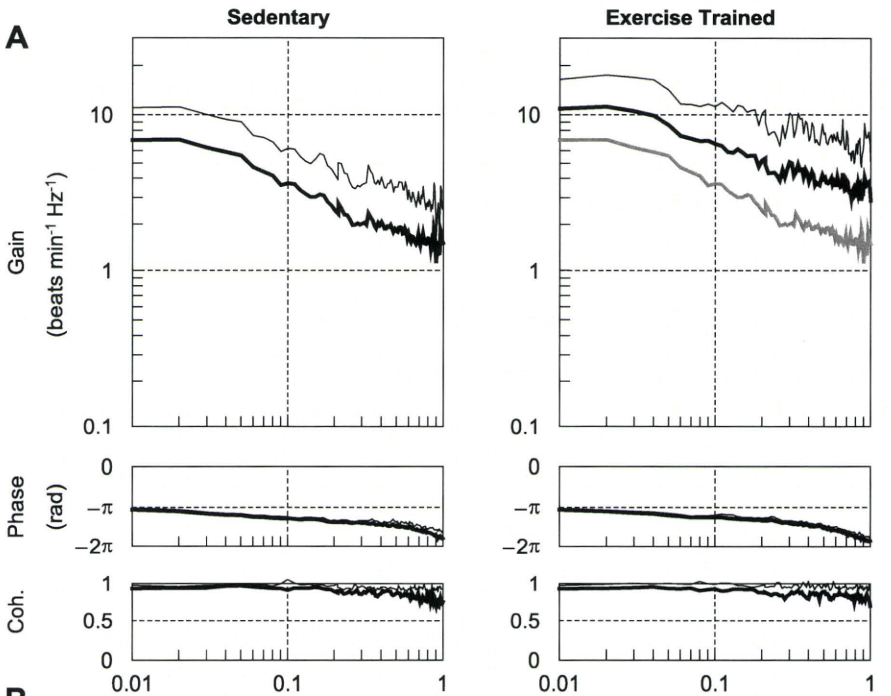

Fig. 3. *A*: transfer function from vagal stimulation to the HR response obtained in sedentary and exercised-trained rats. Gains (*top*), phase shifts (*middle*), and coherence functions (*bottom*) are presented. *B*: calculated step response to 1-Hz tonic vagal stimulation. Thick lines represent the mean, whereas thin lines indicate ± SD values. The gray solid curves in the gain and step response panels (*right*) duplicate the means (*left*).

well as the percentage of HF power (Table 2) were significantly greater in the exercised-trained compared with the sedentary group. These results suggest that the augmentation in HRV induced by exercise training is, at least in part, mediated by augmentations in the peripheral vagal control of HR.

What are the possible mechanisms underlying augmentations in the peripheral vagal control of HR? Danson and Paterson (10) have presented evidence that neuronal nitric oxide synthase may be a key enzymatic protein underlying such training-induced increases in cardiac vagal function. This group has also demonstrated that HR changes in response to vagal stimulation are enhanced by exercise training in wild-type mice but not in heterozygous neuronal nitric oxide synthase knockout mice (9). Another candidate for augmentations in the peripheral vagal control of HR is muscarinic receptors, which play a fundamental role in HR control via vagally mediated regulation. However, the effects of exercise training have been inconsistent among studies, showing both increases (12) and no change (2, 3) in muscarinic receptors in the myocardium of rats. The possibility cannot be dismissed that training-induced changes in the activity of afferent inputs mediating vagal outflow may also contribute to the alterations in HRV (4). Further investigation is needed to clarify these issues.

Table 6. *Vagal transfer function parameters and step response*

	Sedentary	Exercise Trained
Gain, beats·min^{-1}·Hz^{-1}	6.1 ± 3.0	9.7 ± 5.1#
Corner frequency, Hz	0.11 ± 0.05	0.17 ± 0.09
Lag time, s	0.10 ± 0.08	0.17 ± 0.08
Steady-state response, beats/min	−6.7 ± 3.6	−11.2 ± 5.7#
80% Fall time, s	4.3 ± 2.2	4.3 ± 1.5

Values are means ± SD. #P = 0.06 compared with sedentary group. See APPENDIX for transfer function parameters.

Perspectives and Significance

To date, the mechanisms underlying increased HRV after exercise training remain to be elucidated. HRV may reflect both the autonomic outflow from the central nervous system and the peripheral autonomic regulation of atrial pacemaker cells. In human studies, it is difficult to separately examine each factor. The findings of the present study suggest that the augmentation in HRV induced by exercise training is, at least in part, mediated by augmentations in the peripheral vagal control of HR. In other words, even if vagal outflow from the central nervous system remains unchanged after exercise training, HRV could be increased by an enhanced responsiveness in the peripheral vagal, but not sympathetic, regulation of HR.

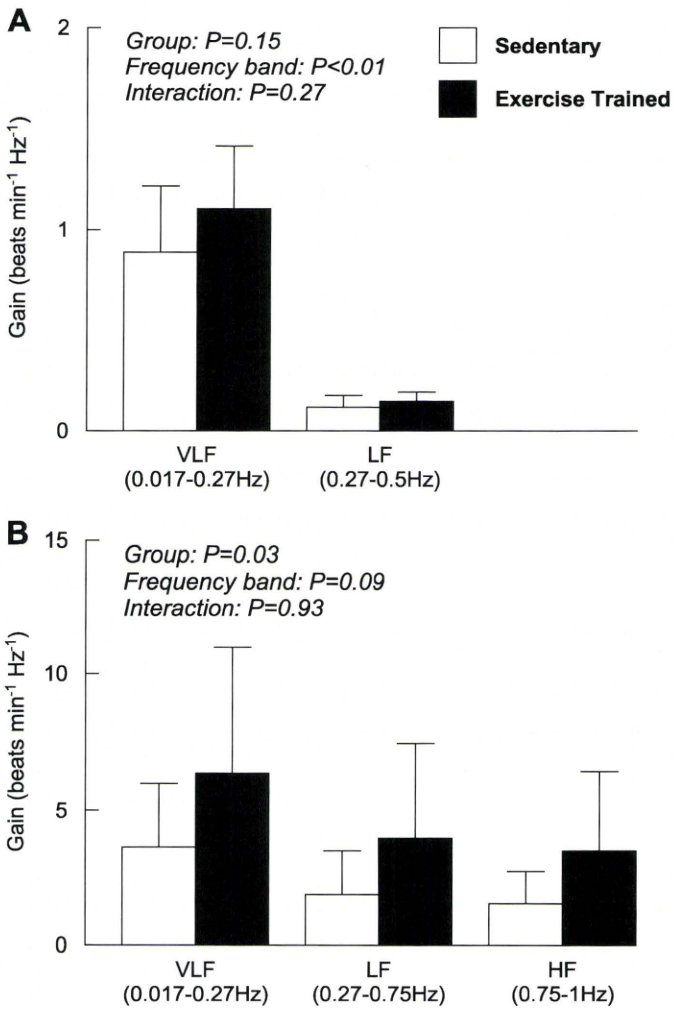

Fig. 4. Averaged sympathetic (*A*) and vagal (*B*) gain calculated from corresponding transfer function in very low frequency (VLF), low frequency (LF), and high frequency (HF) bands.

It has been well documented that decreased HRV is observed in heart failure (18) as well as in a variety of lifestyle-related diseases such as diabetes (16), hypertension (24), and obesity (1). Furthermore, reductions in HRV are related to increases in mortality rates as well as the occurrence of adverse cardiac events (32). Exercise training-induced augmentations in HRV maintain the potential to partially correct or normalize the autonomic dysfunction manifest in these disease states (4). Understanding the mechanisms contributing to the alterations in HRV induced by exercise training may significantly impact the development of novel therapeutic strategies for the treatment of autonomic dysfunction.

Limitations

There are several limitations to this study. First, the rats were slightly hyperventilated throughout the stimulation protocol. We cannot rule out the possibility that the hyperventilation might have affected the results reported. Second, dynamic sympathetic stimulation lowered mean AP in sedentary rats although sinoaortic barodenervation was performed. This may be explained by a possible difference in left ventricular functional capacity. For example, under conditions of equivalent HR, changes in systolic blood pressure were smaller in sedentary rats compared with exercised-trained rats (13). Third, the stimulation amplitude was fixed at 10 V for both sympathetic and vagal nerve stimulation. It should be noted, however, that our preliminary results indicated that 10 V was sufficiently large enough to evoke maximal HR responses. Fourth, transfer function data were obtained from anesthetized animals. This must be taken into account when interpreting the present results as anesthesia may affect the peripheral autonomic regulation of atrial pacemaker cells. Finally, we stimulated the sympathetic and vagal nerves according to a binary white noise signal. Although this method of stimulation is quite different from the physiological pattern of neuronal discharge, the coherence was near unity over the frequency range of interest. This finding indicates that the system properties do not vary considerably in response to different patterns of stimulation.

Conclusion

In the present study, it was demonstrated for the first time that exercise training did not alter dynamic sympathetic control of HR, while it did augment dynamic vagal control of HR. In addition, the group effect was significant with regard to the dynamic gain values for the vagal transfer functions corresponding to VLF, LF, and HF bands. This finding suggests that enhancements in the peripheral vagal control of HR may, at

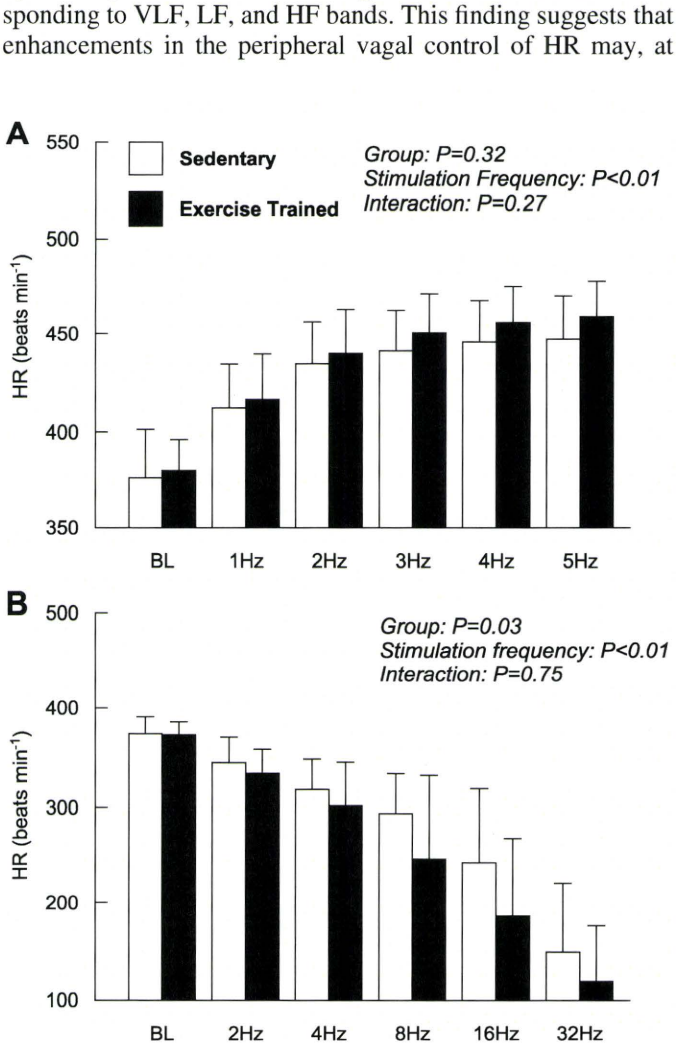

Fig. 5. HR response to stepwise sympathetic (*A*) and vagal (*B*) stimulation obtained in sedentary and exercised-trained rats.

least in part, contribute to the exercise-induced augmentation in HRV in healthy rats.

APPENDIX: TRANSFER FUNCTION ANALYSIS

The dynamic transfer function from binary white noise stimulation to the HR response was estimated based on the following procedure. Input-output data pairs of the stimulation frequency and HR were resampled at 10 Hz to be consistent with our previous study (21). Subsequently, data pairs were partitioned into eight 50% overlapping segments consisting of 1,024 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 [$S_{N\cdot N}(f)$], the power of the HR [$S_{HR\cdot HR}(f)$], and the cross-power between these two signals [$S_{N\cdot HR}(f)$] were ensemble averaged. Finally, the transfer function [$H(f)$] from nerve stimulation to the HR response was determined using the following equation (20).

$$H(f) = \frac{S_{N\text{-}HR}(f)}{S_{N\cdot N}(f)}$$

To quantify the linear dependence of the HR response on vagal or sympathetic stimulation, the magnitude-squared coherence function [Coh(f)] was estimated employing the following equation (20).

$$\mathrm{Coh}(f) = \frac{|S_{\mathrm{N\text{-}HR}}(f)|^2}{S_{\mathrm{N\cdot N}}(f) \cdot S_{\mathrm{HR\cdot HR}}(f)}$$

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.

Since the transfer function from sympathetic stimulation to HR response in rats approximated a second order low-pass filter with pure delay (21), we determined the parameters of the sympathetic transfer function using the following equation.

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

where K is dynamic gain (in beats·min^{-1}·Hz^{-1}), f_N is the natural frequency (in Hz), ζ is the damping ratio, L is lag time (in s), and f and j represent frequency and imaginary units, respectively. These parameters were estimated by means of an iterative nonlinear least squares regression.

Since the transfer function from vagal stimulation to HR response in rats approximated a first-order, low-pass filter with pure delay (21), we determined the parameters of the vagal transfer function using the following equation.

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

where K represents the dynamic gain (in beats·min^{-1}·Hz^{-1}), fc denotes the corner frequency (in Hz), L denotes the lag time (in s), and f and j represent frequency and imaginary units, respectively. The negative sign in the numerator indicates the negative HR response to vagal stimulation. These parameters were estimated by means of an iterative nonlinear least squares regression.

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DISCLOSURES

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

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Involvement of the mechanoreceptors in the sensory mechanisms of manual and electrical acupuncture

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ABSTRACT

The modalities of acupuncture can be broadly classified into manual acupuncture (MA) and electroacupuncture (EA). Although MA has been reported to cause winding of tissue around the needle and subsequent activation of the sensory mechanoreceptors and nociceptors, the sensory mechanisms of acupuncture stimulation are not fully understood. To test the hypothesis that the involvement of the mechanoreceptors in the sensory mechanism is different in MA and EA, we examined the effects of a stretch-activated channel blocker gadolinium on the hemodynamic responses to hind limb MA and EA in anesthetized rats (n=9). Gadolinium significantly attenuated the MA-induced bradycardic response (−22±5 vs. −10±3 bpm, P<0.05) and tended to attenuate the MA-induced depressor response (−30±5 vs. −18±4 mm Hg, P=0.06). On the other hand, gadolinium significantly attenuated both the EA-induced bradycardic (−22±5 vs. −9±4 bpm, P<0.01) and depressor responses (−32±6 vs. −15±5 mm Hg, P<0.01). These results indicate that the mechanoreceptors are involved in the sensory mechanisms for both MA and EA.

1. Introduction

Acupuncture has been used to modulate autonomic nervous activity and cardiovascular function (Kimura and Sato, 1997; Lin et al., 2001). The modalities of acupuncture can be broadly classified into two categories: manual acupuncture (MA) and electroacupuncture (EA). MA and EA induce similar changes in the functional magnetic resonance imaging signal in the human brain (Napadow et al., 2005). Neural mechanisms involved in acupuncture have been the focus of investigations. The effects of EA are considered to be related to stimulation of finely myelinated (group III) and unmyelinated (group IV) fibers, which activate opioid receptors in the rostral ventrolateral medulla to inhibit sympathetic outflow (Chao et al., 1999). Depletion of group IV fibers by neonatal capsaicin treatment reduces the influence of EA on the pressor responses to mechanical stimulation of visceral organs (Tjen-A-Looi et al., 2005). The extensive network of tangential cutaneous axons, coupled with their communications with the large numbers of Merkel cells, might be considered a new division of the autonomic nervous system: the cutaneous intrinsic visceral afferent nervous system (Silberstein, 2009).

Although cardiovascular responses induced by acupuncture-like stimulation are known to be reflexes mediated via somatic afferent nerves, visceral afferent nerves and autonomic efferent nerves (Sato et al., 1994, 2002; Tjen-A-Looi et al., 2005; Uchida et al., 2007; Yamamoto et al., 2008; Silberstein, 2009), the sensory mechanisms of MA and EA that initiate afferent nerve discharge are not fully understood. Langevin et al. (2001) proposed that MA causes winding of tissues around the needle and subsequent activation of sensory mechanoreceptors and nociceptors, and also suggested that changes in extracellular milieu induced by MA are important factors for neuromodulation. Burnstock (2009) proposed that mechanical deformation of the skin leads to the release of ATP from keratinocytes, fibroblasts and other cells; then the sensory nerves are activated through purinergic receptors. Although EA may induce MA-like stimuli via electrical twitching of surrounding tissues, EA may also directly depolarize sensory axons and nerve terminals adjacent to the needle and induce reflex responses. If the direct depolarization is the major sensory mechanism of EA, inhibition of mechanoreceptors would not zsignificantly attenuate the effects of EA. On the other hand, if the mechanical stimulation plays a dominant role in the sensory mechanism of EA, inhibition of mechanoreceptors would significantly attenuate the effects of EA.

Among mechanoreceptors, mechanosensitive ion channels detect mechanical stimuli and transduce these stimuli into electrical signals in sensory neurons. Gadolinium chloride is widely used experimentally as an inhibitor of stretch-activated ion channels and physiological responses of tissues to mechanical stimulation (Adding et al., 2001). To test the hypothesis that the contribution of mechanoreceptors in the sensory mechanism differs in MA and EA, we examined the effects of gadolinium on the hemodynamic responses to MA and EA in anesthetized rats.

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2. Methods

2.1. Surgical preparation

Animal care was provided in strict accordance with the Guiding Principles for the Care and Use of Animals in the Field of Physiological Sciences approved by the Physiological Society of Japan. All protocols were reviewed and approved by the Animal Subject Committee at the National Cerebral and Cardiovascular Center. Male Wister Kyoto rats weighing from 310 to 460 g were anesthetized by an intraperitoneal injection of pentobarbital sodium (50 mg/kg) and ventilated mechanically via a tracheal tube with oxygen-enriched room air. The depth of anesthesia was maintained by continuous intravenous infusion of pentobarbital sodium (20–25 mg kg^{-1} h^{-1}) through a double lumen catheter inserted into the right external carotid vein. Ringer solution (6 mg kg^{-1} h^{-1}) was administered to maintain fluid balance. Arterial blood pressure (AP) was measured using a catheter inserted into the right common carotid artery. Heart rate (HR) was determined from AP using a cardiotachometer. Body temperature was maintained at approximately 38 °C using a heating pad.

2.2. MA and EA stimulations ($n=9$)

With the animal in the supine position, both hind limbs were lifted to obtain a better view of the lateral sides of the lower legs. An acupuncture needle with a diameter of 0.2 mm (CE0123, Seirin-Kasei, Japan) was inserted into a point below the knee joint just lateral to the tibia in the left or right leg. For MA stimulation, the acupuncture needle was inserted to a depth of 5–10 mm and manually twisted clockwise and counter-clockwise, and moved up and down at a frequency of 1–2 Hz for a duration of 120 s. Two to three MA trials were conducted with an intervening interval of more than 5 min within which AP and HR returned to the respective pre-stimulation values. For EA stimulation, another acupuncture needle was inserted into a point approximately 1 cm from the above-mentioned needle toward the ankle joint and used as the ground. EA was applied for 120 s using an isolator connected to an electrical stimulator (SEN 7203, Nihon Kohden, Japan). The pulse width and the stimulus current were set at 500 μs and 5 mA, respectively. The stimulation frequency was set at 10 Hz in six and at 20 Hz in three of the nine rats. The pulse duration was based on previous studies (Tjen-A-Looi et al., 2005; Yamamoto et al., 2008; Uchida et al., 2008). The amplitude and frequency were selected so that the magnitudes of reflex hemodynamic responses became comparable to those induced by MA before gadolinium administration. In each animal, two to three EA trials were conducted with an intervening interval of more than 5 min within which AP and HR returned to the respective pre-stimulation values.

Gadolinium chloride hexahydrate was dissolved in saline at a concentration of 20 mM (Nakamoto and Matsukawa, 2007). After performing MA and EA under control conditions, we administered the gadolinium solution intravenously (2 ml/kg). After 10 min, we repeated MA and EA. The acupuncture needle positions were kept unchanged between MA and EA trials as well as before and after the gadolinium administration.

In a supplemental protocol ($n=7$ additional rats), to examine the possibility that simple insertion of needles caused significant hemodynamic influences, an acupuncture needle (CE0123, Seirin-Kasei, Japan) was only inserted into a point below the knee joint just lateral to the tibia in the left or right leg and placed for a duration of 120 s. Needle was inserted to a depth of 5–10 mm.

2.3. Aortic depressor nerve stimulation ($n=6$)

Using a pair of platinum electrodes, we identified the aortic depressor nerve (ADN) running along the common carotid artery, based on the AP pulse-synchronous nerve activity monitored through a loud speaker. After a depressor response to brief electrical stimulation of the nerve was confirmed, the electrodes and the nerve were fixed and insulated by silicone glue (Kwik-Sil, World Precision Instruments, FL, USA). The nerve fibers caudal to the electrodes were then crushed by a tight ligature so that only the afferent fibers directed to the central nervous system were stimulated. In four of the six rats, the right ADN was stimulated. In the remaining two rats, the left ADN was stimulated because of failure to stimulate the right ADN properly. The ADN was stimulated for 120 s at a frequency of 50 Hz (pulse width: 2 ms, voltage: 2 V). ADN stimulation was repeated with an interval of 5 min until the AP and HR responses appeared to be reproducible under control conditions. We then administered the gadolinium solution intravenously (20 mM, 2 ml/kg). After 10 min, we repeated the ADN stimulation.

2.4. Data analysis

Data were digitized using a 16-bit analog-to-digital converter (Contec, Japan) and stored at 200 Hz on a laboratory computer system. First, AP and HR data were averaged every 10 s. Averaged time courses of AP and HR responses were then obtained from two to three trials of MA, EA or ADN stimulation in each animal. Next, the effects of MA, EA or ADN were examined using repeated-measures one-way analysis of variance (ANOVA) followed by Dunnett's test (Glantz, 2002). The baseline data point immediately before stimulation was treated as a single control point for the Dunnett's test. Finally, the maximum effect of MA, EA or ADN stimulation was quantified by the differences between the minimum and baseline values for AP and HR (ΔAP and ΔHR). The effects of gadolinium on ΔAP and ΔHR were examined by a paired-t test (Glantz, 2002). The differences were considered significant at $P<0.05$. Data are presented in mean ± SE values.

3. Results

Fig. 1 depicts the averaged time courses of AP and HR responses to MA ($n=9$ rats). MA gradually decreased AP and HR under control conditions. The minimum AP and HR were reached near the end of the MA stimulation period. After the cessation of MA, AP and HR gradually returned toward the respective baseline values. Intravenous gadolinium administration significantly decreased baseline AP from 138 ± 5 to 120 ± 5 mm Hg ($P<0.01$) but had no significant effect on baseline HR (379 ± 10 vs. 383 ± 7 bpm). Following gadolinium administration, although MA also decreased AP and HR significantly, ΔAP tended to be attenuated (−30 ± 5 vs. −18 ± 4 mm Hg; 68 ± 16% of the pre-gadolinium; $P=0.06$) and ΔHR was significantly attenuated (−22 ± 5 vs. −10 ± 3 bpm; 57 ± 23% of the pre-gadolinium; $P<0.05$) compared to control conditions.

Fig. 2 depicts the averaged time courses of AP and HR responses to EA ($n=9$ rats). Under control conditions, EA decreased AP and HR. Both responses reached almost a steady state at approximately 1 min of EA stimulation. AP and HR remained decreased during the rest of the EA stimulation period, and gradually returned toward the respective baseline values after the cessation of EA. Intravenous gadolinium administration significantly decreased baseline AP from 140 ± 5 to 123 ± 7 mm Hg ($P<0.01$) but did not affect baseline HR (385 ± 9 vs. 384 ± 7 bpm). Following gadolinium administration, although EA significantly decreased AP, the decrease in HR was only significant at 55 s of EA stimulation. ΔAP (−32 ± 6 vs. −15 ± 5 mm Hg; 38 ± 11% of the pre-gadolinium; $P<0.01$) and ΔHR (−22 ± 5 vs. −9 ± 4 bpm; 37 ± 14% of the pre-gadolinium; $P<0.01$) were attenuated significantly compared to control conditions.

In the supplemental protocol ($n=7$ rats), the insertion of an acupuncture needle alone did not significantly change AP (138 ± 9 vs.138 ± 9 mm Hg) or HR (399 ± 20 vs. 400 ± 20 bpm).

Fig. 3 shows the averaged time courses of AP and HR responses to ADN stimulation ($n=6$ rats). ADN stimulation decreased AP and HR under control conditions. The minimum AP and HR were reached at 15 s of ADN stimulation. Both parameters remained decreased during the rest of the ADN stimulation period, and returned toward the respective

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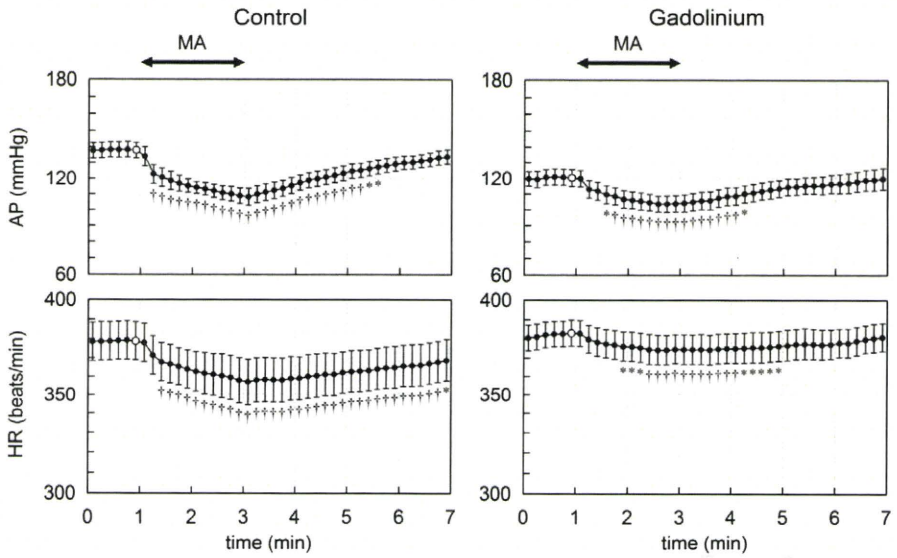

Fig. 1. Time courses of arterial pressure (AP) and heart rate (HR) responses induced by manual acupuncture (MA) averaged from 9 rats. MA gradually decreased AP and HR under control conditions (left) and after gadolinium administration (right). Gadolinium treatment tended to attenuate the AP response and significantly attenuated the HR response induced by MA, compared to control conditions. Data are mean ± SE values. *P<0.05 and †P<0.01 versus the control data point (open circle) immediately before the application of MA.

baseline values after the cessation of ADN stimulation. AP and HR appeared to recover more rapidly compared to those observed after MA and EA. Intravenous gadolinium administration significantly decreased baseline AP from 126 ± 4 to 118 ± 2 mm Hg (P<0.01) but had no significant effect on baseline HR (373 ± 13 vs. 369 ± 11 bpm). Following gadolinium administration, ADN stimulation significantly decreased AP and HR. Neither ΔAP (−43 ± 7 vs. −49 ± 3 mm Hg) nor ΔHR (−27 ± 8 vs. −34 ± 5 bpm) was attenuated compared to control conditions.

4. Discussion

We have shown that ion channels blocked by gadolinium are implicated in the hypotensive and bradycardic effects of acupuncture at the hind limb in rats, irrespective of technique.

4.1. Effects of gadolinium on AP and HR responses to MA and EA

Insertion of acupuncture needle alone did not change AP and HR significantly, indicating that continuous stimulation either by MA or EA was necessary to induce sustained AP and HR responses. Mechanoreceptors are thought to play an important role in the sensory mechanism of MA. Because gadolinium blocks mechanosensitive ion channels in sensory neurons (Cho et al., 2002), we hypothesized that intravenous administration of gadolinium would attenuate the AP and HR responses to MA. As expected, ΔAP tended to be attenuated after gadolinium administration (Fig. 1, top). However, since gadolinium also decreased baseline AP, it is uncertain whether the attenuation of ΔAP was mainly attributable to the inhibition of reflex response to MA or to the decreased baseline AP. On the other hand, gadolinium did not significantly affect baseline HR and

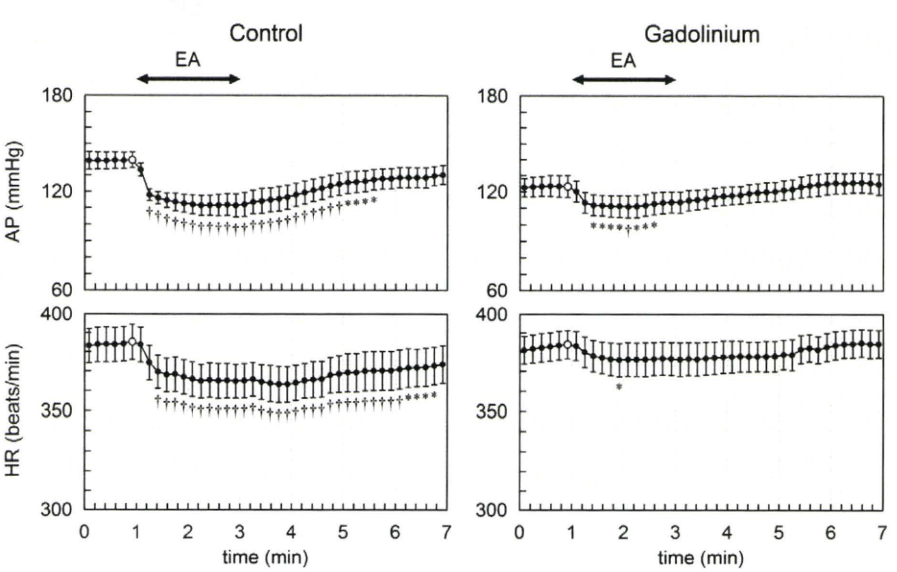

Fig. 2. Time courses of AP and HR responses induced by electroacupuncture (EA) averaged from 9 rats. EA gradually decreased AP and HR under control conditions (left) and after gadolinium administration (right). Gadolinium significantly attenuated both AP and HR responses induced by EA, compared to control conditions. Data are mean ± SE values. *P<0.05 and †P<0.01 versus the control data point (open circle) immediately before the application of EA.

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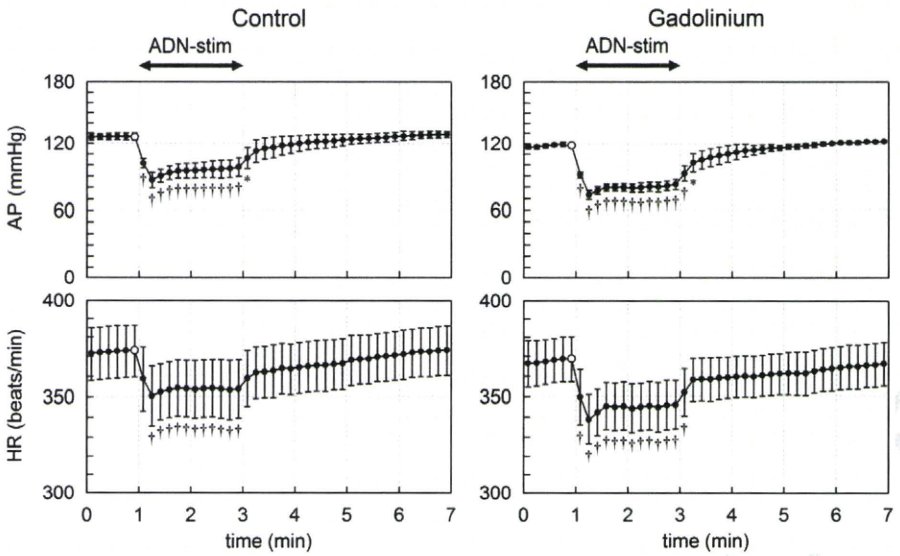

Fig. 3. Time courses of AP and HR responses induced by electrical stimulation of the aortic depressor nerve (ADN-stim) averaged from 6 rats. ADN-stim decreased AP and HR under control conditions (left) and after gadolinium administration (right). Gadolinium did not attenuate the AP and HR responses induced by ADN-stim, compared to control conditions. Data are mean ± SE values. *P<0.05 and †P<0.01 versus the control data point (open circle) immediately before the application of ADN-stim.

significantly attenuated ΔHR induced by MA (Fig. 1, bottom). Judging from the HR response, it is conceivable that gadolinium inhibits the reflex hemodynamic responses to MA.

We assumed that direct depolarization of sensory axons and nerve terminals adjacent to the needle could be the major sensory mechanism of EA. In fact, direct electrical stimulation of muscle afferent fibers evokes a variety of cardiovascular responses similar to those induced by EA (Sato et al., 1981). If direct depolarization is the major sensory mechanism for EA, inhibition of mechanoreceptors would have no significant effect on EA, because the results of the ADN stimulation protocol indicates that the axonal conduction would not be blocked even after gadolinium administration once the afferent nerve is discharged (Fig. 3). Contrary to this assumption, gadolinium significantly attenuated ΔAP and ΔHR induced by EA (Fig. 2), suggesting that the mechanoreceptors play an important role in the sensory mechanism of EA, as in the case of MA. EA probably causes electrical twitching of surrounding tissues and exerts MA-like stimulation through the mechanoreceptors.

Despite the significant contribution of mechanoreceptors to the sensory mechanisms of both MA and EA, the fact that the hemodynamic responses to MA and EA were not entirely abrogated after gadolinium administration indicates the presence of sensory mechanisms other than the mechanosensitive ion channels. Not all capsaicin-sensitive neurons are mechanosensitive, and gadolinium has no effect on capsaicin-induced calcium transient in sensory neurons (Gschossmann et al., 2000). Depletion of group IV fibers by neonatal capsaicin treatment reduces the influence of EA on the pressor responses to mechanical stimulation of visceral organs (Tjen-A-Looi et al., 2005), suggesting an importance of capsaicin-sensitive neurons in the mechanisms of acupuncture. Nociceptive neurons are therefore a likely candidate for the residual sensory mechanism after gadolinium administration. The group IV C-fiber tactile afferents is known to be widely distributed in the skin of mammals (Wessberg et al., 2003). These fibers could be regarded as a cutaneous intrinsic visceral afferent nervous system (Silberstein, 2009). In addition, the present results do not rule out the possibility that direct depolarization of sensory axons or nerve terminals occurs during EA. Albeit this assumption, EA seemed to have received even greater influence from gadolinium than MA (Figs. 1 and 2). Because MA with needle movements can cause greater deformations in the adjacent extracellular milieu compared to EA, MA may have induced signal transductions other than mechanosensitive ion channels, such as integrin-linked signal transduction pathways (Aplin et al., 1998), resulting in the greater residual hemodynamic responses after gadolinium administration. Further studies are required in the future to solve this question.

4.2. Effects of gadolinium on the AP and HR responses to ADN stimulation

Gadolinium decreased baseline AP, suggesting actions other than the inhibition of mechanosensitive ion channels. For instance, gadolinium has been shown to block voltage-gated calcium, sodium and potassium channels (Adding et al., 2001). To exclude the possibility that gadolinium attenuates the reflex hemodynamic responses to MA and EA via nonspecific mechanisms such as the inhibition of central autonomic neurotransmission, we performed the ADN stimulation experiment. Gadolinium did not attenuate ΔAP and ΔHR induced by ADN stimulation (Fig. 3). It is unlikely, therefore, that gadolinium inhibits the central autonomic neurotransmission from afferent to efferent nerve activities or significantly blunted the AP and HR responses to changes in autonomic nerve activities.

4.3. Implication of MA and EA

Although the present results indicate that MA and EA may share a common sensory mechanism, EA may be more flexible than MA in terms of its application for biomedical engineering because the effects of EA can be controlled quantitatively by adjusting the stimulation current and stimulation frequency. As an example, a previous study from our laboratories has demonstrated that servo-controlled hind limb electrical stimulation can reduce AP at a prescribed target level in anesthetized cats (Kawada et al., 2009). EA can be applied continuously using a stimulating device without the attendance of an acupuncturist once the needle is properly positioned. Continuous electrical stimulation of auricular acupuncture points for 48 h/week has been shown to be more effective than auricular acupuncture without electrical stimulation for the treatment of chronic cervical pain in an outpatient population (Sator-Katzenschlager et al., 2003). Although further studies are required, EA delivered via a dedicated stimulating device may be an additional modality to the treatment of cardiovascular diseases.

4.4. Limitations

First, the present study was conducted under pentobarbital anesthesia. Because anesthesia affects the autonomic tone, AP and HR

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responses may differ when different anesthetics are used or when the animals are in a conscious state. However, as we compared the effects of gadolinium on the reflex responses to MA and EA under the same anesthetic conditions, the interpretation of the sensory mechanisms for MA and EA should be valid. Second, we performed EA at frequencies of 10 or 20 Hz in order to obtain AP and HR responses comparable to those observed during MA under control conditions. Because the effects of EA may differ depending on the magnitude of stimulation including pulse duration, current and frequency (Uchida et al., 2008; Kawada et al., 2009), further studies are needed to examine whether the effects of gadolinium on EA-induced hemodynamic responses vary depending on the stimulation intensities.

4.5. Conclusion

Intravenous administration of gadolinium attenuated the AP and HR responses to both MA and EA, suggesting that the mechanosensitive ion channels are involved in the sensory mechanisms of both MA and EA. EA may cause electrical twitching of surrounding tissues and induce MA-like stimulation through mechanoreceptors.

Acknowledgments

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Appendix A

In an attempt to demonstrate that gadolinium does not significantly affect the hemodynamic responses to direct nerve stimulation related to acupuncture at the hind limb, we performed an additional protocol of tibial nerve stimulation in 5 anesthetized rats. The right tibial nerve was exposed and placed on a pair of platinum electrodes, and was stimulated for 120 s (500 μs, 10 Hz, 2 or 5 mA). ΔAP was -10.5 ± 3.5 mm Hg under baseline conditions, which was attenuated to -8.2 ± 4.4 mm Hg after gadolinium administration ($74 \pm 15\%$ of the pre-gadolinium, $P<0.01$). Although the relative reduction seemed smaller than that observed in EA ($38 \pm 11\%$ of the pre-gadolinium, see main text), because the reduction of ΔAP could be partly attributable to the decreased baseline AP after gadolinium administration, we could not judge whether gadolinium had truly inhibited the hypotensive effect of tibial nerve stimulation. Unfortunately, the tibial nerve stimulation did not change HR significantly in our experimental conditions ($\Delta HR = -1.1 \pm 4.4$ bpm before gadolinium vs. $\Delta HR = -1.4 \pm 4.1$ bpm after gadolinium), as opposed to a previous study (Uchida et al., 2008). As a result, we could not judge the effect of gadolinium based on HR either. We think the ADN stimulation protocol in the main text would be a second best surrogate to indicate the inability of gadolinium to block hemodynamic responses induced by direct activation of the afferent nerve.

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Baroreflex Sensitivity Might Predict Responders to Milrinone in Patients With Heart Failure

Takuya KISHI,[1] MD, *and* Kenji SUNAGAWA,[1] MD

SUMMARY

The phosphodiesterase III inhibitor milrinone (MIL) is considered to be effective for "wet and cold" heart failure. In some cases, however, the inotropic effects of milrinone are insufficient. A previous study suggested that baroreflex sensitivity (BRS) predicts the cases in which MIL increases left ventricular *dp/dt*. The aim of this study was to determine whether BRS measured using the spontaneous sequence method predicts the MIL responders. Twenty-four patients with "wet and cold" heart failure whose systolic blood pressure > 100 mmHg were enrolled. At 2 hours MIL improved dyspnea, general fatigue, urine volume, and tricuspid regurgitant pressure gradient in 13 patients (responders; R group), whereas it failed to improve in 11 patients (nonresponders; NR group). BRS in the R group was significantly higher than that in the NR group prior to the MIL infusion. At 2 hours after the MIL infusion, BRS was further increased in the R group, but did not increase in the NR group. The sensitivity and specificity of BRS at a cut-off level of 5 ms/mmHg for the prediction of R group were 0.94 and 0.93, respectively. BRS might be useful for identifying potential responders to milrinone in patients with blood pressure-preserved "wet and cold" heart failure. (Int Heart J 2010; 51: 411-415)

Key words: Heart failure, Baroreflex sensitivity, Milrinone

Milrinone, a phosphodiesterase-III inhibitor (PDEIII-I), has an inotropic and vasodilator effect for "wet and cold" heart failure,[1,2] which is determined as heart failure with congestion and hypoperfusion.[3] In some cases, however, the inotropic effects of milrinone are insufficient and combined treatment with dobutamine is necessary.[1] A previous study suggested that baroreflex sensitivity (BRS) can predict the cases in which milrinone increases left ventricular *dp/dt*.[4] However, whether arterial baroreflex function is related to the inotropic responsiveness to milrinone has not been clarified in human heart failure.

Baroreflex control is one of the key mechanisms responsible for the short-term control of blood pressure.[5-7] Impairment of this reflex has been found in a number of conditions, such as aging,[8] post myocardial infarction,[9,10] hypertension,[7] and heart failure.[11] Baroreflex sensitivity was originally assessed by intra-arterial measurement of the change in pulse interval following a pharmacologically induced change in blood pressure. However, for some time now, noninvasive monitoring of blood pressure using finger plethysmography has been available, and further methods for measuring baroreflex sensitivity have been developed, which assess spontaneous changes in blood pressure and pulse interval, and do not require pharmacological manipulation of blood pressure-spectral analysis.[12-16]

The aim of this study was to determine whether the baroreflex sensitivity measured using the spontaneous sequence method can identify potential milrinone responders or not in patients with sinus rhythm and blood pressure-preserved "wet and cold" heart failure.

METHODS

The present study was approved by the Ethics Committee for Human Research of Kyushu University Graduate School of Medical Sciences. Data collected retrospectively were fully de-identified.

Patient populations: We retrospectively studied patients with symptomatic acute heart failure admitted to Kyushu University Hospital from January 2006 to December 2007 who were treated with intravenous infusion of milrinone. The criteria for enrollment in the study were clinical evidence of acute heart failure diagnosed by Framingham criteria[17] and low cardiac output, which is called "wet and cold" heart failure.[3] We defined low cardiac output from the clinical state of "cold and wet". In those patients, the New York Heart Association (NYHA) functional classification on admission ranged between III and IV. We excluded patients whose systolic blood pressure was < 100 mmHg or who had atrial fibrillation, chronic obstructive pulmonary disease, dehydration, right ventricular myocardial infarction, or right heart failure. Prior medication by intravenous injection of diuretics, nitrates, and morphine was permitted. The dose of milrinone was adjusted according to the condition of each individual patient, and if symptoms of heart failure were not adequately improved by milrinone, concomitant use of or replacement with other agents indicated for the treatment of acute heart failure was

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permitted. Finally, we enrolled 24 patients with sinus rhythm and blood pressure-preserved "wet and cold" heart failure. We did not use the intravenous infusion of digitalis, and none of the patients in the present study were taking oral digitalis. Before the initiation of treatment, all patients underwent blood sampling, and an electrocardiogram, echocardiogram, and chest x-rays.

Measurement of blood pressure and heart rate: Blood pressure monitoring was performed using the TaskForce Monitor 3040i (CNSystems, Austria). The cuff was attached to a finger on the left hand and supported at heart level. Electrocardiogram electrodes were attached to the chest. Once a reading of blood pressure and heart rate had stabilized, 3 consecutive 5-minute recordings were made of the blood pressure and electrocardiogram tracing. Noninvasive brachial blood pressure readings were taken with an appropriate sized cuff.

Measurement of BRS by spontaneous sequence method: Sequence analysis detected sequences of 3 or more beats in which there was either an increase in SBP and pulse interval (Up sequence) or a decrease in SBP and pulse interval (Down sequence). The sequences involving premature ventricular contraction were excluded. BRS was estimated as the mean slope of the up sequences (Up BRS), the down sequences (Down BRS), and also the mean slope of all sequences (Sequence BRS).[14,15] Previous reports showed that this protocol measures BRS accurately in animals compared with standard pharmacological techniques.[14-16]

MIL responders and nonresponders: Blood sampling was performed in all patients, and the severity of tricuspid regurgitation (TR) was evaluated by color-flow Doppler (graded as trivial, mild, moderate, or severe). TR pressure gradient (TRPG) was measured by echocardiography and BRS was measured by the spontaneous sequence method before and 2 hours after the initiation of intravenous infusion of milrinone (0.25 μg/kg/minute). We did not do an initial bolus infusion. The effects of MIL are reported to be stable and plateau at 2 hours after the initiation of MIL.[18] At 2 hours after administration, milrinone improved dyspnea, urine volume (> 100 mL/hour), and the severity of tricuspid regurgitation in 13 patients (responders; R), whereas no improvement was observed in 11 patients (nonresponders; NR) (Figure 1). The degree of dyspnea was assessed by the modified Borg scale.[19] An improvement in dyspnea was defined as a reduction of 2 or more in the modified Borg scale score.[20]

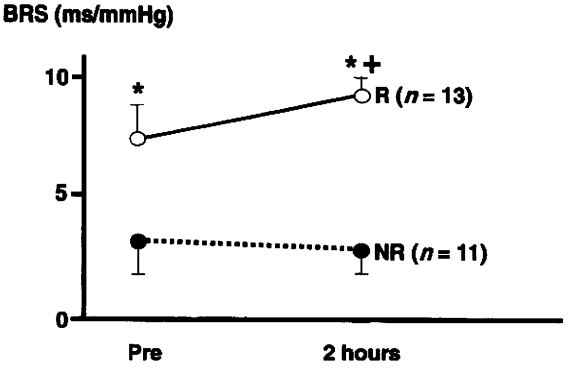

Figure 1. Baseline BRS was significantly lower in the NR group than in the R group. At 2 hours after milrinone treatment, BRS was also significantly lower in the NR than in the R group. Furthermore, in the R group, BRS at 2 hours was significantly higher than that at baseline. *$P < 0.05$ versus NR group. +$P < 0.05$ versus pre.

Statistical analysis: Normally distributed variables are expressed as the mean ± SD. The unpaired t test or Mann-Whitney U test was used to compare the differences in normally distributed variables, respectively, between the R group and NR group. All statistical tests were carried out against the baseline characteristics. Differences were considered significant at a P value of < 0.05. A receiver operating characteristic (ROC) curve was used to determine the discriminatory ability of BRS on the occurrence of response to MIL. An ROC-curve (plot of sensitivity versus 1-specificity) analysis is a powerful tool for assessing a test's ability to discriminate between R and NR groups of subjects.

Results

Patient characteristics at baseline: The patient profiles at enrollment are summarized in Tables I and II. As can be seen in Table I, there were no significant differences in age, gender, or the prevalence of dilated cardiomyopathy, hypertensive heart disease, or valvular heart disease between the R and NR group just before the milrinone therapy. Prior medications were not different either.

As shown in Table II, hemoglobin, brain natriuretic peptide (BNP) measured at discharge, and the estimated glomerular filtration rate (eGFR) did not differ between the two groups. Left ventricular ejection fraction (LVEF), left ventricular end-diastolic dimension (LVEDD), left ventricular end-systolic dimension (LVESD), and TRPG assessed by echocardiography

Table I. Baseline Characteristics

	R	NR	P
n	13	11	
Male	8	7	NS
Age	58 ± 7	59 ± 5	NS
BMI	22 ± 4	23 ± 3	NS
Body weight (kg)	56 ± 7	53 ± 9	NS
Current smoker	4 (31%)	3 (27%)	NS
Causes of heart failure			
Coronary artery disease	4 (31%)	4 (36%)	NS
Dilated cardiomyopathy	4 (31%)	3 (27%)	NS
Hypertensive heart disease	3 (23%)	2 (18%)	NS
Valvular heart disease	2 (15%)	2 (18%)	NS
NYHA functional classification			
III	9 (69%)	8 (73%)	NS
IV	4 (31%)	3 (27%)	NS
Modified Borg scale	6.1 ± 1.8	6.4 ± 2.2	NS
Systolic blood pressure (mmHg)	118 ± 11	121 ± 14	NS
Diastolic blood pressure (mmHg)	78 ± 14	73 ± 9	NS
Heart rate (bpm)	108 ± 9	111 ± 13	NS
Medications			
Diuretics	7 (54%)	6 (55%)	NS
β-Blockers	9 (69%)	8 (73%)	NS
ACE inhibitors	10 (77%)	8 (73%)	NS
Angiotensin receptor blocker	3 (23%)	3 (27%)	NS

Data are presented as number (%) or mean ± SD. BMI indicates body mass index; NYHA, New York Heart Association; and NS, not significant.

Table II. Baseline Characteristics (2)

	R (n = 13)	NR (n = 11)	P
Total cholesterol (mg/dL)	187 ± 33	172 ± 44	NS
LDL cholesterol (mg/dL)	94 ± 31	102 ± 43	NS
HDL cholesterol (mg/dL)	42 ± 8	45 ± 7	NS
Triglycerides (mg/dL)	119 ± 27	120 ± 31	NS
Hemoglobin (g/dL)	9.8 ± 1.4	10.3 ± 2.1	NS
Hematocrit (%)	35 ± 3	34 ± 3	NS
Total bilirubin (mg/dL)	1.3 ± 0.2	1.4 ± 0.3	NS
AST/ALT (U/L)	47 ± 4 / 36 ± 8	57 ± 4 / 45 ± 6	< 0.05 / < 0.05
Serum sodium (mmol/L)	131 ± 3	132 ± 2	NS
FBS (mg/dL)	82 ± 17	99 ± 15	NS
HbA1c (%)	5.2 ± 0.7	5.3 ± 0.4	NS
BNP (pg/mL)	188 ± 27	194 ± 36	NS
eGFR (mL/minute/1.73m^2)	61.4 ± 7.9	62.8 ± 4.9	NS
LVEF (%)	37 ± 7	34 ± 5	NS
LVEDD (mm)	58 ± 6	59 ± 7	NS
LVESD (mm)	42 ± 4	43 ± 7	NS
IVC (mm)	18 ± 4	24 ± 3	< 0.05
TRPG (mmHg)	47 ± 8	51 ± 9	NS
BRS (ms/mmHg)	7.3 ± 1.2	3.2 ± 1.6	< 0.05

Data are presented as number (%) or mean ± SD. LDL indicates low-density lioprotein; HDL, high-density lipoprotein; HbA1c, hemoglobin A1c; eGFR, creatinine-based estimate of glomerular filtration rate; LVEF, left ventricular ejection fraction; LVEDD, left ventricular end-diastolic dimension; LVESD, left ventricular end-systolic dimension; TRPG, tricuspid regurgitant pressure gradient; BRS, baroreflex sensitivity; and NS, not significant.

Table III. Effects of Milrinone at 2 Hours

	R (n = 13)	NR (n = 11)	P
Modified Borg scale	3.3 ± 1.6	6.2 ± 1.9	< 0.05
TRPG (mmHg)	29 ± 4	49 ± 7	< 0.05
Urine volume (mL/hour)	128 ± 18	42 ± 23	< 0.05
SBP (mmHg)	106 ± 15	114 ± 18	NS
Serum sodium level (mmol/L)	135 ± 2	129 ± 3	< 0.05
LVEF (%)	42 ± 3	36 ± 2	< 0.05
HR (bpm)	82 ± 15	106 ± 12	< 0.05

Data are presented as number (%) or mean ± SD. TRPG indicates tricuspid regurgitant pressure gradient; SBP, systolic blood pressure; LVEF, left ventricular ejection fraction; and HR, heart rate.

were not different between the two groups. AST and inferior vena cava diameter were significantly higher in the NR group than in the R group. Median BRS was significantly higher in the R group than in NR prior to the milrinone infusion (7.3 ± 1.2 versus 3.211.6 ms/mmHg, $P < 0.05$).

Effects of MIL at 2 hours: In the R group, the modified Borg scale score and TRPG at 2 hours were significantly lower than in the NR group (Table III). Urine volume per hour at 2 hours was over 100 mL/hour in all patients in the R group and significantly higher than in the NR group (Table III). LVEF and the serum sodium level were significantly higher in the R group than in the NR group (Table III). Systolic blood pressure at 2 hours was not changed. However, heart rate at 2 hours was significantly lower in the R group than in the NR group (Table III).

BRS at 2 hours: At 2 hours after the milrinone infusion, BRS was further increased in the R group, whereas it failed to increase in the NR group (Figure 1). The sensitivity and specificity for BRS at a cut-off level of 5 ms/mmHg were 0.94 and 0.93, respectively. The negative and positive predictive values were 0.98 and 0.71 (Figure 2).

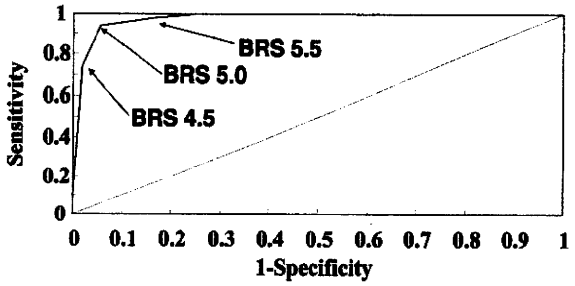

Figure 2. Receiver operator curve of BRS for prediction of responders to milrinone.

Discussion

In the present study, we demonstrated that baroreflex sensitivity measured by a spontaneous sequence method was significantly lower in the nonresponder group to milrinone than in the responder group to milrinone in patients with sinus rhythm and blood pressure-preserved "wet and cold" heart failure. Furthermore, the sensitivity and specificity for BRS at a cut-off level of 5 ms/mmHg was 0.94 and 0.93. These results suggested that BRS might be clinically useful for the prediction of responders to milrinone among patients with sinus rhythm and blood pressure-preserved "wet and cold" heart failure.

Milrinone is recommended for patients with "wet and cold" heart failure,[2] which is determined as heart failure with congestion and hypoperfusion.[3] In cases in which the systolic blood pressure is under 100 mmHg, the combination of milrinone and dobutamine is necessary.[1] However, among patients with "wet and cold" heart failure whose systolic blood pressure is over 100 mmHg, milrinone monotherapy is often insufficient. In the present study, 11 of 24 patients with "wet and cold" heart failure whose systolic blood pressure was over 100 mmHg were nonresponders to milrinone. Based on these results, we believe that systolic blood pressure is not clinically useful for predicting the response to milrinone. This is the first study to demonstrate the cut-off levels of parameters for predicting the response to milrinone, and BRS assessed by a spontaneous sequence method might be a new and novel therapeutic parameter.

A sustained baroreflex-mediated increase in sympathetic activity may contribute to increased end-organ damage and to progression of the underlying disease, and a blunted baroreflex gain is predictive of increased cardiovascular risk in postmyocardial infarction and heart failure patients.[7] Recently, the prognostic value of BRS obtained noninvasively by the modified transfer function method has been assessed in a cohort of 317 mild-to-moderate clinically stable heart failure patients. In 55 of the 228 subjects with a measurable index, a depressed BRS (≤ 3.1 ms/mmHg) was significantly associated with a higher risk of cardiac death. The results of the present study are compatible with a previous study.[16] We believe that BRS might be clinically useful as a parameter with which to determine the severity of heart failure.

The spontaneous sequence method is a noninvasive and easy method for measuring BRS in patients with acute heart failure. The advantages of this method are twofold: (i) computations are automatic and standardized, which virtually eliminates intra- and intersubject measurement variability, and (ii) distinct measurements are obtained for increasing and decreasing arterial pressure values, thus allowing one to take into account the well-known asymmetry of the baroreceptor response.[7] Further studies are necessary to determine whether other noninvasive methods to measure BRS are clinically useful or not.

The mechanisms in which BRS predicts the responses to milrinone were not fully determined in the present study. Previous studies have suggested that arterial baroreflex control of HR is diminished in heart failure,[11,21] and that the contribution of baroreflex control in myocardial contractility is markedly impaired in animals with a lower baseline inotropic state.[22] Furthermore, in heart failure, baroreflex changes in cardiac output are less related to changes in HR and more related to changes in stroke volume.[23] Milrinone is considered to exert a positive inotropic action and to decrease left ventricular end-systolic pressure reflecting the decrease in left ventricular afterload.[24] However, milrinone-induced reductions in effective arterial elastance reflecting the fall in total peripheral resistance and changes in stroke volume are not significant.[24] These previous reports suggested that baroreflex control is important to obtain the inotropic effect of milrinone. Another previous study suggested that BRS predicts the cases in which milrinone increases left ventricular *dp/dt*.[4] The increase in left ventricular *dp/dt* indicates milrinone has an inotropic effect. In the present study, in the R group, the treatment with milrinone for 2 hours was considered to increase cardiac output because LVEF and serum sodium levels were significantly increased. Moreover, AST and inferior vena cava diameter were significantly higher in the NR group than in the R group. These results suggest that right ventricular function was impaired in the NR group to a greater extent than in the R group. Nonresponders to milrinone can be considered to be a higher severity group of heart failure than responders to milrinone. However, we did not have the direct data of cardiac output, chest x-rays, and electrocardiogram in the present study. Further studies monitoring hemodynamic parameters (involving cardiac output) of the patients with acute heart failure treated with milrinone are necessary.

There are several limitations to the present study. First, it is a small and retrospective study. It is necessary to examine the predictive value of BRS in a large-scale population in which the distribution of BRS is normal. Second, we excluded patients with atrial fibrillation. In patients with atrial fibrillation, the spontaneous sequence method is not feasible. However, among patients with acute heart failure, there are many patients with atrial fibrillation. We believe that BRS measured by the spontaneous sequence method can only be used in patients with sinus rhythm. Third, we examined the effects of milrinone for only 2 hours. Furthermore, the present study is retrospective. Fourth, we did not perform the hemodynamic assessment using a Swan-Ganz catheter, and we defined low cardiac output from the clinical state of "cold and wet". There were no variables to show the fact by echocardiography or Swan-Ganz catheter. Randomized study with the assessment of hemodynamic data obtained by Swan-Ganz catheter should be performed.

Conclusions: The results of the present study suggested that baroreflex sensitivity to milrinone measured by the spontaneous sequence method was significantly lower in the nonresponder group than in the responder group in patients with "wet and cold" heart failure and that the cut-off level of BRS is 5 ms/mmHg. We believe that BRS might be clinically useful for the prediction of responders to milrinone in patients with "wet and cold" heart failure.

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Development of artificial bionic baroreflex system

Kenji Sunagawa, *Senior Member, IEEE* and Masaru Sugimachi, *Member, IEEE*

***Abstract*—The baroreflex system is the fastest mechanism in the body to regulate arterial pressure. Because the neural system (i.e., autonomic nervous system) mediates the baroreflex and the system operates under the closed-loop condition, the quantitative dynamic characteristics of the baroreflex system remained unknown until recently despite the fact that a countless number of observational and qualitative studies had been conducted. In order to develop the artificial baroreflex system, i.e., the bionic baroreflex system, we first anatomically isolated the carotid sinuses to open the baroreflex loop and identified the open-loop transfer function of the baroreflex system using white noise pressure perturbations. We found that the baroreflex system is basically a lowpass filter and remarkably linear. As an actuator to implement the bionic baroreflex system, we then stimulated the sympathetic efferent nerves at various parts of the baroreflex loop and identified the transfer functions from the stimulation sites to systemic arterial pressure. We found that the actuator responses can be described remarkably well with linear transfer functions. Since transfer functions of the native baroreflex and of the actuator were identified, the controller that is required to reproduce the native baroreflex transfer function can be easily derived from those transfer functions. To examine the performance of bionic baroreflex system, we implemented it animal models of baroreflex failure. The bionic baroreflex system restored normal arterial pressure regulation against orthostatic stresses that is indistinguishable from the native baroreflex system.**

I. Introduction

Baroreflex is known to be the fastest mechanism in the body to stabilize arterial pressure. The reflex makes use of negative feedback mechanism. The baroreceptors sitting in the arterial wall sense arterial pressure and send the pressure signal to the brainstem through the afferent nerve fibers. The brainstem receives the pressure signal and judges the level of arterial pressure. If the level is low, the brainstem activates the sympathetic system innervating the heart and vascular system to increase arterial pressure. If the level of arterial pressure is high, the brainstem withdraws the sympathetic activation.

The baroreflex system is critically important in animal, particularly in human. This is because, unlike animals with four legs, the position dependent gravitational effect on circulation is most prominent in human. It is well known that once we lose the normal function of baroreflex, we no longer keep sitting and/or standing positions because of position induced profound hypotension and hypoperfusion of the brain. Baroreflex failure destroys normal life and is a devastating pathological state in human. However, since the baroreflex failure is a disease of the neural system, no effective treatment has ever developed to save those patients.

Baroreflex failure could happen under various conditions. In some patients, they lost baroreflex function because they have problems in the baroreceptors, the brainstem and/or the spinal cord. In those patients, if we can develop a mechanism to activate their sympathetic efferent system in response to changes in arterial pressure just like the native brainstem does, in theory, normal baroreflex function can be restored.

The purpose of this investigation is to develop an artificial baroreflex system, so called the bionic baroreflex system, to restore normal baroreflex function to overcome such a serious pathological condition.

II. Bionic Baroreflex System

Shown in Fig. 1 are how we identify the transfer function of the controller of bionic baroreflex system. First we identify the transfer function of the baroreflex open loop (H_{NATIVE}) from baroreceptor pressure to arterial pressure responses. We then electrically stimulate a particular site in the baroreflex loop and identify the transfer function of the actuator from the stimulation to arterial pressure responses ($H_{STM \rightarrow AOP}$). Since the controller will be in series with the actuator, the transfer function of bionic baroreflex system becomes identical to the native baroreflex system when the transfer function of controller (H_{BIONIC}) satisfies the following equation:

$$H_{NATIVE} = H_{BIONIC} \times H_{STM \rightarrow AOP}$$

In theory both H_{NATIVE} and $H_{STM \rightarrow AOP}$ can be experimentally determined. Therefore, H_{BIONIC} can be determined. However whether such a simple approach works or not highly depends on the simplicity of the native baroreflex system including the system linearity. We therefore examined the dynamic characteristics of baroreflex system.

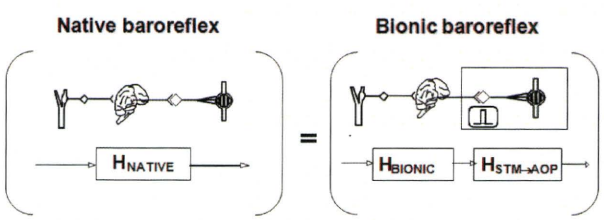

Fig. 1 Native vs. Bionic baroreflex

We vascularily isolated the baroreceptors (carotid sinuses) in rats (n=10) to open the baroreflex feedback loop and connected the carotid sinuses to a servo-controlled piston pump. This preparation allowed us to manipulate the carotid sinus pressure (CSP) independent of arterial pressure. We then perturbed CSP with random binary pressure sequences and identified the transfer function from CSP to arterial pressure. Shown in the left panels of Fig. 2 are the time series of CSP and aortic pressure. As can be seen, aortic pressure changes slowly toward the opposite direction in response to changes in CSP. This becomes even more evident in the transfer function (the right panel). The transfer function has low-pass filter characteristics. The phase response becomes nearly out-of-phase in the low frequency range suggesting the negative feedback nature of baroreflex system. Note that the magnitude squared coherence function is about 0.8 over the frequency range of interest. This is to say that most dominant characteristics of the total baroreflex open loop are captured by the linear transfer function.

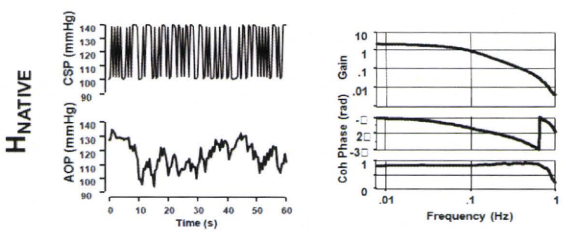

Fig. 2 Dynamic characteristics of native baroreflex system

In order to identify the actuator transfer function, we electrically stimulated the celiac ganglia with random binary pressure perturbations. Illustrated in the left panels of Fig. 3 are the time series of stimulation of celiac ganglia and aortic pressure responses. As can be seen, aortic pressure changes slowly toward the same direction in response to changes in stimulation. As anticipated the transfer function (the right panel) has low-pass filter characteristics. Unlike the total baroreflex loop, however, the phase response becomes nearly in-phase in the low frequency range. The magnitude squared coherence function is about 0.8 over the frequency range of interest. Again, it is reasonable to assume that most dominant characteristics of the actuator are captured by the linear transfer function.

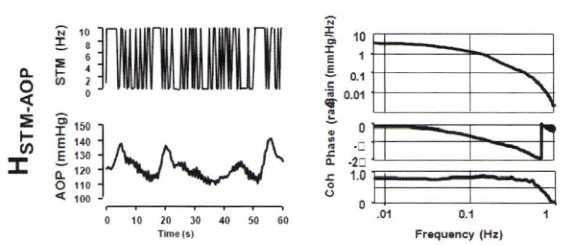

Fig. 3 Dynamic characteristics of sympathetic stimulation

We identified the transfer function (H_{BIONIC}) required for the controller by taking the ratio of H_{NATIVE} to $H_{STM \rightarrow AOP}$. Since both dynamic characteristics of the total baroreflex loop and actuator are well represented by the linear transfer functions, the resultant H_{BIONIC} should reproduce the native characteristics of the baroreflex system when the feedback loop is closed. Shown in Fig. 4 are the changes in arterial pressure in response to orthostatic stresses under the open-loop baroreflex condition (baroreflex failure), the closed-loop baroreflex condition (native baroreflex) and the bionic baroreflex condition. Orthostatic stresses profoundly lowered arterial pressure in the absence of the native baroreflex. Closing the native baroreflex loop markedly attenuated the hypotensive responses. The activation of bionic baroreflex system also attenuated the hypotensive response as much as the native baroreflex system did. Statistical analysis indicated that the pressure regulation achieved by the bionic baroreflex system was indistinguishable from that achieved by the native baroreflex system.

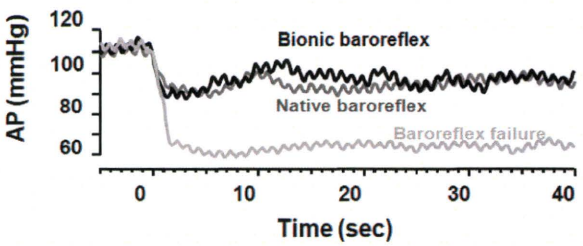

Fig. 4 Native baroreflex system vs. bionic baroreflex system

III. Discussion

We have shown that the bionic baroreflex system was as good as the native baroreflex system in regulating arterial pressure. The dynamic pressure responses to orthostatic stresses were indistinguishable between the native baroreflex system and the bionic baroreflex system. Although the baroreflex system is known to be nonlinear over the wide pressure range, we found that it is remarkably linear in the physiological pressure range. Because of this, the linear transfer function could represent the dominant characteristics of the baroreflex loop, and thereby allowed us to develop the bionic baroreflex system.

We can think of many anatomical sites where we can manipulate the activity of sympathetic system. In 1992, we stimulated the carotid sinus nerve to control the sympathetic system [1]. In 2004, we stimulated the spinal cord to stimulate the sympathetic efferent fibers [2]. The bionic mechanism worked beautifully regardless of the site of stimulation. It equally worked well in rats [3], rabbits [2], and dogs [1]. Although our experience of baroreflex failure in patients is limited, judging from its robustness, the bionic baroreflex system would work in patients as well [4]. If the bionic baroreflex system works in patients, it has a major impact as the treatment of baroreflex failure [5] that has been considered to be an incurable devastating disease.

IV. Conclusion

The bionic baroreflex system restores normal baroreflex function in an animal model of baroreflex failure.

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The pressure-volume relationship of the heart: Past, Present and Future

Kenji Sunagawa, *Senior Member, IEEE*

Abstract—**The pressure-volume relationship of the heart was first reported more than a century ago. It was not widely accepted, however, until the mid-1970s. The pressure-volume diagram became a central theme of cardiac mechanics once it was shown to be a good representation of ventricular mechanics. Early in 1980s, the introduction of the ventricular interaction with afterload using effective arterial elastance made it possible to translate ventricular mechanical properties represented by the pressure-volume relationship to the pumping ability of the heart. Furthermore incorporating the framework of ventricular arterial interaction into the classic Guyton's circulatory equilibrium early in 2000s enabled us to express quantitatively how mechanical properties of the ventricles and vascular systems determine the circulatory equilibrium. Successful quantitative descriptions of circulatory equilibrium using the pressure-volume concept would promote basic cardiovascular physiology and accelerate its clinical applications.**

I. PAST (~1980S)

In 1899 Otto Frank published a theoretical paper [1] entitled "Die Grundform des arteriellen Pulses," in which he characterized contractions of the frog ventricle in a pressure-volume (P-V) diagram. It is a schematic expression based on experimental data published in 1895. This schematic diagram is perhaps the first complete P-V diagram ever published on the heart. For various reasons, however, the approach was not well accepted. The marked loading history dependence of the end-systolic P-V relation (ESPVR) that Frank and his followers observed in the frog heart had left physiologists and cardiologists with a strong negative feeling about the use of the P-V diagram for understanding cardiac mechanics.

Our experience with isolated, blood-perfused canine hearts differed markedly from those earlier observations. In the physiological range, the ESPVR appeared to be linear and insensitive to changes in loading conditions. Encouraged by our own simple findings and by similar findings of other investigators, we proposed early in 1970s to scrutinize this relationship as a potential candidate for an index of ventricular contractility [2].

The discovery of the simple characteristics of the ESPVR in the canine heart was not the only attraction of the P-V analysis. We knew this approach would yield a great deal of information about cardiac pump function that is not explicit in the time function of the ventricular pressure and volume. The systolic P-V area [3] as a measure of the total mechanical energy released per ventricular contraction is an entirely new concept that could emerge only from the P-V diagram. Another essential production of this approach is the notion of effective arterial elastance [4] that functionally represents the mechanical properties of the afterload system in terms of P-V relationship. The concept of effective arterial elastance made it possible to couple the ventricle with the afterload on the P-V plane to analyze ventricular-arterial interaction. The qualitative expression of ventricular arterial interaction using the P-V diagram sharpened as well as deepened our understanding of the mechanism how the mechanical properties of the ventricle and arterial system determine stroke volume, thus cardiac output [5].

II. PRESENT (1990-2010)

Although the ventricular arterial interaction was quantitatively expressed by the P-V relationship, it remained unknown how the ventricle determines cardiac output by interacting with the total vascular system. Guyton established that cardiac output is determined as the equilibrium between the venous return curve and cardiac output curve [6]. In 2004 we developed and experimentally validated an algebraic expression of cardiac output curve by extensively using the ventricular arterial P-V relationship. Incorporating the derived cardiac output curves into a simultaneous expression of systemic and pulmonary venous return (the venous return surface) resulted in a new framework of circulatory equilibrium, i.e., the extended Guyton's model [7]. Since the new framework has an analytical solution of circulatory equilibrium for a given set of ventricular and vascular mechanical properties, it provides us an extremely powerful tool in understanding the mechanisms how cardiac output is determined under a variety of pathological as well as physiological conditions.

III. FUTURE (2010~)

The extended Guyton's model enables us to qualitatively predict a circulatory equilibrium once the mechanical properties of the ventricular and vascular system are known. This helps understand how complex physiological regulatory systems such as baroreflex systems regulate the cardiovascular system. In a