Chronic vagal nerve stimulation improves baroreflex neural arc function in heart failure rats

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Submitted 12 February 2014; accepted in final form 25 March 2014

Kawada T, Li M, Zheng C, Shimizu S, Uemura K, Turner MJ, Yamamoto H, Sugimachi M. Chronic vagal nerve stimulation improves baroreflex neural arc function in heart failure rats. J Appl Physiol 116: 1308–1314, 2014.—We tested whether 6-wk vagal stimulation (VS) treatment improved open-loop baroreflex function in rats after myocardial infarction (MI). The following three groups of Sprague-Dawley rats were examined: normal control (NC, n = 9), MI with no treatment (MI-NT, n = 8), and MI treated with VS (MI-VS, n = 7). Under anesthesia, a stepwise input ranging from 60 to 180 mmHg was imposed on isolated carotid sinus baroreceptor regions, while the responses in splanchnic sympathetic nerve activity (SNA) and arterial pressure (AP) were measured. The response range of percent SNA was greater in the MI-VS than in the MI-NT group (63.8 ± 4.9% vs. 33.1 ± 3.8%, P < 0.01). The slope of the AP response to percent SNA was not different between the MI-VS and MI-NT groups (0.611 ± 0.076 vs. 0.781 ± 0.057 mmHg/%). The difference in the response range of AP between the MI-VS and MI-NT groups did not reach statistical significance (40.7 ± 6.2 vs. 26.4 ± 3.5 mmHg). In conclusion, the 6-wk VS treatment significantly improved the baroreflex control of SNA, but the effect was limited for the baroreflex total-loop function due to the lack of significant improvement in the AP response to percent SNA.

MATERIALS AND METHODS

Animal care was provided in strict accordance with the Guiding Principles for the Care and Use of Animals in the Field of Physiological Sciences, approved by the Physiological Society of Japan. All protocols were reviewed and approved by the Animal Subject Committee of National Cerebral and Cardiovascular Center. The following groups of Sprague-Dawley rats were examined: normal control (NC), MI with no treatment (MI-NT), and MI with VS treatment (MI-VS).

Creation of myocardial infarction and device implantation. For the MI-NT and MI-VS groups, the left coronary artery was ligated under halothane anesthesia in 8-wk male rats (14). One week later, the rats in the MI-VS group underwent a second surgery for implanting a radiocontrolled pulse generator (ISE1010C, Unimec) to stimulate the right vagus and an electrocardiogram transmitter (TA10CA-F10, Data Science International) to monitor heart rate (HR). The total weight of the implanted devices was 17 g. This value was subtracted in the measurement of body weight in the MI-VS group. The VS treatment was started from 1 wk after the device implantation (i.e., 2 wk after the MI induction). The right vagus was stimulated using a rectangular pulse of 0.2-ms duration at 20 Hz for 10 s every minute according to our previous study (14). The intensity of VS was adjusted in each rat to reduce HR by 20–30 beats/min. The VS treatment was continued for 6 wk until the day of the acute experiment described below. The VS was ceased when the rat was removed out of the cage for the acute baroreflex study. Because the intraperitoneal anesthesia took ~30 min to develop its sufficient effect and the preparation for venous and arterial lines took 10–20 min, an acute effect of VS might have been lost before the measurement of baseline AP and HR.

Acute baroreflex study. Each rat was anesthetized with an intraperitoneal injection (2 ml/kg) of a mixture of urethane (250 mg/ml) and α-chloralose (40 mg/ml), and mechanically ventilated with oxygen-supplied room air. Anesthesia was maintained by a continuous intravenous infusion of a diluted solution of the above anesthetic mixture from the right femoral vein. An arterial catheter was inserted...
into the right femoral artery to measure AP and HR. Another catheter was inserted into the left femoral vein to measure central venous pressure. Body temperature of the animal was maintained at ~38°C using a heating pad and a lamp.

A postganglionic branch of the splanchnic sympathetic nerve was exposed retroperitoneally, and a pair of stainless steel wire electrodes (Bioflex wire, AS633, Cooner Wire) was attached to the nerve. This nerve was selected as representing the sympathetic outflow because it was easy to locate even when the surrounding tissue was edematous and carotid sinus baroreceptor regions were isolated from systemic circulation bilaterally (19, 21). The isolated carotid sinus nerves were filled with warmed Ringer’s solution through catheters inserted into the common carotid arteries. Carotid sinus pressure (CSP) was controlled with warmed Ringer’s solution through catheters inserted into the common carotid arteries. Carotid sinus pressure (CSP) was controlled using a servocontrolled piston pump. Heparin sodium (100 U/kg) was given intravenously at the end of the experiment to prevent blood coagulation.

Protocols. After completing the above surgery, the baroreflex-mediated AP response was monitored for more than 30 min. The rat was excluded from the final data analysis in the event that the reflex response to CSP was progressively diminished within this stabilization period. Possible causes for the deterioration include surgical damage to the carotid sinus nerves and brain ischemia due to the bilateral carotid occlusion. Although the vertebral arteries were kept patent, some litters of rats do not tolerate the bilateral carotid occlusion. We occasionally experience diminished baroreflex function even in normal rats. In the present study, three rats assigned to the MI-NT group were excluded from the final data analysis because of diminished baroreflex function (Table 1). This exclusion does not affect our main conclusion, because including the three rats would only exaggerate the impaired baroreflex function in the MI-NT group.

After the stabilization, CSP was decreased to 60 mmHg for 4 min, and then increased stepwise from 60 to 180 mmHg in increments of 20 mmHg every minute (8). The data obtained from two consecutive stepwise CSP inputs were averaged for analysis.

Data analysis. The rats assigned to the MI-NT and MI-VS groups were excluded from the final data analysis when the normalized biventricular weight was less than 2.2 g/kg (based on the mean plus 1 SD of the NC group), because it indicated insufficient development of heart failure due to small MI (Table 1). On the other hand, two rats in the MI-NT group did not survive the acute baroreflex study due to severe heart failure (the normalized biventricular weights were 2.97 and 3.00 g/kg, respectively). Resultantly, 9 rats in the NC group, 8 rats in the MI-NT group and 7 rats in the MI-VS group were used for the final data analysis (Table 1).

Data were acquired at 1,000 Hz using a 16-bit analog-to-digital converter. Mean SNA, AP, and HR values were calculated at each CSP level by averaging the data during the last 10 s of each step. Because the absolute amplitude of SNA varied among animals depending on recording conditions, SNA was presented in percent values in each animal. The SNA value corresponding to the CSP level of 60 mmHg was assigned to 100%. The noise level after the hexamethonium administration was assigned to 0%.

The CSP-SNA, CSP-AP, and CSP-HR relationships approximated an inverse sigmoid curve and were quantified using a four-parameter logistic function as (10):

$$y = \frac{P_1}{1 + \exp(P_2(CSP - P_3))} + P_4$$

where $y$ denotes the output (SNA, AP, or HR); $P_1$ is the response range of $y$ (i.e., the difference between the maximum and minimum values of $y$); $P_2$ is the slope coefficient; $P_3$ is the midpoint of the sigmoid curve on the CSP axis; and $P_4$ is the minimum value of $y$ (i.e., the lower plateau of the sigmoid curve). The maximum gain or the maximum slope of the sigmoid curve was calculated as $P_1/P_4$.

The SNA-AP relationship approximated a straight line and was quantified using a linear regression as:

$$AP = P_a SNA + P_b$$

where $P_a$ and $P_b$ are the slope and intercept, respectively.

Statistical analysis. All data are presented as means and SE values. Each parameter was compared among NC, MI-NT, and MI-VS groups using one-way analysis of variance (ANOVA) followed by Tukey test for simultaneous multiple comparisons. Differences were considered significant when $P < 0.05$ (4).

RESULTS

As shown in Table 2, body weight was not different among the three groups. Biventricular weight was significantly heavier...
in the MI-NT and MI-VS groups compared with the NC group. Central venous pressure, measured just after inserting arterial and venous catheters and before isolating baroreceptor regions, was higher in the MI-NT and MI-VS groups compared with the NC group. Mean AP and HR under the baseline conditions did not differ among the three groups.

Typical time series obtained from the NC, MI-NT, and MI-VS rats are shown in Fig. 1. The gray lines in the AP and HR signals represent 200-Hz resampled data. The gray lines in the SNA signals represent 10-Hz resampled data. The black lines in the SNA, AP, and HR signals indicate 2-s moving averaged data. The increase in CSP decreased SNA, AP, and HR in all groups, but the baroreflex responses in the MI-NT rat were smaller than those in the NC and MI-VS rats.

Static characteristics of the baroreflex neural arc obtained from the NC, MI-NT, and MI-VS groups are illustrated in Fig. 2, A–C. The response range of percent SNA was significantly narrower in the MI-NT group than in the NC group and was significantly wider in the MI-VS than in the MI-NT group (Fig. 2D). Differences in the maximum slope among the three groups were not statistically significant (Fig. 2E). The midpoint pressure was significantly lower in the MI-NT group than in the NC group, but the difference was not observed between the NC and MI-VS group (Fig. 2F). Changes in the lower plateau of SNA were reciprocal to those in the response range of SNA because the maximum SNA was assigned to 100% in each animal (Fig. 2G).

Static characteristics of the baroreflex peripheral arc approximated a straight line in all of the NC, MI-NT, and MI-VS groups (Fig. 3, A–C). The slope was significantly smaller in the MI-NT and MI-VS groups than in the NC group (Fig. 3D). The slope did not differ significantly between the MI-NT and MI-VS groups. The intercept tended to be higher in the MI-VS than in the NC group, but the difference in the intercept was not statistically significant among the three groups (Fig. 3E).

Static characteristics of the baroreflex total loop obtained from the NC, MI-NT, and MI-VS groups are summarized in Fig. 4, A–C. The response range of AP was significantly narrower in the MI-NT and MI-VS groups than in the NC group (Fig. 4D). The maximum gain was significantly smaller in the MI-NT group than in the NC group, but was not significantly different between the NC and MI-VS groups (Fig. 4E). The midpoint pressure and the lower plateau did not differ significantly among the three groups (Fig. 4, F and G).

Static characteristics of the baroreflex control of HR obtained from the NC, MI-NT, and MI-VS groups are depicted in Fig. 5, A–C. The response range of HR was significantly narrower in the MI-NT group than in the NC and MI-VS groups (Fig. 5D). The maximum slope of the HR response was significantly greater in the MI-VS group than in the MI-NT group (Fig. 5E). The midpoint pressure and the lower plateau did not differ significantly among the three groups (Fig. 5, F and G).

DISCUSSION

The present results demonstrated that the 6-wk VS treatment improved the ability of the carotid sinus baroreflex to suppress...
percent SNA in the neural arc (Fig. 2D). On the other hand, the VS treatment did not affect the slope of the AP response to percent SNA in the peripheral arc (Fig. 3D). As a result, the VS treatment did not reveal statistically significant improvement in the response range of AP in the baroreflex total loop (Fig. 4D). No significant difference in the maximum gain between the NC and MI-VS groups may partly reflect an improvement of the baroreflex total-loop function (Fig. 4E).

Effects of VS treatment on the baroreflex neural arc. The response range of percent SNA was narrowed in the MI-NT group compared with the NC group (Fig. 2D), being consistent with our previous study that was performed in rats with longer duration of heart failure (100–200 days after MI) (8). The response range of percent SNA in the MI-VS group was nearly the same as that in the NC group. The improved neural arc function to suppress percent SNA may partly contribute to the sympathetic suppression induced by the VS treatment such as reduced plasma norepinephrine levels (14, 29). Because percent SNA is influenced by basal sympathetic discharge, the improved response range of percent SNA does not necessarily indicate the improved sympathetic control has physiological significance. However, because the VS treatment also increased the response range of HR in the MI-VS group compared with the MI-NT group (Fig. 5D), we speculate that VS might have actually improved the sympathetic control of target organs.

Several mechanisms are considered for the impaired baroreflex control of SNA in heart failure. Plasma angiotensin II levels are increased in heart failure, and VS can suppress the increased plasma angiotensin II levels (29). Angiotensin II can increase the maximum SNA without significantly affecting the response range of SNA in the baroreflex neural arc (7). Cardiac sympathetic afferent activation inhibits the baroreflex control of SNA via a mechanism associated with central angiotensin II (3). On the other hand, vagal afferent stimulation by phenylbiguanide can decrease the maximum SNA without significantly affecting the response range of SNA in the baroreflex neural arc (6). Because VS might have activated not only efferent but also afferent pathways in our study, the vagal afferent stimulation probably antagonized the sympathoexcitation such as that induced by angiotensin II. In addition, pharmacological vagal activation (13) or electrical VS (29) can reduce systemic inflammatory responses in heart failure, which may contribute to sympathetic suppression by reducing the pathological afferent signals from the heart. Aside from the systemic inflammation, increases in proinflammatory cytokines in the brain may also produce sympathoexcitation in heart failure (28). Although a link between the VS treatment and central anti-inflammation seems elusive, the presence of a cholinergic anti-inflammatory pathway (22) suggests a possibility that VS suppresses sympathetic overactivity through the anti-inflammatory mechanism in the central nervous system as well as in the heart.
The slope of the peripheral arc in the MI-VS group was not increased compared with the MI-NT group, indicating that the VS treatment prevents cardiac remodeling and ventricular maximum SNA is decreased in the MI-VS group compared with the MI-NT group, the slope of the peripheral arc assessed by the AP response to the absolute change in SNA could be increased.

Effects of VS on the sympathetic HR control. Because the vagal nerves were sectioned bilaterally in the acute baroreflex study, the CSP-HR relationship represents the sympathetically mediated HR response (Fig. 5). The response range of HR was narrowed in the MI-NT group compared with the NC group, but it was nearly the same between the MI-VS and NC groups. In a previous study using canine heart failure induced by high-rate pacing, Zhang et al. (29) demonstrated that chronic VS improves the baroreflex sensitivity assessed by the RR interval response to a phenylephrine-induced AP increase. In that study, both the sympathetic and vagal limbs might have contributed to the RR interval response. In contrast, the present result indicates that the VS treatment may improve the HR response even in the absence of the vagal control of HR. Sustained sympathetic activation in heart failure results in downregulation and/or desensitization of β-adrenergic receptors (15). In addition to the improved SNA response to the CSP input, the VS treatment may correct the functionality of β-adrenergic signaling through sympathetic suppression and improve the sympathetically-mediated HR response in the MI-VS group compared with the MI-NT group.

Consideration of MI models. Our previous study indicates that the VS treatment prevents cardiac remodeling and ventricular maximum SNA is decreased in the MI-VS group compared with the MI-NT group, the slope of the peripheral arc assessed by the AP response to the absolute change in SNA could be increased.

Effects of VS treatment on the baroreflex peripheral arc. Open-loop static characteristics of the baroreflex peripheral arc approximated a straight line (Fig. 3, A–C). As has been reported in our previous study (8), the slope of the peripheral arc was significantly reduced in the MI-NT group compared with the NC group. Pump failure associated with MI may blunt the AP response to percent SNA. Regarding this point, Feng et al. (2) examined the AP response to preganglionic sympathetic nerve stimulation using pithed rats. In that study, the AP response is decreased in MI rats compared with control rats. They discussed that the pressor response in pithed rats mainly represented the vascular response because cardiac output did not change significantly in response to preganglionic sympathetic nerve stimulation. However, when cardiac output is reduced in the MI rats compared with control rats, the same amount of changes in the peripheral vascular resistance would yield smaller AP response in the MI rats than in the control rats. In this sense, the contribution of pump failure to the decreased AP response may be inevitable.

The slope of the peripheral arc in the MI-VS group was not increased compared with the MI-NT group, indicating that the VS treatment had little effect on the sympathetic control of AP. While these results seem to be consistent with no significant effect of the VS treatment on the normalized biventricular weight (Table 2), we need to be cautious about the fact that the slope of the peripheral arc can be differently estimated depending on how SNA was expressed. For instance, if the absolute maximum SNA is decreased in the MI-VS group compared with the MI-NT group, the slope of the peripheral arc assessed by the AP response to the absolute change in SNA could be increased.

Fig. 4. Open-loop static characteristics of the baroreflex total loop (AP vs. CSP) obtained from the NC (A), MI-NT (B), and MI-VS (C) groups. The response range of AP (D), maximum gain (E), midpoint (F), and lower plateau (G) derived from fitted sigmoid curves. Data are means and SE values. **P < 0.01 and *P < 0.05 by Tukey test following one-way ANOVA.

Fig. 5. Open-loop static characteristics of the baroreflex control of HR (HR vs. CSP) obtained from the NC (A), MI-NT (B), and MI-VS (C) groups. The response range of HR (D), maximum slope (E), midpoint (F), and lower plateau (G) derived from fitted sigmoid curves. Data are means and SE values. **P < 0.01 and *P < 0.05 by Tukey test following one-way ANOVA.
weight was 2.75 ± 0.25 g/kg in a treated group (CHF-VS) and 3.14 ± 0.22 g/kg in an untreated group (CHF-SS). In contrast, normalized biventricular weight was nearly the same between the MI-NT and MI-VS groups (Table 2), and both groups showed normalized biventricular weight similar to that in the CHF-VS group. One possible explanation for the discrepancy between the previous and present studies is the difference in the MI-NT and CHF-SS group characteristics as follows. In the previous study (14), a second surgery was performed for telemeter implantation and dummy stimulator implantation in the CHF-VS group. The second surgery and the weight burden of the implanted devices might have aggravated heart failure and promoted cardiac remodeling. In the present study, only MI was induced in the MI-NT group without the second surgery. Accordingly, heart failure might have been less severe in the MI-NT group than in the previous CHF-SS group.

Although performing the second surgery in the untreated MI group might have been a better protocol, it is confounded by another problem that the rat with too severe heart failure could not survive the acute baroreflex study (see APPENDIX for details). In this sense, there was unintentional bias as to the severity of heart failure in the MI-NT group. It may be worth noting that two rats in the MI-NT group but no rats in the MI- VS group were lost during the acute baroreflex study (Table 1).

**Limitations.** First, the baroreflex static characteristics were examined in anesthetized conditions. Because anesthesia affects autonomic nervous activities, the present results may not be directly extrapolated to interpret the baroreflex function in heart failure under conscious conditions. Second, we were unable to determine whether the VS treatment prevented worsening of the baroreflex neural arc function or restored the baroreflex neural arc function that had been worsened by heart failure before the initiation of the VS treatment. Further studies are required to elucidate the time course of the effects of the VS treatment on the baroreflex function. Finally, basal sympathetic discharge might have been increased in the MI-NT group compared with the NC group, and VS might have ameliorated the elevated sympathetic discharge in the MI-VS group. Our previous study (14) has demonstrated that chronic VS treatment reduces plasma norepinephrine concentration in MI-induced heart failure rats. Thus, if SNA was evaluated in absolute amplitude and/or frequency, the response range of SNA in the MI-NT group might become larger. In other words, the response range of the neural arc in the MI-NT group might be underestimated in Fig. 2, and the slope of the peripheral arc in the MI-NT group might be overestimated in Fig. 3. These points need to be taken into account when interpreting the data.

**Conclusions.** The 6-wk VS treatment significantly improved the ability of the carotid sinus baroreflex to reduce percent SNA. Because sympathetic overactivity promotes the progression of heart failure, the suppression of SNA may be beneficial for the improved prognosis of heart failure. On the other hand, the treatment effect of VS on the baroreflex total-loop function was limited due to the lack of significant improvement in the AP response to percent SNA.

**APPENDIX**

To address the effect of a second surgery in untreated MI rats, we performed an additional experiment, which included a sham operation on the right vagus and implantation of dummy devices in MI rats (MI-SS). Eight rats survived until the day of the acute baroreflex study. One rat showed small normalized biventricular weight (<2.2 g/kg) and was excluded, and the remaining seven rats exhibited normalized biventricular weight of 3.05 ± 0.12 g/kg (mean ± SE), confirming that a second surgery promoted cardiac remodeling compared with the MI-NT group. Among the seven rats, three rats died during the acute baroreflex study, and the remaining four rats exhibited normalized biventricular weight of 2.93 ± 0.13 g/kg. The problem with the MI-SS group was that rats with severe heart failure died during the acute baroreflex study, and we could not create a group of severe heart failure compared with the MI-NT group any further. As a reference, data obtained from the MI-SS group combined with other groups are depicted in Fig. 6. Statistical analyses were not performed due to the small number of successful baroreflex study in the MI-SS group (n = 4). The mean parameter values of the baroreflex total loop and the neural arc indicate that the baroreflex function in the MI-SS group was not worse than that in the MI-NT group.

**GRANTS**

This study was supported by Health and Labour Sciences Research Grants (H20-Katsudo-Shitei-007 and H21-Nano-Ippan-005) from the Ministry of Health, Labour and Welfare of Japan; by Grants-in-Aid for Scientific Research (JSPS KAKENHI Grant Nos. 23592319, 25350909); by a Grant-in-Aid for JSPS Fellows (JSPS KAKENHI Grant Number 23·01705); and by the Hiroshi and Aya Irisawa Memorial Award for Excellent Papers on Research in Circulation in The Journal of Physiological Sciences.

**DISCLOSURES**

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

**AUTHOR CONTRIBUTIONS**

Author contributions: T.K. and M.S. conception and design of research; T.K., M.L., C.Z., and S.S. performed experiments; T.K. analyzed data; T.K., M.L., C.Z., S.S., K.U., M.J.T., and H.Y. interpreted results of experiments; T.K. prepared figures; T.K. and M.S. drafted manuscript; T.K. and M.S. edited and revised manuscript; T.K., M.L., C.Z., S.S., K.U., M.J.T., H.Y., and M.S. approved final version of manuscript.

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