Title

**Optimal titration is important to maximize the beneficial effects of
vagal nerve stimulation on chronic heart failure**

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Running head: Dose response of vagal nerve stimulation in chronic heart failure

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Abstract

Background

Although vagal nerve stimulation (VNS) benefits patients with chronic heart failure (CHF), the optimal dose of VNS remains unknown. In clinical trials, adverse symptoms limited up-titration. In this study, we evaluated the impact of various voltages of VNS which were titrated below symptom threshold on cardiac function and CHF parameters in rat myocardial infarction (MI) models.

Methods and Results

We randomly allocated MI rats to vagal (VNS, n=41) and sham (Sham, n=16) stimulation groups. We stimulated the right vagal nerve with 20 Hz at three different voltages for 4 weeks. We defined Max as the highest voltage that did not evoke any symptom, Half as 1/2 of Max and Quarter as 1/4 of Max. All three groups significantly reduced biventricular weight compared to Sham ($p<0.05$). In contrast, only Half decreased left ventricular (LV) end-diastolic pressure (Half: 17.5 ± 2.0 , Sham: 24.2 ± 1.2 mmHg, $p<0.05$), and increased LV ejection fraction (Half: 37.9 ± 3.1 , Sham: $28.4\pm2.3\%$, $p<0.05$) and LV maximum +dP/dt (Half: 5918.6 ± 2.0 , Sham: 5001.2 ± 563.2 mmHg/s, $p<0.05$). The number of large vagal nerve fibers was reduced with Max (Max: 163.1 ± 43.0 , Sham: 360.0 ± 61.6 counts/bundle, $p<0.05$), indicating the significant neural damage by VNS.

Conclusion

The optimal titration of VNS would maximize benefits for CHF and minimize adverse effects.

Keywords: Vagal Nerve Stimulation, Chronic Heart Failure, Myocardial Infarction

26 **Introduction**

27 Chronic heart failure (CHF) has a poor prognosis and is one of major public health
28 concerns. Although new therapeutic strategies for CHF have been proposed in the last
29 decades, approximately one-half of CHF patients die within 5 years¹. Since the
30 imbalance between sympathetic and parasympathetic nervous systems plays a major
31 role in the pathogenesis of CHF², there is increasing clinical interest in
32 neuro-modulation therapy that alters the autonomic imbalance.

33 Since Li et al.³ reported that vagal nerve stimulation (VNS) markedly improved
34 the survival of CHF rats, many animal studies had shown the favorable effects of VNS
35 on CHF, such as decreases in heart rate and oxygen consumption⁴, reduction in
36 inflammation through activation of nicotinic receptors⁵, attenuation of norepinephrine
37 spillover in the left ventricle (LV)⁶, suppression of free radical generation⁷ and
38 sympatho-inhibition by activating the afferent arm⁸. Subsequent to these animal studies,
39 a few clinical trials of VNS therapy for CHF were conducted but the outcomes were
40 inconsistent among trials^{9–11}. Thus the efficacy of VNS for CHF in humans remains
41 controversial. A non-randomized clinical trial exploring VNS in 32 NYHA classes II-IV
42 CHF patients found that VNS improved quality of life, exercise capacity and LV
43 ejection fraction⁹. In contrast, the first randomized sham-controlled trial that
44 investigated right-sided VNS for CHF patients (NECTAR-HF) failed to demonstrate
45 significant beneficial effects on cardiac remodeling or functional capacity¹⁰. The results
46 of NECTAR-HF raised many questions such as patient selection, synergistic effects of
47 medications and the setting of electrical stimulation. Especially, incomplete
48 understanding of the appropriate dosing of VNS makes it difficult to maximize the
49 beneficial effects, while minimizing the adverse effects.

Introduction

In animals studies, the dose of VNS were chosen at levels that reduce heart rate³. In contrast, the clinical trials showed that VNS-induced symptoms limited up-titration of the stimulating dose; consequently heart rate was not reduced in most cases^{9,10}. On the other hand, a recent investigation in dogs revealed that VNS improved CHF independent of heart rate reduction^{12,13}. This finding suggests that the heart rate-guided up-titration of VNS may be unnecessary and unrealistic in humans. To prove this hypothesis, we need to examine whether VNS below the symptom threshold yields any improvement in CHF. In this study, we investigated the impact of subthreshold VNS that did not induce either symptoms or heart rate reduction on cardiac function and CHF parameters in rats.

Methods

Experiments and animal care were approved by the Committee on Ethics of Animal Experiment, Kyushu University Graduate School of Medical Sciences. The investigation conforms to the *Guide for the Care and Use of Laboratory Animals* published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1985).

Animal preparation

We anesthetized 8 week-old male Sprague-Dawley rats (SLC, Hamamatsu, Japan) with a mixture of isoflurane in oxygen-enriched air. We ligated the left anterior descending coronary artery to create large MI. One week after ligation, we performed echocardiography (SSA-380A, TOSHIBA, Japan) under anesthesia¹⁴. Rats with dilated left ventricle (LV) (LV diastolic dimension ≥ 9.5 mm) and reduced LV ejection fraction (LVEF) were included in subsequent experiments (Table 1). We attached a pair of stainless steel electrodes to the right vagal nerve at the neck level and implanted a pulse generator (ANRE-100, Anpex, Japan) to stimulate the vagal nerve. We also implanted a telemetry system (TA11PA-C40, Data Sciences International, USA) and inserted the pressure sensing catheter in the abdominal aorta to record heart rate (HR) and blood pressure (BP).

Protocol and titration of VNS

One week after implantation, we randomized the rats into a VNS group (n = 41) and a sham group (Sham, n = 16). The VNS groups were divided into 3 groups depending on the voltage of VNS.

Methods

We determined the symptom threshold by observing symptoms such as respiratory twitching and abnormal behaviour while changing the voltage of VNS. We defined the maximum voltage as the voltage just below the threshold (Max; $n = 14$). We defined the half-maximum voltage as 1/2 of Max (Half; $n = 13$) and quarter-maximum voltage as 1/4 of Max (Quarter; $n = 14$). Figure 1 shows the representative HR response of a rat during titration of VNS. VNS at Max, Half and Quarter did not reduce HR during VNS. The voltage just above Max evoked symptoms in all rats without HR reduction, while a higher voltage (Max + 0.5 volts) significantly reduced HR. The frequency was set at 20 Hz, pulse width at 0.18 ms, and duty cycle at 10 s/min. We titrated the VNS voltage once a week in every rat, readjusting the voltage if necessary. Each rat received chronic VNS for 4 weeks.

Echocardiography and hemodynamic studies

At the end of the protocol (after 4 weeks of VNS), we anesthetized the rats with isoflurane and recorded echocardiograms and hemodynamic under closed-chest condition. After performing echocardiography, we inserted a 2F catheter-tipped micromanometer (SPR-320: Millar Instruments, USA) into the LV via the right carotid artery and recorded blood pressure and LV pressure (LVP). We digitized LVP at 1 kHz using a 16-bit analogue-to-digital converter (Power Lab 16/35: AD Instruments, NSW, Australia) and stored in a dedicated laboratory computer system. We calculated the first derivatives of LVP to estimate max dp/dt and max -dp/dt as indexes of systolic function and diastolic function, respectively.

Neurohormonal studies

We sampled blood for measurements of hormone concentrations at the end of the hemodynamic study. Plasma concentrations of norepinephrine and brain natriuretic peptide (BNP) were measured.

Remodeling study and histological studies

After hemodynamic measurements, the heart and lung were excised, weighed, and then dissected and fixed in 10% buffered formalin. The LV was sliced from the apex to base into 4 transverse slices of 4- μ m thickness, and stained by Masson trichrome method. We digitized histological images and estimated the size of MI using the ratio of the total length of MI lesion relative to the total circumferential length in 4 slices¹⁵.

We harvested the right vagal nerve from the site of electrode implantation. All samples were fixed in 2.5% glutaraldehyde, cut into 4- μ m thick sections and stained with toluidine blue. The number and diameter of myelinated fibers in the vagal trunk were analysed under a microscope (Olympus, Japan). The fibers were classified as large ($\geq 3 \mu$ m) or small ($< 3 \mu$ m).

Statistical Analysis

Results are expressed as mean $\pm$ standard error of mean (SEM). One-way ANOVA was used to compare each parameter in four experimental groups (Max, Half, Quarter, and Sham). Post-hoc Dunnett test was then performed to identify which group differences accounted for the significant overall ANOVA. A *p* value less than 0.05 was considered significant.

Results

Alteration of VNS stimulation setting

Shown in Table 2 are weekly alterations of electrode impedance, stimulation voltage, and current. We derived the electrode impedance by applying a test voltage with a fixed amplitude (5 volts) and measured the resultant current. Taking the ratio of the test voltage to the current yielded the electrode impedance. The electrode impedance increased gradually in all groups. In most cases, we had to increase the stimulation voltage for titration. As a result, the stimulation current remained unchanged throughout the observation period in all groups. Although we employed the same electrode and surgical procedure to attach the electrode to the vagal nerve, minute variations such as connective tissue growing between the vagal fibers and the electrode, uncontrollable leakage of electricity and the involvement of sensory nerves in the vagal nerve and/or in the electrode could not be avoided in some rats. In such cases, increasing in the electrode impedance did not parallel the stimulation voltage of symptom threshold.

Effect of chronic VNS on 24-h averaged heart rate and blood pressure

Figure 2 shows the 24-h averaged HR and mean BP during 4 weeks of VNS. Half reduced 24-h averaged HR significantly compared to Sham from 1 to 4 weeks of VNS, while Max reduced HR at 3 and 4 weeks of VNS. The difference between Sham and Half reached 40 bpm at the end of the study ($p = 0.003$). Mean BP averaged over 24 h did not differ among 4 groups (Sham: 86.5 ± 3.8 , Quarter: 84.1 ± 1.6 , Half: 89.7 ± 1.8 , Max: 91.0 ± 2.1 mmHg, not significant).

Effect of VNS on LV function

Figure 3 shows the effect of VNS on LV function. Half did not decrease LVDd (Half: 10.9 ± 0.2 vs. Sham: 11.1 ± 0.1 mm), while significantly decreased LVDs (Half: 9.1 ± 0.2 vs. Sham: 9.8 ± 0.2 mm, $p < 0.05$). As a result, Half significantly preserved LVEF compared to Sham (Half: 37.9 ± 3.1 vs. Sham: $28.4 \pm 2.3\%$, $p < 0.05$). Furthermore, Half reduced LV end-diastolic pressure (LVEDP) by 6.7 mmHg compared to Sham (Half: 17.5 ± 2.0 vs. Sham: 24.2 ± 1.2 mmHg, $p < 0.01$), and showed the highest maximum LV +dP/dt. (Sham: 5001 ± 563 , Quarter: 4988 ± 201 Half: 5918 ± 20 , Max: 5351 ± 189 mmHg/s; Half vs. Sham, $p < 0.05$) and the lowest minimum LV -dP/dt. (Sham: -3632 ± 188 , Quarter: -3595 ± 136 , Half: -4342 ± 309 , Max: -3903 ± 156 mmHg/s; Half vs. Sham, $p < 0.05$).

Effect of VNS on other parameters

Table 3 shows the effect of VNS on infarct size, body weight, organ weights and neurohormonal factors. Infarct size and body weight were not different across groups at the completion of the VNS protocol. Max, Half and Quarter significantly reduced biventricular weight compared to Sham. Only Half significantly decreased lung weight compared to Sham, indicating improvement of pulmonary edema. Both Half and Max markedly decreased plasma BNP, a biomarker of aggravation of heart failure (Half: 157.4 ± 25.7 and Max: 177.8 ± 27.6 vs. Sham: 397.3 ± 37.5 pg/mL, both $p < 0.01$). Half reduced plasma norepinephrine, a marker of sympathetic nerve activity, by more than 50% compared to Sham (Half: 474.8 ± 58.6 vs. Sham: 936.8 ± 126.2 pg/mL, $p < 0.01$).

Results

180 **Histology of vagal nerve**

181 Figure 4 shows the effect of VNS on histological damage of vagal nerve. VNS did not
182 change the number of small fibers (Sham: 1222.7 ± 293.9 , Quarter: 1086.8 ± 151.2 ,
183 Half: 1224.4 ± 108.6 , Max: 1312.5 ± 98.3 counts/bundle). However, Max significantly
184 decreased the number of large fibers (Max: 163.1 ± 43.0 vs. Sham: 360.0 ± 61.6
185 counts/bundle, $p < 0.05$).

Discussion

This is the first study to elucidate the dose response of VNS below the symptom threshold, with respect to the effect on CHF. Major findings of this study are: (1) up-titration of VNS evoked symptoms before the level that reduced heart rate; (2) A VNS voltage at 50% of the symptom threshold (Half) significantly improved cardiac remodelling and heart failure compared to other voltages; and (3) a voltage just below the symptom threshold (Max) induced histological injury to vagal nerve fibers.

Effect of VNS voltages below symptom threshold on heart failure

We compared the effect of chronic VNS at three different voltages below the symptom threshold. Max, Half and Quarter did not elicit symptoms such as pain and respiratory twitching, and all three also did not reduce HR during stimulation, whereas the higher voltage (Max + 0.5 volts) evoked both HR reduction and symptoms. Although all three voltages of VNS did not exhibit direct HR reduction during the period of titration, Half had the lowest 24-h averaged HR among 4 groups all through 4 weeks of chronic VNS. Since HR strongly correlates with the severity of heart failure¹⁶, this result indicates that Half improved heart failure. As shown in Fig. 3 and Table 3, Quarter failed to improve any parameter of CHF, indicating that a certain intensity level of VNS is required to achieve beneficial effects. Half, which was the voltage at 50% of the symptom threshold, clearly improved cardiac function, cardiac remodelling and heart failure, while Max showed no significant improvement in these parameters except for biventricular weight and plasma BNP. These data suggest that optimal stimulus dose is required to maximize the beneficial effects of VNS for CHF. Using a stimulation intensity (current amplitude of 0.1 to 0.13 mA) sufficient to reduce heart rate, Li et al.³ showed that chronic VNS

improved cardiac function and survival in a rat model of heart failure after myocardial infarction. In our study, we were not able to up-titrate VNS because of adverse effects. At Max that did not decrease heart rate, we found no benefit of chronic VNS on ventricular function. The difference in physiological response to VNS observed in Li's and our study may be due to differences in electrode and stimulator (Li et al. used a constant current system). Thus, we cannot directly interpret and compare the two findings.

Several possible mechanisms may explain why Half yielded better outcomes than Max. As shown in Fig. 4, chronic electrical stimulation at the maximum voltage caused significant neural fiber damage. Several modes of injury such as compressive¹⁷, reperfusion^{18,19} and electrical damage to peripheral nerves secondary to implantation and electrical activation of electrodes have been reported^{20,21}. In the present study, physical damages caused by compression and electrode attachment to the vagal nerve were conceivably the same in the four groups. We assume that the higher electrical intensity of Max may have induced neural damage. As for histological investigation, Agnew et al.²⁰ showed that electrical stimulation of feline peroneal nerves reduced the number of large myelinated fibers, whereas the smaller myelinated fibers were spared. Their findings are consistent with our observation that the maximum subthreshold voltage of VNS reduced the number of large fibers but not small fibers (Fig. 4). To clarify if the histological neural damage parallels the function or sensitivity of vagal nerve, we examined the HR response to VNS at the highest voltage (10 volts, supramaximal) during titration as an indicator of loss of vagal nerve function. As shown in Fig. 5, supramaximal VNS in Max significantly attenuated the HR responder compared to other groups indicating that Max induced a functional deficit of vagal

nerve as well as an anatomical deficit. In addition, it is well known that the myelinated, large vagal fibers play a pivotal role in cardio-protective effects of VNS.²² Thus, electrical damage and loss of vagal myelinated fibers might have weakened the beneficial effects of Max. Another possible factor is the sensitivity of vagal nerve. The sensitivity and activity of vagal nerve vary daily or even hourly. Since we fixed the voltage of Max just below the symptom threshold, we cannot rule out the possibility that Max exceeded the threshold transiently. Persisting symptoms may evoke worsening of respiratory rhythm and stress-induced sympathetic activation, and in turn lead to aggravation of CHF.

The need for investigation of other parameters to optimize VNS

Apart from voltage, other stimulation parameters such as frequency, duration and duty cycle should be considered to maximize the impact of VNS for CHF. In particular, frequency should be another important factor. Saku et al.⁸ reported that VNS at 20 Hz induced marked respiratory inhibition via the vagal nerve afferent pathway. In addition, we also evaluated how VNS frequency altered respiration, and clearly demonstrated that VNS at 5 Hz delivered higher voltage without obvious respiratory inhibition compared to VNS at 20 Hz²³. These findings suggest that the frequency of VNS may alter the symptom threshold. Further investigations are needed to decide the optimal combination of stimulation parameters which balance the efficacy and adverse effects.

Clinical implication

Several clinical trials on VNS for CHF have been reported, and stimulation parameters varied among trials. In clinical trials, stimulation-related symptoms limited up-titration

of VNS. De Ferrari et al.⁹ showed that adverse effects (discomfort and pain) prevented up-titration in 72% of the patients. In NECTAR-HF, the current amplitude used in VNS was less than half of the target current amplitude (4 mA) because of side effects, resulting in a low current amplitude of 1.42 ± 0.80 mA¹⁰.

There have been no studies, including animal investigations, which examine the dose–efficacy relationship of VNS below the symptom threshold. We demonstrated that half of the maximum subthreshold voltage outperforms the maximum subthreshold voltage. In the open-labelled ANTHEM-HF study, the VNS current amplitude was set below the symptom threshold with a safety margin (0.25 mA) to insure that the therapy was well tolerated¹¹. This dose setting may be a reason why VNS in patients with CHF achieved significant improvement of LV ejection fraction and exercise tolerance in ANTHEM-HF. Titration of VNS considering both intensity and safety would maximize the beneficial effects and minimize the adverse effects in CHF patients. Taken together, we cannot support the notion that the stronger intensity the better.

Limitation

There are several limitations in this study. First, in studying the dose effects of VNS, many stimulation parameters other than the voltage are involved, such as frequency, duty cycle, timing to the cardiac cycle and efferent/afferent nerve activation. In this study, we focused on the voltage. To develop VNS with maximum efficacy and minimum adverse effects, further investigations are needed to assess the effects of other stimulation parameters.

Second, since we focused on the impact of VNS on cardiac function and remodeling after myocardial infarction, we did not fully evaluate the mechanistic

Discussion

insights of our findings. Zhang et al.¹³ reported that improvement of autonomic balance, inhibition of the renin-angiotensin system and anti-inflammatory effects mediate the therapeutic effects of VNS on CHF. It is conceivable that such mechanisms may also contribute to the beneficial effects of low-dose VNS in this study. Further investigations are needed to clarify the fundamental mechanism of low-dose VNS.

Third, we showed that the loss of vagal large fibers paralleled the beneficial effect of VNS on CHF at Max. Since the design of electrode and stimulus conditions in this study differ greatly from those in human, we should be very careful to extrapolate this finding to VNS in patients. On the other hand, VNS evokes similar physiological responses in rats and in humans. Therefore, the symptom guided up-titration method proposed in this study could serve as a practical indicator in determining the doses of VNS in patients.

Conclusion

Optimal titration maximize the beneficial effects of VNS for CHF. A VNS voltage at 50% of the symptom threshold significantly improved CHF. The proposed titration procedure is potentially a practical clinical tool in determining the optimal dose of VNS.

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315 **Author Contributions**

316 Dr. Akiko Nishizaki contributed to conduct the whole study and wrote this manuscript.
317 Dr. Kazuo Sakamoto contributed to design of the work and revising the manuscript
318 critically for important intellectual content. Dr. Keita Saku contributed to interpretation
319 of data and revising the manuscript critically for important intellectual content. Dr.
320 Kazuya Hosokawa contributed to the design of the work and revising the manuscript
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324 Mr. Takuya Akashi contributed to acquisition of data and drafting the article. Mr.
325 Yoshinori Murayama contributed to analysis and interpretation of data and drafting the
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327 the manuscript. Dr. Tomomi Ide contributed to design of the work, interpretation of data,
328 and revising the manuscript critically for important intellectual content. Dr. Kenji
329 Sunagawa contributed to design of the work, interpretation of data and revising the
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Figure Legends

Figure 1. Direct effect of vagal nerve stimulation (VNS) on heart rate (HR).

Max, voltage just below the symptom threshold; Half, half of Max; Quarter, quarter of Max; Sham, sham-stimulation. Subthreshold VNS up to Max did not reduce HR during stimulation. A higher symptom-evoking voltage (Max + 0.5 volts) decreased HR.

Figure 2. Effects of vagal nerve stimulation (VNS) on 24-h averaged heart rate (HR) and 24-h mean blood pressure (BP) during 4 weeks of chronic VNS.

Max, voltage just below the symptom threshold; Half, half of Max; Quarter, quarter of Max; Sham, sham-stimulation. (A) Half significantly decreased HR compared to Sham during 4 weeks of VNS. (B) Mean BP did not differ among 4 groups. Data are expressed as mean $\pm$ SEM. * $p < 0.05$, ** $p < 0.01$ vs. Sham at the same period; † $p < 0.05$ vs. Sham at 0 week; n.s., not significant

Figure 3. Effects of vagal nerve stimulation (VNS) on left ventricular (LV) function.

Max, voltage just below the symptom threshold; Half, half of Max; Quarter, quarter of Max; Sham, sham-stimulation; LVDd, left ventricular end-diastolic dimension; LVDs, left ventricular end-systolic dimension; LVEF, left ventricular ejection fraction; LVEDP,

Figure Legends

LV end-diastolic pressure; LV, left ventricle. Half significantly increased LVEF. Half decreased LVEDP, and showed the highest maximum +LV dP/dt and the lowest minimum LV -dP/dt. Data are expressed as mean $\pm$ SEM. * $p < 0.05$, ** $p < 0.01$ vs. Sham.

Figure 4. Effect of vagal nerve stimulation (VNS) on histology of vagal nerve.

Max, voltage just below the symptom threshold; Half, half of Max; Quarter, quarter of Max; Sham, sham-stimulation. (A) VNS at all three voltages did not decrease the number of small fibers. (B) Max decreased the number of large fibers of vagal nerve. Data are expressed as mean $\pm$ SEM. * $p < 0.05$ vs. Sham.

Figure 5. The impact of acute supramaximal VNS (10-volts) on heart rate (HR) among four groups.

Max, voltage just below the symptom threshold; Half, half of Max; Quarter, quarter of Max; Sham, sham-stimulation. We defined the HR responder as a rat showing the HR reduction of over 10 bpm from the baseline, and counted the number of responders once a week in each group. We estimated the rate of HR responder among four groups by the Kaplan–Meier method and compared by log-rank test. Max halved the responder rate

Figure Legends

after 4 weeks. On the contrary, the responder rate remained unchanged in Quarter or Half (Sham; 100, Quarter; 100, Half; 93.3 and Max; 52.6% in 4-week after VNS, $p=0.01$).

Figure 1

HR (bpm)

Figure 2

Figure 3

Figure 4

A Small fibers (counts/bundle)

B Large fibers (counts/bundle)

* $p < 0.05$ from Sham

Figure 5

HR responders to supramaximal VNS

Table 1. Body weight and echocardiographic measurements at baseline

	Sham	Quarter	Half	Max
BW, g	275.5±4.8	264.4±7.7	278.2±4.7	268.8±8.4
LVDd, mm	9.7±0.1	9.6±0.1	9.8±0.1	9.6±0.1
LVDs, mm	7.9±0.2	7.9±0.1	8.0±0.2	7.9±0.1
LVEF, %	41.8±1.9	41.7±1.8	42.0±1.4	41.6±1.8

Legend:

In each parameter (BW, LVDd, LVDs and LVEF), there were no significant difference among four groups. Max, voltage just below the symptom threshold; Half, half of Max; Quarter, quarter of Max; Sham, sham-stimulation; BW, body weight (g). LVDd, left ventricular end-diastolic dimension (mm). LVDs, left ventricular end-systolic dimension (mm). LVEF, left ventricular ejection fraction (%). Data are expressed as mean ± SEM.

Table 2. Weekly changes in VNS setting

	Impedance (kΩ)					Stimulation Voltage (volts)					Stimulation Current (mA)				
weeks	0	1	2	3	4	0	1	2	3	4	0	1	2	3	4
Quarter	13.2±1.0	25.0±7.0	31.8±10.1	29.3±7.4	30.2±7.3	0.5±0.1	0.5±0.1	0.7±0.1	0.8±0.1	0.8±0.1	0.03±0.007	0.03±0.003	0.03±0.003	0.03±0.004	0.03±0.003
Half	14.5±1.0	20.9±1.7	23.1±2.0	25.9±4.3	32.0±6.9	1.1±0.2	1.4±0.3	1.9±0.5	1.9±0.4	2.0±0.4	0.08±0.01	0.08±0.02	0.08±0.02	0.07±0.01	0.08±0.02
Max	17.9±1.9	24.3±2.2	26.6±3.2	33.8±4.7	39.6±9.2	1.8±0.3	2.3±0.5	2.9±0.4	3.7±0.7	3.7±0.7	0.11±0.03	0.11±0.03	0.11±0.01	0.10±0.01	0.10±0.01

Legend:

Weekly changes in electrode impedance, stimulation voltage, and current at each titration period. We derived the electrode impedance every week (see text for details). After knowing the electrode impedance, we stimulated the vagal nerve at various voltage amplitudes. The ratio of stimulation voltage to the electrode impedance yielded the stimulation current. Max, voltage just below the symptom threshold; Half, half of Max; Quarter, quarter of Max; Sham, sham-stimulation. Data are expressed as mean ± SEM.

Table 3. Effects of vagal nerve stimulation (VNS) on other parameters.

	Sham	Quarter	Half	Max
MI size, %	46.9±1.2	51.8±2.3	48.0±1.9	48.8±1.6
BW, g	396.4±9.7	382.6±10.8	405.5±13.2	382.6±8.5
Biventricular weight, g/kg	3.7±0.11	3.4±0.06*	3.2±0.11**	3.2±0.12**
Lung weight, g/kg	9.7±0.3	9.4±0.8	6.7±0.8**	8.3±0.5
BNP, pg/mL	397.3±37.5	372.9±48.2	157.4±25.7**	177.8±27.6**
NE, pg/mL	936.8±126.2	932.6±179.9	474.8±58.6**	631.9±74.4

Legend:

The effect of vagal nerve stimulation (VNS) on infarct ratio (MI size, normalized by LV circumferential length), body weight (BW), biventricular weight (normalized by body weight), lung weight (normalized by body weight), plasma brain natriuretic peptide (BNP) and plasma norepinephrine (NE). Infarct size and body weight were not different across groups at the completion of the VNS protocol. Max, voltage just below the symptom threshold; Half, half of Max; Quarter, quarter of Max; Sham, sham-stimulation. Data are expressed as mean ± SEM. *p < 0.05, **p < 0.01 vs. Sham.