

Original Article

Unilateral vagus nerve stimulation improves ventricular autonomic nerve distribution and functional imbalance in a canine heart failure model

Juan Sun^{1*}, Yanmei Lu^{1*}, Yan Huang², Najina Wugeti¹

¹Heart Center, The First Affiliated Hospital of Xinjiang Medical University, Urumqi, Xinjiang; ²Department of Electrocardiogram, Huadong Hospital of Fudan University, Shanghai. *Equal contributors.

Received December 17, 2014; Accepted April 7, 2015; Epub June 15, 2015; Published June 30, 2015

Abstract: Background: Chronic heart failure (HF) is the main complication of various organic heart diseases. An imbalance of the autonomic nervous system has close associations with arrhythmia, ventricular remodeling, and sudden death upon HF. This study investigated whether unilateral vagus nerve stimulation could increase vagal activity, improve autonomic nerve distribution, and cardiac function in a HF model. Methods: Eighteen dogs were randomly divided into 3 groups: sham operation, HF, and HF + vagal stimulation, with 6 dogs per group. The sham operation group had a pacemaker implanted, with no pacing; the HF group was tachypaced at 50 times/min for 37 d to establish the (chronic or congestive) HF model; Vagal stimulation group were implanted with a pacemaker at 250 times/min rapid pacing for 30 d, then the right vagus nerve was stimulated for 7 d (frequency: 20 Hz). Left ventricular ejection fraction (LVEF) and mean arterial pressure (MAP) were examined with an ultrasound diagnostic apparatus. Resting heart rate was recorded with an electrocardiogram monitor. The expression of tyrosine hydroxylase (TH) and choline acetyl-transferase (ChAT) protein was examined by immuno-histochemistry. Results: 30 d after rapid ventricular pacing, MAP < 60 mm Hg, and LVEF < 50%, the HF model was successfully established in canines. The results showed that there were no differences in MAP, LVEF, and resting heart rate at baseline in each group ($P > 0.05$). After 30 d of pacing, MAP and LVEF were reduced and resting heart rate increased in the HF and HF + vagal stimulation group compared to the sham operation group ($P < 0.05$). After 37 d, compared with the sham operation group, MAP and LVEF were significantly reduced and resting heart rate significantly increased in HF group ($P < 0.05$). Compared with the sham operation group, TH and ChAT positive nerve density heterogeneity increased in the HF group but did not change in the HF + vagal stimulation group. TH protein expression was increased and ChAT protein expression reduced in the HF group; TH expression decreased and ChAT expression increased in the HF + vagal stimulation group. Conclusion: Unilateral vagus nerve stimulation increased vagus nerve activity, slowed heart rate, recovered autonomic nerve homogeneity and function imbalance, and improved heart function in HF.

Keywords: Chronic heart failure, vagus nerve stimulation, tyrosine hydroxylase, acetylcholine transferase

Introduction

Chronic heart failure is an end-stage complication of various organic heart diseases. It has a high mortality rate. The majority (40~50%) of deaths from heart failure are caused by ventricular arrhythmia [1]. A great amount of research has shown that: an imbalance of the autonomic nervous system, which has close associations with arrhythmia, and ventricular remodeling [2], plays an important role in the pathogenesis of heart failure. Upon heart failure, increased catecholamine release results in additional stimulation of sympathetic nerves. Meanwhile, the functions of the parasympa-

thetic vagus nerve declines. At the start, this type of adaptive adjustment is beneficial because it increases cardiac output. However, over the long-term the decline of the functions of the vagus nerve, and the excessive activation of sympathetic nerves, induces serious changes to the heart at different sites and levels, forming the cornerstone of ventricular remodeling. A large number of basic and clinical studies have shown a correlation between activation of the vagus nerve and the prognosis of heart failure. Presently, treating heart failure by vagus nerve activation is in the initial stages [3-5]. Two potential directions of treatment have been found recently: One method is to use chronic

electrical stimulation to activate the vagus nerve, and the second method is to prescribe benzothiazepine drugs (calcium antagonists). The present study discussed the mechanism of reversing remodeling of autonomic nerves of ventricular muscle of heart failure dogs by stimulating the unilateral vagus nerve in a heart failure model induced by rapid pacing of dog right ventricle.

Materials and methods

Experimental animals and grouping

Eighteen healthy, male and female, adult mongrel dogs, with weight ranging (18.00 ± 2.66 kg) were used. The quality of the animals reached first class standard (License No.: SCXK-2003-001). The animals were randomly divided into 3 groups; sham operation, heart failure, and vagus stimulation + heart failure (vagus stimulation group), with six animals per group. In the sham operation group, the right vena jugularis external and vagus nerve cord of the dogs were separated and a pacemaker was implanted without pacing stimulation; in the heart failure group, pacemaker stimulation was given for four weeks and then the vagus nerve cord was separated and pacing continued for another 7 d; in the vagus nerve group, the pacemaker stimulation was given for four weeks and then the vagus nerve cord was separated and the electrode was implanted to implement stimulation and the pacing continued for 7 d simultaneously. The design and execution of the experiment received approval from the Animal Ethics Committee of Renmin Hospital of Wuhan University.

Preparation of mongrel dog CHF model [6]

The dogs were anaesthetized through intravenous injection of 30 mg/kg of 3% pentobarbital sodium, which was maintained with an intravenous injection of 4 mg/kg during the operation. The trachea cannula was found. The hair on the right-side of the neck was removed and regular sterilization was performed. A cut of 5 cm was made on the right neck and the vana jugularis externa on the right neck was separated. After that, the vein sheath was peeled off carefully and the distal end was ligated. A 1/4-1/3 diameter of the vein was cut open with ophthalmic scissors. When the wall of vein was lifted via the vein hook, the electrode tip was cautiously

threaded through the vein and the pacing electrode was sent to the right ventricular apex along the venous system. The intracardiac electrophysiological instrument LEAD2000 (Jinjiang Electronics) showed the aVR lead presenting a big R wave. The pacemaker was connected and started (250 times/min), and the skin was then sutured. And then the switches of the pacemaker were opened to carry out pacing rhythm. During, and three days after the operation, 1.6 million u-penicillin were injected intramuscularly 2 times/d to prevent infection. Four weeks later, symptoms of heart failure; loss of appetite, reduction of activities, breathing acceleration, among others occurred in the dogs. Standard mean artery pressure (MAP) in the heart failure dogs was < 60 mm Hg and ejection fraction $< 50\%$, indicating the heart failure model was successfully established. Under the conditions of closure of pacemaker and anesthesia of the dogs, the right vagus nerve stimulator was installed and then the pacemaker was restarted, for seven days with a pacing frequency of 190 times/min.

Methods of stimulating vagus nerves

The vagus nerves in the right neck of dogs was separated and an electrode for stimulating vagus nerves was implanted, the other side of which connected with the stimulator of nerves. The stimulus parameters were: voltage 4~8 V, pulse width 1 ms, and frequency 20 Hz. Stimulation was considered efficient when the heart rate was reduced by at least 30%. When the stimulation failed to reach the standard, the stimulation voltage, frequency, and stimulation sites were adjusted. After the verve stimulators were placed and debugged, the cut was sutured and the stimulation continued for 7 d.

Ultrasonic examination of the heart

The ultrasound instrument DESAOTE and a probe of 10S was applied with a frequency of 10 MHz at a scanning depth of 5 cm. Before the examination, the pacemaker was turned off. The left ventricular ejection fraction (LVEF) was measured.

Immunohistochemical detection of tyrosine hydroxylase (TH) and Cholineacetyl-transferase (ChAT)

After the in vivo experiment of each group finished, left ventricular anterior wall tissue was

Ventricular autonomic nerve in heart failure canine

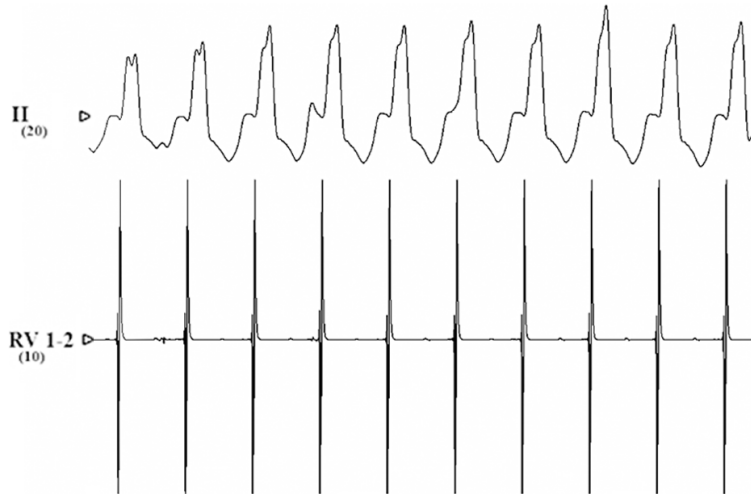


Figure 1. Rapid pacing right atrium induced heart failure model (250 times/min).

taken, embedded in paraffin, cut into slices and then stained according to the SP staining method. For a negative comparison, PBS was used in place of primary antibody. Computer aided image analysis (Motic Images 3000) was applied to calculate the density of nerves. The color of TH and ChAT was represented that the endochylema was stained into brownish yellow, meaning positive results. The color was classified as follows: colorless for a negative result, pale yellow brown for positive and brown for strong positive. The density of nerves was represented by the ratio between the nerve fiber area and the total detection area ($\mu\text{m}^2/\text{mm}^2$). The average density of all the nerves in the field of view was regarded as the average density of never of the slices. In each slice, five visions with maximum density of nerves and five visions with minimum density of nerves was taken, and the difference between the averages of the density of nerves was taken as the heterogeneity of the local distribution of nerves.

Statistical methods

The software SPSS 16.0 was used for statistical analysis. The metering data was represented by mean \pm standard deviation ($\bar{x} \pm s$). The comparison among multiple groups was analyzed with single factor analysis of variance while the comparison between two groups was detected with LSD method, taking the difference as statistically significance when $P < 0.05$.

Results

Completion of rapid pacing atrium induced heart failure model

After four weeks of rapid right atrium pacing, MAP < 60 mm Hg and LVEF $< 50\%$ in both the heart failure group and vagus stimulation group. In consideration of the symptoms of accelerated breathing, fastened heart rate, and loss of appetite in the dogs, the heart failure model was successfully achieved, see **Figure 1**.

Figure 1.

Comparison of MAP, LVEF and resting heart rate of the dogs in the three groups

After comparison, the baseline differences of MAP, LVEF, and resting heart rate in the groups had no statistical significance ($P > 0.05$). 30 d after the experiment, the values of MAP and LVEF in the heart failure group and vagus stimulation group declined as compared to those in the sham operation group. The resting heart rate increased in the heart failure (161 bpm) and vagus stimulation group (165 bpm) as compared to the sham operation group (131 bpm) ($P < 0.05$). There were no differences in MAP, LVEF, and resting heart rate in the heart failure and vagus stimulation group. 37 d after the experiment, MAP and LVEF in the heart failure group decreased remarkably as compared to the sham operation group while the resting heart rate increased remarkably as compared to the sham operation group and vagus stimulation group ($P < 0.05$). Compared with those in the sham operation group, the differences of MAP, LVEF, and resting heart rate in the vagus stimulation group had no statistical significance ($P > 0.05$) see **Tables 1** and **2**.

Comparison of heterogeneity of ventricular muscle nerve distribution of dogs in each group 37 d after the experiment

The comparison of heterogeneity of autonomic nerves showed: compared with those in the sham operation, the heterogeneity density of positive TH and ChAT nerves in the heart failure

Ventricular autonomic nerve in heart failure canine

Table 1. Comparison of MAP and LVEF of Dogs in the Three Groups, before and after pacing ($\bar{x} \pm s$)

Group	MAP (mm Hg)			LVEF		
	Baseline	Experiment 30 d	Experiment 37 d	Baseline	Experiment 30 d	Experiment 37 d
Sham operation group	128.42 ± 9.24	126.18 ± 8.54	127.44 ± 7.57	0.75 ± 0.01	0.72 ± 0.03	0.71 ± 0.01
Heart failure group	129.13 ± 8.65	58.45 ± 3.80 ^a	50.35 ± 6.85 ^a	0.65 ± 0.02	0.45 ± 0.01 ^a	0.43 ± 0.02 ^a
Vagal stimulation group	126.57 ± 10.82	55.32 ± 5.43 ^a	123.56 ± 7.84 ^b	0.73 ± 0.01	0.48 ± 0.01 ^a	0.65 ± 0.02 ^b

Note: ^aCompared with those in the sham operation, $P < 0.05$; compared with those in the heart failure group, ^b $P < 0.05$.

Table 2. Comparison of MAP and LVEF of the Dogs in the Three Groups, before and after pacing ($\bar{x} \pm s$)

Group	Resting heart rate (times/min)		
	Baseline	Experiment 30 d	Experiment 37 d
Sham operation group	133.42 ± 9.24	130.55 ± 7.20	135.92 ± 7.07
Heart failure group	126.20 ± 7.01	160.80 ± 5.10 ^a	164.00 ± 6.50 ^a
Vagal stimulation group	125.57 ± 6.44	165.43 ± 7.22 ^a	130.42 ± 7.48 ^b

Note: ^aCompared with those in the sham operation, $P < 0.05$; compared with those in the heart failure group, ^b $P < 0.05$.

Table 3. Comparison of heterogeneity of ventricular muscle nerve distribution of dogs in each groups 37 d after the experiment ($\mu\text{m}^2/\text{mm}^2$, $\bar{x} \pm s$)

Group	TH	CHAT
Sham operation group	7.50 ± 1.25	10.42 ± 3.40
Heart failure group	21.80 ± 4.03 ^a	4.57 ± 2.14 ^a
Vagal stimulation group	9.25 ± 2.50 ^b	13.24 ± 3.48 ^b

Note: ^aCompared with those in the sham operation, $P < 0.05$; compared with those in the heart failure group, ^b $P < 0.05$.

group increased remarkably (average $P < 0.05$). While no remarkable changes in the heterogeneity of the density of TH and ChAT positive nerves in the vagus stimulation group occurred, as compared to the sham operation group. The nerve fibers of TH and ChAT in the sham operation group were stained brown; while the funicular protein expression of brown TH and pale brown ChAT in the heart failure group decreased; the pale brown funicular TH expression and a large amount of deep brown funicular ChAT protein expression was seen in the vagus stimulation group, see **Table 3** and **Figures 2** and **3**.

Discussions

At present, there are many methods for preparing chronic heart failure animal models, including; pressure or volume load induced heart failure model, ischemic cardiomyopathy model,

cardiac pacing heart failure model, drug induced dilated cardiomyopathy model, myocardial hypertrophy, and transgenic animal heart failure models [7]. The establishment of heart failure animal models plays an important part in the study of the pathology and physiology of heart failure as well as disease prevention and treatment. The heart failure model induced by a long period of rapid right ventricle pacing (> 200 bpm) seems quite similar to the dilated cardiomyopathy model, while the hemodynamics and neuroendocrine levels of the heart induced by pacing are similar to that of humans. The present model has a higher controllability, and can reproduce different models by changing fre-

quency and duration of pacing, and thus can be used for the study of pathological, physiological, and molecular biological features of chronic heart failure at different stages of development. The results of the experiment showed, 30 d after the rapid right ventricle pacing, MAP < 60 mm Hg and LVEF $< 50\%$ in the heart failure group and vagus stimulation group, indicating the preparation of the heart failure dog model was successful. Meanwhile, the values of MAP and LVEF at 30 d and 37 d in the heart failure group lasted shorter than those in the sham operation group, hinting that the severity of heart failure tended to develop gradually along with the elongation of pacing time. On 37 d after the experiment, values of MAP and LVEF in the vagus stimulation group increased compared with those in the heart failure group, but the differences compared with those in the sham operation group had no statistical signifi-

Ventricular autonomic nerve in heart failure canine

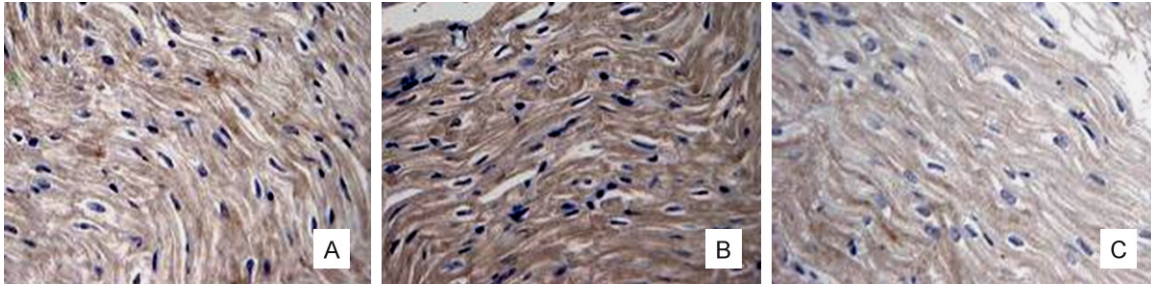


Figure 2. Positive nerve expression of TH at the anterior wall of the left ventricle of dogs 37 d after experiment ($\times 400$ magnification). A: Sham operation group; B: Heart failure group; C: Vagus stimulation group.

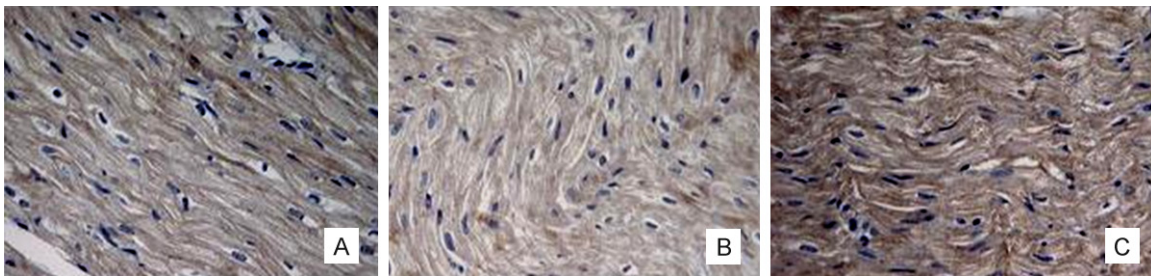


Figure 3. Positive nerve expression of ChAT at the anterior wall of left ventricle of dogs 37 d after experiment ($\times 400$ magnification). A: Sham operation group; B: Heart failure group; C: Vagus stimulation group.

cance, hinting that the stimulation on vagus nerve cords could improve cardiac function and hemodynamics under heart failure.

It is very hard to evaluate the activity of vagus nerves of patients, clinically. Presently, what can be done is to observe the acceleration of resting heart rate (HR) and the reduction of variance of HR, particularly the reduction of high frequency elements or the indirect accomplishment of low frequency and high frequency [8]. Therefore, the resting HR could reflect the functions of vagus nerves and can be used to roughly evaluate the balance of autonomic nerves. Large scales of clinical experiments suggested that, the cardiovascular mortality rate of the crowd with resting HR > 90 bpm is two times higher than those with a resting HR < 60 bpm [9]. The present study also showed, the resting HR in the heart failure group increased remarkably as compared to the sham operation group, whereas the vagus stimulation group had no statistical significance as compared to the sham operation group. The results of the experiment hinted that the functions of autonomic and vagus nerves decreased after heart failure, causing an increase of resting HR. Upon

heart failure, the excitement of sympathetic nerves occurred and the functions of vagus nerves declined. With the reduction of functions of vagus nerves and excessive activation of sympathetic nerves, serious changes occur in cardiac muscle, forming the cornerstone of ventricular remodeling. The actions of sympathetic and vagus nerves on the heart are supplementary and antagonistic. Once the balance between them is broken, ectopic rhythm will be induced, the threshold of ventricular fibrillation would be reduced, and the opportunity of ventricular fibrillation will be strengthened.

In systolic heart failure, both the nerves and humoral system are activated to maintain the functions of the heart. The activation of the nervous system is represented by the excitement of sympathetic nerves and reduction of activation of vagus nerves. There is evidence suggesting that regulation of the vagus nerves of patients with heart failure becomes abnormal. At present, there are already multiple drugs which have a suppressive action on the excessive activation of the sympathetic nerves of patients with heart failure, such as β -adrenergic receptor antagonists, angioten-

sin-converting enzyme inhibitors, and angiotensin II receptor antagonists. However, no effective drugs were found to improve the abnormal regulation of the vagus nerves. Therefore, the present study turns to device therapy for the regulation of vagus nerves. The results of the experiment showed that, heart failure was closely related to the attenuation of activation of vagus nerves. Vagus stimulation may become another effective method to treat heart failure. Zhang, et al. [10] studied the therapeutic effects of vagus stimulation in the right ventricle high frequency stimulation induced heart failure model. The results showed, vagus stimulation over three months can significantly reduce ventricular volume and increase the ejection volume. Gaetano M, et al. [11] conducted a multiple center study using vagus nerve stimulation for (how long?) to 32 patients with heart failure and found: the level of cardiac functions (by NYHA classification) of most patients improved at least one level and the resting heart rate declined remarkably and was easy to maintain over a period of. Living quality, step walking distance for 6 minutes, and ultrasonic cardiogram indexes of these patients significantly increased. The study proved that, homogeneity of autonomic nerve distribution occurred in the heart failure group, the homogeneity of the density of the TH and ChAT positive nerves increased significantly, the expression of TH fibrin increased while the ChAT fiber protein expression reduced. After stimulation of the vagus nerve cord, the imbalance of autonomic nerves of the ventricular muscles was improved, the fiber protein expression of ChAT increased and simultaneously the hemodynamics, ejection fraction, and resting heart rate were restored. The abnormal functional regulation of autonomic nerves in the heart is an important pathological and physiological feature in chronic heart failure. In the early stages of heart failure, the tension of sympathetic nerves raises and the tension of vagus nerves declines; in later stages of heart failure, the tension of both sympathetic and vagus nerves will be damaged. The homogeneity of autonomic innervations of the heart reflects the severity of heart failure. Clinical and basic experiments suggest that vagus nerves have a close relationship with cardiovascular diseases. Therefore, it is crucial to improve the tension of vagus nerves and maintain the balance of autonomic nerves in treating cardiovascular diseases.

Conclusion

Results of this study hint that chronic stimulation of vagus nerves could become a potential clinical therapy for the treatment of heart failure. It could also reverse some aspects of ventricular remodeling such as the heterogeneous distribution of nerves. Unilateral vagus nerve stimulation increased vagus nerve activity, slowed heart rate, recovered autonomic nerve homogeneity and function imbalance, and improved heart function in heart failure canines.

Acknowledgements

This work was supported by grant 2013911119 from Science and Technology Supporting Project of Xinjiang Uygur Autonomous Region.

Disclosure of conflict of interest

None.

Address correspondence to: Dr. Najina Wugeti, Heart Center, The First Affiliated Hospital of Xinjiang Medical University, Xinjiang Urumqi Carp Road No. 137, Xinshi District, Xinjiang 830054, China. Tel: +86 991 4365592; Fax: +86 991 4365592; E-mail: 454726435@qq.com

References

- [1] Hunt SA, Abraham WT, Chin MH. 2009 Focused Update incorporated into the ACC/AHA Guidelines for the Diagnosis and Management of Heart Failure in Adults: a Report of the American College of Cardiology Foundation/American Heart Association Task Force on Practice Guidelines. Developed in collaboration with the International society for Heart and Lung Transplantation. *J Am Coll Cardiol* 2009; 53: e1-e90.
- [2] Ogawa M, Zhou S, Tan AY, Song J, Gholmieh G, Fishbein MC, Luo H, Siegel RJ, Karagueuzian HS, Chen LS, Lin SF, Chen PS. Left stellate ganglion and vagal nerve activity and cardiac arrhythmias in ambulatory dogs with pacing-induced congestive heart failure. *J Am Coll Cardiol* 2007; 50: 335-343.
- [3] La Rovere MT, Pinna GD, Maestri R, Robbi E, Caporotondi A, Guazzotti G, Sleight P, Febo O. Prognostic implications of baroreflex sensitivity in heart failure patients in the beta-blocking era. *J Am Coll Cardiol* 2009; 53: 193.
- [4] Dunlap ME, Bibevski S, Rosenberry TL, Ernsberger P. Mechanisms of altered vagal control

Ventricular autonomic nerve in heart failure canine

- in heart failure: influence of muscarinic receptors and acetylcholinesterase activity. *Am J Physiol Heart Circ Physiol* 2003; 285: H1632-40.
- [5] Sun YX, Wang GG. Research Progress on Animal Models of Heart Failure. *Medical Recapitulate* 2011; 21: 73-77.
- [6] Hui J, Shen ZY, Jiao P. Experimental Heart Failure Using Rapid Right Ventricular Pacing in Meishan Pig. *Chinese Journal of Cardiac Pacing and Electrophysiology* 2004; 18: 470-472.
- [7] Ji M, Zhou QN, Hou YM. Research Progress on vagal nerve activity of Heart Failure. *Chinese Journal of Cardiac Pacing and Electrophysiology* 2012; 26: 547-549.
- [8] Thayer JF, Lane RD. The role of vagal function in the risk for cardiovascular disease and mortality. *Biol Psychol* 2007; 74: 224-242.
- [9] Zhang Y, Popovic ZB, Bibeovski S, Fakhry I, Sica DA, Van Wagener DR, Mazgalev TN. Chronic vagus nerve stimulation improves autonomic control and attenuates systemic inflammation and heart failure progression in a canine high-rate pacing model. *Circ Heart Fail* 2009; 2: 692.
- [10] De Ferrari GM, Schwartz PJ. Vagus nerve stimulation: from pre-clinical to clinical application: challenges and future directions. *Heart Fail Rev* 2011; 16: 195-203.
- [11] Dibner-Dunlap ME, Thames MD. Baroreflex control of renal sympathetic nerve activity is preserved in heart failure despite reduced arterial baroreceptor sensitivity. *Circ Res* 1989; 65: 1526-1535.