Influence of the hypothalamic paraventricular nucleus (PVN) on heart rate variability (HRV) in rat hearts via electronic lesion[1]

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Abstract. Previous literatures have indicated that hypothalamic paraventricular nucleus (PVN) neurons are important for regulating the level of sympathetic and vagal nervous activity. Sympathovagal balance is closely related to heart rate variability (HRV). However, it still requires further elucidation regarding the effect of PVN on HRV by regulating sympathovagal balance. To detect the influence of the PVN on HRV, we evaluated the changes in time domain (including standard deviation of the R-R intervals (SDNN), and the root mean square of successive differences (RMSSD)) and frequency domain (including low frequency (LFnu), high frequency (HFnu) and the ratio of LF/HF) in HRV upon appropriate electronic stimulation, and lesions on the PVN of the rat in vivo. Cardiac vagal modulation was evaluated by HFnu; sympathetic modulation was evaluated by LFnu. Sympathovagal balance was evaluated by LF/HF and SDNN. Upon electronic stimulating (less than 0.6 mA) to the PVN of rats, we found that LFnu and HFnu changed correspondingly but recovered after the stimulation. When the PVN of the rats was injured, the RR intervals were enhanced with the rats' unilaterally or bilaterally injured PVN, especially the bilateral lesion. Meanwhile, LFnu, LF/HF and SDNN decreased gradually, accompanied with an increase of HFnu levels. So these PVN changes may indicate alterations of the sympathovagal balance.

Keywords: Heart rate variability (HRV), paraventricular nucleus (PVN), Rat, electrical lesion

1. Introduction

Automaticity is an intrinsic character of myocardiac cells. Their electrophysiological activity is largely modulated by the autonomic nervous system (ANS) [1, 2]. The sympathetic nervous system (SNS) and parasympathetic (also called vagal in cardiovascular) nervous system (PNS) are the two branches of the ANS. Both branches may influence each other in response to the function of the central nervous system (CNS) [3]. Therefore, the ANS is critical to the physiological and pathological status of the myocardiac cells [4].

The paraventricular nucleus (PVN) of the hypothalamus is inter-connected with the other parts of the brain involved in cardiovascular function. As the central regulator of the cardiovascular system, the PVN is an important site that integrates the automaticity and response of the cardiovascular system [5-7]. Thus, the activities of the PVN and ANS may be tightly connected.

Recent studies demonstrated an effective computational method of heart rate variability (HRV) to reveal both cardiac sympatho-vagal modulation [3, 8-10]. HRV is a noninvasive measurement that assesses the autonomic modulation of cardiac activity. It may analyze beat-to-beat variability in normal R-R (NN) intervals in an ECG. In a normal heart, there are continuous physiological variations of the sinus cycles reflecting a balanced sympatho-vagal state by HRV.

The standard clinically used parameters of HRV consist of the time domain indices, geometric measures and frequency domain indices [11]. The time domain index includes the standard deviation of all NN intervals (SDNN), and the root mean square of successive differences (RMSSD) represents the changes of heart rate over time or over the RR intervals, which is the time between two successive R waves in QRS complexes. Geometric methods are from the conversion of sequences of normalized RR (NN) intervals. The power spectrum of the frequency domain included mainly a very low frequency (VLF), a low frequency (LF) and a high frequency (HF). LF is modulated by sympatho-vagal nervous systems. When the LF is expressed in normalized units (nu), the increase of the LFnu component (including tilt, mental and/or physical stress, and sympathetic pharmacological agents) is generally considered a consequence of sympathetic activity. The HFnu and RMSSD are generally defined as markers of vagal modulation, which is controlled by breathing frequency. The LF/HF ratio and SDNN reflect the global sympatho-vagal balance, so they may be used as a measure for the balance [4, 12]. Although the parameters of HRV may reflect distinctly the influence of sympathetic or vagus nerves on the heart, the relationship between HRV and the ANS mediated by the PVN has not yet been fully elucidated.

To identify the effect of the PVN on HRV, we examined the changes of SDNN, LFnu, HFnu and LF/HF under the conditions of unilateral or bilateral PVN lesions over the course of time. We speculated that the PVN may play an important role on the balance of LF/HF, and it may regulate HRV through the ANS.

2. Material and methods

2.1. Animals

All experiments were performed at China Medical University (CMU) in accordance with the NIH Guidelines of the USA for Care and Use of Laboratory Animals. Protocols for animal handling and treatment procedures were reviewed and approved by the CMU Animal Care and Use Committee. Fifty male Wister rats (200-250 g) were obtained from the Department of Laboratory Animals, CMU.

Two groups of rats were caged under controlled temperature ($24 \pm 2°C$) with a dark/light cycle of 12 h. All efforts were made to minimize animal suffering as well as the number of animals used.

2.2. Successive ECG of rat under awake state

Two days before the operation, all rats in an awake state were detected for successive ECG. Rats were placed in experimental cages. The ECG lead II was recorded with surface electrodes (ADInstruments, Australia). Measurements were collected at baseline, prior to electronic lesion and after the 4th day, 7th day and 14th day of lesions. The conditions for the experiment are as following: sample rate, 1 k/s; range, 10 mv; low pass, 200 Hz; high pass, 10 Hz; and signaling requirement, successively intact ECG of 5 mins for each data.

2.3. Surgical procedure

Rats were individually caged without restraints for 1-2 h to adapt to the experimental conditions. Rats were then anesthetized with sodium pentobarbital (30-35 mg kg^{-1}, i.p.) and additional doses were given as needed. Then they were placed prone in a stereotaxic apparatus (Japan) with the incisor bar 5 mm below the interauaral line. The rats were implanted with two guide cannulas directed to the PVN (1.8 mm posterior to bregma, 0.5 mm lateral to midline and 7.6 mm below the surface). The guide implantation technique was adapted based on a previous work [5, 13].

An electronic stimulated needle was inserted into one of the paraventricular nuclei, as previously described [13, 14]. The PVN was stimulated with 0.2, 0.4 and 0.6 mA for 60 s or was impaired by using DC 1 mA for 60 s, either at the right only or the entire part, according to experimental procedures. A PVN without lesions was used as the control. In the groups of the Sham operation, rats were processed according to above operation, but without electronic stimulation. Rats were allowed recuperation for 3-5 days after the surgery. Successive ECGs for all animals according to standard II lead were collected for a minimum of 5 min in the morning of the 4th day, 7th day and 14th day, from 8:00 to 12:00, when the animals were conscious.

2.4. HRV measurements

Heart rate variability (HRV) parameters were measured over 256 RR intervals as previously described [15], with Powerlab® system (ADInstruments) and Chart 5.0 software over 256 R-R intervals. Any ectopic beats were automatically identified and discarded by the software before the analysis of the captured beats. Small-animal HRV software (nevrokard) was then used to identify HRV data. The parameters of HRV were all proposed by the Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology [11].

The variances of frequency components were transformed to a normalized unit (nu), which is performed by the following formula (VLF is from 0.04 to 0.1 Hz, LF is from 0.1 to 1 Hz and HF is from 1 to 3 Hz).

$$LF \ or \ HF \ norm \ (nu) = \frac{LF \ or \ HF(ms^2)}{Total \ power(ms^2) - VLF(ms^2)} \times 100$$

All samples' ECGs were collected and analyzed by PowerLab® with Chart® software Version 5.0

to obtain the parameters of HRV. The normalized unit (nu) is useful when evaluating the effects of different interventions in the same subject (graded tilting) or when comparing subjects with major differences in total power.

2.5. H & E staining of the coronal brain slice

Rat brains were removed and post-fixed in 4% formalin for 24 h and then washed, dehydrated from alcohol to dimethybenzene, and paraffin-embedded. Coronal brain slices of the rats in unilateral lesion and/or bilateral lesion were made and stained by standard *H&E*. Examination was performed by light microscope (TE2000-S Nikon, Japan) photograph.

2.6. Statistics

Data are analyzed statistically by one-way analysis of variance (ANOVA). The level of significance was set at 95% ($P < 0.05$). The software used for analysis of the data was the Statistical Package for the Social Sciences (SPSS, Chicago, IL, USA), version 13.0 for Windows. The data shown are mean ± SEM of at least ten independent experiments (n=10).

3. Results

3.1. Effect of PVN on power spectral density upon stimulation by electronic currents

Rats' PVN were stimulated with currents of 0.2, 0.4 or 0.6 mA. The normalized auto-power spectral density from right to left showed HF, LF and VLF, respectively, in Figure 1. When the PVN was stimulated with 0.2, 0.4 and 0.6 mA currents, the components of HF, LF and VLF changed correspondingly, and these changes backed down gradually after the stimulation. These results suggest that when the current stimulation was less than 0.6 mA, the changes were reversible.
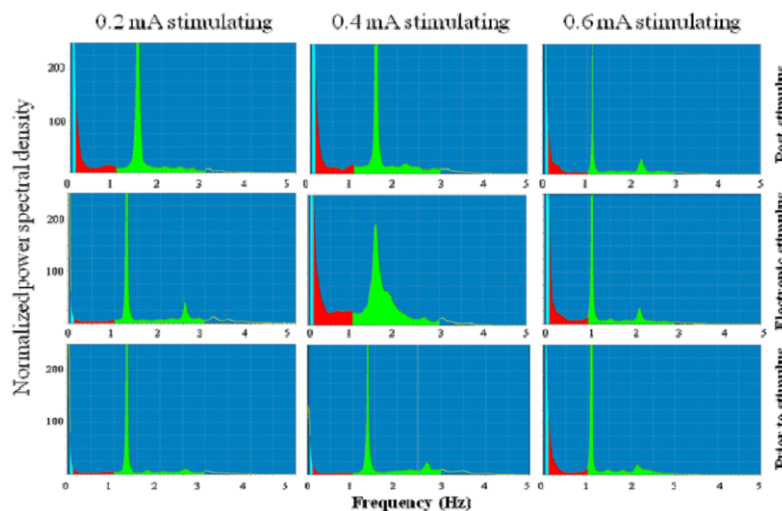

Fig. 1. Autoregressive spectrum of the PVN of the rats stimulated with different electronic currents. The right part is HF as indicated in green. The Middle is LF in red. The left part is VLF in ligh blue. When rat PVN was stimulated with 0.2 mA, 0.4 mA or 0.6 mA, the densities of VLF, LF and HF were shown in the frequence spectrogram, respectively.
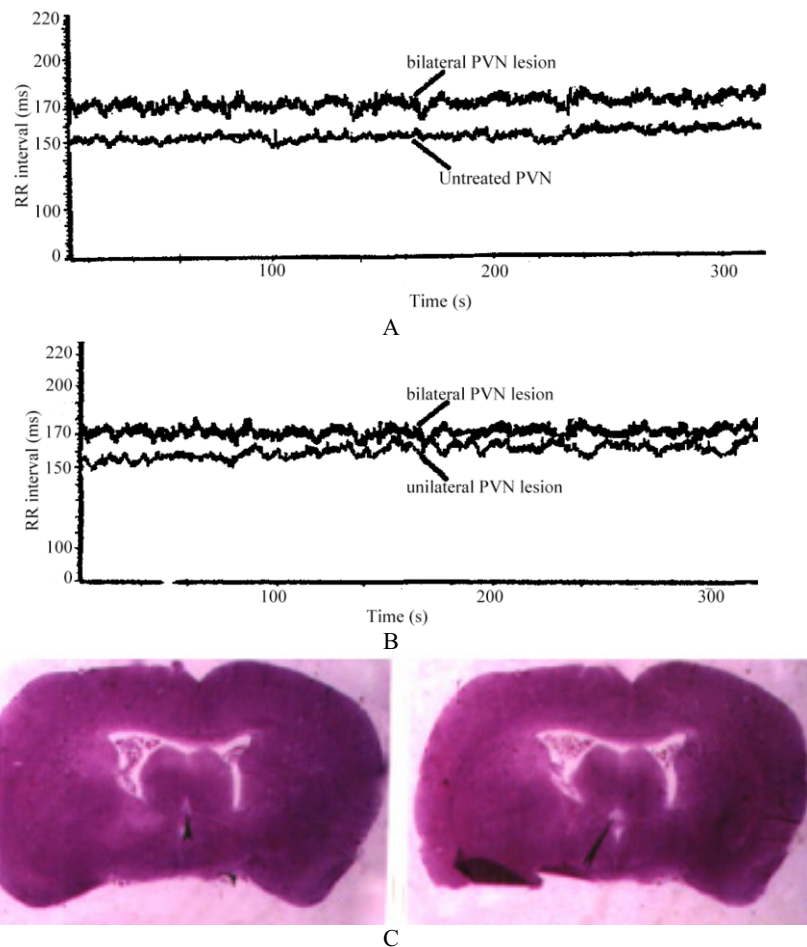
Fig. 2. Influence of PVN damage on RR intervals. A: RR interval in the PVN with bilateral lesion compared with that in the untreated PVN; B: RR interval in the PVN with bilateral lesion compared with that in the PVN with unilateral lesion; C: The localization of PVN lesions. Left: coronalis slice of rat brain at PVN with lesion at right; Right: coronalis slice of rat brain of PVN with bilateral lesion, indicated with the black arrowhead (1× magnification).

3.2. Effect of Lesion of PVN on R-R chart

Under the waking state, the heart rate (HR) of the rat over 5 minutes is about 460 ± 45 min^{-1}. Under a narcotic state, the HR is about 410 ± 53 min^{-1} (data not shown). The data showed that no significant change in the R wave in both the waking state and the narcotic state. However, after the PVN was damaged by electric stimulation, the RR interval changed distinctly. The RR intervals of the normal group were shorter than that of the lesion group, especially that of the bilateral lesion group. The RR interval values of the normal, unilateral lesion, and bilateral lesion groups are 150 ± 10 ms, 155 ± 22 ms and 170 ± 15 ms, respectively (Figures 2A and 2B). The heart rates also decreased in lesion group accordingly.

In the histology of the brain section, we observed that there was a non-staining area in the right of the PVN with a unilateral lesion PVN. In the PVN with a bilateral lesion, there was a triangle non-staining in the left and right areas of the PVN (Figure 2C, indicated by arrow).

3.3. Influence of PVN lesion on HRV

Martins-Pinge et al. reported that the neurons within the PVN may contribute to cardiac autonomic modulation [5]. Our results showed that in the PVN with unilateral and/or bilateral lesions, the LFnu were much lower in postoperative day 4, day 7 and day 14 than in the preoperative day 0 ($P < 0.05$), especially in day 14 for the PVN with a bilateral lesion ($P < 0.05$). During preoperative day 0 to postoperative day 4, the LFnu and SDNN in the PVN with unilateral and/or bilateral lesions had already gradually decreased with a significant difference ($P < 0.05$). After the postoperative day 7, the decrease of LF gradually leveled off. As a result, there was no significant difference in LFun between postoperative day 7 and day 14 ($P > 0.05$). Furthermore, an opposite change in HFnu in contrast to LFnu and SDNN in the PVN with unilateral and/or bilateral lesion was observed (Figure 3). However, LF/HF and SDNN were similar to those of the LFun (Figure 3). Figure 4 is an illustration of the interaction of HRV, PVN and ANS.

4. Discussion and Conclusion

The areas of the central nervous system involved in cardiovascular control include the rostral
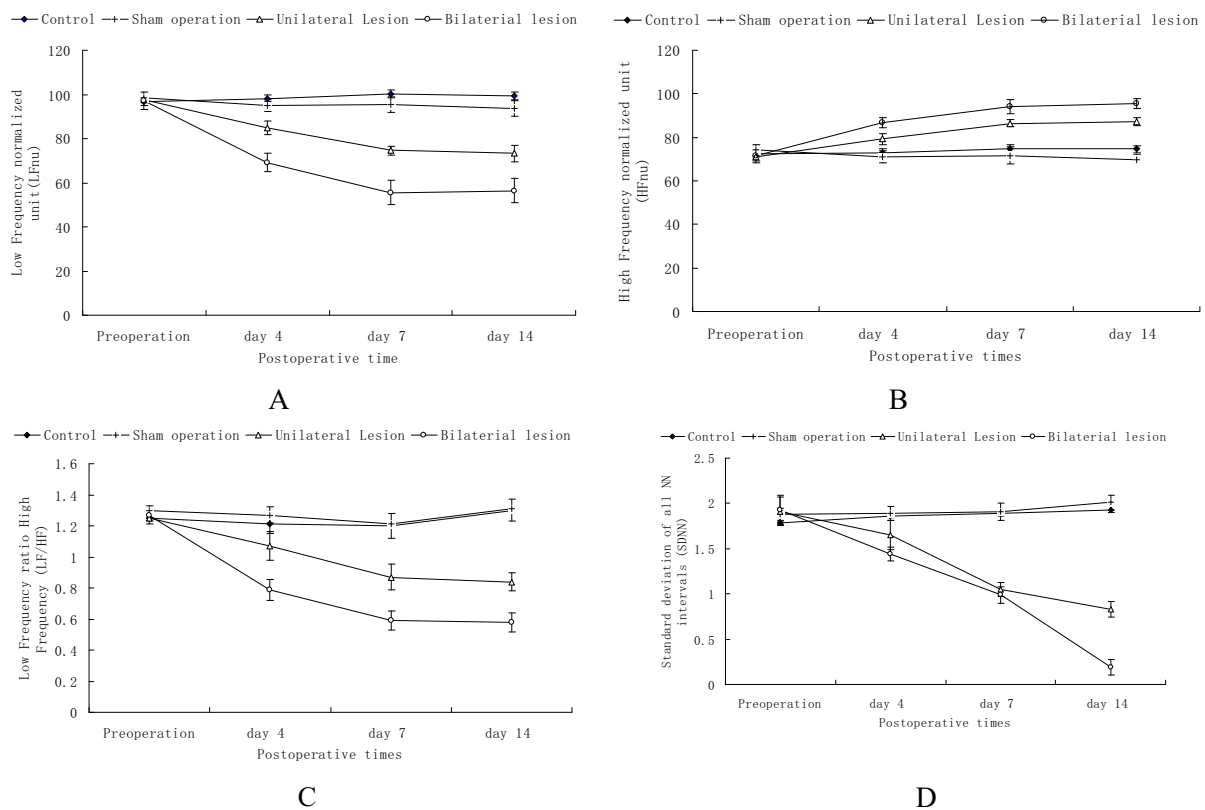

Fig. 3. Influence of PVN damage on HRV parameters in rat hearts. The standard deviation of the normalized RR intervals (SDNN), Low frequency normalized unit (LFnu), High frequency normalized unit (HFnu) and LF/HF were recorded in preoperation, postoperative 4, 7 and 14 days when the PVN was treated with electronic leision, respectively. Preoperation and sham operation groups were used as control.
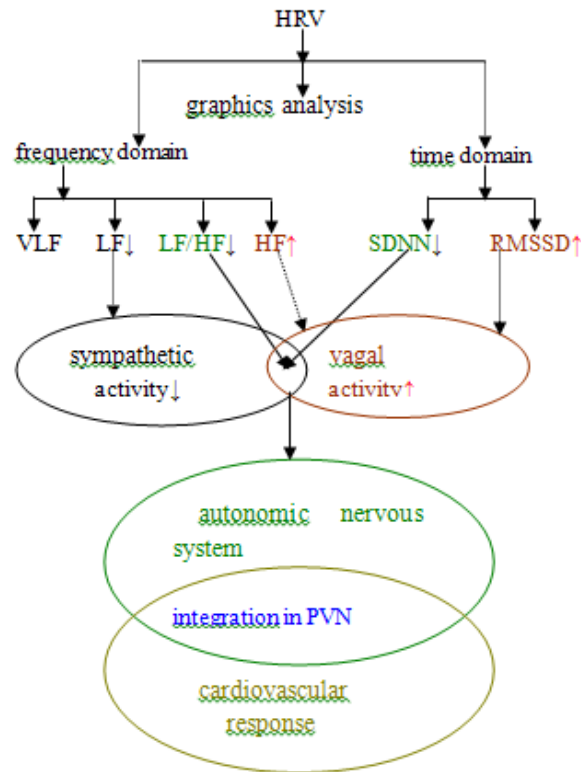
Fig. 4. Proposed diagram of the interactions among HRV, ANS and PVN. The HRV parameters of the heart consist of time domain (mainly including SDNN and RMSSD), geometric and frequency domain (mainly including VLF, LF, HF and LF/HF). LF is related to sympathetic activity. HF and RMSSD are related to vagal activity. LF/HF and SDNN are related to the sympathetic and vagal balance. ANS includes sympathetic and vagal nerves. Its function is integrated in PVN together with cardiovascular response.

ventrolateral medulla (RCLM) [16-24], the nucleus of solitary tract (NTS) [7, 25] and paraventricular nucleus (PVN) [26]. The PVN connects these areas in the brain involved in cardiovascular function, forming important neuroendocrine and autonomic modulation, which further modulates the homeostasis of the body through the brain stem, spinal cord, median eminence and posterior pituitary. A recent report by de Abreu indicated that neurons in the PVN could contribute to cardiac autonomic modulation by changing parasympathetic or sympathetic activity [5, 22, 23, 27]. Also, the interplay between the sympathetic and vagal outflows may be assessed by cardiovascular rhythmicity with appropriate spectral methodologies [28, 29].

The rat is the most simple and natural HRV model among vertebrates that are similar to human. There are few reports about the mechanism of the regulation of the PVN of rats, especially for events related to HRV. Here, we applied electronic stimulation and caused lesions on rats' PVN, and then investigated the changes in the spectral components by special HRV analysis.

We showed that electric-induced lesions in the PVN of rats results in decreases of LFnu, LF/HF and SDNN, but an increase of HF power and RMSSD of HRV, suggesting that these spectral components may be modified by PVN damage. In rats with a PVN lesion, the decrease of LFnu reflected the decline of sympathetic activity, which explains why the HRs slowed down gradually. Our data support the notion that the potential neurons and transmitters in the PVN may be closely related to HRV. A concrete procedure for this assumption should be further studied in this field.

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