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Tragus Nerve Stimulation Suppresses Post-Infarction Ventricular Arrhythmia by Modulating Autonomic Activity and Heterogeneities of Cardiac Receptor Distribution

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Background: Imbalanced cardiac autonomic control and cardiac receptors redistribution contribute to the arrhythmogenic substrate under the myocardial infarction (MI) condition. Stimulating the auricular branch of vagus nerve (AB-VNS) has been proven to reduce post-infarction ventricular arrhythmia (VAs), but its potential mechanisms were largely unknown.

This study aimed to investigate whether long-term intermittent low-intensity AB-VNS could produce a protective effect on modulating autonomic activities and abnormal redistribution of autonomic nerve efferent receptors in a MI canine model.

Material/Methods: Twelve healthy beagle dogs underwent ligation of the left anterior descending coronary artery to establish a MI model and were randomized into 2 groups: an AB-VNS group, (AB-VNS for 4 weeks) and a control group (sham stimulation for 4 weeks). Dynamic electrocardiogram recording, neural recording, catecholamine concentration, and histological studies were conducted subsequently.

Results: Compared to the control group, the AB-VNS group had significantly suppressed post-infarction VAs, reduced low frequency (LF) power and increased high frequency (HF) power. In the AB-VNS group, with the progression of reduced cardiac sympathetic activities and augmented cardiac parasympathetic activities, the catecholamine concentration in heart tissue declined in the peripheral infarction area and right ventricle (RV); tyrosine hydroxylase (TH)-positive neurons decreased in the inferior cardiac sympathetic nerve, and choline acetyltransferase (ChAT)-positive neurons increased in the cervical vagus nerve. Expression of TrkA and P75NGFR were reduced in the peripheral MI (peri-MI) and non-MI area with AB-VNS. The mRNA expression of adrenergic and nicotinic receptors (β_1-AR, β_3-AR, and CHRNA7) significantly declined in the peri-MI and non-MI area of the AB-VNS group.

Conclusions: Chronic intermittent low-intensity AB-VNS effectively suppressed post-infarction VAs by potentially rebalancing extracardiac intrathoracic autonomic activities, reducing excessive cardiac sympathetic denervation, and attenuating the heterogeneities of cardiac efferent nerve receptors distribution.

MeSH Keywords: Arrhythmias, Cardiac • Autonomic Nervous System • Vagus Nerve

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Background

Malignant ventricular arrhythmia (VA) after myocardial infarction (MI) is a major etiology of sudden cardiac death [1,2]. It is well recognized that MI facilitates the formation of cardiac sensitive substrates and imbalances cardiac autonomic control, resulting in the creation of arrhythmogenic triggers [3]. On the one hand, disordered cardiac autonomic control generally includes unbalanced extrinsic or intrinsic cardiac autonomic activities, adverse intrathoracic neural remodeling, and inhomogeneous cardiac innervation, is especially deteriorative in chronic MI phases [4]. On the other hand, differential cardiac receptors distribution, which contributes to the abnormal interface reaction between neurotransmitters and cardiomyocytes surface receptors, is considered to potentially aggravate arrhythmogenesis [5,6].

As a transcutaneous noninvasive therapeutic strategy, the auricular branch of the vagus nerve stimulation (AB-VNS) has been shown to allow for a protective effect in some pathological conditions with sympathetic hyperactivity, such as heart failure, ischemia-reperfusion injury (I/R), and the initial phase of atrial fibrillation [7–9].

In addition, our previous work indicated that chronic intermittent AB-VNS significantly lowered the incidence of post-infarction VAs by enhancing cardiac electrical stability [10], while more potential mechanisms remain unclear. Thus, we hypothesized that AB-VNS could protect against post-infarction VAs through balancing cardiac autonomic activities and ameliorating the receptors redistribution in the canine models with healed MI.

Material and Methods

Ethics approval

This study was implemented in absolute accordance with the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. Experimental protocols were approved by the Animal Experimental Ethics Committee of the First Affiliated Hospital of Xinjiang Medical University (Permit number: IACUC-20150225-60) under the Declaration of Helsinki and conformed to the principle of the Association for Assessment and Accreditation of Laboratory Care (AAALAC).

Animals and study design

All animals were handled in compliance with the guiding principles for their care and use. Twelve male beagle dogs, each weighing 15 to 20 kg, were randomized into 2 groups. One was the AB-VNS group (n=6), which was given chronic intermittent AB-VNS after the preconditioning of MI. The other was the control group (n=6), which was given sham AB-VNS under MI circumstance. In the process of all invasive operations, animals were administered Zoletil 50 at the first dose of 20 mg/kg to induce anesthesia and with sodium pentobarbital (3 mg/100 mL) at the dose of 3 mL/hour to maintain anesthesia. Fentanyl (0.5 mg/kg) was used for intraoperative analgesia. At the end of this study, all dogs were given S1S1 programmed electrical stimulation to induce VT/VF and the stimulus intensity was defined as twice the pacing threshold. Survived dogs were euthanized by intravenously injecting overdosed pentobarbital sodium (80 mg/kg) in the end. All experimental procedures were carried out according to the study protocol (Figure 1A).

MI model

A respirator (UGO BASILE 5025) connected to an oxygen cylinder was used for ventilating in all dogs under anesthesia. A left thoracotomy was carried out in the fifth intercostal. Left ventricle (LV) infarction was produced by permanent ligation of the left anterior descending (LAD) artery above the first diagonal branch using a 4-polyglactin suture. After the surgery, cefazolin (20 mg/kg, intravenous) was injected intravenously to prevent infection for 2 weeks and morphine (4.4 mg/kg) was administered to maintain postoperative analgesia.

AB-VNS

Chronic intermittent stimulation was performed on the bilateral tragus by 2 alligator clips (MLA 250, BNC-alligator clip) in a closed-loop path under anesthesia (Figure 1B). As our previous method indicated [10], incremental voltages were delivered to the bilateral tragus by a Grass-S88 stimulator (Astro-Med, Inc., West Warwick, RI, USA; parameters, 20 Hz, 1-ms pulse width, square wave) until the sinus rate slowed. The range of stimulating voltage was from 15 V to 25 V. The sham stimulation was defined as bilateral tragus stimulation without delivering any voltage. The voltage threshold was defined as the lowest voltage which induced a greater than 20% slowing of the sinus rate. The AB-VNS group was given tragus stimulation at 50% threshold voltage intensity for 1 hour every other day in consecutive 4 weeks. In the control group, the sham stimulation was applied for 1 hour every other day with the same pattern as set forth.

VA and heart rate variability

As in our previous work [11], 24-hour dynamic electrocardiograms (ECGs) were measured for heart rate (HR), incidence of VAs, and HR variability (HRV) index in ambulatory dogs in home-made jackets under a normalized environmental situation (Century 3000 Holter; BMS, USA). HRV was recorded in

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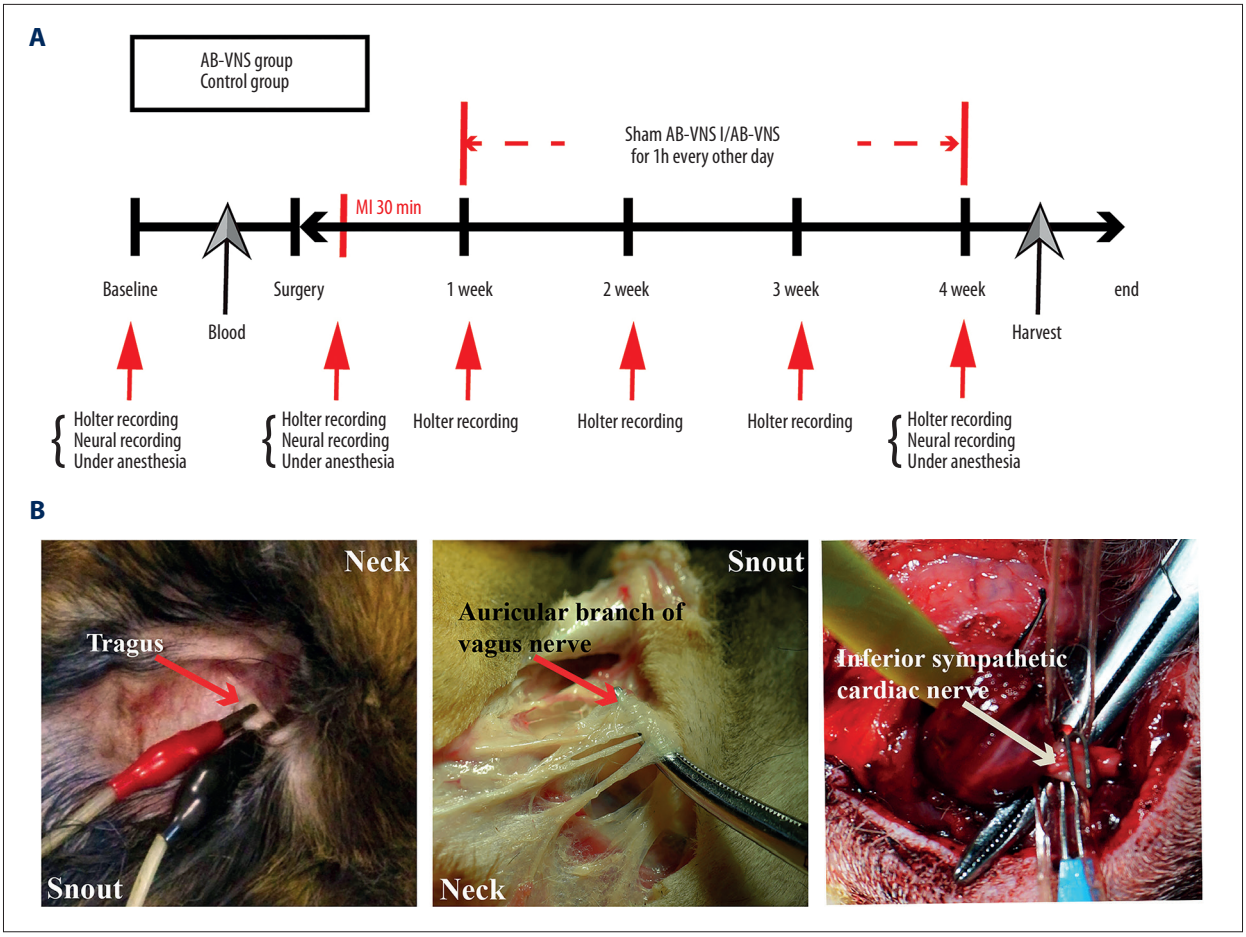

Figure 1. The study protocol for **Panel A** was performed with a particular flow chart. From left to right sequentially, **Panel B** showed the location of tragus stimulation and where to place the 2 alligator clips, the detailed anatomy of auricular branch of vagus nerve and the anatomical location of the subordinate cardiac branch of left stellate ganglion (LSG) for neural recording.

conscious dogs at the baseline condition before surgery and weekly performed when starting AB-VNS. Three main spectral components were determined as high frequency (HF norm, the power range from 0.15–0.4 Hz, a marker of parasympathetic tone), low frequency (LF norm, the power range from 0.04–0.15 Hz, a marker of sympathetic tone), and LF/HF ratio (an interplay of autonomic activities) [12]. Obtained data were analyzed with a DMS CardioScan Holter monitor system (Model Number 1495322841). VAs was classified as premature ventricular contraction (PVC, extra ventricular systole without atrial depolarization), ventricular tachycardia (VT, 3 or more consecutive PVCs) and ventricular fibrillation (VF, asynchronous and uncoordinated ventricular contraction or diastole with heart rate >250 beats/minute) according to the Lambeth conventions [13].

Neural recording

Left stellate ganglion (LSG) is located on the left side of the adipose tissue in front of the seventh vertebra. The inferior cardiac sympathetic nerve (ICSN) ascends from the LSG and descends along the deep part of the cardiac plexus. In accord with our previous work [14], the ICSN and the left cervical vagal nerve (CVN) were covered by a layer of liquid paraffin and attached to a modified bipolar electrode to record autonomic activities for 5 minutes at 3-time points: baseline, 30 minutes after MI, and 4 weeks after AB-VNS (Figure 1B). Bandpass filtering was set at high-pass 200 Hz and low-pass 120 Hz, raw signals were delivered to a PowerLab DP-301 Differential Amplifier (ML866/P; ADInstruments, Bella Vista) and were analyzed by the LabChart 8.0/proV7 software (ADInstruments, Australia). Root mean square (RMS) indicator was calculated to indirectly evaluate the amplitude, frequency, and duration of nerve activities.

Measurement of catecholamine concentrations

Blood samples were harvested from the femoral vein and liquid supernatants were collected after centrifuging immediately. Hearts were excised, rinsed in cold phosphate-buffered

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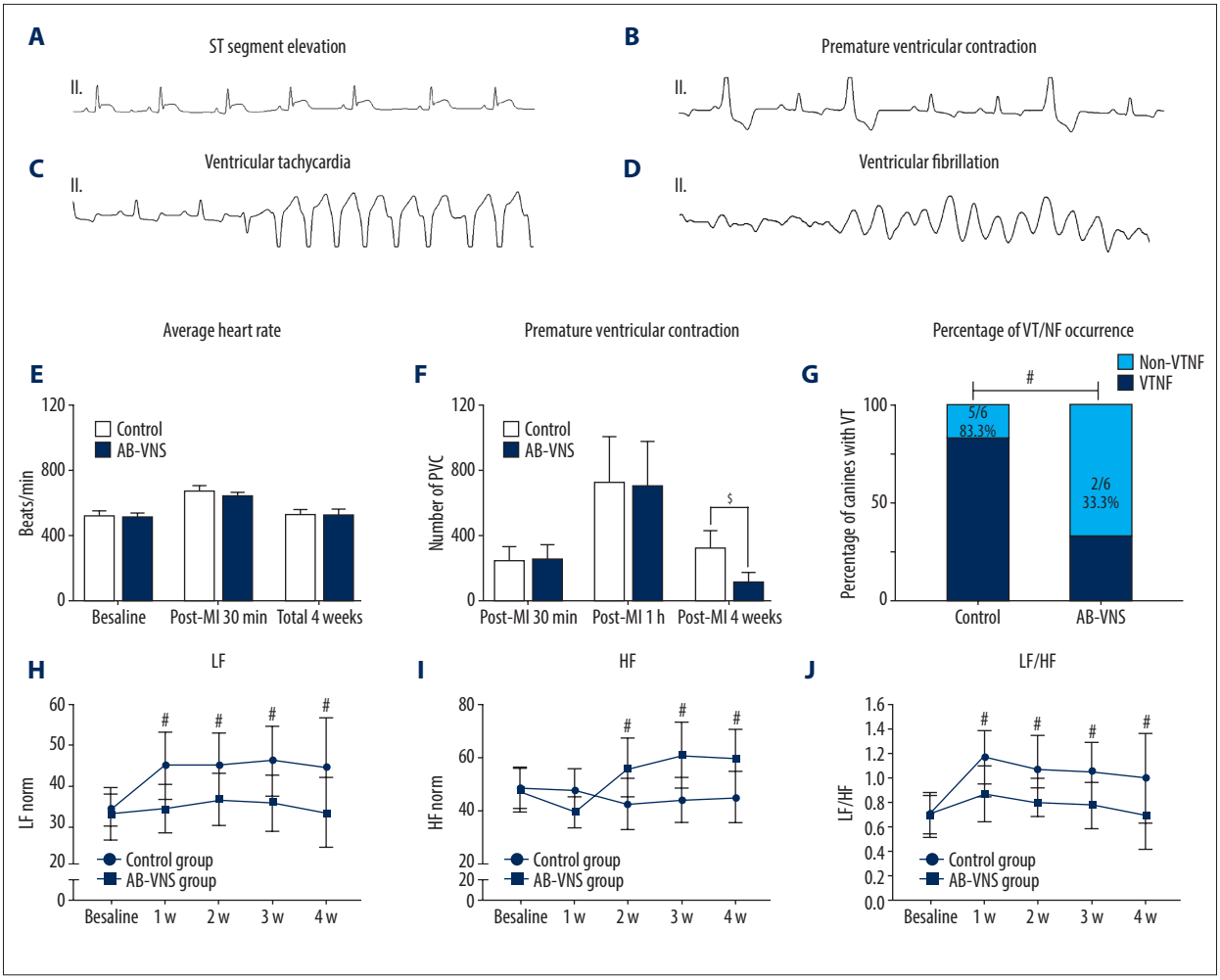

Figure 2. Typical ST segment elevation images and spontaneous VA images from AMI models containing PVC, VT and VF are indicated in **Panels A–D**. Results of average heart rate analysis and VAs burden were showed in **Panels E–G** successively. # $P<0.05$ compared with the control group, $ $P<0.01$ compared with the control group. HRV results were recorded weekly from the baseline to the fourth week after AB-VNS (**Panels H–J**). # $P<0.05$ between the 2 groups at each point in time. LF– low frequency norm; HF – high frequency norm; LF/HF – low frequency norm/high frequency norm. VA – ventricular arrhythmia; AMI – acute myocardial infarction; PVC – premature ventricular contraction; VT – ventricular tachycardia; VF – ventricular fibrillation; HRV – heart rate variability; AB-VNS – auricular branch of the vagus nerve stimulation.

saline and then frozen in liquid nitrogen before storing at –80°C. The ventricle was routinely separated from total heart tissue and cut open to expose the infarction zone for examination under the 6.4× dissecting microscope. Catecholamine concentrations of the blood and tissue, including epinephrine (EPI) and norepinephrine (NE) were measured as previously described [11].

Histology

Immunohistochemistry

Cardiac peri-infarction tissue, ICSN, CVN, and auricular branch of vagus nerve (AB-VN) from all sacrificial animals were immediately transferred to 4% paraformaldehyde for 24 hours and embedded in paraffin for mounted sections. The densities of tyrosine hydroxylase (TH) positive (TH antibody, Abcam, Britain, GR27536-1) and choline acetyltransferase (ChAT) positive neurons (ChAT antibody, Abcam, Britian, GR13308-5) of tissues were measured by immunostaining as previously described [11]. The nerve densities were semi-quantitatively analyzed by fluorescence microscopy and a computer-assisted image analysis system.

Western blotting

Frozen excised specimens containing peri-infarction area, non-infarction area, and remote ventricular tissue were selected for analysis. Total extracted proteins from the samples were

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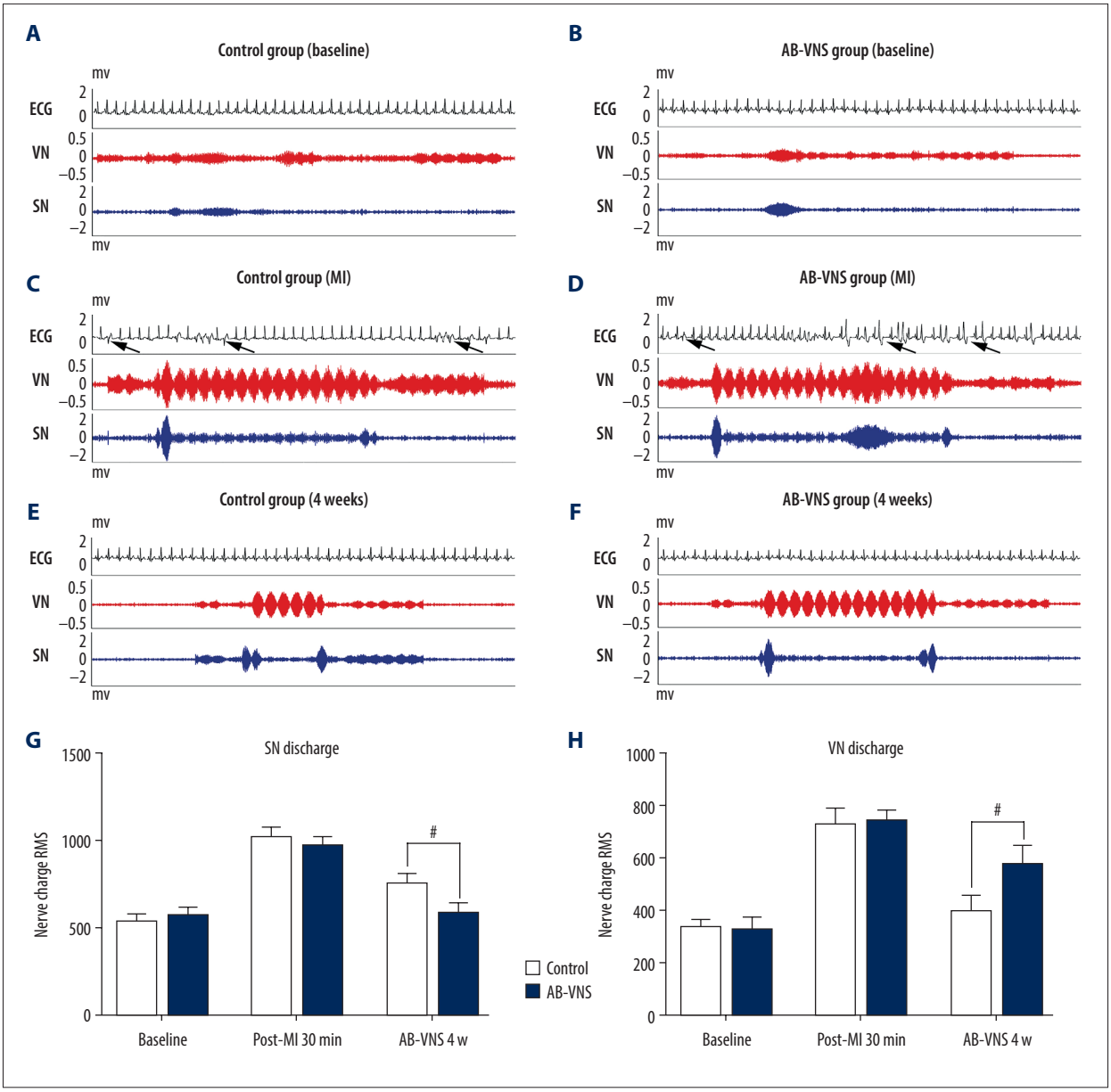

Figure 3. Neural discharge was recorded from ICSN and CVN at baseline status (**Panels A, B**), post-infarction 30 minutes (**Panels C, D**) and after AB-VNS for 4 weeks (**Panels E, F**). Typical ECG and neural recording images were shown above. Black arrows indicated the appearance of spontaneous PVC. **Panels G, H** showed quantitative statistical results of nerve discharge RMS in 3 conditions, including baseline level and the post-MI 30 minutes and after AB-VNS for 4 weeks. # $P<0.05$ compared with the control group. VN – discharge of vagus nerve; SN – discharge of inferior cardiac sympathetic nerve. ICSN – inferior cardiac sympathetic nerve; CVN – cervical vagal nerve; AB-VNS – auricular branch of the vagus nerve stimulation; ECG – electrocardiograms; PVC – premature ventricular contraction; RMS – root mean square; MI – myocardial infarction.

measured by western blots to quantify the expression of neurotrophins and adrenergic receptors using the primary antibody NGF/P75 (mouse monoclonal antibody, Bioss, bs-0193R), NGF/TrkA (rabbit polyclonal antibody, Santa Cruz, Sc-13577) and β1 (β_1-AR, goat polyclonal antibody, Abcam, ab77189). Gray level values on the images of every gel were analyzed by BandScan, and the relative quantities of target proteins were calculated from a fixed formula: the optical density (OD) of the target protein band/OD of the GAPDH band.

Quantitative real-time polymerase chain reaction (qRT-PCR)

The aforementioned samples were measured by qRT-PCR to quantify the mRNA expression levels of adrenergic and nicotinic

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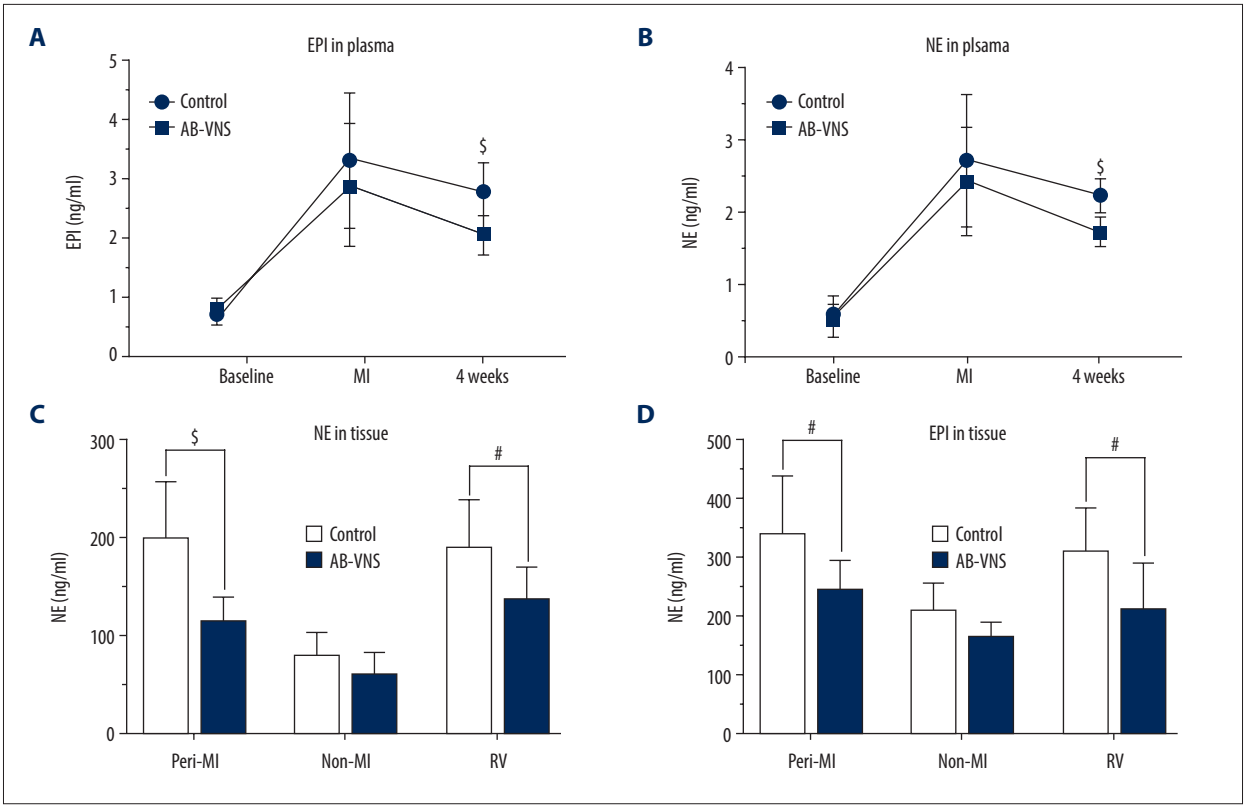

Figure 4. Line graphs (**Panels A, B**) show the variation trend of the EPI and NE concentrations of plasma, which were measured in 3 different periods: baseline, acute MI phase, and after AB-VNS for 4 weeks. (**Panels C, D**) The EPI and NE concentrations in tissue were determined at 3 sites: peri-MI, non-MI area, and RV, # $P<0.05$ compared with the control group, $ $P<0.01$ compared with the control group. EPI – epinephrine; NE – norepinephrine; MI – myocardial infarction; peri-MI – peripheral myocardial infarction; non-MI – non-myocardial-infarction; AB-VNS – auricular branch of the vagus nerve stimulation; RV – right ventricle.

receptors and every sample was tested in triplicate. All molecular biological assays were carried out according to the normative procedures with GAPDH (mouse monoclonal antibody, Abcam, ab9484) as the internal control. The tissue value was adopted by the average intensity value. The primer squences for related gene are presented in Supplementary Table 1.

Statistical analysis

IBM SPSS Statistics v.17.0 software (SPSS, Inc., Chicago, IL, USA) was used for statistical analysis. Qualitative data are expressed as percentages, and quantitative data are illustrated as the means±standard deviations (SDs). For histological analysis, the independent-samples *t*-test was used to compare the mean between the 2 groups. Repeated-measures analysis of variance (ANOVA) was performed for comparisons among the HR, PVC, HRV, RMS, and plasma catecholamine concentrations in 2 groups at different time points. The least significant difference (LSD) test was used as a post hoc test for multiple comparisons. Fisher's exact test was used to compare the incidence of induced VT/VF. A 2-tailed *P* value <0.05 was considered statistically significant.

Results

Efficacy of AB-VNS on spontaneous VAs and HRV

We performed 24-hour dynamic ECG on the study animals at these time points: baseline, post-infarction 30 minutes, and once a week during the AB-VNS period. Typical examples of spontaneous VAs are shown in Figure 2A–2D. There were no significant differences in average heart rate (HR) between the 2 groups at the time nodes aforementioned (Figure 2E). Premature ventricular contractions (PVCs) were comparable between control and AB-VNS group half or one hour after LAD occlusion. The total number of PVCs in the experimental group decreased noticeably compared to the control group from the 1st week to the 4th week (326±102 versus 123±58, $P=0.002$, Figure 2F). The incidence of induced VT/VF by programmed stimulation in the AB-VNS group was decreased in contrast to the control group ($P<0.05$, Figure 2G). As shown in Figure 2H, the LF values of the AB-VNS group were significantly lower than those in the control group in each week (respectively, $P<0.05$). Inversely, significant increases of HF values were shown in the AB-VNS group when compared with

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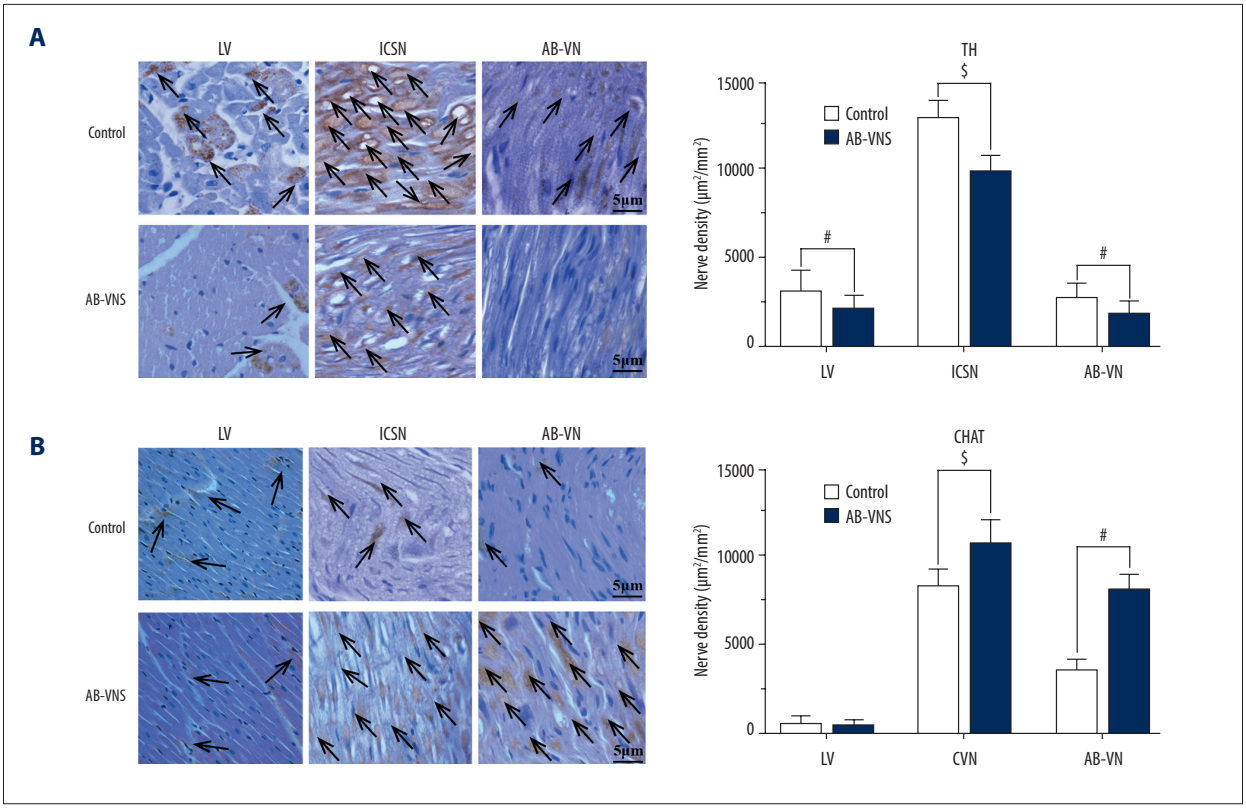

Figure 5. The density of tyrosine hydroxylase (TH)-positive and choline acetyl transferase (ChAT)-positive nerve fibers were depicted in **Panels A, B** sampled in 3 regions: LV – left ventricle; ICSN – inferior cardiac sympathetic nerve; CVN – cervical vagus nerve; AB-VN – auricular branch of the vagus nerve. $^{\$}$ P<0.01 compared with the control group, $^{\#}$ P<0.05 compared with the control group.

the control group from the 2nd week to the 4th week (respectively, P>0.05, Figure 2I). The LF/HF value in the AB-VNS group decreased significantly in comparison with the control group (respectively, P<0.05, Figure 2J).

Effect of AB-VNS on autonomic activities

Representative examples of the neural recording are displayed in Figure 3A–3F. Both at baseline and at 30 minutes after MI, there were no significant differences in DMS values between the 2 groups. However, significant decreases of RMS were shown in the AB-VNS group after a chronic intermittent stimulation for 4 weeks compared to the control group (RMS: 753.88±52.83 versus 582.29±53.53, P<0.05, Figure 3G). As for the vagal nerve activities, discharge RMS of CVN in the 2 groups was comparable at baseline and post-infarction 30 minutes. But the significant increase of CVN discharge RMS was shown in the experimental group by the treatment of AB-VNS when compared to the control group (RMS: 401.38±60.19 versus 580.38±66.84, P<0.05, Figure 3H).

Measurements of the plasma and tissues catecholamine concentration

As shown in Figure 4A, compared with the control group, the plasma EPI levels in the AB-VNS group markedly decreased 4 weeks later (2.15±0.33 versus 2.89±0.46, ng/mL, P=0.009). Moreover, AB-VNS significantly reduced the high NE levels of plasma in contrast to the control group after the intervention for 4 weeks (2.25±0.22 versus 1.75±0.19, ng/mL, P=0.002, Figure 4B). We found that both EPI and NE levels in peri-MI areas and right ventricle (RV) of the AB-VNS group were lower than those in the control group (respectively, P<0.05, Figure 4C), especially NE of tissue in peri-MI area (200.95±55.84 versus 108.82±28.38, P=0.005, Figure 4D). No obvious difference in tissue catecholamine concentrations was seen between the 2 groups at the non-MI area (respectively, P>0.05).

The densities of TH-positive and ChAT-positive neurons in tissue

As demonstrated in Figure 5A, the TH-positive nerve densities in LV of the AB-VNS group showed a significant decrease compared to the control group (TH was 705±321 versus 382±202, ChAT

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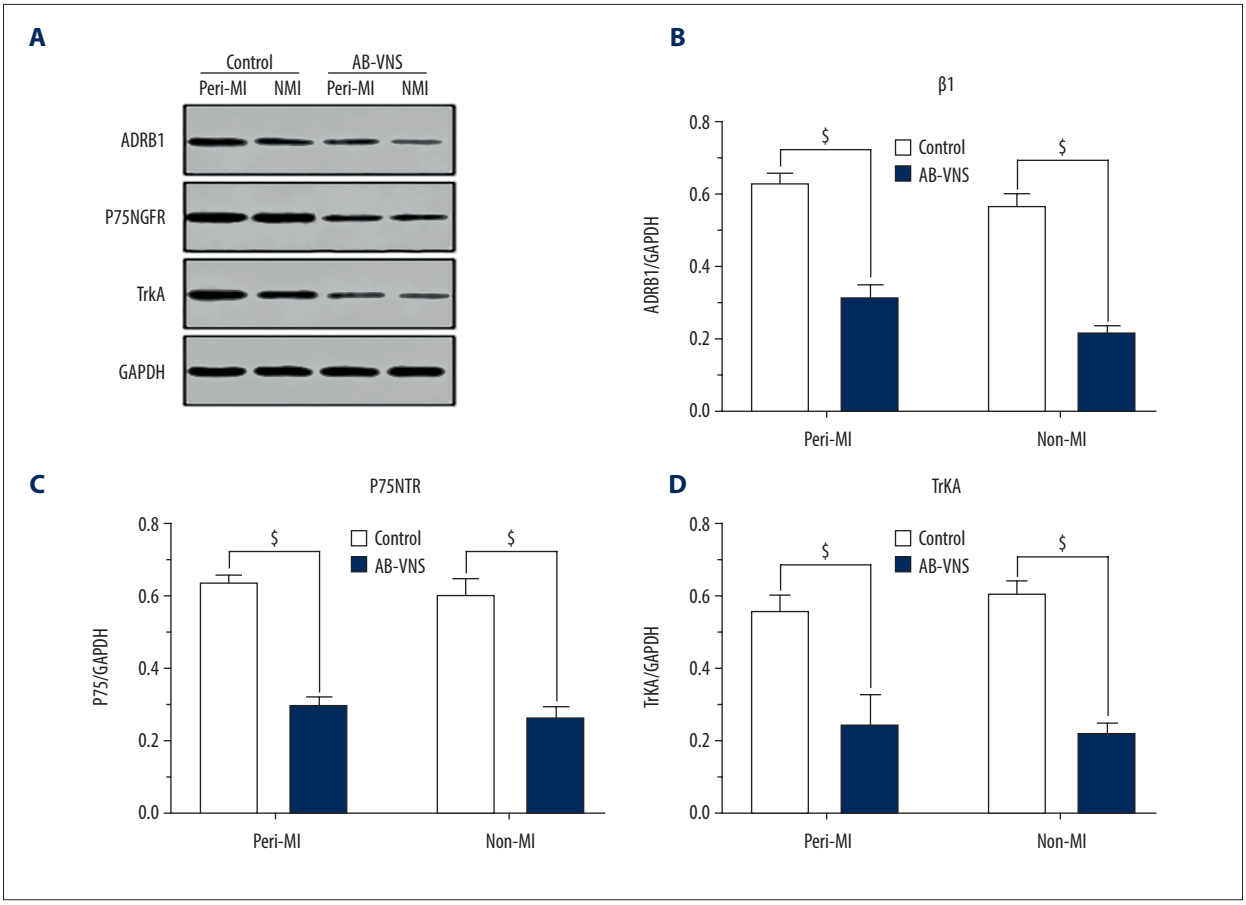

Figure 6. Immunoblotting measurement of sympathetic nerve-related protein (β_1AR, adrenergic receptor β_1) and NGFs (P75NTR, TrkA) were performed by western blot (shown in **Panel A**). GAPDH was the internal reference. Quantitative statistical analysis results were displayed in **Panels B–D**. All tissues were from 2 sampling sites: peri-MI – peripheral myocardial infarction area; non-MI – non-myocardial-infarction area. $^{\$}$ P<0.01 compared with the control group.

was 3214±1030 versus 2070±652, μm²/mm², both P=0.044). In contrast to the control group, there were marked reductions of TH-positive nerves in both ICSN and AB-VN branches in the AB-VNS group (ICSN was 13154±860 versus 10056±781, P<0.01; AB-VN was 2774±742 versus 1910±554, μm²/mm², P=0.045). As shown in Figure 5B, the ChAT-positive nerve densities were significantly higher in both CVN and AB-VN sites (CVN was 8524±803 versus 10934±1205; AB-VN was 3723±542 versus 8322±710, μm²/mm², both P<0.01) compared with the control group. But there was no significant difference of ChAT-positive nerve densities between the 2 groups in LV (705±321 versus 582±202, μm²/mm², P=0.45)

Measurements of the expressions of NGFRs and adrenergic receptor protein

NGFRs mainly comprise the P75 and Trk family. Representative samples of western blots are shown in Figure 6A. The quantitative statistical analysis results, including β_1-AR, P75NGFR, and TrKA, are present in Figures 6B–6D. All 3 indicators decreased significantly in the AB-VNS group compared to those in the control group, in both the peri-MI and non-MI areas (β_1-AR: 0.632±0.025 versus 0.316±0.031 in the peri-MI area, P<0.01/0.572±0.03 versus 0.217±0.015 in the non-MI area, P<0.01; P75NGFR: 0.633±0.02 versus 0.297±0.018/0.601±0.038 versus 0.262±0.024, peri-MI/non-MI area; TrkA: 0.555±0.019 versus 0.353±0.026/0.602±0.031 versus 0.217±0.05, peri-MI/non-MI area, respectively, P<0.01).

Measurements of the mRNA expression of autonomic nervous efferent receptors

As shown in Figures 7A–7C, in the peri-infarction area, the β_1-AR, β_3-AR, and CHRNA7 expression levels in the AB-VNS group were significantly higher than those in the control group (β_1-AR, β_3-AR, CHRNA7, respectively, P<0.01). Compared with the control group, the mRNA expression levels of β_1-AR and β_3-AR were decreased noticeably in the AB-VNS group in the non-infarction area (β_1-AR, β_3-AR, respectively, P<0.01), whereas there was no statistically significant difference in CHRNA7

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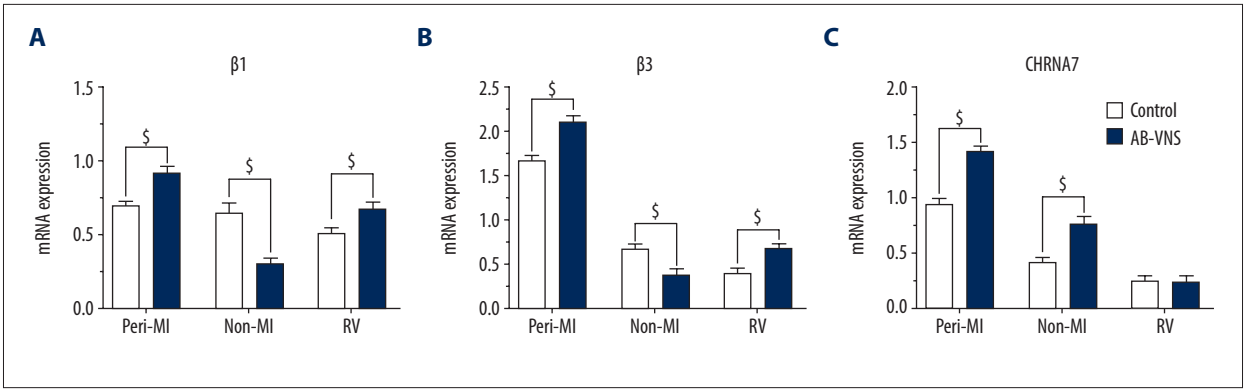

Figure 7. The mRNA levels of autonomic nerve efferent receptors were measured by qRT-PCR in the peripheral MI region (peri-MI), non-MI area (non-MI) and right ventricle (RV), and **Panels A–C** showed the statistical results between the control group and AB-VNS group. $ P<0.01 compared with the control group. qRT-PCR – quantitative real-time polymerase chain reaction; MI – myocardial infarction; peri-MI – peripheral-MI; AB-VNS – auricular branch of the vagus nerve stimulation.

mRNA expression in the RV myocardium between the 2 groups (P=0.64). However, the β_1-AR and β_3-AR mRNA expressions in RV increased in the AB-VNS group in contrast to the control group (β_1-AR and β_3-AR, respectively, P<0.01).

Discussion

Major findings

The present study found that 1) chronic intermittent low-intensity AB-VNS effectively reduced the incidence of VAs induced by MI. 2) AB-VNS rebalanced the extracardiac autonomic activities during the chronic MI phase by reducing ICSN activities and augmenting CVN activities, which was implied by the evidence of neural recording, HRV analysis, and catecholamine concentrations measurements. 3) Its potential mechanisms were accompanied with the alleviative intrathoracic neural remodeling, inhibitory cardiac sympathetic denervation, and ameliorative heterogeneities of receptor distribution.

AB-VNS reduced VAs burden after MI

Early in 1991, vagus nerve stimulation (VNS) was found to reduce the incidence of VAs from 100% to 10% in a healed MI model [15]. As shown in our previous work, 50% voltage below the threshold was choose for cervical vagal stimulation (CVS) and it markedly suppressed VAs in the acute ischemia/reperfusion (I/R) model [16]. Unlike the voltage parameters (80% below the voltage threshold) in other studies [17,18], 50% threshold voltage was used for transcutaneous stimulation at bilateral tragus in our study which indicated that AB-VNS significantly reduced the number of spontaneous PVC after MI as well as the incidence of induced VT/VF 4 weeks later. Even if both parameters above tragus stimulation yield a positive outcome, different parameters of vagus stimulation may produce different neuromodulatory effects by activating central drive by different degrees [19].

AB-VNS rebalanced cardiac autonomic control after MI

During the post-infarction period, imbalanced autonomic control including sympathetic overactivity and vagal withdrawal contributes to the onset of VAs [20,21]. HRV variables had been applied to clinical assessments for autonomic nervous system (ANS) activity [12]. Chen et al. [17] found that low-level VNS effectively decreased LF and LF/HF values of a 5-minute ECG recording during I/R. It also had been proved that intermittent vagal stimulation increased HF component of HRV in rabbits [22]. From our results of all-day HRV, AB-VNS tends to balance the systemic autonomic control by decreasing sympathetic tone and increasing parasympathetic tone. However, there was still controversy regarding whether time-domain HRV analysis can exactly predict autonomic nervous tone [12]. Although our results reached statistical significance, caution is required when explaining the clinical significance.

Extracardiac intrathoracic autonomic nervous components such as LSG and LCVN construct the complex network of cardio-neural hierarchy [23]. A previous study showed that synchronous neural recording of LSG and vagus can reflect the relationship between autonomic activities and ectopic rhythm [24]. As the major cardiac branch of LSG, ICSN conveys signals to spinal segmental sympathetic outflows and indirectly reflects LSG activities [25]. According to our neural recording, the spike discharge of ICSN was commonly accompanied by VAs and clusters of vagus discharge especially in post-infarction 30 minutes to 1 hour. The data of RMS of discharge indicated that AB-VNS can rebalance autonomic control by suppressing excessive ICSN activities and increasing CVN activities. The most likely convincing explanation related to anti-arrhythmic mechanisms

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of AB-VNS was the direct antagonistic effect to sympathetic activity and excitation effect to central vagal outflow [26,27].

Moreover, EPI and NE are released from the sympathetic nerve terminal and are regarded as the semi-quantitative measure of sympathetic excitability [28]. Consistent with our previous results [11], decreased local and circulating NE and EPI levels induced by AB-VNS may produce the elevated arrhythmogenic threshold [21].

In the present study, the immunostaining results provided evidence for excardiac autonomic structural remodeling. Observed data showed a distinct reduction of TH-positive neurons in ICSN and an increase of ChAT-positive neurons in CVS and AB-VN in the experimental group. Furthermore, TH-positive nerve fibers decreased in LV and the possible explanation was that the feedback neuromodulation after AB-VNS reversed neural remodeling. In short, AB-VNS might rebalance cardiac autonomic control via altering systemic autonomic tone, modulating excardiac autonomic activities, and ameliorating intrathoracic autonomic nervous remodeling.

AB-VNS attenuated cardiac sympathetic denervation after MI

It is well-known that cardiac hyperinnervation activated by upregulating expression of NGF at the scar region plays a key role in irritating VAs [29]. Besides, continuous sympathetic denervation of infarction and the border area also contribute to the heterogeneous sympathetic innervation and increased VAs susceptibility [30,31]. P75 neurotrophin (P75NTR), as a member of the neurotrophin family, mediates a TrkA retrograde signaling cascade that coordinates the sympathetic neuronal survival [32]. Previous research has shown elevated expression of P75NTR at cardiac ventricles caused by I/R facilitated sympathetic denervation of the peri-MI area [33]. Our study indicated that AB-VNS remarkably decreased the levels of TrkA and P75NTR both at the peri-MI and non-MI areas. The possible mechanism might be that the synchronic decline of P75NTR and TrkA further inhibited sympathetic axon degeneration as a result of suppressing TrkA-related signaling pathway [34]. Thus, chronic AB-VNS might exert a beneficial effect on attenuating long-term rather than transient sympathetic denervation [35].

AB-VNS alleviated abnormal receptor remodeling after MI

There are 3 types of beta-adrenoceptors (β_1-AR, β_2-AR, and β_3-AR) present in myocardium. The stimulation of β_1-AR/β_2-AR has a proarrhythmic effect, conversely, β_3-AR works as a buffer to resist excessive response of β_1-AR/β_2-AR and exerts an antiarrhythmic effect [36]. In the experimental group in our study, we observed a reduction of β_1-AR in parallel with a decrease of NE/EPI in peri-infarction areas and the RV. A possible explanation was that alleviated sympathetic denervation promoted the reuptake of catecholamine and lessening of NE/EPI residue in tissues [21]. Accordingly, the transcription of β_1-AR increased as a means to compensate for a declining expression of β_1-AR. In normal myocardium, AB-VNS restricted more release of catecholamine by partly inhibiting sympathetic hyperactivities [37]. It also induced the desensitization and reduction of transcription of β_1-AR. Consequently, as a functional antagonist for β_1-AR, the mRNA expression of β_3-AR showed a similar variation tendency in line with β_1-AR.

Many experiments have revealed that a change in macrophage polarization state can lead to an increase in the number of macrophages in the peri-infarction area [38–41]. And it has been well-accepted that nicotine acetylcholine receptor a7 subunit (CHRNa7) in macrophages is an essential component of the cholinergic anti-inflammatory pathway to ameliorate systemic inflammation [42,43]. Our results demonstrated that AB-VNS increased the level of mRNA expression of CHRNa7 in the peri-infarction and non-infarction areas. A potential hypothesis was that AB-VNS upregulated parasympathetic tone and augmented an inherent anti-inflammatory effect through releasing more ACh from the vagus nerve terminal. Increased transcription of CHRNa7 is required to bind to more ACh in this processing. In summary, AB-VNS significantly reversed the proarrhythmic substrate after MI, probably due to the alleviated redistribution of β_1-AR, β_3-AR, and CHRNa7.

Clinical implication

Imbalanced autonomic activity after MI could enhance the vulnerability of cardiac electrophysiology and aggravate the susceptibilities of VAs [28]. Recently, 2 single-center proof-of-concept studies demonstrated that low-level AB-VNS was a feasible and noninvasive treatment to prevent VAs induced by I/R or suppress paroxysmal AF [8,9]. Our present study showed that chronic intermittent low-intensity AB-VNS also effectively decreased VAs after MI potentially by modulating cardiac autonomic control and improving the heterogeneities of receptor distributions. This finding indicated that AB-VNS probably exerted the same therapeutic effect as beta-blockers against neural remodeling to some extent. In addition, stimulation parameters of AB-VNS in our study were 50% of the voltage threshold, and this intensity could be tolerated by conscious patients as demonstrated by clinical evidence [9]. These findings from basic research indicate the potential benefits of AB-VNS for the prevention and treatment of VAs.

Study limitations

There were 4 main limitations to our study. Firstly, time-domain accurate measures of HRV by 24 hours dynamic ECG required an absolute normalized situation. However, it was difficult to

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correct all confounders in the actual experiment by merely isolating canines in cages. Secondly, invasive neural recording was performed in canines under an anesthetic condition, which might change autonomic nervous activities. Thirdly, while our study evaluated the antiarrhythmic function of AB-VNS by chronic intermittent stimulation for only 4 weeks, the longer-term therapeutic effectiveness remains unknown. And lastly, the present study was unable to provide the most optimized combination of stimulation parameters.

Conclusions

Chronic intermittent low-intensity AB-VNS can reduce the incidence of VAs in the post-infarction phase. Its potential neuromodulatory effect is associated with decreased ICSN activity, enhanced vagus activity, mitigated excardiac neural remodeling, ameliorated cardiac sympathetic denervation, and homogeneous distribution of autonomic nerve efferent receptors. AB-VNS might be a feasible approach to alleviate post-infarction VAs.

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Conflict of interest

None.

Supplementary Data

Supplementary Table 1. The supplementary primer sequence table is shown as follows.

Name	Primer	Sequence	Size
Dog GAPDH	Forward	5'- TCTACCCACGGCAAATTCCA -3'	133 bp
	Reverse	5'- CATACTCAGCACCAGCATCAC -3'	
Dog CHrna7	Forward	5'- GGCGTGAAGACTGTTCGTTT -3'	243 bp
	Reverse	5'- CCACCCTCCATAAGACCAGG -3'	
Dog beta1-AR	Forward	5'- CCCATCCTCATGCACTGGT -3'	158 bp
	Reverse	5'- AGGTACACGAAGGCCATGAT -3'	
Dog beta3-AR	Forward	5'-GCCCTGGTCACCAAACGG-3'	217 bp
	Reverse	5'-CCAGAAGCGGAAGGTAGAAGGA-3'	

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