Intermittent Vagal Nerve Stimulation Alters the Electrophysiological Properties of Atrium in the Myocardial Infarction Rat Model

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Abstract—**Intermittent vagal nerve stimulation (VNS) has emerged as a potential therapy to treat cardiovascular diseases by delivering electrical stimulation to the vagus nerves. The purpose of this study was to investigate the electrophysiological changes in the atrium resulting from long-term intermittent VNS therapy in the chronic myocardial infarction (MI) rat model. MI was induced via left anterior descending coronary artery (LAD) ligation in male Sprague-Dawley rats, randomized into two groups: MI (implanted with non-functional VNS stimulators) and MI-VNS (implanted with functional VNS stimulators and received chronic intermittent VNS treatment) groups. Further, a sham group was used as control in which MI was not performed and received non-functional VNS stimulators. At 12 weeks, optical mapping of right atrium (RA) of sinus rhythm was performed. Our results demonstrated that chronic MI changed the electrical properties of the atrium action potentials and resulted in reduced action potential duration at 50% (APD_{50}) and 80% (APD_{80}) repolarization. Chronic right cervical VNS restored the APD back to healthy heart APD values. Additionally, APD heterogeneity index increased as a result of the chronic MI. Chronic VNS was not found to alter this increase. By calculating PR intervals from weekly ECG recordings of anaesthetized rats, we demonstrated that chronic MI and intermittent VNS did not affect the AV conduction time from the atria to the ventricles. From our study, we conclude the MI decreased the APD and increased APD spatial dispersion. VNS increased the APD back to healthy normal values but did change the APD spatial dispersion and the electrical conduction in the RA.**

I. Introduction

Myocardial infarction (MI) is the most common cause of heart failure (HF) [1], [2]. Current therapies designed to treat MI patients include conventional pharmacological and mechanical device-based interventions. While these approaches have improved the prognosis of patients with MI, the mortality rate still remains high [3].

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Research was supported in part by Cyberonics Inc. and the following funding sources: NSF PHY09574683, NSF CMMI-1233951 (E.G.T), NSF CAREER PHY-125541 (E.G.T), UMN IEM Exploratory Grant, and UMN Grant-in-Aid.

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Vagal nerve stimulation (VNS) is an adjunctive procedure involving the stimulation of the vagus nerve with electrical impulses. Clinically, VNS is already currently being used to treat intractable epilepsy and treatment-resistant depression [4], [5].

Recently, modulation of nerve activity through cardiac VNS has emerged as a potential therapy for cardiovascular diseases [6]. It has been shown that chronic VNS can inhibit sudden cardiac death [7] and markedly suppress arrhythmias [8] in MI animal models. Peripheral cardiac nerve stimulation can also modify atrial and ventricular contractile functions [9], [10]. It has been reported that chronic VNS may improve left ventricular function and the quality of life in chronic HF patients with severe systolic dysfunction [6].

It is well known that parasympathetic vagal nerves extensively innervate the atria. The vagus nerve (parasympathetic) system communicates with the heart through the intracardiac ganglia, which are divided into the sinoatrial (SA) and atrioventricular (AV) nodes [11]. It is thought that VNS might have a significant effect on atrial electrophysiological properties due to dense vagal innervations. However, the effects of chronic VNS on the atrium remain unclear. Additionally, there are controversial findings on whether VNS is linked to atrial fibrillation (AF). Specifically, VNS has been shown to shorten the duration of action potential (APD) in atrial myocytes and reduce the atrial absolute refractory period, facilitating the induction of AF by a single atrial ectopic beat and the presence of multiple reentrant circuits coexisting in the atrial myocardium [12], [13]. Furthermore, animal studies involving vagal denervation have suggested that VNS may be proarrhythmic [14], [15]. Nonetheless, there are also studies that have shown that VNS can inhibit spontaneous activities of isolated cardiac myocytes from rabbit pulmonary veins [16] and canine superior vena cava [17]. In addition, it has been shown that the use of phenylephrine to enhance the vagal tone suppresses focal AF originating in the pulmonary veins in patients [13], [18]. Low-level VNS has also been implemented in ambulatory dogs to reduce atrial tachy-arrhythmias [19]. Therefore, further investigations are required to determine the exact role of VNS on the electrophysiological properties of atria.

The objective of this study was to use high resolution optical mapping techniques to evaluate the changes in atrial electrophysiology due to chronic intermittent VNS in a chronic MI rat model. We aimed to identify whether these changes might potentially promote or inhibit the creation of substrates for atrial arrhythmias. To our knowledge, this is the first study to characterize the electrophysiological

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changes in the atria in MI rat hearts after treatment with long-term VNS.

II. Methods

A. MI Rat Model

All experiments conformed to the Guide for the Care and Use of Laboratory Animals (NIH publication No. 85-23, revised 1996) and the University of Minnesota guidelines regulating the care and use of animals.

Male Sprague-Dawley rats (n=6, 250-300g, Charles River Laboratories, Wilmington, MA) were randomized into three groups: Sham (n=2), MI (n=2), and MI-VNS (n=2). Sham rats underwent open chest and cervical sham surgeries only, while MI was induced in both MI and MI-VNS rat hearts. The MI was created through permanent ligation of the left anterior descending coronary artery (LAD) in the MI and MI-VNS groups. Rats were anesthetized with Isoflurane (3%) during the open chest MI surgeries. Rats were intubated with a ventilator (Model 683, Harvard Apparatus) and body temperature was maintained at 37°C throughout the surgeries. LAD surgery was performed by ligation of the proximal LAD artery with 6-0 silk sutures. Full LAD occlusion was visually confirmed when the myocardium of the left ventricle changed color to a pale blue, reflecting the lack of oxygenated blood flow into the region.

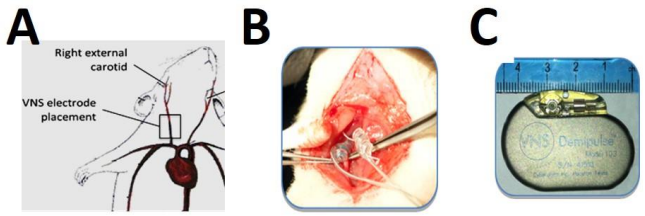

Fig.1. A) VNS lead placement around the cervical vagus nerve and carotid artery bundle. **B)** Placement of the helical bipolar leads. **C)** Cyberonics 103 Vagal Nerve Stimulator

B. VNS Stimulator Implantation

Immediately after MI surgery, VNS stimulators (Fig. 1C. Cyberonics Model 103, Houston TX) were implanted subcutaneously on the lower back of the rats. Active and non-functional VNS stimulators were implanted in MI-VNS and MI rats, respectively. The bipolar cuff electrodes were coiled around the bundle of right cervical vagus nerve and carotid artery as shown in Fig. 1B. Intermittent VNS was given to the MI-VNS rats for 12 weeks. Parameters of VNS stimulation were defined as in previous literature [8]: stimulation frequency of 20 Hz; pulse width of 500 microseconds; and stimulation current of 1 mA; duty cycle of roughly 12% (7 second ON every 1 minute).

C. ECG recordings

ECG recording was performed as described previously [20]. Briefly, ECG recordings was performed in all rats weekly for approximately 20 minutes per animal using the iWorx IX-ECG-12 recording system, while rats were lightly anesthetized with 2% Isoflurane. Custom-made program in Matlab was written to calculate PR intervals and heart rate (HR) from anaesthetized rats' ECG data. For each week and for each rat, mean PR intervals were calculated over 20 minutes of ECG recording. HR was derived from the first 2 minutes of the ECG during which the effects of anesthesia were minimal.

D. Optical Mapping

At week 12, rat hearts were extracted. The aorta was quickly cannulated and perfused in a retrograde manner using Langendorff-perfusion system with warm (37±1ºC) oxygenated Tyrode's solution. A dose of 0.01 ml of the fluorescent voltage-sensitive dye di-4-ANEPPS (10 µmol/L) was administered to the right atrium (RA) directly. Two diode continuous lasers (532 nm, SDL-532-1000 T, Shanghai Dream Lasers Tech., Shanghai, China) were used for excitation, and the fluorescence signal was recorded from the RA surface by fast (1000 frames per second) 14-bit resolution, 80x80 pixels resolution camera (Little Joe, RedShirt Imaging, SciMeasure). The field of view was approximately 6 x 6 mm (Fig. 2A). After stabilization (~30 minutes), optical mapping movies of the whole RA were acquired during sinus rhythm.

E. Data Analysis

We analyzed 12 sinus rhythm responses from each rat, for all three groups. Sinus rhythm APD was measured at both 50% (APD_{50}) and 80% (APD_{80}) repolarization (Fig.2B), and two-dimensional (2D) APD maps (Fig.2C) were constructed to reveal the spatial distribution of APDs on the RA surfaces of the heart. The APD heterogeneity, µ, was calculated as described previously [21]:

$$\mu = \frac{(APD^{95\%} - APD^{5\%})}{APD^{50\%}} \quad (1)$$

where $APD^{95\%}$ and $APD^{5\%}$ represent the 95th and 5th percentiles of the APD distribution, respectively, and

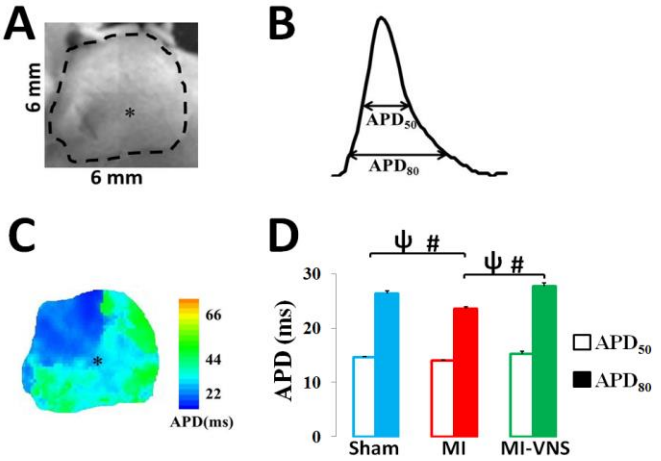

Fig.2. A) Optical mapping field of view (indicated by dashed lines) of RA. **B)** A typical action potential trace from pixel marked as "*" of the RA demonstrating APD_{50} and APD_{80}. **C)** Corresponding 2D APD_{80} maps to **A)**. **D)** Mean values for APD_{50} (open bars) and APD_{80} (filled bars). ψ represents statistical difference between APD_{50} and # represents statistical difference between APD_{80} ($p < 0.05$).

$APD^{50\%}$ is the median APD distribution.

Mean APD and μ were calculated as the following. For each rat, we first calculated mean APD and μ from a single sinus rhythm across the entire RA. Then, we performed averaging of these mean APD and μ values for all 12 sinus rhythm responses. Data are presented as means ± SE. Statistical comparisons among the 3 groups were performed using an ANOVA statistical test. $p< 0.05$ was considered to be statistically significant.

III. Results

A. Effect of VNS on APD and Spatial Heterogeneity

Fig. 2D shows mean values for APD_{50} (open bars) and APD_{80} (filled bars) of Sham (blue), MI (red), and MI-VNS (green) rats during sinus rhythm. Note that MI significantly reduced both APD_{50} and APD_{80} ($p<0.05$) of RA. However, this reduction of APD at both levels of repolarization was restored upon chronic intermittent VNS stimulation back to Sham (healthy) level.

Fig. 3A shows representative sinus rhythm traces as well as examples of 2D APD_{80} maps for Sham, MI and MI-VNS rats. Note the enhanced spatial dispersion of APD during sinus rhythm for both MI and MI-VNS rats. We quantified these data by calculating mean μ values separately for both APD_{50} (open bars) and APD_{80} (filled bars). Fig. 3B and Fig. 3C indicate that MI significantly increased μ in RA ($p<0.05$), but was not reduced with chronic intermittent VNS.

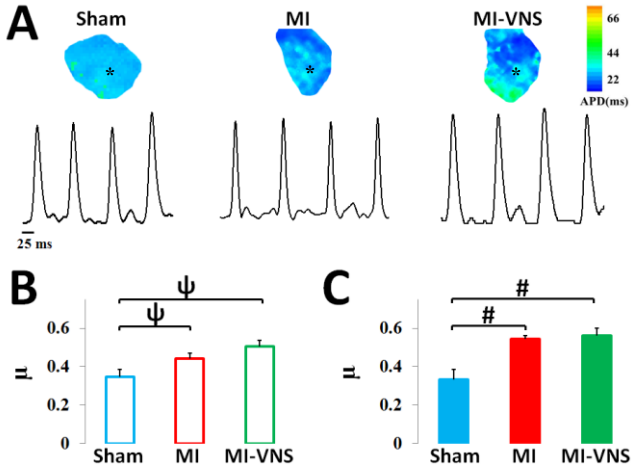

Fig.3 A) Typical samples of 2D APD maps and representative sinus rhythm action potential traces (from pixel marked as "*") from all three groups. Mean spatial heterogeneity index μ for **B)** APD_{50} (open bars) and **C)** APD_{80} (filled bars) are shown for Sham, MI and MI-VNS groups. ψ represents statistical difference between APD_{50} and # represents statistical difference between APD_{80}, $p<0.05$.

B. Effect of VNS on PR interval and Heart Rate

To investigate whether VNS affects the electrical conduction time from atria to ventricles, we calculated PR intervals from weekly ECG recordings of anaesthetized rats. Average data in Fig. 4A illustrates the absence of any differences in PR intervals of Sham (blue), MI (red) and MI-VNS (green) ECG recording over the duration of study. In addition, as seen in Fig. 4B, our chronic VNS treatment did not induce any significant changes in HR of the anaesthetized rats.

IV. Conclusion and Discussion

In this study, we investigated the long-term effects of both MI and intermittent VNS on the electrophysiological properties of the atrium in a MI rat model. The main findings of our study are as follows: 1) chronic MI decreased APD and the VNS restored the APD values during sinus rhythm in rat heart RA. On the other hand, 2) VNS had no effect on APD heterogeneity that was increased due to chronic MI. Finally, 3) neither chronic MI nor intermittent VNS affected the AV conduction system in the heart.

There are only few studies on the effects of chronic MI on atrial electrophysiology. Previous studies showed inconsistent changes in atrial action potential morphology in cardiovascular diseases: APD was increased [22], shortened [23], or unchanged [24].

MI-induced HF is often associated with AF [25] and our study showed that MI decreases both atrial APD_{50} and APD_{80} during sinus rhythm. The shortening of the atrial APD could potentially provide substrates for atrial arrhythmias since reduction in APD can shorten the wavelength of re-entry (the product of both APD and absolute refractory period) and increase the number of wavelets during AF [13], [26]. On the other hand, chronic VNS increased both the APD_{50} and APD_{80} values and restored them back to the healthy animal values, which can be a potentially anti-arrhythmic effect.

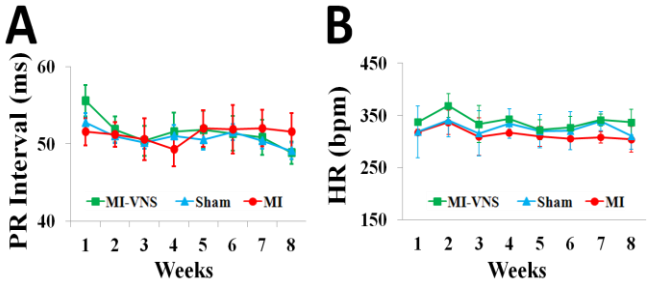

Fig.4 A) Mean PR interval over 8 weeks of study for Sham (blue), MI (red) and MI-VNS (green) hearts. **B)** Mean weekly HR over 8 weeks of study for Sham (blue), MI (red), and MI-VNS (green).

Our results also indicate that MI increased the spatial APD heterogeneity in the RA. However, VNS did not reduce the heterogeneity of the MI hearts towards healthy values. This result may explain the reported conflicting results regarding the anti-arrhythmic effects in the whole heart induced by the VNS. The increased spatial heterogeneity of APD is known to be one of the contributing factors to arrhythmogenesis [21]. This indicates that the reported anti-arrhythmic effects of VNS in the whole heart level are less likely related with the APD spatial heterogeneity in the RA.

The PR interval is one of the many important parameters used to identify cardiovascular diseases. It can be used as an indication of unfavorable alteration in the electrical system of the heart. An abnormal PR interval is often associated with SA nodal and or AV nodal blocks. Our PR interval results showed that both MI and VNS did not affect the PR

intervals among the three groups of rats. This could indicate that there were no SA nodal and/or AV nodal disturbances.

The potential benefits of chronic VNS are now being evaluated in human subjects diagnosed with chronic, symptomatic HF [27]. Previously, data from a small pilot study suggested that VNS alters the natural history of HF [6]. Large, multi-center, controlled clinical studies are underway and should provide additional insights regarding the safety and efficacy of this new therapy for treatment of chronic HF.

A limitation of our study was the limited sample size. Another limitation was the use of general anesthesia to perform ECG recordings from the rats. While it is known that anesthesia changes the heart rate (RR interval) of animals in comparison to conscious animal ECG recordings, we believe that changes to the PR interval are minimal.

V. Acknowledgment

The authors would like to thank Cyberonics Inc. for their support.

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