Acute cardiovascular and hemodynamic effects of vagus nerve stimulation in conscious hypertensive rats

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Abstract— **Hypertension (HTN) affects over 1 billion people worldwide, with a significant number who are unable to control their blood pressure (BP) with conventional therapies. Recently, novel device-based therapies targeting the autonomic nervous system have been evaluated for treating HTN, including vagus nerve stimulation (VNS). Numerous studies have indicated the beneficial effects of chronic VNS in various models of HTN, however the acute effects of VNS on physiological responses have not been widely investigated. To better understand the acute effects of VNS, this study evaluates cardiovascular and hemodynamic responses from conscious hypertensive rats implanted with VNS stimulators and physiological telemeters for simultaneous monitoring of BP and heart rate (HR) as therapy is applied. We demonstrated that there are no acute changes in mean BP, HR and contractility measures as a result of VNS stimulation. However, there were significant increases in both HR variability and BP variability during VNS, which returned to baseline levels immediately at the cessation of therapy. The small acute changes observed during intermittent VNS could be additive, leading to beneficial chronic changes in BP and HR control, and may help in furthering the understanding of beneficial effects demonstrated in chronic use of VNS therapy.**

I. Introduction

Hypertension (HTN) prevalence has been rapidly growing, affecting more than 1 billion people worldwide in 2013 [1]. Common treatment approaches include antihypertensive drugs, diet, and exercise. Currently, the majority of hypertensive patients are treated with antihypertensive drugs to control blood pressure (BP), but many limitations exist including resistant HTN, inability to tolerate therapy, and non-compliance with the medication regime [2-4]. For these patients, new device-based therapies have been investigated, such as vagus nerve stimulation (VNS), which provide an alternative approach to control BP by targeting the imbalance in the autonomic nervous system seen in HTN.

Several researchers have demonstrated the beneficial effects of chronic VNS therapy in treating HTN [5, 6]. There is evidence that stimulating the autonomic nervous system, specifically the parasympathetic branch, has the ability to rebalance autonomic activity which can be used for controlling BP and addressing adverse effects associated with HTN [7]. Specifically, chronic VNS has been shown to attenuate the rise in BP as well as reduce endothelial dysfunction and aortic stiffening in hypertensive rats [5, 6]. In addition, direct beneficial cardiac effects have been demonstrated including altering electrophysiological properties and suppressing ventricular arrhythmias [5, 8-10].

The acute effects of VNS have been investigated in many studies that quantified hemodynamic, autonomic, and inflammatory responses in various animal models. Recently, acute VNS was shown to have anti-inflammatory effects and result in a significant reduction in heart rate (HR) in rats with pulmonary arterial HTN [11]. In addition, VNS has been demonstrated to reduce BP acutely when targeting the aortic depressor nerve within the vagus nerve [12, 13]. However, the majority of studies were conducted with continuous VNS while the animal is under anesthesia. These experimental conditions raise two fundamental questions: (1) are the effects of VNS different when applied continuously versus intermittently, and (2) what are the effects of anesthesia and could the effects of VNS either be diminished or exaggerated?

The aim of this study is to better understand the acute effects of low-level intermittent VNS in hypertensive rats through evaluation of cardiovascular and hemodynamic responses while the rat is conscious. Here, changes in mean and variability measures of BP and HR are quantified to better understand physiological changes induced by acute parasympathetic activation through VNS therapy.

II. Methods

All experiments were approved by the University of Minnesota Animal Care and Use committee and were conducted in accordance with Institutional and NIH guidelines (NIH publication No. 85-23, revised 1996).

A. Experimental Model

HTN was induced in Dahl salt sensitive rats (n=6, 5 weeks old) using high salt 8% NaCl diet (S10001, Research Diets, Inc., New Brunswick, NK, USA), which was maintained for the entire duration of the study. After 5 weeks of high salt diet, the rats underwent surgery to implant physiological telemeters (HD-S11, Data Sciences Inc (DSI)) and vagal nerve stimulators (Model 103, Cyberonics Inc.) as described previously [5]. Briefly, the telemetry device was placed in the abdomen of the rat with a pressure catheter inserted into the left femoral artery for continuous BP monitoring and two leads were placed subdermally on the chest wall of the rat for continuous ECG monitoring. The VNS stimulator was placed under the skin on the back of the rat, and a lead connected to

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the pulse generator was implanted through a midline incision on the neck. The bipolar cuff electrodes were then placed around the right cervical vagus nerve and carotid bundle. The rats were given at least one week to recover prior to the start of VNS therapy.

B. Vagus Nerve Stimulation

The low-level VNS was applied intermittently with a pulse frequency of 20 Hz, a pulse width of 500us, and with an "On" and "Off" time of 14 and 66 seconds respectively. Each stimulation period has a 2-second ramp up and ramp down intervals, which are counted in the "On" period of the stimulation resulting in a 22.5% duty cycle (see Figure 1, top panel). The amplitude of the VNS stimulation was set individually for each rat to influence HR by no more than 5%, resulting in current values of 0.25-0.75mA. Rats were individually housed and free to move about their cages while being continuously monitored for the remainder of the study.

C. Data Analysis

BP and ECG traces were collected at a sampling rate of 500Hz. Four-hour data segments were acquired for day (10am-2pm) and night (10pm-2am) intervals for each rat at Week 6 (Day 1 of VNS therapy). Data was segmented into the following windows: "Pre", "VNS On", "Post 1", and "Post 2" (Figure 1). "Pre" was defined as the 7-second segment prior to the VNS therapy. "VNS On" was defined as the 14 seconds during which VNS was applied, with the exclusion of the 2-second ramp up and ramp down intervals. "Post 1" and "Post 2" were defined as two consecutive 7-second segments immediately after VNS stimulation. For each data segment, the following values were calculated: BP, BP variability (BPV), HR, HR variability (HRV), and the contractility, which was determined as the maximum slope of the derivative of the BP waveform (dP/dt_{max}). BPV was calculated as

$$BPV = \sigma_{BP} / BP, \qquad (1)$$

where σ_{BP} and BP are the standard deviation and mean of the BP data, respectively, and BP was measured as the systolic BP peak value. HRV was calculated as

$$HRV = \sigma_{HR} / HR, \qquad (2)$$

where σ_{HR} and HR are the standard deviation and mean of the BP data, respectively. Due to the noisy nature of the 2-lead ECG data, HR was derived using pulse interval calculated from the BP trace, as described in [14]. Data are presented as percent change relative to the "Pre" stimulation interval. All data analysis was performed using custom MATLAB code.

D. Statistical Analysis

One-way ANOVA was used to compare values at each segment ("Pre", "VNS On", "Post 1", "Post 2") with significance determined at $P < 0.05$. Post hoc analysis was performed with Tukey's multiple comparison correction.

III. RESULTS

The custom MATLAB program was developed to analyze ECG and BP data traces for approximately 400 VNS episodes per rat (see Fig. 1). Day and night intervals were combined together to obtain average values for all measured parameters.

A. Heart Rate Responses

Mean HR derived from the BP waveform was calculated for each interval during Day 1 of VNS therapy and showed no significant changes before, during, and after VNS therapy (see Figure 2A). However, VNS significantly increased HRV during the "VNS On" interval when compared to the "Pre" interval. Nevertheless, the HRV decreased back to baseline levels immediately after VNS was turned off, so that both HRV during "Post 1" and "Post 2" have similar values as during "Pre" interval (see Fig. 2B).

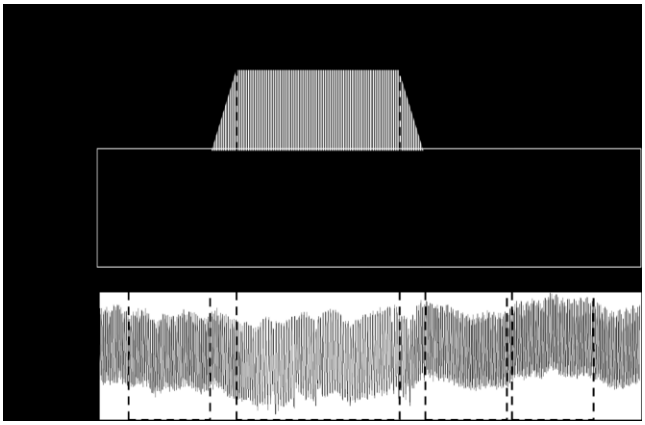

Figure 1. An example of the data segmentation into "Pre", "VNS On", "Post 1" and "Post 2" intervals for one episode of VNS in the rat. The top panel is the representative stimulation including the ramp up and ramp down periods. The middle panel shows the recorded ECG trace with VNS artifacts visible during the "VNS On" segment. The bottom panel shows the corresponding BP trace.

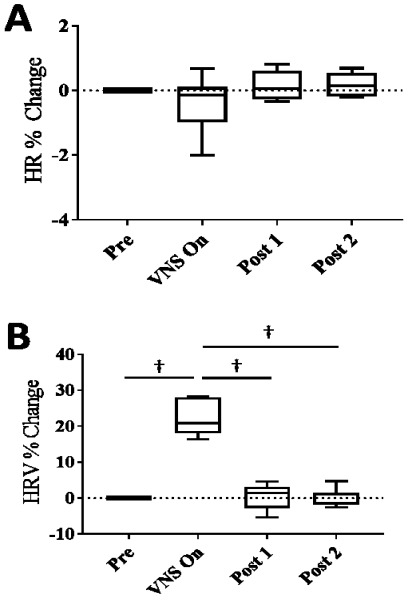

Figure 2. Acute HR (A) and HRV (B) responses during VNS therapy in hypertensive rats for "Pre", "VNS On", "Post 1" and "Post 2" intervals. † indicates statistical significance between mean values between intervals.

B. Blood Pressure Reponses

Similarly, the mean BP response of the VNS rats show no significant changes before, during and after VNS therapy (see Fig 3A). However, there was a significant increase in BPV during "VNS On" period, which returned to "Pre" values immediate after VNS therapy is off (see Fig 3B).

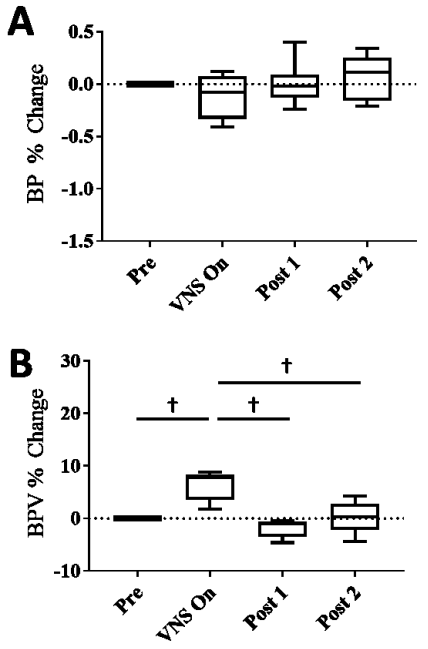

Figure 3. Acute BP (A) and BPV (B) responses during VNS therapy in hypertensive rats for "Pre", "VNS On", "Post 1" and "Post 2" intervals. † indicates statistical significance between mean values between intervals.

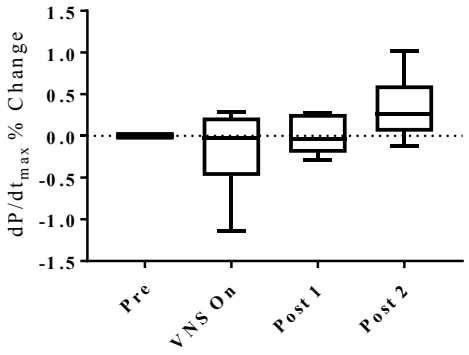

Figure 4. Acute contractility (dP/dt_{max}) response during VNS therapy in hypertensive rats for "Pre", "VNS On", "Post 1" and "Post 2" intervals.

C. Contractility

Contractility was assessed by quantifying the maximum rate of change of the pressure waveform (dP/dt_{max}). With increased vagus activity, contractility is known to decreases, however during low-level VNS therapy in our study, no significant acute changes (less than 1% change) were observed in the intervals analyzed. Figure 4 shows no changes in dP/dt_{max} during any intervals surrounding episodes of VNS therapy.

IV. Discussion

This paper evaluated the acute effects of low-level, intermittent VNS in conscious hypertensive rats. The major findings of this paper were the following: (1) no significant acute changes in HR, BP, and contractility were induced during the "VNS On" period, when compared to "Pre" and "Post" intervals, (2) however both HRV and BPV are significantly increased acutely during the "VNS On" period.

A. The effect of VNS on mean cardiovascular and hemodynamic responses

During VNS stimulation, there were no significant acute changes in HR in the hypertensive rats. Indeed, in this study we used low-level VNS therapy that intentionally does not drastically alter either HR or BP. This effect can be controlled through selection of the stimulation parameters. Specifically, varying amplitude has been shown to induce significantly different acute cardiovascular responses [15, 16]. The stimulation settings and subsequent effects depends on the goal of the therapy. Here, VNS was initially titrated to cause no more than a 5% drop in HR during day time stimulation in order to provide low-level intermittent VNS therapy while reducing side effects. Therefore the short therapy duration in combination with the low-level stimulation likely led to no significant changes in mean HR during acute episodes of VNS therapy.

VNS also did not cause any changes in BP. Previously, several studies have shown an acute decrease in BP due to selective VNS therapy in anesthetized rats, which was accompanied by a reduction in mean HR [12, 13]. This reduction was smaller when applying non-selective VNS using the same stimulation parameters [12]. Here, intermittent VNS stimulation is applied non-selectively at a low level with 22.5% duty cycle. Such a short therapy duration and low amplitude may not have time to elicit a significant change in the mean BP measures before stimulation is turned off. Also, in previous studies, the acute effects of VNS on BP and HR have been demonstrated in healthy rats [12, 13, 17], while here, we have evaluated the effect of acute VNS in hypertensive rats. HTN has been associated with an imbalance in the autonomic nervous system with increased sympathetic nerve activity and a decrease in parasympathetic nerve activity [18]. This imbalance in the autonomic nervous system could lead to a different response to acute stimulation, which may require higher amplitude or longer duration stimulation to elicit a response in this altered autonomic state.

B. The effect of VNS on variability in cardiovascular and hemodynamic responses

The results of the acute stimulation show a strong positive effect on HRV. HRV is a common prognostic indicator used in the clinic to evaluate the cardiac condition of patients. In disease states, such as HTN and heart failure, the overactive sympathetic nervous system reduces the autonomic control of cardiac function, decreasing HRV. Increases in HRV indicate better autonomic balance, through either a suppression of sympathetic activity or activation of parasympathetic activity, and can be indicative of therapeutic efficacy. The acute changes in HRV observed here could have an additive effect, contributing to the long term beneficial effects of VNS therapy.

In addition to changes in HRV, acute changes in BPV were also observed in the hypertensive rats. Traditionally in the clinic, increases in BPV are associated with increased cardiovascular risk [19]. However, the effect on BPV observed here has been shown before, with variability in BP occurring in response to HRV induced through vagal activation [20]. This response observed in this study, returns to baseline values at the cessation of therapy.

C. *The effect of VNS on contractility*

The results show no change in contractility during stimulation. It is commonly known that enhanced parasympathetic activity has a negative inotropic effect on the heart. This effect has been recently demonstrated using various VNS parameters applied for 30 seconds in anesthetized sheep [15]. There are several key differences however that can explain this difference observed in this study including the species, disease state, and duration of stimulation. Short VNS duration in our study may result in a smaller cumulative effect on the contractility of the heart in the conscious hypertensive rats. Increasing duration or amplitude of stimulation may result in a measurable change in contractility along with changes in other cardiac parameters including HR.

D. *Limitations*

There are several limitations for the current data analysis. First, only 4-hour day and night data segments were analyzed from each of the VNS-treated rats. Larger data segments may give more information about the acute changes and whether time of day and related autonomic balance affects the acute cardiovascular and hemodynamic response to VNS. Another limitation of this study is that the acute response was only quantified on Day 1 of VNS therapy. Future work will include analyzing the acute response to VNS over time to see if therapy possibly becomes less effective due to acclimation to stimulation or tissue ingrowth decreasing the magnitude of the stimulation seen by the vagus nerve. In addition, future studies will investigate the effects of various intermittent VNS parameters on the cardiovascular and hemodynamic responses. Finally, further evaluation of HRV using spectral analyses can provide indications of sympathetic and parasympathetic tone.

V. CONCLUSION

Quantifying acute cardiovascular and hemodynamic changes due to VNS in conscious animals can help provide a better understanding of VNS therapy and physiological responses to acute parasympathetic activation. The small acute changes observed during intermittent VNS could be additive, leading to beneficial chronic changes in BP and HR control [5]. Evaluating the acute effects can help further the understanding of VNS in its application as a chronic therapy and assist in parameter selection and optimization for providing beneficial effects for treating HTN.

VI. REFERENCES

[1] N. R. F. Collaboration, "Worldwide trends in blood pressure from 1975 to 2015: a pooled analysis of 1479 population-based measurement studies with 19· 1 million participants," The Lancet, vol. 389, no. 10064, pp. 37-55, 2017.

[2] B. M. Egan, Y. Zhao, R. N. Axon, W. A. Brzezinski, and K. C. Ferdinand, "Uncontrolled and apparent treatment resistant hypertension in the United States, 1988 to 2008," Circulation, p. CIRCULATIONAHA. 111.030189, 2011.

[3] G. Mancia et al., "2007 Guidelines for the management of arterial hypertension - The task force for the management of arterial hypertension of the European society of hypertension (ESH) and of the European society of cardiology (ESC)," European Heart Journal, vol. 28, no. 12, pp. 1462-1536, Jun 2007.

[4] F. L. Ng, M. Saxena, F. Mahfoud, A. Pathak, and M. D. Lobo, "Device-based therapy for hypertension," Current Hypertension Reports, vol. 18, no. 8, pp. 1-10, 2016.

[5] E. M. Annoni et al., "Intermittent electrical stimulation of the right cervical vagus nerve in salt-sensitive hypertensive rats: effects on blood pressure, arrhythmias, and ventricular electrophysiology," Physiological reports, vol. 3, no. 8, p. e12476, 2015.

[6] M. W. Chapleau, D. L. Rotella, J. J. Reho, K. Rahmouni, and H. M. Stauss, "Chronic vagal nerve stimulation prevents high-salt diet-induced endothelial dysfunction and aortic stiffening in stroke-prone spontaneously hypertensive rats," American Journal of Physiology-Heart and Circulatory Physiology, vol. 311, no. 1, pp. H276, 2016.

[7] K. Saku et al., "Afferent vagal nerve stimulation resets baroreflex neural arc and inhibits sympathetic nerve activity," Physiological reports, vol. 2, no. 9, p. e12136, 2014.

[8] B. Akdemir and D. G. Benditt, "Vagus nerve stimulation: An evolving adjunctive treatment for cardiac disease," Anatol J Cardiol, vol. 16, pp. 804-10, 2016.

[9] X. Xie, R. Visweswaran, P. A. Guzman, R. M. Smith, J. W. Osborn, and E. G. Tolkacheva, "The effect of cardiac sympathetic denervation through bilateral stellate ganglionectomy on electrical properties of the heart," American Journal of Physiology-Heart and Circulatory Physiology, vol. 301, no. 1, pp. H192-H199, Jul 2011.

[10] R. W. Myers et al., "Beneficial effects of vagal stimulation and bradycardia during experimental acute myocardial ischemia," Circulation, vol. 49, no. 5, pp. 943-947, 1974.

[11] K. Yoshida et al., "Acute Electrical Vagal Nerve Stimulation Exerts Powerful Anti-inflammatory Effects Through its Nicotinic Action in Rats with Pulmonary Arterial Hypertension," The FASEB Journal, vol. 31, no. 1 Supplement, pp. 697.6-697.6, 2017.

[12] D. T. Plachta et al., "Blood pressure control with selective vagal nerve stimulation and minimal side effects," Journal of neural engineering, vol. 11, no. 3, p. 036011, 2014.

[13] D. T. Plachta, J. Zentner, D. Aguirre, O. Cota, T. Stieglitz, and M. Gierthmuehlen, "Effect of Cardiac-Cycle-Synchronized Selective Vagal Stimulation on Heart Rate and Blood Pressure in Rats," Advances in therapy, vol. 33, no. 7, pp. 1246-1261, 2016.

[14] J. Schultz, E. M. Annoni, and E. G. Tolkacheva, "Isolating low frequency oscillations in baroflex function in rats," in 36th Annual International Conference of the IEEE-Engineering-in-Medicine-and-Biology-Society (EMBC), Honolulu, HI, 2018.

[15] D. Ojeda et al., "Sensitivity analysis of vagus nerve stimulation parameters on acute cardiac autonomic responses: Chronotropic, inotropic and dromotropic effects," PloS one, vol. 11, no. 9, p. e0163734, 2016.

[16] H. M. Stauss, "Differential hemodynamic and respiratory responses to right and left cervical vagal nerve stimulation in rats," Physiological reports, vol. 5, no. 7, p. e13244, 2017.

[17] M. Gierthmuehlen, T. Stieglitz, J. Zentner, and D. T. Plachta, "Haemodynamic responses to selective vagal nerve stimulation under enalapril medication in rats," PloS one, vol. 11, p. e0147045, 2016.

[18] G. Mancia and G. Grassi, "The autonomic nervous system and hypertension," Circulation research, vol. 114, no. 11, pp. 1804-1814, 2014.

[19] C. Höcht, "Blood pressure variability: prognostic value and therapeutic implications," ISRN Hypertension, vol. 2013, 2013.

[20] D. Clement, L. Jordaens, and G. Heyndrickx, "Influence of vagal nervous activity on blood pressure variability," Journal of hypertension. Supplement: official journal of the International Society of Hypertension, vol. 2, no. 3, pp. S391-3, 1984.