Low Level Vagosympathetic Stimulation:
A Paradox and Potential New Modality for the Treatment of Focal Atrial Fibrillation

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Abbreviations:
AF: atrial fibrillation
AF-TH: threshold of atrial fibrillation
ANS: autonomic nervous system
GP: ganglionated plexus
HFS: high frequency stimulation
LL-VNS: low level vagosympathetic nerve stimulation
PV: pulmonary vein
ABSTRACT

Background: We used high frequency stimulation (HFS) delivered during the refractory period of the atrium (A) and pulmonary veins (PVs) to induce focal firing and atrial fibrillation (AF). This study was designed to demonstrate that bilateral low-level vagosympathetic nerve stimulation (LL-VNS) could suppress HFS induced focal AF at atrial and PV sites.

Methods and Results: In 23 dogs, anesthetized with Na-pentobarbital, electrodes in the vagosympathetic trunks allowed LL-VNS at 1 volt below that which slowed the sinus rate or atroventricular conduction. Multi-electrode catheters were fixed at right and left superior and inferior PV (RSPV, RIPV, LSPV, LIPV) and both atrial appendages (AA). LL-VNS continued for 3 hours. At the end of each hour, the HFS algorithm consisting of a 40 ms train of stimuli, (200 Hz, stimulus duration 0.1-1.0 msec) was delivered 2 ms after the atrial pacing stimulus during the refractory period at each PV and AA site. The lowest voltage of HFS that induced AF was defined as the AF threshold (AF-TH). Five dogs without LL-VNS served as sham controls. Six dogs underwent LL-VNS but after transection of bilateral vagosympathetic trunks. LL-VNS induced a progressive increase in AF-TH at all PV and AA sites, particularly significant (p<0.05) at RSPV, RIPV, LSPV and right AA. Bilateral vagosympathetic transection did not significantly alter the previous findings and the 5 sham controls did not show changes in AF-TH at all sites over 3 hours.

Conclusions: LL-VNS may prevent episodic AF due to rapid PV and non-PV firing.

Key Words: arrhythmia; nervous system, autonomic; vagus nerve; Atrial Fibrillation; vagal stimulation
INTRODUCTION

Since the early part of the last century investigators (1) have used vagosympathetic nerve trunk stimulation (VNS) to induce atrial fibrillation (AF). Once initiated, AF can be maintained by continuous VNS (2). The underlying mechanism of action of VNS was postulated to be a marked shortening of refractoriness and a dispersion or heterogeneity of refractoriness, thereby promoting multiple reentrant wave fronts (3, 4). Of interest, even excessive slowing of the heart rate by intense VNS is not sufficient to initiate AF; however, the occurrence of spontaneous or induced atrial premature depolarization or burst pacing in the presence of VNS invariably initiates the arrhythmia (5, 6). There are reports in the basic literature (7, 8) as well as clinical reports (9, 10) suggesting that vagal activation can be anti-arrhythmic. Tai et al reported that focal firing from the PV in AF patients was suppressed by intravenous administration of phenylephrine, possibly through increased vagal tone caused by Baroreflex activation (9). A recent report from our laboratory demonstrated that VNS inhibited the neural activity in the anterior right ganglionated plexus (ARGP), one of the major GP in the mammalian heart (11). Both studies suggest that increased vagal activation may inhibit rapid focal firing or AF and also focused the attention on the interactions between the extrinsic and intrinsic cardiac autonomic nervous system (ANS). The former consists of the neurons and nerves in the brain or spinal cord and the axonal connections to the heart. The latter is composed of the neurons and nerves on the heart itself or the great vessels in the thorax. The purpose of the present study was to use a method previously described (12-14) to induce rapid firing and AF in order to test the hypothesis that lower level VNS (LL-VNS) could suppress this form of AF by inhibiting the intrinsic cardiac ANS in the
METHODS

All animal studies were reviewed and approved by the institutional Animal Care and Use Committee of the University of Oklahoma Health Sciences Center. Twenty-three adult mongrel dogs weighing 20~25 kg were anesthetized with Na-pentobarbital, 50mg/kg, and ventilated with room air by a positive pressure respirator. Core body temperature was maintained at 36.5±1.5°C. Standard ECG and blood pressure were continuously recorded.

Both cervical vagosympathetic trunks were exposed by dissections. A pair of Teflon®-coated silver wires (0.1 mm diameter) was inserted into the cervical vagosympathetic trunks for stimulation. Vagosympathetic nerve stimulation (VNS) was performed by applying high frequency electrical stimulation (HFS; 20 Hz, 0.1 msec duration, square waves) to both vagosympathetic trunks via a stimulator (Grass-S88, Astro-Med; West Warwick, RI). The lowest voltage level of VNS that slowed the sinus rate or AV conduction (measured by the AH interval) was considered the threshold. One volt lower than the threshold was then chosen as the voltage for LL-VNS. For each experiment, LL-VNS was applied to both vagosympathetic nerve trunks. Prior to each hour of LL-VNS, the threshold of VNS was determined again to adjust the voltage for LL-VNS for the next hour. During LL-VNS, the sinus rate and AH interval were monitored to ensure that the stimulation voltage was below the threshold.
After a bilateral thoracotomy, multielectrode catheters were attached to each of the PVs and both atrial appendages (Figure 1A and 1B). On the right side, a bipolar plaque electrode was sutured to the epicardial surface overlying the anterior right ganglionated plexus (ARGP) for HFS (20 Hz, pulse width 0.1 msec). HFS was applied to the ARGP for 1 minute at different voltages up to the voltage that induced AF (N=8). The average sinus rate was determined every five seconds and the lowest average sinus rate induced by ARGP stimulation was used as an indicator of the cholinergic influence of the ARGP (Figure 2). The same procedure was repeated hourly during three hours of LL-VNS.

The Effect of Bilateral LL-VNS on AF Inducibility at Multiple Atrial and PV Sites

Atrial pacing (at 2x diastolic threshold) was performed at cycle lengths of 330 msec. By enslaving the second channel of the Grass S88 Stimulator, a 40 ms train of stimuli, (frequency 200 Hz, stimulus duration 0.1-1.0 msec) was delivered 2 ms after the atrial pacing stimulus during the atrial refractory period. In this way, HFS would stimulate local nerves but not PV or atrial myocardium (12-14). AF was defined as irregular atrial rhythm (>500 beats per minute) lasting ≥ 5 seconds. In 8 dogs, in the baseline state (no bilateral LL-VNS), the lowest voltage of HFS required to induce AF at PV and atrial sites were determined as the AF threshold (AF-TH). LL-VNS was initiated for a total of 3 hours. At the end of each hour, VNS was temporarily discontinued for approximately 20 minutes to allow the determination of AF-TH. In all the 8 dogs, the AF-TH was measured with both vagosympathetic trunks intact. In six other dogs, the AF-TH was measured with both trunks transected at the C3-C4 level and VNS was applied to the distal end of the nerve trunks .In four other dogs, the AF-TH was measured at LL-VNS that was only 50% of the
threshold which induced slowing of the sinus rate or AV conduction. In another 5 dogs, AF-TH was measured hourly for three hours without VNS to serve as a control.

**Statistical Analysis**

Animals were randomly assigned to the control, LL-VNS (90% TH), LL-VNL (50% TH) and the decentralization group. The effects of LL-VNS on the changes in AF threshold (AF-TH; outcome of interest) at each site were evaluated by the linear regression test to evaluate the statistical significance of the trend of changes between the AF-TH in the baseline state and progressively longer application of the intervention (LL-VNS). The voltage of AF-TH at each site was presented as mean ± standard error in different groups at different time points. After the significance of the trend was established by the linear regression test, paired t-test was then used for comparisons of the AF-TH at the end of each hour versus AF-TH at the baseline. P values<0.05 were considered significant.

**RESULTS**

**Effects of LL-VNS on ARGP Stimulation**

The sinus rate slowing response induced by continuous ARGP stimulation for one minute was used as a surrogate for the cholinergic influences of ARGP stimulation (15). Figure 2 illustrates a typical example of the sinus rate response during ARGP stimulation. The lowest average sinus rate that was achieved prior to AF being induced was calculated as the percent change (decrease) of sinus rate at baseline and after 3 hours of LL-VNS. The greatest percent change in sinus rate achieved before and after LL-VNS
was 51±18% (baseline), 46±23% (1 hour; NS), 43±20% (2 hours; NS) and 39±19% (3 hours; p<0.05; N=8).

Effects of LL-VNS on AF Threshold in Dogs with Intact Vagosympathetic Nerve Trunks

To determine if LL-VNS affects the AF inducibility at atrial or PV sites, the AF-TH induced by HFS coupled to atrial pacing at RSPV, RIPV, RAA, LSPV, LIPV and LAA sites was measured in the baseline state and at the end of each hour of LL-VNS in 8 dogs (Figure 3). Before each hour of LL-VNS, the lowest voltage (threshold) that slowed the sinus rate or AV conduction was measured. No significant change in the threshold was found (before 1st hour: 10.1±2.9V; before 2nd hour: 10.2±3.9V; before 3rd hour: 11.1±3.7V; after 3rd hour: 10.6±2.8V, N=8; p>0.05 for all). In 3/8 dogs, LL-VNS stimulation was performed for 5 hours. There was no difference in the AF-TH at all atrial and PV sites between 3-hour and 5-hour LL-VNS (data not shown). Therefore, LL-VNS was performed only for 3 hours for the rest of the study.

Figure 4A demonstrated that there were consistent and statistically significant trends showing a progressive increase of AF-TH at all sites; the AF-TH at right-sided sites (RSPV, RIPV, RAA and LSPV) increased significantly during the first hour and second hour of LL-VNS than at baseline. Also, there was a significant increase in the AF-TH in LIPV at the third hour compared to baseline (p value < 0.05). In 4 other dogs, the voltage of LL-VNS was further reduced to 50% below the threshold that slowed the sinus rate or AV conduction. Similar results were obtained compared to those with LL-VNS at the voltage that was 1 volt or approximately 10% below the threshold (Figure 4B). For the 5
dogs serving as controls (without LL-VNS for 3 hr). The AF-TH at all sites showed no significant difference between the baseline and after three hours of VNS (Figure 4C).

To determine if the effects of LL-VNS resulted from activating the afferent vagal nerve fibers projecting to the brain, both vagosympathetic trunks were transected at the C3-C4 level in 6 other dogs. Then, HFS coupled to atrial pacing as described above was repeated hourly for 3 hrs in the presence of LL-VNS. The results were similar to those presented in Figure 4A, except that the duration of LL-VNS required to induce significant change in the AF-TH at several sites were slightly different (Figure 4A,D).

DISCUSSION
In the present study, we discovered a paradox in the sense that HFS of bilateral vagosympathetic trunks at the voltage that did not slow the sinus rate or AV conduction can suppress rapid firing and AF. This antiarrhythmic effect is not dependent on the activation of the afferent vagal nerve fibers that project to the brain. These findings indicate complex interactions between the extrinsic and intrinsic cardiac ANS since both the proarrhythmic and antiarrhythmic effects can be induced by activation of the extrinsic cardiac ANS.

The discovery that patients with AF, resistant to drugs and cardioversion, had focal firing arising from PV (16, 17) and non-PV (18, 19) sources engendered many basic studies seeking the mechanism (s) underlying this ectopic activity at PV (12, 20-23) or...
non-PV sites (12, 24-27). Many of these studies implicated the intrinsic cardiac ANS as playing a critical role in the initiation and maintenance of the focal form of AF. The prevailing concept of cardiac autonomic innervation envisions the major input to the heart from the brain. The ganglia on the heart itself presumably serve parasympathetic relays for the atria, whereas postganglionic fibers from the sympathetic chain ganglia at the spinal cord supply the sympathetic input to the atria and ventricles. The extensive studies by Randall and his associates over several decades (28) provided evidence that the extrinsic and intrinsic cardiac ANS can function interdependently as well as independently; that is, each system can modulate the activity of the other through efferent and afferent connections (29).

Evidence for GP Mediation of the Increase in AF-TH due to Bilateral LL-VNS

In this study, HFS was delivered at the ARGP and the ability of ARGP stimulation to slow the sinus rate was used to assess the ARGP function under the influence of LL-VNS. We chose the ARGP for these experiments because previous studies have shown that ARGP is the most important “integration center” for the vagal innervation to the sinus node (30, 31). Hou et al studied the effects of sinus rate slowing by stimulation of either the right or left vagosympathetic trunk and found that the sinus rate response was markedly diminished after ablation of the ARGP, suggesting that innervation from both vagosympathetic trunks is integrated at the ARGP; therefore, the effects of bilateral LL-VNS may be mediated by ARGP as well. In the present study, LL-VNS suppressed the sinus rate slowing induced by ARGP stimulation, suggesting that activation of the neural elements within the ARGP was attenuated. Although other major atrial GPs were not
examined in this study, it is conceivable that multiple GPs may also be affected by LL-VNS given the observation that various electrophysiological properties induced by VNS were integrated at GP sites (31).

**Bilateral LL-VNS Suppressed AF Inducibility at Multiple Sites**

To examine the efficacy of LL-VNS in suppressing AF at other atrial and PV sites, we used an AF model that reliably induced rapid firing and AF by delivering HFS during the atrial or PV refractoriness to activate the local autonomic neural elements (12-14). AF induced by this approach could be markedly suppressed by atropine, esmolol or GP ablation, supporting the contention that autonomic activation, not direct myocardial stimulation, was the underlying mechanism for the AF induced in this model (13, 14). The short AF cycle length (average AF cycle length = 50-70 msec; Figure 3) was also consistent with activation of the ANS that shortened the refractory period. It could be argued that HFS might directly stimulate the atrial or PV myocardium to induce AF. Figure 3A and 3B illustrate typical examples of a 60-100 msec latency between the end of HFS and the onset of the rapid firing. If AF were induced by direct myocardial stimulation, AF would have occurred during or immediately after the end of HFS, without exhibiting a latency period. Moreover, if one of the stimuli in this 40-msec train of HFS directly stimulated the myocardium, it should elicit only a single atrial response, not a run of AF since all the rest of HFS would have fallen into the refractory period of the captured single atrial beat.

In the present study, the AF-TH at all sites did not change significantly over the
time frame of the experiments (Figure 4C), indicating that this AF model was stable over
the entire period of experiments and the changes induced by LL-VNS were not a result of
time-dependent drift in AF-TH. Although there was a statistically significant trend of
AF-TH increase at all sites, the duration of LL-VNS required to increase the AF-TH varied
among different sites (Figure 4A). This finding is possibly caused by a relatively small
sample size and also is consistent with heterogeneous autonomic innervation shown
previously (12,28,29,32) that the atrial appendages have the lowest nerve density among
all the sites tested in the present study (33). When the voltage of LL-VNS was further
reduced to 50% threshold, in stead of one volt below the threshold (approximately 90%
threshold), it still induced similar increase in AF-TH. Although we did not lower the
stimulation voltage further, it is possible that voltage lower than 50% threshold may still
suppress AF eliciting much less afferent vagal inputs to the brain.

Afferent vagal nerve fibers, originating from visceral organs such as heart and
lungs, account for approximately 80% of the nerve fibers in the vagosympathetic nerve
trunk (34). These afferent fibers project primarily or secondarily to multiple areas in the
brain, many of which are not known to be excitatory or inhibitory. To discount the possible
inhibitory effects via a reflex that involves the brain, both vagosympathetic trunks were
transected in six other dogs and LL-VNS applied at the distal end of the nerve trunks still
induced similar changes in AF–TH at multiple atrial and PV sites, indicating that the
effects we observed do not require the autonomic activation from the brain. However, the
subtle differences between the results shown in Figure 4A, 4B and 4D are also indicative
that the roles of the afferent vagal nerves cannot be completely ignored.
Limitations

In this study we did not record neural activation within the GP before and after 3 hours of LL-VNS; therefore the conclusion we reached were based on indirect functional evidence. The autonomic neurons in GP form small clusters of ganglia embedded in the epicardial fat in a “raisin-in-bread” fashion. To secure the tip of a microelectrode in the same autonomic ganglia of a beating heart and maintain the fidelity of neural recording for 4-5 hours imposes enormous technical challenges. Loss of the microelectrode position during the course of experiment would lead to the false conclusion in favor of our working hypothesis that the activity of the autonomic neurons is inhibited by LL-VNS. Therefore, we elected to use electrophysiological properties, such as the sinus rate slowing response and AF inducibility, to assess the function of the intrinsic cardiac ANS in the presence of LL-VNS. Although the conclusions we reached were based on indirect functional evidence, the accumulated evidence was consistent in support of our hypothesis that the effects of LL-VNS were acting by inhibition of GP function, known to support focal AF inducibility (12, 22-24).

Clinical Implications:

HFS of the vagosympathetic trunk without inhibiting sinus rate or AV conduction has become an effective therapeutic modality to treat drug refractory epilepsy. The frequency and strength of such stimulation are similar to the LL-VNS described here, except that bilateral VNS was utilized in this study. The side effect profile of VNS in treating epilepsy has already been well documented. Our study suggests a new modality for the treatment of focal AF, probably by attenuating the function of GP. Importantly,
when voltage of LL-VNS was reduced to 50% threshold, it still produced satisfactory results. Future research will be required to determine the optimal frequency, duration, and voltage that exert the most inhibition on AF with minimal interfering with the brain function.

CONCLUSIONS

In this study, we discovered that AF can be suppressed by LL-VNS, possibly through inhibiting the neural activity of the GP. Our results suggest that triggered firing arising from PV sites and non-PV sites (e.g. RAA) can be suppressed by LL-VNS. While similar stimulation frequency, pulse width and strength have been used safely and effectively to treat drug-refractory epilepsy (34), LL-VNS may serve as a new therapeutic modality to treat AF.

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FIGURE LEGENDS

Figure 1. Diagrammatic representation of the atria as seen from a right (A) or left sided (B) thoracotomy. A. Electrode catheters were sutured at the right superior and right inferior pulmonary veins (RSPV, RIPV, respectively) and on the right atrial appendage (RAA). B. A similar arrangement of electrode catheters was made on the left PVs and LAA. Abbreviations: SVC=superior vena cava; ARGP = anterior right ganglionated plexi; CS=coronary sinus; IVC=inferior vena cava; LAA: left atrial appendage; LPA=left pulmonary artery; SLGP=superior left ganglionated plexi; LOM=ligament of Marshall.

Figure 2. A typical example of the sinus rate slowing response induced by high frequency stimulation (HFS) of the ARGP for one minute. Each data point indicates the average sinus rate during a 5-second interval. The slowest sinus rate occurred within 10 seconds after the onset of the HFS and the sinus rate immediately returned to the baseline state with a mild overshoot.

Figure 3. A typical example of AF induced by HFS coupled to RSPV pacing. A. Before LL-VNS the AF-TH was 1.6V. B. AF-TH increased to 6.0V after two hours of LL-VN. Note that HFS was delivered to the atrial refractory period and a latency of 85 msec (panel A) and 95 msec (panel B) existed between the end of HFS and the beginning of rapid firing, indicating that AF was not induced by direct myocardial stimulation. II: ECG lead II. HB: electrogram recorded from the His bundle region. A: atrial pacing at 330 msec. All other abbreviations identical to Figure 1.

Figure 4. AF-TH of multiple PV and atrial sites before and after bilateral LL-VNS. A. LL-VNS, which was one volt or approximately 10% below threshold. B. LL-VNS (50% below threshold). C. no LL-VNS. D. LL-VNS (1 volt or 10% below threshold) but with both vagosympathetic trunks being transected. LR(+) indicates statistical significance (p<0.05) calculated by the linear regression test for the trend of AF-TH increase induced by LL-VNS. * and **: p<0.05 and p<0.01, respectively; compared to baseline (without LL-VNS) using the paired t-test. See “Statistical Analysis” for detail.
Figure 1A.
Figure 2
Figure 3A
Figure 4A
AF-THRESHOLD (volts) N=6

LR(+) LR(+) LR(+) LR(+) LR(+) LR(+)

Baseline 1H 2H 3H

RSPV RIPV RAA LSPV LIPV LAA

Figure 4B
Figure 4C
Figure 4D

AF-THRESHOLD (volts) N=6

- RSPV
- RIPV
- RAA
- LSPV
- LIPV
- LAA

LR(+)

Baseline
1H
2H
3H

*
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