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

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Background—We used high-frequency stimulation delivered during the refractory period of the atrium 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 high-frequency stimulation–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 V below that which slowed the sinus rate or atrioventricular conduction. Multielectrode catheters were fixed at the right and left superior and inferior PVs and both atrial appendages. LL-VNS continued for 3 hours. At the end of each hour, the high-frequency stimulation algorithm consisting of a 40-ms train of stimuli (200 Hz; stimulus duration, 0.1 to 1.0 ms) was delivered 2 ms after the atrial pacing stimulus during the refractory period at each PV and atrial appendages site. The lowest voltage of high-frequency stimulation that induced AF was defined as the AF threshold. Five dogs without LL-VNS served as sham controls. Six dogs underwent LL-VNS after transection of bilateral vagosympathetic trunks. LL-VNS induced a progressive increase in AF threshold at all PV and atrial appendages sites, particularly significant ($P < 0.05$) at the right superior PV, right inferior PV, left superior PV, and right atrial appendage. Bilateral vagosympathetic transection did not significantly alter the previous findings, and the 5 sham control dogs did not show changes in AF threshold at any site over a period of 3 hours.

Conclusions—LL-VNS may prevent episodic AF caused by rapid PV and non-PV firing. (Circ Arrhythm Electrophysiol. 2009;2:645-651.)

Key Words: arrhythmia ■ nervous system ■ autonomic ■ vagus nerve ■ atrial fibrillation ■ vagal stimulation

Since the early part of the last century, investigators have used vagosympathetic nerve trunk stimulation (VNS) to induce atrial fibrillation (AF). Once initiated, AF can be maintained by continuous VNS. The underlying mechanism of action of VNS was postulated to be a marked shortening of refractoriness and a dispersion of heterogeneity of refractoriness, thereby promoting multiple reentrant wave fronts. 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. There are reports in the basic literature as well as in clinical reports suggesting that vagal activation can be antiarrhythmic. Tai et al reported that focal firing from the pulmonary veins (PVs) in patients with AF was suppressed by intravenous administration of phenylephrine, possibly through increased vagal tone caused by baroreflex activation. A recent report from our laboratory demonstrated that VNS inhibited the neural activity in the anterior right ganglionated plexus (ARGP), a major ganglionated plexus in the mammalian heart. 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 to induce rapid firing and AF to test the hypothesis that low-level VNS could suppress this form of AF by inhibiting the intrinsic cardiac ANS in the normal dog heart.

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Methods
All animal studies were reviewed and approved by the institutional Animal Care and Use Committee of the University of Oklahoma.

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Twenty-three adult mongrel dogs weighing 20–25 kg were anesthetized with Na-pentobarbital, 50 mg/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. VNS was performed by applying high-frequency electric stimulation (HFS; 20 Hz, 0.1-ms 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 atrioventricular (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. Before 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 1, A and B). On the right side, a bipolar plaque electrode was suctioned to the epicardial surface overlying the ARGP for HFS (20 Hz; pulse width, 0.1 ms). 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 5 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 3 hours of LL-VNS.

**Effects of Bilateral LL-VNS on AF Inducibility at Multiple Atrial and PV Sites**

Atrial pacing (at 2× diastolic threshold) was performed at cycle lengths of 330 ms. By enslaving the second channel of the Grass S88 Stimulator, a 40-ms train of stimuli (frequency, 200 Hz; stimulus duration, 0.1 to 1.0 ms) 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. 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 6 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 4 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 3 hours without VNS to serve as a control.

**Statistical Analysis**

Animals were randomly assigned to the control group, the LL-VNS group (90% TH), the LL-VNL group (50% TH), and the decentralization group. The effects of LL-VNS on the changes in AF-TH (the 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 ± SEM in different groups at different time points. After the significance of the trend was established by the linear regression test, the paired t test was then used for comparisons of the AF-TH at the end of each hour versus AF-TH at the baseline. Probability 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 1 minute was used as a surrogate for the cholinergic influences of ARGP stimulation. Figure 2 illustrates a typical example of the sinus rate response during ARGP stimulation. The lowest average sinus rate that was achieved before 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 51/11006 18% (baseline), 46/11006 23% (1 hour; NS), 43/11006 20% (2 hours; NS), and 39/11006 19% (3 hours; P<0.05; n=8).

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

To determine whether LL-VNS affects the AF inducibility at atrial or PV sites, the AF-TH induced by HFS coupled to atrial pacing at the right superior PV, right inferior PV, right atrial appendage, left superior PV, left inferior PV, and left atrial appendage 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±2.9 V; before 2nd hour, 10.2±3.9 V; before 3rd hour, 11.1±3.7 V; after 3rd hour, 10.6±2.8 V; n=8; P>0.05 for all). In 3 of 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 demonstrates that there were consistent and statistically significant trends showing a progressive increase of AF-TH at all sites; the AF-TH increased significantly during the first and second hours of LL-VNS than at baseline. Also, there was a significant increase in AF-TH in the left inferior PV at the third hour compared with baseline (P<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 with
those with LL-VNS at the voltage that was 1 V or approximately 10% below threshold (Figure 4B). For the 5 dogs control dogs (without LL-VNS for 3 hours), AF-TH at all sites showed no significant difference between the baseline and after 3 hours of VNS (Figure 4C).

To determine whether 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. HFS coupled to atrial pacing as described above was then repeated hourly for 3 hours 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 and 4D).

**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 because 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 PV12,23 and non-PV18,19 sources engendered many basic studies seeking the mechanisms underlying this ectopic activity at PV12,23 or non-PV sites.12,23–27 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 presumably serve as 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 et al28 over several decades 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

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Figure 4. AF-TH of multiple PV and atrial sites before and after bilateral LL-VNS. A, LL-VNS, which was 1 V or approximately 10% below threshold. B, LL-VNS (50% below threshold). C, No LL-VNS. D, LL-VNS (1 V 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. *P<0.05, **P<0.01, compared with baseline (without LL-VNS), using the paired t test.
Evidence for GP Mediation of the Increase in AF-TH Caused by 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. 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.

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. 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. The short AF cycle length (average AF cycle length, 50 to 70 ms; 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- to 100-ms 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 1 of the stimuli in this 40-ms train of HFS directly stimulated the myocardium, it should elicit only a single atrial response, not a run of AF, because all the rest of the 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 is consistent with heterogeneous autonomic innervation shown previously. The atrial appendages have the lowest nerve density among all the sites tested in the present study. When the voltage of LL-VNS was further reduced to 50% threshold, instead of 1 V below the threshold (approximately 90% threshold), it still induced a 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 input to the brain.

Afferent vagal nerve fibers, originating from visceral organs such as the heart and lungs, account for approximately 80% of the nerve fibers in the vagosympathetic nerve trunk. 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 6 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 that 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 conclusions 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 to 5 hours imposes enormous technical challenges. Loss of the microelectrode position during the course of the 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.

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 used 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 the voltage of LL-VNS was reduced to 50% threshold, it still produced satisfactory results. Future research will be required to deter-
mine 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 (eg, right atrial appendage) can be suppressed by LL-VNS. Although 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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CLINICAL PERSPECTIVE
At present, there are approved devices that are surgically implanted for low-level vagosympathetic nerve stimulation (LL-VNS) to the brain for treating drug-refractory epilepsy. In this study, using an experimental model of induced atrial fibrillation, we have demonstrated that LL-VNS, at approximately 10% below that which slowed the heart rate and applied for up to 3 hours, can significantly suppress AF inducibility. In addition, we found that similar efficacy could be achieved for atrial fibrillation suppression at LL-VNS voltage 50% below that which slows heart rate or slows atrioventricular conduction. Vagal trunk decentralization did not alter the suppressive action of LL-VNS, and additional evidence suggested that these effects appear to be mediated by efferent inhibition of intrinsic autonomic nervous system elements called ganglionated plexi found on the heart. Clinically, atrial fibrillation control requires either drug and/or ablation procedures. Further development of methods to apply LL-VNS, using less invasive approaches than are now used for epilepsy therapy, may allow the emergence of a nonpharmacologic, nonablative treatment for atrial fibrillation.
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