Continuous Low-Level Vagus Nerve Stimulation Reduces Stellate Ganglion Nerve Activity and Paroxysmal Atrial Tachyarrhythmias in Ambulatory Canines

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Background—We hypothesize that left-sided low-level vagus nerve stimulation (LL-VNS) can suppress sympathetic outflow and reduce atrial tachyarrhythmias in ambulatory dogs.

Methods and Results—We implanted a neurostimulator in 12 dogs to stimulate the left cervical vagus nerve and a radiotransmitter for continuous recording of left stellate ganglion nerve activity, vagal nerve activities, and ECGs. Group 1 dogs (N=6) underwent 1 week of continuous LL-VNS. Group 2 dogs (N=6) underwent intermittent rapid atrial pacing followed by active or sham LL-VNS on alternate weeks. Integrated stellate ganglion nerve activity was significantly reduced during LL-VNS (7.8 mV/s; 95% confidence interval [CI] 6.94 to 8.66 versus 9.4 mV/s [95% CI, 8.5 to 10.3] at baseline; P=0.033) in group 1. The reduction was most apparent at 8 AM, along with a significantly reduced heart rate (P=0.008). Left-sided low-level vagus nerve stimulation did not change vagal nerve activity. The density of tyrosine hydroxylase–positive nerves in the left stellate ganglion 1 week after cessation of LL-VNS were 99,684 μm²/mm² (95% CI, 28,850 to 170,517) in LL-VNS dogs and 186,561 μm²/mm² (95% CI, 154,956 to 218,166; P=0.008) in normal dogs. In group 2, the frequencies of paroxysmal atrial fibrillation and tachycardia during active LL-VNS were 1.4/d (95% CI, 0.5 to 5.1) and 8.0/d (95% CI, 5.3 to 12.0), respectively, significantly lower than during sham stimulation (9.2/d [95% CI, 5.3 to 13.1]; P=0.001 and 22.0/d [95% CI, 19.1 to 25.5], P<0.001, respectively).

Conclusions—Left-sided low-level vagus nerve stimulation suppresses stellate ganglion nerve activities and reduces the incidences of paroxysmal atrial tachyarrhythmias in ambulatory dogs. Significant neural remodeling of the left stellate ganglion is evident 1 week after cessation of continuous LL-VNS. (Circulation. 2011;123:2204-2212.)

Key Words: nervous system, autonomic • vagus nerve stimulation • tachyarrhythmias • atrial fibrillation

Animal studies suggest that sympathetic stimulation enhances, whereas vagal stimulation reduces, ventricular tachyarrhythmias and mortality.1,2 Increased sympathetic tone and reduced vagal tone, indicated by baroreflex sensitivity analysis, are associated with increased cardiovascular mortality after myocardial infarction (MI)3 and life-threatening ventricular arrhythmias in patients with heart failure.4 Interventions that increase vagal tone are often antiarrhythmic. For example, increased vagal activity elicited by exercise training is associated with strikingly lower cardiac mortality in post-MI patients.5 Continuous vagal stimulation can prevent ventricular fibrillation and sudden cardiac death in conscious dogs with a healed MI.6 Vagal stimulation can also improve cardiac autonomic control and significantly attenuate heart failure development in both dogs,7 rats,8 and humans.9 Spinal cord stimulation, which enhances parasympathetic activity,10 improves ventricular function and reduces ventricular arrhythmias in a canine postinfarction heart failure model.11 Although most of these previous studies used animal models of ventricular arrhythmia and conducted vagal stimulation with stimulus strength sufficient to reduce heart rate, low-level vagus nerve stimulation (LL-VNS) with stimulus strength 1 V below the threshold needed to reduce heart rate is known to be effective in suppressing atrial fibrillation (AF) induction in open-chest anesthetized dogs.12,13 The mechanisms by which vagal stimulation...
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Methods

The animal protocol was approved by the Institutional Animal Care and Use Committee of the Indiana University School of Medicine and the Methodist Research Institute, Indianapolis, IN, and conforms to the guidelines of the American Heart Association.

Continuous Ambulatory Autonomic Nerve Recordings

Twelve male mongrel dogs (weighing 22.8 to 30.0 kg) were used in this study. Under isoflurane inhalation general anesthesia, a small incision was made on the left anterior side of the neck. The left cervical vagus nerve was identified and isolated from the carotid artery. A bipolar pacing lead was sutured around the nerve and connected to a subcutaneously positioned Medtronic Itrel neurostimulator (Medtronic Inc, Minneapolis, MN). Subsequently, a left thoracotomy was performed through the 4th intercostal space. A Data Sciences International (DSI; St. Paul, MN) D70-EEE radio-transmitter with 3 bipolar recording electrodes was implanted to record nerve activity according to methods described in detail elsewhere.16,17 One pair of bipolar electrodes was sutured onto the caudal half of the left stellate ganglion (LSG) beneath its fascia to record SGNA. Another pair of bipolar electrodes was sutured onto the superior cardiac branch of the left vagal nerve to record vagal nerve activity (VNA). The final pair of electrodes was inserted into the fat pad at the junction of the left superior pulmonary vein and the left atrium18 to record superior left ganglionated plexi nerve activity (SLGPN). The chest was then closed and the dog was allowed to recover. After a recovery period of 2 weeks, the radiotransmitter was turned on and telemetric signals were continuously acquired for 1 week while the dogs were ambulatory.

Continuous Low-Level Vagus Nerve Stimulation in Normal Dogs (Group 1)

In the first 6 dogs (group 1), LL-VNS was administered after the protocol illustrated in Figure 1A. After 1 week of baseline recording, LL-VNS was then commenced while the dog was ambulatory. We first defined the stimulation threshold for each dog by stimulating the left cervical vagus nerve at 13 Hz (450 μs pulse duration). The stimulus amplitude (V) that elicited an abrupt decrease of heart rate by >20% from baseline or caused atrioventricular conduction block was defined as the stimulation threshold. We then programmed the pacemaker output to 1 V below the stimulation threshold12 and confirmed that this stimulus voltage (4±2 V, actual electrical voltage was in the range of 1 to 6 V) did not cause any heart rate changes. The stimulation parameters chosen resulted in no serious adverse reactions. However, transient cough and drooling were observed in 4 dogs, and nausea occurred in 2 dogs. The LL-VNS was kept for 1 week during which continuous nerve signals were acquired. After the LL-VNS was terminated, the dogs were monitored for another week before being euthanized. The stellate ganglia were harvested for histological analysis.

LL-VNS was then commenced while the dog was ambulatory. We performed LL-VNS in a high-yield canine model of atrial tachyarrhythmias16,17 in the remaining 6 dogs (group 2). In addition to the radiofrequency and Itrel neurostimulator, a Medtronic En-Pulse pacemaker (Medtronic Inc, Minneapolis, MN) was implanted with a pacing lead sutured onto the left atrial appendage for intermittent high-rate atrial pacing according to a protocol that is described in detail elsewhere.16,17 After 2 weeks of postoperative recovery, the DSI radiotransmitter was turned on to record baseline rhythm for 1 day. Baseline is defined as the observational period before the onset of pacing. High-rate (640 bpm) atrial pacing was then given for 6 days, followed by 1 day of pacing-free recording. During this recording period only, active or sham stimulation sessions were given on alternate weeks. This protocol was repeated until sustained AF was documented. AF indicates atrial fibrillation.

Low-Level Vagus Nerve Stimulation in Dogs With Intermittent Rapid Atrial Pacing (Group 2)

To determine if LL-VNS has atrial antiarrhythmic effects, we performed LL-VNS in a high-yield canine model of atrial tachyarrhythmias16,17 in the remaining 6 dogs (group 2). In addition to the radiofrequency and Itrel neurostimulator, a Medtronic En-Pulse pacemaker (Medtronic Inc, Minneapolis, MN) was implanted with a pacing lead sutured onto the left atrial appendage for intermittent high-rate atrial pacing according to a protocol that is described in detail elsewhere.16,17 After 2 weeks of postoperative recovery, the DSI radiotransmitter was turned on to record baseline rhythm for 1 day. Baseline is defined as the observational period before the onset of pacing. High-rate (640 bpm, twice the diastolic threshold) atrial pacing was then given for 6 days, followed by 1 day of monitoring during which the atrial pacemaker was turned off. During this recording period, active or sham LL-VNS was performed on alternate weeks as shown in Figure 1B. The rhythm was monitored to determine the presence of paroxysmal atrial tachyarrhythmias. The atrial pacing protocol was repeated until sustained (>48 hours) AF was documented.

Data Analyses

We analyzed recordings from all channels using custom-written software. Nerve activities were considered present if there was a 3-fold increase in the amplitude over baseline noise. We analyzed both the frequency of sympathetic discharge episodes and the corresponding heart rate increments after each discharge from 7 AM to 9 AM at baseline and during LL-VNS. In addition, we also...
determined the occurrence of paroxysmal atrial tachyarrhythmia, which was defined as a tachycardia with a rate of >160 bpm that lasted for >10 seconds.\textsuperscript{19} To reduce the probability of including sinus tachycardia in this analysis, we required the tachycardia to have both an abrupt onset (>50 bpm increment) and a reduction in bipolar electrogram amplitude. The ones with irregular atrial activations are paroxysmal AFs (PAFs) whereas the ones with regular activations are paroxysmal atrial tachycardias (PATs). Quantitative analyses were also performed with the aid of custom-design software to automatically import, filter, and analyze the recordings. To optimize nerve signals, data from SGNA and VNA were high-pass filtered at 100 Hz.\textsuperscript{16,19} Spike-triggered averaging was performed to allow removal of the atrial electrograms from SLGPNA recordings by subtracting an atrial electrogram template obtained from averaged atrial electrograms in the observation window.\textsuperscript{20} The filtered or transformed signals were then rectified, integrated with a 100-ms time constant, and summed to represent integrated nerve activity of 6-second segments over 24 hours of baseline recordings. We did not directly record a surface ECG. Instead, we applied bandpass filtering (5 to 100 Hz) on the VNA recording to obtain an ECG for analysis.\textsuperscript{16,17}

**Immunohistochemistry Studies**

LSG samples of all group 1 dogs were obtained from the recording sites and fixed in 4% formalin for 45 to 60 minutes, followed by storage in 70% alcohol.\textsuperscript{21} The tissues were processed routinely, paraffin embedded, and cut into 5-μm-thick sections. Immunohistochemical staining was performed with antibodies against tyrosine hydroxylase (TH) using mouse monoclonal anti-TH (Accurate Chemical, Westbury, NY). We included LSG samples from 5 normal dogs as controls. The density of TH-positive nerves was expressed as μm\textsuperscript{2}/mm\textsuperscript{2}. A second method of analysis was to determine the number of TH-negative ganglion cells among all ganglion cells in a stained slide. A blinded observer was asked to randomly select 10 high-power (20×) fields with highest ganglion cell density in the LSG from each dog and obtained a digital picture. A second blinded investigator used these digital pictures to manually determine the percentage of TH-negative ganglion cells among all ganglion cells in the stained slides. The mean of those 10 selected fields was used as the value for that LSG.

**Statistical Analyses**

The data are presented as mean and 95% confidence interval (CI). Repeated measures ANOVA followed by the Fisher protected least significance difference test was performed to compare the mean values and daily changes of nerve activity at baseline, during LL-VNS, and after LL-VNS. A paired t test was used to compare the immunostaining of the LSG from group 1 dogs and normal control dogs and to compare episodes of atrial tachyarrhythmias during active LL-VNS and during sham stimulation. A Poisson regression model was used to fit the frequency of paroxysmal atrial tachyarrhythmias for comparisons in group 2 dogs. The regression parameters were estimated through the generalized estimating equation with exchangeable correlation structure to account for correlations of measures from the same dog. Overdispersion parameters were estimated by Pearson residuals. All tests were performed at a 2-tailed significance level of $P=0.05$ and a 95% CI was calculated for each mean value. Online-only Data Supplement Figure I shows that the major variables are normally distributed. The statistics were computed using PASW Statistics (version 18; SPSS Inc, Chicago, IL) and SAS 9.2 (SAS Inc, Cary, NC).

**Results**

**Group 1**

**Immediate Effects of LL-VNS**

After the optimal stimulus strength was chosen, we turned on the neurostimulator for continuous LL-VNS. Figure 2 illustrates an example of the simultaneous nerve activity and ECG recordings before and immediately after the commencement of LL-VNS (arrowhead). The applied voltage was 1 V less than the threshold that immediately reduced the heart rate, and therefore no obvious heart rate deceleration was observed. There were abundant sympathetic discharges (solid arrows) prior to the LL-VNS. However, immediately following LL-VNS, there was suppression of SGNA for approximately 10 seconds (from point a to b) before sporadic SGNA reappeared (dashed arrows). There were no apparent changes in either VNA or SLGPNA in this example. Except for the transmission artifacts produced by pacemaker programmer in the initial 5 seconds of LL-VNS, continuous LL-VNS did not produce any stimulus artifacts.

**Effects of LL-VNS on Integrated Nerve Activity and Heart Rate**

We evaluated the long-term effects of LL-VNS by calculating integrated nerve activity and average heart rate. We analyzed 5 days at baseline and during LL-VNS for each group-1 dog. During LL-VNS, the integrated SGNA (7.8 mV/s [95% CI, 6.94 to 8.66]) was significantly reduced compared to baseline (9.4 mV/s [95% CI, 8.5 to 10.3]; $P=0.033$) with an overall reduction of 17% over 24 hours (Figure 3A). The nerve activity was first integrated over 6 seconds and then averaged over 5 days in each of the 6 dogs. The mean value for each dog was then averaged to get the integrated nerve activity. We also used the same method to obtain an hourly average of integrated nerve activity over 5 days of all 6 dogs. The reduction in integrated SGNA was particularly striking at 8 AM (35% reduction; 12.2 mV/s [95% CI, 5.0 to 17.4] versus 18.8 mV/s [95% CI, 11.7 to 25.8] at baseline; $P=0.002$). Figure 3B shows the hourly average of SGNA over a 24-hour period. The reduction in average heart rate during this period (14% of reduction; 78.1 bpm [95% CI, 68.7 to 87.5] versus 90.6 bpm [95% CI, 80.8 to 100.4] at baseline; $P=0.008$) paralleled the reduction in SGNA. Figure 3B also reveals that SGNA was suppressed to a greater degree during the daytime (from 7 AM to 5 PM) than during the nighttime. The morning surge in heart rate that is normally observed at baseline in
ambulatory dogs (arrowhead in Figure 3D) was considerably attenuated during LL-VNS, as well. The 24-hour average heart rate, however, was not significantly reduced (76.7 bpm [95% CI, 59.9 to 93.5] versus 77.5 bpm [95% CI, 61.9 to 93.1] at baseline; \( P = 0.726 \), Figure 3C). Of note, LL-VNS did not change either the 24-hour integrated VNA (4.2 mV/s [95% CI, 1.3 to 7.1]) versus 4.0 mV/s [95% CI, 1.4 to 6.6] at baseline; \( P = 0.164 \)) or 24-hour SLGPNA (6.6 mV/s [95% CI, 2.1 to 11.1] versus 5.7 mV/s [95% CI, 3.0 to 8.4] at baseline; \( P = 0.241 \)). Hourly averaged VNA and SLGPNA did not show a significant difference in any hour of the 24-hour period (online-Only Data Supplement Figure II).

Effects of Low-Level Vagus Nerve Stimulation on Sympathetic Discharge Episodes
The presence or absence of SGNA within each 60-second window from 7 to 9 AM each day was used to represent the frequency of sympathetic discharge episodes.\(^{19} \) During LL-VNS, the sympathetic discharge episodes were present 31% (95% CI, 18 to 44) of the time, significantly less frequently than at baseline (44% [95% CI, 29 to 59] of the time, \( P = 0.021 \)). The average duration of each sympathetic discharge episode (1.9 seconds [95% CI, 1.4 to 2.3]) was also significantly shortened compared with baseline (3.0 seconds [95% CI, 2.1 to 3.9]; \( P = 0.029 \)). We further analyzed the heart rate responses to each episode by comparing the average heart rate at 5 seconds before the onset of the sympathetic discharge episodes (averaged over 3 seconds; from 8 to 5 seconds before the onset of SGNA, see Online-Only Data Supplement Figure III) with that during the episodes (from onset to 3 seconds after onset). At baseline, the SGNA-induced heart rate acceleration was 129.2 bpm [95% CI, 105.2 to 153.1] (increased from 57.9 to 187.1 bpm, Figure 4A). However, during LL-VNS, the SGNA-induced heart rate acceleration was 107.9 bpm [95% CI, 81.6 to 134.2].

Figure 3. Effects of LL-VNS on SGNA and heart rate (HR). A, Continuous LL-VNS significantly reduced SGNA over 24 hours. The SGNA normalized to baseline level after cessation of LL-VNS. B, Hourly averages of SGNA show that the reduction in integrated SGNA was particularly striking at 8 AM. All values are averaged over 5 days and 6 dogs. C, The administration and cessation of continuous LL-VNS did not change the overall heart rate. D, Hourly averages of heart rate reveal that the morning surge of heart rate (arrowhead) was markedly attenuated during LL-VNS. SGNA indicates stellate ganglion nerve activity; LL-VNS, left-sided low-level vagus nerve stimulation. * \( P < 0.05 \).

Figure 4. Effects of LL-VNS on SGNA-induced heart rate changes. A, Graph of statistical dot plot shows that the SGNA-induced heart rate acceleration was markedly attenuated during LL-VNS compared with baseline. B, An example at baseline shows that a burst of SGNA (black arrowhead) led to heart rate increase from 67 to 196 bpm (129 bpm increment). C, An example during LL-VNS shows that a burst of SGNA (white arrowhead) led to heart rate increase from 63 to 160 bpm (97 bpm increment). SGNA indicates stellate ganglion nerve activity; \( \Delta HR \), change in heart rate; LL-VNS, left-sided low-level vagus nerve stimulation; VNA, vagal nerve activity; and SLGPNA, superior left ganglionated plexi nerve activity.
bpm [95% CI, 84.8 to 131.1] (increased from 57.8 to 166.2 bpm), which was significantly attenuated \((P = 0.041)\). Figure 4B is an example at baseline, in which an episode of sympathetic discharge (black arrowhead) led to a heart rate increase from 67 to 196 bpm (129 bpm increment). This contrasts with Figure 4C, which shows that an episode of sympathetic discharge (white arrowhead) led to a heart rate increase from 63 to 160 bpm (97 bpm increment).

**Normalization of SGNA After the Cessation of Low-Level Vagus Nerve Stimulation**

The group-1 dogs were further monitored for 1 week after cessation of LL-VNS. During this week, SGNA and the frequency of sympathetic discharges normalized to near-baseline level. The integrated SGNA rose to 9.0 mV/s (95% CI, 8.2 to 9.9, \(P = 0.037\), compared with the values during LL-VNS, Figure 3A). The frequency of sympathetic discharges from 7 to 9 AM increased to near-baseline values (44% [95% CI, 31 to 56] of the time during which SGNA was present; \(P = 0.041\) compared with the values during LL-VNS). The heart rate acceleration value induced by SGNA also rose to 130.6 bpm [95% CI, 105.2 to 153.1] (increased from 57.4 to 188.0 bpm), which was almost identical to baseline values \((P = 0.883)\). Despite a trend toward increased SGNA, the 24-hour average heart rate did not significantly differ from the values during LL-VNS (79.2 bpm [95% CI, 63.6 to 94.7] versus 76.7 bpm [95% CI, 59.9 to 93.5]; \(P = 0.281\), Figure 3C). The cessation of LL-VNS did not alter either VNA (4.5 mV/s [95% CI, 2.1 to 7.0] versus 4.2 mV/s [95% CI, 1.3 to 7.1] during LL-VNS; \(P = 0.522\)) or SLGPNPNA (6.4 mV/s [95% CI, 1.6 to 11.3] versus 6.6 mV/s [95% CI, 2.1 to 11.1] during LL-VNS; \(P = 0.379\)). An overview of daily changes of SGNA of all group-1 dogs is provided in Figure 5. The SGNA was significantly decreased on the first day (D1) of LL-VNS (8.0 mV/s [95% CI, 7.1 to 8.9] versus 9.6 mV/s [95% CI, 8.2 to 10.9] on the D5 of baseline; \(P = 0.017\)). On D4 during LL-VNS, the SGNA was further decreased (5.6 mV/s [95% CI, 4.8 to 6.4] versus 7.7 mV/s [95% CI, 6.5 to 8.9]; \(P < 0.001\)). The SGNA remained significantly suppressed 1 day after the cessation of LL-VNS (7.4 mV/s [95% CI, 5.9 to 8.9] versus 9.6 mV/s [95% CI, 8.2 to 10.9] on D5 of baseline; \(P = 0.001\)). Afterward, the SGNA gradually normalized to the near-baseline level. However, there was no rebound increase of SGNA as compared with baseline.

**Immunohistochemical Studies**

We compared the results of immunostaining of the LSG between group-1 dogs that underwent LL-VNS (n=6; Figure 6, top) and normal control dogs (n=5; Figure 6, bottom). For dogs with LL-VNS, there was a significantly decreased density of TH-positive nerves in the LSG (99684 m²/mm² [95% CI, 28 850 to 170 517]) compared to normal dogs (n=5; 186 561 m²/mm² [95% CI, 154 956 to 218 166]; \(P = 0.008\)). There were significantly more ganglion cells without immunoreactivity to TH (as indicated by unfilled and solid arrows in Figure 6) in dogs with LL-VNS (5.5% [95% CI, 4.3 to 6.7] in 60 randomly selected windows, 10 windows for each dog) than in control (1.4% [95% CI, 0.2 to 2.6] in 50 randomly selected windows, 10 windows for each dog; \(P < 0.001\) after Bonferroni correction).
Effects of Low-Level Vagus Nerve Stimulation on Paroxysmal Atrial Tachycardia

Paroxysmal atrial tachycardia episodes are observed in both groups of dogs. In group-1 dogs, most episodes of PAT (104 of total 130 episodes observed; 80%) occurred from 4 AM to 12 PM. During LL-VNS, the frequency of PAT was significantly reduced (1.2/d [95% CI, 0.5 to 1.9] versus 4.3/d [95% CI, 1.1 to 7.6]; \( P < 0.048 \)). The reduction was particularly apparent from 4 AM to 12 PM when most episodes of PAT occurred (0.6/d [95% CI, 0.1 to 1.2] versus 3.9/d [95% CI, 0.5 to 7.1]; \( P < 0.026 \)). No PAF was noted in group 1.

Group 2

Effects of Low-Level Vagus Nerve Stimulation on Paroxysmal Atrial Tachycardia

For group-2 dogs that underwent rapid atrial pacing, the number of PATs significantly increased, along with the occurrence of PAF. We compared the periods with active LL-VNS (n = 12) and those with sham stimulation (n = 14). The frequency of PAF during the periods with active LL-VNS (1.4/d [95% CI, 0.5 to 5.1]) was significantly lower than during the periods with sham stimulation (9.2/d [95% CI, 5.3 to 13.1]; \( P < 0.001 \)) (Figure 7A). This reduction was even more obvious for PAT (8.0/d [95% CI, 5.3 to 12.0] versus 22.0/d [95% CI, 19.1 to 25.5]; \( P < 0.001 \)) (Figure 7C). Along with the decrease of PATs, SGNA was significantly suppressed during active LL-VNS (11.0 mV/s [95% CI, 7.8 to 12.7]) compared with during sham stimulation (14.3 mV/s [95% CI, 11.3 to 17.5]; \( P = 0.030 \)). There were no significant differences in VNA (6.7 mV/s [95% CI, 5.3 to 8.0]) under active LL-VNS versus 6.8 mV/s [95% CI, 5.2 to 8.3] under sham stimulation; \( P = 0.897 \)), SLGPNA (6.9 mV/s [95% CI, 4.5 to 9.2] under active LL-VNS versus 8.6 mV/s [95% CI, 4.8 to 12.3] under sham stimulation; \( P = 0.370 \)) or heart rate (92.6 bpm [95% CI, 84.6 to 103.6] under active LL-VNS versus 88.3 bpm [95% CI, 76.0 to 99.4] under sham stimulation; \( P = 0.285 \)). Figure 7B is an example of PAF. The ECG showed fast and irregular ventricular response, and the left atrial local electrograms recorded from the SLGP revealed fractionated electrograms (inset). Figure 7D is an episode of PAT in which the atrial rate abruptly accelerated to 228 bpm and lasted for more than 20 seconds.

Discussion

New Observations

We showed in ambulatory dogs that continuous left LL-VNS (1) effectively suppresses left SGNA, particularly in the morning; (2) reduces the density of TH-positive ganglion cells and increases the density of TH-negative ganglion cells in the LSG 1 week after cessation of continuous LL-VNS; and (3) significantly reduces the frequency of PAT.

Continuous Low-Level Vagus Nerve Stimulation Suppresses Stellate Ganglion Nerve Activity

It has been proposed that the primary electrophysiological effect of vagal activity is the direct consequence of antagonizing the effects of sympathetic activity. However, this relationship has not been proven through direct recording of nerve activity in conscious ambulatory animals. In this study, continuous LL-VNS was administered to ambulatory dogs, and its ability to suppress sympathetic nerve activity was assessed by directly examining SGNA. Without significantly reducing the heart rate or increasing VNA, LL-VNS was able to suppress the overall SGNA throughout the day, especially in the morning (8 AM). These findings are particularly important because of the circadian variation of sudden cardiac death, with its increased incidence in the morning.

Figure 7. Effects of LL-VNS on paroxysmal atrial tachyarrhythmias. A, Continuous LL-VNS significantly prevented the occurrence of paroxysmal atrial fibrillation (PAF). B, An example of PAF that shows fast and irregular ventricular responses and fractionated atrial electrograms (inset). The PAF episodes followed burst firings of SGNA, VNA, and SLGPNA (arrowheads). C, Left-sided low-level vagus nerve stimulation also significantly prevented the occurrence of paroxysmal atrial tachycardia (PAT). D, An example of PAT shows that after burst firings of SGNA, VNA, and SLGPNA (arrowheads), the atrial rate abruptly accelerated to 228 bpm and lasted for more than 20 seconds. In contrast to PAF, the atrial local electrograms in PAT were regular (inset). SLGP indicates superior left gangionated plexi; SGNA, stellate ganglion nerve activity; VNA, vagal nerve activity; and SLGPNA, superior left gangionated plexi nerve activity. \( * P < 0.05 \) comparing active LL-VNS with sham.
and the role of SGNA as a direct trigger of sudden cardiac death. Furthermore, the heart rate responses to SGNA were significantly blunted during LL-VNS compared with baseline. This may have been due to reduced SGNA duration through prejunctional inhibition of sympathetic nerve terminals or to postjunctional actions of VNA. Myocardium is known to produce antinociceptive neurotransmitters such as chromogranin A and vasostatin-1 (one of the derivatives of chromogranin A). It is possible that the production of these neuromodulators is increased by LL-VNS and exerts direct antinociceptive effects on the myocardium. Alternatively, these substances can be transmitted to the LSG through the retrograde axonal transport to cause neural remodeling in that structure, similar to the mechanisms of LSG remodeling induced by MI.

Because no change in heart rate is observed, the LL-VNS may activate the afferent nerves that result in neural remodeling in the central nervous system. The latter remodeling processes may then cause secondary neural remodeling in the LSG. Finally, after the cessation of LL-VNS, there was a gradual normalization of SGNA back to approximately baseline level. This washout effect further validates the causal relationship between LL-VNS and the suppression of cardiac sympathetic outflow.

**Low-Level Vagus Nerve Stimulation on Paroxysmal Atrial Tachycardia and Neural Remodeling in the Stellate Ganglion**

In addition to the reduced SGNA, we also observed a significant reduction of TH-positive nerve structures in the LSG and an increase of TH-negative ganglion cells. Although it is known that remodeling changes in the LSG occur during MI, the plasticity of the LSG during VNS has not been previously reported. We report in the present study that the LSG has significant plasticity and that the changes are associated with reduced TH-positive nerve structures. However, the significant histological changes inside the LSG were observed in group-1 dogs 1 week after cessation of the pacing, during which SGNA had already normalized. A possible explanation is that the remaining ganglion cells may have increased their activities to compensate for the neural remodeling caused by LL-VNS. When used clinically to suppress seizure disorder, the LL-VNS is administered intermittently. However, the seizures occurring during the VNS off time are also affected. This carry-over effect of continuous LL-VNS is also seen in group-1 dogs of the present study (ie, continued partial SGNA suppression for >24 hours). Structural neural remodeling may explain these carry-over effects of LL-VNS.

**Continuous Low-Level Vagus Nerve Stimulation Suppresses Paroxysmal Atrial Tachycardia**

There has been no clear clinical evidence linking therapeutic vagal stimulation with increased incidence of AF. In anesthetized dogs, vagal stimulation with moderate intensities (producing ≤40% sinus cycle length prolongation) can be used to deliver therapeutic benefits without the risk of atrial arrhythmogenesis. Also in anesthetized dogs, LL-VNS is shown to prevent AF inducibility. The authors attribute the effect to the inhibition of nerve activity of major ganglionated plexi, including the SLGPNA.

The present study for the first time demonstrates that continuous LL-VNS can reduce the frequency of atrial tachyarrhythmias in ambulatory dogs. However, the antiarhythmic mechanism of LL-VNS lies in the significant suppression of SGNA. The SLGPNA was reduced, but the reduction was statistically insignificant. This discrepancy may have stemmed from the design of the present study, in which long-term data were acquired to allow investigating the long-term effects of LL-VNS. It is possible that LL-VNS suppresses SLGPNA in the short term but this effect is not sustained. Furthermore, the ability to record nerve activity in ambulatory animals enables us to record nerve activity without the need of anesthetic agents, which are specifically designed to inhibit nerve activities.

**Clinical Implications**

More than 50,000 patients worldwide have been implanted with the left cervical LL-VNS system for suppression of epilepsy and depression. Its application has generally been safe, with reversible bradycardias related to vagal stimulation therapy reported to be extremely rare at a frequency of about 0.1% of implanted devices. More recently, in a pilot human study, continuous right cervical vagal stimulation was also shown to be feasible, safe, and beneficial for heart failure patients. Therefore, LL-VNS may constitute a safe nonpharmacological approach to control cardiac arrhythmias such as drug-refractory PAT and PAF in which suppression of cardiac sympathetic outflow is desired. It is also possible that LL-VNS may help control ventricular arrhythmias in long-QT syndrome or catecholaminergic ventricular tachycardia without the need for surgical sympathetic denervation. In addition, LL-VNS might also apply to certain clinical conditions (eg, palmar hyperhidrosis in which hyperactivity of the stellate ganglion may be responsible for the clinical manifestations of the diseases.

**Study Limitations**

We used the left rather than the right cervical vagus nerve for LL-VNS for 3 reasons: First, the left vagus nerve has fewer projections to the sinoatrial node. Left-sided stimulation may be less likely to cause heart rate changes. Bradycardia induced by vagal stimulation may lead to reactivation of SGNA that is undesirable in this study. A second reason is that left-sided stimulation is less likely to promote atrial arrhythmogenesis at the same intensity of stimulus when compared with the right. Third, as stated above, left LL-VNS has been widely used clinically and has proven to be safe. However, further studies are warranted to provide information about potential differences in the suppression of SGNA and atrial arrhythmic effects for left- versus right-sided stimulation. In addition, atrial cardiac ganglionated plexi are reported to contain 3 types of neurons: efferent sympathetic, efferent parasympathetic, and afferent ones. These neurons might colocalize with each other. Our recording methods could not differentiate one type of nerve activity from the other. A third limitation is that we only recorded...
from the LSG and the left vagus nerve. The autonomic interactions from the right side remain to be determined.

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Disclosures
Medtronic Inc and St. Jude Inc donated research equipment to our laboratory.

References


### CLINICAL PERSPECTIVE

The present study was conducted in ambulatory dogs with continuous recording of left stellate ganglion nerve activity and left vagus nerve activity before, during, and after low-level vagus nerve stimulation (LL-VNS) of the left cervical vagal nerve. We showed that LL-VNS can effectively suppress stellate ganglion nerve activity while not increasing or decreasing thoracic vagus nerve activity. The most significant stellate ganglion nerve activity reduction occurred in the morning when the sympathetic outflow was the highest. Immunohistochemical studies of the left stellate ganglion showed significant neural remodeling, including reduced sympathetic nerve structures, 1 week after cessation of LL-VNS. We further demonstrated that LL-VNS can suppress paroxysmal atrial tachycardia and paroxysmal atrial fibrillation induced by intermittent rapid atrial pacing. A possible clinical implication is that LL-VNS can be used as a nonpharmacological approach to controlling paroxysmal atrial tachycardia and paroxysmal atrial fibrillation through the suppression of cardiac sympathetic outflow. This method may also apply to other clinical conditions in which hyperactivity of the stellate ganglion and increased sympathetic outflow are responsible for the pathogenesis of the diseases. For example, previous studies have shown that the risk of sudden death is the highest in the morning. Low-level vagus nerve stimulation, which selectively suppresses sympathetic outflow in the morning, may be used to reduce the risk of sudden death. Other possible clinical applications include the suppression of ventricular tachyarrhythmias, including those associated with long-QT syndrome, catecholaminergic polymorphic ventricular tachycardia, or structural heart diseases. It may also be effective in noncardiac diseases caused by increased sympathetic outflow.
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Supplemental Figure 1. Probability-probability plots (P-P plots) of main variables used in the study. The diagonal line fits the data points well, indicating no strong violation of normality. These findings indicate that the main variables are normally distributed. SGNA, stellate ganglion nerve activities. HR, heart rate. TH, tyrosine hydroxylase.
Supplemental Figure 2. Effects of LL-VNS on VNA and SLGPNA. A, Hourly averaged VNA did not show a significant difference in any hour of the 24-hr period. B, Hourly averaged SLGPNA did not show a significant difference in any hour of the 24-hr period, either.
Supplemental Figure 3. Methods of heart rate (HR) calculation. The heart rate response to stellate ganglion nerve activity (SGNA) was determined by comparing the average heart rate at 5-8 sec before the onset of the sympathetic discharge ($HR_{\text{pre}}$) with that within 3 sec after the onset of sympathetic discharges ($HR_{\text{during}}$).