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Prevention and Reversal of Atrial Fibrillation Inducibility and Autonomic Remodeling by Low-Level Vagosympathetic Nerve Stimulation

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Objectives We hypothesized that autonomic atrial remodeling can be reversed by low-level (LL) vagosympathetic nerve stimulation (VNS).

Background Previously, we showed that VNS can be antiarrhythmogenic.

Methods Thirty-three dogs were subjected to electrical stimulation (20 Hz) applied to both vagosympathetic trunks at voltages 10% to 50% below the threshold that slowed sinus rate or AV conduction. Group 1 (n = 7): Programmed stimulation (PS) was performed at baseline and during 6-h rapid atrial pacing (RAP). PS allowed determination of effective refractory period (ERP) and AF inducibility measured by window of vulnerability (WOV). LL-VNS was continuously applied from the 4th to 6th hours. Group 2 (n = 4): After baseline ERP and WOV determinations, 6-h concomitant RAP+LL-VNS was applied. Sustained AF was induced by injecting acetylcholine (ACh) 10 mM into the anterior right ganglionated plexus (Group 3, n = 10) or applying ACh 10 mM to right atrial appendage (Group 4, n = 9).

Results Group 1: The ERP progressively shortened and the ΣWOV (sum of WOV from all tested sites) progressively increased ($p < 0.05$) during 3-h RAP then returned toward baseline during 3-h RAP+LL-VNS ($p < 0.05$). Group 2: 6-h concomitant RAP+LL-VNS did not induce any significant change in ERP and ΣWOV. Group 3 and Group 4: AF duration (AF-D) and cycle length (AF-CL) were markedly altered by 3-h LL-VNS (Group 3: baseline: AF-D = 389 ± 90 s, AF-CL = 45.1 ± 7.8 ms; LL-VNS: AF-D = 50 ± 15 s, AF-CL = 82.0 ± 13.7 ms [both $p < 0.001$]; Group 4: baseline: AF-D = 505 ± 162 s, AF-CL = 48.8 ± 6.6 ms; LL-VNS: AF-D = 71 ± 21 s, AF-CL = 101.3 ± 20.9 ms [both $p < 0.001$]).

Conclusions LL-VNS can prevent and reverse atrial remodeling induced by RAP as well as suppress AF induced by strong cholinergic stimulation. Inhibition of the intrinsic cardiac autonomic nervous system by LL-VNS may be responsible for these salutary results. (J Am Coll Cardiol 2011;57:563–71) © 2011 by the American College of Cardiology Foundation

The pathological consequence of atrial fibrillation (AF) known as atrial remodeling takes the form of electrical, contractile, and ultrastructural changes (1–7). Atrial remodeling has been shown in both animal (1–4) and human hearts (5–7). Conversion to maintained sinus rhythm can reverse these changes, but the longer the duration of AF, the less effective such a reversal becomes. Early conversion of the paroxysmal form of AF, therefore, should prevent the progression of AF to a persistent and long-standing form associated with additional abnormalities caused by remodeling and thereby less chance of restoring the rhythm to normal.

In a recent report from our laboratory (8), we demonstrated that rapid atrial pacing (RAP) up to 6 h was associated with a significant increase in AF inducibility, an acute form of "AF begets AF" originally proposed by Wijffels et al. (1). This type of atrial remodeling resulting from RAP was reversed by ablation of the 4 major left atrial ganglionated plexi and ligament of Marshall, suggesting the important role of the changes in the intrinsic cardiac autonomic nerve system (au-

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Abbreviations and Acronyms

AF = atrial fibrillation
AF-CL = atrial fibrillation cycle length
AF-D = atrial fibrillation duration
ANS = autonomic nervous system
ARGP = anterior right ganglionated plexus
ERP = effective refractory period
HFS = high-frequency stimulation
LAA = left atrial appendage
LA = left atrium
LL-VNS = low-level vagosympathetic nerve stimulation
LSPV = left superior pulmonary vein
PV = pulmonary vein
RA = right atrium
RAA = right atrial appendage
RAP = rapid atrial pacing
RIPV = right inferior pulmonary vein
RSPV = right superior pulmonary vein
ΣWOV = sum of the window of vulnerability at all recording sites

tonomic remodeling) in maintaining and perpetuating AF. In the present report, we describe a nonpharmacologic, nonablative methodology to reverse and prevent *acute* autonomic remodeling as well as to suppress AF induced by strong cholinergic stimulation.

Methods

Animal preparation. All animal studies were reviewed and approved by the Institutional Animal Care and Use Committee of the University of Oklahoma Health Sciences Center. Thirty-three adult mongrel dogs (20 to 30 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 electrocardiogram leads and blood pressure were continuously recorded.

The procedures, including animal preparations, bilateral thoracotomy, and position of multielectrode catheters at several atrial and pulmonary vein (PV) sites have been communicated in detail elsewhere (8) (Fig. 1). All recordings were displayed on a computerized, electrophysiology system (Bard Inc., Billerica, Massachusetts). Electrocardiogram recordings were made with filter settings of 0.1 to 250 Hz, whereas electrogram recordings were filtered between 30 and 250 Hz.

Programmed stimulation. Left atrial appendage was paced at 1,200 beats/min (2× threshold) to induce acute atrial remodeling. After each hour of pacing, RAP was temporarily stopped for 5 to 10 min to measure the effective refractory period (ERP) and AF inducibility. ERP at atrial or PV sites was determined by programmed stimulation that consisted of 8 consecutive stimuli (S1-S1 = 330 ms) followed by a premature stimulus (S1-S2). The S1-S2 intervals were decreased from 150 ms initially by decrements of 10 ms and then 1 ms when approaching ERP (8). Pacing was performed at 10× threshold. ERP dispersion was calculated offline as the coefficient of variation (mean ± SD) of the ERP at all 8 recording sites (8). AF was defined as irregular atrial rates >500 beats/min and a duration >5 s, associated with irregular atrioventricular conduction. The difference between the longest and shortest S1-S2 interval (in ms) at which AF was induced was defined as the window of vulnerability (WOV). The WOV served as a quantitative measurement of AF inducibility. The ΣWOV was the sum of WOVs at all sites in each dog (8).

Low-level vagosympathetic nerve stimulation. A pair of Teflon-coated silver wires (0.1-mm diameter) was inserted into the cervical vagosympathetic trunks after dissection. Vagosympathetic nerve stimulation (VNS) was performed by applying high-frequency electrical stimulation (HFS) (20 Hz, 0.1 ms duration, square waves) to *both* vagosympathetic trunks via a Grass stimulator (AstroMed, West Warwick, Rhode Island). The lowest voltage level of VNS that induced *any slowing* of the sinus rate or AV conduction (measured by the A-H interval) was considered the threshold. A voltage 10% or 50% lower than the threshold was then chosen as the voltage for low-level (LL) VNS. Prior to each hour of LL-VNS, the threshold of VNS was determined again in order to adjust the voltage for LL-VNS for the next hour. During LL-VNS, sinus rate and A-H interval were monitored to ensure that the stimulation voltage was below the threshold.

Experimental protocol 1: 6-h RAP. GROUP 1 (N = 7). At the end of each hour of the 6-h RAP, programmed stimulation as described in the previous text was performed to determine the ERP and WOV at all sites during sinus rhythm. Bilateral LL-VNS 10% below threshold was applied during the last 3 h of the 6-h RAP.

GROUP 2 (N = 4). After determinations of the baseline values of ERP and WOV, RAP and concomitant LL-VNS were applied for 6 h with hourly determinations of the ERP and ΣWOV as described above.

In 3 other animals, LL-VNS was delivered for 3 h without RAP to serve as controls.

Experimental protocol 2: AF induced by injecting acetylcholine into the anterior right ganglionated plexus. GROUP 3 (N = 10). To induce sustained AF, 10 mM acetylcholine (Ach) (0.5 ml) was injected into the fat pad containing the anterior right ganglionated plexus (ARGP) as previously described (9). Continuous monitoring was used to detect changes in heart rate, occurrence of premature depolarizations, initiation of AF, and duration of atrial tachyarrhythmias after ACh injection. If spontaneously AF did not occur in 5 min, AF was induced by delivering an atrial premature beat. The average AF cycle length was determined at the right superior PV (RSPV) site adjacent to the injected ARGP by measuring the average interval encompassing the first 20 electrograms after the onset of AF. After 3 h of LL-VNS (50% below threshold) applied during sinus rhythm, 10 mM ACh was injected into the ARGP again, and all the measurements described above were repeated. In 4/10 animals, we injected 10 mM ACh into the ARGP in the baseline and without 3-h LL-VNS, to serve as controls.

Experimental protocol 3: sustained AF induced by applying ACh-moistened gauze pad to right atrial appendage. GROUP 4 (N = 9). In this group, sustained AF was induced by placing an ACh-moistened (10 mM, 0.5 ml)

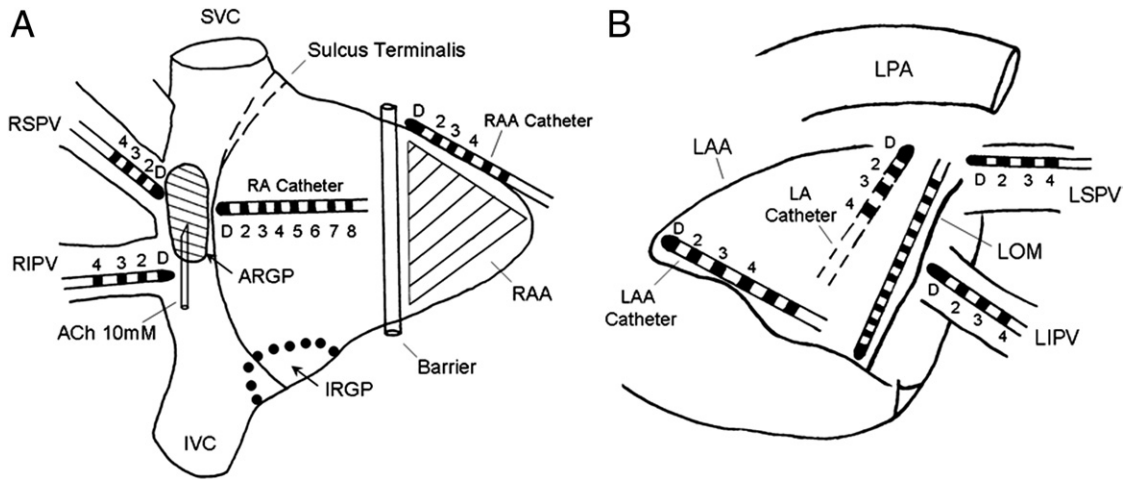

Figure 1 **Schematic Representation of the Right and Left Thoracotomy Approach and Catheter Positions in the Atria and PVs**

(A) Right thoracotomy. Multielectrode catheters were sutured to the epicardial surface of right atrial appendage (RAA), right atrial free wall (RA), and to the adjacent right superior (RSPV) and right inferior (RIPV) pulmonary veins (PVs). For Group 4 experiments, a plastic barrier was used to isolate the RAA from the rest of the atrium. **(B)** Left thoracotomy. Similarly, multielectrode catheters were positioned at the left superior PV (LSPV), left inferior PV (LIPV), left atrium (LA), and left atrial appendage (LAA). Rapid atrial pacing (1,200 beats/min) was applied at the LAA. IVC = inferior vena cava; LOM = ligament of Marshall; SVC = superior vena cava.

gauze pad (area = 240 mm^2) on the right atrial appendage (RAA) in the baseline state and at the end of each hour of the 3-h LL-VNS (50% below threshold) (10). A plastic barrier was sutured to the base of RAA and sealed with tissue glue to prevent ACh from leaking into the rest of the atrium (10). The changes of heart rate, premature depolarization, AF duration, and cycle length were determined as described above. In 3 of 9 animals, an ACh-moistened gauze pad was applied to the RAA at the baseline and the end of each hour for 3 h without LL-VNS to serve as controls.

Statistical analysis. Data were expressed as mean ± SD. Paired *t* tests were used for comparisons of ERP and WOV at the end of each hour versus ERP and WOV: 1) in the baseline state (asterisks, Figs. 2 to 4); or 2) at the end of the 3rd hour of RAP where the largest changes in ERP and WOV were observed (triangles, Figs. 2 to 4). No adjustments for multiple comparisons were made for these tests. In Group 3 and Group 4, ANOVA for repeated measures was used to compare the changes before and after ACh intervention. Values of $p \leq 0.05$ were considered statistically significant.

Results

Throughout the experiment, blood pressure was stable and no evidence of heart failure was found as a result of rapid atrial pacing. There was no significant change in the VNS threshold, indicating that the vagosympathetic trunks were not damaged during the entire period of the experiment (Table 1). In the 3 control animals that received LL-VNS for 3 h without preceding or concomitant RAP, ΣWOV (1.3 ± 1.1 ms, baseline; 0.7 ± 0.6 ms, 3-h, $p > 0.05$) and ERP did not change significantly (baseline: RSPV = 94 ± 17 ms; right inferior PV [RIPV] = 99 ± 11 ms; left superior PV [LSPV] = 90 ± 10 ms; left inferior PV [LIPV] = 89 ± 4 ms; right atrium [RA] = 96 ± 4 ms; left atrium [LA] = 96 ± 5 ms; left atrial appendage [LAA] = 94 ± 14 ms; after 3-h LL-VNS: RSPV = 98 ± 12 ms; RIPV = 100 ± 14 ms; LSPV = 93 ± 9 ms; LIPV = 92 ± 8 ms; RA = 94 ± 7 ms; LA = 97 ± 5 ms; LAA = 92 ± 13 ms; $p > 0.05$ for all).

ERP changes. In Group 1, as a result of RAP, ERP at all recording sites were significantly shortened in the first or the second hour (Fig. 2). After the initiation of LL-VNS (10% below threshold), ERP shortening at all sites was reversed, and returned to the baseline values at the end of the 6th hour. For example, ERP at the LSPV site was shortened from 99.2 ± 12.7 ms in the baseline state to 89.2 ± 10.1 ms at the end of 1st hour, and reached 78.0 ± 10.9 ms at the end of the 3rd hour ($p < 0.05$ for all). LL-VNS reversed these changes (91.2 ± 14.0 ms, and 97.3 ± 6.7 ms at the end of the 5th and 6th hour, respectively; $p < 0.05$ for both, compared to the end of 3-h RAP). For Group 2 animals, no significant ERP shortening was detected at all recording sites when RAP and LL-VNS (10% below threshold) were applied simultaneously (Fig. 3).

WOV changes. When AF was induced by programmed stimulation, AF typically lasted for ≤30 s and terminated spontaneously but was re-inducible by programmed stimulation. The cumulative WOV (ΣWOV) steadily increased during RAP in Group 1 (Fig. 4A). With RAP, the ΣWOV increased significantly to 59.3 ± 25.2 ms at 2 h and 90.7 ± 32.7 ms at 3 h ($p < 0.001$ for both, compared with baseline). After the initiation of LL-VNS, the ΣWOV gradually declined to 20.8 ± 10.3 ms at the end of the 6th hour ($p < 0.001$, compared with the end of the 3rd hour of

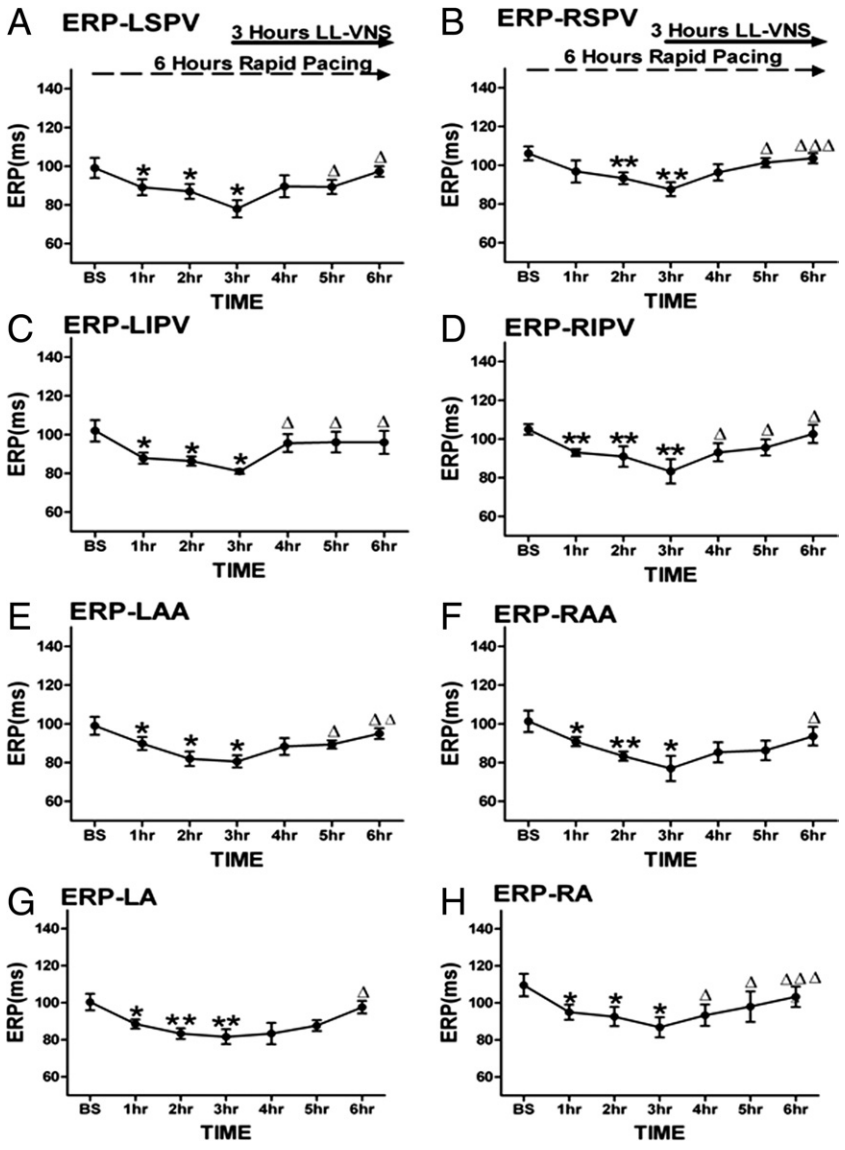

Figure 2 Change of Mean ERP at Different Recording Sites During 6-h RAP Accompanied by LL-VNS in the Last 3 h (Group 1, n = 7)

*p < 0.05 and **p < 0.01 compared with baseline using paired *t* test; △p < 0.05, △△p< 0.01, and △△△p < 0.001 compared with the end of the 3rd hour of rapid atrial pacing (RAP) using paired *t* test. ERP = effective refractory period; LL-VNS = low-level vagosympathetic nerve stimulation; other abbreviations as in Figure 1.

RAP). No increase in the ΣWOV was observed during the entire experimental period when RAP and LL-VNS were applied simultaneously (Fig. 4B).

ERP dispersion. In Group 1, there was a statistically significant increase in the ERP dispersion in the first 3 h (Fig. 4C). When LL-VNS was continuously applied from the 4th to 6th hour, ERP dispersion regressed toward the baseline values. In contrast, concomitant RAP+LL-VNS failed to increase the ERP dispersion (Fig. 4D).

The differences in the changes of ERP, ERP dispersion and WOV between group 1 and 2 at the end of 3-h RAP were also found to be statistically significant (Fig. 5).

AF duration and cycle length. GROUP 3. In the baseline state, injection of ACh into the ARGP resulted in spontaneous occurrence of AF in 4/10 dogs. In the other 6 dogs, AF was easily induced by delivering a premature atrial depolarization. After 3 h of LL-VNS (50% below threshold), spontaneous AF occurred in 2/4 of the control dogs (without LL-VNS) but in none of the 6 dogs receiving LL-VNS. Figures 6A to 6D show a marked decrease in the atrial fibrillation duration (AF-D) and increase in the atrial fibrillation cycle length (AF-CL) after 3-h LL-VNS (baseline: AF-D = 389 ± 90 s, AF-CL = 45.1 ± 7.8 ms; 3-h LL-VNS: AF-D = 50 ± 15 s, AF-CL = 82.0 ± 13.7 ms [both $p < 0.0001$]) but not in the control dogs

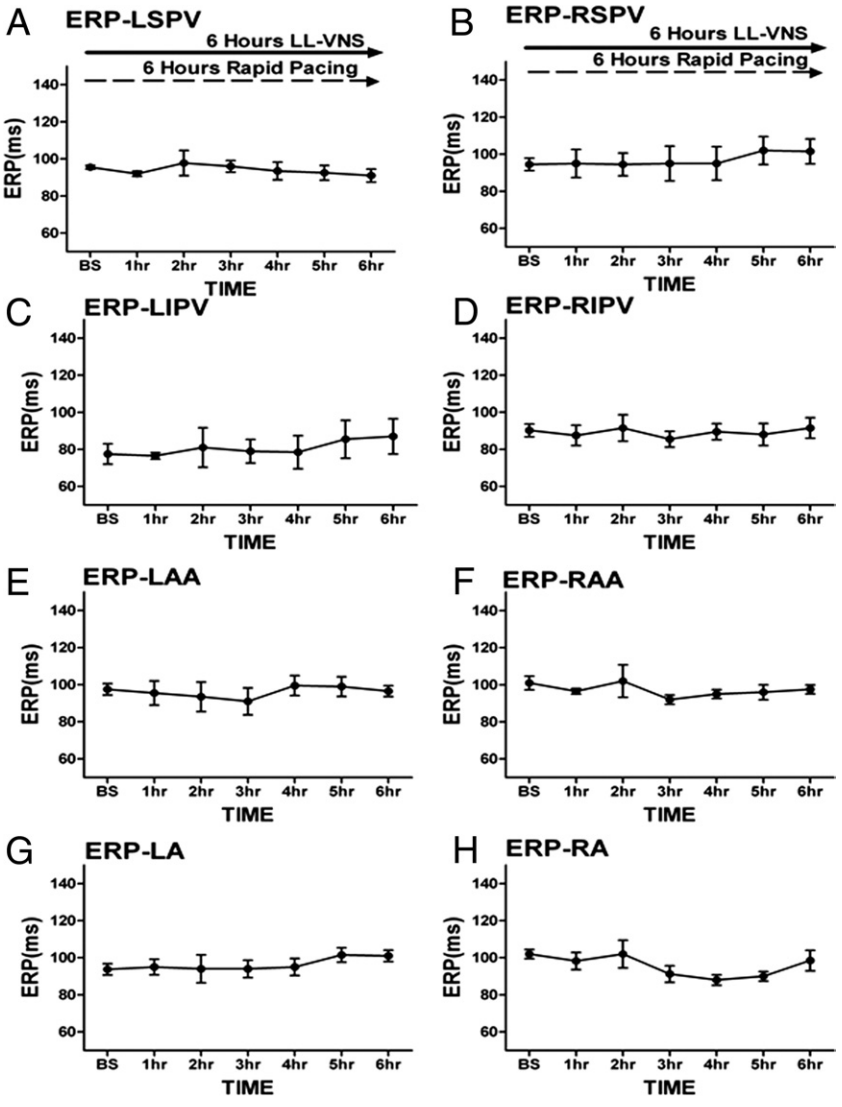

Figure 3 Change in Mean ERP at Different Recording Sites During 6-h RAP and Concomitant 6-h LL-VNS (Group 2, n = 4)

When RAP and LL-VNS were applied concomitantly, the ERP was not altered throughout the 6-h period. Abbreviations as in Figures 1 and 2.

(baseline: AF-D = 486 ± 150 s, AF-CL = 37.1 ± 7.6 ms, without 3-h LL-VNS: AF-D = 471 ± 115 s, AF-CL = 35.6 ± 5.9 ms, $p > 0.05$ for both).

GROUP 4. In the baseline state and at the end of each hour of LL-VNS (50% below threshold), sustained AF was induced by placing an ACh-moistened gauze pad on the RAA. Figures 6E to 6H show that the AF-D was substantially decreased and AF-CL was greatly increased by just 1-h LL-VNS (baseline: AF-D = 505 ± 162 s, AF-CL = 48.8 ± 6.6 ms; 1-h LL-VNS: AF-D = 133 ± 54 s [$p < 0.01$], AF-CL = 71.0 ± 16.4 ms [$p < 0.0001$]; 2-h LL-VNS: AF-D = 79 ± 31 s [$p < 0.01$], AF-CL = 85.3 ± 4.4 ms [$p < 0.0001$]; 3-h LL-VNS: AF-D = 71 ± 21 s [$p < 0.0001$], AF-CL = 101.3 ± 10.9 ms. In contrast, the sham LL-VNS did not induce any change in AF-D or AF-CL (baseline: AF-D = 455 ± 152 s, AF-CL = 44.8 ± 6.0 ms; 3-h LL-VNS: AF-D = 495 ± 124 s, AF-CL = 45.3 ± 12.1 ms ($p > 0.05$ for all).

For Group 3 and 4 animals, the differences in the changes of AF-D and AF-CL between LL-VNS and no LL-VNS were also found to be statistically significant (Fig. 7).

Discussion

Main finding. Since the last century, VNS had been used to induce and maintain AF (11). In the present study, we observed a paradoxical antiarrhythmic effect of LL-VNS to substantially suppress AF in 3 acute animal models. Acutely, RAP induced shortening of ERP, an increase in AF inducibility, and an increase in ERP dispersion. LL-VNS reversed these changes. When 6-h RAP was delivered in the

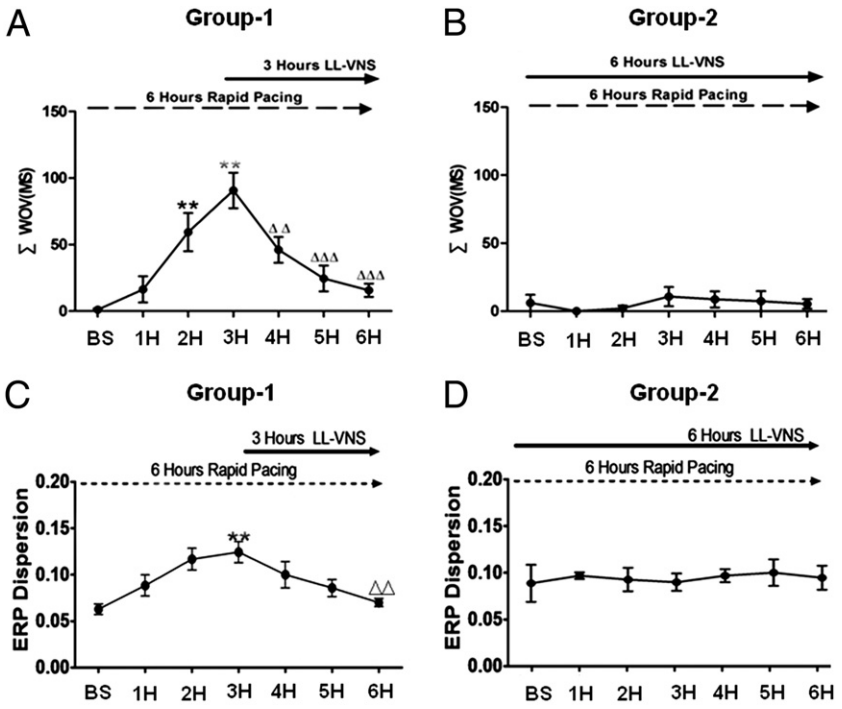

Figure 4 Change of ΣWOV and ERP Dispersion During 6-h RAP

Cumulative WOV (ΣWOV) **(A)** and ERP dispersion **(C)** were increased by 3-h RAP but were reversed by LL-VNS. In contrast, ΣWOV **(B)** and ERP dispersion **(D)** were not altered if LL-VNS and RAP were applied concomitantly. All **symbols** for p values as in Figure 2. Abbreviations as in Figures 1 and 2.

presence of LL-VNS, the aforementioned electrophysiological properties were not altered. Our results indicate that the *acute* electrical remodeling described as part of "AF begets AF" may be reversed and prevented by LL-VNS. In addition, LL-VNS (50% below threshold) markedly shortened the duration and increased the cycle length of AF induced by very strong cholinergic stimulation described in Group 3 and 4 experiments. Particularly, the AF-D was reduced by nearly 80% by only 1 h of LL-VNS that was 50% below the threshold.

"AF begets AF" and cardiac autonomic nerve system. Wijffels et al. (1,12) established an AF animal model by RAP and introduced the concept of "AF begets AF" (atrial remodeling). In the present study, the significant changes of ERP, WOV, and ERP dispersion were detected in the first 3 h with RAP, consistent with the concept that electrophysiological changes lead to a more stable substrate to facilitate the maintenance of AF (1,12). Over the past decade, multiple mechanisms have been proposed to account for atrial remodeling. Chronic RAP induced AF and caused significant reduction of the transient outward K current and L-type Ca current as well as cellular ultrastructure and Ca^{2+} handling abnormalities (13,14). Sustained AF also increased superoxide production in both LA and LAA, which may contribute to atrial remodeling (15). Atrial fibrosis is another important factor. However, increased fibrosis is unlikely a factor in our acute AF model but plays an important role in AF induced by chronic RAP (16).

Another major contributing factor to atrial remodeling is autonomic remodeling. Chronic RAP increases the innervation of the atrial sympathetic nerve system (17). This result suggested that the remodeling of intrinsic cardiac autonomic nervous system (ANS) may be involved in atrial remodeling. A recent study from our institute further highlighted the critical role of the intrinsic cardiac ANS in *acute* atrial remodeling. Inhibition of the intrinsic cardiac ANS by ablating the 4 major atrial ganglionated plexi and

Table 1 The Mean VNS Threshold (in Volts) That Slowed the Sinus Rate or AV Conduction During the Entire Experimental Period

	Baseline	1st Hour	2nd Hour	3rd Hour	4th Hour	5th Hour	6th Hour
Group 1 (n = 7)	0.32 ± 0.14				0.34 ± 0.11	0.37 ± 0.13	0.38 ± 0.07
Group 2 (n = 4)	0.38 ± 0.15	0.39 ± 0.14	0.40 ± 0.08	0.36 ± 0.08	0.38 ± 0.06	0.427 ± 0.12	0.40 ± 0.08

$p > 0.05$ for all.
AV = atrioventricular; VNS = vagosympathetic nerve stimulation.

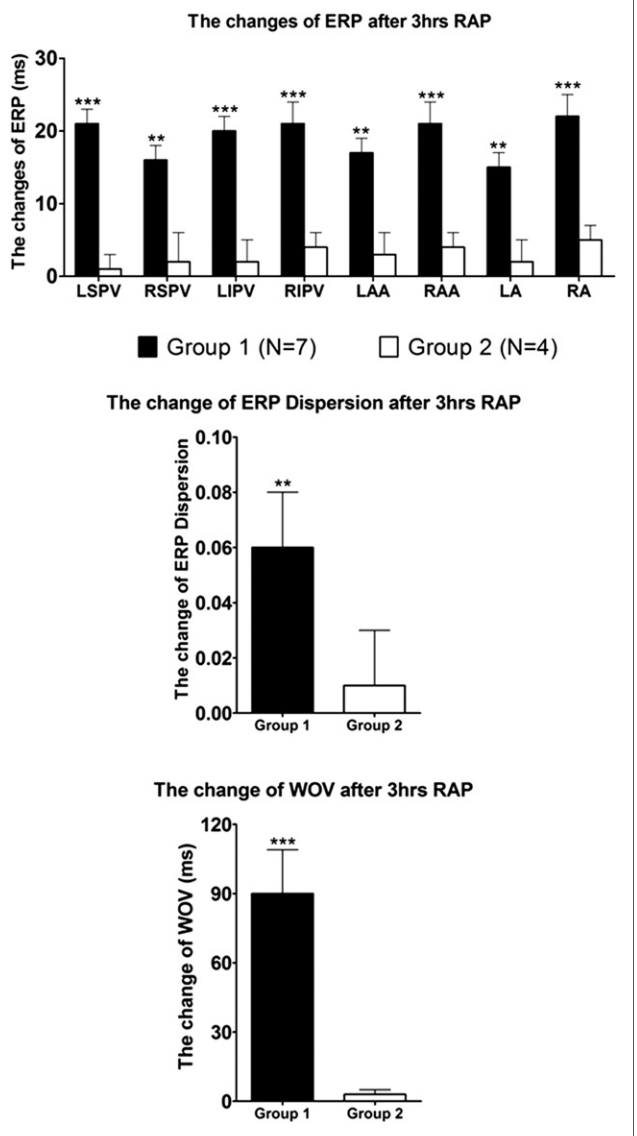

Figure 5 Comparison Between Groups 1 and 2

**p < 0.01 and ***p < 0.001. Abbreviations as in Figures 1 and 2.

ligament of Marshall or by administering autonomic blockade (atropine or esmolol) *before* the initiation of 6-h RAP prevented *acute* atrial remodeling, whereas acute atrial remodeling can be reversed if these interventions were initiated within a few hours after RAP was started (8). That study provides experimental evidence that autonomic remodeling is an important element in the *early phase* of atrial remodeling and inspired us to design the present study to seek an alternative approach to globally suppressing the intrinsic cardiac ANS and in turn prevent the progression of AF.

Explanations for suppression of AF by LL-VNS. The results of our study showed that LL-VNS had a potent inhibitory effect on AF induced by strong cholinergic stimulation as well as acute atrial remodeling induced by RAP, regardless of whether LL-VNS was initiated before or within a few hours of RAP. Prior studies have implied that interactions between the extrinsic and intrinsic cardiac ANS may have a significant impact on cardiac electrophysiology. First, by a combination of microinjection, dual antegrade tracing, and confocal microscopic technique to examine vagal efferent axons and terminals in cardiac ganglia, Ai et al. (18) observed that vagal efferent fibers ramified within cardiac ganglia. They formed a complex network of axons, and innervated cardiac ganglia with very dense basket endings around individual cardiac principal neurons (18). These findings provide strong anatomical evidence for explaining the complex interactions between the extrinsic and intrinsic cardiac ANS. Second, not all VNS is arrhythmogenic. The strength and duration of VNS may dictate its effects. Zhang et al. (19) found that AF inducibility by right cervical VNS was intensity dependent. Strong VNS (>60% sinus rate slowing) facilitated AF inducibility, whereas moderate VNS (slowing the sinus rate

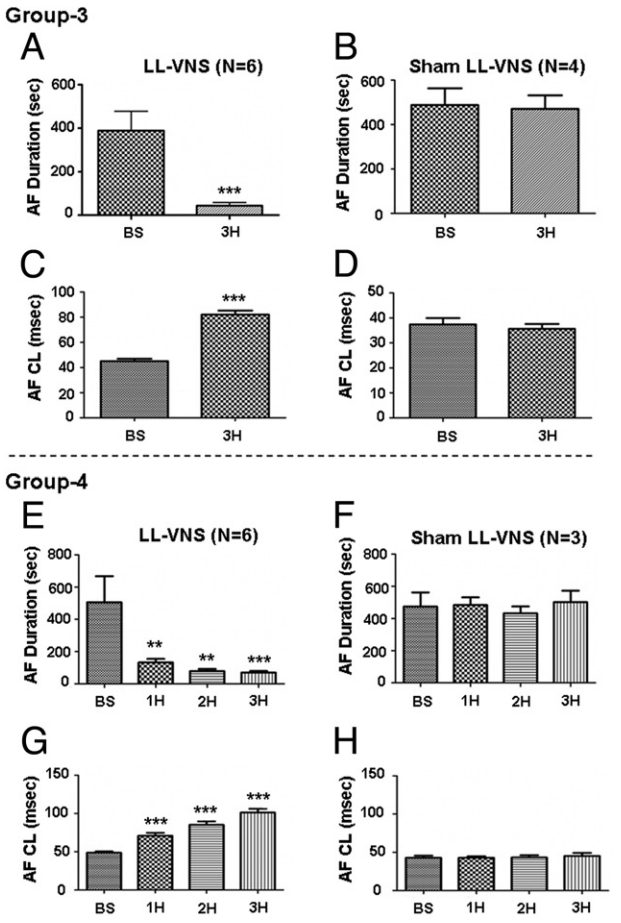

Figure 6 Changes of AF Duration and Cycle Length by 3-h LL-VNS

(A to D) Group 3. **(E to H)** Group 4. (*p < 0.05; **p < 0.01; ***p < 0.0001). See text for details. AF = atrial fibrillation; CL = cycle length; LL-VNS = low-level vagosympathetic nerve stimulation.

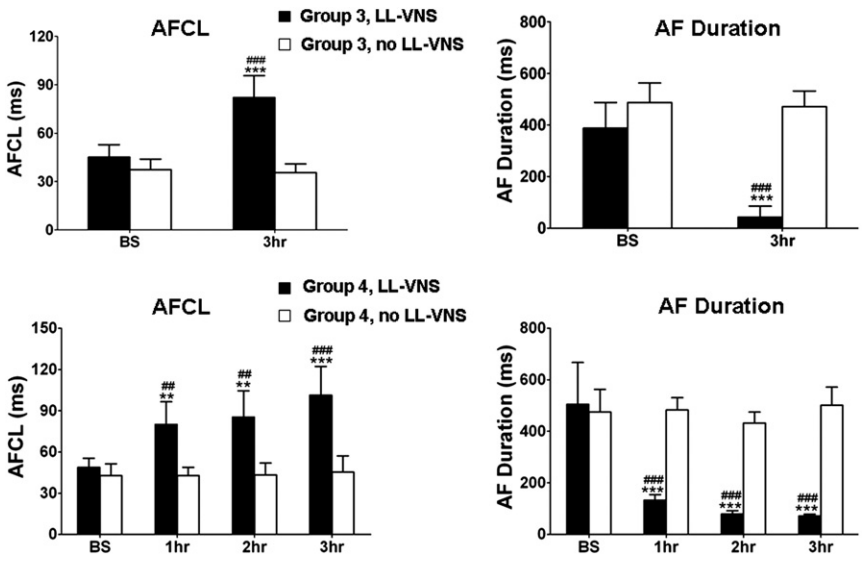

Figure 7 Comparison Between LL-VNS and No LL-VNS (Sham) Among Group 3 and 4 Animals

**p < 0.01 and ***p < 0.001, compared with baseline. ##p < 0.01 and ###p < 0.001, compared with no low-level vagosympathetic nerve stimulation (LL-VNS).

by 10%) for 4 weeks did not affect AF inducibility, and imposed no arrhythmogenic risk (19). Prior to a 7-h RAP, Takei et al. (20) stimulated both vagus nerves, which slowed the sinus rate by ~30%. Surprisingly, ERP did not shorten after 7 h of RAP (20). These observations implied that VNS may be antiarrhythmic. Third, the intrinsic cardiac ANS comprises the "integration center" for the co-ordination of regional cardiac reflex, for which the intrinsic cardiac ANS is considered the "little brain" of the heart (21). The interactions between the extrinsic and intrinsic cardiac ANS are complex and remain poorly understood. Zhang et al. (22) studied the effect of bilateral VNS on tonic firing recorded from the ARGP. Shortly after VNS application, GP neural activity was completely suppressed, and demonstrated that activation of the extrinsic cardiac ANS can suppress the neural activity in the intrinsic cardiac ANS. In a recent study from our group, AF threshold progressively increased during a period of 3 h when LL-VNS (50% below threshold) was applied only to the right vagosympathetic trunk (23). The amplitude and frequency of the ARGP neural activity were also markedly inhibited by LL-VNS of the right vagosympathetic trunk. Taken together (22,23), these results reinforce the notion that the extrinsic cardiac ANS may suppress the activity of intrinsic cardiac ANS and subsequently suppress AF.

It is known that vagosympathetic trunks carry mainly afferent nerve fibers that project primarily or secondarily to multiple areas in the brain, many of which are not known to be excitatory or inhibitory. In a recent study, we transected both vagosympathetic trunks in 6 dogs; LL-VNS was then applied at the distal end of the nerve trunks (24). LL-VNS still induced similar changes in AF threshold at multiple atrial and PV sites as LL-VNS with intact vagosympathetic trunks, indicating that the inhibitory effects of LL-VNS on cardiac electrophysiology involve efferent activation to the heart.

Study limitations. We hypothesize that the mechanism responsible for the atrial antifibrillatory effect of LL-VNS may be due to inhibition of the intrinsic cardiac ANS by the extrinsic cardiac ANS. This hypothesis is based on prior studies (20–24) and the electrophysiological evidence shown in the present study. Although we have no direct evidence indicating an inhibition of neuronal firing within the GP, the accumulated indirect evidence supports the inhibitory hypothesis. This hypothesis will have to be verified in the future when the neural activity from the GP of a beating heart can be reliably recorded over 6 to 8 hours.

Clinical implications. A therapeutic modality that can prevent paroxysmal AF to progress to persistent forms may greatly reduce the resultant mortality and morbidity associated with this arrhythmia. Notably, LL-VNS, which was 50% below threshold, was still capable of reducing the AF duration by >80%. The present study introduced a potential therapy that is less invasive than catheter or surgical ablation, and may limit the duration of or even prevent AF thereby reducing the risk of thromboembolic events or cardiomyopathy.

Conclusions

LL-VNS can prevent and reverse the electrophysiological changes of atrial remodeling induced by 6-h RAP as well as suppress AF induced by strong cholinergic stimulation. Inhibition of the intrinsic cardiac ANS by LL-VNS may be responsible for these salutary results. LL-VNS may serve as

a novel therapeutic modality to treat AF related to acute atrial remodeling or a hyperactive state of the intrinsic cardiac ANS.

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