Low-level transcutaneous electrical stimulation of the auricular branch of the vagus nerve: A noninvasive approach to treat the initial phase of atrial fibrillation

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BACKGROUND We studied the effects of transcutaneous electrical stimulation at the tragus, the anterior protuberance of the outer ear, for inhibiting atrial fibrillation (AF).

OBJECTIVE To develop a noninvasive transcutaneous approach to deliver low-level vagal nerve stimulation to the tragus in order to treat cardiac arrhythmias such as AF.

METHODS In 16 pentobarbital anesthetized dogs, multielectrode catheters were attached to pulmonary veins and atria. Three tungsten-coated microelectrodes were inserted into the anterior right ganglionated plexi to record neural activity. Tragus stimulation (20 Hz) in the right ear was accomplished by attaching 2 alligator clips onto the tragus. The voltage slowing the sinus rate or atrioventricular conduction was used as the threshold for setting the low-level tragus stimulation (LL-TS) at 80% below the threshold. At baseline, programmed stimulation determined the effective refractory period (ERP) and the window of vulnerability (WOV), a measure of AF inducibility. For hours 1–3, rapid atrial pacing (RAP) was applied alone, followed by concomitant RAP+LL-TS for hours 4–6 (N = 6). The same parameters were measured during sinus rhythm when RAP stopped after each hour. In 4 other animals, bivagal transection was performed before LL-TS.

RESULTS During hours 1–3 of RAP, there was a progressive and significant decrease in ERP, increase in WOV, and increase in neural activity vs baseline (all $P < .05$). With RAP+LL-TS during hours 4–6, there was a linear return of ERP, WOV, and neural activity toward baseline levels (all $P < .05$, compared to the third-hour values). In 4 dogs, bivagal transection prevented the reversal of ERP and WOV despite 3 hours of RAP+LL-TS.

CONCLUSIONS LL-TS can reverse RAP-induced atrial remodeling and inhibit AF inducibility, suggesting a potential noninvasive treatment of AF.

KEYWORDS Atrial fibrillation; Autonomic nervous system; Transcutaneous stimulation

ABBREVIATIONS **ABVN** = auricular branch of the vagus nerve; **AF** = atrial fibrillation; **ANS** = autonomic nervous system; **ARGP** = anterior right ganglionated plexi; **AV** = atrioventricular; **CANS** = cardiac autonomic nervous system; **ERP** = effective refractory period; **LL-TS** = low-level tragus stimulation; **LL-VNS** = low-level vagal nerve stimulation; **NTS** = nucleus tractus solitarius; **PV** = pulmonary vein; **RAP** = rapid atrial pacing; **TENS** = transcutaneous electrical nerve stimulation; **WOV** = window of vulnerability

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Introduction

In previous studies from our laboratory, we found that low-level vagus nerve stimulation (LL-VNS), at voltages substantially below that which slowed the sinus rate or atrioventricular (AV) conduction, significantly increases the effective refractory period (ERP) in the atria and in the pulmonary vein (PV) myocardium.[1–5] Furthermore, atrial fibrillation (AF) inducibility at these sites was significantly suppressed and AF duration was also shortened substantially. In those experiments, LL-VNS was applied to both vagal trunks dissected in the neck and the vagal preganglionics at the posterior wall of the superior vena cava.[3] Direct neural recordings also indicate that the antiarrhythmic effects of LL-VNS is mediated by suppressing the activity of the intrinsic cardiac autonomic nervous system (CANS).[3]

Several previous reports have documented the effects of transcutaneous electrical stimulation to reduce the amount of anesthetic used during operative procedures,[6] suppress sepsis in a murine model of endotoxemia[7] or elicit evoked potentials

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in the vagal nucleus in the brain in volunteer subjects.[8,9] In some of these reports, stimulation of the auricular branch of the vagus nerve (ABVN) located at the tragus, the anterior protuberance of the outer ear, was capable of affecting neural pathways at a distance.[6,8–10] The purpose of the present study was to develop a noninvasive transcutaneous approach to deliver LL-VNS to the tragus in order to treat cardiac arrhythmias such as AF. We chose right tragus stimulation because the ABVN is easily accessible[8,9] and LL-VNS of the right vagus nerve had the same antiarrhythmic effects as bilateral vagal stimulation.[3,4]

Methods

All animal studies were reviewed and approved by the Institutional Animal Care and Use Committee of the University of Oklahoma Health Sciences Center. Ten adult mongrel dogs, weighing 22–26 kg, were anesthetized with sodium pentobarbital (30 mg/kg), and general anesthesia was maintained by hourly intravenous injection of 50–100 mg. Dogs were intubated and attached to positive pressure ventilation with a mixture of room air and 100% oxygen. The right and left femoral veins were dissected and 8-F sheaths inserted into each vessel to deliver drugs and saline as well as catheter insertion. An electrode catheter was inserted into the left femoral artery and passed into the aortic root to record the His bundle potential. A sensor-controlled heating pad was used under the dog to regulate body temperature at 37.0°C ± 0.5°C.

Initially, a left thoracotomy was performed at the fourth intercostal space and the left atrium and left superior and inferior PVs were exposed by incising and reflecting t he pericardium as previously described.[1–5] Multielectrode catheters were attached to the PVs and left atrial appendage. The pericardium and thoracotomy were then sutured closed. The dog was then turned to the right side and a similar thoracotomy and pericardiotomy allowed exposure of the right atrium and right superior and inferior PVs. Again, multielectrode catheters were attached at these sites.

Tragus stimulation

The stimulation of the tragus in the right ear (Figure 1) was accomplished by attaching 2 alligator clips side by side on the right tragus or by using a light spring loaded plastic clip with electrodes on opposite sides of the inner and outer portions of the tragus. Incremental voltages were applied to the tragus (20 Hz, 1-ms square wave) until slowing of the sinus rate or AV conduction was achieved. The voltage necessary to achieve a slowing of the sinus rate or AV conduction (measured by the AH interval) was used as the threshold for setting the low-level tragus stimulation (LL-TS) in each experiment. In 6 experiments, LL-TS was set at 80% below the voltage required to slow the sinus rate or AV conduction. In 4 other experiments, LL-TS set at 80% below the threshold was delivered to the right tragus after transection of both vagi at the level just below the junction of the innominate vein and superior vena cava. In all experiments,

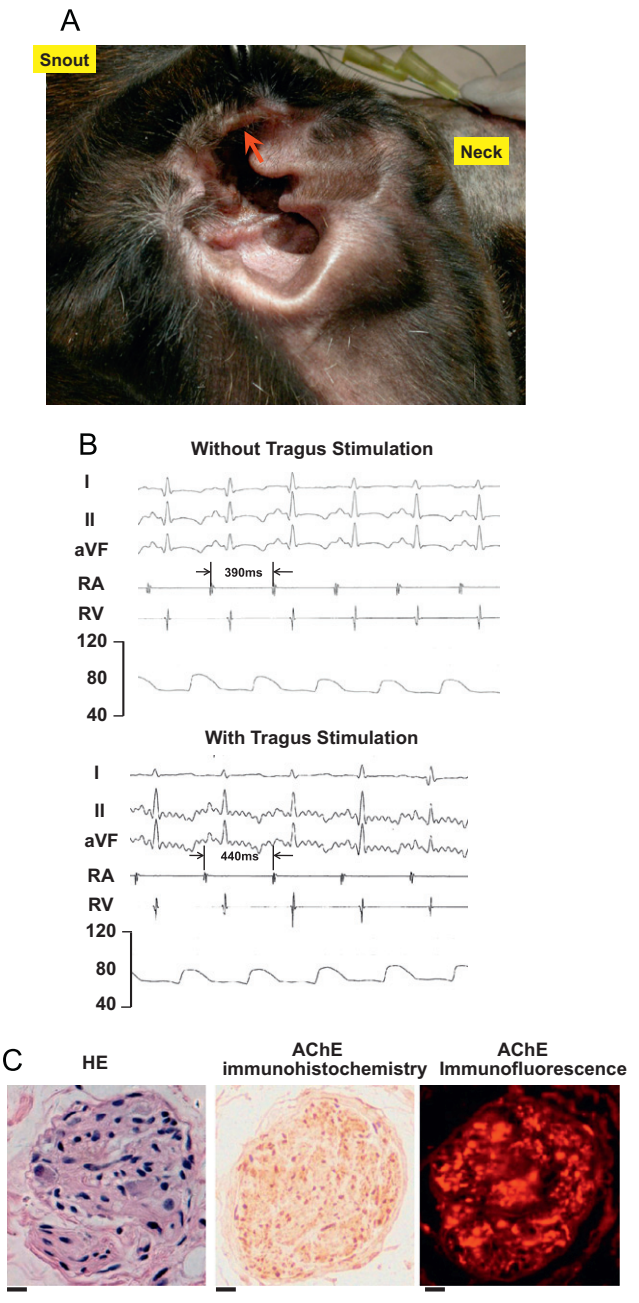

Figure 1 Representative examples of (**A**) the location of the right tragus highlighted by the red arrow; (**B**) suprathreshold tragus stimulation that shortened the sinus rate from 440 to 390 ms, suggesting the activation of the vagus nerve; (**C**) a nerve bundle in the tragus stained with HE (hematoxylin and eosin; left panel) and acetylcholine esterase (AChE; middle and right panels). The dark brown color in the middle panel and the fluorescent spots in the right panel represent sites showing immunoreactivity to AChE.

the stimulation threshold was checked at the end of each hour of rapid atrial pacing (RAP) to ensure that LL-TS was set appropriately.

RAP simulating AF

The left atrial appendage was paced for 6 hours at 1200 beats/min (2× threshold) to induce acute atrial remodeling. After each hour of RAP, pacing was temporarily stopped for 5–10 minutes. After AF terminated and sinus rhythm

resumed, we determined the ERP and AF inducibility. Using programmed stimulation S1–S1 = 330 ms and decremental S1–S2 at 10× diastolic threshold, ERP at atrial and PV sites were determined. The S1–S2 intervals were decreased from 150 ms initially by decrements of 10 ms and then 1 ms when approaching ERP.[5] The difference between the longest and the shortest S1–S2 interval (in ms) at which AF was induced was defined as the window of vulnerability (WOV), which served as a quantitative measurement of AF inducibility. The cumulative WOV ($\sum$WOV) was the sum of WOVs at all sites in each dog.[1–3] ERP dispersion was calculated off-line as the coefficient of variation (standard deviation/mean) of the ERP at all recording sites.[2,5] RAP was performed in the first 3 hours without the application of LL-TS, whereas during the last 3 hours, both RAP and LL-TS were applied simultaneously.

Neural recording

Three tungsten-coated microelectrodes were inserted into the fat pad located at the caudal end of the sinus node containing the anterior right ganglionated plexi (ARGP). The 3 microelectrodes were positioned such that they would contact different areas of the ARGP but would not be displaced by either cardiac or respiratory movement. The 3 microelectrodes were connected by a common lead to a preamplifier (Princeton Applied Research, model 113, Princeton, NJ). Bandpass filters were set between 300 Hz and 10 kHz, with amplification ranging from 100× to 500×. The sampling rate was 1 kHz. Further amplification (50–200×) was obtained by use of a hardwired amplifier (Spike 2, CED, Ltd, Cambridge, England, UK). A 1-minute recording of the ARGP neural activity during sinus rhythm was acquired immediately before LL-TS and after each hour of LL-TS for comparison. The neural activity was characterized by the recorded amplitude and frequency. Neural activity was defined as deflections with a signal-to-noise ratio greater than 3:1 and the amplitude and frequency were manually determined as previously described.[5]

Immunohistochemical staining of the tragus

In 6 animals, the right tragus was excised and 5-μm sections were cut from paraffin blocks of the tragus. The sections were air dried and fixed in acetone for 10 minutes and then washed in Tris-buffered saline. Hydrogen peroxidase block (Dako, Carpenteria, CA) was placed on the sections for 10 minutes, and the slides were washed in Tris-buffered saline. Protein block was placed on the sections for 30 minutes. Primary antibodies were then incubated overnight at 4°C. Antibodies for acetylcholine esterase (Chemicon, Leverkusen, Germany) were used to stain cholinergic nerves. Quantification of the nerve density in the tragus area was assisted by a commercially available software (ImagePro, Media Cybernetics, Inc, Rockville, MD). The nerve density based on the immunoreactivity of each slide was determined by the average of 3 fields with the highest nerve density. The nerve density was expressed as the total area of positive staining per square millimeter ($\mu m^2/mm^2$).

Statistical analysis

Data are expressed as mean ± SD. Repeated measures analysis of variance (ANOVA) was used to examine the effect of each intervention on the respective parameters over time. Post hoc analysis, with the Tukey method to adjust for multiple comparisons, was performed to compare the following parameters measured hourly to the values in the baseline state or the values at the end of the third hour of RAP before the initiation of LL-TS: (a) PV and atrial ERPs (Figures 2 and 3), (b) ERP dispersion and ΣWOV (Figures 4 and 5), and (c) the frequency and amplitude of the neural activity (Figure 6). P values $<.05$ were considered statistical significant.

Results

The average stimulation threshold, which induced any slowing of the sinus rate or AV conduction, was 9.8 ± 2.6 V (N = 10; Figure 1B). In 6 dogs, immunohistochemical studies showed the presence of nerve bundles in the tragus area, which was positive for acetylcholine esterase (Figure 1C). The density of acetylcholine esterase (+) neural elements in the right tragus was 45,658.3 ± 7338.2 $\mu m^2/mm^2$ (N = 6). Figure 2 shows the consistent pattern of a significant decrease in the ERP during the first 3 hours of RAP at all PV and atrial recording sites. With the addition of LL-TS set at 80% below the threshold for the next 3 hours along with RAP, all sites showed a reversal of the ERP decrease and return toward baseline values. In contrast, LL-TS after transection of both vagus nerves failed to reverse the ERP changes (Figure 3). LL-TS did not affect the AF duration or cycle length induced by RAP. After RAP was stopped every hour, AF continued for 28 ± 13, 27 ± 6, and 31 ± 7 seconds after 1, 3, and 6 hours of RAP, respectively (n = 6; $P > .05$). In the presence of LL-TS, the AF duration after RAP was 30 ± 4, 31 ± 6, and 35 ± 2 seconds after 1, 3, and 6 hours of RAP, respectively (n = 6; $P > .05$). The AF cycle after RAP was stopped was 101 ± 11, 101 ± 9, and 99 ± 15 ms after 1, 3, and 6 hours of RAP, respectively (n = 6; $P > .05$). In the presence of LL-TS, the AF cycle length after RAP was 109 ± 6, 103 ± 10, and 106 ± 9 ms after 1, 3, and 6 hours of RAP, respectively (n = 6; $P > .05$).

LL-TS had a similar effect on the dispersion of refractoriness, which increased progressively during the first 3 hours of RAP (Figure 4A). The values at this time were significantly greater than at baseline. With the continued application of RAP+LL-TS for the next 3 hours, there was a reversal of the ERP dispersion toward baseline levels. In contrast, LL-TS after transection of both vagus nerves failed to reverse the changes in ERP dispersion (Figure 4B).

Using the same programmed stimulation protocol to determine ERP curves, the width of $\sum$WOV was determined as a function of the same time periods of 3 hours of RAP and 3 hours of combined RAP+LL-TS. Figure 5A shows the progressive

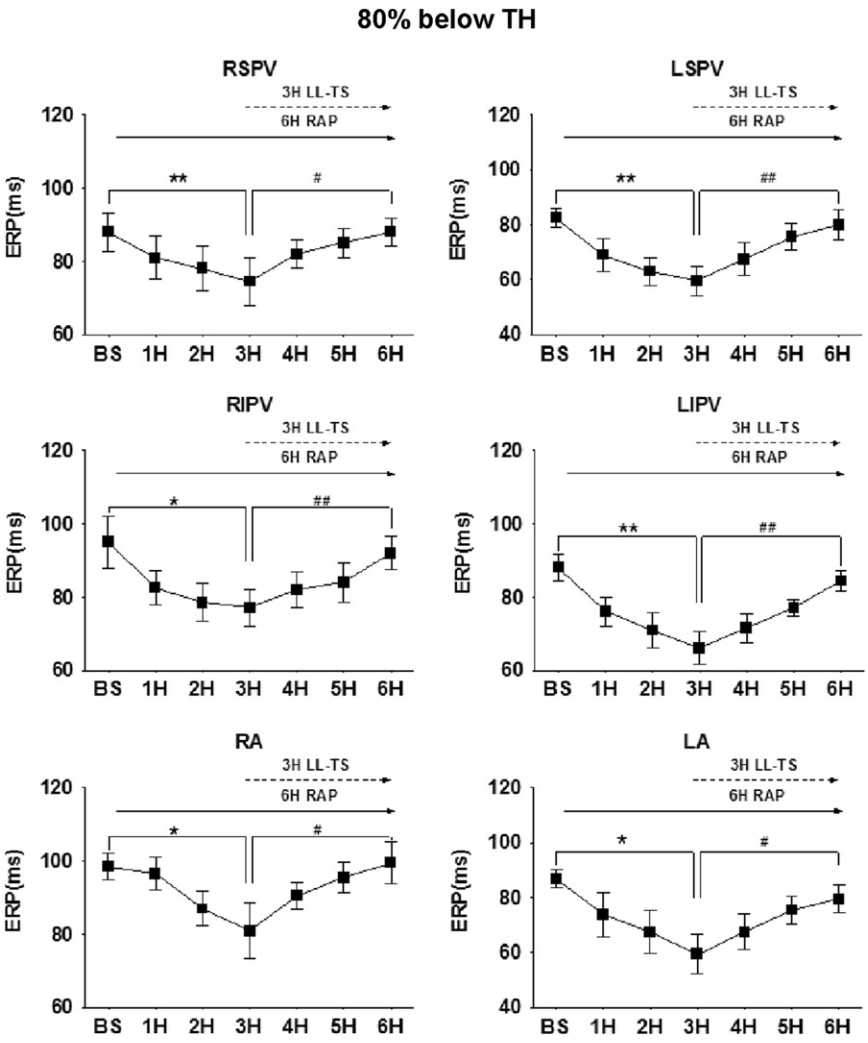

Figure 2 Mean ERP values at PV and atrial sites during 6 hours of RAP. In the last 3 hours, LL-TS set at 80% below the threshold was applied with RAP (N = 6). At all sites, mean ERP decreased significantly after 3 hours of RAP ($^{*}P < .05$, $^{**}P < .01$, compared to baseline). After 3 hours of RAP+LL-TS, mean ERP at all sites showed a significant reversal toward baseline values ($^{\#}P < .05$, $^{\#\#}P < .01$, compared with the end of the third hour of RAP). 3H LL-TS = 3 hours of low-level tragus stimulation; 6H RAP = 6 hours of rapid atrial pacing; ERP = effective refractory period; PV = pulmonary vein; RA and LA = right and left atrium, respectively; RAP = rapid atrial pacing; RSPV, LSPV, RIPV, and LIPV = right superior, left superior, right inferior, and left inferior pulmonary vein, respectively; TH = threshold.

and statistically significant increase in ∑WOV during the first 3 hours and the reversal toward control values during the next 3 hours with the delivery of LL-TS set at 80% below the threshold. Again, LL-TS after transection of both vagus nerves failed to reverse the changes in ∑WOV (Figure 5B).

A typical example of the continuous monitoring of the neural activity recorded from the ARGP for 6 hours of RAP is shown in Figure 6A. Figures 6B and 6C show a progressive increase in the frequency and amplitude of neural firing compared to baseline, which was reversed by LL-TS set at 80% below the threshold introduced during hours 4–6 (n = 6).

Discussion

Major findings

In this report, transcutaneous electrical stimulation of the ABVN at the right tragus was capable of suppressing AF and reversing acute atrial remodeling (eg, shortening of ERP and increase in ERP dispersion) induced by RAP. These salutary effects are likely the result of inhibition of the activity of the intrinsic CANS. Elimination of these effects by transection of both vagus nerves indicates that the vagal efferent fibers are part of the final pathway responsible for the inhibition of the intrinsic CANS.

In the present study, we could not record the vagus nerve activity during LL-TS because of the noise introduced by LL-TS. The antiarrhythmic effects of LL-TS that we attributed to the stimulation of the ABVN thus indirectly supported by the presence of acetylcholine esterase-positive nerve bundles in the tragus. However, the neural connection between the tragus and the atrium was demonstrated by the changes in the neural activity of the ARGP and the associated electrophysiological properties. Three hours of RAP resulted in a progressive and significant increase in AF inducibility as measured by ∑WOV and a concomitant

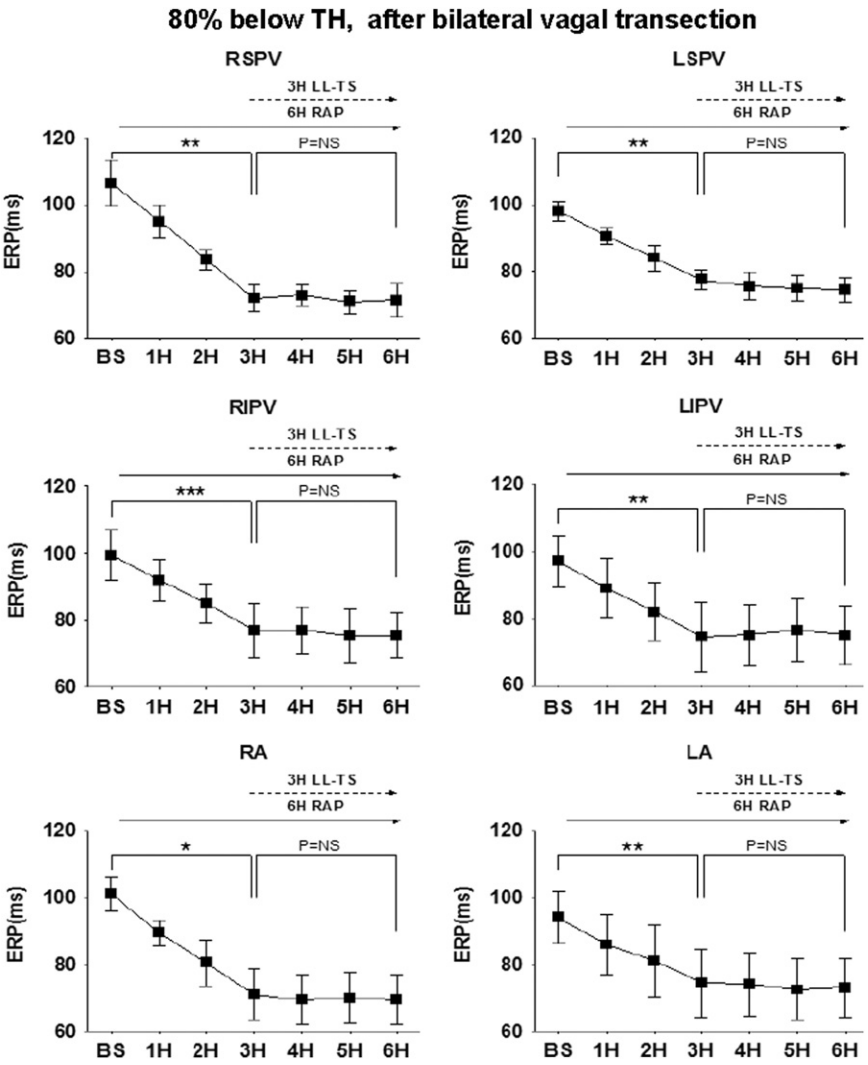

Figure 3 Mean ERP values at PV and atrial recording sites during 6 hours of RAP. In the last 3 hours of RAP, LL-TS was set at 80% below the threshold after transection of both vagus nerves (N = 4). At all sites, mean ERP decreased significantly at 3 hours compared to baseline ($^{*}P < .05$, $^{**}P < .01$, $^{***}P < .001$). LL-TS after vagal transection failed to reverse ERP shortening ($P > .05$, compared with the end of third hour of RAP). All abbreviations as in Figure 2.

significant decrease in the ERP at all tested PV and atrial sites. The initial progressive increase in neural firing was directly associated with these changes. LL-TS during the next 3 hours resulted in a progressive return of ERP and WOV toward baseline values and an associated decrease in neural activity recorded from the ARGP. Notably, the antiarrhythmic effects of LL-TS were eliminated by transection of both vagus nerves at the level of the junction of the innominate vein and the superior vena cava (Figures 3–5), underlying the critical role of the efferent vagal fibers in AF suppression.

Tragus stimulation has been shown to activate the nuclei in the brain. Polak et al[9] showed that vagus somatosensory evoked potentials can be elicited by transcutaneous tragus stimulation at intensities that did not produce perception of pain. Fallgatter et al[8] demonstrated that the stimulation of the tragus area innervated by ABVN-elicited sensory evoked potentials that can be recorded from the scalp overlying the brainstem in human volunteers. These evoked potentials presumably originated from the vagal nuclei in the nucleus tractus solitarius (NTS). In mammals, the ABVN courses through the mastoid canaliculus and then between the internal jugular vein and the bony wall of the jugular foramen through which it reaches the brain stem.[11] Although the anatomy of this nerve had been studied in detail, the physiology of it remains poorly understood. Nomura and Mizuno[12] applied horseradish peroxidase to trace the cranial course of the auricular branch of the vagus nerve and found that the afferent fibers of this nerve terminate mainly in the NTS as well as other brain stem nuclei such as the trigeminal nucleus. It is well known that a large number of autonomic nerve fibers, including the fibers from the heart and lungs, project to the NTS.[10,13,14] The reflex loop formed by the ABVN, NTS, and autonomic nerves of the lungs has been proposed to cause a unique form of cough. This reflex (Arnold's ear-cough reflex) is induced by the stimulation of the posterior or anterior aspect of the inferior wall of the external ear canal. The role of the NTS in this reflex may provide insight into antiarrhythmic effects we observed in our study. It is known that the NTS receives afferent vagal

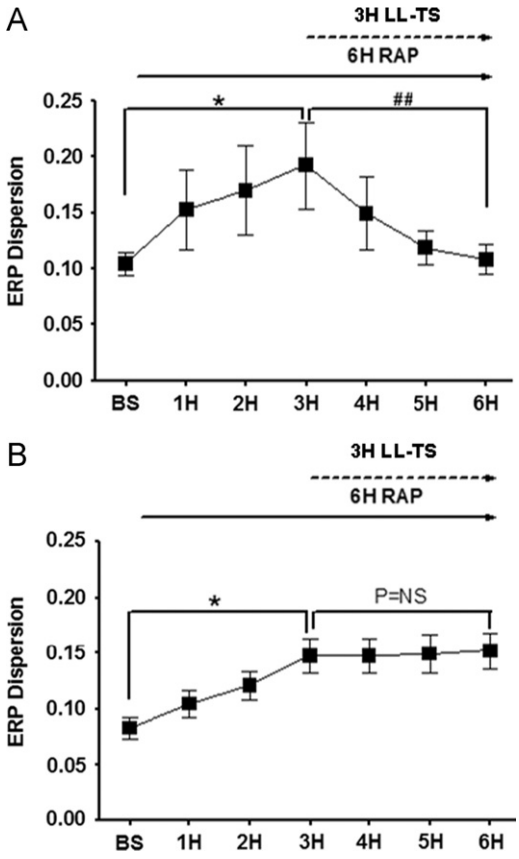

Figure 4 Changes in the dispersion of refractoriness during 6 hours of RAP and the last 3 hours of simultaneous RAP and LL-TS (80% below the threshold). **A:** In the first 3 hours of RAP, ERP dispersion progressively increased but was reversed by LL-TS applied during the last 3 hours (N = 6). **B:** After bilateral vagal transection, LL-TS applied during the last 3 hours failed to reverse the increased ERP dispersion (N = 4). Under both circumstances, the dispersion of refractoriness of the first 3 hours of RAP increased significantly ($^{*}P < .05$, compared to baseline). Then, the values of the fourth to sixth hour of RAP were compared with the end of third hour of RAP ($^{\#\#}P < .01$). Abbreviations as in Figure 2.

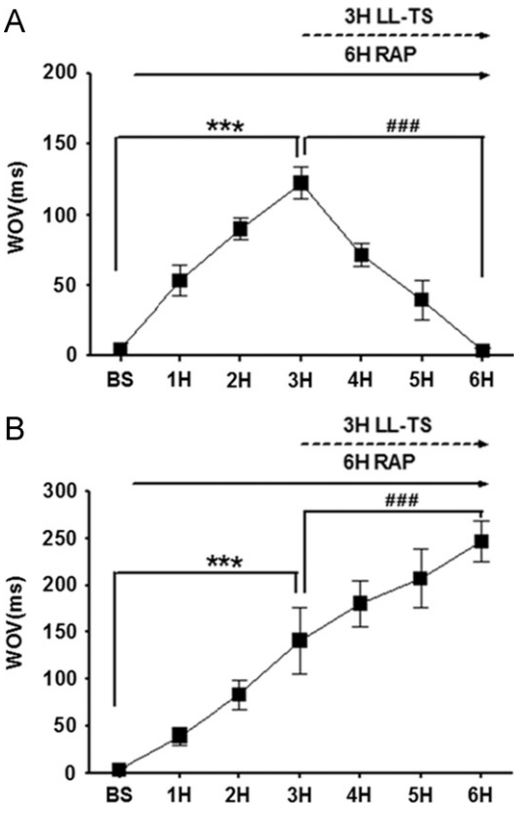

Figure 5 Changes of the cumulative window of vulnerability (∑WOV) for AF during 6 hours of RAP and the last 3 hours of simultaneous RAP+LL-TS (80% below the threshold). **A:** In the first 3 hours of RAP, WOV progressively increased but was reversed by LL-TS applied during the last 3 hours (N = 6). **B:** LL-TS, after bilateral vagal transection, applied during the last 3 hours failed to reverse the increased WOV (N = 4). Under both circumstances, WOV increased significantly in the first 3 hours of RAP ($^{***}P < .001$, compared to baseline). Then, the values of the fourth to sixth hour of RAP were compared to the end of third hour of RAP ($^{\#\#\#}P < .001$). Abbreviations as in Figure 2.

fibers from nearly all the viscera and neurotransmissions from the NTS project to multiple cortical and subcortical areas of the brain as well as the adjacent vasomotor center and the vagal motor nucleus.[8–10,13,14] Since tragus stimulation elicited evoked potential in the brain stem, presumably from the NTS,[8] and transection of both vagus nerves eliminated the antiarrhythmic effects of LL-TS (Figures 3–5), we hypothesize that tragus stimulation activated a series of neurotransmission including the afferent vagal fibers in the ABVN, NTS, other nuclei in the brain participating cardiovascular control and eventually activated the efferent vagal fibers in the vagus nerves.

The frequency and intensity of LL-TS is similar to transcutaneous electrical nerve stimulation (TENS) for pain relief.[13,14] TENS has been shown to decrease the circulating epinephrine level and increase coronary blood flow in patients with coronary artery disease.[15,16] Interestingly, such effects could not be produced in cardiac transplant patients, indicating that intact communication between the brain/spinal cord and the intrinsic cardiac autonomic nervous system (ANS) is required for these effects, similar to the antiarrhythmic effects observed in the present study.[16] Recent reports of the effects of TENS on the ANS have been inconclusive, mainly owing to differences in the stimulation frequency and intensity as well as the location at which TENS was applied among several studies.[14] Despite the controversy, TENS has been shown to alter the balance of the sympathetic and parasympathetic nervous system.[15,17] It is known that vagus nerves contain A, B, and C fibers. Up to 70%–80% of the vagal fibers are unmyelinated C fibers that require higher stimulation strength and lower stimulation frequency (eg, 5 Hz) to activate.[13,18] A direct activation of C fibers typically produces bradycardia. As TENS mainly stimulates A fibers, which in turn suppress the pain transmission mediated by C fibers,[13,14] LL-TS at 20 Hz in the present study most likely activated A fibers in the ABVN and subsequently the NTS. However, we cannot overlook the contribution from C fibers in the vagus nerves since a large proportion of vagal fibers that involve cardiovascular homeostasis are C fibers.

Recent studies identified a series of nonadrenergic, noncholinergic neurotransmitters/neuromodulators in the

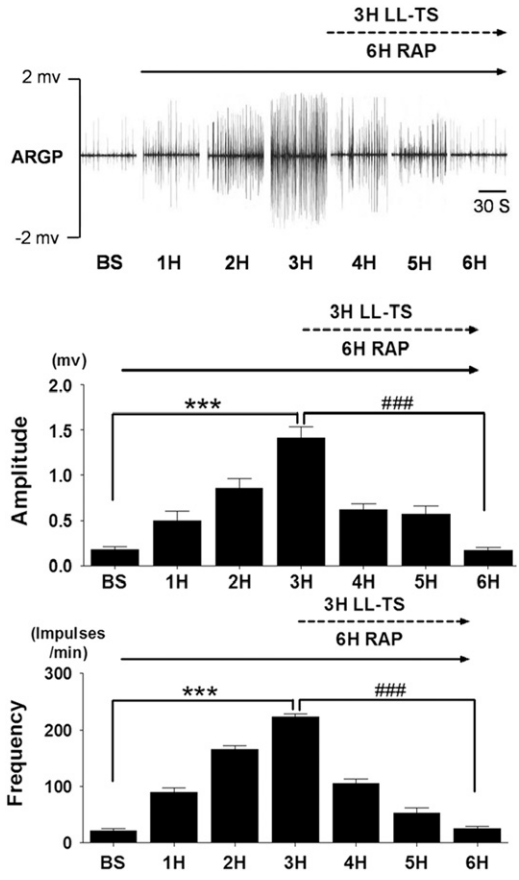

Figure 6 **A:** A typical example of neural recordings from the anterior right ganglionated plexi (ARGP) taken each hour (during sinus rhythm) after 6 hours of RAP. **B, C:** The average amplitude and frequency of neural recordings in 6 animals. During the first 3 hours of RAP, there was a progressive increase in both the amplitude (**B**) and the frequency (**C**) of neural firing in the ARGP. With the addition of LL-TS set at 80% below the threshold, the amplitude and frequency returned toward the initial levels (N = 6). Abbreviations as in Figure 2.

ganglionated plexi.[19–22] Liang et al[20] and Miserez et al[22] applied electrical stimulation to the autonomic nerves in the spleen and found the local release of vasostatin and its precursor chromogranin A. Vasostatin-1 has been shown to have strong antiadrenergic effects without modulating the adrenergic receptors.[19,21] We recently reported that an injection of vasostatin-1 (1–33 nM) into the major atrial ganglionated plexi resulted in effects on the ERP, WOV, and AF duration similar to those of LL-VNS, suggesting that vasostatin-1 may be one of the neurotransmitters/neuromodulators responsible for the antiarrhythmic effects of LL-VNS.[23] An injection of L-NAME (a nitric oxide synthase inhibitor) or wortmannin (a phosphatidylinositol-3 kinase inhibitor) into the anterior right ganglionated plexi and inferior right ganglionated plexi markedly inhibited the antiarrhythmic effects of LL-VNS, indicating that LL-VNS is mediated, at least in part, by the nitric oxide/phosphatidylinositol-3 kinase signaling pathway.[24] Future studies exploring the neurotransmitters that are either anticholinergic or antiadrenergic and their associated signal transduction pathways may elucidate the mechanism underlying the antiarrhythmic actions of LL-VNS and LL-TS.

Clinical implications

Recent reports on the long-term success of catheter ablation for paroxysmal AF showed that despite a significant complication rate, the success rate was lower than 50% after 5 years of follow-up.[25,26] The number of patients with AF in the United States is anticipated to increase to 9.4–11.7 millions in year 2030, many of whom will have drug-refractory AF.[27] A less invasive therapy has to be developed to treat such a large population of patients with AF. LL-TS presented in this study was designed to treat AF shortly after its initiation. If this approach works in patients as well, such a noninvasive treatment can be initiated shortly after AF onset and may prevent AF from progressing to more persistent forms.

The human skin impedance is in the kΩ–MΩ range but greatly depends on the method of impedance measurement, skin-electrode interface, electrode size, moisture of the skin, and the psychological condition of the patients.[28] The average current delivered for TENS therapy is 10–50 mA.[14–16] In the present study, the average threshold voltage was 9.8 ± 2.6 V and the minimal effective voltage for LL-TS is likely even lower than 80% below the threshold, suggesting that the strength of LL-TS may be below the pain threshold of TENS. However, this has to be proven by studies done in awake and ambulatory dogs.

Study limitations

The afferent vagal nerve fibers innervating the tragus area enter the main vagal trunk through the jugular ganglion at the level of the base of the skull. We therefore did not attempt to transect the vagal trunk to eliminate the afferent vagal fibers between the tragus and the brain stem. Whether the effects of LL-TS involve the afferent vagal fibers and brain stem cannot be verified by the present study. Since up to 90% of the vagal fibers are afferent fibers, we hypothesize that LL-TS may activate the vagal afferent fibers and that the neural inputs are subsequently processed at the sensory and motor vagal nuclei in the brain stem. The final inhibitory neural inputs to the intrinsic cardiac ANS are then carried by efferent vagal fibers.

We did not randomly choose a cutaneous site for high-frequency stimulation to serve as a control. Skin is richly innervated by the ANS that modulates the sweat gland secretion, blood flow, and pilomotor activity. It would be nearly impossible to find a cutaneous site lacking autonomic innervation.

In this study, all experiments were conducted under pentobarbital anesthesia, which is known to alter the autonomic tone. It raises the concern as to whether LL-TS would work in awake animals. Since all interventions were compared in the presence of similar background autonomic tone, it is unlikely that anesthesia plays a meaningful role in the effects of LL-TS. To support this claim, a recent report

from Shen et al[29] showed that chronic LL-VNS markedly suppresses AF and tachycardia in awake, ambulatory animals.

Conclusions

We demonstrated the antiarrhythmic effects of a novel, noninvasive approach that combines 2 promising therapies—TENS and LL-VNS—both of which have been shown to provide clinical benefits to patients with various diseases such as intractable pain, epilepsy, and depression. Our study suggests that LL-TS can modulate the interplay between the extrinsic autonomic innervation from the brain/spinal cord and the intrinsic neurons on the heart itself. LL-TS may open a new avenue for noninvasively treating various cardiac arrhythmias (eg, AF).

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References

1. Li S, Scherlag BJ, Yu L, et al. Low-level vagosympathetic stimulation: a paradox and potential new modality for the treatment of focal atrial fibrillation. Circ Arrhythm Electrophysiol 2009;2:645–651.
2. Sheng X, Scherlag BJ, Yu L, et al. Prevention and reversal of atrial fibrillation inducibility and autonomic remodeling by low-level vagosympathetic nerve stimulation. J Am Coll Cardiol 2011;57:563–571.
3. Yu L, Scherlag BJ, Sha Y, et al. Interactions between atrial electrical remodeling and autonomic remodeling: how to break the vicious cycle. Heart Rhythm 2012;9:804–809.
4. Sha Y, Scherlag BJ, Yu L, et al. Low-level right vagal stimulation: anticholinergic and antiadrenergic effects. J Cardiovasc Electrophysiol 2011;22:1147–1153.
5. Yu L, Scherlag BJ, Li S, et al. Low-level vagosympathetic nerve stimulation inhibits atrial fibrillation inducibility: direct evidence by neural recordings from intrinsic cardiac ganglia. J Cardiovasc Electrophysiol 2011;22:455–463.
6. Greif R, Laciny S, Mokhtarani M, et al. Transcutaneous electrical stimulation of an auricular acupuncture point decreases anesthetic requirement. Anesthesiology 2002;96:306–312.
7. Huston JM, Gallowitsch-Puerta M, et al. Transcutaneous vagus nerve stimulation reduces serum high mobility group box 1 levels and improves survival in murine sepsis. Crit Care Med 2007;35:2762–2768.
8. Fallgatter AJ, Neuhauser B, Herrmann MJ, et al. Far field potentials from the brain stem after transcutaneous vagus nerve stimulation. J Neural Transm 2003;110:1437–1443.
9. Polak T, Markulin F, Ehlis AC, Langer JB, Ringel TM, Fallgatter AJ. Far field potentials from brain stem after transcutaneous vagus nerve stimulation: optimization of stimulation and recording parameters. J Neural Transm 2009;116:1237–1242.
10. Dietrich S. A novel transcutaneous vagus nerve stimulation leads to brainstem and cerebral activations measured by functional MRI. Biomed Eng 2001;53:104–111.
11. Tekdemir I, Aslan A, Elhan A. A clinico-anatomic study of the auricular branch of the vagus nerve and Arnold's ear-cough reflex. Surg Radiol Anat 1998;20:253–257.
12. Nomura S, Mizuno N. Central distribution of primary afferent fibers in the Arnold's nerve (the auricular branch of the vagus nerve): a transganglionic HRP study in the cat. Brain Res 1984;292:199–205.
13. Groves DA, Brown VJ. Vagal nerve stimulation: a review of its applications and potential mechanisms that mediate its clinical effects. Neurosci Biobehav Rev 2005;29:493–500.
14. Sluka KA, Walsh D. Transcutaneous electrical nerve stimulation: basic science mechanisms and clinical effectiveness. J Pain 2003;4:109–121.
15. Chauhan A, Mullins PA, Thuraisingham SI, Taylor G, Petch MC, Schofield PM. Effect of transcutaneous electrical nerve stimulation on coronary blood flow. Circulation 1994;89:694–702.
16. Mannheimer C, Carlsson CA, Emanuelsson H, Vedin A, Waagstein F, Wilhelmsson C. The effects of transcutaneous electrical nerve stimulation in patients with severe angina pectoris. Circulation 1985;71:308–316.
17. Stein C, Dal Lago P, Ferreira JB, Casali KR, Plentz RD. Transcutaneous electrical nerve stimulation at different frequencies on heart rate variability in healthy subjects. Auton Neurosci 2011;165:205–208.
18. Castoro MA, Yoo PB, Hincapie JG, et al. Excitation properties of the right cervical vagus nerve in adult dogs. Exp Neurol 2011;227:62–68.
19. Corti A, Mannarino C, Mazza R, Angelone T, Longhi R, Tota B, Chromogranin A. N-terminal fragments vasostatin-1 and the synthetic CGA 7-57 peptide act as cardiostatins on the isolated working frog heart. Gen Comp Endocrinol 2004;136:217–224.
20. Liang F, Dillen L, Zhang XY, et al. Vasostatins, N-terminal products of chromogranin A, are released from the stimulated calf spleen in vitro. Acta Physiol Scand 1995;155:23–30.
21. Pieroni M, Corti A, Tota B, et al. Myocardial production of chromogranin A in human heart: a new regulatory peptide of cardiac function. Eur Heart J 2007;28:1117–1127.
22. Miserez B, Annaert W, Dillen L, Aunis D, De Potter W. Chromogranin A processing in sympathetic neurons and release of chromogranin A fragments from sheep spleen. FEBS Lett 1992;314:122–124.
23. Stavrakis S, Scherlag B, Fan Y, et al. Antiarrhythmic effects of vasostatin-1 in a canine model of atrial fibrillation. J Cardiovasc Electrophysiol 2012;23:771–777.
24. Stavrakis S, Scherlag BJ, Fan Y, et al. Inhibition of atrial fibrillation by low level vagus nerve stimulation: the role of nitric oxide signaling pathway. J Intervent Cariovasc Electrophysiol. 2013, in press.
25. Ouyang F, Tilz R, Chun J, et al. Long-term results of catheter ablation in paroxysmal atrial fibrillation: lessons from a 5-year follow-up. Circulation 2010;122:2368–2377.
26. Weerasooriya R, Khairy P, Litalien J, et al. Catheter ablation for atrial fibrillation: are results maintained at 5 years of follow-up? J Am Coll Cardiol 2011;57:160–166.
27. Miyasaka Y, Barnes ME, Gersh BJ, et al. Secular trends in incidence of atrial fibrillation in Olmsted County, Minnesota, 1980 to 2000, and implications on the projections for future prevalence. Circulation 2006;114:119–125.
28. Pearson S, Colbert AP, McNames J, Baumgartner M, Hammerschlag R. Electrical skin impedance at acupuncture points. J Altern Complement Med 2007;13:409–418.
29. Shen MJ, Shinohara T, Park HW, et al. Continuous low-level vagus nerve stimulation reduces stellate ganglion nerve activity and paroxysmal atrial tachyarrhythmias in ambulatory canines. Circulation 2011;123:2204–2212.