Low-Level Electrical Stimulation of Aortic Root Ventricular Ganglionated Plexi Attenuates Autonomic Nervous System–Mediated Atrial Fibrillation

Hong-Tao Wang, PhD, MD,* Ming Xu, PhD, MD, Boyuan Fan, MD,* Xiong-Tao Liu, PhD, MD,* Fei-Fei Su, PhD, MD,* Di Zeng, PhD, MD,* Jun Ren, PhD, MD,† Qiang-Sun Zheng, PhD, MD*

ABSTRACT

OBJECTIVES This study investigated the effect of electrical stimulation of aortic root ventricular ganglionated plexi (GP) on atrial fibrillation (AF) inducibility.

BACKGROUND The ventricular GP are interconnected with atrial GP to govern heart function, although the effect of ventricular GP modification on control of AF remains unknown.

METHODS Effective refractory periods (ERPs) of test pulmonary veins (PVs) were measured at baseline and during high-level (HL-ES) and low-level (LL-ES) electrical stimulation of the aortic root GP. The arrhythmogenic threshold of acetylcholine and isoproterenol was determined at baseline and during HL-ES and LL-ES. Moreover, AF was induced at PVs by programmed electrical stimulation after HL-ES or LL-ES. Immunohistochemistry staining was performed to examine the autonomic activity from aortic root GP to the PVs.

RESULTS Compared with the baseline group, HL-ES of aortic root GP significantly shortened atrial ERP (95 ± 13 ms vs. 122 ± 9 ms) and PV ERP (104 ± 11 ms vs. 131 ± 12 ms); decreased the threshold concentration of AF by both acetylcholine (1.3 ± 0.2 μmol/l vs. 3.2 ± 0.3 μmol/l) and isoproterenol (0.3 ± 0.1 μmol/l vs. 1.3 ± 0.2 μmol/l); and increased the AF-inducing rate from PVs (90% vs. 30%). In contrast, LL-ES of the GP prevented the shortening of ERP and PV ERP to 125 ± 10 ms and 133 ± 11 ms, respectively; increased threshold levels of acetylcholine and isoproterenol to 5.7 ± 0.4 μmol/l and 3.2 ± 0.3 μmol/l; and decreased the AF-inducing rate to 5%. We also found that the biotinylated dextran amine–containing varicose fibers projected directly from the aortic root GP to the left PVs.

CONCLUSIONS These findings suggest that autonomic innervations of left PVs partly originated from aortic root ventricular GP. Moreover, LL-ES of aortic root ventricular GP suppressed AF inducibility and arose from PVs mediated by the autonomic nervous system. (J Am Coll Cardiol EP 2015;1:390–7) © 2015 by the American College of Cardiology Foundation.
Nonetheless, our recent study found that stimulation of aortic root ventricular GP provoked robust AF in the absence of extrinsic cardiac nerve activity using an isolated perfused heart model (6). This finding suggests that ventricular GP, which are thought to mainly govern the function of the ventricle and/or the initiation of AF with the exception of atrial GP.

Yu et al. (8) found that long-term, low-level vagosympathetic stimulation suppressed AF inducibility by inhibiting the neural activity of the major atrial GP within the intrinsic cardiac autonomic nervous system. However, the precise intrinsic mechanism of low-level electrical stimulation (LL-ES) of the aortic root GP on AF control remains unknown. In an effort to investigate the effect aortic root GP LL-ES has on AF inducibility, we provoked AF by using the perfusion of acetylcholine or isoproterenol during LL-ES of aortic root GP and then initiated AF again from PVs by using programmed electrical stimulation.

METHODS

ANIMAL PREPARATION. All animal protocols were reviewed and approved by the Institutional Animal Care and Use Committee of the Fourth Military Medical University (Xi’an, China). Twenty mongrel dogs of either sex, weighing 16 to 23 kg, were anesthetized with a 0.1-ml/kg intramuscular injection of Sumianxin (a mixed anesthetic including xylidino-thiazoline 2,4-xylazole, ethylenediaminetetraacetic acid, dihydroetorphine hydrochloride, and haloperidol; Military Veterinary Institute, Changchun, China). If necessary, extra Sumianxin was given to maintain anesthesia during the duration of the study. Dogs were ventilated with room air by a constant volume-cycled respirator (model DDH-1, No. 3529, Factory of the People’s Republic of Army, Tianjin, China). Bilateral vagosympathetic nerves were isolated and cut off to avoid excitation from cervical vagosympathetic trunks. The right femoral vein was cannulated and infused with saline at 100 to 200 ml/h to replenish possible spontaneous fluid loss. Lead II on the electrocardiogram was monitored throughout the duration of the study. After thoracotomy, the pericardium was opened and sutured to the chest wall to cradle the heart. Multielectrode catheters (Biosense Webster, Diamond Bar, California) were attached to the left superior PVs.

AORTIC ROOT GP STIMULATION. The aortic root GP are embedded in adipose tissues surrounding the root of the aorta and connected by the mesangial ligament (Figure 1), which is very large and circled around the aortic root (Figure 2). To stimulate the neurons in the aortic root GP, a custom electrode with 8 metal electrode heads (Henan Huanan Medical Science and Technology Co., Zhengzhou, China) was placed tightly on the surface of the aortic root GP. High-frequency electrical stimulation (HL-ES) was defined as a stimulation frequency of 20 Hz, a pulse duration of 0.1 ms, a train duration of 50 ms, and a voltage of 0.6 to 2.4 V (programming stimulator model 5329, Astro Med Inc., West Warwick, Massachusetts). The lowest voltage level that induced any slowing of sinus rate or atrial ventricular conduction (measured by the A-H interval) was considered as the threshold. A voltage at approximately 10% below the threshold was chosen for LL-ES. During LL-ES, the sinus rate and A-H interval were monitored to ensure that the stimulation voltage was below the threshold (8).

MEASUREMENT OF PV EFFECTIVE REFRACTORY PERIODS, ATRIAL EFFECTIVE REFRACTORY PERIODS, AND SINUS CYCLE LENGTH. PV effective refractory periods (ERPs) and atrial ERPs were measured at baseline and during HL-ES or LL-ES. They were recorded by using the extrastimulus technique (basic cycle length 400 ms; final extra stimulus steps 5 ms; 64-channel electrophysiological recorder, Henan Huanan Medical Science and Technology Co.). The longest coupling interval that did not capture the atrium was defined as an atrial ERP. Atrial ERPs were measured at the left atrial appendage, the free wall of the left atrium, the right atrial

ABBREVIATIONS AND ACRONYMS

AF = atrial fibrillation
BDA = biotinylated dextran amine
ERP = effective refractory period
GP = ganglionated plexi
HL-ES = high-level electrical stimulation
LL-ES = low-level electrical stimulation
PV = pulmonary vein

FIGURE 1 Location of the Aortic Root GP (Frontal View)

The aortic root ganglionated plexus (GP) surround the aortic root and connect with the aorta by the mesangial ligament (ML). LV = left ventricle.
appendage, and the free wall of the right atrium, driven by a 2-ms rectangular stimulus twice the diastolic threshold. PV ERPs were measured at the proximal portion of the left superior PV, the left inferior PV, the right superior PV, and the right inferior PV by using the same aforementioned protocols. Sinus cycle length was also measured at baseline, during the aortic root GP stimulation, and after ablation.

**MEASUREMENT OF THE ARRHYTHMOGENIC THRESHOLD OF ACETYLCHOLINE AND ISOPROTERENOL.** The AF induction threshold of acetylcholine and isoproterenol was measured at baseline and during HL-ES and LL-ES of the aortic root GP. Incremental acetylcholine concentrations (1, 3, 6, and 10 μmol/l) were perfused at a flow rate of 9 ml/min with 5- to 10-min intervals between perfusions. Perfusion was terminated at 1 min if no AF occurred or immediately after the onset of AF. Isoproterenol was perfused by incremental concentrations (0.1, 0.5, 1, 2, and 4 μmol/l) at a flow rate of 9 ml/min for 1 min and 10 to 25 min for restoration. The threshold (minimum) of arrhythmogenic concentration of acetylcholine or isoproterenol was determined in triplicate for drug induction (9). Acetylcholine and isoproterenol were perfused through the femoral vein.

**INITIATION OF AF FROM LEFT SUPERIOR PV BY PROGRAMMED ELECTRICAL STIMULATION.** AF was induced at baseline and after HL-ES and LL-ES of the aortic root GP by using a programmed electrical stimulation of the left superior PVs sequentially at basic cycle lengths of 400 and 300 ms with up to 2 extra stimuli (S3). All stimuli were monitored on a 64-channel electrophysiological recorder (Henan Huanan Medical Science and Technology Co.). The first extra stimulus (S2) was introduced with the S1 to S2 interval at 30 ms longer than the atrial ERP. The coupling interval was reduced in 10-ms decrements. If S1 to S2 extra stimuli failed to induce arrhythmia, a second extra stimulus (S3) was introduced while the S1 to S2 interval was set at 80% of the basic cycle length (400 or 300 ms), introducing the extra stimulus with 10-ms scanning decrements. Sustained AF was defined as irregularly repetitive atrial responses lasting >20 s (10). The percentage of hearts (N = 20) in which AF was induced by programmed electrical stimulation was calculated. A 10-min recovery period was allowed between the spontaneous termination of AF and the next pacing sequence (11).

**AUTONOMIC INNERVATION PATHWAY FROM THE AORTIC ROOT GP TO THE PVS.** Tissue preparation. Biotinylated dextran amine (BDA) anterograde tracer has been shown to be a highly efficient and powerful marker for bidirectional tracing of nerve pathways in a wide variety of species. It was used to examine possible nerve fiber connections between aortic root GP and PVs (12). Five additional adult mongrel dogs were anesthetized using the same method described earlier.
After thoracotomy, the pericardium was dissected to expose the aortic root GP, and BDA was then injected into the root of the aorta. Moreover, the dogs were kept alive for 7 days to enable BDA transportation to the PVs and the atrium. Then the dogs were sacrificed, transaortically perfused, and fixed with 4% paraformaldehyde. Both atria and the 4 PVs were cut into 10-μm-thick slices on a cryostat. The series of sections were then processed for BDA, tyrosine hydroxylase, and choline acetyltransferase triple immunofluorescence histochemistry testing. The fluorescein isothiocyanate–conjugated mouse anti-tyrosine hydroxylase (1:500 dilution, Abcam International, Inc., Cambridge, Massachusetts) antibody was used to label adrenergic nerves. The rhodamine-conjugated goat anticholine acetyltransferase (1:100 dilution, Millipore, Billerica, Massachusetts) antibody was used to label cholinergic nerves. Avidin-conjugated Cy5 (1:50 dilution, Southern Biotechnology Associates, Birmingham, Alabama) was used to label BDA for the nerve fiber connection. The triple-labeled slides were examined under a laser-scanning confocal microscope (Olympus Optical Co., Tokyo, Japan).

**Statistical Analysis.** All data are expressed as mean ± SD or percentages. Atrial ERP, PV ERP, and threshold concentrations were compared by using the paired t test and then measured again by analysis of variance (with Bonferroni confidence interval adjustment) tests. The chi-square test was used to evaluate the inducibility of AF. Statistical significance was defined as p < 0.05.

**Results**

**Effect of Different Levels of Electrical Stimulation of Aortic Root GP on Atria, PV Electrophysiological Properties, and Sinus Cycle Length.** A 10-min HL-ES of aortic root GP altered the atrial electrophysiological properties. Compared with baseline values, the ERPs of the left atrial and left PVs were significantly prolonged during HL-ES GP stimulation (p < 0.05 vs. baseline of HL-ES group, n = 20) (Table 1), whereas the ERPs of the right atrium and the right PVs displayed few obvious changes (p > 0.05 vs. baseline of HL-ES, n = 20) (Table 2). Sinus cycle length was prolonged from 400 ± 98 ms at baseline to 527 ± 98 ms; it recovered within seconds (p > 0.05 vs. baseline of HL-ES group, n = 20) (Table 1).

Similar to an earlier study (8), 1 h of LL-ES of the aortic root GP triggered a tendency for prolonged ERP not only in atria but also in PVs, although no statistical significance was attained (p > 0.05 vs. baseline of LL-ES, n = 20) (Tables 1 and 2). Moreover, LL-ES did not overtly affect sinus cycle length (p > 0.05 vs. baseline of LL-ES, n = 20).

**Effects of Different Levels of Aortic Root GP Stimulation on the Arrhythmogenic Threshold of Acetylcholine and Isoproterenol.** All 20 dogs were induced with AF after drug perfusion concentrations were increased. However, 10-min HL-ES of the aortic root GP significantly decreased the arrhythmogenic thresholds of acetylcholine and isoproterenol compared with those of the baseline group (p < 0.01 vs. baseline of HL-ES, n = 20) (Table 3). In contrast, the arrhythmogenic thresholds of acetylcholine and isoproterenol increased significantly after 1 h of LL-ES compared with those of the baseline group (p < 0.01 vs. baseline of LL-ES, n = 20).

**Effect of Different Levels of Electrical Stimulation of Aortic Root GP Stimulation on AF Inducibility.** After the arrhythmogenic threshold test of acetylcholine and isoproterenol, AF was induced from left superior PVs by programmed stimulation at baseline, after 10 min of HL-ES, and after 1 h of LL-ES. AF was initiated in 6 hearts (30% inducing rate) (Table 4) at baseline. HL-ES elicited abrupt AF episodes in 18 hearts (90% rate, p < 0.05) (Figure 3, Table 4), although LL-ES

### Table 2: Electrophysiological Properties of Pulmonary Veins During Low- or High-Level Aortic Root GP Electrical Stimulation

<table>
<thead>
<tr>
<th></th>
<th>Electrocardiographic Properties of Pulmonary Veins, ms</th>
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<tbody>
<tr>
<td></td>
<td>RAA</td>
</tr>
<tr>
<td>BS of HL-ES</td>
<td>121 ± 11</td>
</tr>
<tr>
<td>HL-ES</td>
<td>111 ± 7</td>
</tr>
<tr>
<td>BS of LL-ES</td>
<td>117 ± 12</td>
</tr>
<tr>
<td>LL-ES</td>
<td>122 ± 10</td>
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</tbody>
</table>

Values are mean ± SD. N = 20. RAA = right atrial appendage; RAF = free wall of right atria; RIPV = right inferior pulmonary vein; RSPV = right superior pulmonary vein; other abbreviations as in Table 1.

### Table 3: Differentiated Effects of Low- or High-Level Aortic Root GP Electrical Stimulation on the Arrhythmogenic Threshold Concentration of Ach and IPA

<table>
<thead>
<tr>
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<th>Threshold Concentration, μmol/l</th>
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<tbody>
<tr>
<td></td>
<td>Ach</td>
</tr>
<tr>
<td>BS of HL-ES</td>
<td>3.0 ± 0.4</td>
</tr>
<tr>
<td>HL-ES</td>
<td>1.3 ± 0.2*</td>
</tr>
<tr>
<td>BS of LL-ES</td>
<td>3.3 ± 0.2</td>
</tr>
<tr>
<td>LL-ES</td>
<td>5.7 ± 0.4*</td>
</tr>
</tbody>
</table>

Values are mean ± SD. N = 20. *p < 0.05 versus BS. Ach = acetylcholine; IPA = isoproterenol; other abbreviations as in Table 1.
DIRECT AUTONOMIC INNERVATION FROM AORTIC ROOT GP TO THE LEFT PVs. The BDA anterograde tracer in aortic root GP showed that neurons were scattered among adipose cells and contained adrenergic and cholinergic neurons (Figure 4). Triple immunostaining of tyrosine hydroxylase, choline acetyltransferase, and BDA staining showed that BDA-containing varicose fibers projected directly to the left PVs (Figure 5A), whereas tyrosine hydroxylase (Figure 5B) and choline acetyltransferase–labeled (Figure 5C) axons and varicosities were observed within the same traced varicose fibers. However, the BDA-containing varicose fibers were not observed in the right PVs (Figures 5D to 5G).

DISCUSSION

The salient finding of the present study is that LL-ES of aortic root GP decreased AF inducibility that arose from left PVs by reducing vulnerability to the intrinsic autonomic nervous system.

Previous evidence suggested that autonomic activity led to the local release of both cholinergic and adrenergic neurotransmitters and facilitated the initiation of AF (13). Recently, nonpharmacological, nonablative approaches (especially LL-ES) have been used in the treatment of AF (14). In our study, long-term LL-ES of aortic root GP suppressed AF that arose from left PVs. The mechanism was partly revealed by our histochemistry and immunohistochemistry studies, which showed that autonomic innervation of left PVs directly originated from aortic root GP. Moreover, long-term LL-ES of the aortic root GP changed the electrophysiology of atria and PVs in an antiarrhythmic manner to attenuate the arrhythmogenic threshold of intrinsic cholinergic and adrenergic neurotransmitters. As a result, it is hard to induce AF by the programmed stimulation from PVs. The mechanism involved might be related to the long-term LL-ES–depressed efficacy of synaptic transmission associated with cognitive functions in the central nervous system (15,16). Therefore, the arrhythmogenic effect of

| TABLE 4 Effect of Aortic Root GP Stimulation on AF Inducibility |
|-------------------|-----------------|-----------|
|                   | No. of AF Induced | %         |
| Baseline          | 6               | 30        |
| HL-ES             | 18              | 90*       |
| LL-ES             | 1               | 5*        |

N = 20. Programmed stimulation was performed to provoke atrial fibrillation (AF) from LSPV during Ach or IPA perfusion. % refers to the percentage of AF. *p < 0.05 versus BS. Abbreviations as in Tables 1 and 3.

FIGURE 3 Initiation of AF After HL-ES Aortic Root GP

(A) 10-minute high-frequency electrical stimulation (HL-ES) (frequency 20 Hz; a pulse duration 0.1 ms; a train duration 50 ms; and voltage 0.6 to 2.4 V) of the aortic root ganglionated plexi (GP) was performed first to increase the vulnerability of atrial fibrillation (AF). (B) Programmed electrical stimulation (basic cycle lengths of 400 and 300 ms with up to 2 extra stimuli; the coupling interval was reduced in 10-ms decrements) was used to provoke AF after HL-ES; AF was initiated when dropped to 140 ms.
acetylcholine and isoproterenol was attenuated. Hence, the arrhythmogenic threshold of both acetylcholine and isoproterenol increased, and AF episodes decreased during LL-ES. We found that LL-ES of aortic root GP decreased AF vulnerability mediated by the autonomic nervous system.

LL-ES of vagosympathetic nerves was recently proven to be effective in the control of AF. Li et al. (17) reported that LL-ES of vagosympathetic nerves prevented or reversed rapid PV and non-PV firing. Sheng et al. (18) reported that LL-ES of vagosympathetic nerves prevented or reversed rapid atrial pacing-induced atrial remodeling and suppressed cholinergic stimulation-induced AF. These studies speculated that LL-ES elicited its antiarrhythmic effect by inhibition of the intrinsic cardiac autonomic nervous system. Our study confirmed this notion and further revealed that LL-ES of aortic root GP exhibited antiarrhythmic effects similar to those of LL-ES vagosympathetic nerves.

**FIGURE 4** Neuron Cells in the Aortic Root GP

Biotinylated dextran amine (BDA)-combined neuron cells were scattered among adipose cells and labeled by avidin-conjugated CY5 (A, white arrows). Choline acetyltransferase and tyrosine hydroxylase immunoreactivities were visualized with rhodamine (B, white arrows) and fluorescein isothiocyanate (C, white arrows) antibodies, respectively.

**FIGURE 5** Autonomic Nerve Fibers Innervations of the PVs

Fluorescence photomicrographs showed BDA-labeled autonomic fibers projected from the aortic root GP to the left pulmonary veins (PVs). BDA, choline acetyltransferase, and tyrosine hydroxylase immunoreactivities were visualized with avidin-conjugated CY5 (A, blue; white bars), rhodamine (B, red; white bars), and fluorescein isothiocyanate (C, green; white bars) antibodies, respectively. The overlaid digital image (D) shows a triple stain of choline acetyltransferase, tyrosine hydroxylase, and BDA (white arrows). (E to G) However, BDA-labeled autonomic fibers were not detected in the right PVs. Abbreviations as in Figure 4.
Our data noted prolongation of the transient sinus cycle length during HL-ES. One possible explanation is that the majority of neurons in the aortic GP are parasympathetic (19). The mechanism of action behind the recovery of sinus cycle length may be that aortic root GP plays a trivial role in the modulation of sinus rhythm, whereas the anterior right GP serves as the integration center in this process (20). Moreover, 5 dogs presented with nonsustained ventricular tachycardia during HL-ES, indicating that HL-ES is not safe for clinic application.

It has been well established that epicardial GP is anatomically divided into the atrial and the ventricular GP. However, these 2 types of GPs may cooperate with each other and act as a single functional unit. He et al. (21) found that stimulation of atrial GP may affect the electrophysiological properties of the ventricle, including prolongation of ventricular ERP and delay of action potential duration. Another study conducted by their group showed that atrial GP ablation increased the risk of ventricular arrhythmias under acute myocardial ischemia (22). These studies indicate that activity of atrial GP influenced the electrophysiology of the ventricle and influenced ventricular arrhythmogenic properties. Based on these findings, our study implies that ventricular GP interconnects with atrial GP and collaboratively regulates the function of the atrium and PV.

It is noteworthy that a distinction exists between canine and human in the quantity, size, and location of GP. Whether human ventricular GP may be represented in the canine aortic root ventricular GP remains uncertain. It has been shown that the “anterior GP” is presumably similar to that of the canine superior vena cava and aortic root GP (23). It is, therefore, pertinent to localize the human-equivalent canine superior vena cava aortic root GP for future clinical trials.

**STUDY LIMITATIONS.** The first and perhaps most significant limitation is that we failed to examine the effect of LL-ES of the aortic root ventricular GP on blood flow in the coronary arteries. Second, the long-term impact of LL-ES in both atria and ventricle must be investigated.

**CONCLUSIONS**

Our findings revealed that autonomic innervations of left PVS may partially originate from aortic root ventricular GP. LL-ES of aortic root ventricular GP decreased AF inducibility that arose from PVS mediated by the autonomic nerve system. These findings should shed some light on the implication of ventricular GP modification as a possible target for AF vulnerability.

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