Effect of Epicardial Fat Pad Ablation on Acute Atrial Electrical Remodeling and Inducibility of Atrial Fibrillation

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Background: Atrial electrical remodeling (AER) is the underlying mechanism of atrial fibrillation (AF). The present study investigated the impact of epicardial fat pad (FP) ablation on acute AER (AAER) and inducibility of AF.

Methods and Results: AAER was performed in 28 mongrel dogs through 4-h rapid atrial pacing (RAP). Before RAP, 14 dogs (ablation group) underwent FP ablation, and the other 14 (control group) underwent a sham procedure. The atrial effective refractory period (ERP) and vulnerability window (VW) of AF were measured with and without bilateral cervical vagosympathetic nerve stimulation (VNS) at the high right atrium, ostium of the coronary sinus (CS) and distal CS before and after every hour of RAP. In the control group, ERP was markedly shortened in the first 2h of RAP and then stabilized. AF was only slightly induced. After RAP, the time course of ERP with and without VNS was similar. VNS significantly shortened ERP and increased VW before and after RAP. In the ablation group, ERP was significantly prolonged after FP ablation. Moreover, neither VNS nor RAP shortened the ERP or increased the VW. AF could not be induced (VW=0).

Conclusions: RAP resulted in AAER, which may be mediated and aggravated by autonomic activity. Epicardial FP ablation generated denervation, which not only abolishes AF inducibility but also prevents RAP-mediated AAER. (Circ J 2010; 74: 885–894)

Key Words: Acute atrial electrical remodeling; Atrial fibrillation; Autonomic nervous system; Denervation; Epicardial fat pad ablation
Each dog was ventilated with a constant volume-cycled respirator through a cuffed endotracheal tube, and blood oxygen saturation was maintained at more than 95% throughout the experiment. The chest was opened through a right, then left thoracotomy. The pericardium was cut open and sewn to the chest wall to cradle the heart. Under fluoroscopic guidance (Innova 2000, GE Co, USA), 2 multipolar catheters (Cordis Webster Co, USA) were placed at the coronary sinus (CS) and the high right atrium (HRA) through the right internal jugular vein and right femoral vein, respectively, for the electrophysiological studies. A 6F quadripolar catheter was advanced through the left femoral vein into the right ventricular apex. A temporary pacemaker was applied with a pacing rate of 100 beats/min in case of severe bradycardia. Surface ECG II/aVF and intracardiac electrograms were recorded simultaneously using a Prucka Cardiolab system (Prucka 7000, GE Medical System, Inc, USA). The signals were amplified and filtered between 30 and 500Hz. Every dog underwent rapid atrial pacing (RAP) of the distal

**Figure 1.** Response of the atrial rate to VNS. (A) In the control group, the atrial rate during VNS decreased significantly. Without VNS, the atrial rate was 160 beats/min. With VNS, the atrial rate was 47 beats/min. (B) In the ablation group, VNS could not change the atrial rate after fat pad ablation. With and without VNS, the atrial rate was 158 beats/min. VNS, vagosympathetic nerve stimulation.
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Figure 2. Impact of RAP on ERP at different sites: (A) HRA, (B) CSO, (C) CSD. In the control group, with or without VNS, the ERP decreased significantly after 1 h of RAP, and reached the minimum after 2 h, thereafter remaining relatively stable. In the ablation group, the ERP at each site before ablation was similar to the control group with or without VNS. After epicardial fat pad ablation, the ERP at each site increased significantly (P<0.01) with and without VNS. Additionally, neither VNS nor RAP could shorten the ERP (P>0.05). CSD, distal coronary sinus; CSO, coronary sinus ostium; ERP, effective refractory period; HRA, high right atrium; RAP, rapid atrial pacing; VNS, vagosympathetic nerve stimulation.
Figure 3. Effect of RAP on the VW at different sites. (A) HRA, (B) CSO, (C) CSD. In the control group, without VNS, the VW was close to 0 before or after RAP. With VNS, the VW increased significantly (P<0.01). VW was slightly influenced by short-term RAP. In the ablation group, the VW at each site before ablation was similar to the control group with or without VNS (P>0.05). However, after ablation, the VW was 0 even with VNS. Atrial fibrillation could not be induced. CSD, distal coronary sinus; CSO, coronary sinus ostium; ERP, effective refractory period; HRA, high right atrium; RAP, rapid atrial pacing; VNS, vagosympathetic nerve stimulation; VW, vulnerability window.
CS (CSD) at 600 beats/min for 4 h. Blood pressure was continuously monitored via a pressure transducer positioned in the right femoral artery. An electrical heating pad was placed under the dog, and operating room lamps were used to maintain body temperature at 36–37°C. Intermittent arterial blood gas measurements were taken, and ventilator adjustments were made to correct any metabolic abnormalities.

Vagosympathetic Nerve Stimulation (VNS)
Bilateral cervical vagosympathetic trunks were decentralized by surgical procedures, and the cranial ends of the vagosympathetic nerves were ligated. Two pairs of electrodes were embedded in the caudal ends of the autonomic nerve trunks for VNS. Rectangular electrical stimuli were delivered through a constant voltage stimulator at 20 Hz with a pulse width of 2 ms using a programmable stimulator (RST-2, Huanan-Med Inc, China). The stimulation voltage was set to decrease the drive cycle length (DCL) of 300 ms. The experiments were successfully performed. The systolic and diastolic blood pressures were stable during the entire procedure of experiments.

Irrigated Catheter Ablation of Epicardial FP
In the ablation group, before RAP, an irrigated-tip catheter (3.5 mm electrode tip, 2 bipolar electrode pairs with a distance of 2 mm between electrodes; Biosense Webster, Diamond Bar, CA, USA) was positioned manually on the surface of the epicardial FPs under direct visualization to ensure optimal tissue contact and energy delivery. Irrigated ablation of FPs was performed with an irrigation rate of 17 ml/min and a power output setting between 35 and 45 W delivered from a radiofrequency generator (Cordis Webster Co).

We performed the ablation procedure in the following sequence: superior vena cava–aortic root FP (SVC-AO FP or the third FP), then the right pulmonary vein FP (RPV FP) and finally the inferior vena cava–left atrium FP (IVC-LA FP). Abolishing the VNS-induced decrease in atrial rate was regarded as the endpoint of ablation (Figure 1B).

Electrophysiological Study
An atrial pacing protocol with a single extra stimulus was performed with a programmable multichannel stimulator (model DF-5A, Dongfang Co, China). The pacing amplitude was set at twice the diastolic threshold determined at a basic drive cycle length (DCL) of 300 ms. The atrial effective refractory period (ERP) and vulnerability window (VW) of AF were measured at different atrial sites, including the HRA, the ostium of the CS (CSO) and the CSD, with and without VNS and before and after different time periods (1, 2, 3 and 4 h) of RAP. Additionally, in the ablation group, the ERP and VW were evaluated before and after FP ablation, respectively. During determination of the ERP, the single extra stimulation was at coupling intervals from 200 ms that were progressively shortened by 10 ms with a DCL of 300 ms (S1: S2=8:1). The atrial ERP was defined as the longest coupling interval of the extra stimulus that failed to capture the local atria. The VW of AF was defined as the range of the coupling interval of the extra stimulus at which AF was induced. AF was defined as irregular atrial rates faster than 500 beats/min associated with irregular atrioventricular conduction lasting >5 s after programmable stimulation (Figure 4A).

Histopathological Evaluation
After the electrophysiological study, all dogs were killed, and the hearts were removed for histological examination of the epicardial FPs. Each FP and underlying tissue was excised out VNS, before and after FP ablation) in each group and comparisons between data (before and after RAP, with and without VNS, before and after FP ablation) in each group and between the control and ablation groups were performed with ANOVA. A P-value <0.05 was considered statistically significant. All tests were performed with SPSS 11.0 (Chicago, IL, USA).

Statistical Analysis
All values are expressed as mean ± standard deviation. Comparisons between data (before and after RAP, with and without VNS, before and after FP ablation) in each group and between the control and ablation groups were performed with ANOVA. A P-value <0.05 was considered statistically significant. All tests were performed with SPSS 11.0 (Chicago, IL, USA).

Results
The experiments were successfully performed. The systolic and diastolic blood pressures were stable during the entire procedure of experiments.

Effect of RAP on the Time Course of AAER in the Control Group
After RAP, the ERP at each site was shortened significantly (Figure 2), which indicates that RAP can result in AAER. The ERP decreased markedly after 1 h of RAP (123.71 ± 12.62

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<th>AF Inducibility With VNS in the Control Group</th>
<th>Before RAP</th>
<th>After RAP</th>
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<tr>
<td>HRA AF duration (s)</td>
<td>26.8 ± 18.6</td>
<td>23.7 ± 19.4</td>
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<tr>
<td>CSO AF duration (s)</td>
<td>23.7 ± 19.4</td>
<td>25.1 ± 19.6</td>
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<tr>
<td>CSD AF duration (s)</td>
<td>26.3 ± 16.7</td>
<td>29.7 ± 21.1</td>
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AF, atrial fibrillation; VNS, vagosympathetic nerve stimulation; RAP, rapid atrial pacing; HRA, high right atrium; CSO, coronary sinus ostium; CSD, distal coronary sinus.
vs 105±15.06 ms at HRA, P<0.01; 107.71±14.86 vs 94.29±14.53 ms at CSO, P<0.01; 107.71±14.90 vs 90.71±16.85 ms at CSD, P<0.01), and reached the minimum after 2 h (97.86±32.62 ms at HRA, 93.07±9.35 ms at CSO, 90.0±16.17 ms at CSD); the ERP then remained relatively stable. AF was not induced in any dog before RAP. After RAP, AF was only induced at the CSD in 1 of 14 dogs in the control group (VW: 10 ms after 1 h of RAP, 20 ms after 2 h, 10 ms after 3 h, 10 ms after 4 h). AF duration was 8.7±4.6 s. The VW was slightly influenced by short-term RAP (Figure 3). No differences in the ERP and VW at the different sites were observed (P>0.05).

**Effect of VNS on the Time Course of AAER in the Control Group**

During VNS, the atrial rate decreased significantly (161.36±23.62 vs 63.17±28.65 beats/min, P<0.01) (Figure 1A), which

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**Figure 4.** Programmed stimulation during VNS at the HRA. A train of 8 stimuli (S1) at a cycle length of 300 ms and a premature extrastimulus (S2) followed. (A) In the control group, during VNS, a premature extrastimulus (coupling interval of 70 ms) induced atrial fibrillation. (B) In the ablation group, after fat pad ablation, a premature extrastimulus (coupling interval of 160 ms) during VNS could not capture the atria. The ERP was markedly prolonged. Atrial fibrillation could not be induced. ERP, effective refractory period; HRA, high right atrium; RAP, rapid atrial pacing; VNS, vagosympathetic nerve stimulation.
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indicates vagal predominance in the vagosympathetic nerve modulation of the sinus node. With VNS, the ERP decreased markedly after 1 h of RAP (72.14±23.92 vs 58.57±29.05 ms at HRA, P<0.01; 73.57±26.20 vs 53.57±20.98 ms at CSO, P<0.01; 69.29±24.64 vs 46.43±20.23 ms at CSD, P<0.01), and reached the minimum after 2 h (55.0±24.42 ms at HRA, 51.43±22.48 ms at CSO, 45.71±20.27 ms at CSD); the ERP then remained relatively stable (Figure 2). The modulation of VNS on AAER was similar to the effect of RAP on AAER. Moreover, before and after RAP, VNS significantly decreased the ERP (P<0.01) and increased the VW (P<0.01) (Figures 2, 3). VNS rendered AF inducible (Table, Figure 4A). For instance, before RAP, AF was induced at the CSD during VNS in 10/14 with the VW 25.0±18.29 ms. However, with VNS, there was only a trend towards a wider VW after RAP, and this did not show statistical significance (P>0.05) (Figure 3). No differences in the ERP and VW at the different sites were observed (P>0.05).

FP Ablation Induces Denervation in the Ablation Group

After ablation of the SVC-Ao FP, the VNS-mediated decrease in the atrial rate was remarkably suppressed, which strongly indicates that the SVC-Ao FP is the head station of vagal fibers. Nevertheless, ablation of the SVC-Ao FP did not completely abolish the atrial rate decrease mediated by VNS, which may be related to the fact that a few vagal fibers may bypass the SVC-Ao FP and go directly to the IVC-LA or RPV FP and then innervate the atrial myocardium. After all 3 epicardial FPs were totally ablated (average ablation time 171.15±35.39 s), VNS could not change the atrial rate (Figure 1B), thereby successfully generating denervation. The ablation was limited to the FPs, and the morphology and amplitude of the electrograms at the sites closest to the FPs were not altered after ablation, indicating that collateral tissue damage associated with FP ablation was minimal.

Effect of FP Ablation on the ERP and VW in the Ablation Group

After FP ablation, the ERP increased markedly (without VNS: 125.0±18.29 vs 142.14±17.62 ms at HRA, P<0.01; 106.43±16.92 vs 122.14±20.07 ms at CSO, P<0.01; 105.0±16.53 vs 119.29±15.42 ms at CSD, P<0.01; with VNS: 72.86±15.41 vs 138.57±25.38 ms at HRA, P<0.01; 67.14±26.14 vs 115.71±21.74 ms at CSO, P<0.01; 65.71±19.10 vs 113.57±25.90 ms at CSD, P<0.01) and VW was 0 with and without VNS (with VNS: 27.14±28.94 vs 0 ms at HRA, P<0.01; 26.43±24.68 vs 0 ms at CSO, P<0.01; 27.86±23.26 vs 0 ms at CSD, P<0.01). Neither RAP nor VNS shortened the ERP or increased the VW (Figures 2, 3). AF was not induced (Figure 4B). No differences in the ERP and VW at the different sites were observed (P>0.05). These results indicate that after FP ablation, RAP cannot induce AAER, and AF can be effectively prevented.

Figure 5. (A, C) Sections from the control group stained with H&E and PGP 9.5, respectively. The membrane and cell nuclei are intact. (B, D) Sections from the ablation group stained with H&E and PGP 9.5, respectively. The ganglia plexuses are disconnected with swollen cell nuclei. The membrane is fragmented and the organelles are in disorder. Red cells can be observed in the interstitium. (Amplified size: 20-fold.) H&E, hematoxylin–eosin; PGP 9.5, mouse anti-protein gene product 9.5.
Comparison of the Ablation and Control Groups
Before ablation, the ERP, VW and atrial rate, with or without VNS, in the ablation group were comparable (P>0.05) to those in the control group. After epicardial FP ablation, the ERP of the ablation group at each site increased significantly compared with the control group and the VW dropped to 0 with and without VNS. In addition, neither RAP nor VNS changed the ERP or the VW after ablation in the ablation group (Figures 2,5). Moreover, AF was noninducible (Figure 4B), even with VNS, which implies that epicardial FP ablation can induce vagosympathetic denervation and prevent AAER and AF.

Histopathological Study
In the specimens from the control group, intact ganglia plexuses were found in the epicardial FPs. In the specimens from the ablation group, the architecture of the ganglion plexuses was significantly destroyed (ie, disconnected with swollen cell nuclei) and the membrane was fragmented with disordered organelles. There was a notable absence of nerve fibers running through the ablated FPs (Figure 5).

Discussion
Main Findings
The main findings of the present study are as follows. (1) RAP induced AAER. The ERP decreased markedly after 1 h of RAP and reached the minimum after 2 h, after which the ERP remained relatively stable. (2) The time course of VNS modulation of AAER was similar to that of RAP on AAER. VNS could further shorten the ERP and aggravate AAER. (3) Epicardial FP ablation generated ANS denervation, which not only abolished AAER but also prevented RAP-mediated AAER.

These findings underscore the autonomic nerve mechanism of AAER and suggest that epicardial FP ablation can prevent AAER and AF.

Effect of RAP on AAER and AF Inducibility
In the control group, RAP significantly shortened the ERP and yielded AAER, similar to previous studies. Coincident with the study by Lu et al., the atrial ERP decreased significantly after 1 h of RAP and reached the minimum after 2 h, then remaining relatively stable, which might be associated with the immediate and transient increase in the Kv1.5 gene and protein that is induced by RAP. Wijffels et al also found that the inducibility of AF is dependent on the duration of RAP. However, in the present study, the effect of RAP on the VW was slight, and AF was barely induced without VNS, even after RAP, which might be related to the short duration of RAP. This result leads to the hypothesis that the electrophysiological changes in the atrium may differ between short-term (several hours) and chronic (several days) RAP. Short-term RAP only induced AAER, which might be an underlying mechanism of paroxysmal AF. These results suggest that RAP-mediated AAER might not progress in the short term, which may explain why many patients stay in paroxysmal AF for a long period instead of immediately converting to persistent AF in the absence of other coexisting substrates for AF.

Effect of VNS on AAER and AF Inducibility
Goette et al have previously reported the time course of AAER. But as far as we know there have been no studies on the time course of vagosympathetic nerve modulation of AER. In the present study, before RAP, VNS shortened the ERP and decreased the atrial rate significantly, indicating vagal predominance in the vagosympathetic nerve modulation of the atria. After AAER was induced by RAP, VNS further shortened the ERP, which aggravated AAER. Interestingly, the time course of vagosympathetic modulation of AAER paralleled that of RAP on AAER. Under VNS, the ERP decreased significantly after 1 h of RAP, reached its minimum after 2 h and then remained relatively stable, indicating that vagosympathetic activity can induce and aggravate RAP-mediated AAER. Moreover, VNS rendered AF inducible before and after RAP, whereas AF was only slightly induced without VNS, which might be related to VNS-induced regulation of ion channels, thus aggravating AAER and thereby facilitating the development of AF. Therefore, AF inducibility might be mainly attributed to VNS rather than short-term RAP.

Epicardial FP Ablation Prevents AAER and AF
Both sympathetic and vagal nerve systems have been suggested as potentially responsible for AF. Moreover, vagal and sympathetic nerves are colocalized not only in tissues, but also in cells. Such anatomic colocalization implies that selectively eliminating vagal or sympathetic nerves is virtually impossible. Ganglionated plexuses abundant in the epicardial FPs modulate autonomic innervation to the atria, so in the present study, we ablated the ganglion plexuses in all 3 FPs, which was verified by histopathological examination. FP ablation-induced vagosympathetic nerve denervation rendered several significant changes: a flat response of the atrial rate to VNS, increased ERP, prevention of RAP-induced AAER and noninducibility of AF even under VNS. Considering our observation that the atrial rate decreased as a response to VNS before ablation and the vagal predominance in vagosympathetic modulation of the atria, the lack of a response of the atrial rate to VNS after ablation is most likely because of vagal denervation. Several reports have found that vagal denervation might contribute to suppressing AF. Onorati et al observed that FP ablation during the Maze procedure significantly decreased AF recurrence at discharge and after 12.8 months of follow-up. However, there is no general agreement on the efficacy of ablation of ganglionated plexuses and denervation on AF and AER. Considering the response of the atrial rate during RAP and the vagal predominance in vagosympathetic modulation of the atria, the lack of a response of the atrial rate to VNS after ablation is most likely because of vagal denervation. Several reports have found that vagal denervation might contribute to suppressing AF. In contrast with those 2 studies, we chose to ablate all 3 epicardial FPs, which yielded deep, direct damage to the ganglionated plexuses, including vagal and sympathetic nerves. This procedure may have resulted in a more homogeneous and complete denervation of the atria, thereby not partially but completely eliminating the response of the atrial rate to VNS and preventing AF inducibility and AAER, implying that the AAER induced by RAP may be mainly mediated by cardiac ganglionated plexus activity.

It has been widely accepted that RAP-mediated AAER is associated with alterations in expression of ion channels. Blunting of AAER and AF inducibility may be related to denervation, which might be attributed to a decrease in the modulation of the autonomic nerves on ion channels remodeling. A study in dogs demonstrated that the abbreviation in the atrial ERP induced by short-term RAP can be blunt by verapamil and accentuated by hypercalcemia.
suggesting that intracellular Ca\(^{2+}\) overload may be primarily responsible for AAER.\(^{13,38,39}\) Vagal releases acetylcholine (ACh) can inhibit adenylyl cyclase and cAMP via G proteins, which results in inhibiting Ca\(^{2+}\) ATPase, increased cytosolic calcium and downregulation of L-type calcium currents (I\(_{Ca,L}\)) because of the decreased electrochemical gradient.\(^{30,41}\) Calcium is released from the sarcoplasmic reticulum, which triggers a much larger Ca\(^{2+}\) release via ryanodine receptor channels in the sarcoplasmic reticulum through Ca\(^{2+}\)-induced Ca\(^{2+}\) release.\(^{35}\) Parasympathetic blockade can promote recovery from ERP shortening induced by short-term RAP.\(^{43}\) Norepinephrine released from sympathetic nerve endings enhances the Ca\(^{2+}\) transient and persistent Ca\(^{2+}\) transient elevation in phase 3 of the action potential may activate the Na\(^{+}\)–Ca\(^{2+}\) exchange current and induce late phase 3 early afterdepolarizations. These late phase 3 early afterdepolarizations initiate focal discharge and AF.\(^{3,44}\) So, VNS may induce Ca\(^{2+}\) overload, which accentuates AAER and AF inducibility. In addition, ACh stimulates muscarinic receptors (M-receptors), facilitating activation of the atrial ACh-regulated potassium current I\(_{K,ACh}\) and AF inducibility.\(^{45}\) The resulting abbreviation of the ERP and AF inducibility in response to M-receptor stimulation is mediated by I\(_{K,ACh}\), because in knockout mice lacking this channel, M-receptor stimulation does not induce AF.\(^{45}\) Therefore, denervation induced by FP ablation suppresses the I\(_{K,ACh}\), Ca\(^{2+}\) overload and the Na\(^{+}\)–Ca\(^{2+}\) exchange current, which leads to subsequent prolongation of the ERP of the atrial myocardiun and protects against RAP-mediated AAER and AF induced by VNS. Our data indicate that epicardial FP ablation might be an effective therapy for preventing AAER and AF.

**Clinical Relevance**

Many patients who undergo cardiac surgery have accompanying AF and AF may also occur following cardiac surgery. Epicardial FPs could be ablated simultaneously during surgical procedures. The present study demonstrates that the ANS plays a prominent role in AAER, and epicardial FP/ganglionated plexuses ablation can effectively prevent AAER and AF. This approach might be considered in future clinical studies of the prevention and/or treatment of AF.

**Study Limitations**

We did not puncture the atrial septum or map the left atrium. Additionally, the electrophysiologic characteristics of the left atria were not evaluated. The electrophysiologic change in the HRA, CSO and CSD sites may not completely represent that in both atria, and the difference in the ERP among these sites may not well represent the spatial dispersion of both atria. We did not address ERP dispersion, which also plays an important role in maintaining AF, as well as the shortened ERP, in the pacing-induced AF model. Further studies that include more atrial sites, including the left atrium, for ERP measurement are necessary, as well as evaluating ERP dispersion with or without VNS, before and after FP ablation. No response of the atrial rate to VNS implies functional denervation but does not mean there was complete autonomic nerve denervation to both atria, as ganglionated plexuses modulate autonomic innervation to the whole heart. We did not test the effect of epicardial FP ablation on chronic AER remains controversial. Further studies are needed to clarify these topics.

**Conclusions**

RAP could result in AAER, which might be mediated and further aggravated by ANS. The ANS might be required for AF inducibility. Epicardial FP ablation achieved denervation, which not only abolished AF inducibility modulated by the ANS but also prevented AAER mediated by RAP. This might be an alternative and effective therapy for AF, particularly for patients undergoing cardiac surgery.

**Acknowledgments**

We thank Win-Kuang Shen, MD and Shiwen Yuan, MD, PhD for their valuable suggestions.

**Disclosure**

Conflict of Interest statement: the authors declare that there are no conflicts of interest.

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