Vagal Effects on the Occurrence of Focal Atrial Fibrillation Originating From the Pulmonary Veins

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Background  There is evidence that the autonomic nervous system may be involved in the mechanism of focal atrial fibrillation (AF), so the present study investigated the effects of the parasympathetic nervous system on the occurrence of focal AF originating from the pulmonary veins (PVs).

Methods and Results  In 10 mongrel dogs, programmed stimulation and local burst stimulation (12.5Hz, impulse duration 0.5ms) were performed at each of the PVs. Pacing thresholds at different sites were determined and shown as a terraced distribution. The closer to the ostium of the PV, the lower was the pacing threshold (P<0.05–0.001). The local effective refractory period (ERP), AF induction and AF threshold were measured at baseline and during bilateral vagal nerve stimulation (VNS). VNS led to local ERP shortening at each of the PV sites (P<0.05–0.001), increased the inducibility of AF at all sites in the 4 PVs (P<0.05–0.001), and decreased the AF threshold at most sites, especially in the distal portions of the 4 PVs (P<0.05–0.01).

Conclusions  VNS changes the electrophysiological characteristics of the PVs and facilitates the induction of AF. Interaction between the autonomic nervous system and local cardiac autonomic nerve system may be a potential mechanism.  (Circ J 2009; 73: 48–54)

Key Words:  Atrial fibrillation; Autonomic nervous system; Electrophysiology; Veins

Many investigators have demonstrated that focal activity in the pulmonary veins (PVs) is responsible for focal atrial fibrillation (AF). Radiofrequency catheter ablation of the PVs eliminates these rapid activations, resulting in successful treatment of AF, but the mechanisms of focal AF have not been well characterized.

Previous studies have suggested that increased vagal tone is involved in the onset of AF in patients with a structurally normal heart. It has been recognized that vagal nerve stimulation (VNS) or cholinergic agonists can shorten the atrial effective refractory period (ERP), decrease the reentrant wavelength, increase refractoriness heterogeneity in the atria, and promote the maintenance of AF, but there are less data evaluating the vagal effects on the electrophysiological properties of PVs. Therefore, in the present study, we used a canine model of focal AF to assess the influence of VNS on the occurrence of AF originating from the PVs.

Methods

Surgical Preparation

All animal studies were performed in accordance with the institutional “Guide for the Care and Use of Laboratory Animals” and were approved by the Animal Studies Committee at Beijing University School of Medicine.

Ten male mongrel dogs weighing 14–25kg (Experimental Animal Center, People’s Hospital of Beijing University) were anesthetized with sodium pentobarbital 20mg/kg IV. After intubation and mechanical ventilation, additional doses were injected as needed to maintain anesthesia during the study. The surface ECG and blood pressure were continuously monitored. A catheter introducer was inserted into the right femoral vein, and a 6Fr quadripolar electrode catheter (2mm interpole distance, 10mm interelectrode spacing,

Fig 1. Catheter arrangement for local electrical stimulation. 5Fr quadripolar electrode catheters were sutured to the right atrial appendage (RAA), the left atrial appendage (LAA), the left atrium (LA), the left superior pulmonary vein (LSPV), the left inferior pulmonary vein (LIPV), the right superior pulmonary vein (RSPV) and the right inferior pulmonary vein (RIPV). AO, aorta ascendens; CS, coronary sinus; IVC, inferior vena cava; LPA, left pulmonary artery; LV, left ventricle; RA, right atrium; RPA, right pulmonary artery.
Cordis Webster Corp) was advanced into the right ventricle. After right lateral thoracotomy, the heart was exposed in a pericardial cradle. Three 5Fr quadripolar electrode catheters (2 mm interpolara distance, 5 mm interelectrode distance, Bard Electrophysiology) were sutured to the right atrial appendage (RAA), the right superior PV (RSPV) and the right inferior PV (RIPV). After closure of the thorax, a left intercostal thoracotomy was performed and the pericardium was opened. Four 5Fr quadripolar electrode catheters were sutured to the left atrial appendage (LAA), the left atrium (LA), the left superior PV (LSPV) and the left inferior PV (LIPV) (Fig 1). For local electrical stimulation and electrogogram recording, each PV was divided into 3 portions: the proximal (p) portion (electrode pair PV1–2, positioned at the ostium of each PV), the middle (m) portion (electrode pair PV2–3) and the distal (d) portion (electrode pair PV3–4). The signals were amplified, filtered between 40 and 600 Hz, and recorded simultaneously on a digital mapping system (model GY-6328, Huanan Engineering, Zhengzhou, China).

VNS

For cervical VNS, the cervical vagosympathetic trunks were isolated bilaterally, doubly ligated, and transected. Each stellate ganglion was isolated and crushed at its junction with the ansae subclaviae. These maneuvers remove almost all tonic neural activity to the heart! Bilateral VNS was delivered by a DF-5A stimulator (Oriental Instruments Inc, Suzhou, China) with a pulse width of 0.5 ms at a frequency of 12.5 Hz through custom-made bipolar electrodes embedded in the cardiac end of the cut nerve. The voltage was set to obtain sinus arrest lasting 2 s or complete atrioventricular block. These stimulation strengths were also applied during bilateral VNS and were kept constant during the experiments. After VNS, muscarinic receptor blockade was induced with an initial bolus of atropine (0.04 mg/kg) followed by maintenance infusion (0.007 mg kg\(^{-1}\) h\(^{-1}\)). These doses have been shown to produce complete blockade.

Electrophysiological Study

Prior to the electrophysiological study, the pacing threshold at each site of all the atria and PVs was measured with a 0.5-ms rectangular stimulus, and the pacing rate was 10–20 beats/min higher than the sinus rate. The pacing voltage was decreased by 0.1 V decrements until it failed to capture the atria or PVs. The ERP was determined by the extrastimulus technique with another programmable stimulator (DF-5A, Oriental Instruments Inc). Each test site was driven with a 0.5-ms rectangular stimulus that was twice the diastolic threshold and which was measured during each intervention. A train of 10 stimuli (S1) was followed by a late premature stimulus (S2). The S1–S1 interval was 300 ms and was kept constant throughout the experiment. The S1–S2 interval was shortened in 5 ms steps until S2 failed to produce a propagated response. The ERP was defined as the longest S1–S2 interval at which S2 failed to capture the atrium or PV, respectively. The ERP was determined at least twice at each electrode site. The inducibility of AF at each electrode site was examined by burst pacing using 12.5 Hz (S1–S1=80 ms) with a 0.5-ms width at twice the diastolic threshold. Each pacing sequence was maintained for 30 s. Rapid tachycardias (>500 beats/min) consisting of >10 irregular atrial beats with varying electrogram morphology and activation times were regarded as AF. Sustained AF was defined as lasting more than 30 s. If AF did not terminate after 5 min, electrical cardioversion was used.

The induction of AF was repeated 3 times at each pacing site. A step-down pacing protocol was used to measure the voltage threshold of AF induction. Rapid burst stimulation at a cycle length of 80 ms (12.5 Hz) with 0.5-ms pulse duration was delivered at each site. Each pacing sequence was also maintained for 30 s, with 2 min pauses in between. After each burst, the stimulation voltage was decreased by 0.5 V decrements. We defined the AF threshold as the lowest stimulation voltage that resulted in AF. The ERPs and AF induction were measured at baseline, during bilateral VNS, and during intravenous infusion of atropine.

Statistical Analysis

Data are given as mean±SD. Student’s t-test for paired or unpaired data was used for comparing pacing thresholds, ERPs and AF thresholds between 2 different conditions. The \( \chi^2 \) exact test was used to compare the incidence of AF among the 3 different conditions. The McNemar test was used to compare AF inducibility between baseline and VNS only. A P-value <0.05 was considered statistically significant.

Results

Experiments were performed in 10 dogs and yielded 9 complete protocols.

Pacing Thresholds in PVs

There were no significant differences in the comparison of the pacing thresholds of the RAA, LAA, LA and the p portions of the 4 PVs (P>0.05). In 1 of 4 PVs, the pacing thresholds in the p and m portions were both lower than that in the d portion (P<0.05–0.001). In the LIPV or RSPV, the pacing threshold in the p portion was statistically lower than that in the m portion (P<0.05, Fig 2).

Effects of VNS on ERPs in PVs

Fig 3 shows the ERPs at different sites in the PVs. During bilateral VNS, the ERPs at all sites shortened obviously (P<0.05–0.001). During infusion of atropine, similar to the baseline state, ERPs at the different sites of the 4 PVs remained significantly longer than those during VNS. There was no significant change in the overall ERPs between the baseline condition and infusion of atropine (P>0.05).

Effects of VNS on AF Induction

In the baseline state, premature extrastimuli from most sites in the PVs evoked single or multiple atrial premature depolarizations (APDs) and short runs of atrial tachycardia (AT); only a few sites induced AF. During VNS, premature extrastimuli induced AF more frequently. Representative examples are shown in Fig 4.

In the absence of VNS, local burst stimulation at most sites in the PVs induced AF, but the inducibility was very low. VNS increased the inducibility of AF at all sites in the 4 PVs (P<0.05–0.001). There was also a lower AF inducibility with intravenous infusion of atropine. Table 1 shows the changes in AF inducibility with local burst stimulation at different sites in the atria and PVs under baseline conditions, during VNS and during infusion of atropine.

Effects of VNS on AF Threshold

At lower voltage levels (twice the diastolic threshold), only a few dogs had AF induced by local burst stimulation in the absence of VNS. With a further increase in the stimulation strength, most sites in the PVs evoked AF. During
Fig 2. Pacing thresholds at different sites of 4 PVs. p, proximal; m, middle; d, distal. Other abbreviations as in Fig 1.

Fig 3. Vagal nerve stimulation (VNS) shortened the effective refractory periods (ERPs) at different sites of 4 PVs. Results were obtained at a basic drive train of 300ms. *P<0.05, **P<0.01, ***P<0.001, baseline vs VNS; #P<0.05, ##P<0.01, ###P<0.001, infusion of atro-pine vs VNS. Other abbreviations as in Fig 1.

Table 1  Inducibility of AF at Different Sites in the Atria and PVs [AF Inducible/Tested (%)]

<table>
<thead>
<tr>
<th>Site</th>
<th>Baseline</th>
<th>VNS</th>
<th>Atropine</th>
<th>P*</th>
<th>P value#</th>
</tr>
</thead>
<tbody>
<tr>
<td>RAA</td>
<td>6/27 (22.2)</td>
<td>16/27 (59.3)</td>
<td>6/21 (28.6)</td>
<td>0.012</td>
<td>0.006</td>
</tr>
<tr>
<td>LAA</td>
<td>4/27 (14.8)</td>
<td>15/27 (55.6)</td>
<td>4/21 (19)</td>
<td>0.002</td>
<td>0.001</td>
</tr>
<tr>
<td>LA</td>
<td>5/27 (18.5)</td>
<td>14/27 (51.9)</td>
<td>3/21 (14.3)</td>
<td>0.005</td>
<td>0.004</td>
</tr>
<tr>
<td>LSPV-p</td>
<td>9/27 (33.3)</td>
<td>18/27 (66.7)</td>
<td>5/21 (23.8)</td>
<td>0.006</td>
<td>0.022</td>
</tr>
<tr>
<td>LSPV-m</td>
<td>3/24 (12.5)</td>
<td>12/24 (50)</td>
<td>3/21 (14.3)</td>
<td>0.004</td>
<td>0.004</td>
</tr>
<tr>
<td>LSPV-d</td>
<td>5/21 (23.8)</td>
<td>12/21 (57.1)</td>
<td>2/18 (11.1)</td>
<td>0.005</td>
<td>0.016</td>
</tr>
<tr>
<td>LIPV-p</td>
<td>8/27 (29.6)</td>
<td>19/27 (70.4)</td>
<td>3/21 (14.3)</td>
<td>0.0002</td>
<td>0.003</td>
</tr>
<tr>
<td>LIPV-m</td>
<td>8/24 (33.3)</td>
<td>15/24 (62.5)</td>
<td>2/18 (11.1)</td>
<td>0.003</td>
<td>0.016</td>
</tr>
<tr>
<td>LIPV-d</td>
<td>0/15 (0)</td>
<td>6/15 (40)</td>
<td>0/12 (0)</td>
<td>0.002</td>
<td>0.031</td>
</tr>
<tr>
<td>RSPV-p</td>
<td>6/27 (22.2)</td>
<td>14/27 (51.9)</td>
<td>3/21 (14.3)</td>
<td>0.010</td>
<td>0.008</td>
</tr>
<tr>
<td>RSPV-m</td>
<td>6/21 (28.6)</td>
<td>12/21 (57.1)</td>
<td>0/21 (0)</td>
<td>0.0002</td>
<td>0.031</td>
</tr>
<tr>
<td>RSPV-d</td>
<td>4/18 (22.2)</td>
<td>13/18 (72.2)</td>
<td>3/18 (16.7)</td>
<td>0.001</td>
<td>0.004</td>
</tr>
<tr>
<td>RIPV-p</td>
<td>5/27 (18.5)</td>
<td>12/27 (44.4)</td>
<td>4/21 (19)</td>
<td>0.059</td>
<td>0.016</td>
</tr>
<tr>
<td>RIPV-m</td>
<td>3/24 (12.5)</td>
<td>13/24 (54.2)</td>
<td>3/18 (16.7)</td>
<td>0.003</td>
<td>0.006</td>
</tr>
<tr>
<td>RIPV-d</td>
<td>0/18 (0)</td>
<td>9/18 (50)</td>
<td>0/15 (0)</td>
<td>0.0002</td>
<td>0.004</td>
</tr>
</tbody>
</table>

*Comparing 3 different conditions; #comparing baseline and VNS only.
AF, atrial fibrillation; PV, pulmonary vein; VNS, vagal nerve stimulation; RAA, right atrial appendage; LAA, left atrial appendage; LA, left atrium; LS, left superior; p, proximal; m, middle; d, distal; LI, left inferior; RS, right superior; RI, right inferior.
bilateral cervical VNS, a lower AF threshold was observed at each site, and the changes in the RAA, LAA, LA, LSPV-d, LIPV-p, LIPV-d, RSPV-d and RIPV-d were significant, especially in the d portions of the 4 PVs (P<0.05–0.01, Table 2). There was an increase in the AF threshold with intravenous infusion of atropine compared with VNS, but there was no significant change in the AF threshold between baseline and the infusion of atropine (P>0.05).

Discussion

This study presents evidence that local electrical stimulation in the PVs evokes ectopic activity and induces focal AF originating from the PVs. VNS shortened the ERPs at different sites in the PVs, decreased the AF thresholds and increased the inducibility of AF, results that suggest the parasympathetic nervous system plays an important role in the initiation of focal AF originating from the PVs.

Pacing Thresholds in the PVs

As shown in our study, the pacing thresholds at different sites within the PVs had a terraced distribution. The closer to the ostium of the PV, the lower was the pacing threshold. At the d portion of the PV, there was the highest threshold.

Table 2 Changes in the AF Threshold at Different Sites in the Atria and PVs (V)

<table>
<thead>
<tr>
<th>Site</th>
<th>Baseline</th>
<th>VNS</th>
<th>Atropine</th>
</tr>
</thead>
<tbody>
<tr>
<td>RAA</td>
<td>4.43±2.13*</td>
<td>2.71±1.50</td>
<td>4.71±2.87*</td>
</tr>
<tr>
<td>LAA</td>
<td>4.71±2.08**</td>
<td>3.19±2.38</td>
<td>5.57±2.28**</td>
</tr>
<tr>
<td>LA</td>
<td>4.21±1.47***</td>
<td>2.93±1.21</td>
<td>4.70±2.11***</td>
</tr>
<tr>
<td>LSPV-p</td>
<td>4.07±2.23</td>
<td>3.79±2.29</td>
<td>4.93±2.30</td>
</tr>
<tr>
<td>LSPV-m</td>
<td>3.86±1.73</td>
<td>3.21±1.68</td>
<td>4.43±1.79</td>
</tr>
<tr>
<td>LSPV-d</td>
<td>5.64±2.59*</td>
<td>3.86±1.44</td>
<td>5.79±2.49*</td>
</tr>
<tr>
<td>LIPV-p</td>
<td>3.85±2.01**</td>
<td>2.64±1.82</td>
<td>4.57±1.69**</td>
</tr>
<tr>
<td>LIPV-m</td>
<td>4.36±3.43</td>
<td>3.71±2.16</td>
<td>5.21±1.98</td>
</tr>
<tr>
<td>LIPV-d</td>
<td>6.14±1.46**</td>
<td>4.01±0.76</td>
<td>5.86±1.41**</td>
</tr>
<tr>
<td>RSPV-p</td>
<td>3.79±1.99</td>
<td>2.79±0.91</td>
<td>4.66±1.97</td>
</tr>
<tr>
<td>RSPV-m</td>
<td>4.14±2.36</td>
<td>3.79±1.55</td>
<td>4.79±2.51</td>
</tr>
<tr>
<td>RSPV-d</td>
<td>6.02±2.38**</td>
<td>4.59±1.44</td>
<td>6.57±1.69**</td>
</tr>
<tr>
<td>RIPV-p</td>
<td>3.57±2.59</td>
<td>3.43±2.23</td>
<td>4.98±2.95</td>
</tr>
<tr>
<td>RIPV-m</td>
<td>5.43±2.22</td>
<td>4.57±2.70</td>
<td>5.79±2.12</td>
</tr>
<tr>
<td>RIPV-d</td>
<td>7.36±2.75*</td>
<td>5.79±1.95</td>
<td>7.35±0.99*</td>
</tr>
</tbody>
</table>

*P<0.05, **P<0.01, ***P<0.001, comparing baseline and VNS; #P<0.05, ##P<0.01, ###P<0.001, comparing infusion of atropine and VNS.

Abbreviations see in Table 1.
The architecture of the PV may contribute to such changes. As we know, electrical activity within the PV is only related to the cardiomyocytes of the myocardial sleeves. The longest sleeves are found in the superior veins, with a maximum length of 25 mm in humans. The fascicles of myocytes tended to be broader at the venoatrial junction, branching or becoming thinner distally? The length of the myocardial sleeve in the dog is 4–20 mm, so pacing in the m or d portion of the canine PV fails to capture or needs higher voltage to capture because of the lack of cardiomyocytes in these areas. We observed that some PVs could not be captured in the m or d areas more often in the inferior veins than in the superior veins, especially in the LIPV.

**Local Electrical Stimulation and Focal AF**

PVs contain a mixture of pacemaker cells and myocardial cells. Various types of spontaneous activities, including sinoatrial (SA) node-like spontaneous action potentials, have been recorded from guinea pig and dog PVs. More recently, sinus node cells in the PVs of human patients with AF have been described. It is possible that PVs may induce atrial arrhythmia through focal discharge and spontaneous activity.

Previous studies have shown that rapid atrial pacing (RAP) can alter the atrial electrophysiological properties, induce atrial electrical remodeling, and facilitate AF induction. After long-term RAP, PVs have shown enhanced spontaneous activity or high-frequency irregular rhythms. Likewise, in the present study, ectopic activity from a focus in the PVs was also evoked by local burst stimulation. These focal discharges are most likely related to automaticity and triggered activity, which are known to develop in the PV myocytes as a result of pacing-induced remodeling. Chen et al investigated the action potential and ionic currents in PV cardiomyocytes from long-term RAP dogs and found that the PV cardiomyocytes with pacemaker activity had a higher incidence of delayed or early afterdepolarizations (DAD and EAD, respectively). PV cardiomyocytes have ionic characteristics similar to those of SA nodal cells. Isoproterenol can induce DAD and EAD. In addition, Schauerte et al reported that high-frequency electrical stimulation (HFS) in the LSPV also induced EADs, which triggered ensuing ectopic beats and short runs of AF. Furthermore, in isolated PV specimens, Honjo et al recorded spontaneous action potentials during rapid pacing and showed that a train of rapid stimulation caused self-terminating spontaneous activity in most of the PV preparations after treatment with ryanodine. It is very interesting that spontaneous activity could be induced only in PVs and not in the atrial muscle.

In the process of measuring the AF threshold, we found some evidence of acute electrical remodeling. Non-sustained AF (<30 s) was often induced with threshold stimulation at 1 of the sites in the atria or PVs. When AF terminated, after a few seconds (~3 s), subthreshold stimulation also induced AF. If we performed repeated stimulation after a longer pause (i.e., 2 min), the same subthreshold stimulation could not induce AF. This phenomenon resembles findings in patients with paroxysmal AF. In clinical studies, recurrence of AF tends to occur early after electrical cardioversion, possibly because of high vulnerability to AF as a result of persistence of remodeling-induced changes in the immediate post-cardioversion period. This result suggests that rapid pacing changes the cellular characteristics of PV cardiomyocytes. It has been shown that electrical remodeling usually occurs over a long duration (days or weeks) of AF. Subsequently, the term “electrical remodeling” has also been applied to changes in electrophysiology induced by brief periods of atrial pacing or AF. Daoud et al reported that shortened atrial ERP s were observed within a few minutes of AF in humans. We first showed that shorter duration of AF (<30 s) begets AF and we hypothesize that pacing-dependent changes in intracellular currents are involved in this mechanism. There is evidence that spontaneous pacing-induced activity in PVs is attenuated by either depletion of the sarcoplasmic reticulum of Ca<sup>2+</sup> or blockade of the sarcolemmal Na<sup>+</sup>–Ca<sup>2+</sup> exchanger or Cl<sup>-</sup> channels. Changes in the intracellular Ca<sup>2+</sup> and Ca<sup>2+</sup>-dependent ionic currents may be the cause of the spontaneous activity. Chen et al reported that canine PVs cells with spontaneous activity had smaller slow inward and transient outward current, but larger transient inward currents. It is possible that, in the present study, short-term (within seconds or minutes) AF-induced changes in the electrophysiological properties of the PVs were functional and metabolic.

**Focal AF and Vagal Effects**

In this study, we showed that rapid electrical stimulation from the PVs could induce AF. Even if a PV serves as a focal trigger or driver, AF can not be perpetuated without another AF substrate. Most specialists agree that not only the rapid firing itself, but also the localization of the rapid firing, is important for promoting the arrhythmogenicity of AF. Recent studies have demonstrated that the area around the PV ostium is an AF substrate that generates fibrillatory conduction. Interestingly, Tan et al found that atrial autonomic nerve density is highest in this region, which indicates that the autonomic nervous system is involved in PV arrhythmogenesis.

The parasympathetic nervous system is known to suppress sinus rhythm and induce some type of abnormal automaticity. Schauerte et al found that HFS of ganglionated plexi (GP) may induce triggering activity in the PVs. Scherlag et al showed that stimulation of epicardial fat pads that contain clusters of GP can provide a substrate for the conversion of PV firing into AF. Later, Po et al showed that stimulation of the GP situated at the PV–atrial junction can initiate spontaneous AF. A recent report by Zhou et al demonstrated that stimulation of the right anterior GP converts isolated premature depolarization from the RSPV into AF-inducing premature depolarizations. Our study found that VNS shortened the ERPs at all sites in the PVs. Moreover, we also showed that VNS decreased the AF threshold and increased the inducibility of AF at the different sites in the PVs. These findings suggest that VNS changes the electrophysiological characteristics and arrhythmogenic activity of the PVs.

We speculate that interaction between the autonomic nervous system and the local cardiac autonomic nerve system is the mechanism. The shortening of the ERPs in the PVs during parasympathetic stimulation may result from local release of the autonomic neurotransmitters, acetylcholine (ACh) and catecholamines. These neurotransmitters have been shown to shorten refractoriness in the atrium and cause enhanced automaticity or triggered firing. Our study demonstrated that premature extra-stimuli from the PVs during VNS induced AF more often. Therefore, depending on the degree of heterogeneity of shortening of the local refractory period, VNS may induce local reentrant circuits in the PVs, which then initiate and maintain AF.
The SA node is well known as a normal pacemaker and when it is damaged, the pacemaker will shift to another site outside the SA node, which is considered as a subsidiary or non-physiologic node. Pacemaking activity in cardiac cells at the distal end of the PVs has been demonstrated so it is possible that pacemaker cells in the PV may work as a subsidiary pacemaker and thus trigger AF. There is also evidence that a shift of the leading pacemaker from the SA node to an ectopic focus near the right PV–atrium junction is caused by ryanodine.\(^1\)

As a result of bilateral cervical VNS in this study, vagal protection of latent pacemakers in the PVs may allow them to escape from resetting by the dominant rhythm and thus trigger AF. A local increase in autonomic nerve activity was elicited by HFS in the report of Schauerte et al.\(^2\) It is possible that VNS increases the etopic activity of PVs through enhancement of local autonomic nerve activity. There is evidence that autonomic neurotransmitters act as the “drivers” for PV firing.\(^3\) In a recent study, Po et al found that ACN injected into the GP resulted in spontaneous occurrence of rapid excitation arising from adjacent PVs.\(^4\) On the other hand, heterogeneous parasympathetic innervation or varying cellular responsiveness to vagal stimuli may be another mechanism. If latent pacemakers in the PVs are less sensitive to vagal inhibition than physiologic pacemakers, VNS may unmask them and allow them to participate in tachyarrhythmic events. In a recent study of clinical data, Pappone et al demonstrated that circumferential PV ablation can induce complete vagal denervation and cure paroxysmal AF.\(^5\) Therefore, although the exact role of the parasympathetic nervous system in focal AF is unclear at present, it seems reasonable to speculate that some electrophysiological changes in the PVs during AF may be potentiated by the parasympathetic nervous system.

**Study Limitations**

Using a programmed stimulation protocol, we noted that most sites in the PVs evoked APDs or ATs, but few sites induced AF. Higher AF inducibility was observed during VNS. It is unclear whether APDs and ATs have the same significance for inducing AF. The study also demonstrated that local electrical stimulation in the PVs could induce focal AF. Without VNS, the AF inducibility was very low. AF induction frequency could not be obtained at each site within a PV. It is not entirely clear if localization of electrical stimulation in the PVs correlates with AF induction. Another limitation of the study is the lack of assessment of AF induction in the PVs under provoked variation of local autonomic states. Previous studies have already shown that HFS elicits a local increase in autonomic nerve activity. We do not know whether vagal (cholinergic) nerves alone or a combination of vagal and sympathetic (adrenergic) nerves are involved in the mechanism of PV arrhythmogenesis. In fact, it is impossible to intensively observe vagal effects in patients with focal AF. In addition, because of individual differences, vagal effects in each dog may not have been identical. In 1 dog, we observed that VNS induced AF before local electrical stimulation was performed. When considering the effects of VNS on the PVs, we could not exclude the possibility that VNS directly influenced atrial electrophysiological properties. Although the pattern of onset of the arrhythmia was similar to the induction of clinical AF originating from PVs, our data did not give an exact answer about the mechanism by which ectopic activity arises in these veins.

**Conclusions**

Local electrical stimulation in the PVs can evoke ectopic activity, which resembles findings in patients with focal AF. There is a high degree of correlation between VNS and AF induction. Interaction between the autonomic nervous system and local cardiac autonomic nerve system may be the mechanism.

**Acknowledgments**

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**References**


