Neural Control of Ventricular Rate in Ambulatory Dogs With Pacing-Induced Sustained Atrial Fibrillation

Hyung-Wook Park, MD, PhD; Mark J. Shen, MD; Seongwook Han, MD, PhD; Tetsuji Shionohara, MD, PhD; Mitsunori Maruyama, MD, PhD; Young-Soo Lee, MD, PhD; Changyu Shen, PhD; Chun Hwang, MD; Lan S. Chen, MD; Michael C. Fishbein, MD; Shien-Fong Lin, PhD; Peng-Sheng Chen, MD

Background—We hypothesize that inferior vena cava–inferior atrial ganglionated plexus nerve activity (IVC-IAGPNA) is responsible for ventricular rate (VR) control during atrial fibrillation (AF) in ambulatory dogs.

Methods and Results—We recorded bilateral cervical vagal nerve activity (VNA) and IVC-IAGPNA during baseline sinus rhythm and during pacing-induced sustained AF in 6 ambulatory dogs. Integrated nerve activities and average VR were measured every 10 seconds over 24 hours. Left VNA was associated with VR reduction during AF in 5 dogs (from 211 bpm [95% CI, 186–233] to 178 bpm [95% CI, 145–210]; \( P<0.001 \)) and right VNA in 1 dog (from 208 bpm [95% CI, 197–223] to 181 bpm [95% CI, 163–200]; \( P<0.01 \)). There were good correlations between IVC-IAGPNA and left VNA in the former 5 dogs and between IVC-IAGPNA and right VNA in the last dog. IVC-IAGPNA was associated with VR reduction in all dogs studied. Right VNA was associated with baseline sinus rate reduction from 105 bpm (95% CI, 95–116) to 77 bpm (95% CI, 64–91; \( P<0.01 \)) in 4 dogs, whereas left VNA was associated with sinus rate reduction from 111 bpm (95% CI, 90–1250) to 81 bpm (95% CI, 67–103; \( P<0.01 \)) in 2 dogs.

Conclusions—IVC-IAGPNA is invariably associated with VR reduction during AF. In comparison, right or left VNA was associated with VR reduction only when it coactivates with the IVC-IAGPNA. The vagal nerve that controls VR during AF may be different from that which controls sinus rhythm. (Circ Arrhythm Electrophysiol. 2012;5:571-580.)

Key Words: atrial fibrillation ▪ atrioventricular node ▪ autonomic nervous system ▪ ECG ▪ ventricular rate

Randomized clinical trials have shown that in most patients with atrial fibrillation (AF), rate control is not inferior to rhythm control as a management strategy.\(^1\) However, the mechanisms of ventricular rate (VR) control during AF remain unclear. Commonly accepted mechanisms are that the complex local atrial wavefronts play an important role in modulating atrioventricular (AV) node conduction through irregularity and randomness of the atrial activity itself\(^2\) and through repetitive anterograde concealment and electrotonic modulation of the AV node conduction.\(^3\) In addition, it is also generally accepted that autonomic nervous system inputs, especially the vagal tone, are important in modulating AV node conduction.\(^4\) Based on studies using anesthetized animals, there are parallel yet functionally distinct inputs from right and left vagal nerves to AV node.\(^5\) Left vagal nerve stimulation has been proposed as a method to control VR during AF.\(^6\) In addition to vagal nerves, it is also known that the inferior vena cava–inferior atrial ganglionated plexus (IVC-IAGP; also known as the inferior right or right inferior ganglionated plexi [GP])\(^7\) is important in modulating AV node conduction and that direct electric stimulation of this GP may slow VR during AF in human patients.\(^8\) However, none of these studies was performed in the ambulatory state with direct nerve recording. Therefore, the relative importance of right vagal nerve activity (RVNA), left vagal nerve activity (LVNA), and IVC-IAGP nerve activity (IVC-IAGPNA) in VR control during AF in ambulatory animals remains poorly understood in the ambulatory state. Furthermore, whether VR reduction is achieved by vagal nerve activity (VNA) or by IVC-IAGPNA remains unclear. We have developed methods to simultaneously record nerve activities (NAs) from the extrinsic nervous system and GPs in ambulatory dogs.\(^9\) The purpose of the present study is to use these techniques to simultaneously record RVNA, LVNA, and IVC-IAGPNA in dogs with pacing-induced sustained AF to test the hypothesis that IVC-IAGPNA is primarily responsible for VR control during AF in ambulatory dogs.

Clinical Perspective on p 580

Received August 4, 2011; accepted April 12, 2012.

From the Krannert Institute of Cardiology, Division of Cardiology, Department of Medicine (H-W.P., M.J.S., S.H., T.S., M.M., Y-S.L., S-F.L., P-S.C.), Department of Biostatistics (C.S.), and the Department of Neurology (L.S.C.), Indiana University School of Medicine, Indianapolis, IN; Central Utah Medical Clinic Cardiology, Utah Valley Regional Medical Center, UT (C.H.); Department of Pathology and Laboratory Medicine, University of California, Los Angeles, Los Angeles, CA (M.C.F.); and Chonnam National University, Gwangju, Korea (H-W.P.).

Correspondence to Peng-Sheng Chen, MD, Krannert Institute of Cardiology, Division of Cardiology, Department of Medicine, Indiana University School of Medicine, 1800 N. Capitol Ave, E475, Indianapolis, IN 46202. E-mail chenpp@iupui.edu

© 2012 American Heart Association, Inc.

Circ Arrhythm Electrophysiol is available at http://circcep.ahajournals.org

DOI: 10.1161/CIRCEP.111.967737

571
Methods
The study protocol was approved by the International Animal Care and Use Committee of the Indiana University School of Medicine and Methodist Research Institute, Indianapolis, IN, and conforms to the guidelines of the American Heart Association.

Continuous Ambulatory Nerve Recordings
We simultaneously recorded RVNA, LVNA, and IVC-IAGPNA during pacing-induced sustained (\(≥48\) hours) AF in 6 ambulatory dogs. The dogs were intubated and ventilated artificially using isoflurane inhalation. The chest was opened through a right lateral thoracotomy. A Data Sciences International (St. Paul, MN) D70-EEE radiotransmitter was implanted to record NA according to methods described in detail elsewhere.9,10 Figure 1 shows that 1 pair of electrodes was sutured onto IVC-IAGP beneath its fascia (circle), and an epicardial pacemaker lead was put between the lower right and left atrium (dashed arrow). Two pairs of electrodes were placed in the bilateral cervical vagal nerve through a subcutaneous tunnel. A modified Medtronic EnPulse pacemaker (Medtronic Inc, Minneapolis, MN) was implanted for intermittent high-rate atrial pacing. After 2 weeks of postoperative recovery, the Data Sciences International transmitter was turned on to record baseline rhythm and NA for 1 day. The left atrium was paced at 10 Hz (600 bpm; \(2\times\) the diastolic threshold) for 6 days, followed by 1 day of monitoring during which the pacemaker was turned off. The rhythm was monitored for 24 hours to determine the presence of AF. The alternating pacing-monitoring sequence was repeated until sustained (\(≥48\) hours) AF was documented. The dogs were then monitored for 13±5 (range, 8–21) days before being euthanized.

Immunocytochemical Studies
We harvested the left cervical vagal nerve from 4 normal dogs under isoflurane general anesthesia. The tissues were processed routinely, paraffin embedded, cut into 5-μm thick sections, and stained for tyrosine hydroxylase (TH) to identify adrenergic nerves and choline acetyltransferase (ChAT) to identify cholinergic nerves according to methods described elsewhere.9,10

Data Analysis
We analyzed recordings from all channels using custom-written software. NAs were considered present if there was a 3-fold increase in the signal amplitude over baseline noise. We analyzed the frequency of nerve discharge episodes and the corresponding heart rate change after each discharge. Data from both VNA were high-pass filtered at 100 Hz.11 Spike-triggered averaging was performed to allow removal of the ventricular electrograms from IVC-IAGPNA recordings by subtracting a ventricular electrogram template obtained from averaged ventricular electrograms in the observational window.12 The filtered or transformed signals were then rectified, integrated with a 100-ms time constant, and summed to represent integrated NA of 10-second segments during 24 hours of recordings. We applied bandpass filtering (5–100 Hz) on the VNA recording to obtain an ECG for analysis.9,10

Statistical Analysis
All quantitative data are presented as means±SD. Differences in the continuous variables were estimated with 95% CI, and the \(P\) values of the comparisons were calculated using paired \(t\) test. Post hoc analyses of the differences among multiple groups were adjusted for multiple comparisons using Bonferroni test. Pearson correlation coefficients were used to assess correlations among integrated NAs. Fisher exact test was used to determine the correlation among elevated RVNA, LVNA, IVC-IAGPNA, and slow VR. Slopes of the scattered plots were estimated by linear regression. Linear mixed-effects models with random intercepts were used to analyze circadian variation patterns. A 2-sided \(P\)≤0.05 was considered statistically significant. The statistical analyses were performed using Predictive Analytics Software Statistics (version 18, SPSS Inc, Chicago, IL) and SAS 9.2 (SAS Inc, Cary, NC).

Results
Vagal Control of Sinus Rate at Baseline
Sinus rate averaged 82±19 bpm at baseline (before rapid atrial pacing). In 8640 10-second segments during 24 hours, RVNA and LVNA were detected in 17.7% (1529 segments) and 23.9% (2065 segments), respectively. Intermittent sinus rate reduction or sinus pause (>2 seconds) was observed during RVNA discharge in 4 dogs (Figure 2A). In the remaining 2 dogs, LVNA (but not RVNA) discharge was associated with sinus pauses or heart rate reduction (Figure 2B). In the former 4 dogs, RVNA decreased the sinus rate by 27% (from 105 bpm [95% CI, 95–116] to 77 bpm [95% CI, 64–91] at baseline; \(P<0.01\)). In these dogs, integrated RVNA during sinus pause was significantly higher than that before pause (11% increase; 45 mV-s [95% CI, 38–54] versus 40 mV-s [95% CI, 38–51]; \(P<0.001\)). However, LVNA was not significantly different before and during sinus pauses (38 mV-s [95% CI, 23–54] versus 41 mV-s [95% CI, 25–55]; \(P=0.08\)). In 2 dogs, LVNA but not RVNA was associated with sinus rate reduction. Figure 2B shows an example in which intermittent LVNA discharge in the absence of RVNA discharge was associated with sinus pauses. In these 2 dogs, LVNA was associated with 26% reduction of heart rate (from 111 bpm [95% CI, 90–125] to 81 bpm [95% CI, 67–103]; \(P<0.01\)). RVNA during sinus pauses was significantly lower than RVNA at baseline (37 mV-s [95% CI, 27–48] versus 45 mV-s [95% CI, 31–58]; \(P<0.001\)). In contrast, LVNA during sinus pauses was significantly higher than LVNA at baseline (38 mV-s [95% CI, 27–50] versus 26 mV-s [95% CI, 18–33]; \(P<0.001\)). In all dogs studied, simultaneous RVNA and LVNA reduced spontaneous sinus rate by 11% (from 99 bpm [95% CI, 82–107] to 87 bpm [95% CI, 73–102]; \(P<0.05\)).

IVC-IAGPNA and AV Conduction in Sinus Rhythm
IVC-IAGPNA did not change sinus rate or cause sinus pause. The integrated IVC-IAGPNA was 36±13 mV-s in sinus rate.
rhythm. PR interval with IVC-IAGPNA discharge was not significantly longer than that without discharge (147 ms [95% CI, 135–159] versus 141 ms [95% CI, 127–155]; P=0.12). We did not observe any second- or third-degree AV block in sinus rhythm.

**RVNA, LVNA, IVC-IAGPNA During Sustained AF**

All dogs developed sustained AF after 3.5±1.2 weeks of rapid atrial pacing. Average VR during AF was 184±45 bpm (67–269/min) during 24 hours. The VR was 215±33 bpm when neither RVNA nor LVNA was active. VRs associated

### Table. RVNA, LVNA, and IVC-IAGPNA During Sustained AF

<table>
<thead>
<tr>
<th>VNA Associated With VR Reduction in AF</th>
<th>Dog No.</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average VR during AF, bpm</td>
<td></td>
<td>186±34</td>
<td>182±46</td>
<td>183±32</td>
<td>192±56</td>
<td>183±36</td>
<td>187±51</td>
</tr>
<tr>
<td>Average VR associated with each NA, bpm</td>
<td></td>
<td>RVNA (−), LVNA (−), IVC-IAGPNA (−)</td>
<td>214±25</td>
<td>217±23</td>
<td>227±26</td>
<td>221±27</td>
<td>217±30</td>
</tr>
<tr>
<td></td>
<td></td>
<td>RVNA (+), LVNA (+), IVC-IAGPNA (+)</td>
<td>187±42</td>
<td>186±44</td>
<td>187±59</td>
<td>212±48</td>
<td>193±52</td>
</tr>
<tr>
<td></td>
<td></td>
<td>RVNA (−), LVNA (+), IVC-IAGPNA (+)</td>
<td>139±26</td>
<td>143±24</td>
<td>124±25</td>
<td>129±21</td>
<td>128±20</td>
</tr>
<tr>
<td></td>
<td></td>
<td>RVNA (+), LVNA (−), IVC-IAGPNA (+)</td>
<td>197±34</td>
<td>185±32</td>
<td>182±33</td>
<td>180±30</td>
<td>168±33</td>
</tr>
<tr>
<td></td>
<td></td>
<td>RVNA (+), LVNA (+), IVC-IAGPNA (+)</td>
<td>202±33</td>
<td>195±44</td>
<td>204±38</td>
<td>217±38</td>
<td>220±33</td>
</tr>
<tr>
<td></td>
<td></td>
<td>RVNA (−), LVNA (−), IVC-IAGPNA (+)</td>
<td>164±46</td>
<td>151±40</td>
<td>154±25</td>
<td>169±11</td>
<td>135±33</td>
</tr>
<tr>
<td></td>
<td></td>
<td>RVNA (−), LVNA (+), IVC-IAGPNA (+)</td>
<td>181±56</td>
<td>180±56</td>
<td>176±41</td>
<td>165±38</td>
<td>166±36</td>
</tr>
<tr>
<td></td>
<td></td>
<td>RVNA (+), LVNA (−), IVC-IAGPNA (+)</td>
<td>229±46</td>
<td>219±45</td>
<td>210±47</td>
<td>231±39</td>
<td>209±47</td>
</tr>
</tbody>
</table>

**Integrated NA, mV-s**

<table>
<thead>
<tr>
<th></th>
<th>RVNA</th>
<th>LVNA</th>
<th>IVC-IAGPNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>≥100 bpm</td>
<td>46±31</td>
<td>44±30</td>
<td>36±28</td>
</tr>
<tr>
<td>&lt;100 bpm</td>
<td>28±26</td>
<td>36±28</td>
<td>31±22</td>
</tr>
<tr>
<td>≥100 bpm</td>
<td>42±16</td>
<td>34±14</td>
<td>37±10</td>
</tr>
<tr>
<td>&lt;100 bpm</td>
<td>49±21</td>
<td>48±21</td>
<td>46±19</td>
</tr>
<tr>
<td>≥100 bpm</td>
<td>42±16</td>
<td>39±14</td>
<td>36±13</td>
</tr>
<tr>
<td>&lt;100 bpm</td>
<td>55±15</td>
<td>50±13</td>
<td>53±14</td>
</tr>
</tbody>
</table>

RVNA, right vagal nerve activity; LVNA, left vagal nerve activity; IVC-IAGPNA, inferior vena cava–inferior atrial ganglionated plexus nerve activity; AF, atrial fibrillation; VNA indicates vagal nerve activity; VR, ventricular rate; NA, nerve activity; (+), presence of NA; and (−), absence of NA.
with LVNA alone, RVNA alone, and RVNA-LVNA coactivation were 175±43 bpm, 224±42 bpm, and 198±37 bpm, respectively (Table; $P<0.05$). Among all 10-second segments, 28% of 10-second segments were associated with reduced VR (<100 bpm). Five dogs showed VR reduction with LVNA discharge (Figure 3A; 16.9% reduction of VR; 219 bpm [95% CI, 185–255] versus 182 bpm [95% CI, 133–220]; $P<0.001$). One dog showed VR reduction with RVNA discharge (Figure 3B; 16.6% reduction of VR; 210 bpm [95% CI, 166–240] versus 175 bpm [95% CI, 127–213]; $P<0.001$). IVC-IAGPNA discharge was associated with 13.4% VR reduction in all dogs (194 bpm [95% CI, 142–232] versus 168 bpm [95% CI, 126–207]; $P<0.001$). Exclusive RVNA discharge was observed in 12.6±3.4%, LVNA discharge in 8.6±2.1%, and IVC-IAGPNA discharge in 28.4±4.5%. Coactivation of RVNA and LVNA was seen in 1.5±1.2%, RVNA and IVC-IAGPNA in 0.5±0.4%, and LVNA and IVC-IAGPNA in 10.5±3.8%. Discharge of all nerves was observed in 1.6±2.2% and no discharge in 36.6±5.8%. Among 4 dogs with sinus pauses or bradycardia during RVNA activation, 1 showed VR reduction associated with RVNA discharge during AF. In 2 dogs with sinus pause or bradycardia during LVNA discharge, both showed VR reduction with LVNA activation during AF.

Figure 3 shows the common patterns of VR control by these 3 nerve structures. Figure 3A shows that simultaneous LVNA and IVC-IAGPNA discharges were associated with reduction of the VR. One dog showed that simultaneous RVNA and IVC-IAGPNA discharge was associated with reduction of VR in the absence of LVNA (Figure 3B). IVC-IAGPNA discharge without RVNA or LVNA discharge in the same dog could also reduce VR (Figure 3C). However, RVNA without LVNA or IVC-IAGPNA was associated with rapid VR, not VR reduction (Figure 3D). There was no ventricular tachyarrhythmia during IVC-IAGPNA discharge. Because the atrial pacemakers were turned off at the time of the recording, the VR reduction cannot be attributed to direct electric stimulation of IVC-IAGP.

Integrated NA and Ventricular Response in 5 Dogs With LVNA Controlling VR

The VR was <100 bpm in 32±5% of the 10-second segments during which the integrated NAs were 29±22 mV-s (RVNA), 45±25 mV-s (LVNA), and 52±12 mV-s (IVC-IAGPNA). In comparison, those associated with VR ≥100 bpm were, respectively, 42±29 mV-s ($P<0.01$), 36±13 mV-s ($P<0.01$), and 38±13 mV-s ($P<0.01$). The LVNA reduced VR by 15.3% (from 211 bpm [95% CI, 186–233] to 178 bpm [95% CI, 145–210]; $P<0.001$). VR with and without RVNA discharge (205±31 bpm and 198±35 bpm, respectively) was not different ($P=0.32$). There was a good correlation between IVC-IAGPNA and LVNA in the former 5 dogs (average $r$ of $0.792±0.103$; $P<0.05$ in all).

Integrated NA and Ventricular Response in 1 Dog With RVNA Controlling VR

The VR was <100 bpm in 26% of the 10-second segments, during which the integrated NAs were 47±29 mV-s (RVNA), 38±11 mV-s (LVNA), and 34±11 mV-s (IVC-IAGPNA). In comparison,
those associated with VR≥100 bpm were, respectively, 35±21 mV·s (P<0.01), 41±13 mV·s (P<0.01), and 42±9 mV·s (P<0.01).

The VR during RVNA discharge reduced (from 208 bpm [95% CI, 197–223] to 181 bpm [95% CI, 163–200]; P<0.01), but VR with and without LVNA discharge was not different (209 bpm [95% CI, 191–227] and 197 bpm [95% CI, 179–218], respectively; P=0.18). There was also good correlation between IVC-IAGPNA and RVNA in this dog (r=0.773; P<0.05).

Relationship Between LVNA and RVNA

Five dogs showed an L-shaped relationship between RVNA and LVNA (Figure 4A) during AF. When VR was ≥100 bpm (blue dots), low levels of LVNA and IVC-IAGPNA were observed (Figure 4A and 4B). Significant positive linear correlation was present between LVNA and IVC-IAGPNA (r=0.739; P<0.01; Figure 4C), and negative correlation was present between IVC-IAGPNA and VR (r=−0.406; P<0.05; Figure 4D). However, when VR was <100 bpm (red dots), LVNA and IVC-IAGPNA were elevated (Figure 4A through 4C). In the remaining 1 dog, RVNA and LVNA did not have an L-shaped correlation. Rather, a linear correlation (Figure 5B) was present, indicating that RVNA and LVNA activate together. Coactivation of these 2 nerves may be associated with rapid VR (Figure 5A). In the same tracing, IVC-IAGPNA alone was associated with a reduction of VR. There was a positive linear correlation between RVNA and LVNA in this dog and an L-shaped relationship between VNA and IVC-IAGPNA during AF (Figure 5B through 5D). This is the same dog as that shown in Figure 3B, when coactivation of RVNA and IVC-IAGPNA was associated with VR reduction.

Figure 6A shows VR and VNAs in 5 dogs with LVNA controlling the VR during AF. ANOVA and post hoc test showed that the first group (LVNA without RVNA) was associated with a lower VR than the second group (RVNA without LVNA). There were no significant differences of VRs when RVNA and LVNA were absent (third group) or when they were present (fourth group). Figure 6B shows the distribution of NA patterns with VR≥100 bpm or <100 bpm in 1 of the 5 dogs, and when VR was ≥100 bpm, the most common NA pattern was no NA (purple). However, the most common NA pattern associated with VR<100 bpm is the lone activation of IVC-IAG without either RVNA or LVNA (orange). For all dogs studied, the proportion of IVC-IAGPNA alone (without RVNA or LVNA) increased (from 18.2% [95% CI, 13.6–22.6] at VR≥100 bpm to 42.2% [95% CI, 29.1–55.3] at VR<100 bpm; P=0.024). These findings further confirm the importance of IVC-IAGPNA in controlling VR during AF. Figure 6C shows that the magnitude of RVNA is higher when VR is ≥100 bpm than when VR is <100 bpm. On the contrary, the magnitudes of LVNA and IVC-IAGPNA were higher when VR is <100 bpm than when VR is ≥100 bpm.
Circadian Variation of Correlation of LVNA and IVC-IAGPNA and Changes of NA During Sustained AF

Figure 7A shows typical circadian variation pattern of linear correlation of LVNA and IVC-IAGPNA from 5 dogs that exhibited a linear LVNA-GPNA correlation. These NAs were lowest in the morning and in early afternoon. After sustained AF was induced, VR decreased during 14 days ($P<0.01$; Figure 7B) of recording. RVNA also decreased ($P<0.01$; Figure 7C). But, average LVNA and IVC-IAGPNA gradually increased over the same period ($P<0.01$ for both; Figure 7D and 7E).

Immunocytochemical Studies of the Cervical Vagal Nerve

The cervical vagal nerves contain sympathetic and parasympathetic components. Figure 8 shows examples of TH and ChAT staining of the left cervical vagal nerve. ChAT-positive nerve structures formed a majority of the cervical vagal nerve (Figure 8A and 8B). However, a small amount of TH-positive nerves was also present at the edge of the nerve bundles (Figure 8C and 8D). Most unexpectedly, we identified sympathetic neurons in the vagal nerve (Figure 8E), indicating that the cervical vagal nerve was a source of sympathetic innervation. The same neurons stained negative for ChAT (Figure 8F).

Discussion

In a vast majority of dogs, IVC-IAGPNA and LVNA collaborate to control VR during AF. However, in 1 of the 6 dogs, IVC-IAGPNA collaborates with RVNA to control VR during AF. IVC-IAGPNA was not entirely slaved to the VNA. Rather, it can act alone to reduce VR during AF. We also demonstrated that, although RVNA reduces sinus rate at baseline in a majority of dogs, some dogs use LVNA (but not RVNA) to reduce sinus rate. The VNA that controls the sinus rate may not be the one that controls VR during AF.

Relationship Between VNA and IVC-IAGPNA

Based on our previous observations in ambulatory animals, the intrinsic and extrinsic nervous systems often coordinate their activities to achieve maximal physiological or pathophysiological effects. We reaffirmed in the present study that there is extensive collaboration among bilateral vagal nerves and the IVC-IAGP. However, in some dogs, the LVNA and RVNA often activate independently of each other, leading to an L-shaped correlation. In 1 dog, however, the RVNA and LVNA usually coactivate, leading to a nearly linear correlation. Similar findings also exist between left stellate ganglion NA and LVNA in that 75% of the dogs showed an L-shaped correlation whereas 25% showed linear correlation. In the latter study, the patterns of sympathovagal correlation determine the duration of rapid atrial pacing.
needed to induce sustained AF. In the present study, the dogs with an L-shaped correlation between LVNA and RVNA primarily use the LVNA and IVC-IAGPNA to slow VR during AF. However, the dog with a linear correlation primarily used RVNA and IVC-IAGPNA for VR control during AF. Because VNA that controls the sinoatrial node does not always control the AV node, the functional asymmetry of LVNA and RVNA in controlling 1 structure does not predict that the same asymmetry is present in controlling the other structure. The relationship between VNA and IVC-IAGPNA could be further studied with unilateral electric stimulation of either vagal nerve and the response of IVC-IAGP during surgery could be observed. However, general anesthetic agents may partially suppress NAs, making it difficult to interpret the results.

### Contribution of IVC-IAGP in VR Control During AF

AV node-specific fat pad or GP is inconsistently named as the AV node fat pad, right inferior fat pad, IVC-left atrium fat pad, inferior interatrial GP, and inferior right GP.7,8,13 The presence and location can be confirmed in vivo with neurostimulation-based exploration. Stimulation of that GP impairs AV node conduction, while leaving sinus node and atrial function largely unaltered. In vivo studies show that sympathetic and parasympathetic activation can exert direct and indirect effects on AV node conduction.14 Sympathetic activation shortens AV conduction time directly via electrophysiological changes in the nodal tissue. AV conduction is also affected indirectly, with sympathetic stimulation accelerating sinus rate, and hence, an increase in the input into the AV node. In contrast, parasympathetic stimulation not only directly prolongs AV conduction via electrophysiological changes, but also has indirect effects on AV conduction by slowing sinus rate.15 During AF, it is also possible that the autonomic nervous system activities may affect the AV node conduction by changing the local wavefront characteristics, which indirectly affect the AV node conduction. Therefore, the overall effects of nerve discharges on AV conduction most likely depend on the balance of the direct and indirect effects. It is possible that combined effects of VNA and IVC-IAGP not only directly affect the AV nodal physiology, but also alter the local AF wavefront characteristics and, hence, the inputs to the AV node. These direct and indirect effects work together to slow VR during AF. We noted that the IVC-IAGPNA did not cause AV block when the dogs were in baseline sinus rhythm. However, as AF developed, LVNA and IVC-IAGPNA continued to increase during 2 weeks, whereas RVNA continued to decline. It is possible that significant neural remodeling occurred

---

**Figure 6.** Relationship among right vagal nerve activity (RVNA; R), left vagal nerve activity (LVNA; L), and ventricular rate (VR) in a typical dog in which LVNA and inferior vena cava-inferior atrial ganglionated plexus nerve activity (IVC-IAGPNA; I) are associated with VR reduction. **A,** VR was the lowest when LVNA was activated without RVNA. **B,** Proportion of various nerve activities (NAs) in 1 dog when VR was ≥100 bpm or <100 bpm. In the latter situation, the predominant NA pattern was IVC-IAGP activation without either RVNA or LVNA (orange). **C,** Magnitudes of LVNA and IVC-IAGPNA were higher when the VR was <100 bpm than when VR was ≥100 bpm. The magnitude of RVNA was higher when VR was ≥100 bpm than when it was <100 bpm. (+), presence of NA; and (−), absence of NA.

---
during this period and that the neural remodeling contributes to the importance of IVC-IAGP in VR control. Rapid VR during AF may lead to deterioration of left ventricular function, which may increase sympathetic and vagal nerve discharges. These findings suggest that heart failure–induced remodeling involves both branches of the autonomic nervous system. Because GP contains sympathetic and parasympathetic nerves, the remodeling process may include the IVC-IAGP and increase its discharges. Finally, in addition to interacting with VNA, the IVC-IAGPNA can also activate alone. The ability to activate alone further suggests that the IVC-IAGPNA plays an important and independent role in VR control during AF.

**Effects of Electric Stimulation**

Left vagal nerve stimulation has been proposed as a useful method for controlling VR during AF. The present study confirms that in a majority of ambulatory animals, the LVNA is associated with VR reduction, whereas the RVNA is associated with an increased VR. However, in a minority of animals, the RVNA is at least equally important as LVNA in VR control. Patients in whom RVNA controls the VR may not be responsive to this method of treatment. In addition to vagal stimulation, others have proposed to use direct IVC-IAGP stimulation or AV nodal fat pad stimulation to control the VR during AF. These methods have been tested in animal models and in humans. Chronic high-frequency electric stimulation of intrinsic cardiac neurons not only stimulates neurotransmitter release by neuronal cell membrane depolarization, but also may augment parasympathetic tone by eliciting distinct and diverse neurotrophic effects. The continued neurotrophic effects may be important in the mechanisms of VR control during chronic electric stimulation of the IVC-IAGP.

**Sympathetic Nerve Structures Within the Vagal Nerve**

We found that a majority of the nerve structures within the cervical vagal nerve stained positively for ChAT, implying these nerves are cholinergic. However, we also found that a small portion of the nerves stained positively for TH. Furthermore, TH-positive neurons are also present in the cervical
vagal nerve, indicating that the vagal nerve is also a source of sympathetic innervation. The VNAs may include sympathetic and parasympathetic components. These new findings may prove important in the understanding of autonomic control of cardiac function.

Limitations
We were not able to differentiate afferent and efferent NAs or differentiate parasympathetic and sympathetic NAs within the vagosympathetic trunk. However, the primary finding of the present study is that neither RVNA nor LVNA consistently controls the baseline sinus rate or ventricular response rate during AF. In contrast, IVC-IAGPNAs were consistently associated with VR reduction during AF, indicating the importance of IVC-IAGP in VR control. Because dogs do not eat well after cervical vagal nerve resection, we were not able to test the hypothesis that IVC-IAGP alone (ie, without VNA) can control VR during AF. Because of the limitation of the equipment, we were not able to record additional NAs, such as the stellate ganglion NA and the activity of other GPs within the heart. Therefore, the importance of those NAs in VR control remains unclear. Previous studies have shown that the patterns of local atrial activation wavefronts are important in determining whether atrial activation is conducted to the ventricles via an accessory pathway. The same may also be true for the AV node. Because we did not map the wavefront characteristics near the AV node, it remains unclear whether the NAs changed wavefront characteristics and indirectly affected AV nodal conduction. Finally, because of the small number of animals studied, extrapolating the results of these studies to the general population should be done with caution.

Acknowledgments
We thank Lei Lin, Nicole Courtney, Jessica Warfel, and Janet Hutcheson for assistance and Dr Xiaohong Zhou of Medtronic Inc and Michael Bova of St. Jude Medical Inc for donating the pacemaker equipment used in the study.

Sources of Funding
The present study was supported, in part, by National Institutes of Health grants R01HL78931, R01HL78932, R01HL71140, R21HL106554, a Heart Rhythm Society Fellowship in Cardiac Pacing and Electrophysiology (Dr Shen), a Nihon Kohden/St. Jude Medical Electrophysiology fellowship (Dr Maruyama), a Piansky Endowment (Dr Fishbein), and a Medtronic-Zipes Endowment (Dr Chen).

Disclosures
Medtronic Inc, St. Jude Medical Inc, Cyberonics Inc, and Cryocath Inc donated research equipment used in our laboratory. Dr Peng-Sheng Chen is a consultant to Cyberonics, Inc.
References


CLINICAL PERSPECTIVE

Ventricular rate (VR) control is important in managing patients with atrial fibrillation (AF). However, the mechanisms of VR control during AF remain unclear. We simultaneously and continuously recorded right vagal nerve activity, left vagal nerve activity, and inferior vena cava–inferior atrial ganglionated plexus nerve activity (IVC-IAGPNA) in ambulatory dogs with AF. We then compared the nerve discharges with the VR. Immunohistochemical staining of the cervical vagal nerves with AF. We then compared the nerve discharges with the VR. Immunohistochemical staining of the cervical vagal nerves

was performed. There are several unexpected findings. First of all, cervical vagal nerves contain sympathetic and parasympathetic nerve fibers. The cervical vagal nerves also contain sympathetic ganglion cells. Therefore, vagal nerves can be a source of sympathetic tone. A second unexpected finding is that IVC-IAGPNA is invariably associated with VR reduction during AF. In comparison, right vagal nerve activity or left vagal nerve activity is associated with VR reduction only when it coactivates with the IVC-IAGPNA. Sometimes IVC-IAGPNA alone, without either right vagal nerve activity or left vagal nerve activity, can slow down the VR. These findings suggest that IVC-IAGPNA is primarily responsible for VR control during AF in ambulatory dogs. The clinical implications of these findings are as follows: (1) clinicians should not use the term vagal tone to describe the increased activity of the parasympathetic arm of the autonomic nervous system (vagal tone could be either sympathetic or parasympathetic), and (2) IVC-IAGPNA, not the right vagal nerve activity or left vagal nerve activity, controls VR during AF. Therefore, IVC-IAGPNA is a better therapeutic target than either right or left vagal nerve activity in VR control during AF.
Neural Control of Ventricular Rate in Ambulatory Dogs With Pacing-Induced Sustained Atrial Fibrillation
Hyung-Wook Park, Mark J. Shen, Seongwook Han, Tetsuji Shinohara, Mitsunori Maruyama, Young-Soo Lee, Changyu Shen, Chun Hwang, Lan S. Chen, Michael C. Fishbein, Shien-Fong Lin and Peng-Sheng Chen

Circ Arrhythm Electrophysiol. 2012;5:571-580; originally published online May 14, 2012; doi: 10.1161/CIRCEP.111.967737
Circulation: Arrhythmia and Electrophysiology is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2012 American Heart Association, Inc. All rights reserved.
Print ISSN: 1941-3149. Online ISSN: 1941-3084

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circep.ahajournals.org/content/5/3/571

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation: Arrhythmia and Electrophysiology can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation: Arrhythmia and Electrophysiology is online at:
http://circep.ahajournals.org/subscriptions/