The ligament of Marshall (LOM) contains both nerve and muscle fibers.1–3 Sympathetic nerves from the middle cervical and stellate ganglia pass along the LOM to innervate the left ventricle.4 Parasympathetic nerve fibers from the vagus nerve traverse the LOM and innervate the left atrium, left pulmonary veins (PVs), coronary sinus, and posterior left atrial fat pads.3 The close anatomic association between nerves and muscle fibers suggests that they might work synergistically to generate atrial arrhythmia. This hypothesis was supported by direct vein of Marshall cannulation and recording in patients with atrial fibrillation (AF).5,7 Doshi et al1 showed that the LOM is a source of adrenergic atrial arrhythmia. However, because nerve activities were not recorded in that study, it is unclear whether spontaneous nerve discharges can induce atrial tachyarrhythmias, including atrial tachycardia (AT) and AF, in ambulatory animal models. Recently, we developed methods to continuously record from the stellate ganglion and vagus nerve.8,9 Using these methods, Tan et al10 showed that extrinsic cardiac nerve activity (ECNA) in the form of simultaneous sympathovagal discharges preceded the onset of paroxysmal AT and AF in 73% of the episodes. However, the triggers of the remaining atrial tachyarrhythmias are unclear. In addition to the extrinsic cardiac nervous system, there is also an extensive intrinsic cardiac nervous system that forms ganglionated plexi (GP) in the heart.11 It is possible that these intrinsic cardiac nerves can activate independently of the ECNA and contribute to atrial arrhythmogenesis. To determine the importance of intrinsic cardiac nerve activity (ICNA) in triggering atrial arrhythmias, it is necessary to directly record ICNA in ambulatory animal models of atrial arrhythmias and to correlate the nerve discharges with the onset of arrhythmias.

**Methods and Results**—We implanted radiotransmitters to record extrinsic cardiac nerve activity (ECNA; including stellate ganglion nerve activity and vagal nerve activity) and ICNA (including superior left ganglionated plexi nerve activity and ligament of Marshall nerve activity) in 6 ambulatory dogs. Intermittent rapid left atrial pacing was performed to induce paroxysmal atrial fibrillation or atrial tachycardia. The vast majority (94%) of ligament of Marshall nerve activity were preceded by or coactivated with ECNA (stellate ganglion nerve activity or vagal nerve activity), whereas 6% of episodes were activated alone without concomitant stellate ganglion nerve activity or vagal nerve activity. Paroxysmal atrial fibrillation and atrial tachycardia were invariably (100%) preceded (<5 seconds) by ICNA. Most paroxysmal atrial tachycardia events (89%) were preceded by ICNA and sympathovagal coactivation, whereas 11% were preceded by ICNA and stellate ganglion nerve activity—only activation. Most paroxysmal atrial fibrillation events were preceded only by ICNA (72%); the remaining 28% were preceded by ECNA and ICNA together. Complex fractionated atrial electrograms were observed during ICNA discharges that preceded the onset of paroxysmal atrial tachycardia and atrial fibrillation. Immunostaining confirmed the presence of both adrenergic and cholinergic nerve at ICNA sites.

**Conclusions**—There is a significant temporal relationship between ECNA and ICNA. However, ICNA can also activate alone. All paroxysmal atrial tachycardia and atrial fibrillation episodes were invariably preceded by ICNA. These findings suggest that ICNA (either alone or in collaboration with ECNA) is an invariable trigger of paroxysmal atrial tachyarrhythmias. ICNA might contaminate local atrial electrograms, resulting in complex fractionated atrial electrogram–like activity. (Circulation. 2010;121:2615–2623.)

**Key Words:** atrial fibrillation ▪ tachyarrhythmia ▪ autonomic nervous system ▪ physiology

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**Background**—Little is known about the relationship between intrinsic cardiac nerve activity (ICNA) and spontaneous arrhythmias in ambulatory animals.

**Methods and Results**—We implanted radiotransmitters to record extrinsic cardiac nerve activity (ECNA; including stellate ganglion nerve activity and vagal nerve activity) and ICNA (including superior left ganglionated plexi nerve activity and ligament of Marshall nerve activity) in 6 ambulatory dogs. Intermittent rapid left atrial pacing was performed to induce paroxysmal atrial fibrillation or atrial tachycardia. The vast majority (94%) of ligament of Marshall nerve activity were preceded by or coactivated with ECNA (stellate ganglion nerve activity or vagal nerve activity), whereas 6% of episodes were activated alone without concomitant stellate ganglion nerve activity or vagal nerve activity. Paroxysmal atrial fibrillation and atrial tachycardia were invariably (100%) preceded (<5 seconds) by ICNA. Most paroxysmal atrial tachycardia events (89%) were preceded by ICNA and sympathovagal coactivation, whereas 11% were preceded by ICNA and stellate ganglion nerve activity—only activation. Most paroxysmal atrial fibrillation events were preceded only by ICNA (72%); the remaining 28% were preceded by ECNA and ICNA together. Complex fractionated atrial electrograms were observed during ICNA discharges that preceded the onset of paroxysmal atrial tachycardia and atrial fibrillation. Immunostaining confirmed the presence of both adrenergic and cholinergic nerve at ICNA sites.

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**Key Words:** atrial fibrillation ▪ tachyarrhythmia ▪ autonomic nervous system ▪ physiology
We therefore recorded LOM nerve activity (LOMNA) and superior left GP nerve activity (SLGPNA) to determine the timing and magnitudes of ICNA. We also simultaneously recorded from the ECNA, including both the stellate ganglion nerve activity (SGNA) and vagal nerve activity (VNA). We then used intermittent pacing to facilitate the development of spontaneous paroxysmal AT and AF in the same dogs. The data were analyzed to test the hypothesis that ICNA invariably precedes paroxysmal AT and AF in ambulatory dogs.

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Methods

The animal protocol was approved by the Institutional Animal Care and Use Committee of the Indiana University School of Medicine and conforms to the guidelines of the American Heart Association. Male mongrel dogs (n = 8; 22 to 27 kg) were used in this study. The first 2 dogs were used for a preliminary study during sinus rhythm to test the feasibility of ECNA and ICNA recordings. The remaining 6 dogs underwent rapid atrial pacing to induce paroxysmal atrial tachyarrhythmias.

Implantable Radiotransmitters

We used 2 different types of Data Sciences International (DSI; St Paul, Minn) radiotransmitters in this study: model D70-EEE, which was used in previous studies for SGNA and VNA recordings, and a new DSI SNA-beta transmitter. The latter transmitter has a wider bandwidth and a higher sampling rate (5 KHz) but a shorter battery life than the standard DSI transmitter. To verify that DSI SNA-beta is capable of recording nerve activities, we implanted 2 DSI radiotransmitters in the first dog for preliminary study. The bipolar wires from both transmitters were connected to the same right stellate ganglion. The dog was allowed to recover, and recordings were made while the dog was ambulatory. As shown in Figure 1 of the online-only Data Supplement, both transmitters were adequate in recording SGNA, and SGNA recorded by both transmitters preceded the abrupt onset of sinus tachycardia in 97% of the episodes.

Surgical Procedures

Left thoracotomy was performed through the fourth intercostal space under isoflurane general anesthesia. Figure 1A shows the relative anatomic locations of SLGP, LOM, left superior PV, left inferior PV, pulmonary artery, and left atrial appendage. A pair of bipolar wires was placed in the middle portion of the LOM and connected to a DSI SNA-beta transmitter (which has only 1 recording channel) located in a subcutaneous pocket. A D70-EEE transmitter was used to record SGNA from the left stellate ganglion (Figure 1B), VNA from the superior cardiac branch of the left vagal nerve (Figure 1C), and SLGPNA. A pacing lead was implanted onto the left atrial appendage and connected to a subcutaneously positioned modified Medtronic Kappa pacemaker (Medtronic, Minneapolis, Minn) for intermittent high-rate atrial pacing.

Pacing Protocol

Six dogs underwent rapid atrial pacing to induce paroxysmal atrial tachyarrhythmia. After 2 weeks of postoperative recovery, the DSI transmitters were turned on to record baseline rhythm for 1 day (Figure 1D). Baseline is the observational period before the beginning of pacing. High-rate (640 bpm, twice the diastolic threshold) atrial pacing was then given 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 persistent (>48 hours) AF was documented. The dogs were monitored for an average of 79 ± 20 days (range, 57 to 105 days) before being euthanized. The heart and stellate ganglia were harvested for histological analysis.

Data Analysis

The signals were manually analyzed to determine the temporal relationship among nerve activities, heart rate changes, and occurrence of atrial tachyarrhythmia. In all dogs studied, we manually determined the presence or absence of nerve activities at all channels. Nerve activities were considered present if there was a 3-fold increase in the amplitude over baseline noise. When the activation of 2 nerve structures overlapped with each other, we consider these 2 nerve activities coactivated. The nerve activity was considered to precede the onset of paroxysmal AT or AF if there was nerve activity within 1 second before the onset of these arrhythmias. The nerve activities are considered associated if they either discharge simultaneously (coactivation) or activate alternatively to each other. Actual examples of association are shown in the Results section. Paroxysmal atrial tachyarrhythmia was defined as an abrupt (>50 bpm/s) increase in the atrial rate to >200 bpm that persisted for at least 5 seconds. If tachyarrhythmias were regular, we called them paroxysmal AT (PAT) episodes. The irregular tachycardias were called paroxysmal AF (PAF) episodes. In addition to manual analyses, custom-designed software was used to automatically import, filter, rectify, and calculate the integrated nerve activities (Int-NA). Hilbert transforms were used to convert the filtered ECG signals into instantaneous amplitudes and frequencies. A bandpass (30 to 500 Hz) filter was applied to the atrial electrograms before manual analyses of the complex fractionated atrial electrograms (CFAEs), defined as fractionated potentials exhibiting multiple deflections from the isoelectric line (≥3 deflections) and/or poten-
tials with continuous electric activity without isoelectric periods.\textsuperscript{14} We did not directly record a surface ECG. Instead, we applied bandpass filtering (5 to 100 Hz) on the VNA recording to obtain an ECG for analyses.\textsuperscript{10} We also bandpass filtered the SLGP signals from 5 to 100 Hz to obtain bipolar local left atrial electrograms.

Histology
Tissue samples were obtained from the recording sites and fixed in 4\% formalin for 45 minutes to an hour, followed by storage in 70\% alcohol.\textsuperscript{15} The tissues were paraffin embedded and cut perpendicularly to the atrioventricular groove according to method published by Makino et al.\textsuperscript{16} Then, 5-\mu m sections were stained with antibodies against tyrosine hydroxylase using mouse monoclonal anti–tyrosine hydroxylase (Accurate Chemical, Westbury, NY), choline acetyltransferase using goat anti–choline acetyltransferase polyclonal antibody, and growth-associated protein 43 using mouse anti–growth-associated protein 43 monoclonal antibody (both from Chemicon, Billerica, Mass).

Statistical Analysis
Data are presented as mean\pm SD. Paired \textit{t} test was used to compare the Int-NA or the incidence of arrhythmia before and after rapid atrial pacing. Cosinor analysis was used to test the circadian variation of Int-NA.\textsuperscript{17} A value of \textit{P}\leq 0.05 was considered statistically significant.

Results
Simultaneous Recording of LOMNA, SGNA, and VNA
All dogs survived the surgery without complications. Simultaneous recording of ICNA and ECNA was successful in all dogs studied. Figure 2A through 2C came from dog 2 (preliminary study), in which we attempted to simultaneously record LOMNA with SGNA, VNA, and a subcutaneous ECG during sinus rhythm. Figure 2A shows that a short burst of SGNA (arrows on SGNA) occurred alternatively with burst discharges on LOMNA. Figure 2B shows that simultaneous SGNA and LOMNA discharges were associated with heart rate acceleration to 154\% of the baseline rate. In this episode, SGNA preceded LOMNA by 1 second. In contrast to the simultaneous activations, Figure 2C shows an example of alternating SGNA and LOMNA. The latter was associated with a reduction of heart rate to 46\% of the baseline rate. In spectrum analysis, ICNA at the LOM showed a wide frequency content of up to 1300 Hz.

Figure 2. Examples of intrinsic and extrinsic cardiac nerve activity recorded simultaneously. A, Simultaneous ECG, SGNA, LOMNA, and VNA recordings showing that burst SGNA (arrows on SGNA) occurred alternatively with burst discharges on LOMNA. B, LOMNA preceded (within 1 second) or coactivated with SGNA discharges, suggesting that LOMNA was sympathetic. C, LOMNA activated alternatively with SGNA, leading to a reduction and an increase in HR, suggesting that LOMNA contains parasympathetic nerve activity. D, Continuous activation patterns of LOMNA. After SGNA withdrawal, LOMNA started to fire continuously for 81 seconds, during which there was persistent heart rate reduction. Without SGNA and VNA discharges, cyclic pattern of ICNA alone may be associated with either HR acceleration (E) or deceleration (F).
Simultaneous Recording of LOMNA, SLGPNA, SGNA, and VNA

With the success in dog 2, we added SLGPNA recording and rapid atrial pacing protocol in 6 subsequent dogs (dogs 3 to 8). In these studies, the DSI SNA-beta radiotransmitter was used to record LOMNA and the D70-EEE radiotransmitter was used to record SGNA, VNA, and SLGPNA simultaneously. In addition, we performed intermittent rapid pacing to induce PAF. We found 3 types of LOMNA: continuous (10-second continuous discharges; Figure 2D), sporadic (irregular discharges; Figure 2A, 2B, and 2C), and cyclic patterns (regular intermittent activations that repeat themselves 3 times; Figure 2E and 2F). We analyzed 84 segments of LOMNA activities. Among them, 60 (71%) showed sporadic patterns, 22 (26%) showed continuous patterns, and only 2% showed cyclic patterns. Note that the same cyclic patterns are also infrequently observed in the SGNA.9 Figure 2D shows example of the continuous pattern of LOMNA discharges that occurred immediately after termination of SGNA. The LOMNA was associated with a reduction in heart rate from 108 to 79 bpm (26% decrement).

In randomly selected 1800 thirty-second segments (15 hours) at baseline, LOMNA and SLGPNA were detected in 7.5% (135 events) and 17.1% (308 events) of the 30-second windows, respectively. In 210 selected windows with LOMNA, the vast majority (94%) were associated with ECNA (SGNA or VNA; Figure 3A). The association can be either simultaneous discharges (such as Figure 2B) or alternating discharges (such as Figure 2A, 2C, and 2D). Among them, 161 windows (77%) were associated with sympathovagal coactivation, and 36 (17%) were preceded (<1 second) by SGNA only (no VNA). In no episodes did VNA only precede or coactivate with LOMNA. Figure 3A shows the summary of all different patterns of coactivation. An example of the most common pattern (first column) is shown in Figure 3B. In the remaining 6% of episodes, LOMNA activated alone without concomitant SGNA or VNA discharges (as shown in Figure 2E and 2F). SLGPNA also showed a temporal association with the other nerve activities. Most of the SLGPNA was preceded by or coactivated with ECNA, including sympathovagal coactivation (139 windows, 90%), SGNA only (13 windows, 8%) and VNA only (2 windows, 1%). A summary of all patterns is shown in Figure 3C, and an example of a common pattern (column 2) is shown in Figure 3D. We also noted that in 75 episodes (49%), the SLGPNA coactivated with LOMNA, whereas in 79 events (51%), the SLGPNA did not coactivate with LOMNA.

To validate the above analyses, we randomly selected 72 different 30-second time segments per dog, or 576 segments from 8 dogs. LOMNA and SLGPNA were detected in 7.8% (45 events) and 16.0% (92 events) of the 30-second windows, respectively. This sensitivity was not significantly different from the data presented in the beginning of the previous paragraph. In these time segments, we also found that the vast

Figure 3. Relationship between intrinsic and extrinsic cardiac nerve activities. A, Most (94%) of the LOMNA occurred in association with either SGNA or VNA, but occasionally (6%), the LOMNA occurred in the absence of either SGNA or VNA. B, Examples of LOMNA preceded by sympathovagal and SLGPNA coactivation. C, SLGPNA always occurs with the ECNA. We found no incidence in which SLGPNA acted alone. D, An example in which SLGPNA, SGNA, and VNA occurred together; LOMNA was not observed. Arrows indicate the nerve activity.
majority of LOMNA and SLGPNA were associated with the ECNA.

ECNA and ICNA Preceding the Onset of Paroxysmal Atrial Tachyarrhythmia

Five of 6 dogs developed persistent AF after 39±24 days of rapid pacing (range, 20 to 72 days). The remaining dog was euthanized after 57 days because of pacemaker battery depletion. All dogs displayed spontaneous PATs before the development of persistent AF. The PAT episodes per dogs increased from 6±2 episodes per day at baseline to 14±5 episodes after pacing (P=0.004). The intrasubject change was 8±4 between baseline and after pacing. Of 164 episodes of PAT, 89% were preceded (<5 seconds) by ICNA and sympathovagal coactivation, whereas 11% were preceded by ICNA and SGNA without VNA. Figures 4A and 4B show typical examples of PAT episodes preceded by autonomic nerve activities. The magnified pseudo-ECG shows the differences of P-wave morphologies between sinus rhythm (Figure 4Aa) and PAT (Figure 4Ab). The PAT in Figure 4A was preceded by ICNA coactivated with sympathovagal discharges. The PAT in Figure 4B was preceded by ICNA and SGNA (black arrows) but not VNA. Same as these 2 examples, all PAT episodes were preceded by ICNA. LOMNA alone never induced PAT, although it may induce sinus tachycardia (<200 bpm). If SGNA induced sinus tachycardia, additional ICNA discharges may further accelerate heart rate. The same occurs during PAT episodes. ICNA discharges during tachycardia may influence heart rate. Although heart rate was increased by SGNA, combined SGNA and ICNA can further accelerate the sinus tachycardia (Figure 5A). We also found that LOMNA may exert the same effects on the rate of AT. Figure 5B shows an example in which LOMNA accelerated atrial rate from 472 to 485 bpm, leading to a paradoxical reduction in ventricular rate caused by reduced atrioventricular conduction. The nerve activity patterns of PAT-to-PAF conversion (n=29 episodes) appear to be different from that associated with PAT initiation. We

Figure 4. Induction of PAT by extrinsic and intrinsic cardiac nerve activities. A, An example in which ICNA occurred before ECNA and a PAT episode. The magnified pseudo-ECG shows the different P-wave morphologies during sinus rhythm (Aa) and during PAT (Ab). B, Simultaneous ICNA and SGNA leading to the onset of PAT.

Figure 5. ICNA and heart rate acceleration during preexisting sinus tachycardia. A, Sinus tachycardia after SGNA (dashed arrows). ICNA (downward arrows) induced further shortening of the R-R intervals. B, LOMNA (downward arrow) during pre-existing AT resulted in shortening of atrial cycle length and paradoxical decrement of atrioventricular (AV) conduction. Arrowheads indicate the conducted QRS complexes.
found that 28% of PAT-to-PAF conversions were preceded by coactivation of ICNA and sympathovagal discharges (Figure 6A) and 72% by ICNA alone (Figure 6B).

Increased ECNA and ICNA After Intermittent Atrial Pacing
Intermittent atrial pacing increased 24-hour Int-NA on all channels. The Int-SGNA increased from 2.3 ± 1.3 mV-s at baseline to 2.6 ± 1.3 mV-s after pacing (P = 0.022). The Int-VNA increased from 0.7 ± 0.3 mV-s at baseline to 0.8 ± 0.2 mV-s after pacing (P = 0.001). The Int-SLGPNA increased from 0.7 ± 0.4 mV-s at baseline to 2.2 ± 2.0 mV-s after pacing (P < 0.001). The Int-LOMNA increased from 3.8 ± 2.5 mV-s at baseline to 5.9 ± 1.0 mV-s after pacing (P < 0.001). As shown in Figure II of the online-only Data Supplement, the Int-SGNA increased primarily during daytime. However, the Int-VNA, Int-SLGPNA, and Int-LOMNA showed an overall increase in nerve discharges (P < 0.001), but the increment occurred in both daytime and nighttime. The intrasubject change was 0.2 ± 0.4, 0.1 ± 0.2, 1.6 ± 1.7, and 1.9 ± 1.3 mV-s for Int-SGNA, Int-VNA, Int-SLGPNA, and Int-LOMNA, respectively. Cosinor analysis showed significant circadian variation of SGNA (but not VNA, SLGP, and LOMNA) at baseline and after pacing. We also analyzed the effects of pacing duration on the change of Int-NA (Figure II of the online-only Data Supplement). All Int-NAs increased after pacing, but the ICNA doubled its baseline value earlier than ECNA (SLGPNA and LOMNA with 1 to 2 weeks, SGNA and VNA with 3 to 4 weeks), suggesting earlier remodeling of ICNA than ECNA during rapid left atrial pacing (Figure III of the online-only Data Supplement).

CFAE and ICNA
We analyzed 174 episodes of PAT or PAF for the presence of CFAE. Among them, 27 episodes were at baseline and 147 episodes occurred after pacing. We also analyzed 174 control (non-PAT/PAF) time segments. CFAE was noted in the LOM and SLGP in 141 (81%) and 105 (60%) of the PAT or PAF episodes, respectively. Eighty-five PAT or PAF episodes (49%) were preceded by CFAE at both the LOM and SLGP. In 174 control time segments, however, only 10 of 174 (6%) showed CFAE in LOM (P < 0.001 compared with 141 of 174), and 15 of 174 (9%) showed CFAE in SLGP (P < 0.001 compared with 105 of 174). Figure 7A shows an example that documents CFAE at the LOM (dashed arrows) preceding the onset of a PAT episode by >2 seconds. Rapid nerve activities (solid arrows) were seen before or occurred simultaneously with the CFAE. Figure 7B and 7C shows magnified views of the time segments B and C, respectively. Multiple complex deflections (dashed arrows) in Figure 7C are consistent with CFAE, which occurred simultaneously with the nerve activities in the LOM. Because the SLGP recording showed no fractionated potentials, there is no evidence of cross-talk between recording channels. Figure 7D shows representative CFAE at the SLGP before the onset of PAT. SLGPNA (solid arrows) and CFAE (dashed arrows) preceded the onset of PAT. A second arrow on SLGPNA indicates continuous nerve activity during PAT. Figure 7E and 7F shows enlarged time segments E and F in Figure 7D. The incidence of PAT/PAF episodes with preceding CFAE at the LOM increased after rapid pacing compared with baseline (91% versus 44%; P < 0.001), whereas CFAE at the SLGP did not show significant difference between baseline and the post-pacing period (63% versus 60%; P = 0.509).

Histological Examinations
Immunohistochemical staining of the LOM showed abundant nerve structures colocalized with the Marshall bundle (Figure 8A). There are both adrenergic (tyrosine hydroxylase positive; Figure 8B) and cholinergic (choline acetyltransferase positive; Figure 8C) nerve bundles. Growth-associated protein 43 staining was positive in both adrenergic and cholinergic nerves, consistent with active axonal growth (Figure 8D).

Discussion
This study demonstrated that (1) it is feasible to simultaneously record the ICNA and ECNA in ambulatory canine
models of atrial tachyarrhythmia; (2) most ICNA showed a close temporal relationship with ECNA, indicating communication between these 2 systems; (3) a small number of ICNAs activated without a temporal relationship with ECNAs, suggesting that ICNA may be independently arrhythmogenic; (4) ICNA activation may be associated with either tachycardia or bradycardia, suggesting that the adrenergic and cholinergic nerves within the ICNA can activate independently of each other; (5) ICNA always precedes the onset of PAT and PAF episodes, suggesting that ICNA is an invariable trigger of paroxysmal atrial tachyarrhythmias in this model; and (6) CFAE on the local electrogram occurred coincidentally with ICNA.

**Interaction Between ICNA and ECNA**

The canine extrinsic and intrinsic cardiac nervous systems are known to be anatomically similar to those in humans. It was possible to record ECNA in ambulatory dogs and to correlate the changes of ECNA to arrhythmogenesis. However, whether or not ECNA acted alone or activated together with ICNA to induce arrhythmia remains unclear. Intrinsic cardiac neurons receive inputs from both the spinal cord and medullary neurons. Studies in anesthetized animals suggested that GPs modulate the autonomic interactions between extrinsic and intrinsic cardiac nerve structures. Consistent with these observations, we showed that there are frequent interactions between ECNA and ICNA and that most of the time these 2 nervous systems seem to activate together. In a small percentage of incidences, the ICNA could activate alone without the inputs from ECNA. If ICNA activates alone, then it is possible that ICNA plays an important and independent role in cardiac arrhythmogenesis. Tan et al previously reported that sympathovagal coactivation preceded the onset of PAT.
and PAF in 73% of episodes. In the remaining 27% of episodes, there were no apparent ECNA triggers for the atrial tachyarrhythmia. The present study reproduced those results because 151 of 193 PAT and PAF episodes (78%) were preceded by sympathovagal coactivation. If we do not consider the results of ICNA recordings, 22% of the episodes would not have an apparent autonomic trigger. On the other hand, if we include the ICNA recordings, then 100% of the PAT and PAF episodes were preceded by autonomic nerve discharges. Among the AT episodes, the PAT was more often preceded by simultaneous activation of ECNA and ICNA, whereas PAT-to-PAF conversion was more often associated with ICNA alone. These findings suggest that ICNA is an irreversible trigger of atrial tachyarrhythmias in this model.

Importance of ICNA in AF

AF is characterized by 2 coexisting mechanisms (focal PV discharges and atrial reentry).\textsuperscript{21,22} Ibutilide is effective in suppressing reentry but not focal discharges. However, GP ablation is effective in suppressing the focal discharges, resulting in regularization of electrograms in both atria before termination.\textsuperscript{23} These findings suggest that autonomic remodeling produced by prolonged rapid atrial pacing\textsuperscript{23–25} is important in the mechanisms of AF in this model. In addition to the heterogeneous neural remodeling and nerve sprouting, we demonstrated in the present study a direct temporal relationship between ICNA and the spontaneous onset of AF. Our findings are consistent with previous studies that demonstrated the association between cardiac arrhythmia and nerve activity of the ventral lateral cardiac nerve and the GP near the LOM.\textsuperscript{26–29}

Neural Mechanism of CFAE

CFAE is frequently observed in human AF, and ablation aimed at the CFAE sites may terminate AF.\textsuperscript{14} Lu et al\textsuperscript{30} hypothesized that CFAE may be caused by enhanced activity of the intrinsic cardiac autonomic nervous system. Lelouche et al\textsuperscript{31} reported that a fractionated electrogram in sinus rhythm is strongly associated with parasympathetic responses during AF ablation, suggesting that parasympathetic activation during AF ablation is associated with the presence of preablation high-amplitude fractionated electrograms in sinus rhythm. Local acetylcholine release might explain this phenomenon. Although fractionated electrograms might represent atrial activity induced by underlying nerve structures, it is also possible that ICNA is itself a part of the local atrial electrograms. In the present study, we were able to measure ICNA directly at the sites with CFAE. We found that both CFAE and ICNA were recorded simultaneously at the onset of PAT/PAF episodes. Our findings confirmed an association between CFAE and ICNA as suggested by other investigators.\textsuperscript{30,31} The mechanism of CFAE could be due to rapid activity of the cardiac myocytes or to both the activation of myocytes and the activation of intrinsic cardiac nerves. In the latter situation, the electric activity of the nerves forms a part of the intracardiac electrograms.

Clinical Implications

A recent study by Pokushalov et al\textsuperscript{32} showed that selective GP ablation directed by high-frequency stimulation does not eliminate paroxysmal AF in most patients. This finding suggests that high-frequency stimulation is not a highly accurate method to locate the GPs. To locate the GPs more completely and efficiently, direct nerve recording might be necessary. In the present study, we report the feasibility of GP recordings and show that GP activation is an invariable trigger of paroxysmal AT and AF. These findings suggest that GP recordings followed by ablation might prove more reliable for locating the real substrate and thus confer a better clinical outcome.

Study Limitations

Atrial cardiac GPs are reported to contain 3 types of neurons: efferent sympathetic, efferent parasympathetic, and afferent neurons.\textsuperscript{33} These neurons might colocalize with each other. Our recording methods could not differentiate one type of nerve activity from the other. A second limitation is that we recorded only from the left stellate ganglion. The relationship between right stellate ganglion and ICNA was not determined.

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Disclosures

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References

1. Marshall J. On the development of the great anterior veins in man and mammalia: including an account of certain remnants of foetal structure found in the adult, a comparative view of these great veins in the different mammalia, and an analysis of their occasional peculiarities in the human subject. Phil Trans R Soc Lond. 1850;140:133–169.
Cardiac innervation is derived from both extrinsic and intrinsic sources. These 2 sources of innervation jointly control cardiac function, including heart rhythm and arrhythmogenesis. The intrinsic nerves form ganglionated plexi, which are concentrated in the epicardial fat pads. The present study is aimed at developing a method to record intrinsic cardiac nerve activities in ambulatory dogs and to correlate these nerve activities with the spontaneous onset of paroxysmal atrial tachyrhythmias. We found that it is feasible to record intrinsic cardiac nerve activities from the fat pads in ambulatory dogs and that these nerve activities invariably precede the onset of paroxysmal atrial tachycardia and atrial fibrillation. These findings may explain the results of recent clinical studies that show that radiofrequency catheter ablation aimed at ganglionated plexi improves the long-term outcome of atrial fibrillation ablation. Our findings may also lead to a more comprehensive strategy in developing antiarrhythmic drugs for paroxysmal atrial tachyrhythmias. The existing antiarrhythmic drugs are developed to target the action potential or calcium handling characteristics of the myocardial cells. The antiarrhythmic efficacy of the existing drugs in treating paroxysmal atrial tachyrhythmias is limited. The results of the present study suggest that intrinsic cardiac nerve activities may be the triggers of these arrhythmias. If so, then drugs designed to suppress the intrinsic cardiac nerve activity may provide additional benefit to atrial arrhythmia control.
Intrinsic Cardiac Nerve Activity and Paroxysmal Atrial Tachyarrhythmia in Ambulatory Dogs
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SUPPLEMENTAL MATERIAL

Eue-Keun Choi et al, Intrinsic Cardiac Nerve Activity and Paroxysmal Atrial Tachyarrhythmia in Ambulatory Dogs

Figure 1. Right stellate ganglion nerve activity recorded simultaneously with two different DSI radiotransmitters. In Panel A, the SGNA-1 was recorded by a DSI SNA beta transmitter. The SGNA-2 was recorded by a DSI D70-EEE transmitter. The occurrence of SGNA-1 and SGNA-2 preceded the onset of sinus tachycardia. We randomly selected 30 tachycardia episodes for analyses. The criteria for tachycardia included abrupt onset (>50 bpm) to a rate faster than 120 bpm and persisted for at least 5 s. In 29 of the 30 episodes (97%), the SGNA-1 and SGNA-2 both preceded the onset of tachycardia. However, if we amplify the Y axis, the SGNA-1 recorded nerve activity before the transient shortening of RR interval while the SGNA-2 missed these smaller changes (Panel B). These findings suggest that both of these transmitters are adequate in recording the abrupt onset of sinus tachycardia. However, SGNA-1 is more accurate in determining the smaller changes of nerve discharges associated with transient shortening of RR interval.
Figure 2. Integrated (Int) nerve activity over 24 hr period in all dogs studied. Integrated SGNA (A) showed circadian variation both before and after pacing, whereas integrated VNA (B), SLGPNA (C) and LOMNA (D) did not. Pacing significantly increased the nerve activity at all sites. During day time (8:00 AM to 18:00 PM), the Int-SGNA after pacing (3.0 ± 0.8 mV-s) was significantly higher than that before pacing (2.5 ± 0.7 mV-s, p=0.004). However, at night time (18:00 PM to 8:00 AM), the int-SGNA after pacing (2.2 ± 0.5 mV-s) was not significantly different than that before pacing (2.1 ± 0.9 mV-s, p=0.813). For Int-VNA, Int-SLGPNA and Int-LOMNA, the increase occurred both at day time and at night time.
Figure 3. Change of integrated nerve activities (Int-NA) after pacing. Int-NA of SGNA, VNA, SLGPNA and LOMNA were significantly increased compared to baseline. The increased first occurred in ICNA (SLGPNA and LOMNA) in second pacing period (PP). The increase of ECNA (SGNA and VNA) occurred later (after the 4th pacing period).