Neural Mechanisms of Paroxysmal Atrial Fibrillation and Paroxysmal Atrial Tachycardia in Ambulatory Canines

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Background—The relationship between autonomic activation and the mechanisms of paroxysmal atrial fibrillation remains unclear.

Methods and Results—We implanted a pacemaker and a radio transmitter in 7 dogs (group 1). After baseline recording, we paced the left atrium at 20 Hz for 1 week and then monitored left stellate ganglion nerve activity, left vagal nerve activity, and left atrial electrogram without pacing for 24 hours. This protocol repeated itself until sustained atrial fibrillation (>48 hours) was induced in 3 ± 1 weeks. In another 6 dogs (group 2), we cryoablated left and right stellate ganglia and the cardiac branch of the left vagal nerve during the first surgery and then repeated the same pacing protocol until sustained atrial fibrillation was induced in 7 ± 4 weeks (P = 0.01). There were 4 ± 2 episodes of paroxysmal atrial fibrillation per day and 10 ± 3 episodes of paroxysmal atrial tachycardia per day in group 1. Simultaneous sympathovagal discharges were observed to immediately precede the onset of atrial arrhythmias in 73% of episodes. In comparison, group 2 dogs had no paroxysmal atrial fibrillation (P = 0.046) or paroxysmal atrial tachycardia (P < 0.001) episodes.

Nerve sprouting, sympathetic hyperinnervation, and a massive elevation of transcardiac norepinephrine levels occurred in both groups.

Conclusions—Intermittent rapid left atrial pacing results in sympathetic hyperinnervation, paroxysmal atrial fibrillation, and paroxysmal atrial tachycardia. Simultaneous sympathovagal discharges are common triggers of these arrhythmias. Cryoablation of extrinsic sympathovagal nerves eliminated paroxysmal atrial fibrillation and paroxysmal atrial tachycardia, which suggests that simultaneous sympathovagal discharges and these arrhythmias are causally related. Because cryoablation only delayed but did not prevent sustained atrial fibrillation, autonomic nerve activity is not the only factor that determines atrial fibrillation maintenance. (Circulation. 2008; 118:916-925.)

Key Words: nervous system, autonomic ablation atrium fibrillation arrhythmia

Atrial fibrillation (AF) is a complex arrhythmia that requires a trigger for initiation and a favorable substrate for maintenance; however, the nature of the trigger remains elusive. Coumel1 proposed that both the sympathetic and parasympathetic nervous systems are important in triggering AF. Heart rate variability analyses suggest that sympathovagal activation occurs before the onset of PAF episodes.2 In vitro studies showed that combined sympathetic activation and acetylcholine infusion can facilitate the induction of AF, probably through the mechanism of late phase 3 early afterdepolarizations.3-5 These results suggest that combined sympathovagal discharges may be needed to trigger paroxysmal AF (PAF). We have successfully developed methods for continuous autonomic nerve recordings in ambulatory dogs.6,7 Wijffels et al8 showed that chronic rapid atrial pacing leads to sustained AF. We hypothesized that chronic rapid pacing may also lead to PAF. The first goal of the present study, therefore, was to develop a canine model of PAF by chronic intermittent rapid pacing and to use this model to test the hypothesis that specific patterns of autonomic nerve activity (ANA) precede the onset of PAF. The second objective of the study was to perform cryoablation of the stellate ganglia and superior cardiac branch of the vagal nerve to test the hypothesis that cryoablation of these nerve structures can prevent or reduce PAF episodes. Stellate ganglion ablation has been used in patients to control ventricular arrhythmias.9,10 A possible mechanism of its antiarrhythmic effect is reduced cardiac sympathetic innervation. Therefore, a third objective of the study was to test...
the hypothesis that bilateral stellate ganglion ablation can lead to cardiac sympathetic denervation.

Clinical Perspective p 925

Methods

The study protocol was approved by the institutional animal care and use committees. We studied 13 mongrel dogs (weight 22 to 27 kg) in 2 groups. Group 1 consisted of 7 dogs that did not undergo cryoablation. Group 2 comprised 6 dogs that underwent cryoablation of sympathovagal nerves during the first surgery. The first 7 dogs were assigned to group 1 and the second 6 dogs to group 2. Hearts from an additional 6 normal dogs were studied de novo for histological control.

Continuous Recordings of ANA

We implanted in all 13 dogs a Data Sciences Inc (DSI) D70-EEE radio transmitter to record ANA activity and simultaneous ECG according to methods described in detail elsewhere.7 Briefly, a DSI transmitter was implanted into subcutaneous tissues. Left stellate ganglion nerve activity (SGNA) was registered by suturing 1 pair of bipolar electrodes onto the caudal half of the left stellate ganglion (LSG) beneath its fascia. To record cardiac vagal nerve activity (VNA), another pair of bipolar electrodes was sutured onto the superior cardiac branch of the left vagal nerve. The final bipolar pair was sutured onto the left atrial (LA) epicardium or subcutaneously as the surface ECG. Telemetered signals from the transmitter were acquired continuously (24 hours per day, 7 days per week) while the dogs are ambulatory.

Cryoablation of Autonomic Nerves

Schwartz et al10 reported that ablation of the caudal half of the LSG and T2–T4 thoracic sympathetic ganglia was effective in preventing ventricular arrhythmias; however, atrial sympathetic innervation derives significantly from both LSG and right stellate ganglion (RSG).11 Therefore, we ablated both LSG and RSG according to the Schwartz protocol in group 2. The cranial halves of the stellate ganglion were spared to limit the potential for Horner’s syndrome,10 which includes ptosis and miosis ipsilateral to the site of stellate ganglion damage. For vagal denervation, we ablated the superior cardiac branch of the left vagal nerve. Figure 1A through 1D show the cryoablation procedure. The nerves were cryoablated with the SurgiFrost system (CryoCath Technologies, Inc, Montreal, Quebec, Canada) to achieve a tissue temperature of −146°C for 4 minutes. Subsequently, ANA was recorded from the nerve upstream of the site of ablation.

Surgical Procedures

A left thoracotomy was performed under isoflurane anesthesia in all 13 dogs. Blood was sampled simultaneously from the coronary sinus and aorta before and immediately after LSG stimulation (20 seconds, 10 mA, 20 Hz, 2-ms pulse width). Cryoablation was then performed in group 2 dogs. To confirm adequacy of ablation, we electrically...
stimulated the nerves (20 Hz, 2-ms pulse width, 20 seconds, 15 mA) before and 5 minutes after each ablation. Cryoablation was considered complete when stimulation of each ablated nerve no longer produced any changes of heart rate or blood pressure. Subsequently, DSI recording wires were implanted into the unablated (upper) half of the LSG, the superior cardiac branch of the left vagal nerve cranial to the ablated portion and to the LA epicardium. The wires were connected to a DSI transmitter, which was implanted into a subcutaneous pocket. A pacing lead was implanted onto the LA appendage and connected to a subcutaneously positioned Medtronic Irel neurostimulator (Medtronic, Minneapolis, Minn) for chronic atrial pacing (20 Hz, twice diastolic threshold, 2-ms pulse width). A small right thoracotomy was then performed. The RSG was identified and the caudal half of the RSG cryoablated. The chest was closed, and the animal recovered.

Pacing Protocol and Second Surgery
The Table illustrates the pacing protocol. After a week of postoperative recovery, the DSI was turned on to record baseline rhythm for a week. Atrial pacing was then commenced. Pacing was performed for a week at a time, alternating with nonpaced monitoring periods. When pacing was switched off, the immediate rhythm was nonsustained pacing-induced AF. When pacing-induced AF terminated, the ensuing rhythm was monitored for 24 hours to determine whether there were paroxysmal atrial arrhythmias. After this, pacing was resumed. The alternating pacing-monitoring sequence was repeated until sustained (>48 hours) AF developed. Twenty-four hours of monitoring was performed while the dog was in sustained AF. When sustained pacing-induced AF terminated, a further 24 hours of rhythm was monitored. The dog then underwent second surgery. During the second surgery, blood was sampled with a protocol similar to that in the first surgery. The dog was then euthanized, and the heart was fixed in 4% formalin for 1 hour and stored in 70% alcohol for histology.

Data Analyses
Manual analyses were done for all raw data to correlate ANA with the occurrence of atrial arrhythmia. We identified premature atrial contractions (PACs) and PAF and paroxysmal atrial tachycardia (PAT) episodes. PAT was diagnosed when there was an abrupt (>50 bpm/s) increase in the atrial rate to >200 bpm that persisted for at least 10 seconds.12 Isolated PAC was defined as an isolated premature atrial beat followed by a pause before resumption of a regular baseline atrial rhythm. A second method of analysis was to use custom-designed software to automatically import, filter, and analyze both the ANA and ECG recordings. Hilbert transforms were used to convert the filtered ECG signal into its instantaneous amplitudes and frequencies.13 Activation cycle lengths (RR intervals) and SD of RR intervals (SDR) were determined. Data from SGNA and VNA were high-pass filtered (100 Hz), rectified, integrated with a 100-ms time constant, and then summed to represent averaged nerve activity over (1) 5-second segments for 30 seconds before and after onset of atrial arrhythmias and (2) 10-second segments over 24 hours for averages of ANA.

Immunohistochemistry
Five-micrometer sections were cut from paraffin blocks of the LA and right atrial appendages and free walls, left-sided pulmonary veins, LSG, RSG, and superior cardiac branch of the left vagal nerve. The sections were stained with tyrosine hydroxylase (TH) to label sympathetic nerves, growth-associated protein 43 (GAP43) for growing nerve cones, and choline acetyltransferase for cholinergic nerves.

Catecholamine and Nerve Growth Factor ELISA Assays
Plasma norepinephrine and nerve growth factor were assayed with competitive enzyme immunoassay kits from ALPCO Diagnostics (Salem, NH) and Promega (Madison, Wis), respectively. Transcardiac levels (ng/mL) were defined as coronary sinus minus aortal concentrations.

Statistical Analyses
Data are expressed as mean±SD. Student’s t tests with unequal variance were used to compare the means of 2 groups. ANOVAs with Neuman-Keuls tests were used to compare means of multiple groups. Repeated-measures ANOVA was performed to compare transcardiac norepinephrine. Pearson’s χ² tests were performed to assess association, in contingency table analyses. A probability value ≤0.05 was considered statistically significant. The authors had full access to the data and take full responsibility for its integrity. All authors have read and agree to the manuscript as written.

Results
All dogs successfully completed the study protocol. The total duration of monitoring was 6±2 weeks in group 1 dogs and 2±4 weeks in group 2 dogs. All group 2 dogs had transient Horner’s syndrome after the first surgery that resolved completely after 1 week.

Paroxysmal Atrial Arrhythmias
Compared with baseline, group 1 dogs had a significantly increased incidence of paroxysmal atrial arrhythmias after chronic intermittent atrial pacing. After 3±1 cumulative weeks of pacing, 3 of 7 dogs developed PAF (4±2 episodes per day versus 0 at baseline, P=0.046), and all dogs had PAT (10±3 versus 2±1 episodes per day at baseline, P<0.001) and PACs (4±1 episodes per day versus 0 at baseline, P=0.002). Figure 2A illustrates the circadian variation of arrhythmic episodes. Out of a total of 283 episodes (31 PAFs, 194 PATs, and 58 PACs), 36% occurred between 4 AM and 8 AM, 36% between 8 AM and 12 PM, 16% between 12 PM and 4 PM, 5% between 4 PM and 8 PM, 6% between 8 PM and 12 PM, and 3% between 12 AM and 4 AM. Figure 2B and 2C illustrates examples of PAC (arrow) and PAT, respectively, in group 1 (cryoablated) dogs. Note the increased sympathetic discharge that precedes both PAC and PAT. Figure 3A is a typical example of PAF in group 1 dogs. In the initial 20 seconds, there was relatively quiescent SGNA and VNA associated with a sinus arrhythmia. An abrupt increase in SGNA and VNA immediately preceded (by <5 seconds)
the onset of PAF. We also observed multiple episodes of PAT to PAF conversion, of which Figure 3B is an example. Figure 3C is a 6-second close-up of the same episode shown in Figure 3B, straddling the initiation of PAF. An initial increase in VNA followed by increased SGNA resulted in an acceleration of PAT from 521 to 562 bpm and a paradoxical reduction of ventricular rate (increased RR interval). This phenomenon was probably due to increased repetitive anterograde concealment associated with faster atrial rate and due to vagal effects on the atrioventricular node. A second increase in VNA followed closely by a massive burst of SGNA preceded the onset of PAF by approximately 3 seconds.

There were specific patterns of sympathovagal discharge unique to each arrhythmia type. Figure 4 is a more detailed quantitative analysis of SGNA and VNA 30 seconds before and after the onset of PAT, PAF (Figure 4A), PACs (Figure 4B), and sinus tachycardia (Figure 4C). There were no significant differences in the patterns of ANA that preceded PAT and PAF; hence, the data were combined (Figure 4A). There were distinctive patterns of ANA preceding the onset of different arrhythmias. Sinus tachycardia (Figure 4C) can be distinguished from atrial arrhythmias (PACs, PAT, and PAF) by antecedent sympathetic activation accompanied by withdrawal of VNA. On the other hand, sympathovagal activation, rather than sympathetic activation alone, precedes the onset of paroxysmal atrial arrhythmias (Figure 4A and 4B). A greater duration and extent of the SGNA rise led to tachyarrhythmia (PAT/PAF), whereas a smaller and less sustained increase of SGNA led to a PAC. To further demonstrate a positive association between sympathovagal

Figure 2. Paroxysmal atrial arrhythmias in group 1. A, Circadian incidence of paroxysmal arrhythmias (PAC, PAT, and PAF combined) over a 24-hour period. B, Arrow points to the PAC. C, PAT induced by simultaneous sympathovagal discharge.

Figure 3. Two examples of PAF. A, Sinus rhythm to AF conversion. B, Atrial tachycardia to AF conversion. C, Magnification taken from the center of Figure 3B (line segment above ECG) showing that elevated SGNA and VNA accelerated atrial rate, which led to paradoxical reduction of ventricular rate (increased RR interval) before conversion to AF. An initial increase in VNA (1) followed by increased SGNA (2) resulted in an acceleration of PAT from 521 to 562 bpm and a paradoxical reduction of ventricular rate (increased RR interval). A second increase in VNA (3) followed closely by a massive burst of SGNA (4) preceded the onset of PAF by 3 seconds.
discharge and arrhythmias, we analyzed the frequency with which sympathovagal discharge occurred during periods with and without arrhythmia. Nerve discharge was defined as the presence of a 3-fold increase in averaged integrated nerve activity within a 5-second period over baseline nerve activity. Sympathovagal discharge was observed to immediately (10 to 15 seconds) precede the onset of PAC, PAF, and PAT in 73% of episodes. On the other hand, when periods without arrhythmia were analyzed, sympathovagal discharge occurred only 13.3% (P<0.001) of the time.

Effects of Cryoablation

Figure 1E shows histological sections of the RSG in group 2 dogs. The area enclosed by a square is enlarged in Figure 1F. Both were stained with trichrome, and the black arrows point to fibrous tissue (blue) at the sites of cryoablation. Adjacent to these sites are surviving nerves (marked as “S”). Figure 1F is a similar section to Figure 1F, showing TH-positive staining in the surviving portion of the RSG. Figure 1H is an enlargement of the left portion of Figure 1G. Brown structures (arrows) indicate positively stained nerve structures. There are intact (unablated) ganglion cells in this slide.

Effect of Cryoablation on Upstream ANA

Figure 5 shows 24-hour averages of ANA, mean RR, and SDRR during baseline sinus rhythm (before atrial pacing) on postoperative day (POD) 1 and POD 10. Compared with group 1 dogs, group 2 dogs had significantly lower POD 1 levels of SGNA (2.91±1.46 vs 5.23±1.35 mV, P=0.018), VNA (0.75±0.18 vs 1.12±0.52 mV, P=0.05), mean RR (621±71 versus 750±43 ms, P=0.006), and SDRR (141±63 versus 201±53 ms, P=0.049); however, the levels of SGNA (P=0.041), VNA (P=0.052), mean RR (P=0.002), and SDRR (P=0.003) recovered by POD 10 compared with POD 1, to the point where there was no significant difference between POD 10 levels of SGNA, VNA, and mean RR and SDRR between group 1 and 2 dogs.

Figure 6 illustrates the effects of ANA on heart rate in group 1 and 2 dogs during baseline sinus rhythm (before atrial pacing). Both examples were obtained on POD 10 or later. In group 1 dogs (Figure 6A), an initial period of increased VNA and relatively quiescent SGNA was associated with a sinus arrhythmia. This was followed by a burst of SGNA and VNA withdrawal that led to the onset of a sinus tachycardia. In group 2 dogs (Figure 6B), increased VNA and SGNA appeared to have only small effects on heart rate, which indicates that the LSG and vagal nerve were effectively disconnected from the heart. Infrequently, large bursts of SGNA (Figure 6C) were associated with sinus acceleration in group 2 dogs; however, the change was not as dramatic or as abrupt as in group 1 dogs. The dissociation between ANA and HR in group 2 dogs was persistent throughout the entire study period.

Effect of Cryoablation on Inducibility of Sustained AF

Group 1 dogs developed sustained AF after 3±1 cumulative weeks (2 to 4 weeks), whereas group 2 dogs developed sustained AF after 7±4 weeks (range 3 to 12 weeks) of atrial pacing (P=0.01). However, there was significant individual
variability, as well as overlapping durations, between these 2 groups.

**Effect of Cryoablation on Paroxysmal Atrial Arrhythmias**

Compared with group 1, group 2 (cryoablated) dogs had significantly fewer episodes of PACs (2±1 versus 4±1 episodes per day, \( P=0.011 \)), and no episodes of PAT (\( P<0.001 \)) or PAF (\( P=0.046 \)) after the termination of pacing-induced AF. The PACs in cryoablated dogs were also preceded by a sympathovagal discharge.

**ELISA Studies**

Figure 7 shows the results of plasma nerve growth factor and norepinephrine in group 1 and 2 dogs. There was an increase in transcardiac nerve growth factor in both groups (group 1 \( P=0.047 \); group 2 \( P=0.049 \)) after chronic atrial pacing compared with baseline sinus rhythm. Transcardiac norepinephrine sampled during resting state was increased significantly after chronic atrial pacing compared with baseline sinus rhythm in group 2 dogs (−1.1±1.4 versus 5.0±2.9 ng/mL, \( P=0.006 \)) but was not increased significantly in group 1 dogs (0.1±0.4 versus 1.8±0.6 ng/mL, \( P=0.42 \)). Transcardiac norepinephrine was also sampled immediately after LSG stimulation. Transcardiac norepinephrine (LSG stimulation) levels were significantly higher after chronic pacing than at baseline in group 1 dogs (12.1±4.6 versus 2.2±1.3 ng/mL, \( P=0.007 \)) but were significantly lower in group 2 dogs (4.0±7.0 versus 9.0±9.0 ng/mL, \( P=0.035 \)). These results indicate that sprouted sympathetic cardiac nerve fibers were activated.

**Figure 5.** Effects of cryoablation on upstream ANA, mean RR, and SDRR on POD 1 and POD 10. G1 indicates group 1; G2, group 2.

**Figure 6.** Effects of ANA on heart rate in non-cryoablated dogs (A) and cryoablated dogs (B, C). A, VNA activity (a) is associated with sinus arrhythmia. SGNA burst (b) and VNA withdrawal (c) were associated with sinus tachycardia in a noncryoablated dog. B, No or little change of atrial rate occurred with either VNA (d) or SGNA bursts (e) in a cryoablated dog. C, Large burst of SGNA (f) was associated with atrial rate acceleration in a cryoablated dog.
endings were functionally active but permanently disconnected from the LSG.

Immunohistochemistry Studies

Figure 8 compares the results of immunostaining in the atria between normal control and group 1 and 2 dogs. The atrial nerve density for each group was expressed as a mean of nerve densities in the LA appendage, right atrial appendage, and left pulmonary veins. The densities of TH-positive nerves within the atria were $4249 \pm 1198 \mu m^2/mm^2$ in group 1 and $7724 \pm 1476 \mu m^2/mm^2$ in group 2. Both were significantly higher than the $1521 \pm 488 \mu m^2/mm^2$ density seen in normal control dogs ($P=0.007$ and $P=0.001$, respectively). Group 2 dogs had more TH-positive nerves than group 1 dogs ($P=0.005$). Similarly, the density of GAP43-positive nerves was higher in group 1 ($9218 \pm 2214 \mu m^2/mm^2$, $P=0.007$) and group 2 ($12647 \pm 3293 \mu m^2/mm^2$, $P=0.002$) than in normal control dogs ($2141 \pm 405 \mu m^2/mm^2$). GAP43 nerve density was higher in group 2 than in group 1 dogs ($P=0.044$). There was a trend toward increased choline acetyltransferase–positive nerve densities in group 1 and 2 dogs compared with normal control; however, this was not statistically significant (group 1 $7430 \pm 2110 \mu m^2/mm^2$, $P=0.1$; group 2 $8205 \pm 1823 \mu m^2/mm^2$, $P=0.08$; normal control $4611 \pm 4322 \mu m^2/mm^2$). There was no significant difference between choline acetyltransferase–positive nerve densities between group 1 and 2 dogs ($P=0.89$).

Discussion

In the present study, we demonstrated that intermittent rapid pacing may lead to episodes of PAF and PAT. We also found that simultaneous sympathovagal discharges preceded the onset of these paroxysmal atrial arrhythmias and that specific patterns of autonomic nerve discharge differentiated between PAC, PAF/PAT, and sinus tachycardia. Cryoablation of the stellate ganglia and the superior cardiac branches of vagal nerve eliminated all episodes of PAF and PAT. Surprisingly, these antiarrhythmic effects were associated with cardiac nerve sprouting and sympathetic hyperinnervation in the atria of cryoablated dogs. The data suggest that decentralization rather than denervation of sympathovagal nerves is the antiarrhythmic mechanism of stellate ganglion and vagal nerve ablation.

Sympathovagal Discharge and Paroxysmal Atrial Arrhythmias

In the present study, we found that sympathovagal discharge characterizes the onset of paroxysmal atrial tachyarrhythmia. By contrast, sinus tachycardia is preceded by sympathetic discharge alone. These data highlight the importance of concurrent vagal activation as an arrhythmic trigger, even in
the presence of sympathetic hyperinnervation. In addition, atrial tachyarrhythmias (PAF and PAT) could be distinguished from ectopic beats by a greater and more sustained antecedent elevation of SGNA. Because of such specific ANA patterns associated with different arrhythmias, it is reasonable to conclude that ANA is a trigger for these arrhythmias. The hypothesis is strengthened by (1) consistent findings across a large number of arrhythmic episodes, (2) the close temporal relationship of ANA discharge and arrhythmia onset, (3) the fact that ANA activation preceded, rather than followed, the onset of arrhythmia, and (4) elimination of arrhythmia with sympathetic cryoablation. The cellular mechanism of these observations can be explained by late phase 3 early afterdepolarization due to increased intracellular calcium and shortened action potential during sympathetic discharge.

Effects of Cryoablation on ANA
We demonstrated that cryoablation was an effective method to achieve permanent damage to the stellate ganglion, as evidenced by fibrosis at the site of ablation. The lesion caused by cryoablation was discrete, and there was evidence of surviving neurons upstream of the lesion site. The presence of immunopositivity to TH at the upstream site confirmed that the neurons were functionally viable and enabled successful recording of ANA from the surviving neurons. The fact that SGNA and VNA both recovered to levels similar to those in noncryoablated dogs further supports this conclusion. However, these neurons were effectively disconnected from the heart, because discharges of SGNA or VNA were frequently not associated with any significant changes in heart rate, and LSG stimulation did not trigger cardiac norepinephrine release. Immediately after cryoablation, on POD 1, there was a dramatic reduction of SGNA and VNA, probably due to reversible damage induced by the low temperature. This was associated with a reduction of mean RR and SDRR compared with noncryoablated dogs. Nerve recordings confirmed significant disconnection of both sympathetic and vagal nerves from the heart, because the heart rate failed to follow the changes of ANA. On POD 10, there was recovery of SGNA, VNA, mean RR, and SDRR. At the same time, there was an increased association of SGNA and VNA with appropriate changes in heart rate. These findings suggest reestablishment, at least in part, of neural connections by nerve sprouting between the surviving neurons and the non–stellate ganglion neural pathways that traffic to the heart. Alternatively, these changes could be due to increased circulating catecholamines induced by sympathetic discharges elsewhere in the body. Histological analyses confirmed increased innervation of the heart by sympathetic nerves both in cryoablated and in noncryoablated dogs compared with normal controls. We conclude that cryoablation of extrinsic cardiac nerves effectively disrupted the connection between the central nervous system and the heart.

Effects of Cryoablation on Inducibility of Arrhythmia
Elvan et al found that radiofrequency ablation of the atria eliminated pacing-induced sustained AF, probably through autonomic tone modulation. In the present study, we found that cryoablated dogs took significantly longer periods of atrial pacing to achieve sustained electrically induced AF, a finding compatible with the results of that previous study; however, all dogs eventually developed sustained AF. One possibility is that sustained AF occurred primarily due to the changes of electrophysiological characteristics (eg, effective refractory period) of the atria or the thoracic veins rather than to sporadic ANA. Alternatively, rapid atrial pacing may have induced nerve sprouting of the intrinsic autonomic ganglia. The intrinsic autonomic ganglia are capable of developing spontaneous neural activity independent of the extrinsic control. These neural activities could be a cause of sustained AF. A recent clinical study showed that recipients with orthotopic cardiac transplantation had a very low incidence (0.3%) of AF compared with the 21% incidence of AF in patients who have undergone coronary bypass surgery. Only 3 patients had AF in the former group, and all 3 had bicaval anastomosis. The authors suggested that the complete isolation of thoracic veins in these patients is a reason for a low incidence of AF; however, complete isolation from both the extrinsic and intrinsic nervous system could also contribute to the low incidence of AF in these patients.

Autonomic Nerve Modulation as a New Strategy for AF Management
The long-term efficacy of currently available antiarrhythmic drugs for prevention of AF recurrence is far from ideal because of limited efficacy and potential side effects, particularly proarrhythmia. In the past decade, nonpharmacological electrophysiological therapies have become more popular. Targeted destruction of pulmonary vein foci by radiofrequency catheter ablation suppresses PAF. Pulmonary vein denervation enhances long-term results of circumferential ablation for PAF; however, problems include potential recurrence and a small but nontrivial risk of pulmonary vein stenosis, systemic thromboembolism, pericardial effusion, cardiac tamponade, esophageal perforation, and phrenic nerve paralysis. These limitations stimulate research toward the development of less aggressive and yet effective procedures. We demonstrated in the present study that effective autonomic decentralization can be performed by targeting some but not all extracardiac autonomic nerves. This limited strategy was effective at preventing paroxysmal atrial arrhythmias while sparing the LA. The present findings support the assumption that ablation of extracardiac nerves can be an effective alternative to ablation of intracardiac nerves by LA ablation for the treatment of AF.

Absence of Cardiac Denervation After Stellate Ganglia Ablation
Wijffels et al pioneered the concept that AF begets AF using a sheep model of intermittent pacing. The authors hypothesized that progressive electrophysiological remodeling is the mechanism by which intermittent pacing induces sustained AF. Subsequent studies suggest a contribution of neural remodeling to AF maintenance. An unexpected finding of the present study is that bilateral stellate ganglia ablation failed to either prevent the induction of sustained AF or produce
significant cardiac denervation. Rather, it produced nerve sprouting and sympathetic hyperinnervation above and beyond that induced by rapid atrial pacing. A possible mechanism is the upregulation of cardiac nerve growth factor, which might have induced nerve sprouting from the ganglion cells in the intrinsic autonomic nervous system. The increased transcardiac norepinephrine levels suggest that these TH-positive nerves were functional. These findings suggest the antiarrhythmic effects of stellate ganglion ablation were achieved by disconnection between the nerve discharges upstream of the ablation site and the atria, rather than by induction of cardiac denervation. Because sympathetic nerves are responsible not only for the release but also the reuptake of norepinephrine, the presence of abundant sympathetic nerve terminals might be beneficial by providing a sink to norepinephrine in the heart.

**Study Limitations**

We did not perform cryoablation of sympathetic and vagal nerves alone; therefore, we could not determine the relative importance of sympathetic and vagal nerve activation as a trigger for paroxysmal atrial arrhythmias. It is possible that ablation of stellate ganglia alone could prevent PAF. However, previous studies have shown that partial and/or heterogeneous denervation might promote, rather than impede, the development of electrically induced AF. A more complete ablation that involves both extrinsic sympathetic and parasympathetic nerves, as performed in the study, may be more effective in preventing PAF than ablation of either sympathetic or parasympathetic nerves alone. We ablated only the left vagal nerve, because paroxysmal AF usually originates from the pulmonary veins. Whether or not the intact right vagal nerve contributed to the antiarrhythmic effects is unknown.

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**Disclosures**

None.

**References**

CLINICAL PERSPECTIVE

Paroxysmal atrial fibrillation (PAF) is a common cardiac arrhythmia. In the present study, we developed a canine model of PAF induced by intermittent rapid atrial pacing. Continuous autonomic nerve recordings showed that simultaneous sympathovagal discharge is a common trigger for PAF in this model. Cryoablation of the stellate ganglion and cardiac branch of the vagal nerve prevented PAF. A clinical implication of this study is that similar procedures designed to reduce sympathetic and vagal outflow to the heart might prevent PAF in human patients. Stellate ganglion ablation has been used for more than 30 years to prevent recurrent ventricular arrhythmias in patients with long-QT syndrome, catecholaminergic polymorphic ventricular tachycardia, and coronary artery diseases. The results of the present study suggest that similar procedures might be useful in reducing the frequencies of PAF. Because nerve sprouting and sympathetic hyperinnervation are prominent features of the PAF model used in the present study, we propose that patients with evidence of autonomic mechanisms of PAF might be most likely to benefit from this procedure. In addition to surgical interventions, these studies also have implications for pharmacological therapy of PAF. Because simultaneous sympathovagal discharges are often the triggers of PAF, drugs that inhibit both β-blockers and acetylcholine-sensitive potassium current ($I_{KACb}$) may be more effective in preventing PAF than drugs without these actions. Amiodarone, for example, is a drug that blocks both β-receptors and $I_{KACb}$. Drugs with similar actions but with lower toxicity might improve the management of PAF.