Autonomically Induced Conversion of Pulmonary Vein Focal Firing Into Atrial Fibrillation

Benjamin J. Scherlag, PhD, William Yamanashi, PhD, Utpal Patel, MD, Ralph Lazzara, MD, Warren M. Jackman, MD
Oklahoma City, Oklahoma

OBJECTIVES
This study was designed to determine the mechanism(s) whereby focal firing from pulmonary veins (PVs) is converted into atrial fibrillation (AF).

BACKGROUND
The mechanism(s) whereby PV focal firing or even a single PV depolarization is converted into AF is unknown.

METHODS
In 14 anesthetized dogs a right thoracotomy was performed to expose the right superior pulmonary vein (RSPV). An octapolar electrode catheter was sutured alongside the RSPV so that the distal electrode pair was adjacent to the fat pad containing autonomic ganglia (AG) at the veno-left atrial (LA) junction. An acrylic plaque electrode on the fat pad allowed AG stimulation at voltages ranging from 0.6 to 4.0 V. Multi-electrode catheters were sutured to the atria with their distal electrode pairs at the fat pad-atrial junctions. Right superior pulmonary vein focal firing consisted of S1–S1 referred to as epileptiform discharges (ED). The number of EDs needed to induce AF during AG stimulation was as follows: control (no stimulation) 7 ± 4, 2.4 V; 3 ± 1, p ≤ 0.05. In seven dogs, lidocaine (2%, 0.4 cc), a neuronal blocker, was injected into the fat pad, resulting in the loss of AF inducibility in six of seven dogs at the same AG stimulation levels. Three of seven dogs showed AF inducibility only with AG stimulation ≥ 9.3 V.

RESULTS
Autonomic ganglia stimulation, without atrial excitation, caused a reduction in heart rate (HR): control 142 ± 15/min, 4.0 V; 75 ± 30/min, p ≤ 0.05. The fewest number of APDs from the RSPV required to induce AF during AG stimulation was as follows: control (no stimulation) 7 ± 4, 2.4 V; 3 ± 1, p ≤ 0.05. In seven dogs, lidocaine (2%, 0.4 cc), a neuronal blocker, was injected into the fat pad, resulting in the loss of AF inducibility in six of seven dogs at the same AG stimulation levels. Three of seven dogs showed AF inducibility only with AG stimulation ≥ 9.3 V.

CONCLUSIONS
The effects of AG stimulation at the base of the RSPV can provide a substrate for the conversion of PV firing into AF. (J Am Coll Cardiol 2005;45:1878–86) © 2005 by the American College of Cardiology Foundation

The concepts regarding the mechanistic basis for atrial fibrillation (AF) onset and maintenance have followed a circuitous course for more than 50 years. Scherf (1) in 1947 found that application of aceticum to the atrium in the experimental setting could induce AF. He proposed that a focal firing source could initiate AF. Moe et al. (2) in 1964, on the basis of a computer model, hypothesized that AF could exist as a stable mechanism independent of the triggering event. They postulated that this self-sustaining mechanism could be described as multiple wavelets in randomly rotating circuits within the atrial confines. In 1985, Allessie et al. (3), using an elegant mapping technique, showed that at any given time at least three to six self-perpetuating wavelets could be found wandering over the surface of the atrium during AF. The focal mechanism resurfaced, based on the clinical reports by Jais et al. (4) and Haissaguerre et al. (5), who found that rapidly firing foci within the pulmonary veins (PVs) consistently induced paroxysmal atrial fibrillation (PAF) and that radiofrequency ablation of these “plurifocal sources” eliminated this arrhythmia in the majority of cases treated.

The purpose of the present study was to demonstrate a mechanism whereby ectopy arising from a PV could be transduced into AF. Multiple electrophysiologic mechanisms have been proposed as the basis for focal PV firing, including abnormal automaticity, triggered activity, and micro-re-entry; however, the exact mechanism for conversion of the focal PV activity into AF is still unknown (6).

We investigated whether neural influences present in local autonomic ganglia (AG) at the base of the PVs (7–9) can play a significant role in converting rapid firing or even a single atrial premature depolarization (APD) from the PV myocardial sleeves into AF.

METHODS
Fifteen adult mongrel dogs, weighing 20 to 25 kg, were anesthetized with sodium pentobarbital, 50 mg/kg administered intravenously. Additional doses of 50 mg to 100 mg were administered hourly to maintain a surgical plane of anesthesia. Positive pressure ventilation using a respirator (Harvard Apparatus Co., Natick, Massachusetts) was instituted after the insertion of a cuffed endotracheal tube. Sheaths with side arms were inserted into both femoral arteries and veins in order to record arterial blood pressure and for delivery of drugs. Core temperature was monitored continuously with a flexible thermistor (Yellow Springs tele-thermometer, Yellow Springs Instruments, Yellow Springs, Ohio) inserted via a femoral vein. The temperature
was maintained at 36.5 ± 1.5°C using a heating pad situated under the dog. A quadripolar electrode catheter was introduced via the left femoral artery and positioned in the aortic root to record a His bundle electrogram while another catheter was inserted into a femoral vein and stabilized in the right atrium (RA) to record an RA electrogram and for atrial pacing.

The chest was opened via a right lateral thoracotomy at the fourth intercostal space. The upper lobe of the right lung was reflected and the base of the right superior pulmonary vein (RSPV) was dissected from the visceral pleura. An octapolar electrode catheter was attached to the RSPV by sutures sewn into the visceral pleura, thus avoiding damage to the vein itself (Fig. 1). The distal electrode pair was situated at the base of the RSPV adjacent to the atrial myocardium, whereas the last pair of electrodes contacted the RSPV toward the hilus at the right upper lobe. After the pericardium was opened and reflected, multielectrode catheters (Biosense-Webster Inc., Diamond Bar, California) were sutured to the atrial epicardium as shown in Figure 1. The distal electrode pairs of these electrode catheters were positioned adjacent to the fat pad, which is located between the base of the RSPV and the sulcus terminalis separating the left and right atria (Fig. 1). The fat pad has been shown to contain as many as 200 AG in both the canine and human heart and lies directly on the LA adjacent to the caudal end of the sinus node (10,11).

An acrylic plaque electrode containing two bipolar pairs was attached to the fat pad by fine sutures. The AG within the fat pad was stimulated with low-level voltage (0.6 to 4.0 V; frequency 20 Hz; duration square wave pulses 0.1 ms) via the plaque electrode in order to

**Figure 1.** Diagrammatic representation of the heart viewed through a right thoracotomy at the fourth intercostal space. An acrylic plaque electrode was sutured to the fat pad (FP) (shaded area) on the epicardium containing autonomic ganglia (AG) found at the base of the right superior pulmonary vein (RSPV). Multi-electrode catheters were sutured to the pleura so as to rest against the RSPV, attached to the left atrium (LA) extending along the superior vena cava (SVC) and to the right atrium (RA) extending toward the right atrial appendage (RAAp). In each position the distal bipolar electrode pairs were closest to the FP. SAN = sinoatrial node; IVC = inferior vena cava.

**Table 1.** Effect of Increasing Voltage Applied to the Autonomic Ganglia on Sinus Rate

<table>
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<th>Number</th>
<th>Control</th>
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<td>Average ± SD</td>
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<td>134 ± 20</td>
<td>109 ± 26</td>
<td>95 ± 25</td>
<td>84 ± 29</td>
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AF = atrial fibrillation.
produce a progressive slowing of the sinus rate (7–9). These levels of stimulation did not induce atrial excitation, although higher intensities induced AF, presumably by direct stimulation.

Surface electrocardiogram leads II and aVR (filtered at 0.1 to 250 Hz) were monitored continuously, as was femoral arterial blood pressure. All tracings were amplified and digitally recorded using a computer-based Bard Labsystem (CR Bard Inc., Billerica, Massachusetts). Intracardiac bipolar electrograms were filtered at 30 to 250 Hz.

Procedures. SIMULATION OF PULMONARY VEIN FIRING. In order to simulate focal firing from the RSPV, programmed electrical stimulation was performed at a cycle length of 330 ms (A1–A1) for 8 beats followed by atrial extra-stimuli producing APDs delivered from the RSPV at a coupling interval just longer than atrial refractoriness (2 to 4\times threshold intensity). A progressively increasing number of extra-stimuli, up to 11 APDs (A2–A12), with similar coupling intervals were applied until AF was induced.

LIDOCAINE ADMINISTRATION. In seven dogs, lidocaine (2% solution, 0.4 cc) was injected into the fat pad just beneath the epicardial surface in order to infiltrate the ganglionated plexi. The effects of this agent were determined by comparing the lowest voltage level to induce AF before and after lidocaine infiltration. Higher voltages were tested when lower voltages proved to be ineffective.

STATISTICAL ANALYSIS. All data are expressed as mean ± SD. The means were compared using a Student two-tailed t test. Two times two contingency tables were used to compare AF inducibility without and with stimulation of the AG. Chi-square analysis was applied to determine

Figure 2. The tracings from the top: electrocardiogram lead II; a His bundle recording (Hb); bipolar electrograms recorded from the distal electrode pair (D-2) of the catheter along the left atrium toward the SVC and the RA close to the FP (D-2) toward the RA appendage. In the baseline state (A), with no stimulation applied to the FP containing the AG, 10 atrial premature depolarizations (A2–A11) delivered just outside the local refractory period at the RSPV induced three beats of atrial tachycardia (AT). (B) During the delivery of 1.5 V to the AG, six atrial extra-stimuli applied with the same coupling (120 ms) to the RSPV induced atrial fibrillation (AF). Other abbreviations as in Figure 1.
statistical significance. Probability values \( \leq 0.05 \) were considered significant.

GUIDELINES FOR ANIMAL USE. All animal studies were reviewed and approved by the Institutional Animal Care and Use Committee and the Animal Studies Subcommittees of the University of Oklahoma Health Sciences Center and the Department of Veterans Affairs Medical Center (DVAMC), where the animals were housed. The DVAMC Animal Research Facility where the animals are housed is accredited by the Association for Assessment and Accreditation of Laboratory Animal Care, and the laboratories are regularly inspected by the Animal Studies Subcommittee. The investigations conform to the Guide for the Care and Use of Laboratory Animals published by the U.S. National Institutes of Health (NIH Publication No. 85-23, revised 1996).

RESULTS

Stimulation of the AG with incremental voltage levels induced a progressive slowing of the sinus rate \( (7-9) \). Table 1 shows that the average sinus rate in the baseline state (no AG stimulation) was \( 142 \pm 15/min \) and there was no significant change when 0.6 V was applied to the AG \( (134 \pm 20/min) \). However, at voltage levels of 1.5 V and above there was a significant decrease in heart rate: 1.5 V, \( 109 \pm 26; 2.4 V, 95 \pm 25; 3.2 V, 84 \pm 29; \) and 4.0 V, \( 45 \pm 30, p < 0.05 \) versus baseline. At voltages >4.0 V AF occurred in
more than 50% of cases, presumably due to direct stimulation of atrium underlying the AG.

Simulation of focal RSPV firing by as many as 11 APDs (A$_2$-A$_{12}$) applied just outside the RSPV refractory period without AG stimulation required 10 APDs (A$_2$-A$_{11}$) to initiate a short run of atrial tachycardia (AT) (Fig. 2A). In the example shown in Figure 2B, 6 APDs during AG stimulation at 1.5 V-induced AF. In Figure 3 an increase in the voltage to 3.2 and 7.0 V delivered to the AG resulted in a decrease in the number of APDs required to initiate AF from 4 to 1 APD, respectively.

Table 2 shows the fewest number of APDs delivered during pacing at 180/min from the RSPV that were required to induce AF without (control) and with various voltage levels (0.6 to 2.4 V) applied to the AG at the base of the RSPV. With no AG stimulation a mean of 7 ± 4 APDs were required to induce AF, whereas during AG stimulation at 1.5 and 2.4 V significantly fewer APDs, 3 ± 2 and 3 ± 1 respectively, p < 0.05, were required to induce AF. At the lowest voltage applied, 0.6 V, there was no significant difference compared with control.

**Lidocaine administration.** Immediately after lidocaine infiltration of the fat pad, AG stimulation at the same voltage levels, 0.6 to 2.4 V, produced little slowing of the sinus rate and induced AF in only one of seven dogs with the maximum number of APDs (A$_2$-A$_{12}$). It was possible to induce AF in three of seven dogs, but only at voltage levels ≥9.0 V.

**Mechanisms for AF initiation and maintenance.** We attempted to discern the differences between simulated focal firing that did not initiate AF and those episodes that induced AF that were sustained during AG stimulation and terminated when the AG stimulation was discontinued.

In Figure 4A a single APD (coupling interval S$_1$-S$_2$ = 120 ms) delivered to the RSPV site 3-4 during AG stimulation (4 V) induced a short run of AT (4 non-stimulated beats). The electrograms during AT showed discrete atrial potentials separated by isoelectric intervals that progressively increased in duration (from 98 to 124 ms) before termination of the AT. In contrast, in Figure 4B, delivery of a single APD at the same coupling intervals at a higher voltage (7 V) induced AF, which continued as the stimulation was maintained. Note the appearance of markedly fractionated potentials at two electrode sites (Fig. 4, boxed in areas) with no isoelectric intervals. When AG stimulation was discontinued, the rhythm became more organized, with a return to discrete potentials and isoelectric intervals before termination of AF (not shown).

**DISCUSSION**

The present studies provide the demonstration of a possible mechanism whereby focal firing, single or multiple, arising from a PV can induce PAF. Specifically, the activation of the AG at the base of the RSPV serves a mediating role, or in pathophysiologic terms, a substrate upon which the PV trigger(s) act to induce PAF. That the mediating action of the electrical stimulation of the AG was autonomically based was shown by the following:

1. Incremental voltage levels applied to the AG progressively slowed the heart rate and were directly associated with progressively fewer APDs from the RSPV being required to initiate AF.
2. Lidocaine, a neuronal blocker, injected into the AG inhibited AF inducibility or markedly increased the voltage required for APDs to induce AF.

**Focal versus macro-re-entry AF.** It should be emphasized that in the present study we simulated the clinical syndrome of focal AF initiation (4,5) by delivering APDs from the RSPV while electrically activating the adjacent AG. Hirose et al. (12) initially hypothesized that radiofrequency ablation of the AG at the base of the RSPV would reduce the incidence of AF inducibility. In actuality, they found just the opposite effect; the incidence of AF significantly increased. What factors might account for the differences between the findings of Hirose et al. and the present study? It is important to point out that in the study of Hirose et al. AF was induced by bilateral vagosympathetic trunk stimulation with application of premature atrial stimuli to the left atrium (LA). Ablation of the AG at the base of the RSPV caused an increase in the atrial refractory period in the RA. Under these conditions, maximal vagosympathetic stimulation markedly decreased the refractory period mainly in the LA, creating a sharp repolarization gradient between the left and right atria. Analysis of atrial activation maps showed that premature beats introduced into the LA-induced AF by causing block, particularly in the high RA, and produced macro-re-entrant activation around the block. This macro-re-entrant substrate was associated with increased dispersion of atrial refractoriness during vagosympathetic trunk stimulation. In a previous report (13), the same group employed an arte-
rially perfused canine atrial preparation. Stimulation of the AG was used as a surrogate for vagosympathetic trunk stimulation. Optical mapping revealed two distinct substrates for AF induction: macro-re-entry directly related to large repolarization gradients, i.e., dispersion of refractoriness, and focal patterns of initiation of AF that were not dependent on repolarization gradients.

It has been known since the early part of the 20th century that "vagal" stimulation could occasionally induce AF (14,15). With the addition of atrial stimulation, induction of AF was readily achieved and sustained as vagal stimulation was maintained (15). Only recently has it been shown that localized infusion of acetylcholine and catecholamine into the area of the AG near the sinus node without electrical stimulation could induce AF (16). Moreover, several studies have revealed that the AGs release both cholinergic and adrenergic neurotransmitters (17) among others (18). We postulate that the APDs induced in the RSPV combined with the local release of acetylcholine and catecholamines in high concentrations close to the fat pad caused AF initiation. The acetylcholine markedly shortens the refractory period, and adrenergic neurotransmitter release induces enhanced automaticity and triggered firing to induce and maintain AF as AG stimulation continued. Evidence to support this postulation comes from the recent study by Schauerte et al. (19) indicating that focal AF can be electrically induced more easily at the base of the PVs than elsewhere in the atria and that this effect was blunted by beta-blockade and abolished by atropine.

**Figure 4.** A single extra stimulus at RSPV 3-4 during AG stimulation initiated a short run of AT. Traces from the top: electrocardiogram leads II and aVR; a His bundle recording (Hb); right atrial electrogram (RA); multi-electrode catheter extending from the AG toward the superior vena cava (SVC D2→7-8); pacing site from the second pair of electrodes on the RSPV (3-4); multi-electrode catheter extending from the AG toward the right atrial appendage (RA D2→7-8); arterial blood pressure (BP). (A) AG stimulation at 4 V slowed the heart rate from 126 to 99/min (not shown). The last S1-S1 interval is shown followed by a single atrial premature depolarization (APD) (S1-S2 = 120 ms), which induced a short run of AT. The cycle length of the AT progressively slowed (cycle length 98, 106, 110, and 124 ms) before terminating.
Clinical implications. A recent report has reviewed the clinical literature dealing with the relationships between the autonomic nervous system and AF (20). The relationship between autonomics and AF has been emphasized by the clinical reports of Coumel (21,22), which have shown groups of patients with AF occurring either during sleep or with exercise or activation of the sympathetic nervous system.

It was only very recently that the clinical discovery of focal firing arising in the PVs was directly identified as the major triggers for the induction of PAF (4,5). This finding has led to the application of RF catheter ablative techniques to electrically isolate all of the veins from the LA (23,24). On average, this approach results in a 20% to 30% recurrence rate (23,24).

The present study raises the possibility that ablation of the autonomic elements clustered at the base of the PVs may mitigate or abolish AF inducibility. Pappone et al. (25) found that 101 of 297 patients undergoing left atrial circumferential ablation showed a “vagal” response, i.e., slowing of the heart rate during sinus rhythm or the ventricular response during AF. By identification of so-called hot spots at the base of each PV as the site of vagal response, Pappone et al. (25) found that loss of the vagal response during applied RF current was associated with a 99% success rate in this group during a follow-up of 12 months. These findings bring into question whether PV isolation is a necessary end point for successful AF ablation (26). A recent study by Kumaraswamy et al. (27) concluded that “in patients with recurrent AF after PV isolation, return of PV conduction can be expected.” In repeat studies, almost 80% of previously isolated veins showed return of conduction.

It is interesting to note that another recently published
clinical report by Nademanne et al. (28) cites 302 cases (141 PAF and 161 chronic AF) in whom mapping and ablation were performed. They specifically targeted complex fractionated atrial electrograms very similar to those shown in the present study (Fig. 4, boxed areas). These were localized at the interattrial septum, LA, and PVs. Ablation of these fragmented potentials resulted in termination of AF in all 141 patients with PAF, of which 17% were receiving ibutilide. These investigators considered 89% of chronic AF as successfully ablated. In our study, we found these complex fractionated electrograms on the LA along the sulcus terminalis (interatrial septum) and adjacent to the RSPV (Fig. 4). The mechanistic bases for these complex fractionated electrograms or “type 4” electrograms has been suggested to represent pivot points of re-entry circuits (29).

Other studies have suggested that neurotransmitter release, particularly acetylcholine, can markedly shorten local refractory periods and result in high-frequency firing and consistent type 4 electrograms (30).

Study limitations. It should be noted that the present study was performed in essentially normal hearts and that focal firing from the PVs was simulated by single or multiple APDs. In experimental simulation of AF induced by chronic cardiac pacing (31–33), anatomic and electrophysiologic remodeling have been demonstrated (34); similar remodeling has been reported in patients with chronic AF (35–37). It remains to be shown, under appropriate clinical circumstances, whether electrophysiologic remodeling will change the ability of local autonomic nerve stimulation to induce AF and whether ablation of specific AG at the base of the right and left PVs can abolish AF as well as reverse electrophysiologic remodeling (34). In addition, anatomic remodeling, i.e., the development of interstitial fibrosis and marked enlargement of the atria, may provide a substrate for multiple re-entry circuits based on the multiple wavelet theory not amenable to disruption by autonomic interventions.

Acknowledgments

We thank Andrea Moseley and Barbara Prather for their secretarial assistance and Andrea Moseley, Cameron Hogan, and Abdel Al-Hamamsy for their technical assistance. We thank Dr. J. A. Armour for his helpful advice and encouragement.

Reprint requests and correspondence: Dr. Benjamin J. Scherlag, Cardiac Arrhythmia Research Institute, 1200 Everett Drive, Room ET6E103, Oklahoma City, Oklahoma 73104. E-mail: benjamin-scherlag@ouhsc.edu

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