The Role of Ganglionated Plexi in Apnea-Related Atrial Fibrillation

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Objectives
This study was conducted to simulate sleep apnea-induced atrial fibrillation (AF) in an experimental model and to determine whether neural ablation will prevent AF.

Background
An increasing number of clinical reports have associated sleep apnea and AF, and many possible mechanisms responsible for this relationship have been proposed.

Methods
Thirty dogs anesthetized with Na-pentobarbital were ventilated by a positive pressure respirator. Protocol 1 (n = 14): After a right thoracotomy, atrial and pulmonary vein programmed pacing at 2× and 4× threshold determined the shortest atrial refractory period. Obstructive apnea was induced by turning off the respirator during end expiration for 2 min. During apnea, programmed pacing was performed with S1-S2 = 5 to 10 ms earlier than the atrial refractory period. Neural activity was monitored from the ganglionated plexi (GP) adjacent to the right pulmonary veins. Protocol 2 (n = 16): Electrical stimulation identified the GP at the right pulmonary artery (RPA). Programmed pacing was again instituted, below atrial refractory period, during 2 min of apnea. After radiofrequency ablation of the RPA GP, continuous programmed pacing was again repeated during 2 min of apnea. In 5 dogs, blood gases were determined at baseline and at 2 min of apnea.

Results
Protocol 1: During apnea, S1-S2 induced AF within 85 ± 38 s (9 of 10). In 1 case, AF occurred spontaneously at 1 min 36 s of apnea. Recorded GP neural activity progressively increased before AF onset. Systolic but not diastolic blood pressure rose significantly before AF (149 ± 26 mm Hg to 193 ± 38 mm Hg, p < 0.05). In 4 dogs, autonomic blockade prevented apnea-induced AF. Protocol 2: AF induced by pacing occurred in 8 of 11 dogs within the 2-min period of apnea, before neural ablation. After ablation, 0 of 6 showed AF during 2 min of apnea (p = 0.009).

Conclusions
This experimental model of apnea shows a reproducible incidence of AF. After neural ablation of the RPA GP or autonomic blockade, AF inducibility was significantly inhibited. (J Am Coll Cardiol 2009;54:2075–83) © 2009 by the American College of Cardiology Foundation

In the last few years, there have been an increasing number of clinical reports associating sleep apnea and atrial fibrillation (AF) (1–8). Many possible mechanisms responsible for this relationship have been proposed, including hypoxemia, hypercapnea, pulmonary and systemic hypertension, autonomic factors, stretch-mediated channel activation, and pro-inflammatory factors such as C-reactive protein, among others (9).

The purpose of the present study was to develop an experimental model that simulated obstructive sleep apnea-induced AF. Using this model, we tested the possible underlying autonomic mechanisms and used ganglionated plexi (GP) ablation to prevent AF occurrence under the influence of a 2-min period of apnea.

Methods
Thirty dogs were anesthetized with Na-pentobarbital, 30 mg/kg, and maintained with 50 to 100 mg as needed. Insertion of a cuffed endotracheal tube allowed artificial ventilation by a...
positive pressure respirator (Harvard Apparatus Co., Natick, Massachusetts). The right and left femoral veins were dissected, and 8F sheaths inserted into each vessel. An electrode catheter was inserted into the left femoral artery, and passed into the aortic root to record the His bundle potential. On the right side, the side arm of the sheath in the femoral artery was connected to a pressure transducer to continuously monitor arterial blood pressure (BP). The right femoral vein was used to deliver drugs and for a continuous saline infusion. In the opposite vein, a Thermistor probe (Yellow Springs Tele-thermometer, Yellow Springs, Ohio) was inserted and passed into the inferior vena cava to continually monitor core body temperature.

After a right thoracotomy at the fourth intercostal space and reflection of the incised pericardium, multi-electrode catheters were sutured against the right superior pulmonary vein (RSPV) and onto the right atrial (RA) free wall (Fig. 1). Electrocardiogram lead II was continuously registered on a Bard computerized recording system (Bard Inc., Billerica, Massachusetts). The electrocardiographic lead and intracardiac electrograms were electronically filtered between 0.1 to 250 Hz and 30 to 250 Hz, respectively.

**Recordings of neural activity from the GP.** Neural recordings were obtained in 10 of 14 experiments in Protocol 1 (see the following text). These recordings were made from the anterior right ganglionic plexi (ARGP), located at the caudal end of the sinus node between the RSPV and the right inferior pulmonary vein (PV). A circular plastic ring was held against the surface of the atrium surrounding the ARGP to reduce local movement (10). A coated tungsten microelectrode with an exposed tip of 50 μm and an impedance of 9 to 12 Meg Ω at 1,000 Hz (F. Haer Co., Bowdoin, Maine) was mounted on a micromanipulator, which allowed the electrode to be inserted into the GP. A ground lead was attached to the chest wall. Neuronal activity was recorded only from the ARGP throughout.

The microelectrode signals were fed into pre-amplifiers (Princeton Applied Research, Model 113, Princeton, New Jersey), which were set with bandpass filters between 300 and 10 kHz, with amplification ranging from 100× to 500×. Further amplification (50× to 200×) was obtained by use of a hardwired amplifier (Spire 2, CED, Ltd., Cambridge, England). Neural recordings from the GP were made on the Bard recording system.

**Protocol 1.** In 14 dogs, programmed electrical stimulation was delivered at the RA site at 2× and 4× the diastolic thresholds. Eight S1-S1 = 330 ms cycles were followed by S1-S2, progressively decrementing by 10 and then 1 ms until the atrial refractory period (ARP) was reached. The
S1–S2 interval was fixed at 5 to 10 ms shorter than the refractory period, and the pacing algorithm was continually repeated (every 2 s). Under these conditions, no conducted response was observed during the baseline state. Apnea was induced by switching off the respirator at end expiration so as to simulate obstructive sleep apnea. The apneic period was maintained until AF occurred or 2 min had elapsed. In 4 dogs, before apnea, autonomic blockade was induced by the intravenous administration of esmolol or propranolol (1 mg/kg) and atropine (2 mg) (Fig. 3).

Protocol 2. In all dogs, we dissected a branch of the right pulmonary artery (RPA) that passed beneath the superior vena cava (SVC) and entered the upper lobe of the right lung. Cannulation of this vessel with a short 8-F sheath allowed the placement of a specially designed expandable basket probe (5 splines, each with 3 pairs of close bipolar electrodes) into the RPA adjacent to the SVC (Fig. 2). High-frequency electrical stimulation (frequency, 20 Hz, duration of each stimulus, 0.1 ms, voltage 0.6 to 4.5 V) was used to activate the neural tissues at the RPA, causing heart rate and atrioventricular (AV) conduction slowing [11,12]. An electrode catheter attached to the right atrium allowed programmed stimulation (S1–S1, S1–S2) at 2× and 4× threshold to determine the shortest ARP. Continuous programmed atrial pacing from the RA or RSPV with the S1–S2 set at 5 to 10 ms shorter than the ARP was performed and apnea induced, as described in the preceding text. This procedure was performed before and after RPA neural ablation using a probe with bipolar bar electrodes attached to a radiofrequency generator (AtriCure, Inc., Cincinnati, Ohio). When >1 bout of apnea was applied, the apneic periods were separated by 30-min intervals to allow recovery. In 5 dogs, blood gases were determined at baseline and at 2 min of apnea.

FLOW CHARTS OF THE EXPERIMENTAL PROCEDURES. To clarify the several procedures described in protocols 1 and 2, we have included flow charts (Figs. 3 and 4) elaborating the sequence of steps we followed throughout the experimental protocol.

Statistical analysis. The data are expressed as the mean ± SD of the mean. The Student t test was used for comparison of paired and unpaired data within 2 groups. Differences between interventions were determined using a 2 × 2 contingency table followed by a Fisher exact test. Probability values ≤0.05 were considered to be statistically significant.

Guidelines for animal care. All experiments were approved by the Institutional Animal Care and Use Committee of the University of Oklahoma and the Veterans Affairs Medical Center. The animals were housed at the Animal Research Facility at the Veterans Affairs Medical Center.

Results

Protocol 1. In the initial studies (n = 4), we used apnea for as long as 3 min in an attempt to induce AF. For periods of...
With the introduction of the programmed atrial pacing protocol, described in preceding text (see Methods, Protocol 1), AF was induced in 9 of 10 experiments during a period of apnea lasting ≤2 min. Figure 7B illustrates that, during S1-S1 pacing at 330 ms, an S1-S2 coupling of 100 ms (at 4× diastolic threshold) failed to elicit an atrial response. Note that in this case, neural activity was sparse and intermittent. At 1 min 30 s, S1-S2, with even shorter coupling (90 ms), now initiated AF (Fig. 7D). Note the marked increase in the neuronal firing preceding AF and the increased BP (196/160 mm Hg compared with baseline, 175/152 mm Hg).

In these 9 of 10 experiments, AF was induced by the pacing algorithm at an average time of 85 ± 38 s after the onset of apnea. The entire sequence was associated with a significant increase in systolic BP compared with control

apnea >2 min, there were some episodes that resulted in hypotension, electrocardiographic changes indicative of ischemia, and more prolonged recovery back to baseline conditions. Figure 5 illustrates the characteristic changes that occurred during this prolonged apneic period. In the baseline state, the frequency and level of GP neuronal firing was highly variable (Fig. 5A). However, a consistent finding was the decrease in neuronal activity within 15 s after the start of apnea (Fig. 5B). In this case, the amplitude decreased by 50% of the baseline activity.

As apnea continued, there was a progressive increase in the intensity and amplitude of neuronal firing. At 2.5 min, ventricular ectopic beats were observed as well as ST-segment depression (Fig. 5C). Furthermore, the amplitude of the neuronal firing increased by 2× that recorded at baseline. Figure 6 represents the 1 instance of spontaneous AF during apnea that occurred within 2 min. Although the neural recordings obtained were relatively low level at baseline (Fig. 6A), there was a notable decrease in amplitude within 15 s after apnea was induced (Fig. 6B). At 1 min 36 s, associated with the increased neural firing, a premature atrial depolarization occurred spontaneously, then a pause, a sinus beat, and an earlier coupled premature beat leading to AF (Fig. 6C).

Sequential changes are shown in electrophysiological, blood pressure (BP), and neural activity (anterior right ganglionated plexi (GP) neurons) during apnea progression. A consistent finding was the reduction of intensity and amplitude of neural firing from the anterior right GP within 15 s after the start of apnea (B compared with control, A). However, in this case, as apnea progressed and blood pressure rose, ST-segment depression and ventricular premature beats occurred, concomitant with a marked increase in the intensity and amplitude of neural firing (C). RA = right atrial.
Other significant changes caused by apnea. In most of the cases, there was slowing of the heart rate concurrent with the increased systolic BP soon after the initiation of apnea. Another consistent observation was the onset of mechanical or pulsus alternans manifested in the BP before the onset of AF (Fig. 8A).

Blood gas analysis. To determine the alterations in pH, and blood gases CO₂ and O₂, arterial blood gases were sampled at baseline and after 2 min of simulated obstructive sleep apnea in 5 dogs (I Stat-1 Analyzer, Abbott Point of Care, Inc., East Windsor, New Jersey). Table 3 illustrates the absolute and statistical comparison between these 2 states. Specifically, there were marked alterations in all the measured parameters that showed significant decreases, for example, pH, pO₂, and O₂ saturation (p < 0.05), whereas pCO₂ significantly increased (p < 0.05). In regard to the last effect, toward the end of the apneic period there were invariably attempted respiratory movements despite the surgical level of general anesthesia. That was presumably due to hypercarbia, which stimulates the respiratory center.

Discussion

Major findings. Closing the airway by shutting off the ventilator during end expiration induced a condition simulating obstructive sleep apnea since the valves of the respirator were stopped in a closed position. Within the pre-set interval of 120 ms, AF occurred at an average of 85 ± 38 s (protocol 1). The ablation of the RPA fat pad containing GP inhibited the occurrence of apnea-induced AF even though the period of apnea was extended by 30 s (150 s total apneic period, protocol 2).

Several lines of evidence provided insights into the possible mechanisms whereby apnea induced AF, either due to a premature atrial stimulus that was refractory in the baseline state or, more rarely, occurring spontaneously (Fig. 6C). A marked increase in parasympathetic influence on the heart was indicated by the shortening of refractory period, allowing the previously nonconducted premature beat to not only activate the atrium but also, in many cases, to induce AF. Furthermore, there was a progressive increase in neural firing detected in the ARGP, reaching a crescendo just before the onset of AF. Perhaps more indirect evidence was the consistent appearance of mechanical alternans in the BP recording. Mechanical alternans or pulsus alternans is readily induced by high rates or during a constant heart rate associated with weakened ventricular contractility. Because
the test for AF inducibility consisted of programmed stimulation during which S1-S2 was continually (every 2 s) delivered at a fixed coupling interval, 5 to 10 ms earlier than the baseline ARP (Fig. 8A and 8B), the appearance of mechanical alternans would likely be due to the increased parasympathetic and negative inotropic influence on the ventricles despite the overall increase in systolic BP. Indeed, the latter would also contribute to an increased parasympathetic influence through a baroreflex effect. Recent studies of the mechanisms underlying AF inducibility have clearly shown the direct involvement of both arms of the autonomic nervous system (13–15).

It is interesting to note that the most common occurrence of AF during apnea was initiated by a closely coupled atrial premature stimulus that did not result in a conducted beat in the baseline state. Similarly, atrial premature stimuli are the invariable triggers for AF with delivery of vagosympathetic trunk stimulation, namely, extrinsic autonomic innervation, to the heart. Conversely, autonomic stimulation at the level of the intrinsic cardiac nervous system, namely, GP and associated neural network, directly induces AF, whether from PV or non-PV sites (16–18). Specifically, it has been shown that activation of local parasympathetic nerves, inducing shortening of atrial refractoriness, and stimulation of local sympathetic nerves serve as the direct triggers for AF through early after-depolarizations (17). Taken together, this would suggest that the major autonomic influence for apnea-induced AF is primarily occurring from the extrinsic input to the heart. It could be argued that, during apnea, the atrial capture occurring earlier than the baseline ARP induced AF as a result of falling in the atrial vulnerable period and was unrelated to the associated autonomic changes. However, the finding that, during apnea, after RPA ablation, atrial capture could still occur before the

![Figure 7](AF Induction During Apnea With Delivery of Atrial Premature Stimuli Earlier Than Baseline ARP)

Atrial fibrillation (AF) was induced during apnea with the delivery of atrial premature stimuli 5 to 10 ms earlier than the atrial refractory period (ARP) determined in the baseline state. (A) In this experiment, very sparse neural activity was recorded from the anterior right ganglionated plexi (GP) neurons (arrows) during the baseline state (control). (B) At 1 min 30 s of apnea, programmed stimulation was started with a basic 8-beat drive cycle (S1-S1 = 330 ms). The premature stimulus was delivered with a coupling interval 10 ms earlier than the atrial refractory period (S1-S2 = 110 ms) (C). The premature stimulus now induced atrial capture and a spontaneous atrial premature depolarization (APD) (arrow) followed by AF (D). Note the associated increase in frequency, intensity, and amplitude of neural activity as well as the increased BP. Abbreviations as in Figure 5.

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<tr>
<th>Experiment #</th>
<th>Systolic Blood Pressure</th>
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p value 0.05 0.08
baseline ARP without inducing AF (Table 2) somewhat mitigates this argument.

Consonant with this view was the observation that the ablation of the RPA fat pad containing GP inhibited the occurrence of apnea-induced AF during the 2-min apneic interval. This specific ganglionic cluster has been shown to be the “head-stage” or nexus point for the vagosympathetic trunk (extrinsic) innervation to the heart (11,19). Indeed, the end point for determining the ablation of this GP was the inability to slow the heart rate or AV conduction by stimulation of either right or left vagosympathetic trunks at the voltage that reduced the heart rate by 50% or induced 2:1 AV block before RPA GP ablation.

Another observation that deserves some attention was that the recorded neural activity from the ARGP showed a noticeable diminution. An example is shown in Figure 5, in the first 15 s after the start of apnea. A recent study from our laboratory showed that vagal stimulation under normal circumstances suppressed GP firing (20). However, as apnea progressed, marked increases in parasympathetic and sympathetic activation were reflected in the increased neural firing, and these effects were presumably responsible for the occurrence of AF, either spontaneously or induced by programmed stimulation (18,21).

**Clinical implications.** At the Cleveland Clinic, Natale et al. have been using a combined strategy of PV antrum isolation (22) and SVC isolation (23) by radiofrequency catheter ablation for drug- and cardioversion-resistant AF. These investigators, in describing which patient population would benefit from superior vena cava isolation, stated, “In our experience . . . superior vena cava isolation (SVCI) is important in patients with obesity or sleep apnea” (24). This statement was inserted in that publication as a clinical impression rather than based on data and follow-up studies indicating actual success rates. It is interesting to note that the technique used for SVC isolation consists of “the placement of the circular catheter and ablation is performed just above the RA-SVC junction at the lower border of the pulmonary artery as seen by intracardiac echocardiography” (25). In the present study, this site was the area that, when activated by high-frequency stimuli, manifested slowing of the heart rate and AV conduction, namely, the location of the RPA GP.

**Study limitations.** Neural recordings were made only from the ARGP, assuming that these responses before and during apnea would be representative of the activity of the other GP in the intrinsic cardiac autonomic system. Moreover, we did not determine the mechanism, whether focal or re-entrant, for the apnea-induced AF. However, the finding that the most common method for AF initiation was based on an atrial premature beat suggests that the resulting AF was re-entrant, as shown

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<th>Table 2: Comparison of AF Inducibility and Atrial Refractoriness (Atrial Capture) Before and After Ganglionated Plexi Ablation</th>
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<td>After ablation (n = 6)</td>
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p values determined from 2 × 2 contingency calculator followed by 2-tailed Fisher exact test.
AF = atrial fibrillation.
by Hirose et al. (26) in their study that vagal stimulation and premature atrial beats induced AF. It should be emphasized that our experimental model, in the acute setting of the normal dog heart, is only a simulated form of sleep apnea that has several features similar to patients with chronic obstructed sleep apnea, particularly regarding to the tendency toward associated AF. In this regard, there are 2 similar features between our experimental model and clinically observed obstructive sleep apnea: 1) we specifically turned off the respirator during the end expiration, ensuring that the piston of the respirator had obstructed the airways; and 2) there was a predominance of parasympathetic influence during induced apnea as manifested by the slowing of the heart rate and mechanical alternans. However, sympathetic activation was shown by the increase in the BP. Clinical reports have differentiated obstructive from central sleep apnea by a pattern of heart rate variability, with obstructive sleep apnea showing high-frequency power (parasympathetic influence) and reduced very low-frequency power (sympathetic influence).

Only 6 of 11 dogs were tested for the occurrence of apnea-induced AF after RPA GP ablation; this may have skewed our data since almost the same number of dogs, namely, 5, were not tested. However, the 6 passed a more rigorous test consisting of a longer 2.5-min, rather than a 2-min, apneic period without manifesting AF.

Conclusions

This experimental model of apnea shows a significant and reproducible incidence of AF. After neural ablation of the RPA GP or autonomic blockade, AF inducibility was inhibited.

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References


Key Words: atrial fibrillation • autonomic nervous system • sleep apnea.