Relation Between Ligament of Marshall and Adrenergic Atrial Tachyarrhythmia

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Background—The mechanism of the adrenergic atrial tachyarrhythmia is unclear. We hypothesize that the ligament of Marshall (LOM) is sensitive to adrenergic stimulation and may serve as a source of the adrenergic atrial tachyarrhythmia.

Methods and Results—We performed computerized mapping studies in isolated-perfused canine left atrial tissues from normal dogs (n = 9) and from dogs with chronic atrial fibrillation (AF) induced by 10 to 41 weeks of rapid pacing (n = 3). Before isoproterenol, spontaneous activity occurred in only one normal tissue (cycle length, CL = 1300 ms). During isoproterenol infusion, automatic rhythm was induced in both normal tissues (CL = 578 ± 172 ms) and AF tissues (CL = 255 ± 29 ms, P < 0.05). The origin of spontaneous activity was mapped to the LOM. In the AF tissues, but not the normal tissues, we observed the transition from rapid automatic activity to multiple wavelet AF. Ablation of the LOM terminated the spontaneous activity and prevented AF. Immunocytochemical studies of the LOM revealed muscle tracts surrounded by tyrosine hydroxylase-positive (sympathetic) nerves.

Conclusions—We conclude that the LOM is richly innervated by sympathetic nerves and serves as a source of isoproterenol-sensitive focal automatic activity in normal canine atrium. The sensitivity to isoproterenol is upregulated after long-term rapid pacing and may contribute to the development of AF in this model. (Circulation. 1999;100:876-883.)

Key Words: tachyarrhythmias ■ mapping ■ catecholamines ■ atrial fibrillation

The importance of catecholamines in the generation and maintenance of atrial fibrillation (AF) is recognized clinically; it is demonstrated by the relation of AF to states of increased adrenergic tone1-3 and by the use of β-blockers in prophylaxis of AF after open-heart surgery. 4 Coumel reported a clinical entity of adrenergic-mediated atrial tachyarrhythmia. 5 In these patients, isoproterenol readily induces AF. The mechanism by which sympathetic stimulation facilitates the induction of atrial tachyarrhythmia and AF is unclear 6 because sympathetic stimulation has no effect on atrial refractoriness. 7 Although sympathetic stimulation may not shorten the refractory period, it is known that left cardiac sympathetic nerve stimulation can trigger an ectopic rhythm from the inferior left atrium. 8 However, because only a few bipolar electrograms were registered in that study, the exact source of ectopic activity could not be determined. The purposes of this study were to use high-density computerized mapping techniques to determine the site of catecholamine-sensitive automatic foci in the left atrium of dogs and then study the activity of these automatic foci in a chronic canine AF model induced by rapid atrial pacing. 9,10 The results were used to test the hypotheses that a specific atrial structure serves as the predominant origin of catecholamine-sensitive focal left atrial automaticity, and that in atria after long-term rapid pacing, this focal activity is upregulated and results in the induction of AF.

Methods
The following research protocol was approved by the Institutional Animal Care and Use Committee and conforms to the American Heart Association guidelines.

Protocol 1: Studies of Normal Healthy Dogs In Vitro

Tissue Preparation
Nine mongrel dogs (18 to 25 kg) were placed under general anesthesia with sodium pentobarbital (30 to 35 mg/kg IV), intubated, and ventilated with room air by a respirator. The hearts were rapidly excised. The left circumflex artery was selectively cannulated and perfused with oxygenated Tyrode’s solution at 36.5°C. The Tyrode’s solution has the following ionic concentrations (in mmol/L): Dextrose 5.5, NaCl 125, KCl 4.5, NaH2PO4 1.8, CaCl2 2.7, MgCl2 0.5, and NaHCO3 24. 11 The posterior left atrium along with a small portion of the basal left ventricle was excised. The pulmonary veins were removed and were not included in the preparation. The distal portion of the circumflex artery and all large marginal arteries were
ligated. The cut surface of ventricular tissue was cauterized. The preparation was then placed in the tissue bath and was perfused at 15 mL/min. The endocardial surface was placed on a high-density mapping plaque with 509 bipolar recording channels built into the bottom of the tissue chamber.11,12 A bipolar pacing electrode, a roving bipolar recording electrode and a widely-spaced pair of bipolar electrode (the pseudo ECG) were placed on the epicardial surface. In 3 of the dogs at the end of the study, a smaller high-density plaque was placed on the epicardial surface over the area of earliest activation to perform simultaneous ep- and endocardial mapping. The small plaque contains 96 bipolar electrodes with 3.0 interelectrode distance and covers an area measuring 2.1 cm by 2.1 cm. The tissue was paced at a cycle length (CL) of 500 to 600 ms for 10 minutes before data collection. In 2 of the dogs, the ligament of Marshall (LOM)8,13 was excised at the end of study to determine whether or not the induced rhythm would terminate and if other subsidiary pacemakers were present.

Electrophysiological Study
The tissues were observed for spontaneous activity at baseline for 5 minutes. If no spontaneous activity was seen, an attempt to induce arrhythmia was made with programmed stimulation including 8-beat baseline pacing train (S1) at 300 and 400 ms CL, followed by up to 3 premature stimuli or by burst pacing at CL of 160 to 400 ms. Afterward, isoproterenol (4 μmol/L and 8 μmol/L) was added to the perfusate and the above protocol was repeated. Sustained atrial arrhythmia was defined by atrial activity of >30 beats in duration and a CL of <3000 ms. Attempts were made to overdrive-suppress the rhythm with rapid pacing.

Activation Mapping
The recording electrodes were connected to a computerized mapping system (EMAP, Uniservices).12 Individual bipolar electrograms were analyzed12 and the patterns of activation were visualized either by isochronal maps or by dynamic display.14,15 Continuous recordings of epicardial bipolar electrograms and pseudo ECG were acquired by the AXON TL-1 to 40 A/D system (Axon Instrument).

Protocol 2: Studies of Dogs With Pacing-Induced Chronic Atrial Fibrillation
Three mongrel dogs of either sex (18 to 25 kg) were used. An atrial pacing lead was inserted into the right atrial appendage and connected to a Medtronic Itrel II 7424 neurostimulator implanted in a subcutaneous pocket. After approximately 1 week, the pulse generator was programmed to burst pace at a CL of 50 ms for 3 s with an

Figure 1. Canine posterior left atrial tissue preparation revealing LOM. Panels 1 and 2 show posterior left atrial epicardial surface in 2 of the tissue preparations. Panel 3 illustrates the anatomic location. A indicates left atrial appendage; B, posterior left atrium; C, left pulmonary vein; D, coronary sinus; and E, left ventricle.

Figure 2. LOM recordings. Two pairs of bipolar electrodes were inserted into lower LOM and upper LOM. An additional pair is used to record ventricular electrogram. A, Recording during atrial pacing at baseline. B, Activation during isoproterenol (4 μmol·L⁻¹·min⁻¹) infusion. There is both automatic ventricular (or Purkinje) activity (V) and automatic atrial activity (A), with complete AV dissociation. Each atrial electrogram was preceded by a sharp deflection best seen in upper LOM (upward pointing arrows).
output of 3.0 volts, followed by a 5-s period without pacing. Digoxin (0.25 mg/d) was given to control ventricular rate. The animals were checked periodically for the presence of sustained AF (>24 hours) off pacing. In these 3 dogs, sustained AF was documented at 10, 41, and 11 weeks, respectively, after the first surgery. The animals then underwent the same electrophysiological studies as described in Protocol 1. The third dog also underwent radiofrequency catheter ablation of the left atrial epicardial surface at the LOM before excising the heart. The radiofrequency energy was applied with a standard 4-mm tip ablation catheter connected to a Radionic 3C radiofrequency current generator. The power was set at 20 to 30 Watts, and the duration of each application was 1 minute. Multiple applications were performed along the LOM until the entire ligament was ablated.

Histological Examination
The atrial tissues were fixed in 10% formalin, processed routinely, and stained with trichrome and the antibodies to neurofilament and tyrosine hydroxylase. The tissues were examined under light microscopy for the presence of an insulated atrial tract and the sympathetic nerves within the LOM.

Statistical Analyses
Student’s t tests were used to compare the CL before and during isoproterenol infusion. P ≤ 0.05 was considered significant.

Results
Figure 1 shows the epicardial surface of the posterior left atrium in 2 dogs. The LOM is seen with varying prominence, ranging from a whitish, fatty-fibrous streak (panel 1) to a readily apparent ligamentous structure (panel 2). The sutures were used to tie off the marginal branches of the circumflex coronary artery except on LOM where a suture was used to mark the location of the earliest activation.

Protocol 1
Spontaneous activity was not demonstrated in 7 of the 9 tissues before infusion with isoproterenol. The first dog...
activity. The first tissue revealed only occasional atrial depolarizations, and only after burst pacing did it demonstrate any sustained activity or tachycardia. The sustained rhythm after burst pacing was regular with a CL of 800 ms. The remaining 8 tissues demonstrated sustained atrial rhythms that were either regular or irregular, with the CL between 400 and 2500 ms. Once the rhythm stabilized, the CL varied between 400 and 800 ms (mean ± SD = 580 ± 183 ms). Increasing the concentration of isoproterenol to 8 μmol/L did not significantly change the CL of the spontaneous activity. None of the tissues demonstrated spontaneous or pacing-induced fibrillation-like activity.

**LOM Recording During Pacing and During Automatic Rhythm**

In 3 normal dogs we used 2 bipolar hook electrodes to record from the upper and lower LOM. An additional hook electrode was used to register the activation from the left ventricle. Simultaneous computerized mapping studies were done from the endocardium. Figure 2 shows bipolar recordings from one tissue. Panel A shows a small second potential (arrow) following a large local atrial electrogram. During isoproterenol infusion (B), small sharp potentials (arrows) precede the local atrial electrogram. The CL between these sharp potentials (activation of the LOM) gradually lengthened (rate decreased) soon after the discontinuation of isoproterenol. Also note that the conduction time from the atrial tissue into the LOM is short (small interval between 2 deflections) and reliable (always maintaining a 1:1 conduction) (A). However, the conduction in the reverse direction was slow and unreliable, with long intervals and frequent conduction blocks (B). Similar sharp potentials were also observed in human patients.16,17 The bipolar atrial electrogram recorded by the electrodes on the LOM was as early as any electrogram recorded on the endocardium. In the other 2 tissues studied, isoproterenol resulted in the reversal of the 2 potentials. However, a prolonged pause between the 2 potentials was not observed.

Figure 3 shows a typical example of the atrial activity after the administration of isoproterenol. In the beginning, the activity was both slow and irregular. It then became more regular with significantly shorter CLs (approximately 500
The tachycardia would then persist for up to 5 minutes. The spontaneous activity could be suppressed by overdrive pacing (Figure 4), which is compatible with automaticity. The earliest site of activation corresponded to the inferior portion of the LOM in all tissues studied. A representative isochronal map and bipolar electrograms for the spontaneous rhythm in dog 5 are shown in Figure 5.

In tissues 3 to 5, we performed simultaneous endocardial and epicardial mapping. In all tissues, we documented that the earliest activity at both endocardial and epicardial sites arose from the area marked by LOM. Figure 6 shows an example. In that figure, the upper edge of the epicardial electrode array was placed at the LOM. The earliest epicardial and endocardial activation occurred at the same site, then propagated to the remaining atrial tissues centrifugally. There was no evidence of epicardial reentry during the isoproterenol-induced spontaneous activity.

Excision of LOM in 2 tissues terminated spontaneous activity. An example is shown in Figure 7. The left panel shows the tissue preparation. The numbers 1 to 5 indicate the segments of the LOM excised. The top line of the right panel shows tachycardia with a CL of 400 ms during isoproterenol infusion. Excision of the LOM resulted in the termination of sustained tachycardia.

Protocol 2

No spontaneous activity was observed without pacing. Following infusion with 4 μmol/L isoproterenol, tachycardia occurred in the 2 tissues without ablation. The CL ranged from 220 ms to 300 ms (mean 255±29 ms). This CL was significantly shorter than the tachycardia in normal tissues during 4 μmol/L isoproterenol infusion (P<0.05). As in normal dogs, the tachycardia had their origin in the area marked by the LOM. In the third dog, which underwent in vivo ablation of the LOM, no spontaneous activity was seen with or without isoproterenol infusion.

In the first tissue, short runs of non-sustained fibrillation-like activity was inducible by burst pacing. The duration of these runs increased from 12±9 s (range 3 to 26 s) at baseline to 69±81 s (range 18 to 229 s) during isoproterenol infusion. In the second dog, fibrillation could not be induced before isoproterenol but sustained runs of fibrillation could be induced during infusion. The duration was 77±44 s (range 18 to 126 s). In these 2 tissues, isoproterenol facilitated induction of fibrillation-like activity and increased the duration. In a third tissue that underwent ablation of the LOM, short runs of fibrillation could be induced before isoproterenol (mean 5±2 s, range 3 to 7 s) but not afterward. The ablation lesion was nontransmural.

In addition to induced fibrillation, we registered multiple episodes in which automatic activity originated from the LOM area triggered AF. Figure 8 shows an example. The panel on the left shows consecutive snapshots of the dynamic activation display, initially showing focal activity from the LOM (1460 to 1500 ms), followed by multiple wavelet fibrillation. The right panel shows that spontaneous activity originating from the LOM results in a tachycardia (first 3 cycles) with CL alternans, followed by fibrillatory activity. This episode implies that spontaneous rapid activity originating from the LOM plays a role in the induction of in vitro AF. This phenomenon was seen only in the first 2 tissues from the chronic AF model and not in any other tissues from normal healthy dogs or the chronic AF dog that underwent in vivo ablation of the LOM.

Histological Examination

Histological studies showed that the LOM contains muscle tracts and abundant nerve bundles that are well insulated by fibrofatty tissues. The majority of the nerve bundles stained positive for tyrosine hydroxylase. Figure 9 shows typical examples of the histological staining.
Discussion

This study has 4 major findings: 1) In isolated-perfused normal canine left atrium, isoproterenol infusion can induce a focal source of automatic activity from the LOM. Ablation of the LOM results in abolition of the ectopic focus from that site. 2) This automatic activity is upregulated in the canine model of rapid-pacing induced chronic AF. 3) During isoproterenol infusion, the rapid automatic activity can trigger the onset of in vitro AF. This latter phenomenon occurs only in the left atrium harvested from dogs with long-term pacing-induced AF and is not seen in the normal atrium. 4) Abundant tyrosine hydroxylase-positive nerves are present in the LOM.

Ligament of Marshall and the Left Atrial Ectopic Rhythm

In 1850, Marshall described the presence of a “vestigial fold of the pericardium” which had until then escaped attention. This fold is a developmental vestige of the left primitive veins. The location of the vestigial fold is in the back of the left auricle, running from the coronary sinus upward to the region of left superior pulmonary vein. Marshall reported that “besides a duplicate of a serous layer of the pericardium, including cellular and fatty tissue, the vestigial fold contains some fibrous bands, small blood vessels and nervous filaments.” He also observed that this vestigial structure is connected to the small oblique auricular vein that drains into the coronary sinus. Scherlag et al performed a detailed study on the electrical activity of the left atrial tract within the LOM in dogs. They found that the bipolar electrogram near the LOM showed 2 deflections. During sinus rhythm, the second of the 2 deflections originated from the insulated tract. However, during left cardiac sympathetic nerve stimulation, ectopic rhythm was induced and the activation sequence of these 2 deflections was reversed. Because only a limited number of recording channels were available, the exact location of the ectopic focus was not determined.

In this study, we performed multichannel mapping of the isolated-perfused left atrium. The results showed that the automatic focus in the left atrium was dormant at baseline but became active during isoproterenol infusion. Furthermore, the ectopic rhythm always originated in the area near the LOM. These findings imply that the LOM may have signif-
icant clinical importance. Furthermore, because the LOM is an easily identifiable anatomical structure, it is an ideal target for ablative therapy.

**Left Atrial Ectopic Rhythm and Paroxysmal Atrial Fibrillation**

Mirowski et al.18,19 documented the presence of automatic focus in the left atrium of both dogs and humans. These left atrial automatic mechanisms may sometimes result in clinically important arrhythmias.20 The exact location of the automatic rhythm was not determined. However, one of the possible sites was the sinocaval area in the left atrium where transmembrane potential recordings consistently showed slight diastolic depolarizations.21 The rabbit left sinocaval area used in that study roughly corresponded to the coronary sinus and may include its tributaries.

The development of radiofrequency catheter ablation provided new insights into the mechanisms of atrial tachyarrhythmia. Tracy et al.22 and Kay et al.23 performed radiofrequency ablation in patients with ectopic atrial tachycardia. They found that in a few patients, the sites of origin of ectopic atrial tachycardia were in the left atrium posterior wall, near the orifice of the pulmonary veins. More recently, Haissaguerre et al.17 reported that the ectopic beats originating from the pulmonary veins may serve as a source of ectopic activity that leads to spontaneous AF. Among the 4 pulmonary veins, the left superior pulmonary vein (which is adjacent to the LOM) is by far the most common one that generates ectopic atrial activity. The electrograms registered in our study from the LOM (Figure 2) are similar to that registered from the left superior pulmonary vein by Haissaguerre et al.17 and by us.16 In the latter reports, 2 deflections similar to that shown in Figure 2 were found at the orifice and inside the left superior pulmonary vein. During atrial ectopic activity or at the onset of paroxysmal AF, the morphology of the 2 deflections reversed. The sharp local activity was followed after a long pause (>100 ms) by the local atrial activity, with intermittent Wenckebach-type conduction blocks between the 2. Although the similarity of the electrograms is not a definitive proof of a causal relation, these findings suggest a possible role of LOM in the generation and maintenance of paroxysmal AF in humans.

**Adrenergic Atrial Tachyarrhythmia**

A second clinical implication of our study is that the LOM may serve as a structure that facilitates the induction of AF during adrenergic (sympathetic) stimulation. Coumel et al.5 reported a small group of patients in whom AF occurs predominantly in the daytime, with evidence of adrenergic...
over-activity (such as accelerated heart rate) before the onset of AF. In this latter group of patients, isoproterenol infusion results in the induction of AF. In the present study we demonstrated that the ectopic foci in the left atrium is sensitive to sympathetic stimulation. This finding implies that the LOM, with its rich sympathetic nerve distribution and the proximity of these nerves to an isolated muscle bundle, may serve as a source of adrenergic atrial tachyarrhythmias.

Other possible sources of adrenergic atrial tachyarrhythmias included structures in the right atrium, especially in the tissues near sinus node and the crista terminalis. Although crista terminalis is often the source of focal right atrial tachycardia, it rarely serves as the origin of focal AF. Implications on the Mechanisms of Chronic Atrial Fibrillation

A third clinical implication of our study is that the LOM may play a role in the induction of chronic AF. It was recently demonstrated that intermittent or persistent rapid atrial pacing can result in electrical remodeling of the atria and converting nonsustained AF into sustained AF. Ectopic rhythm from the LOM could serve as the source of the intermittent rapid atrial activity that results in electrical remodeling of the remaining atria. This remodeling may eventually lead to sufficient remodeling and chronic AF. Furthermore, we have also observed that isoproterenol infusion in an atrium already remodeled by rapid pacing can first trigger atrial tachycardia from the LOM, followed by an abrupt transition to AF. These findings imply that the LOM may serve as the source of ectopic atrial tachycardia, which converts sinus rhythm to AF after successful cardioversion.

Limitations

A limitation of this study is that the pulmonary veins were not included in the preparation. It is unclear whether or not pulmonary veins also contain pacemaker cells that are sensitive to adrenergic stimulation.

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