Heterogeneous Atrial Denervation Creates Substrate for Sustained Atrial Fibrillation

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Background—Heterogeneous electrophysiological properties, which may be due in part to autonomic innervation, are important in the maintenance of atrial fibrillation (AF). We hypothesized that heterogeneous sympathetic denervation with phenol would create a milieu for sustained AF.

Methods and Results—After the determination of baseline inducibility, 15 dogs underwent atrial epicardial phenol application and 11 underwent a sham procedure. After 2 weeks of recovery, the animals had repeat attempts at inducing AF and effective refractory period (ERP) testing. Epicardial maps were obtained to determine local AF cycle lengths. ERPs were determined at baseline and during sympathetic, vagal, and simultaneous vagal/sympathetic stimulation. Dogs then underwent PET imaging with either a sympathetic ([11 C]hydroxyephedrine, HED) or parasympathetic (5-[11 C]methoxybenzovesamicol, MOBV) nerve label. None of the animals had sustained AF (>60 minutes) at baseline. None of the sham dogs and 14 of 15 phenol dogs had sustained AF at follow-up. Sites to which phenol was applied had a significantly shorter ERP (136 ±17.6 ms) than those same sites in the sham controls (156 ±19.1 ms) (P=0.01). Although there was no difference in the ERP change with either vagal or sympathetic stimulation alone between phenol and nonphenol sites, the percent decrease in ERP with simultaneous vagal/sympathetic stimulation was greater in the phenol sites (17 ±8%) than in the nonphenol sites (9 ±9%) (P=0.01). There was a significantly increased dispersion of refractoriness (21 ±6.4 ms in the sham versus 58 ±14 ms in the phenol dogs, P=0.01) as well as dispersion of AF cycle length (49 ±10 ms in the sham versus 105 ±12 ms in the phenol dogs, P=0.0001). PET images demonstrated defects of HED uptake in the areas of phenol application, with no defect of MOBV uptake.

Conclusions—Heterogeneous sympathetic atrial denervation with phenol facilitates sustained AF. (Circulation. 1998;98:2608-2614.)

Key Words: fibrillation ■ nervous system, autonomic ■ phenol ■ tomography ■ hydroxyephedrine

The role of the autonomic nervous system in ventricular arrhythmias has been studied extensively in both humans and animal models.1–6 Although the importance of the autonomic nervous system in atrial fibrillation (AF) has been suggested by both experimental and clinical observations, its role in the initiation and perpetuation of AF has not been determined. The autonomic tone before the onset of AF has been used to identify so-called vagal AF in humans.7 In addition, animal studies have demonstrated that parasympathetic stimulation results in AF.8 The role of the sympathetic nervous system and sympathovagal balance is less clear, but they have been shown to affect the action potential and ion currents in cardiac tissue.8–10

Spatial heterogeneity of refractoriness has been demonstrated under a variety of conditions that result in AF.11–14 One potential mechanism for this spatial dispersion of refractoriness is heterogeneity of autonomic innervation.16–20 This heterogeneity has been shown to be an important mechanism in ventricular arrhythmias1–6,21,22 and has been hypothesized to be important in atrial arrhythmogenesis. The purpose of this study is to determine the effects of heterogeneous autonomic atrial denervation produced with topical application of phenol23 on refractory periods and on the induction of AF in dogs.

Methods

Twenty-six mongrel dogs (24 to 30 kg) were anesthetized with isoflurane and artificially ventilated. A quadripolar electrophysiology catheter was advanced through the femoral vein into the right atrium. Burst pacing at a cycle length of 50 ms and an output of 10 mA for 10 seconds was used to induce AF. No vagal or sympathetic stimulation was performed during this induction. The duration of induced AF was timed for each of 10 induction attempts. Five minutes after the reversion to sinus rhythm was allowed between attempts.
After baseline inducibility of AF had been determined, a right thoracotomy was performed and the heart positioned in a pericardial cradle to expose the right atrium. With cotton-tipped swabs, topical phenol (88%) was applied to the right atrial epicardium of 15 dogs, and 11 dogs (shams) received saline. Applications were made to a 2×0.5-cm region on the superior right atrial free wall, a 1.5×1-cm region on the inferior right atrial free wall, and a 1×1-cm region on the anterior base of the right atrial appendage near Bachmann’s bundle. These were chosen to create 3 distinct areas of application. The area was then irrigated with saline and dried with a gauze sponge. After all 3 applications, the chest was closed, and the animals were allowed to recover for 2 weeks.

Follow-up Electrophysiological Testing

Two weeks later, all animals were anesthetized as above and underwent attempts to induce AF by the above protocol. Induction of sustained AF (>60 minutes) ended the protocol. Animals then underwent further electrophysiological study and/or PET imaging as described below.

In 5 phenol and 5 sham dogs, epicardial mapping of the AF was performed via a sternotomy. Four custom-made epicardial plaques (2 right atrium, 2 left atrium) consisting of 240 electrodes with 5.6-mm spacing were placed over the right and left atrial free wall and the anterior surfaces of the right and left atria. Unipolar signals and surface ECG were recorded onto a digital mapping system (CardioMap, Prucka Engineering) at 1 kHz. Maps were obtained during right atrial pacing at a cycle length of 200 ms and during induced AF. Mean AF cycle lengths at each of the electrodes were determined by analysis of 4 seconds of AF. Mean AF cycle lengths were used as an estimate of local refractoriness during AF.24,25 Conduction velocities were determined from isochronal maps.

Five phenol and 5 sham dogs underwent electrophysiology procedures via median sternotomy before PET imaging. Eight intramural unipolar plunge electrodes (30-gauge stainless steel wire) were placed in areas in which phenol had been applied (4 electrodes), in areas of the right atrium not exposed to phenol (2 electrodes), and in areas of the left atrium (2 electrodes). Comparable sites were measured in the sham dogs. Both cervical vagi and thoracic ansae subclaviae nerves were exposed. Each nerve was tied and severed. Custom-made bipolar electrodes were attached caudal to the ligature for bilateral efferent nerve stimulation with a constant-voltage stimulator (Grass Instruments). The voltage and frequency were set to obtain a 50% reduction in sinus rate during vagal stimulation and a 50% increase in sinus rate during sympathetic stimulation.

Atrial effective refractory periods (ERPs) from each site were determined with unipolar pacing at twice diastolic threshold by introduction of a premature stimulus (S2) after a drive train (S1) of 8 at a cycle length of 300 ms. The S1-S2 interval was incremented in steps of 2 ms until atrial capture. Refractory periods were determined at each of the 8 sites at baseline, during vagal stimulation, during sympathetic stimulation, and during simultaneous sympathetic and vagal stimulation. ERP was defined as the longest S1-S2 interval at which the S2 failed to produce an atrial response.

Microelectrode Recordings

In 2 of the dogs from the phenol group, microelectrode epicardial recordings were made from regions to which phenol was applied and from regions to which phenol was not applied. The hearts were rapidly removed and perfused through the aorta with cold cardioplegia solution. Two strips (1×3 cm) of atrial tissue from each heart were removed from the right atrial free wall: 1 exposed to phenol and 1 not. The strips were superfused with Tyrode’s solution bubbled with 95% O₂ and 5% CO₂. The strips were stimulated at 0.5 Hz. Micropipette microelectrodes filled with 3 mol/L KCl were positioned with micromanipulators to record an intracellular action potential within the epicardial surface cell layer of the samples. Signals were amplified and digitized at 50 kHz to a PC (AxonScope). Recordings were made from 5 sites on each strip.

PET Scanning

After the electrophysiological study, 10 dogs in the phenol group underwent PET imaging with FDG as an indicator of myocardial metabolism/viability. Five dogs also received [11 C]hydroxyephedrine (HED) to image sympathetic nerve endings,26 and the other 5 received 5-[18 F]methoxybenzovesamicol (MOBV) to image parasympathetic nerve endings.27 Between 3 and 5 mCi of FDG and 20 to 40 mCi of either HED or MOBV were injected intravenously. The compound was allowed to circulate for 30 minutes, and then the animal was euthanized with pentobarbital (1.2 g IV). The heart was examined and then removed from the animal. The atria were separated from the ventricles, stuffed with gauze, and placed in a custom-built, high-resolution (3-mm) PET scanner for imaging.28,29 With the atria in the same position, another scan was performed 2 hours later. Because 11 C has a shorter half-life (20 minutes) than 18 F (110 minutes), the second scan imaged only the distribution of FDG. Subtraction methods were used to generate individual projection data sets for both 18 F and 11 C. Filtered backprojection image reconstruction algorithms were used to generate tomographic images of the 11 C and 18 F distribution. Therefore, from both compounds, 2 separate 3-dimensional images were obtained: distribution of [18 F]FDG to label myocardial viability and distribution of [11 C]HED (5 animals) or [11 C]MOBV (5 animals) to label sympathetic or parasympathetic nerve terminals, respectively.

After PET scanning, the specimens were fixed in formalin, and blocks were cut from phenol and nonphenol areas, embedded in paraffin, sectioned, stained with hematoxylin-eosin, and examined microscopically.

Statistics

Values are presented as mean±SD. Two-way comparisons were made with t tests, paired when appropriate. Comparison of >2 continuous variables were made with ANOVA. Comparison of noncontinuous variables were made by χ² analysis. A value of P≤0.05 was considered significant.

Results

All 26 animals survived 14 days without difficulty. At the time of follow-up, all animals had spontaneous sinus rhythm.

AF Induction

The results of AF inductions are shown in the Table. No animal had sustained AF (>60 minutes) induced at baseline. Two weeks after phenol application, 14 of the 15 dogs had sustained AF induced (P<0.0001 compared with baseline). The 1 animal in which AF was not sustained had AF for 50 minutes. Sustained AF was induced within the first 5.5±3 attempts (median, 5) after phenol application and required 3 attempts in 5 of the 15 animals. Only 1 animal in the phenol group required 10 induction attempts (the 1 animal in which 50 minutes of AF was observed).

The percentage of animals with sustained AF (>60 minutes) was significantly greater (P<0.0001) in the phenol
group than in the sham group and animals at baseline (Table). The duration of induced AF was significantly longer in the phenol group than in the sham group \( (P < 0.0001) \) and animals at baseline \( (P < 0.0001) \) (Table). There was no difference in duration of AF in the sham group 2 weeks after surgery compared with animals at baseline (Table). The ease with which AF was induced was significantly greater \( (P < 0.0001) \) after phenol application than in either the sham group or animals at baseline, as determined by the number of attempts required to induce sustained AF (Table).

In the 5 phenol dogs in which epicardial mapping of the AF was performed, multiple reentrant wavelets were identified. There was no evidence from these maps that focal activity or stationary block in the area of phenol application was responsible for the AF.

**Electrophysiological Testing**

In 5 of the dogs in which phenol was applied, ERPs were determined from intramural sites exposed to phenol and sites not exposed to phenol (nonphenol). In 5 sham dogs, ERPs were determined from the same anatomic locations as those in the phenol group. Sites to which phenol was applied had a significantly shorter ERP \( (136 \pm 17.6 \text{ ms}) \) at baseline compared with those sites in the sham group, as determined by the number of attempts required to induce sustained AF (Table).

To determine the effects of autonomic stimulation on the ERP in the phenol areas, comparisons within the phenol animal group were made between those sites to which phenol was applied and those to which it was not. Sympathetic stimulation did not significantly affect ERP at sites to which phenol was applied (from \( 140 \pm 14 \text{ ms} \) at baseline to \( 135 \pm 12 \text{ ms} \)) or at nonphenol areas (from \( 128 \pm 17 \text{ ms} \) at baseline to \( 127 \pm 16 \text{ ms} \)) (Figure 1). Vagal stimulation decreased the ERP at both phenol \( (116 \pm 15 \text{ ms}) \) and nonphenol \( (99 \pm 25 \text{ ms}) \) sites compared with baseline \( (P < 0.0001) \) (Figure 1). The ERP during vagal stimulation was significantly \( (P = 0.02) \) shorter for the nonphenol sites than for those exposed to phenol. However, after correction for the higher baseline ERP in the phenol sites, the percent decrease in ERP with vagal stimulation from baseline was not different between phenol sites \( (17 \pm 8\%) \) and nonphenol sites \( (23 \pm 15\%) \) (Figure 2). Superimposing sympathetic stimulation on vagal stimulation returned the ERP toward normal in the nonphenol sites \( (116 \pm 19 \text{ ms}) \) \( (P < 0.0001) \) compared with ERP during vagal stimulation but did not affect ERP in the phenol sites \( (116 \pm 15 \text{ ms}) \) \( (P = \text{NS}) \) compared with ERP during vagal stimulation (Figure 1). The percent increase in ERP with vagal and sympathetic stimulation from that during vagal stimulation alone was significantly greater in the nonphenol sites \( (14 \pm 9\%) \) than in the phenol sites \( (1 \pm 1\%) \) \( (P < 0.0001) \) (Figure 2).

The dispersion of refractoriness (maximum ERP–minimum ERP) during baseline autonomic tone was significantly \( (P = 0.01) \) greater in the phenol group \( (58 \pm 14 \text{ ms}) \) than in the sham group \( (21 \pm 6.4 \text{ ms}) \). In addition, the dispersion of AF cycle length was significantly \( (P = 0.0001) \) greater in the phenol group \( (105 \pm 12.2 \text{ ms}) \) than in the sham group \( (48.7 \pm 10.1 \text{ ms}) \).

Conduction velocities in areas in which phenol was applied was not significantly different \( (1.24 \pm 0.153 \text{ m/s}) \) from conduction velocities in areas in which phenol was not applied \( (1.13 \pm 0.118 \text{ m/s}) \).

**Microelectrode Recordings and Pathology**

Microelectrode recordings revealed normal atrial myocyte action potentials without any evidence of afterdepolarizations. There was no difference in resting membrane potential, action potential amplitude, dV/dt, or action potential duration at 50% or 90% repolarization \( (\text{APD}_{50} \text{ or APD}_{90}) \) between phenol and nonphenol sites.

All hearts appeared normal at removal. On gross inspection, there were no adhesions or fibrotic scars on the epicardial surface of the atria, which was smooth in appearance. Atrial sections from both phenol and nonphenol sites stained with hematoxylin-eosin showed no evidence of inflammation.
or fibrosis and had normal-appearing myocytes on microscopic examination.

**PET Imaging**
Examples of PET images obtained from atria labeled with HED and FDG and 1 labeled with MOBV are shown in Figures 3 and 4. In all of the atria imaged with HED, a defect in uptake was seen in the areas in which phenol was applied (Figure 3A). The remainder of the atrial regions appeared to be appropriately labeled with HED (Figure 3A). Corresponding FDG images in these same atria showed no defects in any areas (Figure 3B). The areas in which an HED defect was seen had normal uptake of FDG in all atria (Figure 3). This indicates that the areas to which phenol was applied were sympathetically denervated without affecting myocardial viability. No defects in either MOBV uptake or FDG uptake were seen in the atria labeled with MOBV and FDG.

**Discussion**
This study has demonstrated that heterogeneous sympathetic denervation of the atria with phenol creates a milieu for sustained AF. Autonomic imaging with PET scanning demonstrated that phenol application resulted in regional sympathetic denervation without producing observable regions of nonviable myocardium. Functional electrophysiology demonstrated that areas to which phenol was applied had a significantly decreased ERP and altered autonomic control of ERP compared with areas not exposed to phenol. This regional denervation resulted in an increased dispersion of refractoriness that facilitated sustained AF.

**Autonomic Influences on Atrial Electrophysiology**
The effects of autonomic innervation on atrial electrophysiology have been extensively studied. However, the interaction of the sympathetic and parasympathetic nervous
systems at the synaptic level is not well understood. Stellate stimulation has been shown to blunt the vagally mediated slowing of sinus rate. The present study has demonstrated that sympathetic stimulation blunts the decrease in ERP induced with vagal stimulation in normal atrial myocardium (Figures 1 and 2). However, in areas that had been sympathetic denervated at the synaptic level with phenol, this interaction did not occur (Figures 1 and 2). Therefore, it appears that the sympathetic nervous system has a negative modulatory effect on the parasympathetic nervous system at the synaptic level, at least during high levels of autonomic stimulation.

Mechanism of AF
Spatial dispersion of functional properties such as refractoriness has been shown to be an important factor contributing to the maintenance of AF. Although many factors may influence the heterogeneity of electrophysiological properties, it has long been hypothesized that autonomic innervation contributes to this at least in part. The present study has demonstrated that when innervation is made heterogeneous by regional sympathetic denervation, dispersion of refractoriness is increased and AF can be sustained without vagal stimulation. The heterogeneous atrial electrophysiological properties (refractory period) produced by regional autonomic denervation create the proper environment for the maintenance of an adequate number of reentrant wavelets, facilitating sustained AF.

Role of Autonomic Nervous System in AF
Although sustained AF cannot be induced in a normal dog, vagal stimulation results in sustained AF as long as the vagus is being stimulated. Both vagal stimulation and direct application of acetylcholine have been shown to result in AF in dogs. Spatially disparate effects of vagal stimulation have been demonstrated in the atria. Although this heterogeneous distribution of vagal effects on electrophysiological properties may contribute to the milieu necessary to maintain multiple-wavelet atrial reentry, it is clearly not sufficient in the baseline state, because spontaneous AF does not occur in the absence of other perturbations (such as vagal stimulation).

The effects of the sympathetic nervous system on AF are less well studied. Smeets et al demonstrated that epinephrine increased the wavelength in atria paced at slow rates and had little effect when the atria were paced rapidly. It appears, however, that sympathetic stimulation has little effect on AF in the experimental setting. In the clinical setting, some forms of AF, such as postoperative AF, may be influenced at least in part by sympathetic tone, because β-blockers appear to be an effective treatment.

The present study has demonstrated that regional sympathetic denervation facilitates sustained AF. It is possible that phenol application resulted in some vagal denervation as well but that this was not detectable with MOBV PET imaging. There was a slight trend toward a blunted vagal effect on ERP shortening in the phenol areas compared with the nonphenol areas (Figure 2). However, this effect was small compared with that on sympathetic innervation. The data suggest that the regional sympathetic denervation indirectly changed the vagal effects on refractoriness through a change in the sympathetic modulation of vagal effects. This interaction may be exaggerated during the rapid rates of AF, because it has been shown that the decrease in refractoriness caused by rapid atrial rates may be due to acetylcholine release. In the present study, because this autonomic imbalance was created heterogenously, AF could be sustained.

PET Imaging
Numerous studies have demonstrated the feasibility of imaging sympathetic innervation in the ventricle by use of HED and PET. This is the first study to use this compound to image the atrium. The PET images with HED demonstrated good uptake throughout the atria, except in regions in which phenol was applied. In these regions, HED activity was absent. FDG is a well-established tracer of metabolism, and its uptake has been shown to be a sensitive marker of myocardial viability. None of the atria in the present study demonstrated any abnormality or regional defect in FDG uptake.
uptake, indicating that the phenol did not destroy myocardial tissue. Even areas with HED defects had normal FDG uptake. Normal atrial myocytes were confirmed on hematoxylin-eosin staining. Therefore, the electrophysiological changes and arrhythmogenesis observed cannot be explained by destruction of myocardium and establishment of anatomic obstacles.

Limitations

Although the most likely explanation for the ability to sustain AF after heterogeneous phenol application is regional sympathetic denervation, other explanations are possible. It is possible that the surgery itself produced inflammation on the epicardial surface. However, no evidence of inflammation was seen either on gross inspection or on histological examination. In addition, sham surgeries did not produce the same results as application of phenol. Another possible explanation is that the phenol damaged myocardial tissue, directly affecting electrophysiological properties or creating anatomic barriers to reentry. This is unlikely, however, because histological examination did not reveal any abnormal myocytes, and PET imaging with FDG did not show any nonviable myocardium. Moreover, single-cell electrophysiological recordings revealed no abnormality in the intrinsic action potential morphology, duration, or rate of rise (dV/dt). In addition, estimates of conduction velocity were not affected by phenol application.

Both MOBV and HED imaging were not performed in all animals. Because of poor signal-to-noise ratio from cardiac motion and relative myocardial mass of the ventricle, the atrial PET images were obtained in the explanted heart. Therefore, it was not possible to image with both compounds in the same heart.

Conclusions

We have demonstrated that heterogeneous sympathetic denervation in the atrium is sufficient to produce sustained AF. This is due to an increase in dispersion of refractoriness, which is a result of heterogeneous changes in the balance of autonomic innervation. Because the animals did not have spontaneous AF, this mechanism is clearly insufficient as a trigger for AF in this animal model. However, heterogeneous denervation may play a role in the process of electrical remodeling.

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References


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