Atrial Fibrillation Produced by Prolonged Rapid Atrial Pacing Is Associated With Heterogeneous Changes in Atrial Sympathetic Innervation

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**Background**—Structural and electrophysiological changes of the atria occur with prolonged rapid rates; however, the effects of sustained atrial fibrillation (AF) on autonomic innervation of the atria are unknown. We hypothesized that electrophysiological remodeling from rapid atrial rates is accompanied by altered atrial autonomic innervation.

**Methods and Results**—Six dogs (paced group) underwent atrial pacing at 600 bpm; 9 dogs (control animals) were not paced. All paced dogs developed sustained AF by week 4 of pacing. All 15 animals underwent positron emission tomography imaging of the atria with [C-11] hydroxyephedrine (HED) to label sympathetic nerve terminals. HED retention in the atria was significantly greater in paced dogs compared with control animals ($P < 0.03$). Tissue samples from the atrial appendages had a greater concentration of norepinephrine in paced animals than in control animals ($P = 0.01$). The coefficient of variation of HED retention was also greater in paced animals ($P = 0.05$) and was greater in the right atrium than in the left atrium ($P = 0.004$). Epicardial activation maps of AF were obtained in the paced animals at baseline and with autonomic manipulation. Mean AF cycle length was longer in the right atrium (109.2 ± 5 ms) than in the left atrium (85.8 ± 5.5 ms) at baseline ($P = 0.005$). AF cycle length did not vary significantly from baseline (97.6 ± 13.4 ms) with stellate stimulation (100.5 ± 6 ms) but lengthened with propranolol (107.5 ± 6.1 ms, $P = 0.03$).

**Conclusions**—Rapid rates of AF produce a heterogeneous increase in atrial sympathetic innervation. These changes parallel disparate effects of rapid pacing–induced AF on atrial electrophysiology. (*Circulation. 2000;101:1185-1191.*)

**Key Words:** atrial fibrillation • electrophysiology • nervous system, autonomic • tomography • hydroxyephedrine

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**Perturbation of normal atrial electrophysiological properties by rapid atrial rates that promotes atrial fibrillation (AF) has been termed electrophysiological remodeling.** Shortening of refractoriness has been described as a hallmark of electrophysiological remodeling. It has been proposed that other electrophysiological properties such as the spatial dispersion of atrial refractoriness and conduction velocity are also important elements of electrophysiological remodeling. An increased heterogeneity of atrial refractoriness has been found in animal models of AF and in humans with paroxysmal AF. Although the underlying cause of such an increase in heterogeneity of atrial refractoriness is unknown, alterations in sympathetic innervation have been implicated in ventricular arrhythmogenesis by producing spatial heterogeneity of electrophysiology. Recently we showed that heterogeneous atrial sympathetic denervation with epicardial phenol increases dispersion of atrial refractoriness and creates a substrate for sustained AF. Whether changes in sympathetic innervation occur during electrophysiological remodeling of the atria and the mechanism by which these changes could alter atrial electrophysiology is unknown. We used a rapid atrial–paced canine model of AF to test the hypothesis that rapid atrial rates are accompanied by altered sympathetic innervation.

**Methods**

This study was approved by the Institutional Animal Care and Use Committee of Indiana University in accordance with the “Guide for Care and Use of Laboratory Animals” (NIH publication No. 86-23, revised 1985).

**Long-Term Pacing**

Fifteen mongrel dogs, each weighing 28 kg, were studied. Of these, 9 dogs served as control animals and underwent no intervention before the study. Six dogs (paced group) underwent transvenous implantation of permanent atrial pacemakers. These animals underwent anesthesia with isoflurane and volume-cycled ventilation. An active fixation pacing lead was positioned in the right atrial appendage to obtain an appropriate pacing threshold. A pulse generator (Itrel, Medtronic) was connected to the lead and positioned in a
subcutaneous pocket over the dog’s right shoulder. After recovery, the pacemakers were programmed “AOO” at 600 bpm, at 4 times diastolic threshold. Pacemakers were interrogated weekly for 6 weeks. At each interrogation, a 4-lead surface ECG was recorded during temporary inhibition of pacing to determine the underlying rhythm and mean ventricular rate. Threshold of atrial capture was determined for dogs in sinus rhythm, and pacemaker output was adjusted if necessary to ensure atrial capture.

Electrophysiological Study
After 6 weeks of pacing, during sustained atrial fibrillation (AF), paced dogs underwent an open chest electrophysiological study under general anesthesia maintained with isoflurane. Arterial blood pressure and cardiac rhythm were monitored during all studies. A median sternotomy was performed, and the pericardium was opened to cradle the heart. Both cervical vagi and thoracic ansae subclaviae nerves were exposed and cut. Custom-made bipolar electrodes were attached to the distal ends of the cut nerves for bilateral stimulation with the use of a constant voltage stimulator (Grass Instruments). Separate stimulators were used to stimulate bilateral vagi and ansae subclaviae. The stimulators were programmed with a voltage and frequency that was sufficient to obtain a 50% reduction in ventricular rate during bilateral vagal stimulation and a 40% increase in ventricular rate during bilateral ansae subclaviae stimulation.

A custom-built set of epicardial plaques was used to map atrial activation during AF. This set of 4 epicardial plaques had a total of 240 electrodes (5.6-mm interelectrode spacing) and covered the entire atrial epicardial surface. Two plaques were placed on the mesial aspect of both the left and right atrial appendages and along Bachmann’s bundle in the transverse sinus (43 and 50 electrodes, respectively). A plaque with 77 electrodes was placed on the lateral aspect of the appendage and free wall of the right atrium and a plaque with 70 electrodes was placed on the lateral aspect of the appendage and free wall of the left atrium. All unipolar recordings were made relative to a reference electrode in the inferior vena cava. Thirty-second epochs of AF were recorded at baseline, during vagal stimulation, and during stellate (ansa subclaviae) stimulation. Ventricular rate was allowed to return to baseline before and subsequent to stellate and vagal stimulation. Propranolol hydrochloride was then infused with an intravenous bolus dose of 0.2 mg/kg followed by infusion at 0.04 mg/kg per hour. Data were acquired with the use of a digital acquisition system at 1-kHz sampling frequency (Cardiomapp, Prucka Engineering). Post-acquisition analysis was performed with the use of MATLAB (Math Works Inc) and C programming languages. Four-second epochs from each recording were analyzed. The first rapid deflection of each atrial electrogram was marked to time local atrial activation and the mean AF cycle length (AFCL) for each electrode determined. Time of the QRS by putting on the surface ECG was used to exclude ventricular electrograms. Mean AFCL was used to estimate atrial refractoriness at each electrode.13

Positron Emission Tomography
After 6 weeks of rapid atrial pacing, all animals in both groups underwent positron emission tomography (PET) imaging to determine sympathetic innervation. The feasibility and methods of using [C-11]-labeled hydroxyephedrine (HED) to label atrial sympathetic nerve terminals have been previously described by our group.15,16 HED was injected intravenously and arterial blood samples were drawn over a 30-minute period to calculate the available blood pool of HED. Two minutes before euthanasia, [F-18]-labeled microspheres of hydroxyapatite (20- to 40-μm diameter) were injected into the left ventricular cavity and a continuous blood sample was drawn from the femoral artery at a predetermined rate to determine total atrial blood flow by the reference sample technique.15,16 The [F-18]–labeled microspheres were used to determine atrial perfusion to correct for regional variability in delivery of HED to atrial tissue and partial volume effect caused by variable wall thickness. The animal was euthanized with pentobarbital (1.2 g). The atria were separated from the ventricles by cutting along the tricuspid and mitral annuli. Left and right atrial cavities were filled with gauze, and the entire atrium was placed in the imaging chamber of a custom-built, high-resolution (3.2 mm, full width at half maximum) PET scanner. An initial scan was performed immediately. Without moving the heart, a second scan was performed 2 hours later. Because [C-11] has a much shorter half-life (20 minutes) than [F-18] (110 minutes), the second scan only imaged the distribution of the [F-18]-labeled microspheres. Subtraction methods were used to generate individual projection data sets for both [F-18] and [C-11]. Filtered backprojection image reconstruction algorithms were used to generate tomographic images of the [C-11] and [F-18] distribution. Individual tomographic slices of the atria were stacked and linear interpolation was used to generate 3D image volumes for data analysis.

PET Analysis
Qualitative differences in HED uptake were compared between paced dogs and control dogs. For quantitative analysis of regional differences in sympathetic innervation, the reconstructed atrial images were divided into regions of interest. Each atrial image was divided into 10 axial sections. Each axial section was divided into 13 predetermined regions of interest that included the right and left atria and interatrial septum. Thus there were 130 regions of interest for both atria combined. The HED retention fraction was calculated as the ratio of the HED concentration in the tissue at the time the heart was removed, divided by the integral of the HED concentration in the blood from the time of injection until the heart was removed:

$$RF = \frac{\text{Concentration of HED in tissue at (t=m)}}{\int_{t=0}^{t=m} \text{Concentration of HED in blood(t)} \, dt}$$

where RF is retention fraction, t=m is time at which the heart was explanted, and t=0 is time at injection of HED. HED retention fraction values were then divided or normalized by regional blood flow values to account for regional differences in tracer delivery and partial volume effects caused by variable atrial wall thickness:

$$RF_{\text{NORM}} = \frac{\text{Concentration of HED in tissue at (t=m)}}{MBF \int_{t=0}^{t=m} \text{Concentration of HED in blood(t)} \, dt}$$

where $RF_{\text{NORM}}$ is HED retention fraction normalized by myocardial blood flow (MBF) and is a value without units, (t=m) is time at which the heart was explanted, and t=0 is time at injection of HED. All values of HED retention fraction reported below are normalized by blood flow ($RF_{\text{NORM}}$).

Norepinephrine Content
Biopsy samples of atrial muscle (88±4 mg) were obtained from the appendages of left and right atria. Samples were weighed and stored at −70°C. Samples were subsequently thawed and homogenized with 5 to 10 vol of 0.1N perchloric acid and centrifuged for 15 minutes. The acid supernatant was diluted with 1 mmol/L HCL. A radioenzymatic assay with purified phenylethanolamine N-methytransferase was used to determine norepinephrine content of the supernatant.17

Statistical Analysis
Values are presented as mean±SD. Two-way comparisons were made with t tests, paired when appropriate. A value of $P=0.05$ was considered significant.

Results
By the fourth week of pacing, the underlying rhythm during temporary inhibition of pacing was sustained AF in all dogs (n=6) in the paced group. At 6 weeks, with pacemakers programmed off, all dogs remained in sustained atrial fibrillation during the electrophysiological study. Mean ventricular rate was similar ($P=NS$) in control (105±18 bpm) and paced animals (115±25 bpm). All control dogs maintained sinus rhythm throughout the study.
PET Images
An example of positron emission tomography (PET) images from a control dog is shown in Figure 1A, demonstrating increased [C-11]–labeled hydroxyephedrine (HED) retention in the region of the sinus node and crista terminalis, consistent with previously described increased sympathetic innervation of the sinus node region.18 In all paced dogs, extensive redistribution of HED retention was observed (Figure 1B).

Quantitative Analysis
The mean HED retention fraction of both atria was significantly greater ($P=0.03$) in paced dogs (0.877±0.36) than in control dogs (0.223±0.10). This was also true of individual left atria [paced (0.814±0.25) vs control (0.215±0.06), $P=0.03$] and right atria [paced (0.922±0.45) vs control (0.205±0.08), $P=0.03$] (Figure 2). There was no significant difference in mean HED retention fraction between left and right atria in control animals or paced dogs (Figure 2).

The spatial variation of sympathetic innervation, measured by the coefficient of variation of HED retention, was significantly greater in the paced group (0.57±0.34) compared with control dogs (0.36±0.12, $P=0.05$) (Figure 3). Also, while the variability of sympathetic innervation was similar in the left (0.32±0.03) and right (0.41±0.05) atria of control animals ($P(NS)$), in AF dogs the variability of sympathetic innervation in the right atria (0.66±0.20) was significantly greater than that of the left atria (0.39±0.03) ($P=0.004$) (Figure 3).

Electrophysiological Studies
The effect of autonomic stimulation on mean atrial fibrillation cycle length (AFCL) measured from all electrodes is shown in Figure 4. Mean AFCL at baseline was 97.6±13.4 ms. Vagal stimulation shortened mean AFCL to 85.3±11.6 ms ($P=0.09$). Compared with baseline, stellate stimulation did not result in significant change in overall mean AFCL.
whereas infusion of propranolol resulted in a significant lengthening of mean AFCL to 107.5 ± 6.1 ms (P < 0.03).

Mean AFCL was also compared between the left and right atria at baseline and with autonomic manipulation, as shown in Figure 5. At baseline, mean AFCL was significantly shorter (P < 0.0005) in the left atrium (85.8 ± 5.5 ms) than in the right atrium (109.2 ± 5.0 ms). Vagal stimulation shortened mean AFCL to a greater extent in the right than in the left atrium and therefore resulted in similar mean AFCL in the right (88.5 ± 15.7 ms) and left (82.1 ± 6.5 ms) atria (P = NS). During stellate stimulation, similar to baseline state, mean AFCL remained significantly longer in the right atria (112.2 ± 7.5 ms) than in the left atria (88.1 ± 6.2 ms, P = 0.009). Similarly, with propranolol, mean AFCL remained significantly longer in the right than in the left atria (P = 0.005) (Figure 5). Also with propranolol when compared with baseline, mean AFCL was longer both in right atria (121.7 ± 8.4 ms, P = 0.02) and left atria (91.4 ± 4.9 ms, P = 0.001).

Dispersion of atrial refractoriness, as determined by the coefficient of variation of AFCL, was measured during autonomic manipulation (Table). For both atria combined, the coefficient of variation of AFCL was unchanged from baseline with stellate stimulation, vagal stimulation, or infusion of propranolol (Table). The coefficient of variation of AFCL was also compared between the left and right atria at baseline and during autonomic manipulation. At baseline, the mean coefficient of variation of AFCL was significantly greater in the right atrium than in the left atrium (P = 0.005) (Table). This finding of a greater dispersion of AFCL in the right atrium than in the left atrium was also observed with vagal stimulation.

Figures 2, 3, 4, and 5 are graphs showing the comparison of HED retention, coefficient of variation of sympathetic innervation, and mean AFCL between control dogs (sinus rhythm) and paced dogs (AF) at 6 weeks. The graphs indicate significant differences in HED retention, sympathetic innervation, and AFCL between the control and paced groups. For example, Figure 2 shows that HED retention fraction of the entire atrium and individual left and right atria was significantly greater in the paced group than in the control group (P = 0.03). Similarly, Figure 3 shows that the coefficient of variation of sympathetic innervation was significantly greater in the paced group than in the control group (P = 0.004). Figure 4 demonstrates that with bilateral vagal stimulation, there was a trend to a shorter mean AFCL (P = 0.07). Bilateral stellate stimulation resulted in no significant change in overall mean AFCL. Infusion of propranolol resulted in a lengthening of mean AFCL (P = 0.03). Figure 5 compares mean AFCL between left and right atria with autonomic manipulation. At baseline autonomic state, mean AFCL was shorter in the left atrium. Mean AFCL shortened to a greater extent in right than in left atrium with vagal stimulation and was unchanged from baseline with stellate stimulation. With propranolol, mean AFCL lengthened in both left and right atria (P = 0.001, **P = 0.027). AFCL is in milliseconds.
spatial dispersion of atrial refractoriness (AFCL) as the result of autonomic remodeling and rapid pacing. Altered sympathetic function has been implicated in the pathogenesis of AF in animal models and humans. Increased vagal tone has been demonstrated to cause AF in animals and humans. Although increased vagal tone has been demonstrated to cause AF in animals and humans, the role of the sympathetic nervous system in AF is less understood. Previous investigations of the effect of sympathetic stimulation on atrial refractoriness have found varied results. In humans, an increased incidence of AF has been observed in states of increased sympathetic activity. The mechanisms underlying the provocation or sustenance of AF as the result of an altered sympathetic state have not been clarified. Although our study has not demonstrated a causal link to the sustenance of AF as the result of sympathetic stimulation, we have demonstrated that sympathetic heterogeneity is spatially related to heterogeneity of atrial refractoriness.

Discussion

Major Findings

We found that rapid atrial pacing–induced AF in the dog results in both autonomic and electrophysiological remodeling of the atria. This was confirmed by PET imaging of atrial sympathetic nerve terminals as well as the finding of increased tissue norepinephrine content. Autonomic remodeling was characterized by increased sympathetic innervation of the atria as well as increased heterogeneity of atrial sympathetic innervation. These autonomic findings correlated with electrophysiological changes in the atrium. Dispersion of AFCL was greater in the right atrium compared with the left atrium, which correlated with an increased heterogeneity of sympathetic innervation in the right atrium. Although stellate stimulation did not affect the mean duration of AFCL or the dispersion of AFCL, β-receptor blockade lengthened mean AFCL significantly.

Role of Sympathetic Innervation in Electrical Remodeling and AF

Altered autonomic function has long been implicated in the pathogenesis of AF in animal models and humans. Although increased vagal tone has been demonstrated to cause AF in animals and humans, the role of the sympathetic nervous system in AF is less understood. Moreover, previous investigations of the effect of sympathetic stimulation on atrial refractoriness have found varied results. In humans, an increased incidence of AF has been observed in states of increased sympathetic activity. The mechanisms underlying the provocation or sustenance of AF as the result of an altered sympathetic state have not been clarified. Although our study has not demonstrated a causal link to the sustenance of AF, we have demonstrated that sympathetic remodeling occurs with the rapid rates of AF.

Previous studies have shown that autonomic blockade (β-blockade and vagolysis with atropine) does not prevent a short-term decrease in atrial effective refractory period from rapid pacing in animals or humans. Wijffels et al. have shown that propranolol or atropine have little effect on AFCL length during the first 7 days of sustained AF. This is in contrast to our study, which demonstrated an increase in AFCL after the infusion of propranolol after 6 weeks of rapid pacing induced sustained AF. Thus our study suggests that significant autonomic remodeling may occur only with more prolonged episodes of rapid rates.

We also hypothesized that heterogeneity of autonomic innervation promotes heterogeneity of atrial refractoriness. Normal atria exhibit a heterogeneous distribution of vagal and sympathetic innervation. An increased dispersion of atrial refractoriness has been observed in animal models of AF and humans with paroxysmal AF. Increased regional dispersion of AFCL appears to be an important independent determinant of electrophysiological remodeling and is observed even after shortening of atrial refractoriness caused by rapid pacing has stabilized. Whereas vagal stimulation results in regional differences in shortening of refractoriness, promoting both induction and sustenance of AF, sympathetic stimulation appears to have a less pronounced effect on the dispersion of atrial refractoriness, at least in autonomically decentralized animals. It is also of note that these studies were performed in normal animals in sinus rhythm. However, in recent studies we found that heterogeneous sympathetic denervation of the atria with the epicardial application of phenol in dogs resulted in increased heterogeneity of atrial refractoriness and a milieu for sustained AF. In the present study, we found that heterogeneity of sympathetic innervation increases with prolonged rapid rates. We also found that this sympathetic heterogeneity is spatially related to heterogeneity of atrial refractoriness (as reflected by AFCL).

Autonomic Remodeling

To the best of our knowledge, the present study is the first to assess changes in sympathetic innervation during the process of electrophysiological remodeling and sustained AF. HED is taken up by an energy-dependent uptake-1 mechanism of postganglionic presynaptic sympathetic nerve terminals and is not metabolized and thereby labels functioning sympathetic nerve terminals. Extraneuronal HED retention is low and the extraction of HED from blood to the neuronal axoplasm is high. This suggests that the increase in HED retention in paced dogs represents either an increased number of sympathetic nerve terminals with an intact uptake-1 mechanism or an upregulation of the uptake-1 mechanism. Because our technique corrected for spatial variations in blood flow and myocardial blood flow was in fact reduced in paced dogs, altered flow could not have accounted for increased HED retention.
The triggers and mechanisms underlying these autonomic changes are unknown. Recent findings of reduced atrial myocardial blood flow and histological similarities between chronically ischemic ventricular myocardium and atrial myocardium in sustained AF suggest the possibility of ischemia as a trigger. Myocardial infarction causes sympathetic denervation in the ventricle; however, the effects of long-term ischemia on ventricular sympathetic innervation are unknown. In diabetic cardiac autonomic neuropathy, regional hyperinnervation (increased HED retention) can accompany sympathetic denervation. The structural myopathy of long-term AF may also play a role in these alterations, akin to ventricular sympathetic changes in nonischemic dilated cardiomyopathy.

**Electrophysiological Effects of Autonomic Remodeling**

The present study suggests significant electrophysiological effects of sympathetic activity in the electrically remodeled atrium. It is possible that unopposed α-adrenergic activity in the presence of β-blockade with propranolol in this model prolongs atrial refractoriness, whereas “balanced” α- and β-adrenergic activity as the result of stellate stimulation effects no net change in refractoriness, as in the healthy canine atrium. Unfortunately, the electrophysiological effects of α-adrenergic–blocking agents could not be assessed in the present experiment because they affect the uptake of HED used for sympathetic imaging.

**Study Limitations**

With the use of the above model, the exact time course of the onset and development of changes in sympathetic innervation could not be assessed because the technique of high-resolution atrial imaging in dogs does not permit sequential in vivo studies. The effects of α-receptor blockade on autonomic remodeling could not be assessed in this study because these drugs interfere with PET imaging with the use of HED. This also precluded the assessment of the effect of sympathomimetic agents such as epinephrine or dopamine on atrial electrophysiology. Samples of tissue for norepinephrine content were only obtained from the atrial appendages to allow preservation of atrial architecture for PET imaging. However, because changes in HED uptake were widespread, such sampling may not have significantly biased our results. Atrioventricular junction ablation and ventricular pacing were not performed to control ventricular rate out of concern that ablation at this site would affect atrial innervation. However, ventricular rates were not significantly different between paced dogs and control dogs; furthermore, no animal developed congestive heart failure by clinical or echocardiographic estimation.

**Conclusions**

“Autonomic remodeling” occurs during electrophysiological remodeling of the atria produced by rapid atrial pacing. The possible triggers for this phenomenon and modalities of intervention in preventing their electrophysiological impact during the process of electrophysiological remodeling need further investigation.

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