Alterations in Atrial Electrophysiology and Tissue Structure in a Canine Model of Chronic Atrial Dilatation Due to Mitral Regurgitation

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Background—Clinically, chronic atrial dilatation is associated with an increased incidence of atrial fibrillation (AF), but the underlying mechanism is not clear. We have investigated atrial electrophysiology and tissue structure in a canine model of chronic atrial dilatation due to mitral regurgitation (MR).

Methods and Results—Thirteen control and 19 MR dogs (1 month after partial mitral valve avulsion) were studied. Dogs in the MR group were monitored using echocardiography and Holter recording. In open-chest follow-up experiments, electrode arrays were placed on the atria to investigate conduction patterns, effective refractory periods, and inducibility of AF. Alterations in tissue structure and ultrastructure were assessed in atrial tissue samples. At follow-up, left atrial length in MR dogs was 4.09 ± 0.45 cm, compared with 3.25 ± 0.28 cm at baseline (P < 0.01), corresponding to a volume of 205 ± 61% of baseline. At follow-up, no differences in atrial conduction pattern and conduction velocities were noted between control and MR dogs. Effective refractory periods were increased homogeneously throughout the left and right atrium. Sustained AF (>1 hour) was inducible in 10 of 19 MR dogs and none of 13 control dogs (P < 0.01). In the dilated MR left atrium, areas of increased interstitial fibrosis and chronic inflammation were accompanied by increased glycogen ultrastructurally.

Conclusions—Chronic atrial dilatation in the absence of overt heart failure leads to an increased vulnerability to AF that is not based on a decrease in wavelength. (Circulation. 2003;107:2615-2622.)

Key Words: fibrillation ■ electrophysiology ■ tissue

Atrial fibrillation (AF) is the most commonly occurring arrhythmia in clinical practice. Among the risk factors predisposing to AF are congestive heart failure (CHF), hypertension, and mitral valve disease (both stenosis and insufficiency).1 Clinically, atrial dilatation is also associated with an increased occurrence of AF.2 Various animal models of AF have been developed. In goat and dog models, prolonged rapid atrial pacing (RAP) leads to sustained AF.3,4 In a dog model of CHF due to rapid ventricular pacing, inducibility of AF was increased.5 Structural as well as electrophysiological alterations have been reported for RAP6,7 and CHF models.5,8 Although both models produce LA dilatation,4,9 additional influences on atrial electrophysiology exist in these models (ie, the neurohumoral effects of CHF and rapid rates). Work on atrial dilatation in animal models has focused primarily on the effects of acute atrial dilatation on atrial electrophysiology.10–12 The role of chronic LA dilatation in itself on atrial remodeling remains unclear.

The present study investigates the effects of chronic atrial dilatation on the atrial myocardium. To this end, we have studied atrial electrophysiology and structure in a model of chronic atrial dilatation due to mitral regurgitation (MR).

Methods

Animals

Adult mongrel dogs weighed 25 to 30 kg (LBL Kennels, Reelsville, Ind). In total, 13 control and 19 MR dogs were studied. Studies were performed according to National Institutes of Health guidelines and locally monitored by the Animal Studies Subcommittee at Indiana University School of Medicine.

Surgical Procedure for the MR Model

Before the procedure, transthoracic echocardiography (TTE) was performed. Dogs were anesthetized with isoflurane. The procedure was monitored with fluoroscopy and transesophageal echocardiography (TEE). A long 10-F sheath was inserted through the femoral artery and guided into the left ventricle (LV). A custom catheter with a stainless steel hook on the tip was advanced through the sheath and attached to a mitral chorda. Subsequently, the catheter was advanced over the hook to prevent damage to the aortic valve during pull back. After the catheter was pulled back to rupture chordae, the degree of MR was determined by TEE. This procedure was repeated until MR was moderate to severe, as judged by the size, penetration, and flow...
velocity of the regurgitant jet, flow reversal in the pulmonary veins, and acute dilatation of the LA and LA appendage (LAA). Creation of moderate to severe MR required 1 to 4 passes with the catheter, after which animals were allowed to recover.

Monitoring of the MR Model
All MR dogs were subjected to weekly physical examinations and TTE in the 4-chamber view to measure LA size (LA length from mitral valve to LA roof) and LV function (LV fractional shortening at the papillary muscle level). Each measurement was repeated 5 times and averaged. In 6 dogs, 24-hour Holter recordings were obtained in the week before surgery and in subsequent weeks. MR dogs were followed up at 32±9 days after creation of MR.

Open-Chest Follow-Up Studies
At follow-up, control and MR dogs were anesthetized with isoflurane, and the chest was opened by midline sternotomy. A pericardial cradle was created, and epicardial multielectrode plaques were placed on the posterior and anterior surfaces of the right anterior (RA) and left anterior (LA), with a total of 512 electrodes, as described previously. Unipolar electrode signals were recorded with a CardioMapp system (Prucka Engineering Inc). Pairs of electrodes on the plaques were also used for bipolar stimulation at an amplitude of ×2 diastolic threshold. ERPs were measured at 6 LA and 6 RA sites with the S1S2 method, where S2 was incremented in 2-ms steps after 8 beat drive trains with basic cycle lengths (BCLs) of 200 to 450 ms. At the same BCLs, during stimulation of the contralateral atrium, conduction velocity (CV) was calculated between pairs of electrodes (2 to 3 interelectrode distances apart) perpendicular to the activation wavefront, near the center of the

Figure 1. Creation of MR. TEE images from the transgastric view 5 minutes after partial avulsion of the mitral valve. A, Color Doppler revealed a large regurgitant jet (Ao indicates aorta). B, Continuous-wave Doppler at the level of the mitral valve showed high-velocity holosystolic regurgitant flow.

Figure 2. Time course of LA dilatation and LV fractional shortening. A, LA length as percent of baseline (bsln) was significantly increased in the first week after creation of MR (P<0.01, n=16). B, LV fractional shortening showed a nonsignificant increasing trend after the procedure (n=16).

Figure 3. AF inducibility in open-chest studies. A, Longest observed AF episode duration in control and MR dogs. B, Relationship between the longest observed AF episode duration in individual MR dogs and LA dilatation (% increase in LA length with respect to baseline).
regions indicated. AF inducibility was tested by burst pacing with 1200 bpm at 1 LA and 1 RA site. In total, 8 6-second and 4 12-second bursts were applied. In each animal, the duration of AF was measured; AF was considered to be sustained when an episode lasted longer than 1 hour. After AF vulnerability testing, tissue was harvested for histology and electron microscopy.

### Histology
Transmural tissue samples from the LA and RA appendage and free wall of 5 control and 5 MR dogs were fixed in 10% neutral buffered formalin. Tissue was processed, embedded in paraffin, and sectioned in 4- to 5-μm-thick sections. Serial sections were stained with either H&E, Masson’s trichrome, or Sirius red.

### Electron Microscopy
Small tissue samples from the LA and RA from 4 control and 4 MR dogs were fixed in 3% glutaraldehyde and postfixed with 2% osmiumtetroxide. Sections of 100 nm were stained with uranylacetate and lead citrate. Ultrastructure was visualized using a Philips CM 10 transmission electron microscope. Myocyte widths were determined as the minor axis of the circumference of transversely sectioned myocytes.

### Statistical Analysis
Comparisons between groups were made using ANOVA with post hoc Newman-Keuls test or a χ² test (AF inducibility). *P*<0.05 was considered to be significant. Data are presented as mean±SD, unless mentioned otherwise.

### Results

#### Creation of MR
During the operative procedure, TEE in the transgastric view was used to monitor MR. Figure 1 illustrates MR minutes after partial avulsion of the mitral valve. Marked dilatation of the LA and LAA was noted, and LAA flow became turbulent after creation of MR (not shown). From these criteria, MR was qualified as severe in this particular animal. At this early time point, the LA showed significant acute dilatation; at approximately 10 minutes after mitral valve avulsion, atrial width on TEE had increased from 2.6±0.5 to 3.6±0.6 cm (n=9, *P*<0.01).

#### Development of LA Dilatation and LV Function
From the baseline TTE and weekly postoperative TTE, LA length and LV fractional shortening were determined. The time course of these parameters is shown in Figure 2. LA length was already significantly increased in the first week after creation of MR (Figure 2A). LA length continued to increase slowly until reaching a plateau after 3 weeks. LV fractional shortening showed an increasing trend in the first weeks (Figure 2B), correlating with the hyperdynamic LV that was evident in TTE examinations. The heart rate, 131±23 ms at baseline versus 143±22 ms before follow-up, was not significantly increased (*P*<0.16). No physical signs of heart failure were observed during weekly examinations in any of the dogs.

#### Vulnerability to AF
In 24-hour Holter recordings on 6 dogs, no episodes of spontaneous AF were noted before or in the weeks after creation of MR. In open-chest follow-up experiments, when inducibility of AF was tested by burst pacing, sustained AF (>1 hour) was not observed in any of the 13 control dogs. In the MR group, sustained AF was inducible in 10 of 19 dogs (χ², *P*<0.01). For control and MR dogs, the average longest AF episode durations were 9±2 and 1761±400 seconds, respectively (when an episode had lasted 3600 seconds, the experiment was terminated).

Figure 3A plots data points for the longest observed AF episode in individual dogs. Within the MR group, AF was either sustained, lasting 3600 seconds, or nonsustained, with
episode durations comparable to those observed in the control group. In Figure 3B, AF episode durations in the MR group are plotted against atrial dilatation. MR dogs in which no sustained AF was inducible tended to have less atrial dilatation. In MR dogs with inducible sustained AF, atrial length was 134±11% of baseline, compared with 120±5% of baseline for dogs in which no sustained AF was inducible (P=0.02, corresponding with estimated LA volumes of 243±63% and 173±21% of baseline, respectively).

Atrial Conduction and Refractoriness
At sinus rhythm and during pacing of the contralateral atrium with BCLs between 500 and 250 ms, no systematic differences in conduction pattern were noted in the MR RA and LA compared with control (Figure 4).

Atrial ERP was determined as the average ERP from 6 sites in each atrium. In Figures 5A and 5B, the average LA and RA ERP for control and MR dogs is plotted against the BCL. In both the LA and the RA, ERP was significantly higher in MR dogs compared with control at most BCLs. Cycle length dependence was similar for control and MR dogs. The coefficient of variation (standard deviation/mean) of the ERP in each atrium, a measure for dispersion of refractoriness, was not significantly different at any BCL for control LA versus MR LA (0.07±0.02 and 0.08±0.03, respectively, at a BCL of 300 ms, P=0.43) and control RA versus MR RA (0.10±0.04 and 0.10±0.04, respectively, at a BCL of 300 ms, P=0.88).

At BCLs between 450 and 200 ms, average LA and RA CVs were not significantly different between the control and MR group (Figures 5C and 5D). The wavelength (Figure 5E and 5F) or product of CV and ERP showed an increase that was significant in the LA at higher BCLs.

Figure 6A depicts the regions of the LA and RA for which the ERP and CV were determined. The increase in ERP for the MR group was seen in all atrial regions investigated (Figure 6B) and was significantly higher than control in most regions, providing additional evidence that dispersion of refractoriness was not increased as a result of MR. In all regions investigated, CV in the control and MR groups was similar (Figure 6C), leading to a trend toward increased wavelength (Figure 6D).

Histology
Transmural tissue sections from the RA and LA were stained using H&E (Figures 7A through 7D), Sirius red (Figures 7E and 7F), or trichrome stain (Figures 7G through 7J) to compare tissue structure and the distribution of fibrous tissue in the control and MR groups. In the MR LA, regions with a relatively normal tissue organization occurred alongside re-
regions in which tissue structure was disrupted (Figure 7C). Comparable affected areas were not observed in the control LA (Figure 7A), control RA, and MR RA (not shown). At higher magnification, H&E staining in the MR LA revealed infiltrates of inflammatory cells indicative of chronic inflammation (Figure 7D). In Sirius red–stained (Figure 7F) and trichrome-stained (Figure 7J) sections of the MR LA, regions with increased interstitial fibrosis (red color in panel F, blue color in panels I and J) were present. However, in many areas in which fiber separation and inflammatory infiltrates were visible, interstitial fibrosis was moderate, as in Figures 7F and 7I. Similar regions were not encountered in the control LA (Figures 7G and 7H), control RA, and MR RA (not shown). The gross morphology of myocytes in MR LA (Figures 7D, 7F, and 7J) was similar to that in control LA (Figures 7A, 7E and 7H), without signs of cellular necrosis.

Electron Microscopy
Atrial myocardial ultrastructure due to MR was examined by electron microscopy. At lower magnification, MR LA and RA myocytes (Figure 8B) showed no signs of myofibrillar disarray, myolysis, alterations in mitochondrial structure, or changes in nuclear structure or other indications of myocyte dedifferentiation or necrosis. Measurement of myocyte width showed no evidence of cellular hypertrophy (cellular widths were $12.3 \pm 5.0$ µm for control LA and $13.6 \pm 4.1$ µm for MR LA [$P=0.85$] and $13.0\pm 3.2$ µm for control RA and $12.5\pm 2$ µm for MR RA [$P=0.42$]).

At higher magnification, both control LA (Figure 8C) and MR LA (Figure 8D) showed a normal myofibrillar and mitochondrial structure. However, myocytes in the MR LA showed a dramatic increase in monoarticular glycogen compared with control LA, as evidenced by the increased density of glycogen storage vesicles (Figure 8D, arrows). Increased glycogen accumulation was not present in control or MR RA (not shown).

Discussion
In this study, we describe changes in atrial structure and electrophysiology in a canine model of chronic LA dilatation. Although true atrial size cannot be measured from TEE, a
major component of the effect of MR on LA size was observed within minutes of the creation of MR.

TTE showed an atrial length of 113±25% of baseline within the first week, followed by an additional increase to 126±12% of baseline before follow-up. Over a time course of months, the hemodynamic changes resulting from chronic severe MR may eventually lead to LV heart failure.14 Clinically, CHF is a known predisposing factor for AF.1 In a canine model of CHF, changes in atrial structure and an increased vulnerability to AF have been reported.7 However, at the time of follow-up in this study, LV fractional shortening was not significantly different from baseline, indicating that LV function was preserved. In addition, MR dogs presented no physical signs of heart failure. Therefore, we are confident that the changes in the atrial structure and physiology in the present study are due to chronic LA dilatation in the absence of overt heart failure.

Earlier studies have shown that the LA size is increased both in the CHF model and to a lesser extent in the RAP model.3,9 However, in these models, or in a model of combined dilatation and rapid pacing,15 the contribution of atrial dilatation per se to the remodeling process cannot be determined. In dogs with naturally occurring mitral stenosis, Boyden et al16 reported an increased incidence of AF with chronic atrial dilatation. However, the mitral stenosis in the 23 dogs included in this study was of unknown cause and duration, and the study group had a heterogeneous background, which may have included CHF.

In this study, we report that in dogs with chronic LA dilatation due to MR, sustained AF was inducible in 53% of MR dogs. The distribution of longest AF episode durations in individual dogs was bimodal, showing either inducible sustained AF or short nonsustained AF episodes. Few MR dogs presented episodes of intermediate duration; the longest AF episode observed in a MR dog without inducible sustained AF measured 400 seconds. MR dogs with inducible sustained AF had more LA dilatation than MR dogs in which no sustained AF could be induced. Thus, there seems to be a discrete threshold to the extent of atrial pathology in the MR model necessary for sustenance of AF. Holter recordings in MR dogs did not reveal spontaneous episodes of AF, suggesting that although a substrate for AF was present in these dogs, initiating triggers did not occur within the first month of atrial dilatation.

To investigate the mechanism of the increased AF vulnerability in MR dogs, the distribution of atrial ERP (AERP) was assessed. Several studies have shown a decrease in AERP and increased dispersion of refractoriness, generally recognized to be proarrhythmic for AF, in the RAP model.3,4,17 In a canine model of CHF, no change in AERP was observed.9 Diverging effects of acute atrial dilatation on AERP have been reported. Some studies have reported a shortening of AERP in response
to acute dilatation, whereas others have shown an increase or no change in AERP. Here, we report that AERP was increased both in the chronically dilated LA and in the nondilated RA in MR dogs. These results corroborate observations in patient populations, where an increase in AERP was found to be correlated with atrial dilatation. Dispersion of refractoriness in the MR group was not different from control; the prolongation in ERP was observed to a similar extent in all LA and RA regions tested.

In the multiple wavelet theory, the wavelength of a reentrant wave equals the product of ERP and CV. According to this theory, the prolonged AERP in the absence of increased dispersion observed in MR dogs would in itself be antiarrhythmic. In combination, the increased AERP and the lack of difference in CV mean that wavelength tended to increase in MR atria during normal pacing. Therefore, our data imply that the increased AF inducibility cannot be explained by a decrease in wavelength, suggesting that the mechanism underlying AF in RAP and MR dogs is distinctly different.

In goat and dog RAP models, studies on myocardial ultrastructure have shown severe loss of myofibrillar structure, glycogen accumulation, changes in mitochondrial morphology, and cellular hypertrophy, indicative of myocyte dedifferentiation. In a dog CHF model, extensive interstitial fibrosis in the LA was reported, along with cellular hypertrophy, loss of myofibrils, and signs of necrosis. Similar changes were also present in the RA of CHF dogs, but to a lesser extent. In the present study, alterations in tissue structure were confined to the MR LA. We have not found signs of cellular hypertrophy, myolysis, necrosis, or other degenerative changes; at the ultrastructural level, only increased glycogen accumulation was observed in the MR LA. Histologically, the MR LA displayed areas of chronic inflammation and increased interstitial fibrosis. Comparable chronic inflammation has not been reported for RAP and CHF models. The extent of atrial fibrosis in the MR LA was less than that observed in the CHF LA and was more regional, occurring alongside regions with apparently normal tissue structure.

Limitations of This Study

Our data indicate the presence of regions of chronic inflammation and fibrosis in the MR LA. It is conceivable that inflammatory infiltrates and interstitial fibrosis would affect transverse propagation more than longitudinal propagation, leading to increased conduction anisotropy. In a dog model of CHF, profound fibrosis was reported for the LA, accompa-
nied by local LA conduction abnormalities. In contrast, our epicardial conduction maps do not reveal abnormalities in the affected MR LA. One possible explanation for the difference between MR and CHF LA is that because tissue alterations in the MR LA are more modest, the resulting conduction abnormalities have escaped detection in our epicardial mapping experiments and might be revealed using a higher spatial resolution.

In a clinical setting, MR often develops slowly, and patients with resulting atrial dilatation present at a later stage. In this study, MR dogs were followed up at a relatively early time point after acute valvular damage, before the onset of CHF. At this point, sustained AF was inducible in half of the animals. It is unclear whether sustained AF would have been inducible in a larger proportion of MR dogs at a later time point. In general, we cannot provide information on the time course of structural and electrical alterations due to chronic LA dilatation. Chronic LA dilatation could ultimately lead to the more profound atrial fibrosis and the degenerative cellular changes observed in the canine CHF model. However, it is clear that the extent of atrial pathology present after 1 month of chronic LA dilatation can provide a substrate sufficient for the sustenance of AF.

Conclusions
Chronic LA dilatation due to MR in the absence of heart failure increases vulnerability to AF. The homogeneous increase in AERP and diverging structural alterations suggest that the underlying substrate for AF in the chronically dilated LA is distinctly different from that of the RAP model. The differences in atrial pathology in RAP, CHF, and MR animal models could parallel divergent substrates for AF in humans and would indicate the desirability of differential treatment strategies.

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