Altered Atrial Electrical Restitution and Heterogeneous Sympathetic Hyperinnervation in Hearts With Chronic Left Ventricular Myocardial Infarction
Implications for Atrial Fibrillation

Yasushi Miyauchi, MD; Shengmei Zhou, MD; Yuji Okuyama, MD; Mizuho Miyauchi, MD; Hideki Hayashi, MD; Akira Hamabe, MD; Michael C. Fishbein, MD; William J. Mandel, MD; Lan S. Chen, MD; Peng-Sheng Chen, MD; Hrayr S. Karagueuzian, PhD

Background—The substrates for the increased incidence of atrial fibrillation (AF) in hearts with chronic left ventricular myocardial infarction (MI) remain poorly defined. We hypothesized that chronic MI is associated with atrial electrical and neural remodeling that enhances AF vulnerability.

Methods and Results—We created MI in 8 dogs by permanent occlusion of the left anterior descending (LAD) coronary artery. Seven dogs (3 with thoracotomy) that had no LAD occlusion served as controls. Eight weeks after surgery, the incidence and duration of pacing-induced AF in the open chest anesthetized state were significantly higher in the MI than in control dogs. Multisite biatrial monophasic action potential (MAP) recordings showed increased heterogeneity of MAP duration (MAPD) and MAPD restitution slope. AF in the MI groups was preceded by significantly higher MAPD (P<0.01) and MAP amplitude (P<0.05) alternans in both atria compared with controls. Epicardial mapping using 1792 bipolar electrodes (1-mm spatial resolution) showed multisite wavebreaks of the paced wavefronts leading to AF in MI but not in control dogs. Multiple wavelets in MI dogs were associated with significantly higher incidence and longer duration of AF compared with control. The density of biatrial tyrosine hydroxylase (TH) and growth-associated protein43 (GAP43) nerves were 5- to 8-fold higher and were more heterogeneous in MI compared with control dogs.

Conclusions—Chronic ventricular MI with no atrial involvement causes heterogeneous alteration of atrial electrical restitution and atrial sympathetic hyperinnervation that might provide important substrates for the observed increased AF vulnerability. (Circulation. 2003;108:360-366.)

Key Words: nervous system ▪ myocardial infarction ▪ remodeling ▪ atrium ▪ fibrillation

Myocardial infarction (MI) is associated with an increased incidence of atrial fibrillation (AF) in patients with chronic MI, independent of heart failure and hypertension.1 The substrates for the increased AF vulnerability during the chronic, healed phase of MI, however, remain poorly explored. Studies in a canine model of AF produced by prolonged rapid atrial pacing suggested the induction of heterogeneous sympathetic atrial hyperinnervation2,3 and altered transmembrane ionic currents (electrical remodeling)4,5 as a potential substrate for increased AF vulnerability. The increased sympathetic activity was suggested6 to result in decreased heart rate variability observed during chronic MI.8,7 However, no morphological proof of sinus nodal (SN) sympathetic hyperinnervation has been provided. One aim of the present study was to test the hypothesis that chronic MI promotes sympathetic hyperinnervation of the atria and the sinus node. AF is often associated with rapid pulmonary vein discharge that could induce or maintain AF.8,9 The second aim of the present study was to determine if chronic MI causes pulmonary vein (PV) sympathetic hyperinnervation and rapid focal discharge. Our hypothesis is that chronic MI increases AF vulnerability and that increased atrial dispersion of repolarization and heterogeneous atrial sympathetic hyperinnervation provide important substrates for AF.

Methods

Surgical Preparation

This study protocol was approved by the Institutional Animal Care and Use Committee and followed the guidelines of the American Heart Association. Fifteen mongrel dogs of either sex, weighing 21
preceding diastolic interval13) was fitted to a single exponential

declined, and the MAP duration at 90% repolarization and amplitude (MAPD90 and MAPA, respectively) were measured using a custom-written program. The restitution curve (MAPD90 versus tude (MAPD90 and MAPA, respectively) were measured using a
decrement, and the MAP duration at 90% repolarization and ampli-

 Three of these 7 dogs underwent thoracotomy and were studied 8

 weeks after surgery (sham-operated).

 to 27 kg, were studied (USDA-registered class A dealer, Mo). A

 relatively small MI was created by permanent occlusion of LAD

 below its first diagonal branch in 8 open-chest and anesthetized
dogs31 (mean weight of 24±2 kg). In 3 of these 8 dogs, the left

 stellate ganglion was removed before LAD occlusion. Eight weeks

 after the surgery (healed phase of MI), the dogs were reanesthetized

 with propofol (7.4 mg/kg) followed by inhalation of isoflurane (1.5%

 to 3.0%) and studied in the open-chest state. Seven dogs (mean

 weight of 23±2 kg) with no LAD occlusion served as controls.

 Three of these 7 dogs underwent thoracotomy and were studied 8

 weeks after surgery (sham-operated).

 **AF Vulnerability**

 To test AF vulnerability, we used burst atrial pacing at cycle lengths

 (CLs) of 50 to 20 ms, the extrastimulus method at basic CL of 200

 ms, and pacing at progressively shorter CLs from 280 ms with a

 10-ms decrement until loss of 1:1 captures. Pacing was performed

 from right atrial (RA) and left atrial (LA) appendages, respectively,

 with twice the diastolic current threshold and a pulse width of 5 ms.

 The number of induced AF episodes (incidence) and the duration of

 AF was measured in each dog in both groups.

 **Atrial Monophasic Action Potential and Electrical Restitution**

 Monophasic action potentials (MAPs) were recorded from the RA

 and LA (up to 5 sites in each dog) using contact electrodes12 during

 progressively shorter pacing CLs starting at 280 ms with a 10-ms

decrement, and the MAP duration at 90% repolarization and ampli-
tude (MAPD90 and MAPA, respectively) were measured using a

 custom-written program. The restitution curve (MAPD90 versus
 preceding diastolic interval13) was fitted to a single exponential
 curve using Origin 5.0 (Microcal Software, Inc). The maximum
differences between consecutive MAPDs and MAPAs that preceded
AF were also calculated. Biatrial effective refractory periods (ERPs)
determined by the S1S2 method using twice diastolic threshold
current and 5-ms duration at a CL of 400 ms.

 **High-Density Computerized Mapping of AF**

 AF was mapped in 4 dogs with chronic MI and 3 control dogs using
computerized 1792 bipolar electrodes with 1-mm interelectrode
distance distributed equally over 4 plaques of 1.5 by 2.7 cm
(UNIMAP, Uniservices).14 We simultaneously mapped the LA free
wall at the junction of the left superior pulmonary vein (LSPV), the
Bachmann’s bundle (BB), and the lateral RA free wall (Figure 1A).

 **Immunocytochemical Staining**

 Tissue samples were taken from the RAAs and LAAs, the RA and LA
free walls, the SN region, and the LSPV (longitudinal sections).2 The
density of nerves positive for the growth-associated protein43
(GAP43) and tyrosine hydroxylase (TH) that stain growing and
sympathetic nerves were determined as we described previously.2

 The investigators were blinded to the specimen’s source. We
analyzed 13 to 37 fields at 20×1.7 mm apart to cover the entire
region of the section. We calculated the average of all fields (mean
nerve density). We chose the 3 highest and the 3 lowest nerve density
fields in tissue section, and the difference of these averages was also
calculated and defined as regional heterogeneity. The entire SN
region was analyzed using 12 to 22 fields at ×20. Nerve density is
expressed as the nerve area divided by the total area examined
(µm²/mm²).

 **Macroscopic and Histological Evaluation**

 RA and LA chamber sizes in millimeter, percent atrial interstitial
fibrosis (Masson trichrome stain), and left ventricular infarct size
were measured as described previously.11

 **Statistical Analysis**

 Mann-Whitney U test was used for comparison of AF duration
between the 2 groups, and AF duration data were expressed as
mean±SEM. All other comparisons were made using unpaired t
tests, and data are expressed as mean±SD. P<0.05 was considered
significant.
Results

There was no significant difference between MI and MI plus left stellectomy dogs with respect to AF (incidence and duration, \( P=0.6 \)), restitution parameters (\( P=0.7 \)), and nerve density (\( P=0.37 \)). Consequently, the results of the 8 MI dogs were pooled (MI group). Similarly, because the results of AF, restitution, and nerve counts in the sham-operated dogs were not significantly different (\( P>0.4 \)) from nonsurgery dogs, the results of these 7 dogs were pooled (control group).

AF Vulnerability

The incidence of pacing-induced AF was significantly (\( P<0.01 \)) higher in the MI (83±14%) than in the control dogs (28±17%), respectively. The duration of the induced AF was also significantly (\( P<0.05 \)) longer in the MI dogs (41±23 seconds) than in control dogs (3.2±1.2 seconds).

AF Activation Maps

Figure 1 shows activation map of the onset of AF induced by a RA extrastimulus in a dog with MI (Figure 1B). During the basic drive at a pacing CL of 200 ms (panel C1), a large activation wavefront propagates from the right to the left atrium during a paced beat at a CL of 200 ms (B1). The integrity of the large wave is maintained after the S2 (B2). The activation map during the 3rd induced repetetive atrial activity (B3) also shows a single large wavefront in the entire mapped region. Abbreviations are as in Figure 1.

Figure 2. Initiation repetitive atrial activity by a premature stimulus in a control dog. All panels are organized in the same fashion as in Figure 1. As in the dog with MI, a single large wavefront propagates from the right to the left atrium during a paced beat at a CL of 200 ms (B1). The integrity of the large wave is maintained after the S2 (B2). The activation map during the 3rd induced repetitive atrial activity (B3) also shows a single large wavefront in the entire mapped region. Abbreviations are as in Figure 1.

Figure 3. Isochronal maps of RAFW mapping during rapid pacing in a dog with MI (A) and in a control dog (B). Two electrode plaques were located side by side on the RAFW. The RAFW (just left of the plaques) was paced (stimulus symbol). Up to the 3rd beat pace at 150 ms CL (A1), a smooth propagation was observed; however, nonuniform slowing of propagation (bunched isochrones) developed at left lower portion of the left plaque (arrow in A2), which then leads to AF. No epicardial conduction slowing or wavebreak develops in the control dog using the same pacing protocol (B1 through B4).
Heart rate at the start of the study was not significantly different in the control and MI groups (114 ± 11 versus 116 ± 13 bpm, respectively, P = 0.64). In addition, no significant difference in the MAPD dispersion could be detected in the sham-operated versus nonoperated dogs. MAPD and MAPA alternans of progressively increasing magnitude developed during pacing at CLs shorter than 190 ms in all dogs with MI. In control dogs, however, relatively smaller and stable amplitude alternans (i.e., absence of progressively increasing alternans amplitude) were observed (Figure 4). The magnitude of the MAPD and the MAPA alternans were significantly (P < 0.05) larger in the MI than in control dogs (Table). There was no significant difference in the mean MAPD and the mean ERP at RA and LA in the 2 groups (Table). Regional differences of the MAPD, measured at a CL of 200 ms and determined by the differences of maximum and minimum MAPD (dispersion) in each atrium, were significantly (P < 0.01) greater in both atria of the MI compared with control dogs. The distribution of the maximum slope of the MAPD restitution curves was highly heterogeneous and significantly (P < 0.01) greater than controls (i.e., intra-atrial difference of maximum–minimum of the maximum slope of the restitution curve) (Table). The range of diastolic intervals over which the slope > 1 was significantly (P < 0.05) higher in both atria in MI compared with control dogs (Table). Finally, the MAPD and MAPA alternans ratio just before the onset of AF was significantly higher in the MI compared with control dogs (Table).

Atrial Nerve Density
At all atrial sites, the density of nerves positive for TH and GAP43 were significantly (P < 0.01) higher (5- to 7-fold) in MI than in control dogs at all 4 atrial sites (Figure 5). Similarly, TH- and GAP43-positive nerve density in the sinus node and the LSPV were significantly (P < 0.01) higher (3- to 5-fold) in the MI than in control dogs (Figure 6). Regional heterogeneity, assessed by the difference of the 3 highest and 3 lowest fields in a given slide, was significantly higher in MI dogs than in control dogs (Figure 6).

Histopathologic Findings
Myocardial infarction was confined exclusively to the LV with no atrial involvement in all dogs. The mean percent LV infarct was 14 ± 6% total. No signs of infarction or pathological changes indicative of ischemia or necrosis could be detected in either atria. The amount of atrial fibrosis (1.4 ± 0.3% versus 1.3 ± 0.3%) and atrial chamber sizes were not significantly different in the 2 groups.

Discussion

New Findings
The results of the present study show that experimental chronic left ventricular MI with no atrial involvement creates atrial substrate for AF and increases the incidence of inducible AF and its duration. The simultaneous development of heterogeneous alteration of electrical restitution and sympathetic hyperinnervation provide important substrates that might contribute to the observed increase in AF vulnerability in this model.

Electrical Remodeling as AF Substrate
The present study showed increased difference of the maximum versus minimum MAPD within each atrium in dogs...
with MI. Increased heterogeneity of refractoriness is a known substrate for AF.\textsuperscript{15} The demonstration of wavebreak at multiple sites by a premature stimulus is consistent with heterogeneity in MAPD. Wavebreak, defined as splitting of the wavefront to 2 or more independent wavefronts, promotes disorganized pattern of activation leading to fibrillation.\textsuperscript{16} In addition, the observed increase in atrial heterogeneity (dispersion) of electrical restitution slopes may also contribute to wavefront breakup at multiple sites.\textsuperscript{16} AF was initiated by a simultaneous critical increase in the magnitude of alternans MAPD and MAPA, a phenomenon that increases the excitability gradient between 2 consecutive beats leading to wavebreak. Immediately preceding the onset of AF, the alternans ratio was at its maximum for both MAPD and MAPA in the MI. No such scenario developed in control dogs. In some of the control dogs, however, short-duration AF could be induced in the absence of progressively increasing alternans. The ionic mechanisms of increased slope remain undefined. It is possible that downregulation of atrial L-type calcium channels, a phenomenon that commonly occurs in remodeled atria,\textsuperscript{4,5} may allow greater shortening of the APD, causing steeper slope of electrical restitution. Atrial stretch may yet be another mechanism that may contribute to additional increase in the slope of the MAPD restitution.

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<th>LA</th>
<th>RA</th>
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<tr>
<td></td>
<td>Control</td>
<td>MI</td>
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<tr>
<td>Mean MAPD (CL 250 ms)</td>
<td>127±11</td>
<td>135±11</td>
</tr>
<tr>
<td>Mean MAPD (CL 200 ms)</td>
<td>121±9</td>
<td>120±11</td>
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<tr>
<td>Mean MAPD (CL 150 ms)</td>
<td>102±7</td>
<td>Alternans</td>
</tr>
<tr>
<td>Max-min MAPD (CL 250 ms)</td>
<td>9±6</td>
<td>19±13</td>
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<td>Max-min MAPD (CL 200 ms)</td>
<td>8±4</td>
<td>20±10†</td>
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<tr>
<td>Max-min MAPD (CL 150 ms)</td>
<td>9±5</td>
<td>Alternans</td>
</tr>
<tr>
<td>Max slope Mean</td>
<td>1.2±0.3</td>
<td>1.5±0.3</td>
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<tr>
<td>Max slope Max</td>
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<td>2.5±0.6†</td>
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<tr>
<td>Di slope &gt;1 (max), ms</td>
<td>14±8</td>
<td>26±7†</td>
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<tr>
<td>ERP, ms</td>
<td>132±7</td>
<td>135±13</td>
</tr>
<tr>
<td>Max MAPD alternans ratio</td>
<td>1.1±0.1</td>
<td>1.6±0.3†</td>
</tr>
<tr>
<td>Max MAPA alternans ratio</td>
<td>1.6±0.2</td>
<td>3.1±1.0†</td>
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*P<0.05 vs control, †P<0.01 vs MI.

Di indicates diastolic interval; Di slope >1, diastolic intervals, in ms, within which the slope remains >1; and Max MAPD and Max MAPA alternans ratios, ratios of maximum consecutive differences in the MAPD and MAPA that precede AF.

Figure 5. Atrial immunostaining of TH-positive (A) and GAP43-positive nerves (B) at different atrial sites. Increased TH- and GAP43-positive nerves at the 4 atrial sites are evident in the dog with MI. C, Histograms of mean TH- and GAP43-positive nerve density at all 4 atrial sites. D, Graphs show regional atrial heterogeneity (ie, the difference between maximum and minimum nerve density). *P<0.05, †P<0.01. Abbreviations are as in Figure 1.
Although atrial chamber size was not different in the 2 groups, rapid atrial pacing in hearts with diseased ventricles might increase atrial pressure (stretch), increasing the slope of the APD restitution curve. More work is needed to clarify the relation of atrial chamber pressure to atrial electrical restitution in hearts with chronic MI. Increased atrial sympathetic hyperinnervation might also contribute to the increased slope of the MAPD restitution curve via its catecholamine-releasing influence.

Sympathetic Hyperinnervation as AF Substrate
Heterogeneous sympathetic denervation of the atria by topical phenol application results in heterogeneity of refractoriness and promotes AF. Because sympathetic hyperactivity has been proposed as a mechanism of decreased heart rate variability during MI, the demonstration of sympathetic hyperinnervation in the sinus node in the present study is compatible with this proposal and suggests, but does not provide proof, that the TH-positive nerves may be functional. To the extent that these nerve endings are functional, it is possible that the observed increase in electrical restitution heterogeneity may result from the increased heterogeneity of atrial sympathetic nerve endings via local releases of catecholamine. Sympathetic hyperinnervation in the PV may also provide a substrate for the trigger of rapid focal sources, causing some of the AF episodes observed in the present study.

Possible Mechanisms of Atrial Sympathetic Nerve Sprouting
Ischemic myocardial injury results in nerve degeneration followed by sympathetic regeneration (nerve sprouting). The presence of increased atrial GAP43, a marker of nerve sprouting, indicates that active nerve sprouting is occurring in the atria of dogs with chronic MI. Because postganglionic sympathetic neurons project to different areas of the heart, including the atria, it is likely that some of the atrial neurons trafficking through the ventricle may be injured by the MI in the ventricle, promoting nerve sprouting in the atria. Alternatively, atrial nerve sprouting might result from circulating cytokines and growth factors that become elevated during chronic MI. This alternative mechanism is suggested by our demonstration of lack effect of left stellectomy on atrial nerve density.

Study Limitations
It may be argued that thoracotomy could in and of itself cause inflammation, atrial nerve sprouting, and AF irrespective of the underlying MI. However, the absence of any change in AF vulnerability or nerve sprouting in the 3 sham-operated dogs refutes this possibility. We do not know the functional impact, if any, of the atrial GAP43-positive nerves. These nerves may not be mature enough to express TH, or alternatively these nerves may be parasympathetic.

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References


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