Mechanisms of Atrial Tachyarrhythmias Associated With Coronary Artery Occlusion in a Chronic Canine Model

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**Background**—Coronary artery disease predisposes to atrial fibrillation (AF), but the effects of chronic atrial ischemia/infarction on AF-related substrates are unknown.

**Methods and Results**—Regional right atrial myocardial infarction (MI) was created in 40 dogs by ligating an artery that supplies the right atrial free wall and not the ventricles; 35 sham dogs with the same artery isolated but not ligated were controls. Dogs were observed 8 days after MI and subjected to open-chest study, in vitro optical mapping, and/or cell isolation for patch-clamp and Ca\(^{2+}\) imaging on day 8. Holter ECGs showed more spontaneous atrial ectopy in MI dogs (eg, 662±281 on day 7 versus 34±25 ectopic complexes per day at baseline; 52±21 versus 1±1 atrial tachycardia episodes per day). Triggered activity was increased in MI border zone cells, which had faster decay of caffeine-evoked Ca\(^{2+}\) transients and enhanced (by ≈73%) Na\(^+-\)Ca\(^{2+}\) exchange current. Spontaneous Ca\(^{2+}\) sparks (confocal microscopy) occurred under β-adrenergic stimulation in more MI dogs (66±9%) than in control cells (29±4%; P<0.01). Burst pacing induced long-lasting AF in MI dogs (1146±259 versus 30±14 seconds in shams). Increased border zone conduction heterogeneity was confirmed by both bipolar electrode mapping in vivo and optical mapping. Optical mapping demonstrated stable border zone reentry in all 9 MI preparations but in none of 6 shams. Border zone tissue showed increased fibrous tissue content.

**Conclusions**—Chronic atrial ischemia/infarction creates substrates for both spontaneous ectopy (Ca\(^{2+}\)-release events, increased Na\(^+-\)Ca\(^{2+}\) exchange current) and sustained reentry (conduction abnormalities that anchor reentry). Thus, chronic atrial infarction in dogs promotes both AF triggers and the substrate for AF maintenance. These results provide novel insights into potential AF mechanisms in patients with coronary artery disease. *(Circulation. 2011;123:137-146.)*

**Key Words:** atrial fibrillation ■ calcium ■ electrophysiology ■ ischemic heart disease ■ myocardial infarction

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Atrial fibrillation (AF) is an extremely common cardiac arrhythmia associated with increased cardiovascular morbidity and mortality.\(^1,2\) However, our understanding of AF pathophysiology remains incomplete. An improved comprehension of mechanisms underlying AF may lead to the development of novel therapeutic options.\(^3\) Coronary artery disease is a significant risk factor for AF.\(^4,5\) Short-term (several hours) acute atrial ischemia creates a substrate for AF maintenance.\(^6,7\) However, no data are available on the atrial electrophysiological and arrhythmic changes caused by longer-term atrial ischemia/infarction, as might occur in patients with chronic coronary artery disease.

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**Clinical Perspective on p 146**

Atrial myocardial infarction (MI) is considered unusual because it is rarely diagnosed; however, it is often undetected. The incidence of atrial MI in autopsy series varies from 0.7% to 42%, depending largely on whether or not the atria were specifically examined.\(^8\) The largest series of autopsy-assessed atrial infarctions indicated an incidence of 17%.\(^9\) Although challenging, clinical diagnosis of atrial infarction is possible.\(^10,11\) Patients with atrial MI have increased supraventricular arrhythmia risk.\(^11\) To address the atrial electrophysiological consequences of chronic atrial ischemia/infarction, we studied the consequences of ligating an atrial-specific coronary artery branch over a 1-week time period.
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... by: (1) occurrence of spontaneous atrial arrhythmias; (2) effects on AF-maintaining substrates; and (3) underlying mechanisms.

Methods
A detailed Methods section can be found in the online-only Data Supplement.

Atrial MI Model
Animal-handling procedures were approved by the local Animal Research Ethics Committee. Seventy-five mongrel dogs (weight, 26.2 to 37.6 kg) were anesthetized with ketamine (5.3 mg/kg IV), diazepam (0.25 mg/kg IV), and isoflurane (1.5%), intubated, and ventilated. In 40 dogs (MI group), isolated right atrial (RA) MI was created by double ligation of the right intermediate atrial artery (RIAA) and location of infarct zone. RA indicates right ventricle; SNA, sinoatrial node artery; and RCA, right coronary artery. B, Electrode arrays. Electrode sites are indicated by dots. AVR indicates atrioventricular ring; SVC, superior vena cava; IVC, inferior vena cava; PV, pulmonary vein; BB, Bachmann bundle; PW, posterior wall; and IW, inferior wall. The RA free wall electrode array (dashed border) was used for detailed conduction analyses.

Electrophysiological Studies
Serial closed-chest electrophysiological studies were performed in 5 MI dogs. Two days before surgery, dogs were anesthetized with ketamine (5.3 mg/kg IV), diazepam (0.25 mg/kg IV), and isoflurane (1.5%), and a bipolar electrode was inserted into the RA appendage. A baseline closed-chest electrophysiological study was performed after 24 hours of recovery, atrial MI was created the following day, and a closed-chest electrophysiological study was repeated on days 1, 3, and 7. The effective refractory period (ERP) (longest S1-S2 interval failing to capture) of the RA appendage was measured (2× threshold current, 2-ms pulses, mean of 3 determinations) at basic cycle lengths (BCLs) of 150, 200, 250, 300, and 360 ms with 10 basic stimuli (S1-S10), followed by premature extrastimuli (S1-S10) with 5-ms decrements. AF was induced with 1 to 10-second burst pacing (10-Hz, 4× threshold current). Mean AF duration in each dog was based on 10 AF inductions for AF duration <20 minutes and 5 inductions for AF duration 20 to 30 minutes. Prolonged AF (>30 minutes) was terminated by direct current electric cardioversion.

A terminal open-chest electrophysiological study was performed in 9 MI and 6 sham dogs 8 days after surgery. Dogs were anesthetized with morphine (2 mg/kg SC) and α-chloralose (120 mg/kg IV, followed by 29.25 mg/kg per hour) and ventilated. A median sternotomy was performed, and bipolar electrodes were hooked into the RA and left atrial (LA) appendages for recording and stimulation. Silicon sheets containing 240 bipolar electrodes were attached to the atria as described previously. Atrial ERPs were measured at multiple BCLs in the RA and LA appendage at 1 BCL (300 ms) at 6 additional sites: RA and LA posterior wall, RA and LA inferior wall, and RA and LA Bachmann bundle (Figure 1B). AF vulnerability was determined as the percentage of sites at which AF (>1 second) was induced by single extrastimuli.

Phase Delay Analysis
To evaluate conduction properties, phase maps were constructed for the RA electrode array (white plaque with dashed border in Figure 1B), as reported previously. Data were obtained separately in normal zones and border zones. Contiguous areas with electrogram voltages <0.5 mV were defined as the infarct zone, and the surrounding RA was the border zone. Activation time differences between each electrode site and adjacent neighbors were divided by respective interelectrode distances; the largest activation time/distance ratio indicated the phase delay at that site. Phase delay histograms provided median phase delays (PS0) reflecting average conduction velocities, differences between 5%-shortest (PS5) and 5%-longest (PS5) delays (PS5-PS5) indicating phase delay range, and conduction heterogeneity indices (PS5–PS95).

Cardiomyocyte Isolation
Single atrial cardiomyocytes were isolated as described previously. On day 8 after surgery, dogs were anesthetized (morphine 2 mg/kg SC; α-chloralose 120 mg/kg IV). RA tissue was coronary artery perfused with Tyrode solution (37°C, 100% O2), then with Ca2+-free Tyrode solution followed by the same solution containing collagenase and bovine serum albumin. RA tissues were collected from the border zone (viable tissue adjacent to the MI scar) of MI dogs and the corresponding control region of sham dogs. After isolation, cells were kept in storage solution (for contents, see Methods in the online-only Data Supplement) at 4°C for current and action potential (AP) recordings in 200 μmol/L-containing Tyrode solution for Ca2+-imaging experiments.

Cellular Electrophysiology
Recordings were obtained at 37°C. Perforated patch was used to record APs in current-clamp mode, and tight-seal patch-clamp was used to record currents in whole-cell voltage-clamp mode. Nystatin-free intracellular solution was placed in pipette tips by capillary action, then pipettes were back-filled with nystatin-containing (600-μg/mL) solution. Cell capacitances averaged 94±6 and 91±8 pF in MI dog border zone cells and control dog cells, respectively. Junction potentials averaged 15.9 mV and were corrected for APs only.

Tyrode solution contained the following (mmol/L): NaCl 136, CaCl2 1.8, KCl 5.4, MgCl2 1, NaH2PO4 0.33, dextrose 10, and HEPES 5 (pH 7.4, NaOH). The pipette solution for AP recording contained the following (mmol/L): GTP 0.1, potassium aspartate 110, KCl 20, MgCl2 1, Mg2-ATP 5, HEPES 10, sodium-phosphocreatine 5, and EGTA 0.05 (pH 7.4, KOH).

Na+-Ca2+ exchange (NCX) current was recorded as described previously as current sensitive to Li+ replacement for Na+.
extracellular solution containing (mmol/L) NaCl 140, CaCl$_2$ 2, MgCl$_2$ 1, dextrose 10, HEPES 10, niflumic acid 0.1, and nifedipine 5 µmol/L. Internal solution for NCX current measurement contained CsCl 130, NaCl 5, MgATP 4, and HEPES 10 (pH 7.3, CsOH).

Ca$^{2+}$ Transients
Atrial cardiomyocytes were incubated with indo-1-AM (5 µmol/L) in 100 µmol/L pluronic F-127 and 0.5% dimethyl sulfoxide for 3 to 5 minutes, then superfused with Tyrode solution. Ultraviolet light passing through a 340-nm interference filter was applied for excitation. Emitted light was detected by matched photomultiplier tubes. Fluorescence signal ratios were digitized and converted to [Ca$^{2+}$] as described previously. Cells were field stimulated (1.5 times threshold voltage) at 0.1, 0.5, 1.0, and 2.0 Hz.

Ca$^{2+}$-Release Events
Atrial cardiomyocytes were incubated with fluo-4-AM for 20 minutes (23°C) and then placed in a perfusion chamber followed by Tyrode solution perfusion for another 10 minutes. Confocal microscopic assessment was performed with an Olympus IX-81 microscope and confocal laser-scanning unit (Olympus Fluoview FV-1000). Fluorescence emission was collected between 500 and 550 nm, with excitation at 488 nm. Ca$^{2+}$-release events were detected under unpaced conditions over a 20-second period after 2-minute 1-Hz pacing and after 2-minute 2-Hz pacing with isoproterenol stimulation (0.5 µmol/L). Line scanning had an optical resolution of 512×512 pixels and temporal resolution of 1.8 to 2.0 ms per line. Ca$^{2+}$ sparks were defined by increased signal mass of 3-µm sections without detectable increases in adjacent 3-µm sections.

Optical Mapping
Optical mapping was performed in coronary artery–perfused RA preparations from 6 sham and 9 MI dogs. On day 8 after surgery, dogs receiving morphine (2 mg/kg SC) and nifedipine 100 µmol/L were mechanically ventilated. Excised hearts were placed in cardioplegic solution (mmol/L): 50 KH$_2$PO$_4$, 8 MgSO$_4$-7 H$_2$O, 10 HEPES, 6 adenosine, 140 glucose, and 100 mmnitol; pH 7.4, KOH) saturated with 100% O$_2$. The RA was coronary artery perfused at 37°C with modified Krebs’ solution (mmol/L): 120 NaCl, 4 KCl, 1.2 MgSO$_4$-7 H$_2$O, 1.2 KH$_2$PO$_4$, 25 NaHCO$_3$, 11 glucose, and 1.25 CaCl$_2$, saturated with 95% O$_2$/5% CO$_2$ at 20 mL/min and 37°C.

Optical recordings were made in border zone regions of MI preparations and corresponding control regions of sham preparations in the presence of the motion uncoupler 2,3-butanedione-monoxime (15-mmol/L, Sigma-Aldrich, St Louis, MO) and the potentiometric dye di-4-ANEPPS. A charge-coupled device camera (800×800 pixels, RedShirt Imaging) recorded fluorescence at 1 kHz. Bipolar electrodes were used for pacing and recording reference. Optical maps were recorded during 2× threshold current RA appendage stimulation at BCLs of 500, 300, and 250 ms and during tachyarrhythmias. To estimate mean atrial tachyarrhythmia duration, tachyarrhythmia was induced 10 times with burst pacing at 100-ms BCL and 4× threshold current in the presence of isoproterenol (0.5 µmol/L).

Conduction data were analyzed with custom-made software. Activation maps were obtained by averaging the fluorescence signal segment of each beat segmented with the use of an extracellular electrode recording. A moving mean (10 samples) was then applied to increase the signal-to-noise ratio. Maximum df/dt was used to define the activation time. Phase analysis was performed on the mean activation map with a grid of 20×20 points (every 4 points of the charge-coupled device matrix).

Tachyarrhythmia data were analyzed with custom-made scripts in Matlab (Mathworks, Inc). Raw fluorescence data from each 80×80 pixels were filtered by a moving mean (10-sample window), normalized, and inverted for the time window of interest. Raw fluorescence change (ΔF=F$_{max}$-F$_{min}$) was calculated for each pixel. Pixels with ΔF/|max(ΔF)| <0.01 were discarded. The timing of local activation was determined on the basis of maximum normalized fluorescence criteria. Infarct zone areas were defined by maximum nonnormalized fluorescence amplitudes <13% of mean maximum value of all signals. Reentry cycles were defined as repetitive return of an impulse into the same atrial area with a constant rotation pattern over at least 2 cycles.

Histology
RA tissues were collected from border zone regions of MI dogs and corresponding control regions of sham dogs. Tissue blocks were sectioned along longitudinal and transverse planes. Sections (5-µm thickness) were cut at room temperature and stained with either Masson trichrome or hematoxylin-phloxine-saffron. Trichrome-stained images were digitized (Scion Image Software, Frederick, MD), and connective tissue content was analyzed with Sigmascan 4.0 (Jandel) as percent surface area, excluding blood vessels.

Data Analysis
Continuous variables are expressed as mean±SEM. For time-dependent comparisons, 1-way (serial electrophysiological studies) or 2-way (Holter studies) repeated-measures ANOVA with time as a repeated factor was used. For other comparisons, Fisher exact test (for proportions), unpaired t test, or Mann-Whitney nonparametric test (in case the Kolmogorov-Smirnov test failed) for single comparisons between MI and sham group measurements, or 2-way repeated-measures ANOVA with BCL as a repeated factor were used. Two-tailed P<0.05 indicated statistical significance. The authors had full access to and take full responsibility for the integrity of the data. All authors have read and agree to the manuscript as written.

Results
Spontaneous Atrial Ectopy
MI dogs had substantially increased spontaneous ectopic activity, manifested as premature atrial complexes and atrial tachyarrhythmias (Figure 2). Representative recordings are shown in Figure 2A through 2D. The number of premature atrial complexes per day increased >20-fold with MI (Figure 2E). The number of atrial tachyarrhythmias increased ≈20-fold versus shams on day 2 and ≈50-fold on day 7 (Figure 2F). Atrial tachyarrhythmia beats per 24 hours increased ≈25-fold and >100-fold, respectively (Figure 2G). One MI dog showed repetitive atrial tachyarrhythmias throughout day 7 (Figure I in the online-only Data Supplement). Ventricular arrhythmia occurrence did not differ between sham and MI dogs (Table I in the online-only Data Supplement).

Atrial Cardiomyocyte Spontaneous Activity and Ca$^{2+}$-Related Mechanisms
To assess triggered firing, we monitored atrial APs from single cardiomyocytes after a 1-minute period of pacing at 2 Hz. Figure 3A shows a representative control cell response: very limited spontaneous discharge after drive train cessation. Figure 3B shows the response of a border zone cell from a MI dog, with unstable oscillating diastolic potentials and repeated firing. Overall data (Figure 3C) indicate significantly increased spontaneous activity in MI dogs.

We then investigated the possible cellular basis. We first assessed AP characteristics in border zone cells. Resting potential, AP amplitude, and AP durations (APD50 and APD90) did not differ between border zone and control cells (Figure II in the online-only Data Supplement). We then investigated Ca$^{2+}$-handling properties because abnormal Ca$^{2+}$ handling is a prominent cause of triggered activity. Figure 4A and 4B show examples of Ca$^{2+}$ transients in
control and border zone cells. The Ca\textsuperscript{2+} transient is somewhat smaller in the border zone cell. Overall, border zone cells showed smaller Ca\textsuperscript{2+} transients (Figure 4C), particularly at faster rates, and slightly lower diastolic Ca\textsuperscript{2+} concentrations (Figure 4D) versus controls. A decreased Ca\textsuperscript{2+} transient could result from decreased sarcoplasmic reticulum (SR) Ca\textsuperscript{2+} stores or from impaired Ca\textsuperscript{2+}-release mechanisms. To assess SR Ca\textsuperscript{2+} stores, we used rapid exposure to 10-mmol/L caffeine via a laminar flow rapid-perfusion system that exposes the cell studied to the desired concentration within a 500-ms interval, causing a brisk release of SR Ca\textsuperscript{2+}. Figure 4E and 4F show representative caffeine-induced Ca\textsuperscript{2+} transients from a control and a border zone cell, respectively. The border zone transient is smaller and decays more rapidly. Mean data confirm significant decreases in the amplitude (Figure 4G) and decay time constant (Figure 4H) of caffeine-induced Ca\textsuperscript{2+} transients in border zone cells.

The results in Figure 4 indicate that triggered activity in border zone cells is not due to Ca\textsuperscript{2+} overload. A possible explanation of the reduced border zone cell SR Ca\textsuperscript{2+} load and accelerated caffeine-induced Ca\textsuperscript{2+} transient decay is enhanced NCX function. We therefore assessed NCX current directly, as shown in Figure 5A and 5B. The lithium-sensitive component representing NCX current was smaller in control cells (Figure 5A) versus border zone cells (Figure 5B; mean data in Figure 5C). Overall, NCX current was increased by \(~73\%\) in border zone cells versus control cells from sham dogs. Figure 5D and 5E show examples of spontaneous Ca\textsuperscript{2+} sparks. In these examples, Ca\textsuperscript{2+} sparks were absent at baseline. Isoproterenol caused spontaneous activity and contractions in the control cell but did not induce spontaneous Ca\textsuperscript{2+} sparks (Figure 5D), whereas in the MI dog, border zone cell isoproterenol (0.5 \(\mu\)mol/L) administration induced spontaneous Ca\textsuperscript{2+} sparks, associated with Ca\textsuperscript{2+} waves (Figure 5E). Overall, spontaneous Ca\textsuperscript{2+} sparks were more common in MI dog cells than in control cells, but the difference was not statistically significant under baseline conditions (Figure 5F). In the presence of isoproterenol and 2-Hz pacing to increase Ca\textsuperscript{2+} loading, spontaneous Ca\textsuperscript{2+} sparks were significantly more common in border zone cells from MI dogs than in cells
from corresponding regions in sham dogs (Figure 5G). Thus, both enhanced NCX current and spontaneous Ca\textsuperscript{2+} release events are potential contributors to spontaneous ectopic activity in MI dogs.

### Effects on Refractoriness and AF Promotion

Body weight, heart rate, and hemodynamic indices were similar in both groups on day 8 (Table II in the online-only Data Supplement). Atrial infarction substantially increased the substrate for AF maintenance, as indicated by AF duration during open-chest study (1146 ± 259 seconds in MI dogs versus 30 ± 14 seconds in shams; \( P < 0.01 \); Figure 6A). Atrial vulnerability to premature extrastimulation was not affected by atrial infarction (Figure 6B). LA (Figure 6C) and RA (Figure III in the online-only Data Supplement) ERPs did not differ between sham and MI dogs over a wide range of frequencies, nor did regional ERP (Figure 6D). Time-dependent changes in AF duration and RA ERP during serial closed-chest studies are shown in Figure IV in the online-only Data Supplement. AF duration increased to steady state by day 1 and remained at ≈50 times baseline values for the full follow-up period. The atrial ERP was prolonged at short BCLs on day 1 and then returned to baseline, whereas no significant changes occurred at longer BCLs.

### Effects on Conduction and Structure

Atrial conduction indices were analyzed in the border zone by both optical mapping in coronary artery–perfused preparations and in vivo extracellular electrode epicardial mapping. Figure 7 shows optical activation maps at 3 BCLs in a sham (Figure 7A) and MI (Figure 7B) dog. Conduction is clearly more heterogeneous in MI dogs. Mean data indicate statistically significant increases in conduction heterogeneity indices (Figure 7C). Corresponding results from in vivo epicardial mapping (Figure V in the online-only Data Supplement) are similar. Both P5–95 and the heterogeneity index (P5–95/P50) were significantly increased in MI dogs, indicating localized areas of slowed conduction and increased spatial conduction heterogeneity.

Figure 4D and 4E show representative examples of atrial histopathology. Sham dogs displayed grossly normal atria with small amounts of interstitial fibrous tissue. MI dogs showed irregular zones of recent infarction characterized by granulation tissue, sometimes with central mummified cardiomyocytes. Infarct edges were irregular, with surrounding viable cardiomyocytes, some with abnormalities suggesting persistent ischemia, and adjacent regions of normal myocardium. Substantial fibrosis was observed in MI atria, evident as blue band-like zones on Masson trichrome stains. The border zone connective tissue area was significantly increased in MI dogs (3.5 ± 0.3% to 10.8 ± 1.1%; \( P < 0.001 \); n = 6 control, 9 MI dogs).

### Reentry in MI Dogs

The results in Figures 6 and 7 showed the development of an AF-maintaining substrate after coronary artery occlusion. We used optical mapping to assess potential mechanisms underlying the AF substrate. Figure 4 and Movie I in the online-only Data Supplement show representative optical mapping recordings during atrial tachyarrhythmia in the border zone of a MI dog. A single consistent border zone reentry circuit was seen throughout the entire 4000-ms recording period. Stable reentry (mean cycle length, 145 ± 5 ms; range, 125 to 160 ms) was frequently observed in border zone regions of MI preparations but not in corresponding regions of sham preparations (12 ± 5 versus 0 cycles per second in 9/9 versus 0/6 dogs; \( P < 0.05 \) and \( P < 0.001 \), respectively).

### Discussion

Our study demonstrated that chronic coronary artery occlusion creates substrates for both spontaneous atrial ectopy and prolonged AF in dogs. We found that likely candidate mechanisms to underlie arrhythmogenesis include triggered activity (related to increased NCX current and spontaneous Ca\textsuperscript{2+} release events) that generates spon-
taneous atrial firing and border zone reentry (anchored by focal conduction abnormalities) that promotes AF-maintaining reentrant sources.

Previous Studies of Experimental Atrial Infarction
Very little work has been published on the electrophysiology of atrial ischemia/infarction. Lammers et al. observed that hypoxia causes inhomogeneous refractory periods, decreased conduction velocity and wavelength, increased conduction heterogeneity, and reentrant arrhythmias in isolated superfused rabbit LA. They did not study ischemia per se. Jayachandran et al. demonstrated that proximal right coronary artery occlusion in dogs causes atrial ERP shortening after several hours, which was unaffected by ATP-dependent K⁺-channel blockade (glibenclamide) but was prevented by Na⁺-H⁺ exchanger inhibition. Because of significant ventricular infarction in their model, secondary effects due to hemodynamic changes and neurohumoral responses to ventricular MI could not be excluded. We previously reported that acute atrial ischemia produces local conduction abnormalities and enhances AF perpetuation over the subsequent 5 hours. Acute ischemic conduction slowing and AF promotion can be suppressed by β-adrenoceptor blockade, Ca²⁺-current inhibition, heat-shock protein induction, and gap junction conduction enhancement but not Na⁺- or K⁺-channel blockade.

We are not aware of any previous reports of the electrophysiological and arrhythmic properties of hearts subjected to atrial ischemia or infarction for 24 hours. In the present study, we analyzed in detail the atrial arrhythmias occurring in dogs with chronic occlusions of coronary arteries specifically supplying the RA. We observed increased spontaneous atrial ectopy and stabilization of inducible AF beginning as early as 24 hours after MI and continuing for a week thereafter. The principal underlying pathological change was atrial infarction, which was spatially nonhomogeneous and associated with surrounding areas of myocardium that in some cases showed evidence of continued ischemia.

Relation to Previous Studies of Arrhythmia Mechanisms in Ventricular Infarction
In contrast to the very limited information available about the electrophysiology of atrial ischemia, there is a vast literature dealing with ventricular ischemia/infarction. Tissue fibrosis causes “zigzag” activation, leading to slow conduction and maintenance of reentry. Lateralization of connexin43 occurs at an earlier stage and stabilizes reentry, due in part to loss of scaffolding related to interactions between phosphorylated c-Src tyrosine kinase and zonula occludens-1 tight-
Optical mapping studies have shown reentrant rotors with stable phase singularity points and functional lines of block. Increased NCX current and decreased ryanodine receptor expression have been observed after longer-term (6 weeks to 6 months) infarction in rats and rabbits, whereas SR Ca\(^{2+}\)-ATPase and phospholamban expression remained unchanged. Increased Ca\(^{2+}\)-spark activity and Ca\(^{2+}\)-wave activity have been observed in infarcted ventricular tissue at 48 hours and 3 weeks. The electrophysiological consequences of atrial infarction in the present study are similar to those described previously with ventricular infarction, including tissue fibrosis, abnormal Ca\(^{2+}\) handling, and increased NCX activity (which induce Ca\(^{2+}\) sparks/waves and delayed afterdepolarization-induced triggered activities).

Potential Underlying Mechanisms

We found evidence for stable reentrant sources at the border zone of atrial MIs associated with significant peri-MI fibrosis. Fibrosis was likely an important factor in stabilizing reentrant rotors, as described previously in ventricular MI and nonischemic AF models involving atrial fibrosis. Our observations add to accumulating evidence for the ability of single rotors with fibrillatory conduction to maintain AF and suggest chronic coronary artery disease affecting the atria as a context in which such pathophysiology is particularly likely to occur.

We identified increased triggered activity as a likely candidate to underlie spontaneous atrial activity in MI dogs. Triggered activity most commonly results from depolarizing NCX currents, typically occurring in response to spontane-
ous diastolic Ca\(^{2+}\) leaks from the SR.\(^{18–20}\) We noted enhanced NCX current and increased Ca\(^{2+}\) release events in border zone cardiomyocytes, potentially implicating these abnormalities in triggered activity.

**Potential Significance**

Ischemic heart disease and MI are among the most frequent predisposing factors for AF.\(^{43}\) However, there is very little information in the literature about the potential AF-promoting properties of atrial ischemic disease, and atrial ischemia/infarction per se is rarely considered a direct contributor to AF pathophysiology. To our knowledge, our study is the first to demonstrate a direct relationship between chronic atrial ischemia/infarction and atrial arrhythmogenesis, as well as to study potential underlying mechanisms. The efficacy of specific forms of AF therapy may be related to underlying mechanisms. A better understanding of AF pathophysiology in patients with coronary artery disease may help to develop improved patient-tailored treatment.

**Limitations**

We studied atrial electrophysiological and arrhythmic properties over an 8-day period in dogs with occluded RA-perfusing arteries. Different features may develop with longer-term (months or years) atrial ischemia/infarction. As for any animal model, extrapolation to humans should be done with caution. Optical mapping was performed with a motion uncoupler, 2,3-butanedione-monoxime, which has the potential to affect cellular electrophysiology.

Although this study was quite comprehensive in involving observations at multiple levels (in vivo, isolated intact atrial preparations, single atrial cardiomyocytes), many potentially important areas were not addressed and remain to be investigated. The autonomic nervous system likely plays an important role in AF occurrence\(^{44}\) and was not specifically examined in the present study. A potentially significant role for autonomic function is suggested by 2 observations in our study: (1) Ca\(^{2+}\) release events only occurred in MI dog cells in the presence of β-adrenoceptor stimulation, suggesting that background adrenergic stimulation is important for the genesis of triggered activity; and (2) the occurrence of triggered activity in vitro, although enhanced in MI dog cells, was less than might have been expected on the basis of the relative occurrence rates of spontaneous atrial ectopy in MI dogs versus sham controls (Figure 2E through 2G). This notion is also consistent with observations in a rabbit model of ventricular arrhythmias caused by pressure-overload congestive heart failure, in which triggered activity involves a combination of β-adrenergic drive (which enhances diastolic Ca\(^{2+}\) leak), NCX current enhancement, and ionic current remodeling.\(^{45}\) In addition, detailed analyses of changes in a host of other systems would be of interest, including potential remodeling of Na\(^{+}\), Ca\(^{2+}\), and K\(^{+}\) currents, the function of other pumps and exchangers, the expression of electrogenic ion-channel, pump, and exchanger subunits, connexin distribution and function, and the complex properties of SR Ca\(^{2+}\) handling, all of which contribute to arrhythmogenesis in a variety of paradigms, including ventricular ischemia/infarction.\(^{46}\) However, such studies, although clearly pertinent, are potentially extremely vast and beyond the scope of the present study.

**Acknowledgments**

The authors thank Nathalie L’Heureux, Chantal St-Cyr, and Audrey Bernard for technical help and France Thériault for secretarial assistance.
Sources of Funding
This study was supported by the Canadian Institutes of Health Research (MGP 6957), Quebec Heart and Stroke Foundation, MI-
TACS Network, Japanese Heart Rhythm Society/Nihon-Koden/St.
Jude Medical Fellowship, Japan Heart Foundation/Japanese Society
of Electrocardiology Scholarship, St. Jude Medical Scholarship of
the German Cardiac Society, German Federal Ministry of Edu-
cation and Research (Atrial Fibrillation Competence Network
grant 01G0204), and Fondation Leduq (ENAFRA Network,
07/CVD/03).

Disclosures
None.

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Coronary artery disease is an important risk factor for atrial fibrillation (AF), but the underlying mechanisms have not been explored extensively. In this study, we considered the hypothesis that chronic obstructive coronary artery disease can create a substrate for AF initiation and maintenance by altering atrial properties. In a chronic dog model, we ligated a small coronary artery that provides blood flow only to a portion of the right atrial free wall. After coronary artery ligation, dogs showed increased occurrence of spontaneous atrial ectopy and atrial tachyarrhythmias, along with a substrate for AF maintenance. Cellular studies showed that spontaneous ectopy was related to triggered activity caused by Ca\(^{2+}\)-handling abnormalities, including enhanced Na\(^{+}\)-Ca\(^{2+}\) exchange current and spontaneous quantal Ca\(^{2+}\) releases (sparks) occurring during adrenergically driven Ca\(^{2+}\) loading. AF maintenance was related to stable rotors located in the border zone of the atrial infarction resulting from coronary artery occlusion. Local conduction abnormalities in the heterogeneous border zone of the infarction provided the substrate for reentry. These results show that chronic occlusive disease of coronary arteries supplying atrial tissue produces atrial abnormalities that provide both triggers for AF initiation and a substrate for AF maintenance. They indicate a potential mechanism for the observed association between coronary artery disease and AF. These mechanistic concepts may be useful for understanding AF pathophysiology and developing new mechanism-based approaches to patient-specific therapeutics.
Mechanisms of Atrial Tachyarrhythmias Associated With Coronary Artery Occlusion in a Chronic Canine Model

Kunihiro Nishida, Xiao Yan Qi, Reza Wakili, Philippe Comtois, Denis Chartier, Masahide Harada, Yu-ki Iwasaki, Philippe Romeo, Ange Maguy, Dobromir Dobrev, Georghia Michael, Mario Talajic and Stanley Nattel

_Circulation_. 2011;123:137-146; originally published online January 3, 2011;
doi: 10.1161/CIRCULATIONAHA.110.972778

_Circulation_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circ.ahajournals.org/content/123/2/137

Data Supplement (unedited) at:
http://circ.ahajournals.org/content/suppl/2010/12/03/CIRCULATIONAHA.110.972778.DC1
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Supplemental Material
Detailed Supplemental Methods

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Animal-handling procedures were approved by the local Animal Research Ethics Committee following National Institutes of Health guidelines. Seventy-five mongrel dogs (26.2 to 37.6 kg) were anesthetized with ketamine (5.3 mg/kg i.v.), diazepam (0.25 mg/kg, i.v.), and isoflurane (1.5%), intubated, and ventilated. A right lateral thoracotomy was performed to expose the right atrium. In 40 dogs (MI group), isolated right-atrial (RA) MI was created by double ligation of the right intermediate atrial artery, which supplies only the RA free wall and not the ventricles (Figure 1A). In 35 sham-dogs, the same procedures were performed, but the thread positioned beneath the artery was left without ligation. Buprenorphine (0.02 mg/kg, s.c.) was given before and 12-hourly for 3 days post-surgery. Twenty-four-hour Holter ECGs were recorded on day 2 and 7 after MI or sham surgeries in 6 MI and 6 sham dogs to assess spontaneous atrial arrhythmia.

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RA-appendage was measured at basic cycle lengths (BCLs) of 150, 200, 250, 300, and 360 ms with 10 basic stimuli (S1s) followed by premature extrastimuli (S2s) with 5-ms decrements. The longest S1S2-interval failing to capture defined the ERP, and the mean of 3 ERP determinations at each BCL was used. AF was induced with 1-10 s burst pacing (10-Hz, 4×threshold-current). Mean AF duration in each dog was based on 10 AF-inductions for AF-duration<20 min and 5 inductions for 20-30 min AF. Prolonged AF (>30 min) was terminated by direct-current electrical cardioversion. A 20-min rest period was then allowed. If prolonged AF was induced twice, no further AF induction was performed.

Terminal open-chest EPS was performed in 9 MI and 6 sham dogs 8 days after MI or sham procedures. Dogs were anesthetized with morphine (2 mg/kg, s.c.) and α-chloralose (120 mg/kg, i.v., followed by 29.25 mg/kg/h), and ventilated. Body temperature was maintained at 37°C. A median sternotomy was performed, and bipolar electrodes were hooked into the RA and left atrial (LA) appendages for recording and stimulation. Silicon sheets containing 240 bipolar electrodes were attached to the atria as previously described.¹⁻³ Electrophysiological mapping was conducted with the Cardiomap system (Research Center, Sacré-Coeur Hospital and Biomedical Engineering Institute, École Polytechnique and Université de Montréal, Montreal, Quebec, Canada). Atrial ERPs were measured at multiple BCLs in the RA and LA appendage, and at a BCL of 300 ms at 6 additional sites: RA and LA posterior wall, RA and LA inferior wall, RA and LA Bachmann’s bundle (Figure 1B). AF-vulnerability was determined as the percentage of atrial sites at which AF (>1 second) was induced by single extrastimuli.
Phase-Delay Analysis

To evaluate conduction properties, phase-maps were constructed for the RA electrode array (white plaque with dashed border in Figure 1B) as previously reported. Conduction indices based on phase-delay histograms were used to evaluate local conduction abnormalities. Data were obtained separately in each normal non-infarct zone (NZ, predetermined RA-appendage electrodes). Contiguous areas with electrogram-voltages<0.5 mV were defined as the infarct zone (IZ), the surrounding RA defined the border zone (BZ). Activation-time differences between each electrode-site and adjacent neighbors were divided by respective interelectrode distances; the largest activation time/distance ratio was taken to indicate the phase-delay at that site. Phase-delay histograms were then constructed. Median phase-delay (P50) reflects average conduction-velocities, the phase-delay corresponding to the 5% slowest-conducting zones reflects slowest local-conduction values, difference between 5%-shortest (P5) and 5%-longest (P95) delays (P5-P95) indicates phase-delay range, and P5-P95 divided by median phase-delay (P5-95/P50) is a conduction-heterogeneity index independent of conduction velocity-changes.

Cardiomyocyte Isolation

Single atrial cardiomyocytes were isolated with previously-described methods. On day 8 post-surgery, dogs were anesthetized with morphine (2 mg/kg s.c.) and α-chloralose (120 mg/kg i.v.). The heart was removed after intra-atrial injection of heparin (10,000 U) and the RA removed. RA-tissue was coronary-artery perfused with Tyrode solution (37°C, 100% O₂), then with Ca²⁺-free Tyrode (~10 minutes), followed by ~60-minute perfusion with the same solution containing collagenase (~0.5 mg/mL, CLSII, Worthington) and 0.1% bovine serum albumin (BSA, Sigma). RA-tissues were collected from the BZ (viable tissue adjacent to the MI-scar) of
MI dogs and the corresponding control region of sham-dogs. Tissues were minced and atrial cardiomyocytes harvested. After isolation, cells were kept in storage solution (mmol/L: 20 KCl, 10 KH$_2$PO$_4$, 10 dextrose, 40 mannitol, 70 L-glutamic acid, 10 β-OH-butyric acid, 20 taurine, 10 EGTA, and 0.1%-BSA; pH 7.3, KOH) at 4°C and studied within 12 hours.

**Cellular Electrophysiology**

Recordings were obtained at 37°C. Perforated-patch technique was used to record action potentials (APs) in current-clamp mode and tight-seal patch-clamp to record currents in whole-cell voltage-clamp mode. Nystatin-free intracellular solution was placed in pipette-tips by capillary action, then pipettes were back-filled with nystatin-containing (600-µg/mL) pipette solution. Currents are expressed as densities (pA/pF). Cell-capacitance averaged 94±6 and 91±8 pF in MI-dog BZ-cells and control-dog cells respectively. Junction potentials between bath and pipette solutions averaged 15.9 mV and were corrected for APs only.

Tyrode solution contained (mmol/L): NaCl 136, CaCl$_2$ 1.8, KCl 5.4, MgCl$_2$ 1, NaH$_2$PO$_4$ 0.33, dextrose 10, and HEPES 5; pH 7.4 (NaOH). The pipette solution for AP recording contained (mmol/L): GTP 0.1, potassium-aspartate 110, KCl 20, MgCl$_2$ 1, Mg-ATP 5, HEPES 10, sodium-phosphocreatine 5, and EGTA 0.05 (pH 7.4, KOH).

$\text{Na}^+/\text{Ca}^{2+}$-exchange (NCX) current was recorded as previously described, with extracellular solution containing (mmol/L) NaCl 140, CaCl$_2$ 2, MgCl$_2$ 1, dextrose 10, HEPES 10, niflumic acid 0.1 and nifedipine 5 µmol/L and internal solution containing CsCl 130, NaCl 5, MgATP 4, and HEPES 10 (pH 7.3, CsOH).
**Ca²⁺-Transients**

Atrial cardiomyocytes were incubated with Indo-1-AM (5 μmol/L) in 100-μmol/L pluronic F-127 and 0.5%-DMSO for 3-5 minutes, then superfused with Tyrode solution. Ultraviolet light passing through a 340-nm interference filter was reflected into a ×40 oil-immersion fluor objective for excitation of intracellular Indo-1. Emitted light was reflected into a spectral separator, passed through parallel filters at 400 and 500 nm (±10 nm), detected by matched photomultiplier tubes, and electronically filtered at 60 Hz. Fluorescence-signal ratios were digitized and converted to [Ca²⁺] as previously described. Cells were field-stimulated at 0.1, 0.5, 1.0 and 2.0 Hz (1.5-times threshold-voltage pulses).

**Ca²⁺-Release Events**

Atrial cardiomyocytes were incubated with the Ca²⁺-indicator fluo-4-AM for 20 minutes (23°C), then placed in a perfusion chamber followed by Tyrode-solution perfusion for another 10 minutes (de-esterification-time >30 minutes). Confocal microscopic assessment was performed along the lines used in previous work with an inverted microscope (Olympus IX-81) equipped with a and confocal laser-scanning unit (Olympus Fluoview FV-1000). Fluorescence emission was collected between 500 and 650 nm, with excitation at 488 nm. Ca²⁺-release events were detected under non-paced conditions over a 20-second period following 2-min 1-Hz pacing before isoproterenol-stimulation and following 2-min 2-Hz pacing after isoproterenol-stimulation (0.5 μmol/L). Line-scanning was performed with an optical resolution of 512×512 pixels and a temporal resolution of 1.8-2.0 ms/line. Ca²⁺-sparks were defined by an increase in the signal mass of a 3-μm section without any detectable increase in an adjacent 3-μm section.
Optical Mapping

Optical mapping was performed in coronary-perfused RA-preparations from 6 sham and 9 MI dogs. On day 8 post-surgery, dogs were administered morphine (2 mg/kg, s.c.) and α-chloralose (120 mg/kg, i.v.) and mechanically ventilated. Following left lateral thoracotomy and heparin-administration (5000 units, i.v.), hearts were excised and placed in cardioplegic solution (mmol/L: 50 KH₂PO₄, 8 MgSO₄ 7H₂O, 10 HEPES, 5 adenosine, 140 glucose, and 100 mannitol; pH 7.4, KOH) saturated with 100%-O₂. The RA was perfused via the right coronary artery in a tissue chamber (37°C) with modified Kreb’s solution (mmol/L: 120 NaCl, 4 KCl, 1.2 MgSO₄ 7H₂O, 1.2 KH₂PO₄, 25 NaHCO₃, 11 glucose, and 1.25 CaCl₂, saturated with 95%-O₂/5%-CO₂) at 20 mL/min and 37°C.

Optical recordings were made in BZ-regions of MI-preparations and corresponding control regions of sham preparations, from an epicardial surface-area of ~2×2 cm² in the presence of the motion uncoupler 2,3-butanedione-monoxyime (15-mmol/L, Sigma-Aldrich) and the potentiometric dye, di-4-ANEPPS. Di-4-ANEPPS (300 μg of 1 mg/mL solution) was infused via the coronary perfusate over 10-15 minutes, during which the perfusion-rate was reduced to 10 mL/min. A CCD camera (80×80 pixels, RedShirt Imaging) recorded fluorescence (long-pass >695 nm filter, Omega Optical) at 1 kHz to obtain 4-second acquisitions with light emitting diodes (523-nm peak wavelength, NTE). Bipolar electrodes were used for pacing and recording-reference. Optical maps were recorded during 2×threshold-current RA-appendage stimulation at BCLs of 500, 300, and 250 ms, and during tachyarrhythmias. To estimate mean atrial tachyarrhythmia (AT) duration in each preparation, tachyarrhythmia was induced 10 times with burst-pacing at 100-ms BCL and 4×threshold-current in the presence of isoproterenol (0.5
µmol/L). Prolonged AT (>10 min) was electrically terminated. If prolonged AT was induced twice, no further induction was performed.

Conduction-data were analyzed with custom-made software in Matlab (Mathworks Inc.). Activation maps were obtained after averaging the fluorescence signal-segment of each beat segmented using an extracellular-electrode recording. A moving mean (10 samples) was then applied to increase the signal-to-noise ratio. Maximum |dF/dt| was used to define the activation time. Phase-analysis\(^4\) was performed on the mean activation map with a grid of 20×20 points (every 4 points of the CCD-matrix).

Tachyarrhythmia-data were analyzed with custom-made scripts in Matlab (Mathworks, Inc.). Raw fluorescence-data from each 80×80 pixels was filtered by a moving mean (10-sample window), normalized, and inverted for the time window of interest. Raw fluorescence change (ΔF(pixel number) = F_{max}-F_{min}) was calculated for each pixel. Pixels having a ΔF(pixel number) / (max(ΔF(pixel number))) < 0.01 were discarded. The timing of local activation was determined based on maximum normalized fluorescence criteria. IZ-areas were defined by maximum non-normalized fluorescence amplitudes <13% the mean maximum value of all signals. Reentry-cycles were defined as repetitive return of an impulse into the same atrial area with a constant rotation-pattern over at least 2 cycles.

**Histology**

RA-tissues were collected from BZ-regions of MI dogs and corresponding control regions of sham-dogs. Tissue-blocks were sectioned along longitudinal and transverse planes. Sections (5-µm thickness) were cut at room temperature and stained with either Masson’s Trichrome or hematoxylin-phloxine-saffron (HPS). Trichrome-stained images were digitized (Scion Image
Software, Frederick, MD) and connective-tissue content analyzed with Sigmascan 4.0 (Jandel) as percent surface area, excluding blood vessels.

**Data Analysis**

Continuous variables are expressed as mean±SEM. For time-dependent comparisons within a group, one-way or two-way repeated-measures ANOVA with time as a repeated factor was used. For serial EPSs, which were performed only in MI-dogs, one-way ANOVA was used. For Holter-recording arrhythmia studies two-way ANOVA, with group (sham vs MI) and time as main-effect factors, was used. For other between-group comparisons, Fisher's exact test (for proportions), unpaired t-tests (for single comparisons between MI and sham-group measurements) or two-way repeated measures ANOVA with BCL as a repeated factor was used. Two-tailed $P<0.05$ indicated statistical significance. The authors had full access to and take responsibility for the integrity of the data. All authors have read and agree to the manuscript as written.
References


## Supplemental Table I. Holter ECG Indices

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<th>Sham</th>
<th>Infarction</th>
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<tr>
<td></td>
<td>Day 2</td>
<td>Day 7</td>
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<tr>
<td>Total QRS complexes</td>
<td>113667±8571</td>
<td>125639±5838</td>
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<td>Average heart rates (bpm)</td>
<td>80.7±6.6</td>
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<td>53.3±4.0</td>
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<tr>
<td>Maximum heart rates (bpm)</td>
<td>160.0±16.7</td>
<td>210.0±9.3 *</td>
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<tr>
<td>Ventricular arrhythmia</td>
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<tr>
<td>Total beats</td>
<td>12.2±5.5</td>
<td>24.7±23.7</td>
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<tr>
<td>Isolated premature beats</td>
<td>9.2±4.7</td>
<td>24.7±23.7</td>
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<tr>
<td>Couplets (episodes)</td>
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<td>0.0±0.0</td>
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<tr>
<td>Runs and tachycardias</td>
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<tr>
<td>Episodes</td>
<td>0.3±0.3</td>
<td>0.0±0.0</td>
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<tr>
<td>Total beats</td>
<td>1.0±1.0</td>
<td>0.0±0.0</td>
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<td>Atrial tachyarrhythmia</td>
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<td>Total beats</td>
<td>49.2±32.8</td>
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<tr>
<td>Isolated premature beats</td>
<td>4.36±34.3</td>
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<td>Episodes</td>
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<tr>
<td>Total beats</td>
<td>4.0±4.0</td>
<td>3.7±3.7</td>
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</table>

*P<0.01 and **P<0.005 versus Day 2; †P<0.05, ††P<0.01, and †††P<0.001 versus sham.
Supplemental Table II. Body Weight, Sinus Rate, and Hemodynamic Indices

<table>
<thead>
<tr>
<th></th>
<th>Sham dogs (n=6)</th>
<th>Infarction dogs (n=9)</th>
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</thead>
<tbody>
<tr>
<td><strong>Body weight, kg</strong></td>
<td></td>
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<tr>
<td>Before surgery</td>
<td>31.3±1.8</td>
<td>32.1±1.3</td>
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<td>Open-chest EPS</td>
<td>32.0±1.4</td>
<td>31.8±1.3</td>
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<tr>
<td><strong>Sinus Rate, bpm</strong></td>
<td>153.4±10.3</td>
<td>160.6±9.0</td>
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<tr>
<td><strong>Pressures, mmHg</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BPs</td>
<td>95.0±3.5</td>
<td>98.9±4.4</td>
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<tr>
<td>BPd</td>
<td>60.8±1.8</td>
<td>63.3±3.9</td>
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<tr>
<td>LVEDP</td>
<td>2.7±0.7</td>
<td>2.6±0.8</td>
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<tr>
<td>LAP</td>
<td>4.0±0.8</td>
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</tr>
<tr>
<td>RAP</td>
<td>3.5±0.4</td>
<td>4.1±0.4</td>
</tr>
</tbody>
</table>

BPs, systolic arterial blood pressure; BPd, diastolic arterial blood pressure; LVEDP, left ventricular end-diastolic pressure; LAP, mean LA pressure; and RAP, mean RA pressure.
Supplemental Figure Legends

Supplemental Figure I. Recurrent atrial tachycardia recorded by 24-hour Holter ECG in an MI dog. A, 4-minute ECG showing repeated atrial tachycardias. Dashed lines indicate atrial tachycardias and atrial ectopic complexes. B, Expanded 10-s ECGs corresponding to boxes a and b in panel A, respectively. Panel a shows the spontaneous initiation of atrial tachycardia. Panel b shows atrial tachycardia with AV block. The negative P waves in leads I and III during tachycardia are consistent with an ectopic focus in the lower right atrium near the infarct zone.

Supplemental Figure II. Action potential properties. A, Resting potential. B, Action potential (AP) amplitude. C, AP duration to 50% repolarization (APD50). D, AP duration to 90% repolarization (APD90). CTL indicates control cells (n=17 cells from 5 sham dogs). BZ indicates infarct border zone cells (n=22 cells from 8 MI dogs).

Supplemental Figure III. Effect of atrial MI on atrial effective refractory periods (ERPs) during open-chest study in RA-appendage (RAA) at various basic cycle lengths (BCLs). n=6 and n=9 dogs for sham and MI.

Supplemental Figure IV. Time-dependent changes in mean duration of induced AF (A) and atrial ERP (B-D) in MI dogs (n=5). AF duration prolonged from day 1 post-surgery and then remained constant until day 7 (A). Atrial ERP prolonged at shorter BCLs on day 1 and then recovered gradually (B and C). ERP was not different from baseline on day 7.
(B-D). BL indicates baseline; D1, day 1; D3, day 3; D7, day 7.

*P<0.05 versus and **P<0.01 versus baseline.

**Supplemental Figure V.** A and B, Representative example of multi-electrode epicardial mapping during constant pacing in sham and MI preparations. C, Median local phase delays (P50), phase delays corresponding to slowest conduction zone (P95), absolute phase delay heterogeneity (P5-95), and heterogeneity index (P5-95/P50) as a function of pacing cycle length in normal zone (NZ) of sham (n=6) and MI (n=9) preparations. D, Corresponding values in CTL/BZ region of sham/MI preparations. **P<0.01 and ***P<0.001. In sham preparations (A), conduction was smooth and homogeneous throughout the right atrium. In MI preparations (B), no significant electrogram signals were observed in the infarct zone (IZ), and conduction was irregular and heterogeneous in the BZ. Indices of conduction heterogeneity were significantly increased in BZ of MI preparations (D).
Supplemental Figure I

A Lead I

B a

b

Supplemental Figure I
Supplemental Figure II
Supplemental Figure III

- ○ Sham (n=6 dogs)
- ● MI (n=9 dogs)

ERP (ms) in RAA vs BCL (ms)
Supplemental Figure IV

(A) AF duration (s)

(B) ERP (ms) at BCL 300 ms

(C) ERP (ms) at BCL 150 ms

(D) ERP (ms) at BCL 300 ms
Supplemental Figure V
Supplemental Movie Legend

The movie shows activation sequences during 4 successive cycles of atrial tachyarrhythmia in a coronary artery-perfused right-atrial free wall preparation from an MI dog. The tissue was loaded with di,4-ANEPPS and optical mapping of transmembrane potential performed. Colors indicate transmembrane potential, with dark blue corresponding to resting potential, red to the activation wave front (fully depolarized tissues), and colors between indicating grades of depolarization. The field of view has the infarct zone at the lower left and the border zone situated above and to the right. Note that a clockwise reentry circuit located in the border zone (upper right quadrant) maintains reentry. The tissue in the lower left quadrant, corresponding to the infarct zone, remains in shades of lighter blue and is never activated.
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Detailed Supplemental Methods

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Terminal open-chest EPS was performed in 9 MI and 6 sham dogs 8 days after MI or sham procedures. Dogs were anesthetized with morphine (2 mg/kg, s.c.) and α-chloralose (120 mg/kg, i.v., followed by 29.25 mg/kg/h), and ventilated. Body temperature was maintained at 37°C. A median sternotomy was performed, and bipolar electrodes were hooked into the RA and left atrial (LA) appendages for recording and stimulation. Silicon sheets containing 240 bipolar electrodes were attached to the atria as previously described.1-3 Electrophysiological mapping was conducted with the Cardiomap system (Research Center, Sacré-Coeur Hospital and Biomedical Engineering Institute, École Polytechnique and Université de Montréal, Montreal, Quebec, Canada). Atrial ERPs were measured at multiple BCLs in the RA and LA appendage, and at a BCL of 300 ms at 6 additional sites: RA and LA posterior wall, RA and LA inferior wall, RA and LA Bachmann’s bundle (Figure 1B). AF-vulnerability was determined as the percentage of atrial sites at which AF (>1 second) was induced by single extrastimuli.
**Phase-Delay Analysis**

To evaluate conduction properties, phase-maps were constructed for the RA electrode array (white plaque with dashed border in Figure 1B) as previously reported.\(^1\)\(^-\)\(^3\) Conduction indices based on phase-delay histograms were used to evaluate local conduction abnormalities. Data were obtained separately in each normal non-infarct zone (NZ, predetermined RA-appendage electrodes). Contiguous areas with electrogram-voltages<0.5 mV were defined as the infarct zone (IZ), the surrounding RA defined the border zone (BZ). Activation-time differences between each electrode-site and adjacent neighbors were divided by respective interelectrode distances; the largest activation time/distance ratio was taken to indicate the phase-delay at that site.\(^4\) Phase-delay histograms were then constructed. Median phase-delay (P50) reflects average conduction-velocities, the phase-delay corresponding to the 5% slowest-conducting zones reflects slowest local-conduction values, difference between 5%-shortest (P5) and 5%-longest (P95) delays (P5-P95) indicates phase-delay range, and P5-P95 divided by median phase-delay (P5-95/P50) is a conduction-heterogeneity index independent of conduction velocity-changes.

**Cardiomyocyte Isolation**

Single atrial cardiomyocytes were isolated with previously-described methods.\(^5\) On day 8 post-surgery, dogs were anesthetized with morphine (2 mg/kg s.c.) and \(\alpha\)-chloralose (120 mg/kg i.v.). The heart was removed after intra-atrial injection of heparin (10,000 U) and the RA removed. RA-tissue was coronary-artery perfused with Tyrode solution (37°C, 100% O\(_2\)), then with Ca\(^{2+}\)-free Tyrode (~10 minutes), followed by ~60-minute perfusion with the same solution containing collagenase (~0.5 mg/mL, CLSII, Worthington) and 0.1% bovine serum albumin (BSA, Sigma). RA-tissues were collected from the BZ (viable tissue adjacent to the MI-scar) of
MI dogs and the corresponding control region of sham-dogs. Tissues were minced and atrial cardiomyocytes harvested. After isolation, cells were kept in storage solution (mmol/L: 20 KCl, 10 KH$_2$PO$_4$, 10 dextrose, 40 mannitol, 70 L-glutamic acid, 10 β-OH-butyric acid, 20 taurine, 10 EGTA, and 0.1%-BSA; pH 7.3, KOH) at 4°C and studied within 12 hours.

**Cellular Electrophysiology**

Recordings were obtained at 37°C. Perforated-patch technique was used to record action potentials (APs) in current-clamp mode and tight-seal patch-clamp to record currents in whole-cell voltage-clamp mode. Nystatin-free intracellular solution was placed in pipette-tips by capillary action, then pipettes were back-filled with nystatin-containing (600-µg/mL) pipette solution. Currents are expressed as densities (pA/pF). Cell-capacitance averaged 94±6 and 91±8 pF in MI-dog BZ-cells and control-dog cells respectively. Junction potentials between bath and pipette solutions averaged 15.9 mV and were corrected for APs only.

Tyrode solution contained (mmol/L): NaCl 136, CaCl$_2$ 1.8, KCl 5.4, MgCl$_2$ 1, NaH$_2$PO$_4$ 0.33, dextrose 10, and HEPES 5; pH 7.4 (NaOH). The pipette solution for AP recording contained (mmol/L): GTP 0.1, potassium-aspartate 110, KCl 20, MgCl$_2$ 1, Mg-ATP 5, HEPES 10, sodium-phosphocreatine 5, and EGTA 0.05 (pH 7.4, KOH).

Na$^+$/Ca$^{2+}$-exchange (NCX) current was recorded as previously described,$^6$7 with extracellular solution containing (mmol/L) NaCl 140, CaCl$_2$ 2, MgCl$_2$ 1, dextrose 10, HEPES 10, niflumic acid 0.1 and nifedipine 5 µmol/L and internal solution containing CsCl 130, NaCl 5, MgATP 4, and HEPES 10 (pH 7.3, CsOH).
**Ca^{2+}-Transients**

Atrial cardiomyocytes were incubated with Indo-1-AM (5 μmol/L) in 100-μmol/L pluronic F-127 and 0.5%-DMSO for 3-5 minutes, then superfused with Tyrode solution. Ultraviolet light passing through a 340-nm interference filter was reflected into a ×40 oil-immersion fluor objective for excitation of intracellular Indo-1. Emitted light was reflected into a spectral separator, passed through parallel filters at 400 and 500 nm (±10 nm), detected by matched photomultiplier tubes, and electronically filtered at 60 Hz. Fluorescence-signal ratios were digitized and converted to \([\text{Ca}^{2+}]_i\) as previously described.8 Cells were field-stimulated at 0.1, 0.5, 1.0 and 2.0 Hz (1.5-times threshold-voltage pulses).

**Ca^{2+}-Release Events**

Atrial cardiomyocytes were incubated with the Ca^{2+}-indicator fluo-4-AM for 20 minutes (23°C), then placed in a perfusion chamber followed by Tyrode-solution perfusion for another 10 minutes (de-esterification-time >30 minutes). Confocal microscopic assessment was performed along the lines used in previous work9 with an inverted microscope (Olympus IX-81) equipped with a and confocal laser-scanning unit (Olympus Fluoview FV-1000). Fluorescence emission was collected between 500 and 650 nm, with excitation at 488 nm. Ca^{2+}-release events were detected under non-paced conditions over a 20-second period following 2-min 1-Hz pacing before isoproterenol-stimulation and following 2-min 2-Hz pacing after isoproterenol-stimulation (0.5 μmol/L). Line-scanning was performed with an optical resolution of 512×512 pixels and a temporal resolution of 1.8-2.0 ms/line. Ca^{2+}-sparks were defined by an increase in the signal mass of a 3-μm section without any detectable increase in an adjacent 3-μm section.
Optical Mapping

Optical mapping was performed in coronary-perfused RA-preparations from 6 sham and 9 MI dogs. On day 8 post-surgery, dogs were administered morphine (2 mg/kg, s.c.) and α-chloralose (120 mg/kg, i.v.) and mechanically ventilated. Following left lateral thoracotomy and heparin-administration (5000 units, i.v.), hearts were excised and placed in cardioplegic solution (mmol/L: 50 KH2PO4, 8 MgSO4 7H2O, 10 HEPES, 5 adenosine, 140 glucose, and 100 mannitol; pH 7.4, KOH) saturated with 100%-O2. The RA was perfused via the right coronary artery in a tissue chamber (37°C) with modified Kreb’s solution (mmol/L: 120 NaCl, 4 KCl, 1.2 MgSO4 7H2O, 1.2 KH2PO4, 25 NaHCO3, 11 glucose, and 1.25 CaCl2, saturated with 95%-O2/5%-CO2) at 20 mL/min and 37°C.

Optical recordings were made in BZ-regions of MI-preparations and corresponding control regions of sham preparations, from an epicardial surface-area of ~2×2 cm² in the presence of the motion uncoupler 2,3-butanedione-monoxime (15-mmol/L, Sigma-Aldrich) and the potentiometric dye, di-4-ANEPPS. Di-4-ANEPPS (300 μg of 1 mg/mL solution) was infused via the coronary perfusate over 10-15 minutes, during which the perfusion-rate was reduced to 10 mL/min. A CCD camera (80×80 pixels, RedShirt Imaging) recorded fluorescence (long-pass >695 nm filter, Omega Optical) at 1 kHz to obtain 4-second acquisitions with light emitting diodes (523-nm peak wavelength, NTE). Bipolar electrodes were used for pacing and recording-reference. Optical maps were recorded during 2×threshold-current RA-appendage stimulation at BCLs of 500, 300, and 250 ms, and during tachyarrhythmias. To estimate mean atrial tachyarrhythmia (AT) duration in each preparation, tachyarrhythmia was induced 10 times with burst-pacing at 100-ms BCL and 4×threshold-current in the presence of isoproterenol (0.5
Prolonged AT (>10 min) was electrically terminated. If prolonged AT was induced twice, no further induction was performed.

Conduction-data were analyzed with custom-made software in Matlab (Mathworks Inc.). Activation maps were obtained after averaging the fluorescence signal-segment of each beat segmented using an extracellular-electrode recording. A moving mean (10 samples) was then applied to increase the signal-to-noise ratio. Maximum |dF/dt| was used to define the activation time. Phase-analysis was performed on the mean activation map with a grid of 20×20 points (every 4 points of the CCD-matrix).

Tachyarrhythmia-data were analyzed with custom-made scripts in Matlab (Mathworks, Inc.). Raw fluorescence-data from each 80×80 pixels was filtered by a moving mean (10-sample window), normalized, and inverted for the time window of interest. Raw fluorescence change \( \Delta F(\text{pixel number}) = F_{\text{max}} - F_{\text{min}} \) was calculated for each pixel. Pixels having a \( \Delta F(\text{pixel number}) / \left( \text{max}(\Delta F(\text{pixel number})) \right) < 0.01 \) were discarded. The timing of local activation was determined based on maximum normalized fluorescence criteria. IZ-areas were defined by maximum non-normalized fluorescence amplitudes <13% the mean maximum value of all signals. Reentry-cycles were defined as repetitive return of an impulse into the same atrial area with a constant rotation-pattern over at least 2 cycles.

**Histology**

RA-tissues were collected from BZ-regions of MI dogs and corresponding control regions of sham-dogs. Tissue-blocks were sectioned along longitudinal and transverse planes. Sections (5-\( \mu \text{m} \) thickness) were cut at room temperature and stained with either Masson’s Trichrome or hematoxylin-phloxine-saffron (HPS). Trichrome-stained images were digitized (Scion Image...
Software, Frederick, MD) and connective-tissue content analyzed with Sigmascan 4.0 (Jandel) as percent surface area, excluding blood vessels.

**Data Analysis**

Continuous variables are expressed as mean±SEM. For time-dependent comparisons within a group, one-way or two-way repeated-measures ANOVA with time as a repeated factor was used. For serial EPSs, which were performed only in MI-dogs, one-way ANOVA was used. For Holter-recording arrhythmia studies two-way ANOVA, with group (sham vs MI) and time as main-effect factors, was used. For other between-group comparisons, Fisher's exact test (for proportions), unpaired t-tests (for single comparisons between MI and sham-group measurements) or two-way repeated measures ANOVA with BCL as a repeated factor was used. Two-tailed $P<0.05$ indicated statistical significance. The authors had full access to and take responsibility for the integrity of the data. All authors have read and agree to the manuscript as written.
References


## Supplemental Table I. Holter ECG Indices

<table>
<thead>
<tr>
<th></th>
<th>Sham Day 2</th>
<th>Sham Day 7</th>
<th>Infarction Day 2</th>
<th>Infarction Day 7</th>
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<tr>
<td><strong>Total QRS complexes</strong></td>
<td>113667±8571</td>
<td>125639±5838</td>
<td>125083±4685</td>
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<tr>
<td><strong>Average heart rates (bpm)</strong></td>
<td>80.7±6.6</td>
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<td><strong>Minimum heart rates (bpm)</strong></td>
<td>53.3±4.0</td>
<td>55.0±3.4</td>
<td>55.3±3.3</td>
<td>55.0±3.4</td>
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<tr>
<td><strong>Maximum heart rates (bpm)</strong></td>
<td>160.0±16.7</td>
<td>210.0±9.3 *</td>
<td>178.5±7.8</td>
<td>224.2±7.6 **</td>
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<td><strong>Ventricular arrhythmia</strong></td>
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<tr>
<td><strong>Total beats</strong></td>
<td>12.2±5.5</td>
<td>24.7±23.7</td>
<td>9.5±9.3</td>
<td>0.2±0.2</td>
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<tr>
<td><strong>Isolated premature beats</strong></td>
<td>9.2±4.7</td>
<td>24.7±23.7</td>
<td>9.5±9.3</td>
<td>0.2±0.2</td>
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<tr>
<td><strong>Couplets (episodes)</strong></td>
<td>1.0±0.7</td>
<td>0.0±0.0</td>
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<td>0.0±0.0</td>
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<td><strong>Runs and tachycardias</strong></td>
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<td></td>
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<td></td>
</tr>
<tr>
<td><strong>Episodes</strong></td>
<td>0.3±0.3</td>
<td>0.0±0.0</td>
<td>0.0±0.0</td>
<td>0.0±0.0</td>
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<tr>
<td><strong>Total beats</strong></td>
<td>1.0±1.0</td>
<td>0.0±0.0</td>
<td>0.0±0.0</td>
<td>0.0±0.0</td>
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<tr>
<td><strong>Atrial tachyarrhythmia</strong></td>
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<tr>
<td><strong>Total beats</strong></td>
<td>49.2±32.8</td>
<td>37.8±24.9</td>
<td>1136.8±382.4 ††</td>
<td>1600.2±729.7 ††</td>
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<tr>
<td><strong>Isolated premature beats</strong></td>
<td>4.36±34.3</td>
<td>33.8±25.3</td>
<td>678.8±388.1 †</td>
<td>652.2±339.0 ††</td>
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<tr>
<td><strong>Couplets (episodes)</strong></td>
<td>0.8±0.6</td>
<td>0.2±0.2</td>
<td>33.8±10.4 ††</td>
<td>10.0±4.9 ††</td>
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<tr>
<td><strong>Runs and tachycardias</strong></td>
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<td></td>
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<tr>
<td><strong>Episodes</strong></td>
<td>1.0±1.0</td>
<td>1.0±1.0</td>
<td>27.4±15.2 ††</td>
<td>52.0±25.6 †††</td>
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<tr>
<td><strong>Total beats</strong></td>
<td>4.0±4.0</td>
<td>3.7±3.7</td>
<td>123.6±70.7 ††</td>
<td>928.0±798.5 ††</td>
</tr>
</tbody>
</table>

*P<0.01 and **P<0.005 versus Day 2; †P<0.05, ††P<0.01, and †††P<0.001 versus sham.
Supplemental Table II. Body Weight, Sinus Rate, and Hemodynamic Indices

<table>
<thead>
<tr>
<th></th>
<th>Sham dogs (n=6)</th>
<th>Infarction dogs (n=9)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Body weight, kg</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before surgery</td>
<td>31.3±1.8</td>
<td>32.1±1.3</td>
</tr>
<tr>
<td>Open-chest EPS</td>
<td>32.0±1.4</td>
<td>31.8±1.3</td>
</tr>
<tr>
<td><strong>Sinus Rate, bpm</strong></td>
<td>153.4±10.3</td>
<td>160.6±9.0</td>
</tr>
<tr>
<td><strong>Pressures, mmHg</strong></td>
<td></td>
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</tr>
<tr>
<td>BPs</td>
<td>95.0±3.5</td>
<td>98.9±4.4</td>
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<tr>
<td>BPd</td>
<td>60.8±1.8</td>
<td>63.3±3.9</td>
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<tr>
<td>LVEDP</td>
<td>2.7±0.7</td>
<td>2.6±0.8</td>
</tr>
<tr>
<td>LAP</td>
<td>4.0±0.8</td>
<td>4.4±0.4</td>
</tr>
<tr>
<td>RAP</td>
<td>3.5±0.4</td>
<td>4.1±0.4</td>
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BPs, systolic arterial blood pressure; BPd, diastolic arterial blood pressure; LVEDP, left ventricular end-diastolic pressure; LAP, mean LA pressure; and RAP, mean RA pressure.
Supplemental Figure Legends

**Supplemental Figure I.** Recurrent atrial tachycardia recorded by 24-hour Holter ECG in an MI dog. A, 4-minute ECG showing repeated atrial tachycardias. Dashed lines indicate atrial tachycardias and atrial ectopic complexes. B, Expanded 10-s ECGs corresponding to boxes a and b in panel A, respectively. Panel a shows the spontaneous initiation of atrial tachycardia. Panel b shows atrial tachycardia with AV block. The negative P waves in leads I and III during tachycardia are consistent with an ectopic focus in the lower right atrium near the infarct zone.

**Supplemental Figure II.** Action potential properties. A, Resting potential. B, Action potential (AP) amplitude. C, AP duration to 50% repolarization (APD50). D, AP duration to 90% repolarization (APD90). CTL indicates control cells (n=17 cells from 5 sham dogs). BZ indicates infarct border zone cells (n=22 cells from 8 MI dogs).

**Supplemental Figure III.** Effect of atrial MI on atrial effective refractory periods (ERPs) during open-chest study in RA-appendage (RAA) at various basic cycle lengths (BCLs). n=6 and n=9 dogs for sham and MI.

**Supplemental Figure IV.** Time-dependent changes in mean duration of induced AF (A) and atrial ERP (B-D) in MI dogs (n=5). AF duration prolonged from day 1 post-surgery and then remained constant until day 7 (A). Atrial ERP prolonged at shorter BCLs on day 1 and then recovered gradually (B and C). ERP was not different from baseline on day 7.
(B-D). BL indicates baseline; D1, day 1; D3, day 3; D7, day 7.

*\(P<0.05\) versus and **\(P<0.01\) versus baseline.

**Supplemental Figure V.** A and B, Representative example of multi-electrode epicardial mapping during constant pacing in sham and MI preparations. C, Median local phase delays (P50), phase delays corresponding to slowest conduction zone (P95), absolute phase delay heterogeneity (P5-95), and heterogeneity index (P5-95/P50) as a function of pacing cycle length in normal zone (NZ) of sham (n=6) and MI (n=9) preparations. D, Corresponding values in CTL/BZ region of sham/MI preparations. **\(P<0.01\) and ***\(P<0.001\). In sham preparations (A), conduction was smooth and homogeneous throughout the right atrium. In MI preparations (B), no significant electrogram signals were observed in the infarct zone (IZ), and conduction was irregular and heterogeneous in the BZ. Indices of conduction heterogeneity were significantly increased in BZ of MI preparations (D).
Supplemental Figure I

A  Lead I  

B  a  

Supplemental Figure I
Supplemental Figure II

A. Resting potential (mV)

B. AP amplitude (mV)

C. APD50 (ms)

D. APD90 (ms)

CTL BZ

ns

ns

ns

ns
Supplemental Figure III

BCL (ms)

ERP (ms) in RAA

- Sham (n=6 dogs)
- MI (n=9 dogs)

ns
Supplemental Figure IV

(A) AF duration (s)

(B) ERP (ms) at BCL 300 ms

(C) ERP (ms) at BCL 150 ms

(D) ERP (ms) at BCL 300 ms

Supplemental Figure IV
Supplemental Movie Legend

The movie shows activation sequences during 4 successive cycles of atrial tachyarrhythmia in a coronary artery-perfused right-atrial free wall preparation from an MI dog. The tissue was loaded with di,4-ANEPPS and optical mapping of transmembrane potential performed. Colors indicate transmembrane potential, with dark blue corresponding to resting potential, red to the activation wave front (fully depolarized tissues), and colors between indicating grades of depolarization. The field of view has the infarct zone at the lower left and the border zone situated above and to the right. Note that a clockwise reentry circuit located in the border zone (upper right quadrant) maintains reentry. The tissue in the lower left quadrant, corresponding to the infarct zone, remains in shades of lighter blue and is never activated.