Atrial Ischemia Promotes Atrial Fibrillation in Dogs

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Background—Coronary artery disease is a significant risk factor for atrial fibrillation (AF), but the basis for this association is incompletely understood. The present study evaluated the hypothesis that atrial ischemia can create a substrate for AF maintenance.

Methods and Results—Atrial ischemia was induced by occlusion of an atrial arterial branch that did not provide blood flow to the ventricles. Atrial-arterial occlusion increased the duration of AF induced by burst pacing from 57±32 seconds (control) to 803±214 seconds (P<0.001) after 0.5 hour of occlusion and to 887±209 seconds (P<0.001) after 3 hours of occlusion. Prolonged AF (>20 minutes) was induced in 0 of 16 dogs (0%) under control conditions, 7 of 16 (44%, P<0.01) after 3 hours of ischemia. Refractoriness was initially unaffected but was prolonged 5 hours after occlusion. Phase-delay analysis and high-density mapping confirmed severe conduction slowing in the ischemic zone. Histological examination confirmed the location of ischemic regions and revealed extensive ischemia-induced necrosis at sites of conduction delay.

Conclusions—Experimental atrial ischemia creates a substrate for AF maintenance, apparently by causing local conduction slowing that promotes reentry. These results suggest that atrial ischemia may significantly promote AF, and may be relevant to AF mechanisms in association with coronary artery disease. (Circulation. 2003;107:1930-1936.)

Key Words: fibrillation ■ arrhythmia ■ coronary artery disease

Atrial fibrillation (AF) is a very common rhythm disturbance for which treatment remains problematic. Our understanding of AF pathophysiology has evolved greatly over the past 10 years but remains incomplete. An improved understanding of AF pathophysiology may lead to improved therapeutic approaches.

Coronary artery disease (CAD) is a significant risk factor for AF. Congestive heart failure, an obvious potential mediator between CAD and AF, appears to be present in only a minority of patients with AF and CAD. In addition, although the incidence is declining, AF remains a common complication of acute myocardial infarction. The possibility that atrial ischemia may contribute to the occurrence of AF has received relatively little attention, and we were unable to identify any published experimental studies examining the effects of isolated atrial ischemia on atrial electrophysiology or the maintenance of AF. We therefore developed an approach to creating isolated acute atrial ischemia in the dog and applied it to (1) evaluate effects on atrial electrophysiology, (2) study changes in the duration of AF induced by atrial burst pacing, and (3) evaluate atrial activation to gain insights into potential underlying mechanisms.

Methods

General Methods

Twenty adult mongrel dogs weighing 25 to 32 kg were anesthetized with morphine (2 mg/kg IM) and α-chloralose (100 mg/kg IV) and ventilated with oxygen-enriched air. A median sternotomy was performed, and body temperature was maintained at 37.5±1°C. Bipolar Teflon-coated stainless steel plunge electrodes were inserted into the right atrial (RA) appendage (RAA) and left atrial (LA) appendage (LAA) for recording and stimulation. Arterial pressure was monitored, and femoral vein catheters were used for drug and fluid administration.

Ischemic Model

We doubly ligated a branch of the right coronary artery (the right intermediate atrial artery, RIAA) perfusing the RA free wall (FW) (Figure 1A). In some cases, the RIAA was too deep in the myocardium to ligate directly, and we ligated 2 primary RIAA branches. To delineate the area of atrial hypoperfusion, 6% thioflavin-S in 0.9% NaCl was injected into a femoral artery at the end of the experiments. Two minutes later, the RA, LA, and right ventricle (RV) were removed. Hypoperfused regions (nonfluorescing areas under ultraviolet light) were traced.

Electrophysiological Measurements

Single atrial extrastimuli (S₅ⱽ) were introduced after every 10 basic S₁s (2-ms twice-diastolic threshold pulses), with 5-ms S₁-S₂ decre-
ments from 200 ms until capture failed (atrial effective refractory period, ERP). Global epicardial mapping was performed with 5 arrays containing 240 bipolar electrodes (Figure 1B).5,6 High-density RA free-wall mapping (208 unipolar electrodes) was performed in 4 additional experiments. Conduction was assessed by (1) measuring conduction time between adjacent electrodes across the ischemic-zone border, (2) use of phase-delay histograms (based on conduction times between each electrode and its neighbors, as detailed in Reference 5), and (3) high-density RA mapping. AF was defined as a rapid (>400 bpm) atrial arrhythmia with irregular atrial electrograms. To determine AF duration, AF was induced by burst pacing (10 Hz, 4× threshold current). AF was induced at least 10 times for AF<5 minutes, 5 times for AF 5 to 30 minutes, and 2 times for AF>30 minutes. “Prolonged AF” was defined as AF lasting >20 minutes, and AF>30 minutes was terminated by QRS-synchronized DC cardioversion. Rest periods before reinduction of AF were provided after AF episodes, and no carryover effects of AF on the duration of subsequent AF episodes were observed. Conduction and ERP measurements were obtained before ischemia (control) and 0.5, 3, and 5 hours after occlusion. Because of the time required to obtain AF-duration measurements (particularly with prolonged episodes), results are presented for each of the 0.5 to 3-, 3 to 5-, and >5-hour periods after occlusion.

Histology
Atria and RVs were immersed in 10% neutral buffered formalin for >24 hours. Myocardial necrosis was identified by a trained pathologist (T.K.L.) in sections stained with hematoxylin-phloxin-saffron and Gomori trichrome.

Statistical Analysis
Group data are presented as mean±SEM. Repeated measures ANOVA was used to test the significance of differences between group means. Differences in AF duration were analyzed statistically with a nonparametric test (Wilcoxon’s signed-ranks) because of the nonnormal distribution of AF duration. A McNemar test was used to compare percentages of AF at various times after occlusion. Two-tailed tests were used, with a value of $P<0.05$ indicating statistical significance.

Results
Compared with normal tissue (Figure 2A), the ischemic zone showed typical necrotic changes, including pyknotic nuclei, contraction bands, and disrupted intercalated disks (Figure 2B). In the center of the hypoperfused zone (“C” in Figure 2F), necrosis spread across most of the atrial wall (Figure 2C), with layers of surviving subendocardial tissue (3 to 10 cells thick, Figure 2D). At the periphery (Figure 2E), necrosis was patchy, with both subepicardial and subendocardial sparing. LA and RV tissues were normal.

Electrophysiological Changes
Atrial ischemia substantially increased AF duration (Figure 3A) for up to 5 hours, with the duration of AF decreasing thereafter. Prolonged AF was absent under control conditions but became much more common at 0.5 to 3 and 3 to 5 hours
after occlusion (Figure 3B). The AF cycle length averaged $94 \pm 7$ ms under control conditions, compared with $107 \pm 5$, $107 \pm 10$, and $101 \pm 10$ ms after 0.5, 3, and 5 hours of ischemia. Premature atrial complex frequency was low, averaging $<15$ per hour, and was unchanged by atrial ischemia (Figure 3C).

Ischemia increased excitability threshold (eg, in the RAFW, from $0.49 \pm 0.05$ under control conditions to $1.91 \pm 0.4$ mA, $P<0.01$ at 3 to 5 hours). Excitability did not change in nonischemic tissue. Figure 3 (bottom) shows ERPs before and after occlusion in a nonischemic region (RAA) and in 2 ischemic-zone areas (RAFW and RA inferior wall). ERP increased significantly in RAFW (center of the ischemic zone) at $>5$ hours. No significant ERP changes occurred in other areas or in the RAFW at earlier time points.

In contrast to the limited ERP alterations, ischemia changed atrial conduction importantly. Figure 4 shows RA activation maps from 2 dogs at basic cycle lengths (BCLs) of 150 and 300 ms. The ischemic zone (determined histologically) is delineated by the shaded areas. Ischemia-related activation delays tended to increase at shorter BCLs. A quantitative analysis of ischemic-zone activation delay in all dogs is provided in Figure 5. We selected adjacent electrodes in the direction of longitudinal propagation under control conditions that were located across either side of the ischemic boundary and calculated conduction times between these electrodes before and after occlusion. Activation delay increased progressively after occlusion.

The activation delay analysis in Figure 5 is based on data from a very limited number of sites and is susceptible to a quantitative influence of propagation-pattern changes, particularly with ischemia. We therefore performed an analysis of changes in conduction at all RA sites with the use of phase-delay histograms. Maximum activation-time differences between each electrode and adjacent sites were normalized to interelectrode distance to provide local phase delays. Typical phase-delay maps in 1 dog are shown in Figure 6A. Control phase delays are 1 to 2 ms/mm. With ischemia, there is a progressive increase in phase delays within hypoperfused tissues. Corresponding phase-delay histograms were obtained by binning results at all sites, as illustrated in Figure 6B. Control histograms are narrow, reflecting symmetrical and rapid conduction. With ischemia, histograms show progressive skewing, with tails at larger values containing an increasing proportion of all values and indicating increased regional conduction slowing. At 3 and 5 hours, tails are marked, with longer values recorded in the
zone delineated as hypoperfused at the end of the experiment (open bars). Statistical analysis of the phase-delay histograms provides a median phase delay ($P_{50}$) and values of the distribution corresponding to the 5% lowest delays ($P_{5}$) and the 95% lowest delays (the $P_{95}$). $P_{50}$ is a reflection of average conduction velocity, whereas $P_{95}$ reflects the slowest conduction in each data set, and ($P_{5}-P_{95}$)/$P_{50}$ is a conduction-heterogeneity index independent of mean conduction changes. $P_{50}$ prolonged slightly ($\sim 50\%$ at 5 hours) with ischemia (Figure 7A), whereas $P_{5}$ and ($P_{5}-P_{95}$)/$P_{50}$ were greatly increased by ischemia (Figure 7, B and C), indicating regions of extremely slow and heterogeneous conduction.

To evaluate conduction changes further, high-density mapping of the RAFW was performed in 4 additional experiments, with maps obtained separately during stimulation at anterior (Figure 8A), superior (Figure 8B), and posterior (Figure 8C) sites. Figure 8 shows electrograms from 6 atrial sites and corresponding activation maps for control and 3-hour postocclusion conditions in 1 dog at a BCL of 150 ms. Under control conditions, activation is rapid and symmetrical. With ischemia, zones of marked slowing appear, with local electrograms becoming smaller, broader, and occasionally quite fractionated.

Atrial activation mapping showed regions of marked conduction slowing or block in the RA ischemic zone during AF. The data were consistent with reentry around slow-conduction zones; however, complete reentry circuits could not be delineated over the epicardial surface, possibly because of transmural reentry involving zones of patchy necrosis, as shown in Figure 2E.

**Discussion**

We have shown that isolated atrial ischemia strongly promotes the persistence of AF in the dog. Atrial ischemia has relatively little effect on refractoriness but causes strong conduction slowing in the ischemic zone, which may stabilize atrial reentry that maintains AF.

**Relationship With Previous Studies of Arrhythmias Associated With Experimental Myocardial Ischemia**

The literature on mechanisms of ventricular tachyarrhythmias related to acute myocardial ischemia and infarction is vast. Acute ventricular ischemia tends to reduce ERP by decreasing action potential duration but to increase ERP by inducing postrepolarization refractoriness, with net effects depending on the balance between these opposing actions. Ventricular conduction is markedly slowed by acute ischemia, tending to promote reentry. Ventricular arrhythmias include ectopic complexes and more sustained tachyarrhythmias because of both abnormal impulse formation and abnormal impulse propagation/reentry.

The strong local atrial conduction slowing and unchanged ERP that we observed with acute atrial ischemia are consistent with previous observations at the ventricular level. In contrast to the spontaneous ventricular ectopy frequently caused by acute ventricular ischemia, we did not observe a
significant increase in premature atrial complex prevalence with atrial ischemia. Conversely, atrial ischemia produced a substrate for atrial reentry and AF. Both focal and reentrant mechanisms are involved in ventricular tachyarrhythmias associated with the early phases of acute myocardial ischemia. In the present study, reentrant mechanisms most likely played a predominant role in the substrate for atrial tachyarrhythmia.

We were unable to identify in the literature any other studies of the electrophysiological effects of isolated atrial ischemia on the in situ heart. Lammers et al evaluated the effects of hypoxia on isolated superfused rabbit atrial preparations. They observed a transient increase in ERP over 15 minutes, along with a sustained decrease in conduction velocity. Conduction properties became more heterogeneous, and premature stimuli readily induced reentry around arcs of functional conduction block. The effects of homogeneous hypoxia on isolated superfused atrial preparations in vitro cannot be extrapolated directly to the effects of ischemia (which includes the consequences of hypoxia, substrate, and oxygen deprivation, as well as reduced metabolite removal) on intact atria in situ. Nevertheless, many of the phenomena observed by Lammers et al are quite similar to changes that we observed as a result of isolated atrial ischemia in vivo. Jayachandran et al showed that proximal right coronary artery occlusion, which causes both posterior left ventricular and LA ischemia, reduces atrial ERP after several hours, an effect not altered by the ATP-dependent K\(_{ATP}\)-channel blocker glibenclamide. Atrial arrhythmias were not reported.

**Potential Significance of Our Observations**

Our study is the first of which we are aware to examine directly the effects of acute atrial ischemia per se on the electrophysiological properties of the atria and their ability to maintain AF. Our results point to important profibrillatory effects of atrial ischemia, probably mediated (at least in part) by impairment of atrial conduction.

A recent detailed review of the pathophysiology and prevention of AF indicates that myocardial infarction is one of the most frequent causes of AF and that ischemic heart disease is one of the most common clinical settings; however, acute atrial ischemia per se is rarely considered as a direct contributor to AF pathophysiology. There is extensive indirect evidence for a significant clinical role of atrial ischemia in the AF associated with acute myocardial infarction. Several studies have suggested that atrial infarction is relatively common, observed in up to 17% of autopsy-proven cases of myocardial infarction, with >20% of cases constituting isolated atrial infarction. Isolated atrial infarction is difficult but possible to diagnose clinically, and atrial tachyarrhythmia is a characteristic manifestation. The pathophysiological role of atrial ischemia in clinical infarct-related AF was recently highlighted by a patient with inferoposterior infarction in whom angioplasty reperfusion of occluded atrial coronary branches led to spontaneous termination of AF.

AF is a common complication of cardiac surgery (10% to 65% prevalence) that increases morbidity and hospital costs. Stenosis of the right coronary artery, which provides significant RA blood flow, and retrograde cardioplegia predispose patients to postoperative AF. In addition, stenosis...
of the sinoatrial nodal artery, supplying an important atrial territory, is a particularly important contributor.\textsuperscript{19,20} Thus, there is substantial circumstantial evidence for a role of atrial ischemia in AF after coronary artery bypass surgery. Our results show that atrial ischemia induces a substrate that supports AF maintenance and provides information about the potential pathophysiological basis.

Our understanding of the mechanisms of AF is undergoing rapid evolution, with important contributions from recently developed experimental models that mimic clinical conditions.\textsuperscript{1,11} Experimental work has provided insights into the self-perpetuating nature of AF caused by atrial tachycardia remodeling\textsuperscript{1,11,21} and into the role of conduction abnormalities\textsuperscript{8} and ionic remodeling\textsuperscript{22} caused by congestive heart failure. The present study adds an additional clinically relevant paradigm, that of acute atrial ischemia, to the set of experimental conditions shown to promote AF maintenance.

Our results speak directly to the potential mechanisms of AF associated with coronary artery occlusion causing severe atrial ischemia. It remains to be determined whether less severe forms of atrial ischemia, as might complicate chronic CAD, could also promote atrial arrhythmogenesis and contribute to the increased prevalence of AF associated with chronic coronary disease. The role of healed prior atrial infarction as a potential atrial arrhythmogenic factor also remains to be examined. The involvement of atrial ischemia in specific cases of AF might have therapeutic implications, given the specific responses of arrhythmias related to acute myocardial ischemia and infarction to pharmacological interventions.\textsuperscript{23}

Potential Limitations
We did not observe an increase in spontaneous atrial arrhythmias in response to acute atrial ischemia. The absence of spontaneous arrhythmias may have been a result of the relatively small area of the atria rendered ischemic. To delineate clearly the atrial ischemic zone and to avoid a contribution of ventricular ischemia, we chose to occlude only 1 atrial branch of the right coronary artery. This resulted in distinct and clearly delineated atrial hyperperfusion and ischemic changes, but the size of the ischemic territory was relatively small, possibly accounting for the lack of spontaneous arrhythmias. Although AF is a relatively common clinical complication of acute myocardial infarction, frequent atrial ectopy is not, suggesting that acute atrial ischemia may act more to increase atrial ability to sustain AF than to induce atrial ectopic activity per se.

Our mapping data suggest that atrial conduction slowing played an important role in the stabilization of RA reentry and the maintenance of AF. However, the analyses of conduction properties shown in Figures 4 to 8 indicate that conduction slowing was maximal at 5 hours, whereas effects on AF maintenance were greatest at 0.5 and 3 hours (Figure 3). These results suggest that the extent of conduction slowing cannot be the only factor determining the persistence of AF in the face of atrial ischemia. We measured ERP at only 2 sites in the ischemic zone, and ERP increased in the most ischemic region (the RAFW) 5 hours after occlusion. It is possible that increases in ERP counteracted conduction slowing, making reentry less likely to be maintained at 5 hours. Alternatively, other (currently unidentified) electrophysiological consequences of acute atrial ischemia may contribute importantly to governing the maintenance of AF.

Our epicardial mapping data, along with the absence of spontaneous arrhythmias, point to reentry around areas of ischemic conduction slowing or block as a mechanism of prolonged AF induced in the presence of acute atrial ischemia. However, the apparent reentry circuits we observed at the epicardial surface did not cover the entire cycle. Our inability to map complete circuits may have been a result of endocardial or intramural components. We have previously performed combined atrial endocardial and epicardial mapping in situ\textsuperscript{24}; however, the need for an atriotomy, the limited resolution of the 64-electrode endocardial balloon array, and the hemodynamic effects of prolonged mapping with the system made it unsuitable for the present study. More detailed mapping work will be needed to define the precise mechanisms of prolonged AF during atrial ischemia. We demonstrated that acute atrial ischemia produces a substrate that can maintain AF; however, in the absence of an appropriate trigger, such a substrate might remain latent, and AF would not necessarily result.

Conclusions
We have demonstrated that isolated atrial ischemia causes important localized atrial conduction slowing and promotes the maintenance of AF. These findings are potentially relevant to understanding the mechanisms of AF in patients with CAD and to understanding the response to antiarrhythmic interventions.

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References


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