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Acute Regional Left Atrial Ischemia Causes Acceleration of Atrial Drivers during Atrial Fibrillation

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Abstract

Background—The mechanisms by which acute left atrial ischemia (LAI) leads to AF initiation and perpetuation remain unclear.

Methods and Results—LAI (90-minute ischemia) was obtained in isolated sheep hearts by selectively perfusing microspheres into the left anterior atrial artery. Two CCD cameras and several bipolar electrodes enabled recording from multiple atrial locations: with a dual-camera set-up (Protocol 1, n=10; and 1', n=4; for bi-atrial or atrio-ventricular camera set-ups respectively), in the presence of propranolol/atropine (1μM) added to the perfusate after LAI (protocol 2, n=3) and after a pre-treatment with glibenclamide 10 μM (protocol 3, n=4). Spontaneous AF occurred in 41.2% (7/17) of the hearts that were in sinus rhythm before LAI. LAI caused APD shortening in both the ischemic (IZ) and non-ischemic (NIZ) zones by 21±8 and 34±13%, respectively (pacing, 5Hz, p<0.05 compared to baseline). Apparent impulse velocity was significantly reduced in the IZ but not in the NIZ (−65±19% and +9±18%, p=0.001 and n.s., respectively). During LAI-related AF, a significant NIZ maximal dominant frequency (DF_{max}) increase from 7.4±2.5 to 14.0±5.5 Hz; p<0.05, was observed. Glibenclamide, an IKATP channel blocker, averted LAI-related DF_{max} increase (NIZ: LAI vs Gli, 14.0±5.5 vs. 5.9±1.3 Hz, p<0.05). Interplay between spontaneous focal discharges and rotors, locating at the IZ-NIZ border zone, maintained LAI-related AF.

Conclusions—LAI leads to an IKATP conductance-dependent APD shortening and spontaneous AF maintained by both spontaneous focal discharges and reentrant circuits locating at the IZ border zone.

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Keywords

Atrial ischemia; atrial fibrillation; dominant frequency; IKATP

Atrial fibrillation (AF) is the most common arrhythmia and affects more than 4 million Americans.¹ Furthermore, AF is a frequent complication of acute coronary syndromes, with a post-myocardial infarction incidence ranging between 5 to 18%.^{2,3} Acute regional atrial ischemia/infarction has been observed after a *ventricular* myocardial infarction but also in isolation.^{4–6} Atrial ischemia/infarction translates into PQ segment depression or elevation on the electrocardiogram and often associates with atrial tachy-arrhythmias.^{4,5,7} In an experimental work, Sinno et al. indicated that right atrial coronary branch occlusion resulted in severe conduction slowing and in an increased duration of AF episodes.⁸ Also in a canine model, it was shown that acute occlusion of the right coronary artery led to atrial effective refractory periods shortening.⁹ Recently, Nishida et al. also demonstrated that the border zone of an 8-day right atrial myocardial infarction region is an elective area for rotor anchoring and spontaneous focal discharges following an up-regulation of the sodium-calcium exchanger current in cells from the border zone.¹⁰ Still, the electrophysiological mechanisms of short-term atrial ischemia-induced AF remain unclear, especially when ischemia involves the left atrial muscle. Previous anatomical studies in humans and a study in sheep by our group have indicated that 3 main branches provide the coronary blood supply to the atria: the left anterior atrial artery (LAAA) which arises from the proximal segment of the left circumflex artery, the right anterior atrial artery (RAAA)-also known as right sinus node artery- and the branches of left circumflex artery (LCX).^{11–13} Here, we implemented a newly developed model of acute left atrial regional ischemia (LAI) in isolated ovine hearts to demonstrate that regional impairment in atrial coronary perfusion is conducive to action potential duration (APD) shortening, AF initiation as well as an acceleration and increased complexity of AF drivers.

Methods

Langendorff-perfused Sheep Heart and Regional Left Atrial Ischemia Model

All animal experiments were carried out according to National Institutes of Health guidelines. Twenty one sheep (45–50 kg) were anesthetized with propofol (0.4 mg/kg), and then heparinized (200U/kg, IP). After heart removal, the heart were Langendorff-perfused with warm oxygenated Tyrode's solution (pH 7.4; 95% O₂, 5% CO₂, 36 to 38 °C). During all experiments and to obtain a controlled and physiological level of intra-atrial pressure of 3–5 cmH₂O, we perforated the inter-atrial septum, sutured venous orifices and connected the inferior vena cava to a cannula which enabled to maintain a constant level of intra-atrial hydrostatic pressure as described previously.^{13,14} We initiated ventricular fibrillation (VF) as soon as the heart was perfused and VF was maintained for the entirety of the experiment. After having identified the course of the main atrial coronary branches on the atrial epicardium, the left anterior descending artery was punctured with a 21 gage needle and a 0.36 mm angioplasty wire was retrogradely inserted into the left anterior atrial artery. Then, we deployed an over-the-wire balloon catheter (1.5×9 mm; Ranger, Boston scientific Inc.) or a metal needle (1.5 mm) into the LAAA through the left anterior descending artery (Figure

1A). To generate a regional impairment in atrial coronary perfusion and also avoid coronary collateral flow from other perfusion territories, we first inflated a balloon and then injected 40–100µm microsphere (1.5 ml) into the LAAA. Finally, we ligated this artery. Thereafter, we waited 90 minutes before obtaining optical mapping and electrical recordings as described below. At the end of the experiment, to differentiate the ischemic zone (IZ) and non-ischemic zone (NIZ), we delineated the boundaries of the IZ with an injection of Evans blue (1–5 ml, 2 mg/ml, Sigma, Inc.) into the LCX and Congo red (1–5 mL, 4 mg/mL, Sigma, Inc.) into the right coronary artery as previously reported¹³. The ischemic border zone (BZ) was defined as a 4 mm band peripheral around the IZ. As an example, a representative snapshot is presented in Figure 1B. Evans blue was perfused into the LCX perfusion territory that corresponded to the lower left atrial appendage (LAA) region, while the ischemic region remained unstained.

Experimental Set-up and protocols

In 21 LAI sheep, we performed and analyzed optical mapping recordings as follows (Figure 2 and online supplement table 1). In 14 sheep, a right atrium-left atrium dual-camera set-up (Protocol 1) while in 4 atrial-left ventricle dual-camera set-up was implemented (protocol 1'). In 3 sheep propranolol (1µM) and atropine (1µM) were added to the perfusate after LAI (protocol 2) and in 4 sheep, LAI was performed in the presence of glibenclamide 10 µM (protocol 3). Importantly, during atrial ischemia the perfusion was maintained by a closed-loop recirculating circuit and after 90 minutes ischemia, the Tyrode's solution was changed for washout (see Figure 2B). Optical movies during AF were also recorded after 90-minute ischemia and after washout in 3 animals from protocol 1. Optical and electrodes mapping techniques as well as statistical analysis methods are described in the online supplement.

Results

APD and apparent conduction velocity reductions after 90-minute regional ischemia

Figure 3A presents a post-experiment photographic snapshot of the LAA from one LAI heart, depicting the IZ, BZ and NIZ regions (see methods). Representative single pixel time sequences from the upper LAA and the lower LAA illustrate that before and after LAI, APD80 was substantially shortened in *both* the IZ and NIZ regions of the left atrium. Importantly however, AP upstroke was noticeably shallower in the IZ vs. NIZ—compare the red IZ AP with the blue NIZ AP. Panels 3B and 3C present the corresponding activation and APD80 maps from the same heart. An apparent conduction velocity (CV) decrease was found at the IZ, while CV was unchanged at the BZ and NIZ. This is reflected by the presence of tight isochrones at the IZ region, while inter-isochrone distance was normal in the NIZ region (figure 3B lower map). Results in 6 analyzed experiments are summarized in figure 3D which shows that after LAI, apparent CV was significantly reduced at the IZ, but was unchanged at the BZ and NIZ. In comparison, APD80 was significantly reduced in all regions (figure 3D, middle panel) but in a more heterogeneous fashion within the IZ than the NIZ and BZ. This was indicated by the calculation of APD dispersion which average values were significantly larger in the IZ than in the BZ and NIZ. In summary, after regional left atrial ischemia, we observed a regional CV reduction in the IZ region *only*, and a *pan-atrial* APD shortening in IZ, BZ and NIZ regions.

Spontaneous AF occurrence

As shown in a representative example in Figure 4A, episodes of spontaneous AF were observed after regional LAI. In 17 hearts, LAI led to spontaneous AF initiation in 7/17 experiments and following 45.7 ± 31.4 minutes (range 1–90 minutes) of LAI, see figure 4B. Interestingly, AF never spontaneously occurred in the control condition before LAI (0/17, see Figure 4C). Of note, a short-lasting episode of spontaneous AF occurred in the presence of glibenclamide after LAI (Figure 4D). In addition, it should be mentioned that burst pacing enabled sustained AF initiation in all groups thus allowing recording of AF episodes and fibrillation dynamics comparisons.

Regional DF increases after 90-minute LAI

In all experiments, we observed that after 90-minute LAI, DFs in NIZ regions increased very significantly. Figure 5A shows a representative bipolar electrogram DF time-course before and after 90-minute LAI in one heart. In this heart, AF was initiated before LAI and was maintained during the entire duration of the experiment. A bipolar electrode positioned at the NIZ within the distal coronary sinus recorded a sharp DF increase, which started about 50 minutes after LAI. From a baseline value of 8.0 Hz, DF value progressively increased at the rate of 2.5 Hz every 15 minutes, to reach a maximal value of 20 Hz, about 100 minutes after LAI (Figure 5 B). Importantly optical mapping and DF analysis of IZ, BZ and NIZ regions that were conducted in 10 hearts from protocol 1 confirmed that *NIZ* average DF values significantly increased after 90-minute LAI, see Figure 5C right. In comparison left atrial IZ DF values *did not* change after LAI (Figure 5C left, red column). Importantly, these DF increases were reversible after renewing the perfusate solution in 3 hearts from protocol 1, see washout in Figure 5C. We then tested the hypothesis that local release of catecholamines and/or acetylcholine after LAI was the cause of DF acceleration. Figure 5D demonstrates that the perfusion of atropine 1 μ M + propanolol 1 μ M *did not* prevent this acceleration. Figure 5E, however, shows that pre-incubation with an inhibitor of IKATP channels opening, glibenclamide 10 μ M, *did prevent* post-LAI DF increases (Figure 5E). At BCL=400 ms, NIZ APD80 in Glibenclamide pre-treated preparations was 137 ± 10.6 ms while it was 132.5 ± 13.6 ms after 90-minute LAI in the absence of glibenclamide pre-treatment (Figure 5F, $n=4$, n.s.). Besides, the sinus rate was not significantly different before and after glibenclamide perfusion: 465 ± 26 vs. 487 ± 45 ms ($n=4$, n.s., $p=0.34$). In summary, 90-minute LAI led to sharp increases in *NIZ-only* DF values and to *pan-atrial* APD shortening, both of which did not occur in the presence of glibenclamide.

Atrial DF distribution after 90-minute LAI

Figure 6A shows representative LAA and right atrial appendage (RAA) DF maps obtained from movies recorded during AF after 90-minute LAI. As shown in three different hearts (heart 1 to 3) in Figure 6A, left atrial DFs were higher than right atrial DFs after LAI. Also, regions harboring the highest frequency of excitation (DFmax) constantly spanned over the NIZ region or even, in some hearts (heart 2 for example), the BZ region. Most importantly in all experiments, DFmax regions extended to the PLA, encompassing one or several PVs. For instance in heart 1, the 18.0 Hz DFmax region located at the lower LAA and extended to the neighboring left inferior pulmonary vein, where a maximal DF value of 14.3 Hz was

recorded. This observation is summarized in 10 hearts in Figure 6B, which shows an histogram of DFmax locations. In 9/10 hearts, DFmax located at the left atrium and specifically at the PLA in 6/10 hearts, while it located at the RAA in only one heart. Also left atrial DF values distribution indicates that AF electrical sources preferentially located at the BZ/NIZ region, rather than at the IZ region. DF values were indeed significantly higher at the left atrial BZ/NIZ compared with the left atrial IZ (see Figure 6C). These results are reminiscent of DF distribution after regional *ventricular* ischemia as reported by Zaitsev et al.¹⁵

AF dynamics after 90-minute LAI

AF dynamics were drastically different after LAI. Before LAI, the activity was organized and consistent on a beat-to-beat basis. After LAI, patterns of activation were changing on a beat-to-beat basis and resulted in complex fibrillatory dynamics. This is exemplified in figure 7A that shows sequential phase movie snapshots and corresponding DF maps before and after LAI. Before LAI, while a one-way propagation wave was repeatedly crossing the LAA in the posterior-to-anterior direction, the activity appeared more complex after LAI. Figure 7A lower panel indeed illustrates that LAI-related wave patterns were changing on a beat-to-beat basis: two focal discharges within the IZ-BZ region at 75 ms evolved into a counter-clockwise reentry at 168 ms that was interrupted by additional IZ-BZ focal discharges at 347 ms, to finally give rise to a clockwise rotor. In summary, figure 7B shows that an index of beat-to-beat pattern of activation variability (see methods and online supplement figure 1) was significantly increased after LAI. Besides, spontaneous focal discharges predominantly originated in the IZ-BZ but not in the NIZ.

Discussion

Our results are as follows:

- i. 90-minute regional LAI leads to a well-delimited ischemic left atrial region that includes variable area of the left atrial free wall, left atrial appendage, and PLA-PV region.
- ii. LAI causes spontaneous AF initiation and maintenance.
- iii. LAI leads to *pan-atrial* APD shortening, i.e both IZ-BZ and NIZ regions experience a similar level of APD reduction. However, when LAI occurs in the presence of glibenclamide, APD does not change.
- iv. LAI causes a major reduction in apparent CV in the IZ region *only*. This LAI-related apparent CV decrease is also observed in the presence of glibenclamide.
- v. LAI leads to an acceleration of AF frequency of activation in the NIZ-BZ *but not* in the IZ.
- vi. After LAI, AF evolves into complex fibrillatory dynamics, reflecting a large increase in beat-to-beat variation in patterns of activation.

Electrophysiological changes after acute left atrial ischemia: Previous studies

AF commonly occurs during the acute phase of ventricular myocardial infarction.^{2,16–18} While an increase in atrial pressures after ventricular infarction is a likely cause of AF, several studies support that impaired atrial perfusion *per se*-with or without ventricular injury-may directly cause AF. Sino et al.⁸ demonstrated that a 3–5-hour regional acute right atrial ischemia associates with an increased burst pacing-induced AF duration, and regional impairment in conduction. Lin et al.¹⁹ suggested that a 60-minute global atrial hypoxia leads to a shorter pulmonary vein APD and an increased number of early and delayed after-depolarizations. Also, Jayachandran et al. showed that a 15-minute atrio-ventricular ischemia, obtained after right coronary ligation, leads to a right atrial APD shortening prevented by a Na/H exchanger blocker, HOE642.⁹ However, to our knowledge, the impact of a *regional acute left atrial ischemia* on atrial electrophysiology and fibrillation dynamics had not been explored. Here, we present results obtained in a clinically-relevant left atrial ischemia ovine model, which show for the first time that an acute perfusion impairment of a region of the left atrium is reproducibly conducive to spontaneous AF initiation and maintenance (see figure 4). We demonstrate that two additive LAI-related electrophysiological mechanisms-one *pan-atrial* and the other one *regional* - are underlying our observations. First, 90-minute LAI associates with pan-atrial APD shortening following an increase in IKATP channels conductance (see figures 3 and 5). Second and only in the atrial IZ, apparent CV decreases and APD dispersion increases after LAI (figure 3). We thus propose that the association between a shortened APD everywhere in the atria together with a well-delineated impairment in conduction at the IZ only, led to AF initiation and maintenance. We speculate that a shortened APD increased the likelihood of reentrant circuits to perpetuate, while a more positive resting membrane potential in the IZ favored abnormal automaticity and after-depolarizations. Accordingly, we show that LAI led to a strikingly large (>50%) AF frequency acceleration together with an increased dynamics complexity (see figures 5, 6, 7). This increased complexity resulted from beat-to-beat changes in patterns of propagation, with several instances of reentrant activities interrupted by the onset of spontaneous focal discharges which manifested as breakthrough waves (see figure 7). In fact, our analysis of the patterns of propagation during LAI-related AF shows that *both* spontaneous focal discharges and rotors were more likely to be formed after LAI. In addition, several mechanisms known to be at play during myocardial ischemia could have contributed to our observations. For instance, changes in cell-to-cell coupling, depolarization-dependent inactivation of the sodium current may also have led to an increased complexity in fibrillation dynamics after ischemia.

Pan-atrial IKATP channels opening

Unexpectedly after LAI, APD shortened everywhere in the atrial parenchyma, i.e at both the NIZ and the IZ-BZ regions. This APD shortening was mediated by an increased IKATP channels conductance, as indicated by the absence of APD shortening and DF increase when LAI was obtained in the presence of glibenclamide. The ADP/ATP ratio in the online supplement tended to increase in all atrial regions after LAI, albeit *not significantly*. As shown in figure OS2, we demonstrate that ischemia-related biofactors had no significant effect on VF frequency, while at the same time, we observed a sharp AF acceleration. This

corroborates previous reports indicating a differential IK_{ATP} kinetics and ATP sensitivity between the atrial and the ventricular myocardium.^{20,21} Thus we propose that one or several biofactors released after atrial ischemia into the perfusate may have increased IK_{ATP} conductance in all atrial regions. It is highly plausible that ischemic substances released by the ischemic zone were re-circulated in the non-ischemic region. Our argument is mainly constructed on the following observations: DF acceleration and APD shortening in the non-ischemic regions were partially reversible after washout. Upstroke slope was unchanged in the non-ischemic region while it was markedly shallower in the ischemic zone. Previously, it was shown that acute myocardial ischemia leads to the release of several biofactors into circulation, some of which having been suggested to modulate IK_{ATP} channels conductance. For instance, Downar et al.²² demonstrated that coronary venous blood from an ischemic heart can rapidly produce shortening of action potential duration and post-repolarization refractoriness in the non-ischemic myocardium from the same heart. Fatty acids,^{23–26} fatty acid metabolites,²⁷ or cytokines,²⁸ have been shown to be released after myocardial ischemia. A spike in free fatty acid serum levels occurs within hours after myocardial ischemia and is strongly associated with atrial and ventricular arrhythmias.²³ Besides it has also been reported that IK_{ATP} conductance run-down may be fully prevented by long-chain acyl-coenzyme A esters, which are fatty acid metabolites.^{29–31} We submit that the mechanisms described here may be relevant to the acute phase of a ventricular and/or atrial myocardial infarction during which a large amount of ischemia-related biofactors is released in the circulation.^{23–31} However, we acknowledge that the determination of the biofactor(s) predominantly active will require a separate investigation, preferably *in vivo*.

Limitations

First, we cannot exclude that VF per se could have been responsible for biofactor release. However, the fact that APD shortening was reversible after washout, and in the presence of continuing VF, is an indication that the atrial phenomena described here are distinct. Next, *in vivo*, Jayachandran et al.⁹ showed IK_{ATP} blockade with glibenclamide did not prevent atrial APD shortening. Instead, the role of the Na/H exchanger was demonstrated. Thus, it is likely that *in vitro* short-term ischemia-related electrophysiological changes may translate into different mechanisms. In particular, the role of the autonomic input has been recognized after myocardial ischemia.³² Last, we submit that the mechanisms described here may be relevant to the acute phase of a ventricular and/or atrial myocardial infarction during which a large amount of ischemia-related biofactors is released in the circulation.^{23–31} However, we acknowledge that confirmation by an *in vivo* evaluation is warranted.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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ABBREVIATIONS

AF	atrial fibrillation
LAAA	left anterior atrial artery
RAAA	right anterior atrial artery
LCX	left circumflex artery
LAI	left atrial regional ischemia
APD	action potential duration
VF	ventricular fibrillation
RAA	right atrial appendage
LAA	left atrial appendage
IZ	ischemic zone
NIZ	non-ischemic zone
BZ	ischemic border zone
CV	conduction velocity
DF	dominant frequency
DFmax	highest dominant frequency

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Figure 1. Regional atrial ischemia model

A, Schematic: A guide wire was inserted retrogradely into the left anterior atrial artery (LAAA, red) via the left anterior descending artery (LAD). Then, an over-the-wire balloon catheter was introduced and the balloon inflated. Subsequently microspheres were perfused into the LAAA. After removal of the balloon catheter, the LAAA was ligated.

LMT, Left main trunk, RCA, Right coronary artery, LAD, Left anterior descending artery, LCX, Left circumflex artery, RAAA, Right anterior atrial artery, RV, Right ventricle, LV, Left ventricle, RSPV, Right superior pulmonary vein, LSPV, Left superior pulmonary vein.

B, Photographic snapshot of the left atrial free wall after injection of Evans blue in the LCX which perfuses the NIZ in this example. The BZ is defined as a 4 mm band peripheral around the IZ. Red triangles indicate the LAAA course.

Figure 2.
Experimental protocols 1–3

Figure 3. APD and apparent CV

A: Representative single pixel recording showing an optical action potential before (control) and after 90-minute ischemia in the IZ (red) and NIZ (blue) during pacing at 200 ms CL.

B and C: In the same preparation, representative LAA activation and ADP80 maps are shown.

D: Average conduction velocity, APD 80 and APD80 dispersion (APDmax-minimum)(n=6).

Figure 4. Spontaneous AF initiation after LAI with and without the presence of glibenclamide in the perfusate (protocols 1 and 3 respectively)

A, Example of spontaneous AF initiation during LAI (protocol 1). **B**, Time to spontaneous AF initiation (protocol 1). **C**, % of spontaneous AF initiation after LAI (protocol 1), and after LAI in the presence of glibenclamide (protocol 3). **D**, Example of a spontaneous but non-sustained AF episode after LAI in the presence of glibenclamide (protocol 3).

Figure 5. LAI-related DF changes in the ischemic zone (IZ) and non-ischemic zone (NIZ)

A: Example of DF time-dependent changes before and during LAI. **B:** Roof and CS-D bipolar electrograms before and during LAI. **C:** DFs at baseline, 90min-LAI and after washout. **D:** Atropine and propranolol (protocol 2). **E:** In the presence of glibenclamide (protocol 3), DFs were unchanged. **F:** APD80 during constant pacing (400 ms) before LAI and after LAI with glibenclamide.

Figure 6. Spatial DF distribution during AF after LAI

A: (Left) Representative DF maps and single-pixel recordings at the Roof-IZ, LAA-NIZ and RAA-NIZ and corresponding CS-NIZ bipolar electrograms during LAI (bip. ECG). In heart 1, DFmax after LAI localized at the LAA lower region (LAA-NIZ). **(Right)** In heart 2, DFmax was confined to a small area between ischemic and non-ischemic zone at the border zone (BZ). In heart 3, the DFmax was found in the CS-NIZ region. **B:** Histogram of DFmax atrial locations in 10 hearts. **C:** Average DFs at the LAA-IZ, LAA-BZ, LAA-NIZ, PLA-NIZ and RAA-NIZ. The LAA-IZ average DF value was significantly lower than at any other atrial locations ($p < 0.05$, $n = 10$). Abbreviations as in figure 1.

Figure 7. LAI-related changes in AF activation patterns

A: Four representative phase movie snapshots before and after LAI during AF and corresponding DF map are shown. See results section for description. **B:** Activation variability index before and after LAI.