Chronic myocardial infarction promotes atrial action potential alternans, afterdepolarizations, and fibrillation

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1. Introduction

Atrial fibrillation (AF) is increased in patients with heart failure resulting from myocardial infarction (MI). We aimed to determine the effects of chronic ventricular MI in rabbits on the susceptibility to AF, and underlying atrial electrophysiological and Ca\(^{2+}\)-handling mechanisms.

Methods and results

In Langendorff-perfused rabbit hearts, under β-adrenergic stimulation with isoproterenol (ISO; 1 μM), 8 weeks MI decreased AF threshold, indicating increased AF susceptibility. This was associated with increased atrial action potential duration (APD)-alternans at 90% repolarization, by 147%, and no significant change in the mean APD or atrial global conduction velocity (CV; n = 6–13 non-MI hearts, 5–12 MI). In atrial isolated myocytes, also under β-stimulation, L-type Ca\(^{2+}\) current (I\(_{\text{CaL}}\)) density and intracellular Ca\(^{2+}\)-transient amplitude were decreased by MI, by 35 and 41%, respectively, and the frequency of spontaneous depolarizations (SDs) was substantially increased. MI increased atrial myocyte size and capacity, and markedly decreased transverse-tubule density. In non-MI hearts perfused with ISO, the I\(_{\text{CaL}}\)-blocker nifedipine, at a concentration (0.02 μM) causing an equivalent I\(_{\text{CaL}}\) reduction (35%) to that from the MI, did not affect AF susceptibility, and decreased APD.

Conclusion

Chronic MI in rabbits remodels atrial structure, electrophysiology, and intracellular Ca\(^{2+}\) handling. Increased susceptibility to AF by MI, under β-adrenergic stimulation, may result from associated production of atrial APD alternans and SDs, since steady-state APD and global CV were unchanged under these conditions, and may be unrelated to the associated reduction in whole-cell I\(_{\text{CaL}}\). Future studies may clarify potential contributions of local conduction changes, and cellular and subcellular mechanisms of alternans, to the increased AF susceptibility.

Keywords

Atrial fibrillation • Myocardial infarction • Action potential alternans • Afterdepolarization • β-Adrenergic stimulation • T-tubule • Calcium

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electrophysiological and Ca\(^{2+}\)-handling remodelling from chronic MI were studied in rabbits\(^{11,12}\) and, although atrial enlargement occurred, no atrial electrophysiological or Ca\(^{2+}\)-handling parameters were measured.

HF, in patients, is associated with increased adrenergic tone and elevated circulating levels of catecholamines.\(^{13}\) Catecholamines promote atrial arrhythmias, mainly by increasing L-type Ca\(^{2+}\) current (\(I_{\text{CaL}}\)) and Ca\(^{2+}\)-transient amplitude.\(^{14}\) A reduced contractile response to \(\beta\)-adrenoceptor stimulation to \(I_{\text{CaL}}\) and Ca\(^{2+}\)-transient responses are also blunted,\(^{9}\) but corresponding atrial data are sparse. In the rat atrium, chronic MI reduced \(I_{\text{CaL}}\) and potentiated the \(I_{\text{CaL}}\) increase from \(\beta\)-stimulation.\(^{15}\) \(\beta\)-Stimulation may also affect APD alternans.\(^{16}\) APD alternans precede AF episodes in patients,\(^{17}\) and alternans are associated with Ca\(^{2+}\)-handling abnormalities.\(^{18}\) \(\beta\)-Stimulation enhances Ca\(^{2+}\) cycling and excitation–contraction coupling, and either favours\(^{19}\) or protects against\(^{16,20}\) alternans, depending on whether Ca\(^{2+}\) sequestration or fractional release of Ca\(^{2+}\) from the sarcoplasmic reticulum (SR) predominates.

Atrial conduction disturbances associated with structural changes, including fibrosis, chamber enlargement, and hypertrophy are well established, leading to increased vulnerability to AF.\(^{1,21,22}\) Decreased conduction velocity (CV) from fibrosis could promote re-entry by decreasing re-entry wavelength (\(\lambda\)), since \(\lambda = CV \times ERP\) (effective refractory period). Altered atrial gap junction organization and connexin expression could also disturb intercellular connectivity, with the potential to decrease or increase CV.\(^{23}\) Furthermore, disruption of atrial transverse-tubules (t-tubules), as shown in a sheep VTP model of AF,\(^{24}\) may contribute to hypocontractility, Ca\(^{2+}\)-handling changes, and an increased propensity to arrhythmia in these diseased hearts. However, the effects of MI on atrial CV or the t-tubular network are also unknown.

We hypothesized that chronic MI would promote AF under \(\beta\)-stimulation, associated with any or all of the following: decreased atrial \(\lambda\), increased APD alternans, increased DADs, altered Ca\(^{2+}\)-handling remodelling caused by chronic ventricular MI in rabbits; secondly, to investigate potential mechanisms of AF promotion by MI, by associating changes in propensity to AF with atrial APD, CV, APD alternans, DADs, \(I_{\text{CaL}}, [\text{Ca}^{2+}]_{i}\) and t-tubule density.

2. Methods

2.1 Animal model of MI

Procedures conformed to the Guide for the Care and Use of Laboratory Animals published by US National Institutes of Health. Project license: 60/4206. Adult male New Zealand White rabbits (3.0–4.0 kg) were premedicated with intramuscular Hypnorm (0.3 mg/kg) and anaesthetized with midazolam (0.17–0.3 mg/kg) via the marginal ear vein. Ventilation was with N\(_2\)O and O\(_2\) (1:1) containing 1% isofluorane. A thoracotomy was performed and the left descending coronary artery ligated to produce an ischaemic area of 30–40% of the left ventricle (LV) and subsequent apical infarction (Figure 1Ai). Post-surgery analgesia: intramuscular

![Figure 1](https://academic.oup.com/cardiovascres/article-abstract/99/1/215/324918)

**Figure 1** LA cellular hypertrophy and t-tubule loss resulting from ventricular MI. (A) Rabbit model of MI (i), LVEF (ii), LV end-diastolic dimension (LVEDD) (iii) \(n = 13\) non-MI hearts, 32 MI. (B) Cardiomyocyte capacitance (i); \(n = 52\) cells (13 non-MI hearts) and 56 cells (15 MI), width (ii), and length (iii); \(n = 110\) cells (6 non-MI hearts) and 191 (10 MI). (C) T-tubule (TT) density. Representative confocal images (i) LV non-MI cell (a), LA non-MI (b), LA MI (c); TT density (ii); \(n = 14\) LV cells (six hearts), 18 LA cells (seven non-MI hearts), 30 LA cells (five MI). *\(p < 0.05\), **\(p < 0.01\), ***\(p < 0.001\).
cellular APs, microelectrodes (~5–6 MΩ) contained (mM): KCl (20), K-aspartate (110), HEPES (5), MgCl2 (1), Na2ATP (4), disodium creatine phosphate (1), K2EGTA (0.15), pH 7.25. Cells were stimulated with 5 ms, 1 nA current pulses at 1 Hz for 90 s using an Axcoplamp-2B amplifier and Clampex 9.2 software. In each cell, APs were recorded both with and without current clamping the resting membrane potential (RMP) (to ~80 mV), since some cells in each group had an unclamped RMP more positive than ~60 mV and thus an inactivated \( I_N \), APD50–90, and the occurrence of inter-AP spontaneous depolarizations (SDs), were monitored. For recording \( I_{CaL} \) and \( [Ca^{2+}]_i \), microelectrodes contained (mM): KCl (20), K-aspartate (100), TEA-Cl (20), HEPES (10), MgCl2 (4.5), Na2ATP (4), Na2-creatine phosphate (1), K2EGTA (0.01), and Fura-2 (0.1 mM); pH 7.25, pCa ~7.2. Extracellular 4-AP (5 mM), niflumic acid (0.1 mM) and TTX (5 μM) were used to eliminate contaminating currents. A voltage clamp protocol (Figure 5A) was cyclic at 1 Hz for 90 s to record steady-state \( I_{CaL} \) and \( Ca^{2+} \) transients. Fluorescence was measured at 340 and 380 nm every 15 ms using a Cairn Optoscan. Minimum (\( R_{min} \)) and maximum (\( R_{max} \)) fluorescence ratios (340/380 nm) were measured, and cytoplasmic \( [Ca^{2+}]_i \) calculated as \( K_0 \times [(R_{max} - R_{min})/(R_{max} - R)] \), with \( K_0 \) of 1.2 μM.27 The SR \( Ca^{2+} \) content was assessed by rapid application of 10 mM caffeine (within ~1 s of the end of the 90 s voltage-pulse train, using a 4 mm diameter perfusion pen (Cell Micro Controls, Norfolk, USA) in close proximity to the cell) to fully release SR-Ca2+ stores, and estimated as the absolute time-integral of the resultant \( I_{Na/CaL} \) expressed in coulombs normalized to capacitance.27

### 3. Results

#### 3.1 MI caused LV dysfunction, and LA cellular hypertrophy and detubulation

Ventricular MI decreased LVEF by 31% (Figure 1Aii) and increased LVEDD by 9% (Figure 1Aiii), with no significant effect on dimensions (mm) of LA (10.6 ± 0.2 vs. 11.4 ± 0.3, MI), atrioventricular valve (3.1 ± 0.3 vs. 2.7 ± 0.1), or aorta (9.0 ± 0.3 vs. 8.6 ± 0.2). MI increased atrial cardiomyocyte capacitance by 56% (Figure 1Bii), width by 18% (Figure 1Biii), and length by 14% (Figure 1Biv). T-tubule density was lower in LA cells than LV cells (Figure 1Ci), and MI decreased LA t-tubule density by ~60% (Figure 1Cii).

#### 3.2 In the presence of β-stimulation, MI promoted AF, SDs, and APD alternars

Figure 2A shows an original electrogram recording of AF induction in an intact non-MI heart. Under β-stimulation, AFT was decreased by MI, by ~1 s after AF induction in the LA (2.5 vs. 3.1 ms). MI increased atrial global CV by ~15% (Figure 2Bi), MI decreased LA t-tubule density by ~60% (Figure 2Ci).
stimulation), in non-MI or MI, with or without β-stimulation, suggesting that MI + β-stimulation converted subthreshold SDs to threshold. Figure 3A shows an original recording of APD alternans, from an intact, paced, MI heart, in the absence of β-stimulation. In the unpaced hearts, the mean magnitude of APD alternans was not significantly affected by MI, either in the absence or presence of β-stimulation (Figure 3B). However, in the paced hearts, MI significantly increased APD alternans (Figure 3C), particularly, at APD90, by 147% in the presence of β-stimulation, and by 103% in its absence (Figure 3Ciii).

3.3 Under β-stimulation, APD was not altered by MI

β-Stimulation increased intrinsic heart rate by 50–60% in non-MI and MI hearts (Figure 4A). In unpaced (Figure 4A) and paced (Figure 4B) hearts, under β-stimulation, MI had no significant effect on optically recorded atrial AP morphology or APD50–90. In contrast, in the absence of β-stimulation, MI increased APD50 and APD90, in unpaced (Figure 4A) and paced (Figure 4B) hearts. Figure 4C shows corresponding AP data obtained from isolated cardiomyocytes, comparable except for the lower stimulation rate (1 Hz), which perhaps accounts for the ‘spikier’ AP phase 1 (Figure 4C). Neither ISO nor MI affected RMP (Figure 4Cii) and, in agreement with the intact atrial AP data, MI had no significant effect on APD in the presence of β-stimulation (Figure 4Ciii–v), and tended to prolong APD in its absence.

3.4 Under β-stimulation, I_{CaL} and systolic [Ca^{2+}]i were decreased by MI

Figure 5 shows that in the presence of β-stimulation, but not in its absence, I_{CaL} density and Ca^{2+} flux through I_{CaL} (Figure 5A and B), and the systolic Ca^{2+} transient (Figure 5C and D), were each significantly and similarly decreased by MI, while diastolic [Ca^{2+}]i was unaffected (Figure 5Dii). Both the rate of decay of the Ca^{2+} transient (Figure 5Div), and the SR Ca^{2+} content (Figure 5Dii), were increased by ISO, and not significantly affected by MI.

3.5 Pharmacological inhibition of I_{CaL}, to mimic the I_{CaL} reduction from MI, did not promote AF

In atrial cardiomyocytes from non-MI rabbits, nifedipine inhibited I_{CaL} in a concentration-dependent manner, with the inhibition significantly attenuated by ISO (Figure 6Ai and ii). Using the dose–response curve in the presence of ISO (Figure 6Aii, triangles), two concentrations of nifedipine were chosen, 0.02 and 2 μM (which inhibited I_{CaL} by 35 and 41%, respectively), to test effects of I_{CaL} reduction, in non-MI hearts and cells, on APDs and AFT under β-stimulation. Nifedipine at 0.02

Figure 2 Under β-stimulation, atrial arrhythmia is provoked by MI. (A) Original electrogram recording of AF induction in a non-MI heart; pacing protocol shown beneath. (B) AFT (i) and AF duration (ii); n = 7 non-MI hearts, 7 MI. CV (iii); n = 6 non-MI, 5 MI. (C) SDs: original AP trace recorded in an MI atrial cell, showing SDs (*) during stimulation (†); bar = 250 ms (i); mean number (ii) and incidence (iii) of SDs occurring during AP trains. n = 23–31 cells, 12–13 hearts. ISO = 1 μM ISO.
and 2 μM shortened atrial cellular APD₉₀, by 11 and 65%, respectively (Figure 6Aiii), and intact atrial epicardial APD₉₀, by 30 and 42%, respectively (Figure 6Bi). The higher concentration (2 μM) markedly and significantly reduced the AFT, by 85% (Figure 6Bii). However, 0.02 μM nifedipine, which caused an equivalent I_{CaL} reduction to that produced by MI under β-stimulation (35%), did not affect AF susceptibility, despite significantly decreasing APD.

4. Discussion

This is the first study, to our knowledge, to characterize the effects of chronic LV MI, rather than chronic VTP, on atrial Ca²⁺ handling and electrophysiology. We recorded from intact atria, and isolated cardiomyocytes, with and without a β-agonist. The key findings are that under β-stimulation, MI promoted AF, APD alternans, and cellular SDs, associated with reduced I_{CaL}, Ca²⁺ transient and t-tubule density. β-Stimulation was used to represent in-vivo sympathetic activation (particularly in HF, with catecholamine elevation¹³), with consequent phosphorylation/activation of numerous ion channels and Ca²⁺-handling proteins.¹⁴ We used ISO at supra-maximal concentration to ensure full adrenergic activation, but recognize that this is also supra-physiological.

The APD alternans could contribute to the increased AF susceptibility, by promoting spatial electrical heterogeneity and wavebreak.¹⁰ Since the SDs were not observed in intact atrium, this might suggest the alternans as the more likely contributor to the increased AF susceptibility. However, although isolated cardiomyocytes are more prone to afterdepolarizations than tissues, due to the absence of current sink, we cannot exclude the possibility that SDs occurred also in the intact hearts, remote from the myocardial region sensed by the fibre-optic detector. In support, chronic VTP-induced HF promoted DADs in atrial cells⁵ and triggered-activity characteristic of DADs in intact atria.⁴ Moreover, since MI increased APD alternans with or without ISO, yet MI increased AF susceptibility only with ISO, this argues against APD alternans as the sole potential electrophysiological mechanism, with the accompanying increase in SDs, as resulted from MI exclusively under β-stimulation, perhaps required.

MI affected neither atrial APD₉₀ nor CV, under β-stimulation, and thus might not affect re-entry wavelength. However, although APD at late repolarization is a major determinant of ERP, the ERP has yet to be measured in this model. Furthermore, the MI is likely to increase atrial fibrosis, as in rats.⁹,²⁸ In rabbits, atrial fibrosis and inter-atrial conduction time were increased by VTP,²⁹ and VTP-induced fibrosis in dogs was associated with spatial heterogeneity of CV, and local conduction abnormalities.²¹ Increased atrial myofibroblast density²⁵ could promote AF via multiple arrhythmia mechanisms since, when coupled to cardiomyocytes, myofibroblasts can act as current sources and/or sinks, depending on myofibroblast density, distribution, and coupling strength, thereby modulating AP characteristics and CV, including production of SDs and alternans.²⁰ Such fibrosis had a greater bearing on atrial CV disturbances and AF than gap junction remodelling (altered connexin subtype expression ratios, phosphorylation status, and cellular distribution), from chronic VTP.³¹ Nevertheless, whether atrial gap junctions are altered in the present model is unknown and the potential for such...
Figure 4 Under β-stimulation, APD is not changed by MI. (A) Effects of MI and/or 1 μM ISO on rabbit isolated heart rate (i) and, in un paced hearts, optically recorded atrial APs in non-MI (ii) and MI (iii), and on mean APD_{50–90} (iv–vi). (B) Corresponding data (i–v) obtained from paced (5 Hz) hearts. n = 13 non-MI, 12 MI. (C) Atrial isolated cardiomyocyte APs (i), at 1 Hz-stimulation, in non-MI (a) and MI (b). Effects of MI and/or ISO on RMP (ii) and, in cells with RMP clamped (-80 mV), APD_{50–90} (iii–v). n = 33 cells, 14 non-MI hearts, 22 cells, 11 MI.
remodelling to disturb CV and cause arrhythmias, as proposed in failing ventricle, should not be excluded. We recorded CV at a fixed location, in the direction of fastest signal propagation, and acknowledge that extensive spatial, high-resolution analysis would be required to establish the contribution of local changes in CV and its heterogeneity to AF inducibility.

The present lack of effect of MI on atrial APD, under β-stimulation, contrasts with a canine model of chronic MI and sympathetic stimulation by tyramine, in which APD was decreased. We found that without β-stimulation, atrial APD was increased by MI. Previous studies of atrial electrophysiological remodelling by HF in rabbits used chronic VTP rather than MI, without β-stimulation, and showed increased APD, consistent with the present data, as well as increased ERP and altered K+ channel expression. The present APD increase is also consistent with several canine VTP studies, e.g. APD increase may enhance Ca2+, transients as a result of AP plateau elevation from tyramine, in which APD was decreased. We found that without β-stimulation, atrial APD was increased by MI.

**Figure 5** Under β-stimulation, I_{CaL} and systolic [Ca^{2+}] are decreased by MI. Effects of MI and/or ISO on: (A) I_{CaL} stimulated with voltage-pulse (i) in non-MI (ii) and MI (iii) atrial cardiomyocytes. (B) I_{CaL} density (i) and Ca2+ flux via I_{CaL} (ii). (C) [Ca^{2+}] and I_{CaL} recordings from non-MI (i) and MI (ii) cardiomyocytes. (D) Diastolic (i) and peak systolic (ii) [Ca^{2+}] and Ca2+-transient amplitude (iii), and rate constant (RC) of decay (iv). n = 13 non-MI hearts, 12 MI. (E) Caffeine-evoked [Ca^{2+}] increase (i) and resulting h_{N/Ca} integral (ii), representing SR Ca2+ content. n = 7–10 non-MI hearts, 6–11 MI.
decreased $I_{TO}$. Atrial APD decrease also occurs, after long-term (4 months) VTP in dogs, and with LV systolic dysfunction in patients. The ionic mechanism of the APD increase from chronic MI in rabbits is unknown, whereas in dogs, VTP decreased atrial $I_{Ca,L}$, $I_{Ks}$, and increased $I_{Na,Ca}$. $I_{TO}$ is prominent in the rabbit atrium, and since VTP decreased atrial $Kv4.3$, and $I_{TO}$ decrease prolonged APD$_{90}$ in rabbit atrial cells. $I_{TO}$ reduction might contribute to the APD increase here. However, rabbit atrial $I_{TO}$ is carried primarily by $Kv1.4$, not measured in the VTP study, which confers slow $I_{TO}$ reactivation and could limit the contribution of $I_{TO}$ to APD at physiological and supraphysiological rates.

Since MI provoked AF under $\beta$-stimulation, we measured $I_{Ca,L}$ and the $Ca^{2+}$ transient under those conditions, and found both to be decreased. The reduced $Ca^{2+}$ transient likely resulted from the $I_{Ca,L}$ reduction, since the SR $Ca^{2+}$ content was unchanged. The mechanism of the $I_{Ca,L}$ decrease is unclear, but may not involve altered $I_{Ca,L}$ channel expression, since chronic MI in rats did not affect $\alpha_{1C,15}$. Another candidate is down-regulation of atrial $\beta$-adrenoceptors. We did not measure $\beta$-adrenergic density, but previous studies showed reduced atrial $\beta_1$- and/or $\beta_2$-expression in HF. $I_{Ca,L}$ decrease prolonged APD$_{90}$, transient, and $I_{Ca,L}$ inhibition, while inducing $Ca^{2+}$ alternans in rat ventricular cells, suppressed it in human atrial cells.

However, both APD alternans and SDs result from a complex interaction between membrane potential, ion currents including $I_{Ca,L}$ and $I_{Na,Ca}$, $Ca^{2+}$, and $Ca^{2+}$ cycling, each of which may be altered by myocardial disease as well as stimulation rate. Simultaneous measurement of $[Ca^{2+}]$, and APs in ventricular myocytes has suggested that APD alternans under rapid pacing is driven by $Ca^{2+}$ alternans. $Ca^{2+}$ alternans also occurred under tachypacing of cat atrial cells, although APDs were not measured. In rabbit atrial cells, $Ca^{2+}$ alternans induction depended on refractoriness of SR $Ca^{2+}$ release. Studies of effects of myocardial disease on atrial alternans are sparse. In dogs, chronic MI, and chronic atrial tachypacing, promoted AF, which was preceded by increased atrial APD alternans. However, in neither report nor the present study was $Ca^{2+}$ alternans studied. Future studies of alternans in the present rabbit MI model of AF should attempt to measure $[Ca^{2+}]$, and APs simultaneously, in single cells and intact atrium, under supra-physiological and physiological stimulation rates.

We found that chronic MI caused atrial t-tubule loss, consistent with previous studies of atrial remodelling from chronic VTP and chronic AF. The concurrent increase in cell capacitance, as with chronic MI in rats, infers cell size increase; confirmed here and in studies of chronic VTP or AF. It is unclear whether t-tubule loss contributes to the development of AF in HF, although t-tubule disorganization may promote atrial $Ca^{2+}$ alternans, with the potential to promote arrhythmia. The consequences of t-tubule loss for $Ca^{2+}$ cycling have been...
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9. In conclusion, chronic MI in rabbits remodels atrial structure, electrophysiology and Ca2+ handling. Increased susceptibility to AF by MI, under β-stimulation, may result from associated production of atrial alternans and SDs, since steady-state AP and global CV were unchanged under these conditions, and may be unrelated to the associated reduction in whole-cell ICaL. Further studies in this model are warranted, to clarify potential contributions of local conduction changes, and cellular and subcellular mechanisms of alternans, to the increased susceptibility to AF.

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