Ion Channel Remodeling in Pulmonary Vein Arrhythmogenesis for Atrial Fibrillation

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1. Introduction

Atrial fibrillation (AF) is the most common cardiac arrhythmia seen in clinical practice and can induce cardiac dysfunction and strokes. Mechanisms of AF involve a triggering mechanism from thoracic vein and/or a substrate mechanism in the atria. Studies showed that elimination of ectopic focus from pulmonary vein (PV) or non-PVs by catheter ablation could cure AF. As noted, PVs are the most important focus in the initiation of paroxysmal AF. The mechanisms of AF are quite complex because of the fact that AF results from a variety of clinical conditions, autonomic tone modulation, and the self-perpetuation phenomenon “AF begets AF.” Likewise, there is a wide range of changes in the function and expression of ion channels (electrical remodeling). Ion channels involved in AF triggers include those mediating calcium homeostasis and non—calcium ion channels. Abnormal calcium homeostasis and heterogeneous expression of potassium channels, pacemaker channels, and stretch- and swelling-activated chloride channels may promote the electrical activity of PV and consequently the occurrence of AF. Shortening of action potential duration in response to decreases in inward currents and/or increases in outward currents can facilitate the genesis and maintenance of AF. Additionally, different underlying diseases may yield different patterns of electrical remodeling. This review summarizes the recent findings in this area of research.

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PV non-pacemaker cardiomyocytes. Patterson et al further showed that an increased Ca\(^{2+}\) transient and Na\(^+\)–Ca\(^{2+}\) exchange current (NCX) may enhance EAD formation in canine PVs. Similarly, Wongcharoen et al also found that PV electrical activity can be reduced by an NCX inhibitor (KB-R7943, Tocris Cookson Inc., St. Louis MO, USA) (Figure 1), which reduced the [Ca\(^{2+}\)]i transient's amplitude and sarcoplasmic reticulum (SR) Ca\(^{2+}\) stores. Because of Ca\(^{2+}\) influx from inward NCX, L- and T-type Ca\(^{2+}\) currents can trigger a large Ca\(^{2+}\) release from the SR; these findings suggest that calcium homeostasis plays an important role in PV arrhythmogenesis.

Dysfunction of ryanodine receptors (RyRs) induces diastolic Ca\(^{2+}\) leak and activates the transient inward current, leading to membrane depolarization and generating DADs. Honjo et al reported that a low dose of ryanodine can induce PV firings, suggesting that enhancement of the Ca\(^{2+}\) leak also contributes to PV arrhythmogenesis. Similarly, Wongcharoen et al showed that FK-506 (Sigma-Aldrich Co., St. Louis, MO, USA) (Figure 2), which dissociates the RyR–FKBP12.6 complex and inhibits calcineurin activity, can induce PV burst firings, which also suggests that RyR dysfunction has an arrhythmogenic potential in the PVs. Moreover, aging was shown to express more RyR in PVs, which takes the potential to enhance Ca\(^{2+}\) leak (Figure 3). In contrast, K201 (Aetas Pharm Co., Tokyo, Japan) may reduce the PV firing rates, DADs, and transient inward currents through the stabilization of the RyRs, allowing the reduction in the diastolic calcium leak.

Comparisons of intracellular Ca\(^{2+}\) ([Ca\(^{2+}\)]i) among left atrial cardiomyocytes and PV cardiomyocytes showed larger [Ca\(^{2+}\)]i transients, Ca\(^{2+}\) sparks, and SR Ca\(^{2+}\) stores in PV cardiomyocytes with pacemaker activity, which suggest that distinctive [Ca\(^{2+}\)]i regulation may contribute to the spontaneous activity of PV cardiomyocytes. Although Bay K 8644 (Sigma-Aldrich Co., St. Louis, MO, USA), a calcium channel activator, increased [Ca\(^{2+}\)]i, it alone did not induce automaticity in PV non-pacemaker cardiomyocytes. In contrast, the coadministration of Bay K 8644 and BaCl\(_2\) (an inhibitor of inward rectifier potassium currents) could induce automaticity in PV non-pacemaker cardiomyocytes. Therefore, resting membrane potential and inward rectifier potassium currents also play a role in the PV spontaneous activity. PV cardiomyocytes with pacemaker activity have been shown to have smaller inward rectifier K\(^+\) current and less negative membrane potential. Jones et al reported that cells from the PV myocardial sleeve showed large elevations in diastolic calcium during activation at physiological rates in rabbit model. Cells from the PV share some features with cells from the sinoatrial node but also have distinctly unique features that predispose them to the development of spontaneous activity with a higher stimulated steady-state diastolic calcium.

The SR Ca\(^{2+}\)-ATPase (SERCA2a) is critical in uptaking [Ca\(^{2+}\)]i in cardiomyocytes to maintain SR Ca\(^{2+}\) content. Lee et al found that the tumor necrosis factor-alpha–treated PV cardiomyocytes had a significant decreased SERCA2a with a compensatory increased NCX, which may induce larger DAD, large transient inward currents, and an enhanced PV arrhythmogenesis. These findings

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**Figure 1** KB-R7943 decreased the firing rate of a pulmonary vein pacemaker cardiomyocyte in a concentration-dependent manner. Reproduced with permission from Oxford University Press.

**Figure 2** FK-506 induced burst firing in a pulmonary vein (PV) pacemaker cardiomyocyte from an aged animal. Reproduced with permission from Elsevier.

**Figure 3** Quantification of the expression level of ryanodine receptor (RyR) in pulmonary veins from young and aged animals. (A) Summary of densitometry quantification. (B) Representative Western blot images (β-actin as an internal control to confirm even loading). Reproduced with permission from Elsevier.
may contribute to inflammation or heart failure–related AF. Similarly, aging also reduces SERCA2a, which may underlie the higher AF during aging.22

1.2. Non-Ca\(^{2+}\) ionic currents in PVs

Potassium currents have been suggested to contribute to the determination of pacemaker activity in cardiomyocytes. PV cardiomyocytes differ from atrial cardiomyocytes in manifesting more depolarized resting membrane potentials because of a lower level of inward rectifier potassium currents (I\(_{K_{R}}\)). Therefore, PV cardiomyocytes are prone to trigger arrhythmia because of a reduced threshold for excitation.27 Moreover, the hyperpolarization–activated time-dependent current (I\(_{K_{A}}\)) has been observed in a subset of PV cardiomyocytes and has been suggested to play a role in PV electrical activity. Chen et al27 demonstrated that the fast-firing (>2.5 Hz) PV pacemaker cardiomyocytes had less negative maximum diastolic potential and steeper slope of diastolic depolarization than those of slow-firing (<2.5 Hz) PV pacemaker cardiomyocytes. Moreover, the PV beating rates are linearly correlated with the slope of diastolic depolarization and maximum diastolic potential. The fast-firing PV pacemaker cardiomyocytes had smaller transient outward current (I\(_{to}\)) and I\(_{K_{R}}\) but similar sustained delayed rectifier K\(^{+}\) current (I\(_{K_{sus}}\)), and rapid delayed rectifier currents (I\(_{Kr}\)), as compared with slow-firing PV pacemaker cardiomyocytes (Figure 4). After suppression of I\(_{K_{A}}\) and I\(_{K_{R}}\) with barium, fast-firing PV pacemaker cardiomyocytes manifest a higher incidence and current density of pacemaker currents (I\(_{f}\)) than slow-firing PV cardiomyocytes. However, the percentage of I\(_{f}\)-positive PV cardiomyocytes was significantly less than that of sinoatrial and atrioventricular nodal cardiomyocytes.

Cell swelling secondary to myocardial ischemia might be the underlying mechanism of ischemia-related AF genesis. Lee et al28 found that hypotonic solution induced larger swelling–activated chloride current (I\(_{Cl\text{,swell}}\)) in PV pacemaker cardiomyocytes than in PV non-pacemaker cardiomyocytes or atrial cardiomyocytes. Compared with atrial cardiomyocytes, hypotonic solution shortened the action potential duration and increased the cell width to a greater extent in the PV cardiomyocytes. Moreover, hypotonic solution decreased the PV firing with a decrease in the transient inward currents and DADs. These findings suggest that the I\(_{Cl\text{,swell}}\) plays an important role in the electrical activity of the PV cardiomyocytes. On the other hand, hypertonicity can increase the spontaneous beating rates.29 In addition, hypertonicity increased the transient inward currents (I\(_{to}\)) and NCX to a greater extent in PV cardiomyocytes than in atrial cardiomyocytes. These findings suggest that cell morphology was associated with PV electrical activity. In support of this, external stretch of PV tissue preparations could increase PV spontaneous activity and triggered activity, which was suppressed by stretch channel inhibitors of gadolinium or streptomycin.30

<table>
<thead>
<tr>
<th>AF-related intervention</th>
<th>APD</th>
<th>EAD</th>
<th>DAD</th>
<th>I(_{Ca,L})</th>
<th>I(_{to})</th>
<th>I(<em>{K</em>{R}})</th>
<th>Beating rates</th>
</tr>
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<tbody>
<tr>
<td>Rapid atrial pacing</td>
<td>↓</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
<td>↓</td>
<td>↓</td>
<td>↑</td>
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<tr>
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<td>↑</td>
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<td>↑</td>
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<tr>
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<tr>
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<td>↓</td>
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<tr>
<td>Tumor necrosis factor-alpha</td>
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AF = atrial fibrillation; APD = action potential duration; EAD = early afterdepolarization; DAD = delayed afterdepolarization; I\(_{Ca,L}\) = L-type Ca\(^{2+}\) current; I\(_{to}\) = transient outward currents; I\(_{K_{R}}\) = transient outward currents; I\(_{K_{sus}}\) = inward rectifier potassium currents; I\(_{f}\) = pacemaker currents; ↑ = increase; ↓ = decrease; — = unchanged; ? = unknown.

Figure 4  I–V relationship of I\(_{to}\), I\(_{K_{sus}}\), I\(_{K_{R\text{-tail}}}\), and I\(_{K_{R}}\) in fast and slow pacemaker cardiomyocytes from PV. *p < 0.05 and **p < 0.01 versus the slow PV pacemaker cardiomyocytes. Reproduced with permission from John Wiley and Sons. PV = pulmonary vein.
Table 2: Effects of pharmacological agents on action potential and ionic currents in pulmonary vein cardiomyocytes

<table>
<thead>
<tr>
<th>Pharmacological agents</th>
<th>APD</th>
<th>DAD</th>
<th>L-type Ca(^{2+})</th>
<th>Na(^{+})</th>
<th>K(^{+})</th>
<th>Beating rates</th>
</tr>
</thead>
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<td>1</td>
<td>1</td>
<td>1</td>
<td>?</td>
<td>?</td>
</tr>
<tr>
<td>Losartan</td>
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<td>1</td>
<td>1</td>
<td>1</td>
<td>?</td>
<td>?</td>
</tr>
<tr>
<td>Endothelin-1</td>
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<td>1</td>
<td>1</td>
<td>1</td>
<td>?</td>
<td>?</td>
</tr>
</tbody>
</table>

APD = action potential duration; DAD = delayed afterdepolarization; L-type Ca\(^{2+}\) = transient inward current; Na\(^{+}\) = transient outward current; K\(^{+}\) = inward rectifier potassium currents; Beating rates indicate the potential target therapy for atrial tachyarrhythmias.