Identifying and understanding the role of pulmonary vein activity in atrial fibrillation

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Abstract

The perception of atrial fibrillation development has changed drastically over the last decade. The pulmonary veins have been targeted as the source of arrhythmogenic activity involved in the initiation of atrial fibrillation. This activity appears to be localized in the myocardial sleeves of the vessels. Extensive study of cells within this tissue has helped create a new model for atrial fibrillation. This review attempts to show how the development, architecture and electrophysiologic properties of the pulmonary veins influence the initiation and perpetuation of atrial fibrillation. It also examines the potential long-term effects of pulmonary vein activity on arrhythmia development.

Keywords: Pulmonary veins; Atrial fibrillation; Atrial remodeling; Atrial fibrosis

1. Introduction

Atrial fibrillation (AF) is the most common sustained cardiac arrhythmia. The Framingham study showed that it severely increases the risk of mortality in affected patients, particularly by causing stroke [1].

AF is thought to be initiated in the myocardial sleeves of the pulmonary veins (PVs) [2]. Chen et al. [3] identified the presence of automaticity in cells within the myocardial tissue of the PVs. Pacemaker activity from these cells is thought to result in the formation of ectopic beats that initiate AF.

PVs are also thought to be important in the maintenance of AF. The chaotic architecture and electrophysiologic properties of these vessels provides an environment where AF can be perpetuated [4–7].

Finally, AF has been associated with remodeling of the atria, which, in turn, promotes the development of chronic AF [8]. The activity of PVs is likely to play a role in the structural and electrophysiologic changes seen in “atrial remodeling.”

This article aims to provide a clearer model for the location, mechanism and possible consequences of AF initiated in the PVs.

2. The development of AF

AF is a rhythmic perturbation of the heart that primarily affects the atria. In this phenomenon, the heart is taken out of normal sinus rhythm due to the production of electrical impulses originating from atrial myocytes. With up to 600 pulses being generated every minute, syncytial contraction of the atria is replaced by irregular atrial twitches. The result, in turn, is ineffective transport of blood to the ventricles.

Three mechanisms are commonly associated with AF development: enhanced automaticity, triggered activity and re-entry [9]. The first, automaticity, involves the ability of a cell to spontaneously depolarize. Arrhythmia can arise due to enhanced automaticity from ectopic sites. Increased automaticity is generally associated with less negative...
Triggered activity is pacemaker activity that arises only after an initial impulse has been generated within the cell. Triggered activity manifests as either early or delayed afterdepolarizations. Early afterdepolarizations (EADs) generally follow prolonged action potentials. Calcium currents during the plateau phase of action potentials may be responsible for the generation of EADs [10]. Similarly, delayed afterdepolarizations (DADs) arise in the presence of cellular calcium overload. The electrogenic Na+/Ca2+ channel removes calcium, in exchange for three sodium ions [11]. There is therefore a net movement of positive charge into the cell, bringing it closer to depolarization. Thus, anything that causes a greater than expected rise in intracellular calcium levels during systole will result in the increased likelihood of DADs after sodium–calcium exchange. Both types of afterdepolarizations may potentially reach threshold levels, generate action potentials and be the source of ectopic beats (Fig. 1).

After depolarization, the sodium channels of cardiocytes are inactivated. The duration of sodium channel inactivity is known as the refractory period. During the refractory period, action potentials cannot be generated within these cells should an ectopic beat arise nearby. However, if the ectopic beat is able to propagate electrical impulses via another pathway, the impulse may return to the initially inactive cardiocytes. Sodium channels will have now had time to reactivate, therefore action potential may be generated within these cells and impulse may be propagated further. The phenomenon of re-entry arises when two separate pathways continuously activate one another in this manner.

Longer refractory periods of cardiocytes makes re-entry more difficult because electrical impulses in a re-entrant pathway may meet cells that cannot generate action potentials (i.e. due to the fact that they are still refractory). Thus, decreased refractory periods will obviously lend themselves more to electrical impulse propagation and therefore to AF. Similarly, the slower the conduction velocity of the electrical impulse in a re-entrant pathway, the less likely it is to encounter cardiac cells that are still refractory within the pathway. Therefore, slower conduction also favours maintenance of re-entry and the development of AF.

Current research suggests that AF is caused by a single ectopic focus and re-entry pathway [12]. As mentioned, studies have implicated the myocardial tissue of PVs as a possible origin for ectopic impulses [2].

3. PV myocardial tissue as a possible source of arrhythmogenicity

Nathan and Eliakim showed the presence of striated muscle within the tunica media of PVs [13]. Cheung [14] demonstrated that action potentials could be produced within these myocardial sleeves. Haissaguerre et al. [2] were the first to discover that PVs were a source of ectopic beats possibly involved in the initiation of AF. In fact, this landmark study found that 94% of ectopic beat foci in patients with drug-refractory AF originated in the PV myocardium. They also found that ablation of the PVs using radio-frequency energy resulted in the disappearance of triggering ectopic beats. Localization of arrhythmogenicity to the PVs has subsequently been shown in a number of studies [15,16].

4. Gross anatomical differences between the PVs of patients with and without AF

In a post-mortem anatomical study, Hassink et al. [6] showed that PV myocardial sleeves were found in 100% of patients with AF, whereas similar muscle tissue was seen in only 85% of patients without AF. In addition, patients with AF had significantly longer muscle sleeves present. Similarly, muscle bundles in the superior veins were substantially longer than those in the inferior veins. Along with increased length, Guerra et al. [17] using intravascular ultrasound, found that patients with AF had considerably thicker PV myocardial tissue, and that arrhythmogenicity could only be localized to these areas of thickening. Using PV angiography, Lin et al. [18] suggested that patients with paroxysmal AF had superior PVs that were dilated as compared to those without paroxysmal AF. Dilatation was most prevalent at the ostium of the pulmonary veins. Magnetic resonance imaging has showed similar patterns of dilation [19,20].

5. Specialized conduction cells may initiate PV activity: histologic, embryologic and electrophysiologic evidence

For many years, the existence of pacemaker cells within PVs was questioned. However, Chen et al. [3] were the first to discover the presence of spontaneously
depolarizing cells in the PVs of dogs. Recently, P cells, normally found in the AV and SA node, have been localized to the PVs [21]. These were found in markedly greater numbers near the ostium of the vessels. The same study also identified purkinje and transitional cells in PVs. These cells are commonly found in the Bundle of His and AV node. The current belief is that spontaneous depolarization in P cells may lead to the production of electrical impulses that are propagated to the left atrium through purkinje cells [22].

Embryological evidence supports the idea of specialized conduction cells within the PV myocardium. Using human embryos, Joengbloed et al. [23] demonstrated that cells of the developing cardiac conduction system could be found in PVs prior to birth. The study observed the addition of CCS-lacZ expressing cardiomyocytes to developing PVs. CCS-lacZ gene expression is known to occur only in cells that are part of the developing cardiac conduction system [24]. Presence of CCS-lacZ expression was also noted in other tissue involved in arrhythmia, including Bachmann’s Bundle. Developing PV myocytes that express CCS-lacZ could be precursors to the specialized conduction cells found in mature PVs by Perez-Lugones et al. [21] and they may therefore be a source of arrhythmogenicity.

Histological and embryological findings correlate with electrophysiological evidence. Recent studies have found PV myocytes with spontaneous phase 4 depolarizations, along with slowed phase 0 rates, both characteristic features of pacemaker cells in the SA node (Fig. 2) [3]. In addition, other studies have recorded PV cells with prolonged plateau phases that show triggered activity [25].

The unique distribution of ion channels in the PV myocyte membrane may explain its distinctive electrophysiological properties. Chen et al. [26] discovered that PV myocyte membranes have a much lower density of IK1-channels as compared with typical atrial tissue. Of significance is the fact that these channels are totally absent in SA nodal cells. IK1-channels are thought to contribute to cardiocyte resting potential (−85 mV), and there absence could be a reason for the raised (less negative) maximum diastolic potential of pacemaker cells within PV myocytes (−70 mV). Therefore, decreased levels of IK1-channels within PV myocytes may facilitate pacemaker depolarizations.

SA nodal cells also have a characteristic I\textsubscript{f}-current, whose presence was recently demonstrated within PV myocytes [26]. I\textsubscript{f}-current is a mix of Na\textsuperscript{+}- and K\textsuperscript{+}-current that is activated after cellular repolarization. It is thought to contribute to and enhance depolarization in myocytes following repolarization, allowing for increased pacemaker potential in PV cells.

Chen et al. [27] have discovered the presence of I\textsubscript{Ca\textsubscript{2\textperiodcentered}L-t}\textsuperscript{+}-channels within PV myocytes. The Ca\textsuperscript{2\textperiodcentered}+-current from these channels has been shown to aid in the production of spontaneous depolarizations by causing the release of Ca\textsuperscript{2\textperiodcentered}+ from the sarcoplastic reticulum at low voltages (−60mV), allowing for the generation of pacemaker activity. These channels may also increase the plateau phase of action potentials, raising the possibility of EADs. Application of nickel, a specific inhibitor of I\textsubscript{Ca\textsubscript{2\textperiodcentered}L-t}\textsuperscript{+}-channels, to PV tissue drastically reduced both triggered activity and automaticity in PV myocytes [28].

6. Potential effects of disease on PV activity and the generation of AF

The electrophysiologic properties of PV cardiomyocytes may change in the presence of disease. Using a ryanodine receptor blocker, Honjo et al. [29] demonstrated that alteration of intracellular calcium dynamics results in rapid firing of cardiocytes in the PVs. In fact, treatment with ryanodine caused PV myocytes, undergoing typical atrial action potentials, to develop pacemaker depolarizations. This study notes that pathologic conditions, such as heart failure, similarly change the ability of the cell to handle calcium and therefore may cause AF by this mechanism.

Chen et al. demonstrated that incubation of thyroid hormone with PVs led to the production of increased triggered activity in the myocardial sleeves of these vessels [30]. The study suggested that thyroid hormone caused an increase in the transient inward currents (most likely due to increased \textsubscript{Iglass+}Ca\textsubscript{2\textperiodcentered}L-current) of PV cardiocytes leading to production of DADs and EADs. Paroxysmal AF associated with hyperthyroidism may develop in this manner.

Temperature appears to also play an important role in the arrhythmogenicity of PVs. Chen et al. [31] found that afterpotentials in cardiomyocytes of PVs could only be generated at higher temperatures (>38 °C). In the study, cardiocytes incubated in the highest temperatures (40–41

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Fig. 2. A comparison between model-generated SA nodal cell action potentials (A) and suspected pacemaker potentials localized within PV myocardial tissue (B). Both show spontaneous phase 4 depolarizations and a decreased phase 0 slope.
significant increase in the function of the IKs-channel, mutation of KvLQT1 in a Chinese family caused a

significant increase in the likelihood of re-entry associated with AF

AF is characterized by the presence of re-entry pathways. Shorter refractory periods facilitate the maintenance of AF by increasing the prospect for re-entry. Tada et al. [4] first noted that the cardiocytes of PVs had varying refractory periods that lent themselves well to the development of AF. Jais et al. [5] found similar patterns, identifying reduced refractory periods within the PVs of patients with AF. In fact, the refractory periods in the aforementioned study were the shortest to have ever been documented within the heart.

The effective refractory period (ERP) of a cell is determined by the flow of ion currents across its membrane. \( I_{K1-}, I_{Ks-} \) and \( I_{Kr} \)-channels are all involved in myocyte repolarization and therefore increased activity of these channels results in decreased ERP and action potential duration (APD). Contrastingly, \( I_{Ca}^{2+} \)-channel activity increases ERP and APD length. Ehrlich et al. [34] reported the increased density of \( I_{Ks-} \) and \( I_{Kr} \)-channels on PV cell membranes, as well as reduced \( I_{Ca}^{2+} \)-current, as compared to neighbouring left atrial myocytes. In addition, Melnyk et al. [35] found that HERG protein (associated with \( I_{Kr} \)-channel) and KvLQT1 protein (pore-forming component for \( I_{Ks} \)-channel) were in greater concentrations within PV myocytes. This distribution of ion channels on PV cellular membranes obviously helps to allow for the maintenance of AF. In addition, it also indicates that individuals with inheritable defects in PV myocyte channel protein expression and function may be predisposed to AF. In fact, Chen et al. [36] have already shown, via linkage analysis, that mutation of KvLQT1 in a Chinese family caused a significant increase in the function of the \( I_{Ks} \)-channel, resulting in familial AF.

Autonomic neural regulation appears to significantly influence the maintenance of cardiac arrhythmias, as well. Both sympathetic and vagal stimulation cause reductions in ERP [37]. Acetylcholine activates \( I_{KACH} \)-channels via G-protein second messengers, resulting again in decreased ERP and APD [38]. It is postulated that norepinephrine lowers ERP by activating IP3 and DAG leading to an initial influx of \( Ca^{2+} \). The presence of the two second messengers along with \( Ca^{2+} \) enhance protein kinase C function, which, in turn, reduces \( I_{Ca}^{2+} \)-channel activity. In addition, \( I_{KIH} \), an inward \( K^- \)-conductance current, has also shown to be increased under both sympathetic and parasympathetic stimulation [39]. As mentioned, there is quite an extensive degree of innervation to the PVs [33]. Therefore, increased autonomic tone localized in the PVs may be involved in the development of PAF.

8. Distinctive structural organization of myocardial tissue in PVs facilitates AF maintenance

Myocardial architecture plays an integral role in determining conduction velocity. The principles of anisotropic conduction emphasize that electrical impulses are propagated more quickly over longitudinal rather than transverse paths. This is primarily due to the fact that more cell boundaries per unit distance are encountered within the latter pathway [40]. A greater number of boundaries imply increased resistivity and slower impulse conduction. In addition, longitudinal paths offer maximal intracellular electrical conduction, rather than conduction through gap junctions, which again provide greater resistivity. Hamabe et al. [41] observed that conduction delays occurred in the left superior PV where myocyte direction changed rapidly (Fig. 3). Hocini et al. [7] also found that sudden changes in PV myocyte fiber direction correlated with conduction delays. Additionally, de Bakker et al. [42] noted that a sharp change in the orientation of a myocyte may cause the disorganized propagation of an impulse to neighbouring tissue, resulting in chaotic rather than focal, linear conduction.

Hassink et al. [6] demonstrated patients with AF have greater amounts of hypertrophy and fibrosis in the myocardial tissue of PVs compared with those without AF (Fig. 4). Fibrotic tissue offers greater resistance to traveling electrical impulses, slowing down conduction velocity within cardiac tissue. In addition, fibrous septae are thought to impair side-to-side electrical conduction in myocytes. This so-called “cellular uncoupling” was first documented by Spach and Dolber who observed decreased transverse conduction velocities in isolated cardiac tissue associated with fibrotic septae [43]. Accordingly, quantitative mapping analysis of the left atrial-PV ostium junction indicated impulse conduction block or delay at sites noted for the presence of connective tissue barriers [41]. The fact that connective tissue septae separating myocyte tissue islands are much more common in the PVs as compared with the atria may be why the PVs are the predominant source of AF development.
Mechanisms by which fibrosis develops within the PVs are not as yet known. However, in the atria, angiotensin II has been known to cause significant fibrosis. Recent studies indicate that the PV endothelial cells contain angiotensin II receptors [44]. Binding of this hormone to its receptors initiates a calcium-dependent second messenger cycle that results in the production of extracellular kinases (Erks), which in turn cause the proliferation of fibroblasts and extracellular matrix. This results in significant fibrosis. Angiotensin-converting enzyme (ACE) and Erk levels have been found to be significantly raised in patients with PAF and CAF [45]. Also, studies have already indicated that RAS gene polymorphism is correlated to non-familial AF [46]. Inherent excess production of angiotensin II may therefore predispose patients to AF because of its effects on PV architecture.

In all likelihood, angiotensin II does not act alone in developing PV fibrosis. Recently, Veurheule et al. [47] found that transgenic mice with increased levels of TGF-B1 had increased fibrosis within their atria and were consequently predisposed to AF development. TGF-B1 plays a major role in mesenchymal cell differentiation during angiogenesis of the pulmonary vasculature. Although no direct mechanism of action has been proposed for the actions of TGF-B1 on the heart, this compound has been shown to stimulate the release of collagen and fibronectin from atrial fibroblasts. In addition, Chen et al. [48] have shown that TGF-B1 is one of the most potent stimulators of connective tissue growth factor (CTGF) expression in cardiac myocytes and fibroblasts. Increases in CTGF levels correlated with greater levels of cellular fibronectin and collagen I and III mRNA levels, which again implied greater fibrosis in cardiac tissue.

Fig. 3. (A) Schematic Reconstruction of a PV. The ostium has a chaotic structural arrangement, with fibers traveling in different directions. At the distal end, fibers are less prevalent and run in parallel along the long axis of the vein. (B) Longitudinal cross-section of a PV at the level of the ostium showing the non-axial course of muscle fibers within the myocardial sleeve of the vessel.

Fig. 4. Longitudinal cross-section of a PV at the level of the ostium demonstrating the histologic appearance of a myocardial sleeve within the vessel. Note the fibrous tissue clearly interdispersed throughout the myocardial sleeve. Increased expression of a variety of genes (such as ccn2) may promote fibrous tissue production in this area.
Correlations between TGF-B1 and CTGF within the heart introduce another possible genetic component to AF, as well as to other fibrotic cardiac diseases. The ccn2 (CTGF) gene is known to be overexpressed in a number of fibrotic diseases [49]. Blom et al. [50] have found that polymorphisms in the promoter regions of the ccn2 gene led to increased transcription of the gene and higher levels of myocyte CTGF in patients with ischemic heart disease. Thus, inherent overexpression of CTGF during PV angiogenesis (prior to birth) may cause the development of fibrous patches that separate myocyte networks within the PVs. Immunostaining has identified the presence of CTGF in the lung, heart and vasculature of mouse embryos [51]. Myocardial tissue in the PVs may be greatly affected because of its proximity to angiogenic sources in these areas.

Along with fibrosis, the reduced synthesis of gap junction proteins, connexins, has been suggested to contribute to arrhythmia development. Most experimental work has focused on the correlation between changes in the cellular level of connexin-43 (Cx-43) and ventricular dysrhythmia. Differences in Cx-43 expression levels between PV myocytes and other cardiac cells have not been significant. However, recently, Verheule et al. [52] reported that the PVs had a reduced density of connexin-40 (Cx-40) within their myocardial sleeves as compared with adjoining left atrial tissue. The implied decreased electrical coupling of cardiocytes would result in lowered conduction velocity, promoting AF maintenance. Evidence of this came from the study by Dupont et al. [53], where they found that patients who develop AF after surgery had reduced levels of Cx-40. In addition, mice lacking Cx-40 have been shown to have both conduction block and increased AF development [54,55].

9. If “AF begets AF,” then does “PV-induced paroxysmal AF beget PV-induced chronic AF”?

Wijffels et al. [8] introduced the concept of “atrial remodeling” caused by the presence of arrhythmias. Subsequent studies identified slowing of impulse conduction and reduced ERP in atrial cells after AF [56,57]. Atrial tachycardia has been shown to reduce ERP primarily through a decrease in the ICaL-current of myocytes. In addition, sustained fibrillation has also been associated with structural changes such as myocyte hypertrophy, myocyte death and atrial stretch [58,59], which act to reduce conduction velocity. Both electrophysiological and structural changes of the atria are thought to be involved in the development of chronic AF, and so the theory that “AF begets AF” has been introduced. If this concept is dissected further, then one notes that PVs are the primary source for ectopic foci during AF. Therefore, it is likely that the arrhythmogenic activity of PVs may be an important cause of “atrial remodeling”. Thomas et al. showed that the “Star” model for radio-frequency ablation, where PVs are electrically isolated, resulted in “reverse atrial remodeling” in patients with AF [60]. The study found that atrial size and function was restored after surgery. In addition, structural changes in the atria after remodeling, such as stretch, may also result in increased PV activity. Kalifa et al. showed that atrial stretch led to increased intra-atrial pressure causing a rise in the rate and spatio-temporal organization of electrical waves originating in the PVs [61]. Rapid atrial pacing (RAP) has also been shown to reduce ERP and APD within PV myocytes. Chen et al. showed that RAP reduces the density of ICa2+L- and ICaT-channels, both involved in the plateau phase of action potentials [26]. The same study found that PV myocytes had increased ICaL-currents after RAP. These changes imply that electrical and structural remodeling increase the likelihood of ectopic PV automaticity and AF maintenance.

Therefore, rather than “AF begets AF;” we can have a variation on that theme where “PV-induced paroxysmal AF begets PV-induced chronic AF.”

10. Conclusion

Despite extensive study into the involvement of PV electrical activity in AF, many questions remain unanswered. For example, it is as yet unknown why AF, likely generated by focal activity in the PVs, causes changes in the structural and electrophysiological properties of atrial tissue, which further increase the likelihood of chronic AF development.

In addition, thus far, a variety of different types of ionic currents have been shown to be involved in the generation of pacemaker potential within PV myocytes. Determining which of these is the predominant cause of arrhythmogenicity will help in improving our current model for PV-initiated AF. Such knowledge may also lead to further development of pharmacological interventions for AF. Chen et al. [3] have already documented that beta-adrenergic blockers, ACh, calcium-channel blockers and adenosine reduce spontaneous activity in PVs.

Along with understanding the development of pacemaker potentials, it is extremely important to fully elucidate the biological pathway by which fibrotic tissue is deposited in the PVs. Recent evidence has shown that the effects of angiotensin II in the heart are partially mediated through myocyte production of CTGF [62]. In addition, there is also evidence that angiotensin II increases the production of TGF-B1 within cardiac tissue. As TGF-B1 is the most potent stimulator of CTGF, it may be that all three of these molecules increase the amount of fibrous tissue within the heart via a common sequential pathway (angiotensin II → TGF-B1 → CTGF). However, to determine the exact contributions of CTGF and TGF-B1 to AF, their individual effects on PV myocardial tissue must be examined. Knowledge of these specific effects is imperative as correlations between AF and CTGF and TGF-B1, both known to be involved in a variety of fibrotic disorders (pancreatitis,
glomerulosclerosis, etc.), may allow for the implication of AF as one component of a larger systemic disease(s). If a common link between these disorders can be elucidated, treatment methods for PV-derived AF may have significant impact on the therapy of systemic conditions.

In regards to current therapy, AF has been noted even after successful electrical isolation of PVs [63]. This may be due to the presence of arrhythmogenic tissue in other areas of the heart. Myocardial sleeves, similar to those found in PVs, have been identified in the walls of the coronary sinus. The anatomy of this vessel should be further examined in the hope of definitively identifying pacemaker potential cells as a source of ectopic electrical firing. Electrophysiologic studies have recently shown the importance of the cells as a source of ectopic electrical firing. Electrophysiologic studies have recently shown the importance of the coronary sinus in the perpetuation of AF [64].

More generally, clarifying the role of the PVs in AF may allow for more specific, less invasive treatment and may make prevention increasingly viable.

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References


