Current Perspective

Pulmonary Vein Myocardium as a Possible Pharmacological Target for the Treatment of Atrial Fibrillation

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Abstract. The pulmonary vein has a unique electrophysiological property showing an autonomic electrical activity, and this phenomenon has been further focused on as a source of triggers of atrial fibrillation. The pulmonary vein cardiomyocytes have shorter action potential duration, less negative resting membrane potential, and smaller maximum upstroke velocity than those in the left atrium, whose underlying cellular mechanisms may generate arrhythmogenic substrates such as abnormal automaticity and triggered activity. In diseased conditions including sustained atrial tachycardia or chronic volume overload, its arrhythmogenic profile can be further modified through abbreviation of action potential duration of the pulmonary vein myocardium, which may become a cause of reentry. Recently, antiarrhythmic effects of various drugs have been extensively investigated in isolated pulmonary vein preparations. The present review article highlights the recent advances in our understanding of electrophysiological and pharmacological profiles of the pulmonary vein.

Keywords: pulmonary vein, atrial fibrillation, automaticity, triggered activity, reentry

1. Introduction

In human anatomy, there are a total of 4 pulmonary veins, and all of them are connected to the left atrium of the heart (Fig. 1A). Interestingly, the pulmonary veins have a unique electrophysiological property showing an autonomic electrical activity, as shown in Fig. 1B. Such spontaneous and independent pulsation of pulmonary veins was firstly described in 1876 (1). About 120 years later, this phenomenon has been further focused on as a source of triggers of atrial fibrillation, which was first reported by Haïssaguerre et al. (2). As shown in the photomicrographs (Fig. 1C), vascular smooth muscle is detected on the luminal face of the pulmonary vein, whereas the myocardial sleeve is observed mostly at mid-layer of the pulmonary vein as a circular muscle layer (3). It has been postulated that the circumferential layer of musculature around the pulmonary veins may work as a sphincter-like mechanism, preventing backflow of blood during atrial contraction (4). The origin of the pulmonary myocardial cells has been proposed by gene expression profiles, in which 2 possible pools of cells for the pulmonary myocardium are hypothesized: i) atrial cells that migrate around the pulmonary vein and ii) mesenchymal cells that differentiate into myocardium (5–7). Recently, a biphasic model has been indicated for the development of the pulmonary myocardium that unites both hypotheses: a myocardial population forms de novo at the connection of the pulmonary vein and the atrium, and the pulmonary myocardium expands by proliferation and expansion to form the pulmonary myocardial sleeve (7).

The pulmonary vein myocardium has different electrophysiological properties from those of the working myocardium, which makes it possible to easily generate arrhythmogenic substrates; abnormal automaticity and triggered activity (8, 9). Also, it is suggested that the combination of reentrant and non-reentrant mechanisms is the underlying arrhythmogenic mechanisms of atrial fibrillation from the pulmonary veins (10). Here we provide an overview of arrhythmogenic properties of
the pulmonary vein to discuss possible pharmacological targets for the treatment of atrial fibrillation.

2. Electrical conduction in the pulmonary vein

The electrophysiological property of the pulmonary vein myocardium has been characterized in comparison with that of the left atrium. The pulmonary vein cardiomyocytes have shorter action potential duration, less negative resting membrane potential, and smaller maximum upstroke velocity than those in the left atrium in dogs (8). These characteristics can be explained by lower density of inward rectifier K⁺ current (Iₖ₁) and L-type Ca²⁺ current (IₖCa) in the pulmonary vein myocardial cells than in the atrial ones, whereas there is no significant difference in the density of Na⁺ current (Iₙa) between the two regions (8, 11).

The conduction velocity in the pulmonary vein has been demonstrated to be about half of that in the left atrium (1.11 ± 0.09 vs. 0.42 ± 0.05 m/s at 1 Hz) in our study using the isolated guinea-pig heart (12). Cardiac cells are electrically coupled extensively in the longitudinal direction and to a lesser extent in the transverse direction, and anisotropic trabecular structures are generally known as the major determinants of electrical impulse propagation (13). Histologically, myocardial cells were aligned as a circular muscle layer in the proximal region of the pulmonary vein connected to the left atrium (3). Conduction velocity is hardly affected by elevation of resting membrane potential up to about −70 mV (14). Since the resting membrane potential of the pulmonary vein myocardium in the guinea pig is around −71 to −74 mV (3, 12, 15), the difference in the function of each ion channel between the pulmonary vein myocardium and left atrium (8, 11) may not be a major determinant of the slow conduction in the pulmonary vein preparation. In patients with atrial fibrillation, decremental conduction in the pulmonary vein was more frequent than those in control subjects (93% vs. 56%) (16), which may result in the appearance of 2:1 conduction or Wenckebach conduction patterns. In the isolated pulmonary vein preparation from guinea pigs, we have also observed a 2:1 conduction block in the presence of the class I antiarrhythmic drug pilsicainide (12).

3. Automaticity in the pulmonary vein

In 1968, Tasaki reported that isolated pulmonary vein preparations from guinea pigs were capable of indepen-
dent pace-making activity (17). Later, Chen et al. have demonstrated using the canine pulmonary vein myocardium that several types of spontaneous action potentials with various configurations are identified in the area surrounding the ending of the myocardial sleeve (18). Certain spontaneous action potentials have a slow rate of phase-0 upstroke with a conspicuous diastolic depolarization, similar to those of sino-atrial nodal cells. Some spontaneous action potentials have a prominent phase-0 depolarization and a rapid phase-3 repolarization without plateau phase. Our previous study using micro-electrode techniques has demonstrated that 25 out of 141 isolated guinea-pig pulmonary vein preparations (18%) showed spontaneous electrical activity (9). Since the firing frequency has been reported to be about 1 Hz in the guinea-pig pulmonary vein (9), the automaticity cannot be observed under the sinus rhythm. The spontaneous electrical activity is effectively suppressed by the Na+/Ca2+ exchange inhibitor SEA0400 or ryanodine, suggesting that forward-mode Na+/Ca2+ exchange activated by Ca2+ from the sarcoplasmic reticulum is involved in the automaticity of the pulmonary vein myocardium (9).

Tactics to raise the intracellular Ca2+ concentration of the pulmonary vein myocardium is known to elicit spontaneous electrical beats. Even in the electrically quiescent pulmonary vein preparations, spontaneous electrical activity with a higher frequency of about 5 Hz appears after the application of ouabain at a concentration producing positive inotropic effects (1 μM) (9). It is expected that the higher frequency of spontaneous activity can be detected in the pulmonary vein of in vivo animals after administration of ouabain. However, there is no published data found. Since the ouabain-induced electrical firing can be effectively suppressed by SEA0400 or ryanodine, oscillatory Ca2+ release from the sarcoplasmic reticulum and transient depolarization after completion of myocardial repolarization may be involved in the mechanisms (9).

4. Triggered activities in the pulmonary vein

Triggered activity is one of the well-recognized mechanisms of ectopy aggravated by an increased rate of beating. In the ventricular tissues, tactics to raise the intracellular Ca2+ concentration, such as treatment with digitalis or a low K+/ high Ca2+ extracellular environment, causes an oscillatory Ca2+ release from the sarcoplasmic reticulum and transient depolarization after completion of ventricular repolarization. To better understand the arrhythmogenic activity of the pulmonary vein itself, we analyzed electrophysiological characteristics of triggered activity elicited in the isolated pulmonary vein from the guinea pig under the normal experimental condition consisting of a standard physiological solution without cardiac glycoside (3). Train stimulation was used to induce triggered activity. Immediately after the termination of train stimulation, spontaneous activities accompanied with phase-4 depolarization were detected in the pulmonary vein preparations. Such triggered activities were not observed in the isolated left atrium. Interestingly, the arrhythmogenicity of the pulmonary vein myocardium may be closely associated with its lower resting membrane potential. It is because application of carbachol to the pulmonary vein myocardium decreased cell excitability via hyperpolarization of the membrane potential through increment of G protein–activated inward-rectifier currents, which might counteract the train stimulation–induced triggered activity (3). These observations suggest that the pulmonary vein preparation has more arrhythmogenic characteristics than the ventricular and the left atrial tissues due to lower resting membrane potential.

5. Electrophysiology of the pulmonary vein in diseased conditions

Long-term atrial tachycardia induced by an implanted pacemaker has been known to produce arrhythmogenic remodeling including marked shortening of atrial effective refractory period, which promotes atrial fibrillation and contributes significantly to its pathogenesis (19). Thus, the term “atrial fibrillation begets atrial fibrillation” is often used by researchers. The phenomenon may explain clinical observations that it becomes difficult to keep a patient with AF in sinus rhythm with time. Cha et al. compared the electrical remodeling between the left atrium and pulmonary vein in dogs with atrial tachycardia using implanted pacemakers for 24 h (20). The atrial tachycardia reduced the resting membrane potential and action potential duration both in the isolated left atrial cells and pulmonary vein cardiomyocytes, which may be explained by qualitatively similar reduction of transient outward K+ current (Ito), inward rectifier K+ current (IK1), and L-type Ca2+ current (ICa) in the two regions (Fig. 2). However, differences of action potential parameters between the left atrium and pulmonary vein became smaller in the atrial tachycardia model than in control dogs, suggesting that pulmonary veins may not be necessarily essential for atrial tachycardia–induced atrial tachyarrhythmia promotion.

Atrial stretch has been known to contribute to the maintenance of atrial fibrillation in humans by stabilizing high-frequency sources (21). Indeed, atrial fibrillation can be frequently observed in patients with atrial enlargement. Importantly, there is evidence that the diameters of the superior pulmonary vein ostia are markedly dilated.
in patients with paroxysmal atrial fibrillation, and intratrabial pressure increases rate and organization of waves emanating from the superior pulmonary veins during atrial fibrillation (22, 23). Chronic atrioventricular block is known to produce a moderate degree of sustained volume overload to the atrium (24, 25). In our study, whereas the action potential duration of the left atrium was comparable between chronic atrioventricular block dogs and sham animals, that of the pulmonary vein myocardium in the diseased dog was much shorter than that in the sham, which may provide an arrhythmogenic substrate of reentry (26). These results indicate that the pulmonary vein is more sensitive to sustained volume overload than the atrium, leading to generation of a larger pulmonary vein–left atrial difference in action potential duration during the chronic atrioventricular block (Fig. 2).

The mechanisms of shortened action potential duration in the pulmonary vein of the chronic atrioventricular block dog were analyzed pharmacologically and immunohistologically using peptidic blockers for Ca\(^{2+}\)-activated K\(^+\) channels and antibodies against KCa3.1/KCNN4 (Ca\(^{2+}\)-activated K\(^+\) channel of intermediate conductance; IK channel), where IK channels are expressed and workable in the myocardial sleeve of the chronic atrioventricular block dog (26). Since IK channels are generally considered to be abundantly expressed in vascular smooth muscle cells and myofibroblasts as well as immune cells or epithelial tissue especially when they are in the dedifferentiation or proliferating mode (27–29), the finding may be the first example of its expression in myocardial tissue, suggesting that the pulmonary vein of the atrioventricular block dogs is under remodeling and is shifted towards a dedifferentiated state. Therefore, IK channel inhibitors may be useful for preventing both shortening of APD and pathophysiological remodeling in the diseased pulmonary vein (Fig. 2).

### 6. Antiarrhythmic effects of drugs in the pulmonary vein

Class I or class III antiarrhythmic drugs are often used for termination of atrial fibrillation in patients by oral or intravenous administration. Whereas electrophysiological and antiarrhythmic effects of antiarrhythmic drugs on the atria have been widely investigated in clinical and experimental examinations, information is limited regarding their effects of on electrophysiological parameters of the pulmonary vein myocardium itself. We have assessed effects of pilsicainide and bepridil on the conduction velocity, effective refractory period, and action potential of the isolated pulmonary vein preparation from the guinea pig (12, 15), since the drugs are often used for patients with persistent atrial fibrillation in Japan (30). As shown in Fig. 3A, the effect of pilsicainide for conduction velocity was relatively greater in the pulmonary vein than those in the left atrium, which corresponded to its effects on the maximum rate of phase 0 depolarization in the pulmonary vein and left atrium (12). The effective refractory period was similarly prolonged by pilsicainide in the pulmonary vein as well as the left atrium, suggesting that pilsicainide has suppressive effects on reentrant arrhythmias in the pulmonary vein (12). Since its inhibitory action on the
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Conduction in the pulmonary vein was greater than in the left atrium, suppressive effects of pilsicainide on reentry within the left atrium will be estimated to be greater than within the pulmonary vein. On the other hand, bepridil prolonged the effective refractory period with little effect on the conduction velocity in the pulmonary vein, whereas the drug failed to affect the electrophysiological parameters in the left atrium (15). The drug prolonged the action potential of the pulmonary vein more potently than that of the left atrium, suggesting that antiarrhythmic effects of bepridil on reentry within the pulmonary vein are estimated to be greater than within the left atrium. The observations may provide important information for their clinical usage.

Rapid train stimulation–induced triggered activities can be suppressed by pilsicainide and verapamil, as well as ryanodine in the isolated guinea-pig pulmonary vein preparation (3), as shown in Fig. 3B. In the canine pulmonary vein sleeve preparation, drugs with a Na⁺ channel–blocking property such as ranolazine and vernakalant effectively suppressed delayed afterdepolarizations and triggered activities induced by rapid train stimulation in the presence of isoproterenol and high-Ca²⁺ concentrations (31). Furthermore, in the isolated canine pulmonary vein, the angiotensin-converting enzyme inhibitor enapril or the angiotensin II–receptor blocker losartan also suppressed delayed afterdepolarizations and triggered activities (32), which shows a “direct” antiarrhythmic effect by suppressing triggers responsible for the genesis of atrial fibrillation in addition to their “upstream” effects to reduce atrial structural remodeling. However, since losartan is found to affect neither Ca²⁺ nor K⁺ channels (32), its mechanism is incompletely understood at present.

Concerning the pharmacological treatment of atrial fibrillation, stretch activated cation channels have received attention as a specific target under chronic atrial dilation. This strategy aims at blockade of the Ca²⁺ overload and the resulting generation of delayed afterdepolarizations that may act as an arrhythmogenic trigger. In fact, such blockers as Gd³⁺ or a tarantula peptide GsMtx-4 have been reported to suppress stretch-induced atrial fibrillation without affecting the effective refractory period (33, 34).

7. Conclusions

The pulmonary vein myocardium has a unique electrophysiological property compared with the working myocardium, which makes it possible to easily generate arrhythmogenic substrates: abnormal automaticity and triggered activity. This may be associated with the origin of the pulmonary myocardial cells. In disease conditions such as sustained atrial tachycardia or chronic volume overload, its arrhythmogenic profile can be further modified through abbreviation of action potential duration of the pulmonary vein myocardium, which may become a cause of reentry. Antiarrhythmic effects of various drugs have been extensively investigated in the isolated pulmonary vein preparations. On the other
hand, since information is still limited regarding their in vivo electrophysiological effects on the pulmonary vein in the normal as well as diseased experimental conditions, systematic investigations are expected to find revolutionary pharmacological targets for the treatment of atrial fibrillation that originates from pulmonary veins.

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Conflicts of Interest

The authors indicated no potential conflicts of interest.

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