Current Topics
Pathophysiological and Pharmacological Research in Cardiology

Electrophysiological and Pharmacological Properties of the Pulmonary Vein Myocardium

Iyuki Namekata, Yayoi Tsuneoka, and Hikaru Tanaka*
Department of Pharmacology, Faculty of Pharmaceutical Sciences, Toho University;
Funabashi, Chiba 274–8510, Japan.
Received May 23, 2012

The pulmonary vein contains a myocardial layer extending from the left atrium, which is receiving attention as the source of ectopic electrical activity underlying atrial fibrillation. Electrophysiological and pharmacological analysis of the pulmonary vein myocardium performed in various experimental animal species have revealed characteristics such as presence of intracellular Ca\(^{2+}\) oscillations and low repolarizing potency. The automaticity of the pulmonary vein myocardium is affected by various neurotransmitters, hormones and pharmacological agents. Clarification of the mechanisms and regulation of pulmonary vein automaticity would lead to the development of novel therapeutic strategies and pharmacological agents for atrial fibrillation.

Key words  pulmonary vein myocardium; automaticity; intracellular Ca\(^{2+}\)

1. INTRODUCTION

In the late 19th century, the pulmonary vein, the blood vessel for the return of blood flow from the lung to the heart, was found to undergo spontaneous pulsation independent of the cardiac cavity.\(^1\) The pulmonary vein contains a myocardial layer, which is a continuation from the left atrial myocardium and is capable of generating spontaneous or triggered action potentials.\(^2,3\) Some studies revealed that paroxysmal atrial fibrillation is initiated by trains of rapid discharges from the pulmonary veins.\(^4,5\) Since then, the electrical activity of the pulmonary vein myocardium is considered to play a central role in the generation and maintenance of atrial fibrillation. The myocardial layer of the pulmonary vein is composed of circumferential and parallel longitudinal fibers, which produce non-uniform anisotropy and discontinuities\(^6–9\) resulting in lower conduction velocity than in the left atrium.\(^10,11\) These properties provide a histological basis for microreentry. The length and thickness of the myocardial sleeve in the pulmonary vein appeared to correlate with the patient’s history of atrial fibrillation.\(^12\) The pulmonary vein receives both sympathetic and parasympathetic innervations,\(^9,13\) which are considered to play important roles in the generation of atrial fibrillation through their effects on the pulmonary vein cardiomyocytes. In this review, we will summarize the characteristics of spontaneous and induced electrical activity in the pulmonary vein myocardium as revealed by electrophysiological and Ca\(^{2+}\) imaging analyses.

2. ACTION POTENTIAL AND MEMBRANE CURRENT PROPERTIES

The action potential parameters and ionic current properties of the canine pulmonary vein myocardium were studied by voltage clamp and microelectrode experiments, and were compared with the atrial myocardia.\(^14\) The pulmonary vein myocardium had a less negative resting membrane potential, which could be explained by a lower density of the inwardly rectifying K\(^{+}\) current \(I_{\text{Ks}}\). The maximum upstroke velocity of the rapid depolarization phase of the pulmonary vein myocardium was less than half of that in the atria. As the density of the Na\(^{+}\) channel current was not different between the two regions, the difference in upstroke velocity was probably the result of larger degree of voltage-dependent inactivation of the channel due to less negative resting membrane potential. The action potential duration was shorter in the pulmonary vein myocardium which could be explained by the lower density of L-type Ca\(^{2+}\) channel current and a larger density of the delayed rectifier K\(^{+}\) channel currents \(I_{\text{Kr}}\) and \(I_{\text{K1}}\). The difference in membrane current densities between the canine pulmonary vein and atrial myocardium was supported by western blot and immunohistochemical analyses.\(^15\)

Among the isolated cardiomyocytes from rabbit pulmonary vein, some showed spontaneous pacemaker activity while others did not.\(^16\) The cardiomyocytes showing pacemaker activity had a lower density of \(I_{\text{Kr}}\), which appeared to allow spontaneous depolarization and pacemaking.\(^16\) The densities of the hyperpolarization activated inward current \(I_{h}\) and the T-type Ca\(^{2+}\) current, which can probably contribute to spontaneous depolarization, were larger in pacemaking cells than in non-pacemaking cells.\(^17,18\) Ivabradine, a selective \(I_{f}\) inhibitor, suppressed the spontaneous activity.\(^19\) The density of the delayed rectifier K\(^{+}\) current \(I_{\text{K1}}\) was also larger, while that of the transient outward current \(I_{\text{O2}}\) was smaller in pacemaking cells than in non-pacemaking cells.\(^19\) No difference in the L-type Ca\(^{2+}\) current density was observed.\(^16\)

Microelectrode impalement of the guinea-pig pulmonary vein myocardium was conducted as early as in the 1960s,\(^2\) and was repeated by many other researchers. About a half of the isolated pulmonary vein tissue preparations showed automatic electrical activity, and the average frequency of continuous firing was about 1 Hz.\(^20\) Most of the quiescent...
preparations showed automatic activity after noradrenaline treatment (Fig. 1A). The pulmonary vein myocardium had a less negative resting membrane potential, smaller maximum upstroke velocity and amplitude of the action potential, and shorter action potential duration than the atrial myocardium.\(^3\)\(^,\)\(^20\) The difference from the atria were more prominent in pulmonary myocardium regions distal to the atria than in the proximal. The difference in action potential upstroke velocity of the myocardial regions within the pulmonary vein correlated with the action potential conduction velocity.\(^2\)

Automatic electrical activity was also observed in the rat pulmonary vein myocardium, although the incidence was very low in the absence of adrenergic influence. The electrical activity appeared as repetitive bursts\(^21\),\(^22\) (Fig. 1B). The burst type waveform suggests that a periodic change in the depolarizing vs repolarizing power balance is taking place. The maximum diastolic potential gradually shifted to the negative direction during the burst. It is probable that during the bursts, accumulation of intracellular Ca\(^{2+}\) gradually activates some Ca\(^{2+}\)-dependent hyperpolarizing currents, which eventually inhibits the generation of action potentials.

The mouse pulmonary vein myocardium showed diverse electrical activity.\(^23\) Automatic activity was observed in about half of the preparations, which appeared in three different waveform types. The first was a constant firing which was similar to those observed in the guinea pig, and the second was a repetitive burst with a similar maximum diastolic potential during and between bursts resembling the waveform observed in the rat. The third was also a repetitive burst but with a more negative maximum diastolic potential (>5 mV) between the bursts than during the bursts and thus an early afterdepolarization-like waveform (Fig. 1C). In all three waveform types, the frequency of the repetitive firing was higher than those reported in other experimental animal species such as the guinea-pig, rabbit and canine in which the delayed rectifier K\(^{+}\) current serves as the major repolarizing current.\(^16\),\(^20\),\(^24\) The mouse myocardium relies on I\(_{to}\) as the major repolarizing current, which is involved in the constitution of an extremely rapid early repolarization phase of the action potential. As the activation-inactivation kinetics of the Na\(^{+}\) and Ca\(^{2+}\) currents were similar among these animal species,\(^14\),\(^16\),\(^25\),\(^26\) the high-frequency firing in the mouse could be attributed to the rapid kinetics of I\(_{to}\).

3. INVOLVEMENT OF INTRACELLULAR CALCIUM OSCILLATIONS

The generation of electrical activity in pulmonary vein cardiomyocytes appears to be closely related to intracellular Ca\(^{2+}\) oscillations. In case of the rabbit, pulmonary vein cardiomyocytes with pacemaker activity had higher diastolic Ca\(^{2+}\) concentrations,\(^27\) higher Ca\(^{2+}\) spark incidence and amplitude,\(^28\) and a higher incidence of spindle/bifurcated morphology\(^29\) when compared with those without pacemaker activity. Ryanodine (0.5 \(\mu\)M), which probably caused an increase in cytoplasmic Ca\(^{2+}\) concentration, resulted in appearance of diastolic depolarization in the pulmonary vein cardiomyocytes. Further application of rapid pacing resulted in generation of spontaneous electrical activity, which was inhibited by cyclopiazonic acid,\(^30\) an inhibitor of sarcoplasmic reticulum Ca\(^{2+}\)
The spontaneous activity of the rabbit pulmonary vein myocardium was inhibited by KB-R7943\textsuperscript{31} and K201,\textsuperscript{22} compounds with inhibitory action on multiple ion channels including the Na\textsuperscript+-Ca\textsuperscript{2+} exchanger and sarcoplasmic reticulum Ca\textsuperscript{2+} release channel, respectively.

In the isolated guinea-pig pulmonary vein myocardium, microelectrode experiments revealed the presence of spontaneous electrical activity in about half of the tissue preparations.\textsuperscript{20,23} Interventions which increase intracellular Ca\textsuperscript{2+} load such as ouabain\textsuperscript{20} or high-frequency pacing\textsuperscript{33} induced electrical activity in quiescent preparations. The spontaneous as well as pacing-induced electrical activity was completely inhibited by carbachol, which activates the acetylcholine-activated K\textsuperscript{+} current and increases the repolarizing K\textsuperscript{+} current density. In ouabain-treated preparations, generation of action potentials was preceded by oscillation of the resting membrane potential; ouabain induced an increase in intracellular diastolic Ca\textsuperscript{2+} concentration and Ca\textsuperscript{2+} waves and Ca\textsuperscript{2+} sparks preceding the generation of Ca\textsuperscript{2+} transients.\textsuperscript{20} SEA0400, a selective inhibitor of the myocardial Na\textsuperscript+-Ca\textsuperscript{2+} exchanger,\textsuperscript{34} inhibited the spontaneous electrical activity without affecting the resting intracellular Ca\textsuperscript{2+} concentration or the Ca\textsuperscript{2+} oscillations. Ryanodine completely inhibited intracellular Ca\textsuperscript{2+} oscillations as well as spontaneous electrical activity in the pulmonary vein myocardium.

Results obtained in the rabbit,\textsuperscript{30} rat\textsuperscript{21,26} and guinea pig\textsuperscript{20} suggest the involvement of intracellular Ca\textsuperscript{2+} in the generation of spontaneous action potentials in the pulmonary-vein myocardium (Fig. 2). Elevated intracellular Ca\textsuperscript{2+} concentration, either uniform elevation throughout the cytoplasm or localized elevation in the form of Ca\textsuperscript{2+} sparks and Ca\textsuperscript{2+} waves, activate the forward-mode Na\textsuperscript+-Ca\textsuperscript{2+} exchanger which extrudes Ca\textsuperscript{2+} from the cytoplasm generating an inward current, and slowly depolarizes the cell membrane. This diastolic depolarization drives the membrane potential to reach the threshold level and generates action potentials. Concerning the source of intracellular Ca\textsuperscript{2+}, the effectiveness of ryanodine in the rabbit and guinea pig suggests the involvement of ryanodine receptor channels. In the rat, however, the inhibitory effect of ryanodine on pulmonary vein automaticity was limited.\textsuperscript{21} The automaticity was blocked by U73112 and 2-APB, inhibitors of phospholipase C and the inositol 1,4,5-triphosphate receptor, respectively, which suggests that Ca\textsuperscript{2+} released from the inositol 1,4,5-triphosphate receptor plays an important role.\textsuperscript{26} Thus, there may be some species difference in the mechanisms for the intracellular Ca\textsuperscript{2+} oscillation underlying pulmonary vein automaticity.

The functional components of the intracellular Ca\textsuperscript{2+} dependent automaticity, such as the sarcolemmal Na\textsuperscript+-Ca\textsuperscript{2+} exchanger and the sarcoplasmic reticulum, are present not only in pulmonary-vein cardiomyocytes but also in atrial and ventricular cardiomyocytes. Thus, the question arises, what is the mechanism for manifestation of ectopic pacemaking in the pulmonary vein myocardium? One possibility is that the Ca\textsuperscript{2+} handling properties of the pulmonary vein myocardium may be different from those of the working myocardium. Observations in canine pulmonary vein cardiomyocytes, however, did not support such view.\textsuperscript{35} Cardiomyocytes from the pulmonary vein and atrium from dogs subjected to 7-d rapid pacing were not different in their Ca\textsuperscript{2+} transient amplitude, half-decay time of, beat-to-beat regularity, propensity to alternans and β-adrenergic influence. Incidence of Ca\textsuperscript{2+} sparks by Ca\textsuperscript{2+} loading of the cells and caffeine-induced Ca\textsuperscript{2+} transient amplitudes were also not different. These results do not support the hypothesis that intrinsic Ca\textsuperscript{2+} handling differences account for the occurrence of ectopic pacemaking only in the pulmonary vein myocardium.

A possible explanation for the manifestation of automaticity in the pulmonary vein myocardium is that the lower repolarizing power of the pulmonary vein myocardium plays a permissive role. The density of the inwardly rectifying K\textsuperscript{+} current (I\textsubscript{K1}), the major current to maintain the resting membrane potential, was significantly smaller in pulmonary vein cardiomyocytes than in atrial cardiomyocytes,\textsuperscript{14} and in pacemaking pulmonary vein cardiomyocytes than in non-pacemaking.\textsuperscript{16} Consistent with this view, the electrical activity induced by pacing was completely inhibited by carbachol, which increases the repolarizing K\textsuperscript{+} current density through activation of the acetylcholine activated potassium channel.\textsuperscript{13} The rabbit pulmonary vein myocardial action potential could be well computer-simulated based on existing data from electrophysiological experiments.\textsuperscript{36} In the model, the pulmonary vein cardiomyocytes had a minimal density of I\textsubscript{K1}, and the major currents contributing to pacemaking depolarization were the L-type Ca\textsuperscript{2+} current, the rapid component of the delayed rectifier K\textsuperscript{+} current (I\textsubscript{Ks}), Na\textsuperscript{+}–K\textsuperscript{+} pump current and a background current.

4. EFFECT OF AUTONOMIC NEUROTRANSMITTERS

Both sympathetic and parasympathetic nerve activity is known to greatly influence the automaticity of the pulmonary vein myocardium, as well as atrial fibrillation. In the canine pulmonary vein myocardium, isoproterenol, a β-adrenergic agonist, induced a diastolic depolarization but it was not sufficient to trigger spontaneous action potentials.\textsuperscript{37} When applied after spontaneous action potentials were induced by Ba\textsuperscript{2+}, the frequency was increased by isoproterenol, and decreased by acetylcholine.\textsuperscript{37} An interesting observation with acetylcholine is that transient application in the presence of isoproterenol
rather “induced” automatic electrical activity.

In the rabbit pulmonary vein, either α- or β-adrenergic stimulation induced automatic electrical activity; both effects were inhibited by KN-93 which suggests the involvement of calmodulin kinase II. \(^{39}\) In the guinea-pig pulmonary vein, application of noradrenaline to quiescent preparations induced a gradual depolarization of the resting membrane potential followed by generation of automatic activity (Fig. 1A). Noradrenaline also induced an increase in cytoplasmic Ca\(^{2+}\) concentration and generation of spontaneous Ca\(^{2+}\) oscillations, which were completely inhibited by ryanodine.\(^{21,22}\)

Most of the rat pulmonary vein preparations were electrically quiescent after isolation. Application of noradrenaline induced a transient hyperpolarization followed by a gradual depolarization of the resting membrane potential, which lead to generation of automatic activity\(^{21,22}\) (Fig. 1B). Pharmacological analyses revealed that hyperpolarization and depolarization were mediated by β- and α-adrenergic receptors, respectively, and that simultaneous activation of both receptor types are necessary for the generation of automatic activity.\(^{22,26,39}\)

In the case of the mouse pulmonary vein myocardium,\(^{23}\) application of noradrenaline to quiescent preparations induced automatic activity (Fig. 1C), which were often preceded by hyperpolarization or depolarization of the resting membrane potential. Application of noradrenaline to preparations showing active automatic activity resulted in an increase in the firing frequency and/or a change in the waveform type. Acetylcholine and adenosine, which induced a negative shift in the resting membrane potential, inhibited both spontaneous and noradrenaline-induced automatic activity.

Results obtained in pulmonary vein myocardia from various animal species indicate that sympathetic neuronal influence on the pulmonary vein myocardium leads to the generation of automatic activity through activation of both α- and β-adrenergic receptors. Acetylcholine, which appear to have inhibitory influence on automatic activity when simply applied, may be have stimulatory effects when its concentration is altered in the presence of adrenergic influence. These mechanisms may be involved in the generation and maintenance of atrial fibrillation of pulmonary vein origin under autonomic nerve influence.

5. EFFECT OF PATHOPHYSIOLOGICAL STATUS AND HUMORAL FACTORS

The automaticity of the pulmonary vein myocardium, as well as atrial fibrillation, is known to be greatly influenced by the pathophysiological status of the myocardium. Acute mechanical stretch to the atrium was reported to increase the excitability of the pulmonary vein myocardium and lead to the generation of atrial fibrillation.\(^{40}\) Membrane currents activated by hypotonicity\(^{41}\) or stretch\(^{42}\) have been reported to be present on the cell membrane in the pulmonary vein cardiomyocytes, and to play important roles in their automaticity. In chronic atrioventricular block goat and dog models,\(^{43,44}\) atrial dilatation induced by chronic volume overload was shown to produce a substrate of atrial fibrillation. In the chronic atrioventricular block dog, the action potential of the pulmonary vein myocardium became significantly shorter while that of the left atria did not.\(^{45}\) This indicated that the pulmonary vein is more sensitive to volume overload than the atrium. Furthermore, the difference in action potential duration between the pulmonary vein and left atria became larger, which may be somehow related to the generation of atrial fibrillation. Charybdotoxin, but not iberiotoxin, prolonged the action potential duration in the pulmonary vein after chronic atrioventricular block, which suggested that the volume-overload-induced electrical remodeling of the heart involved expression of the intermediate Ca\(^{2+}\)-activated K\(^+\) channels in the pulmonary vein cardiomyocytes. This channel is generally considered to be abundantly expressed in immune cells or epithelia tissue especially when they are in the proliferating mode. Thus, the pulmonary vein myocardium may be in the process of remodeling and shifted towards a dedifferentiated state under certain pathological conditions.\(^{35}\)

The pulmonary vein automaticity is reported to be affected by various hormones and locally released humoral factors. Thyroid hormone was reported to change the electrophysiological properties of the pulmonary vein cardiomyocyte to increase the arrhythmogenetic activity of the pulmonary vein myocardium.\(^{46}\) Hyperthyroid pulmonary vein cardiomyocytes had shorter action potential duration and higher incidences of early and late afterdepolarizations. Tumor necrosis factor α, a proinflammatory cytokine which is known to induce cardiac arrhythmias, was reported to increase the arrhythmogeneity of the pulmonary vein myocardium through enhancement of abnormal intracellular Ca\(^{2+}\) homeostasis.\(^{47}\) Treatment of the pulmonary vein cardiomyocytes with tumor necrosis factor α resulted in an increase in the amplitude of delayed afterdepolarization, an increase in the Na\(^+-\)Ca\(^{2+}\) exchanger current density, and a decrease in sarcoplasmic reticulum Ca\(^{2+}\) ATPase expression level. Hypoxia reduced the pulmonary vein beating rate in the rabbit; this effect was mimicked by the ATP-sensitive K\(^+\) channel opener, pinacidil, and was attenuated by the ATP-sensitive K\(^+\) channel blocker, glibenclamide.\(^{48}\) Thus, increased repolarization power could suppress the pulmonary vein automaticity. On the contrary, adenosine, which hyperpolarized the canine pulmonary vein myocardium, restored the dormant conduction, which could be explained by restoration of the excitability through removal of voltage-dependent inactivation of the sodium channel.\(^{49}\) Thus, increased repolarization power may either suppress or enhance pulmonary vein automaticity depending on the situation.

6. PHARMACOLOGICAL STRATEGIES AGAINST PULMONARY VEIN AUTOMATICITY

The present understanding of the electrical activity in the pulmonary vein myocardium leads to several possible strategies for the pharmacological therapy of atrial fibrillation. Compounds which affect the Na\(^+-\)Ca\(^{2+}\) exchanger,\(^{20}\) the ryanodine receptor channel\(^{22}\) and the IP\(_3\) receptor channel\(^{26}\) may inhibit the intracellular Ca\(^{2+}\) based mechanisms of depolarization and reduce the electrical automaticity of the pulmonary vein cardiomyocytes. The selectivity of these agents must, however, be carefully investigated as the functional proteins involved in automaticity are present not only in pulmonary vein myocardium but also in the working myocardium. Inhibition of the pathways to provide Ca\(^{2+}\) to the pulmonary vein cardiomyocytes, such as the stretch activated channels, may inhibit pulmonary vein electrical activity through reduction of intracellular Ca\(^{2+}\) load. Increasing
the repolarization power of pulmonary vein cardiomyocytes might be effective in suppressing their automaticity\cite{14,15,16}, this should be achieved without shortening the refractory period of the working myocardium. Modifying the effect of various neurohumoral factors may also be effective. Targeted Gi protein inhibition,\cite{17} as well as inhibitors of the acetylcholine-activated K⁺ channel,\cite{18,19} was reported to have curative effects against experimental atrial fibrillation. Certain antiarrhythmic drugs, such as pilocicainide, appear to have some selectivity towards the pulmonary vein myocardium probably due to the less negative resting membrane potential.\cite{20} Further studies on the factors regulating pulmonary vein automaticity would lead to the discovery of novel therapeutic strategies and pharmacological agents for the treatment of atrial fibrillation.

REFERENCES


29) Yu MC, Huang CF, Chang CM, Chen YC, Lin CL, Chen SA. Diverse cell morphology and intracellular calcium dynamics in


