Review

Basic and clinical electrophysiology of pulmonary vein ectopy

Jacques M.T. de Bakker\textsuperscript{a,\*}, Siew Y. Ho\textsuperscript{b}, Mélèze Hocini\textsuperscript{c}

\textsuperscript{a}The Heart Lung Center Utrecht, University Medical Center, Utrecht and the Interuniversity Cardiology Institute of the Netherlands, Utrecht, The Netherlands

\textsuperscript{b}Paediatrics, National Heart and Lung Institute, Imperial College School of Medicine, London, UK

\textsuperscript{c}Hôpital Cardiologique du Haut-Leveque, Pessac, France

Received 31 August 2001; accepted 2 November 2001

Abstract

In a subset of patients, atrial fibrillation is caused by rapidly firing foci that are often located in the pulmonary veins especially when fibrillation is paroxysmal. Histologic data show that myocardial tissue of the left atrial wall extends into the pulmonary venous walls. Both in dog and human pulmonary veins, arrangement of the myofibers is complex. Clinical and animal studies reveal both double potentials and fractionated electrograms in the pulmonary veins, which are related to the complex architecture of the myocardial sleeves in the veins. Such a structure supports the occurrence of reentry. As well, the reduced coupling of cells at sites with abrupt changes in fiber direction could facilitate the escape of a focus and subsequent activation of surrounding tissue. Intracellular recordings made in the pulmonary veins of guinea pig and dog hearts showed that spontaneous activity can occur. Spontaneous action potentials with phase 4 depolarization as well as early after depolarizations were observed in these animal models. In non-spontaneously active preparations, spontaneous activity could be provoked by pharmacologic interventions. The cycle length of bursts of ectopic beats arising in the pulmonary veins of man is often irregular, supporting a focal mechanism of the ectopic beats. The anisotropic characteristics of the myocardial sleeves in the veins may increase the ability of a focus to become evident.

Keywords: Arrhythmia (mechanism); Impulse formation; Supraventr. arrhythmia

1. Introduction

Atrial fibrillation (AF) is the most common atrial arrhythmia in man. Clinical and experimental studies have shown that multiple wavelet reentry is a major mechanism of AF [1,2]. Recently, it has been shown that rapidly firing atrial foci may be responsible for AF in a selected group of patients [3]. These foci may either trigger the initiation of the arrhythmia or be so rapid that the rest of the atrium cannot follow in a one-to-one ratio, thus giving the appearance of AF (fibrillatory conduction). The driving wavelet may be stable, but remaining atrial tissue does not exhibit a stable response. That focal activity may initiate AF, is confirmed by its eradication after radio frequency ablation of the focus [4–7]. The pulmonary veins (PVs) are the main source of focal activity, although other sites of focal activity have been found in the ligament of Marshall, the crista terminalis, the ostium of the coronary sinus and intratrial septum as well as the atrial free wall [7–10].

Pulsation of the pulmonary veins, suggestive for electrical activity, was already observed in 1872 in the rabbit heart by Brunton and Fayer [11]. Later anatomical studies confirmed the presence of myocardial tissue within the veins. The distance over which cardiac muscle extends from the left atrium into the PVs varies considerably from species to species, but may be several centimeters long in man. Later studies showed that muscle fibers in the PVs were indeed excitable, that they resembled atrial muscle in their action potential characteristics and that electrical activity could spread into the PVs [12,13].

Recent clinical studies suggest that paroxysmal AF is

*Corresponding author. Department of Experimental Cardiology, Academic Medical Center, Meibergdreef 9, 1105 AZ Amsterdam, The Netherlands. Tel.: +31-20-566-3265; fax: +31-20-697-5458.
E-mail address: j.m.debakker@amc.uva.nl (J.M.T. de Bakker).

Time for primary review 31 days.
virtually always initiated by trains of rapid discharges from
the pulmonary veins [3,9]. The mechanism of the focal
activity leading to AF is, however, unknown. Abnormal
automaticity, triggered activity and microreentry have been
suggested. The architecture of the PVs may play a role,
either by setting up microreentry due to structural com-
plexity [14] or by facilitating extinction of activation out of
a focus [15]. Fractionated electrograms, which are often
associated with discontinuous propagation due to enhanced
anisotropy, have been described in the PVs of both human
and animal hearts.

In this review we describe the characteristics of sponta-
neous and induced electrical activity in the pulmonary
veins of the human and animal heart. Possible substrates
for arrhythmogenesis in the PVs and the relation between
structural, ultrastructural characteristics and electrical acti-
vation are discussed.

2. Structural characteristics of the PVs

Myocardial continuity from the left atrial wall extending
to the outer surface of the pulmonary venous walls is well
recognized as a normal anatomical feature (Fig. 1A). Burch and Romney, followed by Nathan and Eliakin, drew
attention to the potential function of these muscular sleeves
in regulating flow [16,17]. Spontaneous pulsations of the
pulmonary veins have been observed as long ago as 1876
(Brunton and Fayer) and electrical activity recorded in the
veins since 1972 [13,18,19]. The ultrastructural studies of
Spach et al. showed conclusively that the myocardial
sleeves are atrial myocardium [13]. They extend from the
left atrium beyond the venous orifice to line the outside of
the venous media. Sectioning longitudinally, the histologi-
cal study of Burch and Romney [16] in 1954 revealed
overlap between venous wall and atrial myocardium with

Fig. 1. (A) Heart specimen viewed from behind. The epicardium has been removed to show the extension of atrial musculature from the left atrium
continuing like sleeves over the pulmonary veins. The sleeve over the right superior pulmonary vein extends beyond the cut. The broken lines mark the
extent of the sleeves over the other three veins. The circles mark the veno–atrial junction, the site of the venous orifices as seen from within the left atrium.
(B) This longitudinal section through a right superior pulmonary vein shows the myocardial sleeve lining the outer side of the venous wall. The
myocardium can be traced to the main branches (*) of the pulmonary vein. Trichrome stain. (C) This transverse section shows myocardium (dark gray)
around 75% of the right superior vein compared to a lack of myocardium around the inferior vein. Trichrome stain. This section was kindly provided by
Professor Damian Sanchez-Quintana, Badajoz, Spain.
myocardium extending well into the lung hila. The sleeves, however, are of varying lengths and degrees of encirclement along the vein (Fig. 1B and C) [17,20–22]. Several studies have reported longer sleeves in the superior veins but markedly shorter sleeves in the inferior veins [17,20,22]. Longer sleeves corresponding to the relatively higher number of foci identified in clinical series suggest a relationship between extent of myocardial sleeves and the source of initiation of ectopic beats causing atrial fibrillation [3,23]. The concept of node-like specialized cells producing atrial automaticity, reported in experimental studies [24,25], has not been supported by morphological studies of human pulmonary veins [21,22]. According to a recent study of human embryos, myocardium around the developing common pulmonary vein expressed HNK-1, an antigen that is a general marker for the developing atrioventricular conduction tissues [26]. Thus, although the myocardial sleeves in the developed hearts do not show histological specialization, they may retain electrophysiologic characteristics that become activated by some triggering mechanism later in life.

2.1. Nonuniform anisotropy

Reconstructions of histological sections reveal a complex arrangement of the myofibres in the sleeves in the majority of cases, both in patients with history of atrial fibrillation and patients without atrial arrhythmias [21,22]. Circumferential fibers with crossover diagonal and parallel longitudinal fibers produce non-uniformity which, together with fibrotic changes, may play a role in the genesis of electrical instability.

Enhanced non-uniform anisotropy and discontinuities in axial resistivity are thought to be associated with conduction disturbances that can lead to reentrant arrhythmias. Although direct evidence is lacking, the structure of the PV myocardium may indeed support the occurrence of reentry. On the other hand, computer simulations suggest that reduced coupling may also affect the initiation of a propagating wave from an ectopic focus. Wilders et al. [15] showed that in a two-dimensional sheet of ventricular cells with an ectopic focus incorporated as the central element, the focus region is spontaneously active at low values of intercellular coupling conductance, but the limited intercellular current prohibits propagation. Loading effects of surrounding cells suppress automaticity at high coupling conductance, but for intermediate values, intercellular current flow can be sufficient and ectopic activity may propagate to surrounding tissue. The effect of loading is facilitated by increased anisotropy, a feature of myocardial tissue that is certainly present in the PVs.

2.2. Stretch and dilation

Although the observation that patients with paroxysmal atrial fibrillation originating from the superior pulmonary veins have dilation of the orifices and proximal portions of the corresponding veins suggest a stretch mechanism in its initiation [27], the dilation may also be the consequence of disorganized contractility.

3. Extracellular recordings

There is ample evidence that potentials recorded within the PVs reflect activation of muscular bands extending from the left atrium to the venous wall. In man, these potentials can be recorded for up to 5 cm into the superior veins and for a lesser distance into the inferior veins [13,17,19].

Several investigators determined the characteristics of extracellular electrograms in the experimental and/or clinical setting. Spach et al. in 1972 determined electrical activity in the thoracic and pulmonary veins of the human and dog heart, using a unipolar epicardial approach [13]. Patients without AF underwent cardiac surgery for various reasons such as repair of valvular stenosis and septal defects. Intact dog preparations were used to study shape and wave forms under normal volume conductor conditions. In the dog hearts electrical activity was found for 1–4 cm from the ostium. The left superior PV consistently demonstrated electrical activity for greater distances than the inferior PV. Unipolar electrograms recorded during sinus rhythm from the right PV usually revealed two deflections. The first one was associated with activity in adjacent atrial myocardium, whereas the second was caused by activation in cardiac muscle of the PV beneath the recording electrode. Configuration of the electrograms recorded in the dog pulmonary veins were similar to those observed in the patients. Amplitudes of electrograms from the PVs were usually smaller than those recorded in the superior vena cava and right atrium.

3.1. Double potentials

Clinical studies in patients with AF revealed both double potentials and fractionated electrograms (Fig. 2). Multi-component electrograms were, however, not confined to patients suffering from AF. Hwang et al. recorded double potentials in the left superior PV in two groups of patients [8]. Group I included patients with an accessory pathway, whereas group II consisted of patients with idiopathic paroxysmal AF. These investigators showed that, in both groups, double potentials were recorded inside the left superior PV during sinus rhythm and premature atrial contractions. The first potential was due to activation of the atrium, whereas the second was caused by activation underneath the recording electrode within the pulmonary veins. The increased separation of the double potentials in the distal part of the PVs was caused by late activation of
the muscular structure in this part of the vein. The time relation between remote and local deflections reverses when activation starts at the distal end of the PV (Fig. 3). Data from Fig. 3 have been obtained from a Langendorff-perfused dog heart, in which electrical activity of the left inferior PV was recorded with a 247-point plaque electrode during stimulation from the ostium (Fig. 3A) or the distal end (Fig. 3B). Laplacian signals, highlighting deflections generated by local activation, have been calculated and show that the sequence of local (PV) and remote (atrial) activation reverses when the site of stimulation is changed.

In a clinical study, Hocini et al. recorded PV electrograms in 20 patients with drug-resistant paroxysmal AF and compared them to those of control patients without AF and PV ectopy [28]. A comparable number of PV potentials was found in both groups. However, PV activity was more complex in patients with AF (3.1±1.4 vs. 2.4±0.6 deflections). In addition, the interval between atrial and PV potentials was longer in patients with AF than in controls (34±0.8 vs. 21±11 ms). A similar difference was observed for the number of late PV potentials (interval between atrial and PV potentials ≥25 ms).

### 3.2. Fractionated electrograms

In the human heart, characteristics of electrograms vary between the different PVs. Hsieh et al. analyzed PV electrograms from 169 ectopic foci both during sinus rhythm and ectopic beats and concluded that electrogram characteristics were different among the four PVs [6]. During ectopic beats, electrograms in the PVs were either double, showing spikes followed by atrial deflections, fragmented, or fused. The incidence of conduction block in the PVs during initiation of AF was higher in the inferior PV as compared to the superior PV. In addition, conduction block occurred more frequently in the distal part of the PVs, which is compatible with the reduced amount of myocardial tissue at the distal ends of the PVs. It was also found that the amplitude of the electrograms in the PVs was larger in the left compared to the right PV and that electrogram duration in the superior PV exceeded that of the inferior PV during sinus rhythm.

In another study, the same group determined extracellular electrograms at the ostia of the left and right superior pulmonary veins in patients with and without paroxysmal AF [29]. Duration of the fractionated electrograms significantly changed when the site of stimulation was changed from the high right atrium to the coronary sinus. This observation, together with the observed complex fiber orientation at the ostium of the superior pulmonary vein [17] suggests that non-uniform anisotropic electrical activity is responsible for the fractionated electrograms in the pulmonary veins. This is compatible with observations we made in Langendorff-perfused dog hearts, where we determined spread of activation within the PVs using high resolution extracellular mapping. Stimulation was performed from three sites within the pulmonary veins: the ostium, the apex and a site in between. Activation maps during basic cycle length and premature stimulation were correlated with histology. Comparison of electrophysiologic and histologic data revealed that abnormal conduction, evidenced by fractionated electrograms, is related to structural complexity at sites in the PVs where muscle bundles branch or join with other bundles [30]. The functional role of structural complexities for propagation of the electrical impulse has been extensively studied by
Fig. 3. Double potentials recorded in the pulmonary vein of a Langendorff perfused dog heart. Recordings were made with a 247-point multiterminal electrode (19×13 grid; interelectrode distance 0.3 mm). The activation maps show spread of activation during stimulation at BCL 500 ms. Recordings show electrograms recorded at the black dot. Tracings marked U are unipolar electrograms. Tracings marked L are Laplacians, determined by subtracting the weighted sum of surrounding electrograms from that recorded at the central (black dot) electrode. Deflections generated by remote activity are suppressed. In contrast to bipolar recordings, the Laplacian is direction-independent. The panels at right are maps of activation of the pulmonary vein during stimulation at the ostium (A) and distal end (B). Lines are isochronal lines at 2-ms intervals. Numbers are activation times referenced to the time of stimulation. Arrows show main spread of activation. The unipolar tracing in (A) shows a deflection caused by remote atrial activation (marked a) preceding the local deflection p caused by activation in the atrial sleeve of the pulmonary vein. This is evidenced by the Laplacian recording, which shows only one deflection, corresponding to the second, local, component in the unipolar tracing. Tracings in (B), obtained during stimulation at the apex of the pulmonary vein, show that the remote atrial electrogram (a) arises after the local deflection of the pulmonary vein (p). The inset shows the location of the plaque electrode. L, Laplacian signal; U, unipolar electrogram; a, atrial deflection; p, deflection caused by activation of the myocardial sleeve in the pulmonary vein; s, stimulus; PV, pulmonary vein.

Spach and co-workers, and this architecture might well explain the conduction abnormalities and complex electrograms in the PVs [14,31].

4. Intracellular recordings

Masani showed that the myocardial layer in the PVs of rats revealed ordinary myocardial cells resembling those of atrial myocardium, but also cells with structural features of the fine structure of sinus node pacemaker cells [25]. These cells were present in intrapulmonary preterminal portions of the PVs and were intermingled, singly or in small groups, with myocardial cells. The investigator did not carry out electrophysiologic measurements to confirm the nodal characteristics of these cells.

Cheung showed that spontaneous activity of the PVs of the guinea pig could occur [24]. In this model, differences in action potential characteristics were present between cells at the distal end of the PVs and those at the ostium. Cardiac muscle was found at the proximal end of the PVs, whereas smooth muscle was present at the distal intrapulmonary end. Intracellular recordings from the smooth muscle cells showed stable membrane potentials of −56 to −60 mV, but action potentials could not be elicited in these cells. Thus, the smooth muscle cells of the PVs play no role in the generation of ectopic beats and/or slow conduction of the impulse in the myocardial sleeves of the PVs.

The cardiac muscle had resting membrane potentials of −66 mV at the distal end and −71 mV near the ostial side. In these cells, action potentials could be elicited. Action potentials recorded from cells at the proximal part of the veins resembled those of the atrium and duration was
longer compared to those recorded at the distal end. These differences in action potential characteristics are compatible with observations we made in the dog pulmonary veins [30] and recordings of the refractory period made by Chen and coworkers in patients with AF [23,29]. These authors showed that the effective refractory period at the proximal sites of the right superior PVs and left superior PVs was significantly longer than refractory periods determined at the distal sites.

Spontaneous activity in the PVs of the guinea pig, was observed in about half of the hearts [24]. Spontaneous activity occurred through automaticity and was seen at the distal end of the PVs. Depolarization started at a maximal diastolic potential of $-66 \text{ mV}$ and transition from diastolic depolarization to the upstroke of the action potential was gradual. In non-spontaneously active preparations addition of noradrenaline initiated spontaneous action potentials in cardiac cells at the distal end of the PVs. No activity was elicited in the smooth muscle portion of the pulmonary veins. Spontaneous activity of cardiac cells at the distal end could also be inhibited by perivascular nerve stimulation, which resulted in hyperpolarization of the membrane.

Recently, Chen et al. studied transmembrane potentials in superfused pulmonary vein preparations of the dog heart. Pulmonary veins were harvested from normal healthy dog hearts after 6–8 weeks rapid atrial pacing [32]. Cells near the distal end of the veins, which they presumed to be smooth muscle cells, had resting membrane potentials of $-76 \text{ mV}$ and were unable to generate action potentials upon stimulation. Cells at the ostium of the pulmonary veins revealed action potentials similar to those of atrial muscle cells. At the distal end of the myocardial sleeves spontaneously occurring action potentials were recorded. These action potentials either showed diastolic depolarization as observed in pacemaker cells of the sinus node or a fast depolarization followed by a rapid phase 3 repolarization without a plateau. Some of the spontaneously occurring action potentials revealed early after depolarizations. Action potentials recorded in the pulmonary veins of dog hearts that were chronically paced for 6–8 weeks were significantly shorter than those of healthy dogs and the incidence of early after depolarizations was greater. These investigators found spontaneous activity with a frequency ranging from 0.2 to 6 Hz, in 71% of the healthy dogs. In preparations that were not spontaneously active they were able to induce activity by the application of isoproterenol. Propranolol, acetylcholine, nifedipine and adenosine depressed the spontaneous activity. Early after-depolarization-related, high-frequency, irregular rhythm in the pulmonary veins was suppressed by TTX and $\beta$-sotalol.

In contrast to the observations made by Cheung and Chen, we were unable to elicit spontaneous activity or after depolarizations in our dog hearts, either by burst pacing, or by addition of norepinephrine or ouabain [30]. Differences in the experimental setup, in vitro versus Langendorff-perfusion, might explain this discrepancy.

Clinical studies by Chen et al. showed that $\beta$-blockers, calcium blockers and procainamide could suppress spontaneous ectopic beats and AF from PVs, suggesting abnormal automaticity or triggered activity as a mechanism for ectopic activity in patients [23]. Abnormal automaticity or triggered activity is further supported by observations that show: (1) provocation of ectopy by postpacing pauses with or without supplemental isoprenaline infusion, (2) dissociation of the local PV spike from atrial activity as a slow automatic rhythm or rapid dissociated rhythm at cycle lengths as short as 160 ms after radiofrequency ablation.

### 5. Ectopic beats

Characteristics of typical PV ectopic electrograms may vary from sharp deflections with short-duration to wide, highly fractionated electrograms [3,23]. During ectopic beats, PV activity precedes the far field atrial potentials. Both intra PV conduction delay and block may occur. Conduction time to the left atrium has been observed to be as large as 160 ms, and decremental conduction occurred with increasing prematurity [33]. Spikes closely coupled to the previous sinus beat were not conducted to the left atrium and were thus not followed by an ectopic P-wave (‘concealed’ PV extrasystole, Fig. 4). In most cases, the spike is also identified in sinus rhythm at the end of the multicomponent atrial activity, with this sequence inverting during extrasystoles in the source vein of ectopy.

Differences in the refractory period between the proximal and distal parts of the PVs may account for the occurrence of conduction block. Chen et al. demonstrated conduction block in 34% of the patients of which 70% had block in the right superior PV [23]. They attributed this high percentage to the more complex arrangement of the myocardial sleeve with more anisotropic conduction properties in the right superior PV as compared to the left superior PV. Among the four pulmonary veins, ectopic atrial activity is generated most commonly in the left superior pulmonary vein, followed by the right superior PV [23]. In contrast to PV potentials recorded in the right PV, potentials at the ostial side of the left PV may be masked because of superimposed left atrial deflections during sinus rhythm. However, during ectopy activity, double potentials are visible. It has been shown that in nearly 2/3 of the patients, PV potentials appeared to be absent in the left veins during sinus rhythm, whereas they were consistently visible in the right superior veins [34]. Distal coronary sinus or left atrium pacing unmasked and separated left PV potentials from left atrial potentials.

Ectopic beats that induce AF may be single or occur in bursts, the majority being single [35]. Using a circumferential PV catheter equipped with ten electrode terminals, Hocini et al. showed that multiple sources and variable activation patterns occurred in 53% of the cases they studied, indicating that multiple arrhythmogenic foci were
shown that the mean cycle length was shorter in the veins as compared to those in the left atrial free wall [39]. This might suggest that the ligament of Marshall and PVs were the sources of rapid activation in this model. The investigators could, however, not exclude multiple wavelet reentry as the mechanism for AF, because the veins were not electrically isolated. The veins could have been activated by a source in the atrium having a lower rate. The highly anisotropic regions in the veins can produce more disorganized electrograms than the left atrial free wall and therefore suggest a higher rate in the veins.

6. Conclusions

Histologic studies in human and animal hearts have shown that atrial myocardium extends into the pulmonary veins at variable distances. Electrograms recorded in the PVs reveal double and often fractionated characteristics. Double potentials reflect remote atrial activation together with activation of the atrial sleeves within the PVs. Fractionation of the electrograms can be attributed to the anisotropic characteristics of the atrial myocardium within the PVs. This anisotropic structure, together with the observation that the refractory period is shorter at the distal end of the PVs compared to the proximal end, may explain the frequent occurrence of conduction block in the pulmonary veins. Few studies address the mechanism of ectopic beats in the pulmonary veins. Animal experiments together with pharmacologic interventions in patients suggest that abnormal automaticity or triggered activity is the most likely candidate. The anisotropic characteristics of the PVs may enhance the ability of a focus to activate the PVs and the atrium and lead to atrial fibrillation.

References

[7] Chen SA, Tai CT, Yu WC et al. Right atrial focal atrial fibrillation: