

Electrophysiological Characteristics of Arterially-Perfused Canine Pulmonary Veins: Role of the Delayed Afterdepolarization-Induced Triggered Activity

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ABSTRACT

Background and Objectives : The mechanism responsible for the generation of ectopic beats in pulmonary veins (PVs) remains to be well defined. The present study examines the electrophysiological characteristics of the PVs and other regions of the canine left atrium (LA) under low dose (300 μ M) caffeine condition. **Materials and Methods :** Transmembrane action potentials were recorded from the left superior PVs, PV-LA junctions (PLJ, atrium <5 mm from the PV ostium), LA appendage (LAA) or Bachmann's bundle (BB) in arterially perfused canine LA-PV preparations, using floating glass microelectrodes. Rapid atrial pacing (cycle lengths 140-300 ms, 10 sec) was used to induce delayed afterdepolarizations (DAD) at the baseline and under low dose (300 μ M) caffeine conditions. **Results :** Spontaneous diastolic depolarization or triggered activity (TA) was not observed in any of the recording area under the baseline condition. DAD and TA were induced by caffeine in 4/8 PVs and in 3/8 PLJs, but in no LAA (0/6) or BB (0/5). These TA and DAD were also observed after termination of pacing-induced atrial tachyarrhythmia. DAD was abolished by pretreatment of the atria with verapamil or propranolol (1.0 μ M). **Conclusion :** Spontaneous diastolic depolarization was not present in perfused canine left atria or proximal PV. Pulmonary veins and adjacent areas displayed an increased susceptibility to develop DAD-induced TA under low dose caffeine condition. This distinctive electrophysiological property of the PV and PLJ area may contribute to the arrhythmogenic substrate responsible for the ectopic activity that initiates atrial fibrillation. (Korean Circulation J 2005;35:643-648)

KEY WORDS : Pulmonary veins ; Calcium ; Atrial fibrillation.

Introduction

Atrial fibrillation (AF) can originate from rapidly firing foci in pulmonary veins (PVs).^{1,2)} The cellular electrophysiological mechanism responsible for the initiation of AF remains poorly understood.^{1,2)} In the present study, the basic electrophysiological characteristics of the pulmonary vein myocardial sleeves were evaluated in arterially perfused canine LA-left superior pulmonary vein (LSPV) preparations. Rapid atrial pacing was used to provoke PV ectopic discharges under the baseline and

cellular Ca overload conditions using low dose caffeine.

Materials and Methods

Arterially perfused canine LA-LSPV preparation

Animals were handled according to the National Institutes of Health guidelines. Eight mongrel dogs (Martin Creek Kennels, Williford, Ark), weighing 20 to 25 kg, were anticoagulated with heparin and anesthetized with pentobarbital (30 to 35 mg/kg IV). The chest was opened via a left thoracotomy, and the heart excised, placed in a cardioplegic solution, consisting of cold (4°C) Tyrode's solution containing 8.5 mM [K⁺]_o, and transported to a dissection tray. After removal of the ventricles, the ostium of the left coronary artery was cannulated with polyethylene tubing (ID, 1.75 mm; OD, 2.1 mm), and the preparation perfused with cold Tyrode's solution (12°C to 15°C) containing 8.5 mM [K⁺]_o. With con-

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tinuous coronary perfusion, all ventricular branches of the left coronary artery were immediately clamped with metal clips. To simplify preparations and to minimize the number of sutures, the left circumflex coronary artery was ligated in between the ostium and the ligament of Marshall. With this limited left atrial perfusion, only a block of left atrial tissue, which consisted of the LA appendage (LAA), Bachmann's bundle (BB) and left superior PV (LSPV), could be perfused consistently (Fig. 1). Ventricular branches of the left circumflex coronary artery and the cut atrial branches were ligated with silk thread. The left atrium was carefully dissected to remove unperfused tissues. The preparation was placed in a temperature-controlled bath ($8 \times 6 \times 3$ cm) and perfused, at a rate of 8 to 12 mL/min, with Tyrode's solution ($36.5 \pm 0.5^\circ\text{C}$). The perfusate was delivered to the artery by a roller pump (Cole Parmer Instrument Co). An air trap was used to avoid bubbles in the perfusion line.

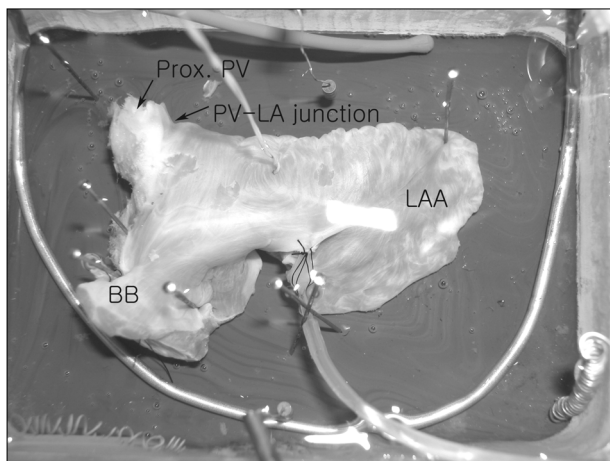


Fig. 1. Photograph of the LA-LSPV preparation. A block of LA tissue containing BB, LAA and LSPV is shown. The junctional area between the PV and LA is marked with an arrow. LA: left atrial, LSPV: left superior pulmonary vein, BB: Bachmann's bundle, LAA: left atrial appendage, PV: pulmonary vein.

The composition of the Tyrode's solution was (in mM): NaCl 129, KCl 4, NaH_2PO_4 0.9, NaHCO_3 20, CaCl_2 1.8, MgSO_4 0.5 and D-glucose 5.5, buffered with 95% O_2 and 5% CO_2 . Transmembrane action potential recordings were obtained with floating glass microelectrodes (2.7 M KCl, 10 to 25 M Ω DC resistance) connected to a high-input impedance amplification system (World Precision Instruments). The signals were displayed on oscilloscopes, amplified, digitized (Cambridge Electronic Design) and stored on a computer hard drive or to compact disk. A pseudo-ECG was recorded using 2 AgCl half-cells placed in the bath solution, 1.0 to 1.2 cm from opposite ends of the atrial preparation.

Drugs

Caffeine, propranolol and verapamil (dissolved in distilled water) were prepared fresh as stocks of 100 mM (caffeine) and 1.0 mM (propranolol, verapamil) before each experiment. The final concentration of caffeine was 300 μM , and those of propranolol and verapamil were 1.0 μM .

Experimental protocols

Coronary-perfused, spontaneously beating atrial preparations were equilibrated in the tissue bath until electrically stable, usually 30 minutes, and then paced at a basic cycle length (CL) of 2,000 ms, with an insulated pair of thin silver electrodes, with the exception of at their tips. Stimuli were biphasic rectangular pulses of 2-ms duration and three times the diastolic threshold intensity. The effect of caffeine was measured after 10-15 minutes of exposure. The rapid atrial pacing interval started at 500 ms, and was then progressively shortened, in 100 to 50 ms increments to 200 ms, and then in 20-ms increments down to 140 ms. Each pacing train was continued for 10 seconds, with an interval of 20-30 se-

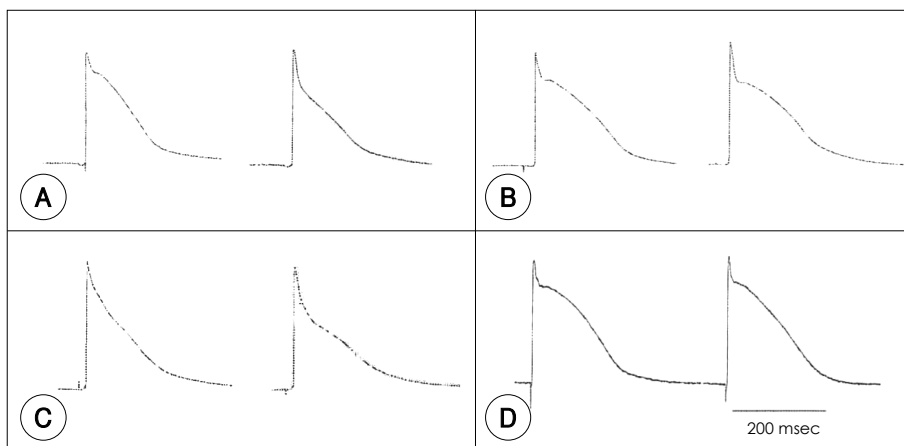


Fig. 2. Action potentials (APs) recorded from pulmonary vein myocardial sleeves (PV), the PV-LA junctional area (PLJ), left atrial appendage (LAA) and Bachmann's bundle (BB) in perfused canine left atrial (LA)-LSPV preparations (Fig. 2A-D). In each panel, the AP in the left is recorded at the baseline, and that in the right was recorded after caffeine (300 μM) infusion. Median APD_{90} of the PV, PLJ, LAA and BB areas at a pacing cycle length of 2,000 ms were not significantly different, while the median APD_{50} tended to abbreviate slightly after caffeine infusion, APD_{90} : AP duration at 90% of repolarization.

conds between pacing trains. The effect of propranolol or verapamil was measured after 10 minutes of exposure.

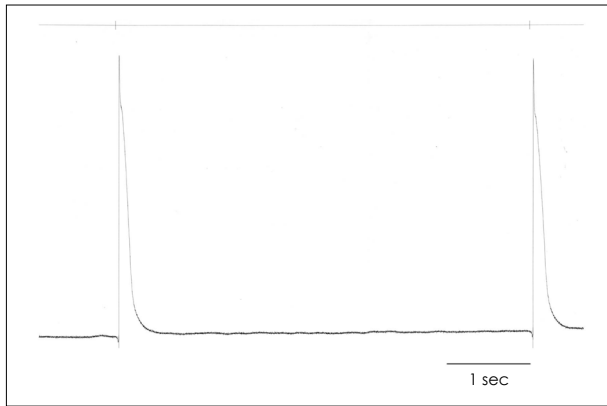


Fig. 3. Absence of spontaneous phase 4 depolarization. Action potential was recorded from the proximal pulmonary vein. Spontaneous phase 4 depolarization was not recorded at the baseline or after a low dose ($300 \mu\text{M}$) caffeine infusion. The horizontal bar in the upper part of the figure represents the pacing protocol.

Statistics

Statistical analysis was performed with SPSS ver. 11.0. Kruskal-Wallis test was used for the analysis of the AP duration in different atrial regions and the PV. Data are expressed as the median (25, 75 percentile). A $p < 0.05$ was considered significant.

Results

Electrophysiological characteristics of PV myocardial sleeve in LA-LSPV preparations

The action potentials were recorded randomly from the PV (proximal LSPV), PV-LA junction (PLJ, approximately within 5 mm from the border of PV and LA), LA appendage (LAA) and Bachmann's bundle (BB) area in 8 dogs. During baseline pacing at 2,000 ms, the AP durations at 90% repolarization (APD_{90}) were not significantly different among the proximal PV, PLJ and LAA areas. After infusion of low dose caffeine, the action potential plateau decreased and the APD_{50} tended to ab-

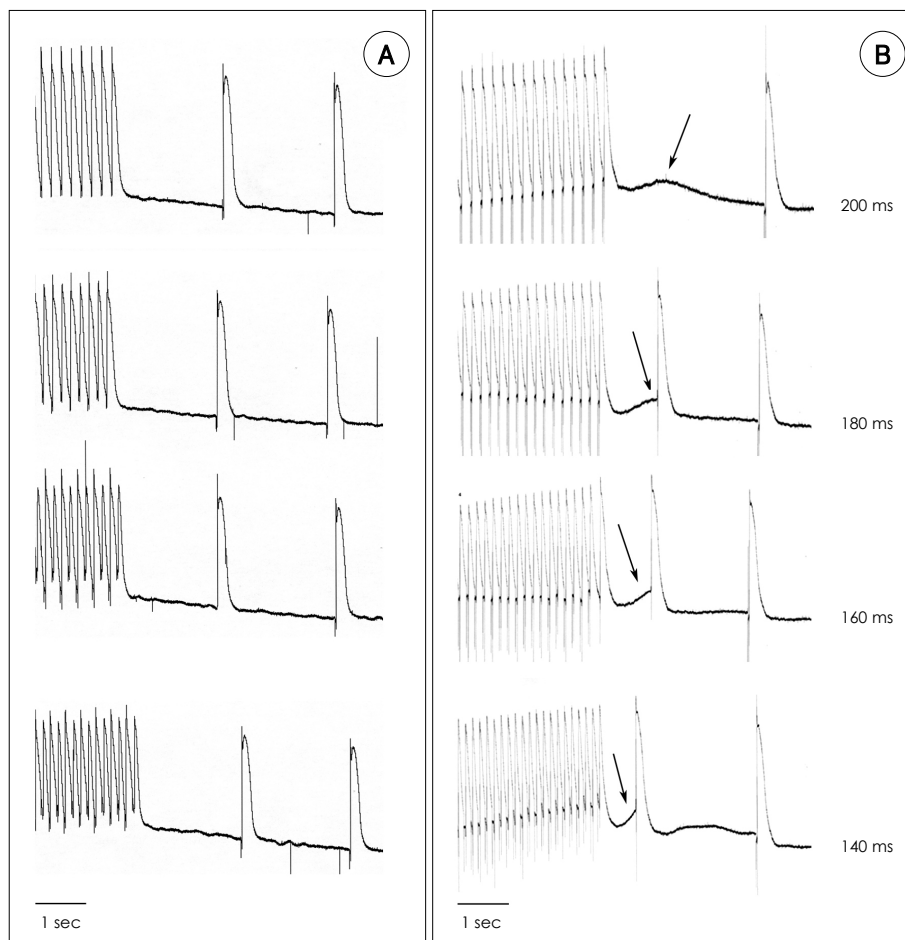


Fig. 4. Delayed afterdepolarization (DAD) and triggered activity (TA) induced by rapid pacing under the low dose caffeine conditions. A: to assess the presence of delayed afterdepolarization (DAD), the LA-PV preparation was paced at rapid rates (CL 200 to 140 ms) for 10 seconds. DAD or triggered activity was not recorded in the PV (0/8), PLJ (0/8), LAA (0/6) or BB (0/5) recording areas. B: under the conditions of low dose caffeine ($300 \mu\text{M}$), the pulmonary vein (PV) or PV-LA junctional area (PLJ) showed delayed afterdepolarizations (DADs) after rapid atrial pacing. These began to develop at PCL of 200 ms, and became prominent at PCL of 180-140 ms. After reaching a threshold, the DADs produced action potentials. DAD was not recorded in the LA appendage or Bachmann's bundle at PCL down to 140 ms. CL: cycle length, LAA: left atrial appendage, BB: Bachmann's bundle, PCL: pacing cycle length, LA-PA: left atrial-pulmonary vein.

breviate, but the APD₉₀ did not change significantly (Fig. 2A-D). To record the presence of spontaneous depolarization, preparations were paced at slow rates (CL 5,000-2,000 ms). At the baseline or under low dose caffeine conditions, spontaneous phase 4 depolarization was not observed in any of the PV, PLJ, LAA or BB regions (Fig. 3). Pacing at cycle lengths of 500 to 140 ms did not yield DAD or TA in the PV (0/8), PLJ (0/8), LAA (0/6) or BB (0/5) recording areas (Fig. 4A).

DAD-induced TA under low dose caffeine condition

Under the condition of low dose caffeine (300 μ M), rapid atrial pacing induced DADs, both with and without TAs, usually at pacing CLs of <200 ms. These DADs were recorded in 4/8 PVs and 3/8 PLJs, but in no LAA (0/6) or BB (0/5) recording areas (Fig. 4B). In most

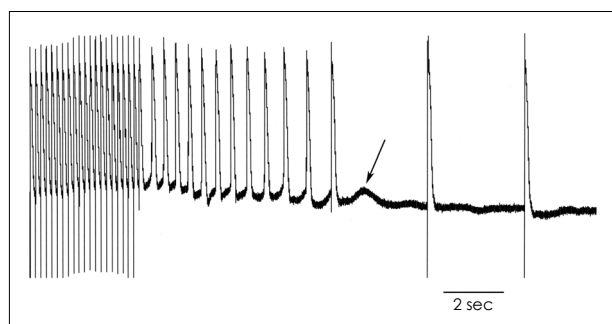


Fig. 5. Delayed afterdepolarization (DAD) in a pulmonary vein after spontaneous termination of pacing-induced atrial tachycardia under low dose caffeine conditions. After brief atrial pacing, at a pacing cycle length of 180 ms, in the presence of caffeine (300 μ M), DADs that reached the threshold potential induced a nonsustained atrial tachycardia. The last beat of the induced tachycardia was followed by a small DAD (arrow), which failed to produce a triggered beat. The horizontal bar in the upper part of the figure represents the pacing protocol.

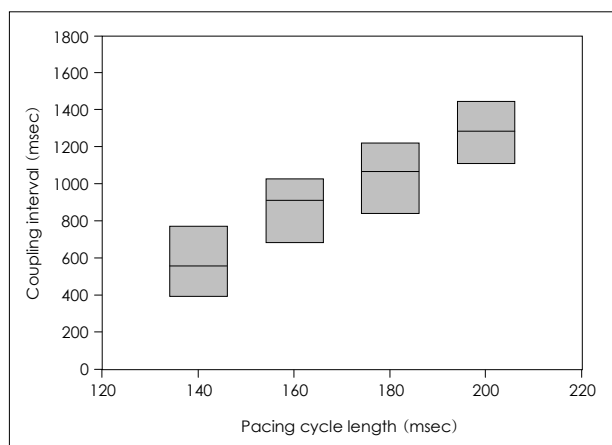


Fig. 6. Relationship between pacing cycle length (PCL) and coupling interval (CI) of triggered activity. Under the low dose caffeine conditions, brief atrial pacing induced a delayed afterdepolarization (DAD) and triggered activity (TA). The amplitudes of the DADs, which were initially below the threshold of action potentials, produced triggered activity as the pacing rate progressively increased. The CI of the induced triggered activity showed a direct relationship with PCL. Bar graphs represent the median (25, 75 percentile) CI values vs. CL obtained from 4 experiments. CL: cycle length.

cases, the DADs were followed by TA during the pacing protocol. In some rare cases, these DADs induced triggered beats, in the form of nonsustained atrial tachycardia. After spontaneous termination of the induced atrial tachycardias, a small afterdepolarization, which failed to reach the membrane threshold, was observed (Fig. 5). The amplitudes of DADs, which were initially below the threshold of action potentials, produced triggered activity as the pacing rate was progressively increased. The coupling intervals of the triggered beat showed a direct relationship with pacing CLs (Fig. 6). Verapamil (1.0 μ M, n=2) or propranolol (1.0 μ M, n=2) completely abolished the DAD and TA in preparations where caffeine induced DAD and TA had occurred before addition of the drugs (Fig. 7).

Discussion

Recent recognition of PVs as a major source of ectopic activity in AF has renewed interest in the mechanism of AF. A re-entry mechanism, facilitated by slow conduction and heterogeneous repolarization in PV myocardial sleeves, has been proposed.^{3,4)} However, the role of afterdepolarization and triggered activity in the precipitation of ectopic beat or maintenance of AF has been largely unknown. The present study has demonstrated, in arterially perfused canine atrial preparations, the presence of DAD-induced TA under conditions of Ca⁺⁺-overload, and an increased susceptibility of the PV and PLJ areas to develop DAD-induced ectopic beats compared to the LAA or BB areas.

Mechanism of arrhythmogenicity in PVs

Rapid, repetitive firing from PVs underlie the mechanism for the initiation or maintenance of AF.^{1,5,6)} From the clinical electrophysiological data, all of the three mechanisms of cardiac arrhythmia, i.e., re-entry, triggered activity and automaticity, have been implicated in PV arrhythmogenesis.^{1,4)} The complex tissue geometry in PV myocardial sleeves, together with its heterogeneous repolarization characteristics, are believed to facilitate re-entrant tachyarrhythmias.^{4,7)} Recently, re-entrant circuits in PVs have been demonstrated in arterially perfused canine atrial preparations using optical mapping techniques.³⁾ The possibility of triggered activity, especially in the first beats initiating these rapid paroxysms, has been suggested from clinical tracings¹⁾ and in single cell studies.⁵⁾ Experiments performed in single PV myocytes demonstrated pacemaker activity and delayed after depolarization (DAD)-like membrane oscillations.⁵⁾ In addition, cells from pacing-induced, remodeled atria showed larger transient inward current than those in the control dogs.⁵⁾ Arora et al.³⁾ also observed sustained focal discharge in the presence of isoproterenol in coronary-perfused, isolated whole-atrial preparations. These

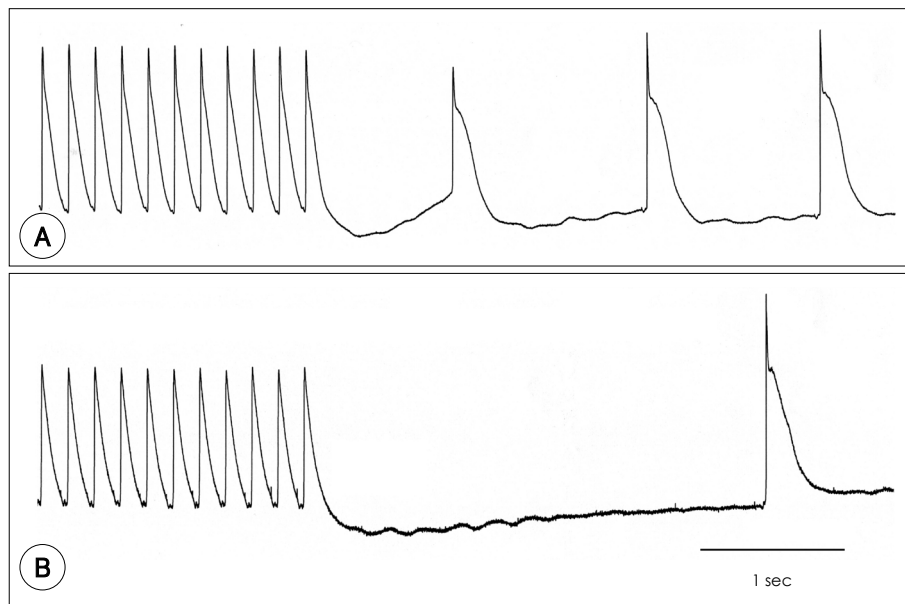


Fig. 7. Response to verapamil. A: rapid atrial pacing (RAP), at a pacing cycle length (PCL) of 180 ms, in the presence of caffeine (300 μ M) induced delayed afterdepolarization (DAD) in the PV area. B: after pretreatment with verapamil (1.0 μ M), RAP at the same pacing cycle length failed to induce DAD or TA. PV: pulmonary vein, TA: triggered activity.

results suggest that DAD-induced TA could play a significant role in the initiation of AF, especially under catecholamine stimulation or in electrically remodeled atria. However, isolated myocytes lack intercellular coupling, and are potentially subject to damage during the cell isolation procedure. In addition, the experimental results from superfused canine atria are not uniformly consistent.^{7,8)} In these regards, the presence of DADs in electrically-coupled, perfused atrial preparations remains largely unknown.

Because of the complex arterial supply in the left atrium and PV regions, a simplified left atrial preparations, consisting only of the LAA, BB and LSPV, was devised in this study. Our study has demonstrated that PVs and the adjacent junctional area show DAD-induced TA under conditions of Ca^{++} -overload, while other areas (LAA or BB) failed to show DADs. Caffeine has been known to induce DADs and TAs by increasing the intracellular concentration of Ca^{++} .^{9,10)} The mechanism for this increased susceptibility of the PV to develop DAD and TA is not clear from the present study, but may be attributable to the differences in the constitution of the ionic currents in the PV and LA. Ehlich et al.¹¹⁾ reported that canine PV myocytes showed a decreased density of inward rectifier K^+ current (I_{K1}) compared with cells in the left atrium. The reduced I_{K1} lowers the threshold for I_{ti} -triggered AP by allowing I_{NaCa} to produce greater depolarization.¹²⁾ Although the diastolic potential could not be directly measured in this study, due to the limitation of the floating microelectrodes, it is likely that the cells in the PV myocardial sleeve in our study had reduced resting membrane potentials. The density of I_{NaCa} was

not reported to be significantly different.¹¹⁾ In addition, an increased tissue anisotropy in PV myocardial sleeves could have facilitated the development of ectopic focal activity by enhancing its propagation to the surrounding tissue.¹³⁾ A difference in the SR (Sarcoplasmic Reticulum) Ca^{++} handling between the PV and atrial myocytes is also a possibility for this differential response, and is an area for future research.

Previous studies have shown that PV myocytes show spontaneous phase 4 depolarizations.¹⁴⁾ The diastolic potentials of the canine PVs in the present study were extremely stable. Spontaneous phase 4 depolarization was not recorded in any of our perfused atrial preparations. The reason for the absence of automaticity in our study is unclear. The PV action potentials were recorded at the proximal half of the veins; while the spontaneous depolarization has been shown to be more prominent in the distal part of the PVs. Inhibition of diastolic depolarization due to tight electrotonic coupling between cells in our perfused atrial preparations could also have been operative.

Clinical implications

Our experimental conditions paralleled the clinical situations of the burst PV firing¹⁷⁾ in acute tachycardia-induced atrial remodeling. In the acute stage of atrial fibrillation or tachycardia, cellular Ca^{++} -overload develops. As the PV and adjacent area are more prone to develop DAD-induced TA, they are expected to initiate ectopic activities shortly after termination of AF. The importance of the PV in the reinitiation of AF after cardioversion has been demonstrated in a recent clinical

cal study.⁶⁾ The second clinical implication of the present study is the importance of the PV-LA junctional area. Our results suggest the peri-PV area is as important as the PV in the development of DAD-induced TA. The current ablation strategy focuses on the dissociation of PV muscular inputs from their connection to the left atrium. Our data support the results of recent ablation trials, emphasizing the importance of the peri-PV area in the maintenance of sinus rhythm after AF ablation. Radiofrequency ablation performed 1-2 cm away from the ostium of PV was significantly more effective than segmental PV isolation in the maintenance of sinus rhythm.¹⁵⁾¹⁶⁾

Study limitations

Arterial supply in the left atrium was more complex than that of the right atrium. There were many small branches from the left circumflex coronary artery, and ligation of all the branches was extremely difficult, especially in the whole left atrial preparations. For this reason, our preparation was modified from whole left atrial to LAA-PV preparation, which minimized, by reducing the preparation size, any possible ischemic insults during the experiments. In addition, triangular, ischemic action potentials were excluded from the analysis. The changes in the ionic currents were not evaluated, and future studies should be directed at the subcellular level to find the possible ionic mechanisms of this action potential study.¹⁸⁾

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