ELSEVIER

Cardiovascular Research 48 (2000) 265–273

Cardiovascular Research

www.elsevier.com/locate/cardiores
www.elsevier.nl/locate/cardiores

Arrhythmogenic activity of cardiac muscle in pulmonary veins of the dog: implication for the genesis of atrial fibrillation

Yi-Jen Chen[a], Shih-Ann Chen[a], Mau-Song Chang[a], Cheng-I Lin[b,*]

[a]*Department of Medicine and Institute of Clinical Medicine, National Yang-Ming University School of Medicine, Veterans General Hospital-Taipei, Taipei Medical University, Affiliated Wan-Fang Hospital, Taipei, Taiwan*
[b]*Institute of Pharmacology and Department of Biomedical Engineering, National Defense Medical Center, Taipei, Taiwan*

Received 10 February 2000; accepted 23 June 2000

Abstract

Objective: Pulmonary veins are important foci of ectopic beats to initiate paroxysmal atrial fibrillation. The purpose of this study were to investigate the electrophysiological characteristics of excitable cells in canine pulmonary veins obtained from healthy and chronic rapid atrial pacing dogs and their responses to cardioactive agents. **Methods:** Transmembrane action potentials (APs) were recorded from multiple sites of pulmonary veins isolated from 17 healthy dogs and 14 dogs with chronic (6–8 weeks) rapid atrial pacing (780 bpm). **Results:** In normal superfusate, several types of electrical activities were identified, including silent electrical activity, fast response APs driven by electrical stimulation, and spontaneous fast or slow response APs (with or without early afterdepolarizations). The incidences of AP with an early afterdepolarization (93% versus 41%) was greater in chronic pacing dogs. The spontaneous activities were depressed by beta-adrenoceptor blocker, calcium channel blocker, adenosine and acetylcholine. High frequency (>8 Hz) irregular rhythms occurred spontaneously or were induced by cardioactive agents or electrical stimuli. The incidence of spontaneously occurring tachyarrhythmias was much higher in preparations from chronic pacing dogs (93%) than from control (12%). The tachyarrhythmias were suppressed by sodium channel blocker, potassium channel blocker or magnesium. **Conclusions:** Pulmonary veins have arrhythmogenic ability through spontaneous activities or high-frequency irregular rhythms. The higher incidence of spontaneously occurring high-frequency irregular rhythms in chronic rapid atrial pacing dogs may account for the increased risk of atrial fibrillation in these dogs.

Keywords: Supraventr. arrhythmia; Membrane potential; Arrhythmia (mechanisms); Impulse formation

1. Introduction

Recent studies have shown that pulmonary veins (PVs) are important sources of ectopic beats which could initiate paroxysmal atrial fibrillation [1,2]. PVs have also been demonstrated to be the foci of ectopic atrial tachycardia and focal atrial fibrillation [3,4]. Atrial fibrillation can be cured through successful radiofrequency ablation of the ectopic foci in the PVs [1,2,4]. Previous studies have demonstrated that PVs contain cardiac muscle connecting to the left atrium [5,6]. However, the electrophysiological and pharmacological characteristics of PVs are not clear. Moreover, knowledge about the reasons why PVs become arrhythmogenic and which antiarrhythmic agents were useful in suppressing the arrhythmogenic activity is also limited. It may be caused by triggered activity, enhanced automaticity, or micro-re-entry.

Chronic rapid atrial pacing has been demonstrated to be a useful model for the genesis of atrial fibrillation [7,8]. It is known that rapid atrial pacing may shorten the atrial refractoriness and facilitate the occurrence of atrial fibrillation [9–11]. In addition, slow conduction velocity and the increase of heterogeneity also promote the maintenance of atrial fibrillation [7,12]. However, whether rapid atrial

*Corresponding author. Tel.: +886-2-8792-3100 ext. 18655; fax: +886-2-8792-3900.
E-mail address: bme03@ndmctsgh.edu.tw (C.-I Lin).

Time for primary review 28 days.

0008-6363/00/$ – see front matter
PII: S0008-6363(00)00179-6

pacing could trigger the initiation of atrial fibrillation is not clear. Moreover, the roles of rapid atrial pacing in the arrhythmogenic activity and the changes of electrophysiological characteristics of PVs have not been evaluated yet. Therefore, the purpose of the present studies was to (a) investigate the electrophysiological characteristics of excitable cells in isolated canine PVs and their pharmacological responses to cardioactive agents; and (b) evaluate the effects of chronic rapid atrial pacing on the arrhythmogenic activity of PVs.

2. Materials and methods

2.1. Animal preparation

The investigation conforms with the institutional *Guide for the Care and Use of Laboratory Animals*. For the dogs subjected to chronic rapid atrial pacing, healthy mongrel dogs (weight, 15–20 kg) were anesthetized with sodium pentobarbital (30 mg/kg, i.v.). Additional doses were given as necessary to maintain anesthesia during the study. The dogs were artificially ventilated with room air by the use of a cuffed endotracheal tube and a constant volume cycled respirator. A circulation water blanket and controller were used to maintain body temperature at 37±1°C. The chest was opened through the right fifth intercostal space. The pericardium was incised to expose the heart which was then suspended in a pericardial cradle. A bipolar pacing wire (O Flexon, Davis Geck) was positioned in the high right atrium for high-frequency pacing at a rate of 780 bpm, 2-ms pulse duration and an output of 5 mA (Activitrax or Spectrax, Medtronics). After the experimental protocols, the animals were allowed to recover with proper care. The dogs received atrial pacing while conscious and freely moving for 6–8 weeks.

2.2. Tissue preparation

Electropharmacological investigations were conducted on preparations isolated from 17 healthy dogs and 14 dogs with chronic rapid atrial pacing. The chronic pacing dogs had sinus rhythm after stoppage of pacing. After the dogs were anesthetized with sodium pentobarbital (30 mg/kg, i.v.), the hearts were rapidly removed through a thoracotomy and dissected at room temperature in normal Tyrode solution with the composition (in mM) of 137 NaCl; 4 KCl; 15 $NaHCO_3$; 0.5 NaH_2PO_4; 0.5 $MgCl_2$; 2.7 $CaCl_2$, and 11 dextrose. Tyrode solution was equilibrated with a gas mixture of 97% O_2–3% CO_2, with a pH of around 7.4.

For dissection of the PVs, the left atrium was opened by an incision extending from the coronary sinus. The PVs (left superior in 16 healthy and 14 pacing dogs and one left inferior PV in one healthy dog) were separated from the left atrium about 5 mm proximal to the junction between PVs and left atrium. The veins were separated from the lung parenchyma through the incisions about 15 mm distal to the ending of myocardial sleeve. After dissection, proximal segments (close to left atrium, diameter around 4 mm) of the PVs were cut longitudinally. One end of the preparation (about 4 mm in width and 12 mm in length) consisting of PV and left atrial muscle was pinned to the bottom of a tissue bath. The other end was connected to a Grass FT03C force transducer with silk thread. An initial tension of 350 mg was applied to the preparation. The adventitia of the PVs and the epicardial surface of the left atrium faced upward. The tissue was superfused at a constant rate (3 ml/min) with Tyrode solution which was saturated with the 97% O_2–3% CO_2 gas mixture. Temperature was maintained constant at 37°C and the preparations were allowed to rest for 1 h before electrophysiological study.

Transmembrane action potential (AP) was recorded by means of machine-pulled glass capillary microelectrodes filled with 3 M KCl and connected to a WPI Duo 773 electrometer. Electrical and mechanical events were displayed simultaneously on a Gould 4072 oscilloscope and a Gould ES1000 recorder. Measurements were made of AP amplitude and AP duration (APD) at 50% (APD_{50}) and 90% repolarization (APD_{90}). The maximal rate of phase-0 depolarization (V_{max}) was determined by electronic differentiation as previously described [13]. Electrical stimuli of 2-ms duration with a suprathreshold strength (30% above the threshold) were provided by a Grass S88 stimulator through a Grass SIU5B stimulus isolation unit. Early afterdepolarization (EAD)-related high-frequency rhythms were defined as the cells generating oscillatory potentials at a depolarized level, as the AP failed to repolarize to the maximal diastolic potential.

2.3. Electrophysiological and pharmacological study

All preparations were studied initially in Tyrode solution. Isoproterenol (Sigma Chemical, St. Louis, MO, USA), and rapid stimulation with or without extrastimulation were used to induce oscillatory potentials or spontaneous activity. After spontaneous activities or tachyarrhythmias were recorded, adenosine (Sigma), acetylcholine (ACh, Sigma), propranolol (Sigma), nifedipine (Sigma), $MgCl_2$ (10 mM), tetrodotoxin (TTX, Sigma) or D-sotalol (a gift from Bristol–Myers Squibb) were used as pharmacological tools to explore the possible therapeutic interventions for tachyarrhythmias originated from PVs. These agents were randomly and separately perfused for 10 min to test pharmacological responses.

In 16 experiments, two microelectrodes were impaled simultaneously to record APs: one at atrial muscle and another at PV or at two different sites in the same PV, to observe presence or absence of conduction block.

2.4. Statistical analysis

All quantitative data are expressed as mean±S.D. The

differences between the before and after drug administration were analyzed by a paired Student's *t*-test. An unpaired *t*-test was applied to compare the differences between dogs with and without chronic rapid atrial pacing. Chi-square test with Yates' correction or Fisher's exact test was used for non-parametric data. A P value of <0.05 was considered to be statistically significant.

3. Results

3.1. Histological examination

The medial layer of the canine PVs were made up of both smooth muscle and cardiac muscle cells at the junction of the left atrium and the PVs (proximal end), and predominantly smooth muscle cells at the distal end. The myocardial sleeve was growing from the left atrium and covering the PVs from the outer layer, beneath the adventitia. Fig. 1 shows an example of the longitudinal section of a dog left superior PV.

3.2. Action potential characteristics of pulmonary veins in control and chronic pacing dogs

Several types of electrical activities were identified in the isolated canine PVs at a depth of 15–50 μm underneath the adventitia. Impalement of the microelectrode in 22 cells (presumably smooth muscle cells) near the distal end revealed stable resting membrane potentials of -76 ± 3 mV. Electrical stimulation at these sites did not induce APs. At the junction between the left atrium and PVs, fast response APs of atrial cells could be detected in response to suprathreshold electrical stimulation (Fig. 2A), similar to those of typical atrial myocardial muscle fibers. In the area surrounding the ending of the myocardial sleeve, spontaneous APs with various configurations were recorded. Certain spontaneous APs had a slow rate of phase-0 upstroke and a conspicuous diastolic depolarization (Fig. 2B), similar to those of pacemaker cells in the right atrium. Some spontaneous APs had a prominent phase-0 depolarization and a rapid phase-3 repolarization without plateau phase (Fig. 2C). Some of these spontaneous APs had EAD during plateau phase or phase-3 repolarization.

Table 1 summarizes AP parameters of 66 cells in preparations from 17 control dogs and 72 cells in preparations from 14 chronic pacing dogs. The APD_{50} and APD_{90} of fast response APs elicited by electrical stimulation in PVs of chronic pacing dogs were significantly shorter than those of healthy dogs ($P<0.05$). Also, the percentage of APs with an EAD (13 of 14, 93%, versus 7 of 17, 41%, $P<0.005$) was greater in the PVs of chronic pacing dogs than in those of healthy dogs.

3.3. Electropharmacological properties of spontaneous action potentials

As illustrated in Fig. 2B and C and characterized in

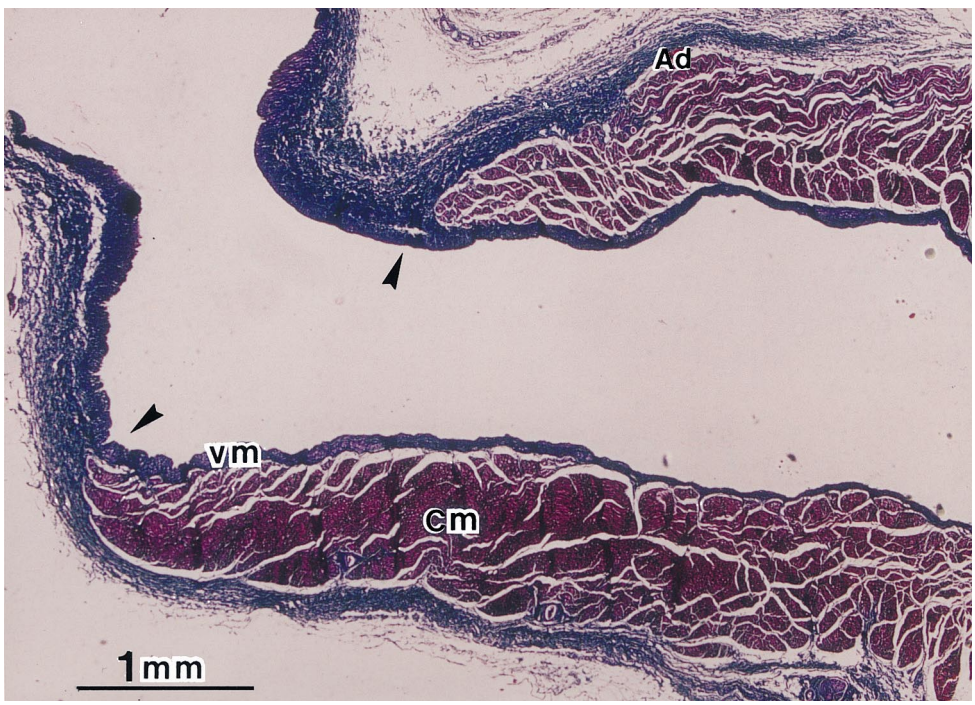

Fig. 1. A longitudinal section of dog left superior pulmonary vein near the myocardial sleeve. The preparation was stained with Masson's trichrome stain. Arrowheads indicate the junction of the sleeve and the vein. The proximal end is close by the left atrium and the distal end, the lung parenchyma. The vessel wall was composed of cardiac muscle (cm, stained dark red) and vascular smooth muscle (vm, stained dark blue), underneath the loosely packed adventitia (Ad).

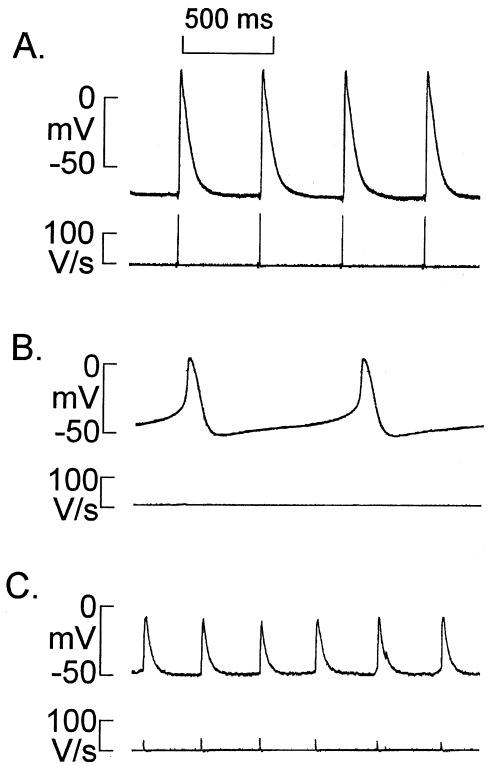

Fig. 2. Various action potentials (APs) recorded in pulmonary veins. (A) Fast response APs driven by electrical stimuli at a frequency of 2 Hz. (B) Spontaneous APs with a conspicuous phase-4 depolarization ($\dot{V}_{max}$ about 15 mV/s). (C) Spontaneous APs with a fast phase-0 upstroke and a rapid phase-3 repolarization. In each panel, traces of APs and their first derivatives are shown.

Table 1, cardiac muscle cells in the PVs were capable of developing spontaneous depolarization and impulse initiation. Table 2 compares the incidence of these electrical activities in PVs from 17 healthy versus 14 chronic pacing dogs. Spontaneous activity was recorded in 71% of PVs of healthy dogs, with a frequency varying from 0.2 to 6 Hz.

Table 2
Rhythms of isolated pulmonary veins in control and chronic pacing dogs[a]

	Control *n*, 17	Chronic pacing *n*, 14	*P* value
Spontaneous activities	12 (71%)	13 (93%)	NS
Occurring automatically	9 (53%)	12 (86%)	NS
Isoproterenol-induced	3 (18%)	1 (7%)	NS
High frequency irregular rhythm	12 (71%)	13 (93%)	NS
Occurring automatically	2 (12%)	13 (93%)	<0.0001
Isoproterenol-induced	4 (24%)	0	NS
Electrical stimulation	4 (24%)	0	NS
Washout of acetylcholine	1 (6%)	0	NS
Washout of propranolol	1 (6%)	0	NS
EAD-related tachyarrhythmias	2 (12%)	11 (82%)	<0.0005

[a] EAD, early afterdepolarizaton. Incidence of rhythms expressed in number (*n*) and percentage (in parenthesis) of healthy control and chronic pacing dogs was compared by χ^2 analysis. NS, not statistically significant.

Nine of the 12 spontaneous activities were observed in preparations not subjected to any experimental intervention. Such automatically occurring spontaneous activity was observed immediately after setting up the preparations. In three silent preparations, spontaneous activities occurred after addition of 1~10 μM isoproterenol.

Thirteen of the 14 PVs of chronic pacing dogs had spontaneous activities (0.3–6 Hz). Twelve of the spontaneous activities occurred automatically without intervention and one occurred after superfusion of isoproterenol. The incidences of spontaneous activity were similar between the PVs of the healthy and chronic pacing dogs (71% versus 93%, $P>0.05$). In addition, the incidence of automatically occurring spontaneous impulse was also similar between the PVs of the dogs with and without chronic pacing (86% versus 53%, $P>0.05$) (Table 2).

Propranolol ($n=7$), ACh ($n=9$), nifedipine ($n=6$), and

Table 1
Action potential (AP) characteristics of pulmonary veins in healthy dogs and chronic pacing dogs[a]

	Cells (*n*)	APA (mV)	MDP (mV)	$\dot{V}_{max}$ (V/s)	APD_{50} (ms)	APD_{90} (ms)
Healthy dogs						
Fast response AP	18	99±13	−82±8	158±76	76±30	173±29
Slow response with a prominent phase-4	27	50±10	−64±16	26±26	52±51	118±88
Spont. AP with a rapid phase-3	21	56±9	−68±9	53±41	23±23	91±46
Chronic pacing dogs						
Fast response AP	18	95±13	−83±13	112±59	49±17[b]	141±25[b]
Slow response with a prominent phase-4	27	52±21	−62±21	11±10	46±31	128±78
Spont. AP with a rapid phase-3	27	57±16	−63±16	70±31	35±26	111±73

[a] APA, action potential amplitude; APD_{50}, APD_{90}, action potential duration at 50% and 90% repolarization; MDP, maximum diastolic potential; Spont. AP, spontaneous AP; $\dot{V}_{max}$, maximum rate of depolarization. Values are mean±S.D.
[b] $P<0.05$ pacing versus healthy dogs by Student's *t*-test.

adenosine ($n=7$) depressed the spontaneous activities. However, after the initial depressant effects during superfusion of adenosine ($n=4$) and ACh ($n=5$), some PVs developed high-frequency irregular rhythms after washout of the drugs. The spontaneous activities did not change after superfusion of TTX ($n=6$), or D-sotalol ($n=6$) (Table 3).

3.4. Electropharmacological properties of high-frequency irregular rhythms

EAD developing at a plateau phase or during phase-3 repolarization could lead to bursts of high-frequency firings as illustrated in examples shown in Fig. 3. Table 2 also compares the incidence of high-frequency irregular rhythms in healthy and chronic pacing dogs. These irregular rhythms (10–24 Hz) were recorded in PVs from 12 of the 17 healthy dogs. Two of the high-frequency irregular rhythms occurred in normal Tyrode solution without any intervention (automatically), four occurred after electrical stimulation, four occurred after the perfusion of isoproterenol, one occurred after washout of ACh and one occurred after washout of propranolol. Fig. 4A illustrates an example of the arrhythmogenic action of isoproterenol (1 μM) in a preparation active spontaneously at a frequency around 2 Hz. Exposure to isoproterenol induced irregular rhythms with a frequency around 14 Hz. Addition of 1 μM propranolol antagonized the arrhythmogenic action of isoproterenol, and regular rhythms recurred at a frequency of 3 Hz. After washout of the drugs, irregular rhythms occurred again and lasted for several minutes (Fig. 4B), presumably due to the residual effect of isoproterenol.

Thirteen of 14 PVs of the chronic pacing dogs had high-frequency irregular rhythms varying from 10 to 20 Hz, which occurred automatically without intervention. The difference in the incidence of high-frequency irregular rhythms between the dogs with and without chronic pacing was not statistically significant (93% versus 67%, $P>0.05$). However, there was a higher incidence of automatically occurring high-frequency irregular rhythms in the PVs of chronic pacing dogs than in healthy dogs (93% versus 12%, $P<0.0001$). In addition, the incidence of EAD-induced high-frequency rhythms was also higher in

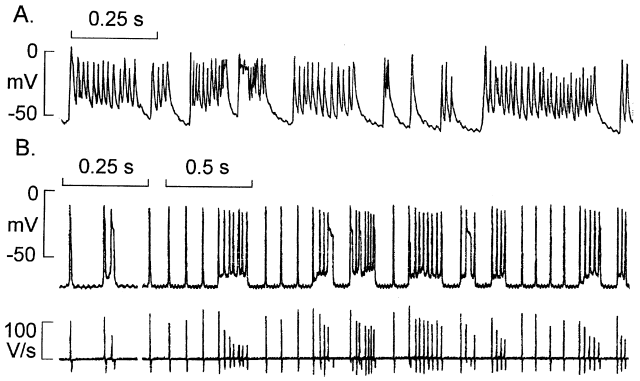

Fig. 3. Spontaneous action potential (APs) with early afterdepolarization (EAD) at high and low level of repolarization in the pulmonary veins. Panel A shows traces of spontaneous APs with incomplete repolarization. Often the phase-3 repolarization stopped at a potential level less negative than −50 mV and a series of high-frequency (cycle length around 20 ms) small amplitude APs developed near plateau level. In panel B in another dog pulmonary vein, the spontaneous APs had a fast phase-0 upstroke and EAD, with the consequent high-frequency irregular rhythms arising from a potential level more negative than −60 mV.

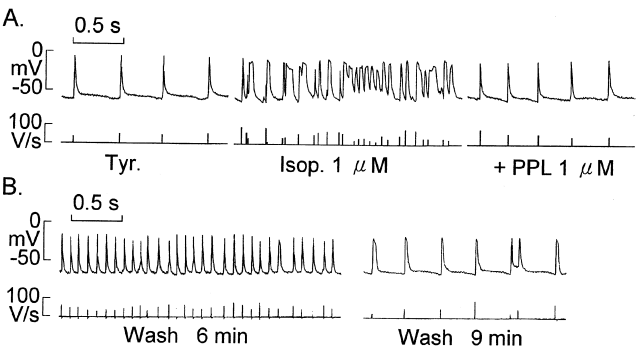

Fig. 4. Propranolol (PPL) suppressed the high-frequency irregular rhythms induced by isoproterenol (Isop) in a pulmonary vein. In panel A, traces of action potentials and their first derivatives were recorded in normal Tyrode solution (Tyr., left panel), during 1 μM Isop exposure (middle panel) and Isop plus 1 μM PPL (right panel). Panel B shows traces after washout of drugs for 6 and 9 min.

Table 3
Pharmacological responses to spontaneous activities and high-frequency irregular rhythms[a]

Drugs	Spontaneous rhythms (Hz)			High-frequency irregular rhythms (Hz)		
	n	Before	After	*n*	Before	After
ACh 5.5 μM	9	3.1±2.4	1.5±1.2[b]	3	9.3±2.8	10.3±2.9
Adenosine 10 μM	7	3.0±2.1	1.3±1.3[b]			
D-Sotalol 100 μM	6	2.4±0.7	2.3±0.5	5	13.2±3.4	4.2±1.6[b]
Nifedipine 3 μM	6	4.9±2.0	3.6±2.7[b]	5	9.8±2.9	9.5±2.7
PPL 0.1 μM	7	2.9±1.6	1.4±0.8[b]			
TTX 1 μM	6	2.6±2.0	2.6±1.7	8	12.0±5.4	3.6±3.1[b]
$MgCl_2$ 10 mM				6	11.2±3.4	3.4±1.5[b]

[a] ACh, acetylcholine; PPL, propranolol; TTX, tetrodotoxin. Values are mean±S.D. *n*, number of experiments.
[b] $P<0.05$, significantly different from values before drug treatment by paired Student's *t*-test.

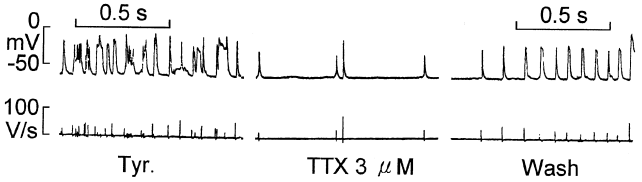

Fig. 5. Sodium channel blocker suppressed early afterdepolarization-related high-frequency irregular rhythms in a pulmonary vein. In the left panel, high-frequency irregular rhythms (around 20 Hz) were recorded during superfusion with normal Tyrode solution (Tyr.). The middle and right panels show traces of action potentials and their first derivatives during tetrodotoxin (TTX, 3 μM) exposure and recovery after washout (Wash), respectively.

chronic pacing dogs than in healthy dogs (82% versus 12%, $P<0.0005$) (Table 2).

TTX (3 μM, $n=8$) and D-sotalol (10 μM, $n=5$) suppressed the high-frequency irregular rhythms as shown in a preparation illustrated in Fig. 5. Magnesium (10 mM) also suppressed the high-frequency irregular rhythms following EAD. In contrast, the high-frequency irregular rhythms did not change after superfusion of ACh ($n=3$) or nifedipine ($n=5$) (Table 3).

3.5. Conduction of impulses in pulmonary veins

When close bipolar electrodes were used to stimulate the left atrium or proximal end of PVs, excitation of atrial cells at this site propagated to the ending of myocardial sleeve in PVs, but did not propagate to the distal end of PVs both in the dogs with and without rapid atrial pacing. In contrast, distal PVs were difficult to excite by stimuli, and the impulses only excited by high current stimuli did not propagate to the left atrium or proximal end of PVs. Furthermore, simultaneous recordings at left atrium and PVs showed that spontaneous activities (0.3–18 Hz) in PVs did not propagate to the left atrium (Fig. 6), and there were asynchronized rhythms at different sites of a PV. Nevertheless, in one experiment on PV of a chronic pacing dog, the high-frequency irregular rhythms recorded at distal site conducted to proximal site after administration of isoproterenol (Fig. 7).

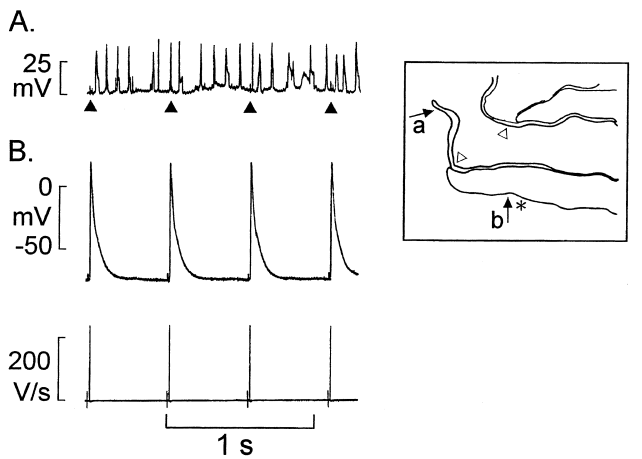

Fig. 6. Simultaneous recordings of action potentials (APs) at sites proximal and distal to the ending of myocardial sleeve in a pulmonary vein. There were non-early afterdepolarization related high-frequency irregular rhythms at point **a** (2 mm distal to the ending of myocardial sleeve) and fast response APs (phase-0 $\dot{V}_{max}=240$ V/s) driven by electrical stimuli at point **b** (2 mm proximal to the ending of sleeve). Electrical stimuli (indicated by solid triangles under tracings in panel (A) were applied at the location* of sleeve as illustrated in the boxed insert (schematic drawing of the histological picture shown in Fig. 1). Open triangles indicate ending of the sleeve.

4. Discussion

4.1. Major findings

This study demonstrates that isolated canine PVs contain atrial cells with or without spontaneous electrical activities. The APs of myocardial cells in PVs consisted of slow response and fast response. The spontaneous activities were suppressed by beta-adrenoceptor blocker, calcium channel blocker, adenosine and ACh. There were high-frequency irregular rhythms, which occurred spontaneously or were induced by brief electrical pacing or after exposure to certain cardioactive agents in these preparations. Chronic pacing dogs had a higher incidence of spontaneously occurring tachyarrhythmias in PVs than healthy dogs. The tachyarrhythmias were suppressed by sodium channel blocker, potassium channel blocker or magnesium.

4.2. Electrical activity of pulmonary veins

This experiment showed that PVs near the left atrium have fast response APs similar to those of normal atrial myocardial muscles. Similar to Cheung's findings in guinea pigs [14], this study also demonstrated that PVs in dogs with and without chronic rapid atrial pacing have spontaneous impulse initiation. The slow response APs and the spontaneous activity resembled characteristics of pacemaker cells. The depressant effects of ACh, adenosine, propranolol and nifedipine further confirmed the pacemaker activity in PVs. Previous study by Chen et al. also demonstrated that calcium channel blocker can abolish the occurrence of ectopic foci of PVs [2]. The histological study has shown that PVs have nodal-like cells in the myocardium [15], which may contribute to the pacemaker activity in this study. Recent embryologic study further demonstrated that PVs contain pacemaker cells, thus suggesting that PVs can work as a subsidiary pacemaker of normal hearts [16]. In addition, PVs might provide the foci of abnormal atrial automaticity.

In this study, we identified various types of APs in the adventitial sites of PVs. It was known that the intimal sites of PVs contain several layers of smooth muscle cells. Thus, it would be difficult to record APs or to differentiate

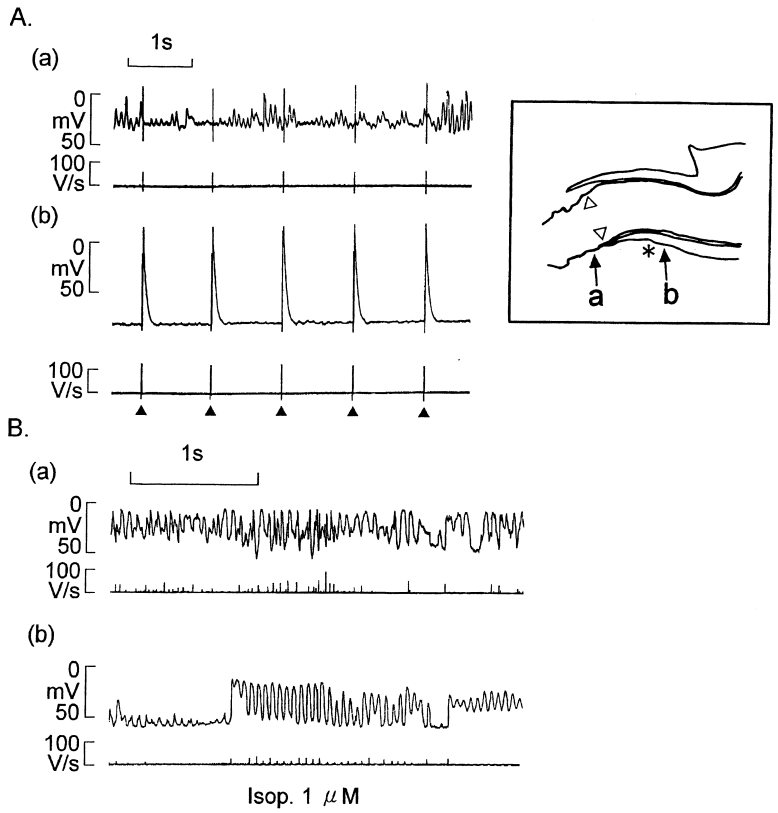

Fig. 7. Conduction of irregular rhythms from distal to proximal sites in a pulmonary vein. Action potentials (APs) were recorded simultaneously at two different sites (distal point **a** and proximal point **b**). In panel A, there were spontaneous irregular rhythms at point **a** and fast response APs driven by electrical stimulation (indicated by solid triangles) at point **b** in normal Tyrode solution. In panel B, recorded 17 min after exposure to 1 μM isoproterenol (Isop.), high-frequency irregular rhythms (18 Hz) occurred at point **b**, suggesting conduction of impulse between two sites in the vein after Isop treatment. Boxed insert shows the locations of the recording sites **a** and **b**, respectively, and the stimulation site*. The open triangles indicate ending of the sleeve.

cardiac APs from electrical activity of smooth muscle cells through intimal approach. These findings were similar to the Arita's study in guinea pig pulmonary trunk [17]. Our observations also suggest that creation of transmural lesions could be very important for successful ablation for ectopic foci in PVs [18].

4.3. Mechanism of high-frequency irregular rhythms

High frequency irregular rhythms were identified in PVs of the healthy and chronic pacing dogs. Several possibilities (enhanced automaticity, triggered activity or re-entry) may account for the tachyarrhythmic rhythms; nevertheless the high-frequency of discharges in this tachyarrhythmia (up to 24 Hz) suggests that enhanced automaticity is not likely to be the underling mechanism. In contrast, from the results of pharmacological study, suppression of tachyarrhythmias by the administration of TTX and D-sotalol (but not by the administration of ACh, adenosine or nifedipine) supports that re-entry is a possible mechanism. Recent work from Chen's laboratory also suggests that sodium channel blocker could inhibit the spontaneous activities in PVs [2].

This study demonstrated the spontaneous occurrence of EAD leading to bursts of high-frequency irregular discharges resembling atrial Torsades de Pointes observed in intact dogs [19]. These findings suggest that triggered activity arising from EAD also contributes to the arrhythmogenic activity in PVs. Previous clinical studies likewise found that triggered activity may play a role in the occurrence of ectopic atrial beats in PVs [2]. In this study, suppression of high-frequency irregular rhythms in PVs by magnesium suggests that triggered activity may account for the tachyarrhythmias in PVs. These findings were similar to previous observation in ventricle, whereas Torsades de Pointes arises from a combination of triggered activity and re-entrant excitation [20].

It is known that ligament of Marshall has arrhythmogenic activity after isoproterenol infusion [21]. In the present study, the recording sites were different from the previous reports of Marshall ligament. Moreover, our specimen clearly did not identify the presence of Marshall ligament, suggesting the low possibility that Marshall ligament could confound the experiments.

Several in vivo or in vitro experimental models have been used for studies of atrial fibrillation. High-frequency irregular rhythm in PVs seems to be a good experimental model for studying atrial fibrillation. There were very

similar electrophysiological characteristics between the high-frequency irregular rhythms in canine PVs and the atrial fibrillation observed in clinical study, whereas ectopic beats in PVs always discharged randomly [1]. The highly irregular electrical activities occurring during tachyarrhythmia shown in the present experiments were in contrast to atrial flutter which, although it could have very high-frequency firings similar to those of atrial fibrillation [22], consisted of rather uniform APs [13,23].

4.4. Conduction of impulse in pulmonary veins

Cheung reported that there was conduction block between atrium and PVs of guinea pigs in the presence of ouabain-induced spontaneous activity [24]. Similar findings have been found in clinical study, whereas there was conduction block within the PV or at the junction of the left atrium and PVs [1,2]. In this study, we showed that pacing at left atrium propagated to the ending of the myocardial sleeve, but did not propagate to the distal end of the PVs. The impulse initiation at the distal end of the PVs also did not propagate to the proximal end of the PVs or left atrium. These findings may result from both the geometric arrangements of the fibers in the PVs or from the voltage–time course of AP in the muscle fibers of the PVs. There was a relatively large volume of muscle fibers near the junction of the left atrium and PVs. With increasing distance from the junction, the density of muscle fibers decreases progressively and only a few individual fibers remain [5,6]. Therefore, stimuli applied to the distal end of the PVs probably excite only a relatively few myocardial fibers. As the impulse spreads towards the atrial side, the impulse may not be able to generate sufficient current to excite the fibers of the proximal end of the PVs because of a large increase of myocardial fibers and the membrane area. Moreover, sparse contacts of myocardial cells due to the dense accumulations of smooth muscle cells and connective tissue may further contribute to conduction block and asynchronized rhythms in the PVs.

The other possible factor affecting the conduction properties of the PVs is the spontaneous electrical activity of myocardial cells. Because pacemaker cells with spontaneous diastolic depolarization may be hardly driven by electrical stimuli or nearby tachyarrhythmias, these cells would slow conduction in the PVs and result in the conduction block. Furthermore, transitional cells also have slow conduction velocity, which also contributes to the conduction block in the PVs.

In contrast, this study demonstrated that administration of isoproterenol occasionally facilitated the conduction of high-frequency irregular rhythms from distal to proximal PVs in the dogs with chronic pacing. This finding is consistent with clinical finding wherein isoproterenol promotes the conduction in PVs [2].

4.5. Effects of chronic rapid atrial pacing

Changes of atrial refractoriness, dispersion and conduction velocity which result from rapid atrial pacing are known to facilitate the occurrence and maintenance of atrial fibrillation [7–10,12]. The effects of rapid atrial pacing on the triggered points of PVs are not clear. In this study, there was a higher incidence of spontaneously occurring high-frequency irregular rhythm in PVs of chronic pacing dogs. Chronic atrial pacing has been demonstrated to produce atrial fiber disarray and early hypertrophy. Disorganization of atrial fiber orientation may slow conduction and facilitate reentrant activity [7]. In addition, there was a higher incidence of EAD in PVs of chronic pacing dogs, which also may facilitate the occurrence of triggered activity. Therefore, enhanced triggered and re-entrant activity may account for the high incidence of spontaneously occurring high-frequency irregular rhythms in PVs of chronic pacing dogs. However, sinus rhythms resumed after stoppage of pacing in the situ atria in the paced dogs. These findings suggest that conduction block between PV and left atrium may prevent atrium from the arrhythmogenic activity of PVs.

Acknowledgements

The present work was supported by grants NSC 89-2314-B-010-017 (SA Chen) and NSC89-2320-B016-014 (CI Lin) from the National Science Council, Taipei, Taiwan, R.O.C.

References

[1] Haissaguerre M, Jais P, Shah DC et al. Spontaneous initiation of atrial fibrillation by ectopic beats originating in the pulmonary veins. N Engl J Med 1998;339:659–666.
[2] Chen SA, Hsieh MH, Tai CT et al. Initiation of atrial fibrillation by ectopic beats originating from the pulmonary veins: Electrophysiological characteristics, pharmacological responses, and effects of radiofrequency ablation. Circulation 1999;100:1879–1886.
[3] Walsh EP, Saul JP, Hulse JE et al. Transcatheter ablation of ectopic atrial tachycardia in young patients using radiofrequency current. Circulation 1992;86:1138–1146.
[4] Jaïs P, Haïssaguerre M, Shah DC et al. A focal source of atrial fibrillation treated by discrete radiofrequency ablation. Circulation 1997;95:572–576.
[5] Burch GE, Romney RB. Functional anatomy and 'throttle valve' action of the pulmonary veins. Am Heart J 1954;47:58–66.
[6] Nathan H, Eliakim M. The junction between the left atrium and the pulmonary veins: An anatomic study of human hearts. Circulation 1966;34:412–422.
[7] Morillo CA, Klein GJ, Jones DL, Guiraudon CM. Chronic rapid atrial pacing: structural, functional and electrophysiological characteristics of a new model of sustained atrial fibrillation. Circulation 1995;91:1588–1595.
[8] Gaspo R, Bosch RF, Talajic M, Nattel S. Functional mechanisms

underlying tachycardia-induced sustained atrial fibrillation in a chronic dog model. Circulation 1997;96:4027–4035.
[9] Daoud EG, Bogun F, Goyal R et al. Effect of atrial fibrillation on atrial refractoriness in humans. Circulation 1996;94:1600–1606.
[10] Yu WC, Chen SA, Lee SH et al. Tachycardia-induced change of atrial refractory period in humans: rate dependency and effects of antiarrhythmic drugs. Circulation 1998;97:2331–2337.
[11] Yue L, Feng J, Gaspo R, Li GR, Wang Z, Nattel S. Ionic remodeling underlying action potential changes in a canine model of atrial fibrillation. Circ Res 1997;81:512–525.
[12] Fareh S, Villemaire C, Nattel S. Importance of refractoriness heterogeneity in the enhanced vulnerability to atrial fibrillation induction caused by tachycardia-induced atrial electrical remodeling. Circulation 1998;98:2202–2209.
[13] Lin CI, Yiu MY, Hwang HR, Lin CL, Chen KY. Reentrant tachyarrhythmias in right atria of cardiomyopathic versus healthy Syrian hamster. J Biomed Sci 1999;6:399–408.
[14] Cheung DW. Electrical activity of the pulmonary vein and its interaction with the right atrium in the guinea-pig. J Physiol (Lond) 1981;314:445–456.
[15] Masani F. Node-like cells in the myocardial layer of the pulmonary vein of rats: an ultrastructural study. J Anat 1986;145:133–142.
[16] Blom NA, Gittenberger-de Groot AC, DeRuiter MC et al. Development of the cardiac conduction tissue in human embryos using HNK-1 antigen expression: possible relevance for understanding of abnormal atrial automaticity. Circulation 1999;99:800–806.
[17] Arita M, Kawata H, Goto M. Measurement of spontaneous electrical activity in the guinea pig pulmonary bulbus by a microelectrode. Jpn J Physiol 1969;19:403–419.
[18] Wilber DJ, Arruda M, Wang ZG, Patel A. Circumferential ablation of pulmonary vein ostia with an ultrasound ablation catheter: acute and chronic studies in a canine model. Circulation 1999;100:1–373, (abstract).
[19] Satoh T, Zipes DP. Cesium-induced atrial tachycardia degenerating into atrial fibrillation in dogs: atrial torsades de pointes? J Cardiovasc Electrophysiol 1998;9:970–975.
[20] El-Sherif N, Caref EB, Yin H, Restivo M. The electrophysiological mechanism of ventricular arrhythmias in the long QT syndrome: Tridemensional mapping of activation and recovery patterns. Circ Res 1996;79:474–492.
[21] Doshi RN, Wu TJ, Yashima M et al. Relation between ligament of Marshall and adrenergic atrial tachyarrhythmia. Circulation 1999;100:876–883.
[22] Gaspo R, Sun H, Fareh S et al. Dihydropyridine and beta adrenergic receptor binding in dogs with tachycardia-induced atrial fibrillation. Cardiovasc Res 1999;42:434–442.
[23] Lin TC, Hou ZY, Liu HW, Wu HS, Lin CI. Atrial tachyarrhythmias induced by acetylcholine in isolated tilapia (*Oreochromis* sp) atria. Clin Exp Pharmacol Physiol 2000;27:330–338.
[24] Cheung DW. Pulmonary vein as an ectopic focus in digitalis-induced arrhythmia. Nature 1981;294:582–584.