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Full Paper

Electrophysiological Effects of the Class Ic Antiarrhythmic Drug Pilsicainide on the Guinea-Pig Pulmonary Vein Myocardium

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Abstract. The pulmonary vein is known as an important source of ectopic beats, initiating frequent paroxysms of atrial fibrillation. We compared effects of the class Ic antiarrhythmic drug pilsicainide on the electrophysiological parameters in the isolated pulmonary vein preparation from guinea pigs with those in the left atrium. Three pairs of bipolar electrodes were attached to the left atrium, pulmonary vein, and junctional region of the left atrium and pulmonary vein to measure intra-atrial and intra-pulmonary vein conduction velocity and effective refractory period. Pilsicainide (10 μM) decreased the conduction velocity in the pulmonary vein as well as the left atrium, whose effect on the pulmonary vein was relatively greater than that on the left atrium. The drug prolonged the effective refractory period in the pulmonary vein as well as the left atrium, and the effect of the drug on the pulmonary vein was less than that on the left atrium. The currently observed electrophysiological property of pilsicainide suggests that its effects on reentry within the pulmonary vein are estimated to be weaker than within the left atrium, which may be one of the key considerations for understanding its antiarrhythmic mechanisms in the atrium and pulmonary vein.

Keywords: pilsicainide, pulmonary vein myocardium, conduction velocity, effective refractory period, action potential

Introduction

Atrial fibrillation is known as the most common cardiac arrhythmia in adult populations (1). Whereas the arrhythmia has been recognized to be perpetuated by reentrant wavelets propagating in an abnormal atrial-tissue substrate, the origin of atrial ectopic beats is clinically demonstrated to be localized in the pulmonary vein myocardial sleeve of patients with drug-resistant atrial fibrillation (2). The pulmonary vein myocardium has different electrophysiological properties from those of the working myocardium, such as lower density of inward rectifier current (I_{K1}) or a less negative resting membrane potential, which makes it possible to easily generate arrhythmogenic substrates: abnormal automaticity and triggered activity (3 – 5). Recently, it is suggested that the combination of reentrant and non-reentrant mechanisms is the underlying arrhythmogenic mechanisms of atrial fibrillation from the pulmonary veins (6).

The class Ic antiarrhythmic drug pilsicainide is often used for termination of atrial fibrillation in patients by oral or intravenous administration, which is also applied to pharmacological isolation of the pulmonary veins (7). To date, electrophysiological and antiarrhythmic effects of pilsicainide on the atria have been widely investigated in clinical and experimental examinations (8 – 10). However, information is limited regarding effects of pilsicainide on electrophysiological parameters of the pulmonary vein myocardium itself (11). In this study, we recorded the conduction velocity, effective refractory period, and action potential of the isolated pulmonary vein preparation from the guinea pig and compared effects of pilsicainide on these parameters in the pulmonary vein with those in the left atrium to better understand the antiarrhythmic action of pilsicainide.

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Materials and Methods

All experiments were approved by the Ethics Committee of Toho University, and performed in accordance with the Guiding Principles for the Care and Use of Laboratory Animals approved by The Japanese Pharmacological Society. The heart and adjunct lungs were isolated from male or female Hartley guinea pigs weighing 350 – 450 g and incubated with the Krebs-Henseleit solution of the following composition: 118.4 mM NaCl, 4.7 mM KCl, 2.5 mM $CaCl_2$, 1.2 mM $MgSO_4$, 1.2 mM KH_2PO_4, 24.9 mM $NaHCO_3$,11.1 mM glucose, gassed with 95% O_2 / 5% CO_2 (pH 7.4 at 37°C).

Histological examinations

The left superior pulmonary vein at the proximal region connected to the left atrium was fixed with 10% formalin neutral buffer solution, and the segments were processed into paraffin blocks. The paraffinized tissue blocks were cut into 4-μm-thick sections and mounted on charged slides. For each paraffin block, one slide each was stained with Masson trichrome to accentuate muscle and connective tissues. Serial section was incubated with antibodies against α-smooth muscle actin (α-SMA, 1:500; Dako, Glostup, Denmark) followed by consecutive incubations with universal immuno-peroxydase polymer (Histofine®, Simple Stain Rat MAX PO MULTI; Nichirei Bioscience, Tokyo). Antibody binding was demonstrated by staining with 3,3′-diaminobenzidine tetrahydrochloride.

Measurement of intracardiac conduction and effective refractory period

Left atrium and adjunct pulmonary veins were mounted in the organ bath. Bipolar stimulating electrodes were attached onto the left atrial appendage and right inferior pulmonary vein, whereas three sets of bipolar recording electrodes were attached on the left atrial appendage, left atrium-pulmonary vein junction region and right inferior pulmonary vein. Electrograms were amplified with a bioelectric amplifier (AB-621G; Nihon Kohden, Tokyo) and fed into a waveform analysis system (PowerLab; ADInstruments, Castle Hill, Australia). The preparation was electrically driven using an electrical stimulator (SEN-7203, Nihon Kohden) and an isolator (SS-104J, Nihon Kohden) with rectangular pulses (about 1.5 times of the diastolic threshold voltage and 3-ms width). The effective refractory period was assessed by a pacing protocol consisted of ten beats of basal stimuli in a cycle length of 100, 300, 500, or 1000 ms followed by an extra stimulus of various coupling intervals. All experiments were performed at 36.5 ± 0.5°C.

Microelectrode recording of cardiac action potentials

The pulmonary veins were separated from the left atrium and lung at the end of the pulmonary vein myocardium sleeve. The luminal side of the pulmonary vein at the middle region between the ostium and the distal end of myocardial sleeve or the endocardial surface of the left atrium was impaled with glass microelectrodes filled with 3 M KCl to record transmembrane potential using a microelectrode amplifier (Intra 767; World Precision Instruments, Sarasota, FL, USA). The preparation was electrically driven using an electrical stimulator (SEN-7203, Nihon Kohden) and an isolator (SS-104J, Nihon Kohden) with rectangular pulses (about 1.5 times of the diastolic threshold voltage and 3-ms width). The action potential signals were monitored by an oscilloscope (CS-5135; Kenwood, Tokyo) and fed into a waveform analysis system (DSS98-type IV; Canopus, Tokyo). All experiments were performed at 36.5 ± 0.5°C.

Drugs

Pilsicainide hydrochloride (Sigma-Aldrich, St. Louis, MO, USA) was dissolved in distilled water and small aliquots were added to the organ bath to obtain the desired final concentration. All other chemicals were commercial products of the highest available quality.

Statistical analyses

The statistical significances within a parameter were evaluated by one-way repeated-measures analysis of variance (ANOVA) followed by Contrasts for mean values comparison, whereas those of unpaired data within a parameter were evaluated by the unpaired *t*-test. A *P*-value less than 0.05 was considered significant.

Results

Histology of the pulmonary vein

Typical photomicrographs of horizontal sections of the left superior pulmonary vein obtained from the guinea pig are shown in Fig. 1. Vascular smooth muscle was detected on the luminal face of the pulmonary vein, whereas the myocardial sleeve was observed mostly at the mid-layer of the pulmonary vein as a circular muscle layer.

Left atrium-pulmonary vein conduction

Figure 2A shows typical tracings of electrograms obtained from the left atrial appendage, left atrium-pulmonary vein junction region, and right inferior pulmonary vein, whereas the conduction velocity and effective refractory period in the left atrium and pulmonary vein at a pacing cycle length of 100, 300, 500, or 1000 ms

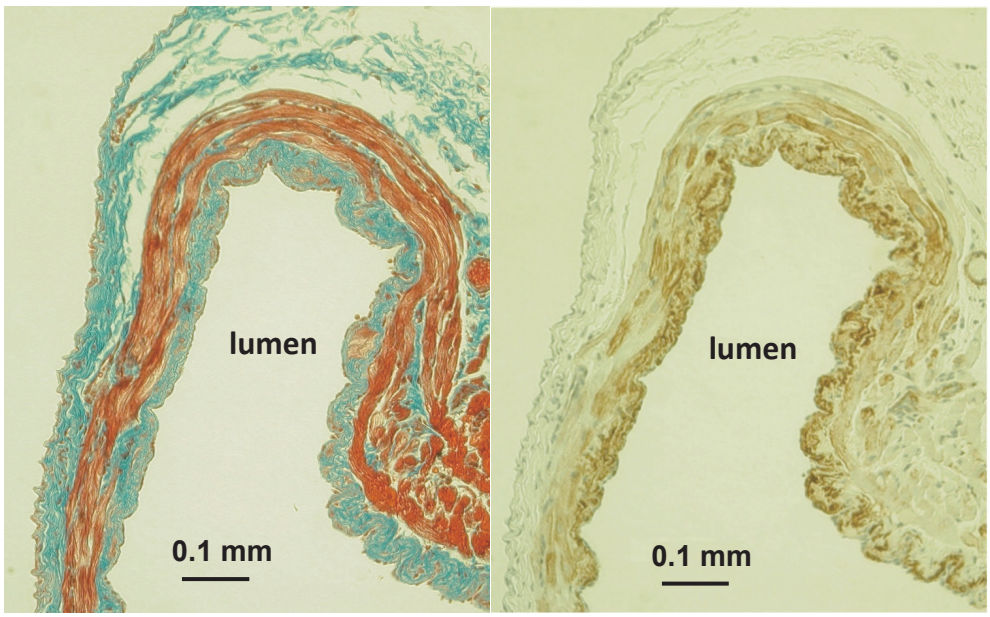

Fig. 1. Photomicrographs of horizontal sections of the left superior pulmonary vein of the guinea pig. Left panel: Masson trichrome staining. Right panel: immunostaining for α-smooth muscle actin (α-SMA).

(n = 16) were summarized in Fig. 2B. The conduction velocity in the pulmonary vein was significantly less than that in the left atrium at each pacing cycle length. The effective refractory period in the pulmonary vein was significantly longer than that in the left atrium at each pacing cycle length.

Effects of pilsicainide on the left atrium-pulmonary vein conduction

Figure 3 summarizes the effects of pilsicainide on the conduction velocity and effective refractory period in the pulmonary vein and left atrium (n = 5). In the presence of pilsicainide at a concentration of 1 μM, no significant change was detected in the conduction velocity or effective refractory period. Thirty minutes after application of 10 μM, the conduction velocity significantly decreased both in the pulmonary vein and left atrium. Meanwhile, activation failure was observed during a constant pacing cycle length of 100 ms. The decrements of the conduction velocity by the drug in the pulmonary vein were 0.08 ± 0.02, 0.07 ± 0.02, and 0.09 ± 0.02 m/s at a pacing rate of 1000, 500, and 300 ms, respectively, whereas those in the atrium were 0.14 ± 0.03, 0.17 ± 0.04, and 0.24 ± 0.04 m/s, respectively. At a pacing cycle length of 1000 ms, the conduction velocity after application of 10 μM pilsicainide was 81.2 ± 5.0% and 87.2 ± 2.3% of the corresponding control values in the pulmonary vein and left atrial preparation, respectively. On the other hand, the effective refractory period significantly increased both in the pulmonary vein and left atrium. The increments of the effective refractory period by the drug in the pulmonary vein were 15 ± 3, 14 ± 1, and 19 ± 4 ms at a pacing rate of 1000, 500, and 300 ms, respectively, whereas those in the atrium were 20 ± 10, 21 ± 8, and 22 ± 6 ms, respectively.

When the preparation was electrically driven at a pacing cycle length of 100 ms, conduction block within the pulmonary vein appeared as shown in the Fig. 4, which was observed about 15 min after application of pilsicainide (10 μM).

Effects of pilsicainide on the action potential configuration

The resting membrane potential in the pulmonary vein was significantly smaller than that in the left atrium, and action potential duration at 90% repolarization (APD_{90}) in the pulmonary vein was significantly greater than that in the left atrium. In the pulmonary vein preparation, pilsicainide at a concentration of 10 μM significantly decreased overshoot and maximum rate of phase 0 depolarization ($\dot{V}_{max}$) and prolonged APD_{90}. In the left atrial preparation, the same concentration of pilsicainide significantly decreased overshoot and $\dot{V}_{max}$. The $\dot{V}_{max}$ after application of 10 μM pilsicainide in the pulmonary vein and left atrial preparation was 65.3 ± 2.3% and 75.1 ± 2.4% of the corresponding control values, respectively, at a pacing cycle length of 1000 ms.

Discussion

In the proximal region of the pulmonary vein connected to the left atrium, myocardial cells were observed mostly as a circular muscle layer, as shown in Fig. 1. Since cardiac cells are electrically coupled extensively in

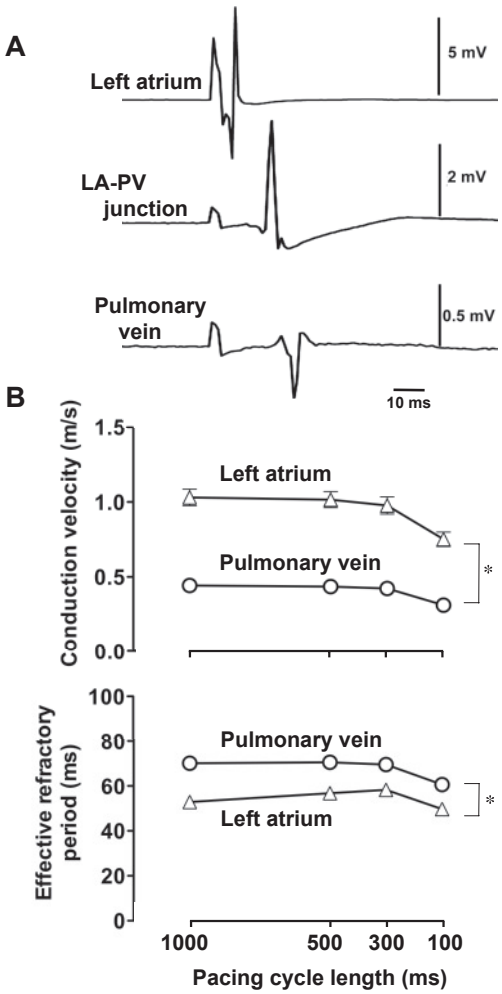

Fig. 2. Electrophysiological property of pulmonary vein and left atrium. A: Typical tracings of electrograms obtained from the left atrial appendage, left atrium–pulmonary vein junction region and right inferior pulmonary vein. B: Conduction velocity and effective refractory period in the left atrium and pulmonary vein at a pacing cycle length of 100, 300, 500, and 1000 ms (n = 16). Data are means ± S.E.M.

the longitudinal direction and to a lesser extent in the transverse direction, anisotropic trabecular structures are generally known as the major determinants of electrical impulse propagation (12). Indeed, the conduction velocity in the pulmonary vein was about half of that in the left atrium, as shown in Fig. 2B, which was similar to a previous study using an optical mapping system (11). Studies using the isolated cardiomyocytes from the pulmonary vein myocardium have demonstrated that Na^+ current density is similar to that of the left atrial cells under voltage-clamp conditions (3). More importantly, it has been shown that conduction velocity is hardly affected by elevation of resting membrane potential up to about −70 mV in the guinea-pig myocardium (13). Thus, it is supposed that the difference of electrophysiological properties between the pulmonary vein myocardium and left atrium, as shown in Table 1, might not be the major determinant of the slow conduction in the pulmonary vein preparation.

Pilsicainide has been recognized as a pure Na^+-channel blocker with little effect on the action potential duration, Ca^{2+} currents, delayed rectifier K^+ currents, inward rectifier K^+ currents, acetylcholine-induced K^+ currents, or ATP-sensitive K^+ currents (14). In this study, the conduction velocity was significantly decreased by 10 μM pilsicainide in the pulmonary vein as well as the left atrium, as shown in Fig. 3. Also, the $\dot{V}_{max}$ was significantly decreased by the same concentration of pilsicainide in the pulmonary vein and left atrium, as shown in Table 1. It has been demonstrated that relative changes in $\dot{V}_{max}$ by the Na^+-channel blocker tetrodotoxin correlated well with the square of those in the conduction velocity in the guinea-pig ventricular preparation (13). In this study, the effect of pilsicainide for conduction velocity was relatively greater in the pulmonary vein than those in the left atrium (81.2% and 87.2% of the corresponding control values, respectively, at a pacing cycle length of 1000 ms), whereas a similar relationship was observed in the effects of pilsicainide on the $\dot{V}_{max}$ in the pulmonary vein and left atrium (65.3% and 75.1% of the corresponding control values, respectively). The correlation of extent of suppressive effects of pilsicainide on the conduction velocity with that on $\dot{V}_{max}$ nearly reflected the previous study using tetrodotoxin (13), which may suggest that the conduction delay within the pulmonary vein is potentially associated with its Na^+ channel–blocking action. Class I antiarrhythmic drugs including pilsicainide generally inhibit $\dot{V}_{max}$ or Na^+ currents of the cardiomyocytes in a voltage-dependent manner; namely, the drugs cause a greater $\dot{V}_{max}$ reduction at less negative conditioning membrane potential (15, 16), which may be associated with the current results of the relatively greater inhibitory effect of pilsicainide on the conduction in the pulmonary vein than in the left atrium.

The effective refractory period in the pulmonary vein was significantly greater than that in the left atrium (Fig. 2), which may be associated with longer action potential duration, as shown in Fig. 5 and Table 1. The effective refractory period was significantly prolonged by 10 μM pilsicainide in the pulmonary vein as well as the left atrium, as shown in Fig. 3, suggesting that pilsicainide has suppressive effects on reentrant arrhythmias in the pulmonary vein. Interestingly, as shown in Fig. 4, conduction block within the pulmonary vein was observed about 15 min after application of pilsicainide (10 μM) at a pacing cycle length of 100 ms, which is thought to be caused by prolongation of effective refractory period to

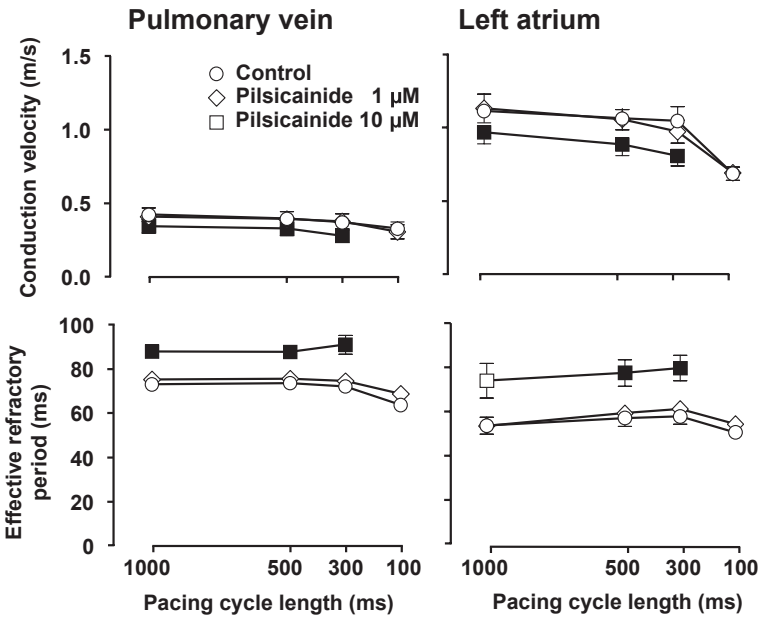

Fig. 3. Effects of pilsicainide on the conduction velocity and effective refractory period in the pulmonary vein and left atrium (n = 6). All parameters were obtained before and 30 min after application of 1 or 10 μM of pilsicainide. At the measurement period of 10 μM pilsicainide, activation failure was observed at a pacing cycle length of 100 ms, probably due to prolongation of the effective refractory period to > 100 ms. Data are means ± S.E.M. Closed symbols represent significant differences from the corresponding pre-drug values (Control) by $P < 0.05$.

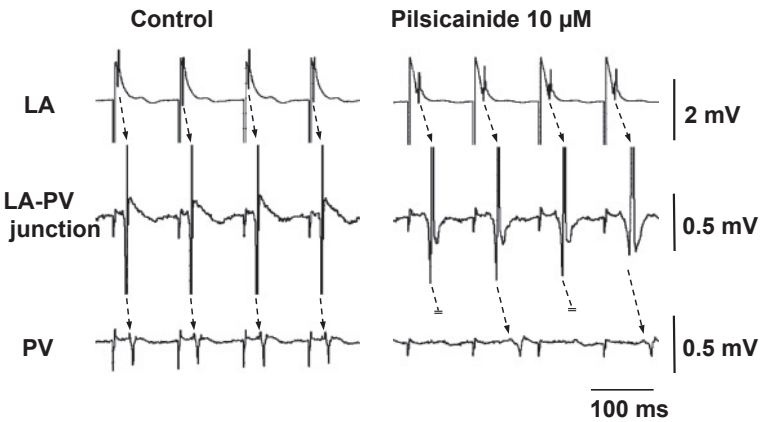

Fig. 4. A typical example of conduction block in the region of pulmonary vein about 15 min after application of pilsicainide (10 μM). The preparations were electrically driven at 10 Hz.

Table 1. Effects of pilsicainide on the action potential parameters of the pulmonary vein and left atrium

	Pulmonary vein		Left atrium	
	Control	Pilsicainide	Control	Pilsicainide
RP (mV)	−74.2 ± 1.4$^{\#\#}$	−73.2 ± 1.2	−80.4 ± 0.7	−79.2 ± 0.8
OS (mV)	34.1 ± 1.2	29.5 ± 1.5**	32.0 ± 0.5	29.2 ± 0.7*
APD_{50} (ms)	41.9 ± 4.2	42.5 ± 4.2*	37.3 ± 1.5	36.3 ± 1.3
APD_{90} (ms)	100.2 ± 3.7$^{\#\#}$	104.7 ± 3.2**	76.4 ± 2.1	76.7 ± 2.1
$\dot{V}_{max}$ (V/s)	194.1 ± 23.7	127.7 ± 16.9**	216.1 ± 15.3	162.4 ± 13.0**

The preparations were electrically driven at 1 Hz. Parameters were obtained before (Control) and 20 min after application of 10 μM of pilsicainide. Resting potential (RP), overshoot (OS), action potential duration at 50% (APD_{50}) and 90% (APD_{90}) repolarization, and maximum rate of phase 0 depolarization ($\dot{V}_{max}$). Data are means ± S.E.M. of 5 experiments. *$P < 0.05$, **$P < 0.01$, compared with the corresponding control values; $^{\#\#}P < 0.01$, compared with the corresponding values in the left atrium.

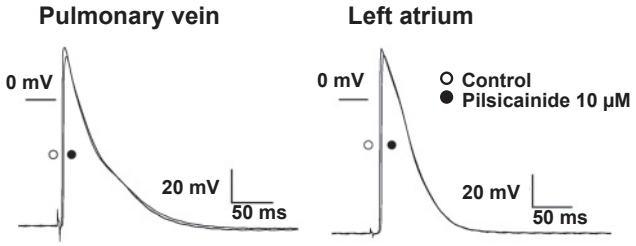

Fig. 5. Typical tracings of effects of pilsicainide (10 μM) on the action potential configuration in the pulmonary vein and left atrium. The preparations were electrically driven at 1 Hz.

> 100 ms within the pulmonary vein only. This property may partly explain the mechanisms of pharmacological isolation of the pulmonary vein by pilsicainide in patients with atrial fibrillation (7). On the other hand, Fig. 3 indicates that the block occurred in the left atrium at a cyclic length of 100 ms, and the extent of prolongation of the effective refractory period by pilsicainide was relatively less in the pulmonary vein than in the left atrium at each pacing cycle length. Since its inhibitory action on the conduction in the pulmonary vein was greater than in the left atrium, suppressive effects of pilsicainide on reentry within the left atrium will be estimated to be greater than those within the pulmonary vein. These electrophysiological profiles may be more important for totally understanding the action of pilsicainide on atrial fibrillation.

In conclusion, pilsicainide decreased the conduction velocity and prolonged the effective refractory period in the pulmonary vein as well as the left atrium. The currently observed electrophysiological property of pilsicainide suggests that its effects on reentry within the pulmonary vein are estimated to be weaker than within the left atrium, although conduction block can be seen within the pulmonary vein during shorter cycle length of atrial fibrillation, which may be one of the key considerations to understand its antiarrhythmic mechanisms in the atrium and pulmonary vein.

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