

A Sodium Channel Blocker, Pilsicainide, Produces Atrial Post-Repolarization Refractoriness through the Reduction of Sodium Channel Availability

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Atrial fibrillation (AF) is the most common tachyarrhythmia. Shortening of atrial action potential duration (APD) and effective refractory period (ERP) is one of the crucial factors in the occurrence and maintenance of AF. ERP is usually shorter than APD, but ERP can be prolonged beyond action potential repolarization in some situations. It is termed as post-repolarization refractoriness (PRR) that is thought to be one of main anti-arrhythmic mechanisms of class I sodium channel blockers (SCBs). Most of anti-arrhythmic agents, including SCBs, have multi-channel blocking effects. It is unknown whether atrial PRR with SCBs is associated with the reduction of sodium channel availability. We therefore explored the relationship between the reduction of sodium channel availability with a pure SCB (pilsicainide or tetrodotoxin) and atrial PRR using the left atrial appendage from male guinea pigs (each group, $n = 3\sim 10$). Employing a standard microelectrode technique, we evaluated APD measured at 90% repolarization (APD_{90}) and the sodium channel availability, judged from the maximal rate of rise of action potential (V_{max}). At a 500-msec basic cycle length (BCL), pilsicainide prolonged atrial ERP assessed by a single extra-stimulus in response to the decrement of the V_{max} in a dose-dependent manner without affecting APD_{90} . Furthermore, pilsicainide increased the ERP and decreased the V_{max} in a rate-dependent manner without APD_{90} prolongation at a shorter BCL (200 msec). Importantly, tetrodotoxin reproduced the effects of pilsicainide on atrial ERP, APD_{90} , and V_{max} . These results indicate that SCBs produce atrial PRR through the reduction of sodium channel availability.

Keywords: action potential duration; anti-arrhythmic agents; atrial fibrillation; effective refractory period; sodium channels

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Atrial fibrillation (AF) is one of the important arrhythmias as it causes various pathological conditions, including peripheral arterial embolism, especially cerebral embolism (Wolf et al. 1978; Cairns and Connolly 1991). The atrium is under the condition of high frequency excitation during AF (over 300 beats per a minute). An action potential duration (APD), which represents the duration of the myocardial excitation, increases at slower rates and decreases at higher rates in physiological conditions. Sustained AF or chronic rapid atrial pacing reduces atrial action potential duration (APD) and deteriorates the APD adaptation to heart rate (Hara et al. 1999). Reduction in APD leads to a shortening of effective refractory period (ERP), which is the maximal interval between the prior stimulation and the following stimulation that cannot develop the next action potential. The shortening of ERP enhances a vulnerability to extra

impulse and shortens the wavelength that is the multiplication between ERP and conduction velocity, allowing the presence of multiple wavelets in atrium. This phenomenon is called as the electrical remodeling that facilitates the maintenance of AF (Yue et al. 1997). An alteration in atrial effective refractory period (ERP) is one of the crucial factors for the occurrence and maintenance of AF (Wijffels et al. 1995; Gaspo et al. 1997). Thus, ERP prolongation can be a major target for pharmacological termination of AF (Kirchhof et al. 1991; Namba et al. 1999).

Although ERP is usually dependent of and shorter than APD (Costard-Jackle and Franz 1989; Nademanee et al. 1990), it can be prolonged beyond the repolarization of action potential in some situations. Lazzara et al. (1975) reported this phenomenon in the ventricular myocardium and termed it post-repolarization refractoriness (PRR).

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PRR is unique and worth for investigation because it prolongs ERP without calcium overload due to APD prolongation, which may cause QT prolongation and torsade des pointes (Tdp) (Jackman et al. 1988; Myktysey et al. 2007). Class I sodium channel blockers can exert anti-arrhythmic effects through this PRR, which was originally reported in the ventricular myocardium (Gettes and Reuter 1974). Many class I agents, which mainly block sodium channels, also have the effects on other ion channels. There are few reports on the effect of a pure sodium channel blocker on atrial PRR. Kanki et al. (1998) reported the atrial PRR by pilsicainide, a pure sodium channel blocker, as the possible anti-AF target. Those studies used the monophasic action potential recordings to evaluate PRR. It enables us to get accurate information on the action potential duration and configuration, but does not give us the precise amplitude or the maximal rate of rise of transmembrane action potentials (V_{max}), which has a comparative relationship with sodium current (Cohen I. et al. 1981) and was calculated as an index of sodium channel availability.

In the present study, using a standard microelectrode technique and analyzing V_{max} , we investigated the effects of the substantial amount of sodium channel blockade on atrial PRR in the presence of a pure sodium channel blocker, pilsicainide (a class Ic clinical-used anti-arrhythmic agent). In addition, we performed the present study with tetrodotoxin (a extensively-studied pure sodium channel blocker) to reconfirm the relationship between sodium channel blockade and atrial PRR.

Methods

Preparations

Male guinea pigs (300 to 500 g) were anesthetized with an intraperitoneal injection of 20 mg/kg pentobarbiture sodium and 250 IU heparin and the hearts were rapidly excised. The left atrial appendage was removed from the heart, opened and mounted in a chamber (volume 3 ml). The preparation was superfused with oxygenated (100% oxygen) Tyrode's solution at rate of 10 ml/min. The composition of Tyrode's solution (mM) was as follows; NaCl 126, KCl 5.4, NaH_2PO_4 0.33, MgCl_2 1, HEPES 10, CaCl_2 1.8, Glucose 10 ($34 \pm 0.3^\circ\text{C}$, pH 7.4 with NaOH). The present study was performed according to the protocols approved by the Institutional Committee for Use and Care of Laboratory Animals of Tohoku University and the Guide for Care and Use of Laboratory Animals published by the U.S. National Institutes of Health (NIH Publication No. 85-23, revised 1996).

Electrophysiological Techniques

The preparations were stimulated with silver bipolar electrodes using rectangular stimuli (1 msec duration, 2-fold amplitude of diastolic threshold). The stimuli were produced by an electronic stimulator (Nihon Kohden: SEN-7103, Tokyo, Japan) and were delivered through an isolation unit (Nihon Kohden SS-302, Tokyo, Japan). Atrial ERP was recorded with a pair of electrodes made of flexible tungsten wires, 80 μm in diameter and amplified by a DC amplifier (AB-620G, Nihon Kohden, Tokyo, Japan).

The recording electrodes were positioned as close to the stimu-

lation electrodes as possible to avoid any effect on atrial conduction delay. Transmembrane action potential (AP) recordings were obtained from standard glass microelectrodes. The microelectrode filled with 3 M KCl had the 20-30 M Ω tip resistance. The recording potentials were connected to a high-input impedance and variable capacity neutralization amplifier (MEZ-8301, Nihon Kohden, Tokyo, Japan) and displayed on a storage oscilloscope (MS-5100A, Iwatsu, Tokyo, Japan). A microelectrode was inserted between the 2 silver stimulation electrodes. A reference electrode was positioned more than 0.5 mm away from the recording site. Duration of action potential was determined at 90% repolarization (APD_{90}). The maximal rate of rise of action potential upstroke (V_{max}) has a comparative relationship with sodium current (Cohen I. et al. 1981), and V_{max} was calculated as an index of sodium channel activity. For data acquisition, the output signals from the amplifier were digitized at 10 kHz using a 16-bit A/D converter and stored in a personal computer with an Axoscope program (Digidata 1200 Axon Instruments, Foster, CA, USA).

Experimental Protocols

After equilibrium by stimulation at a cycle length of 500 msec for 2 hours, atrial ERP was measured using extra-stimuli (S2) with varied coupling intervals (S1-S2) at basic cycle lengths (S1-S1) of 500 msec or 200 msec (rapid pacing). S2 was continuously introduced every tenth beat at each basic cycle length. ERP was defined as the longest S1-S2 interval failing to produce an atrial excitation. In the APD measurement series, action potentials were also recorded with the same pacing protocol as the ERP measurement series. The same measurements were repeated after application of pilsicainide hydrochloride (1.0, 5.0 and 10 μM , kindly supplied by Suntory Inc., Tokyo, Japan) or tetrodotoxin (0.3, 1.0 and 2.0 μM , Wako Pure Chemical Industries Ltd., Tokyo, Japan). The number of guinea-pigs in each group was $n = 3\sim 10$. The therapeutic plasma concentration of pilsicainide is 0.6-2.8 μM (0.2-0.9 $\mu\text{g/ml}$) when it was administrated orally, and the peak plasma concentrations was elevated by $1.7 \pm 0.9 \mu\text{g/mL}$ (5.3 μM) when pilsicainide was intravenously administrated at a dose of 1 mg/kg in clinics. In addition, we could see higher plasma concentration of pilsicainide (over 10 μM) in toxic cases (Ozeki et al. 1999). So we selected as the closest concentration of pilsicainide as we clinically experienced. Each concentration of the drugs was randomly applied and the measurements were performed 15 min after starting the drug administration.

Statistical Analysis

All data were presented as mean \pm s.d. Comparisons between 2 means were made by Student's paired *t*-test or unpaired *t*-test. Statistical analysis of the values between 3 observations was carried out by one-way analysis of variance (ANOVA). A value of $P < 0.05$ was considered to be statistically significant.

Results

Development of post-repolarization refractoriness by pilsicainide

Table 1 summarizes the effects of pilsicainide on ERP, APD_{90} and V_{max} at a cycle length of 500 msec. The percent change in ERP was significantly increased in the presence of 10 μM pilsicainide more than 1 μM pilsicainide or in the absence of pilsicainide (control) (Fig. 1). Fig. 2A

shows the representative tracings of action potentials at a basic cycle length of 500 msec. Pilsicainide did not change action potential duration regardless of the remarkable decrease in V_{max} (Fig. 2B). These results indicate that the substantial amount of sodium channel blockade with pilsicainide increases ERP without prolongation of APD, indicating that it produces post-repolarization refractoriness (PRR).

Rate-dependent blocking effect of pilsicainide

In the presence of 5 μM pilsicainide, the decrease of pacing cycle length from 500 msec to 200 msec increased ERP from 136 ± 11 msec to 156 ± 17 msec, but decreased APD_{90} from 109 ± 11 to 85 ± 3 msec, and V_{max} from 17 ± 8 msec to 11 ± 8 msec. Consequently, % change in ERP increased, whereas those in APD_{90} and V_{max} decreased (Fig. 3). These results suggest that atrial PRR induced by pilsicainide is enhanced in a rate-dependent manner.

Table 1. Effects of pilsicainide on electrical parameters in guinea-pig left atrial preparations.

	ERP msec	APD_{90} msec	V_{max} V/s
Control	117 ± 7	109 ± 7	22 ± 9
Pilsicainide 1 μM	119 ± 10	109 ± 8	22 ± 10
Pilsicainide 10 μM	137 ± 19	107 ± 8	13 ± 6

Data were obtained from the muscle region of the left atrial appendage at pacing cycle length of 500 msec ($n = 3\sim 10$). ERP, effective refractory period; APD_{90} , duration of action potential at 90% repolarization; V_{max} , maximum rate of rise of the action potential upstroke.

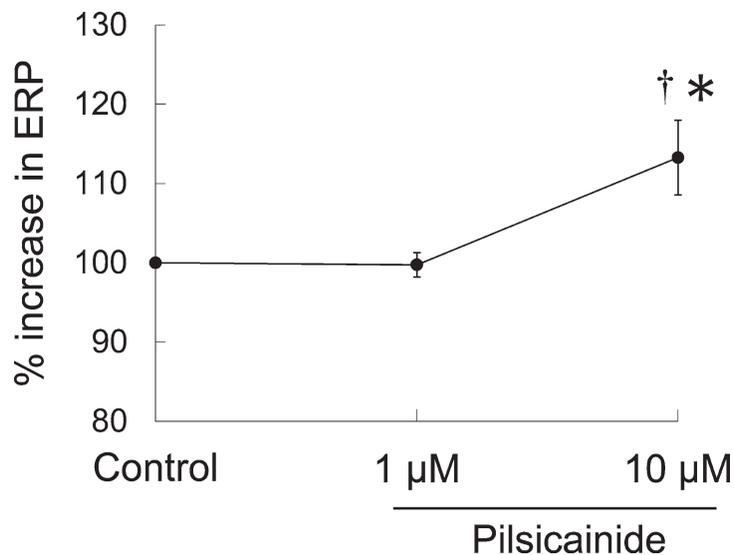


Fig. 1. Effect of pilsicainide on effective refractory period.

Pilsicainide (10 μM) significantly increased % change in ERP. Data were obtained from the left atrial appendage at pacing cycle length of 500 msec ($n = 3\sim 10$).

ERP = effective refractory period, * $P < 0.01$ vs. control, † $P < 0.05$ vs. pilsicainide 1 μM .

Post-repolarization refractoriness in the presence of tetrodotoxin

We then performed the same pacing protocols with tetrodotoxin, a pure sodium channel blocker to reconfirm the role of sodium channel blockade on the PRR phenomenon. ERP was prolonged in proportion to the reduction in V_{max} in a dose-dependent manner although APD_{90} remained unchanged in the presence of tetrodotoxin (Fig. 4). Tetrodotoxin could also reproduce a rate-dependent manner effects on PRR as does pilsicainide. At a faster cycle length of 200 msec, ERP was further increased although APD_{90} and V_{max} were decreased in the presence of 1 μM tetrodotoxin (rate-dependent block) (Fig. 5). These results support the significance of sodium channels blockade on PRR.

Discussion

The present study showed that exclusive sodium channel blockade using two pure sodium channel blockers enhanced atrial ERP without the prolongation of APD. In addition, the ERP increased in response to the reduction of the V_{max} in dose- and rate- dependent manners. These results provide the solid evidence of the correlation between the substantial amount of sodium channel blockade and atrial PRR.

Mechanisms of post-repolarization refractoriness as an anti-arrhythmic target

At the beginning of cardiac excitation, the opening probability of sodium channels increases abruptly since depolarized membrane potential goes beyond a certain threshold, and then sodium current through the channels develops the upstroke of an action potential (phase 0). Sodium channels immediately transform from open state to

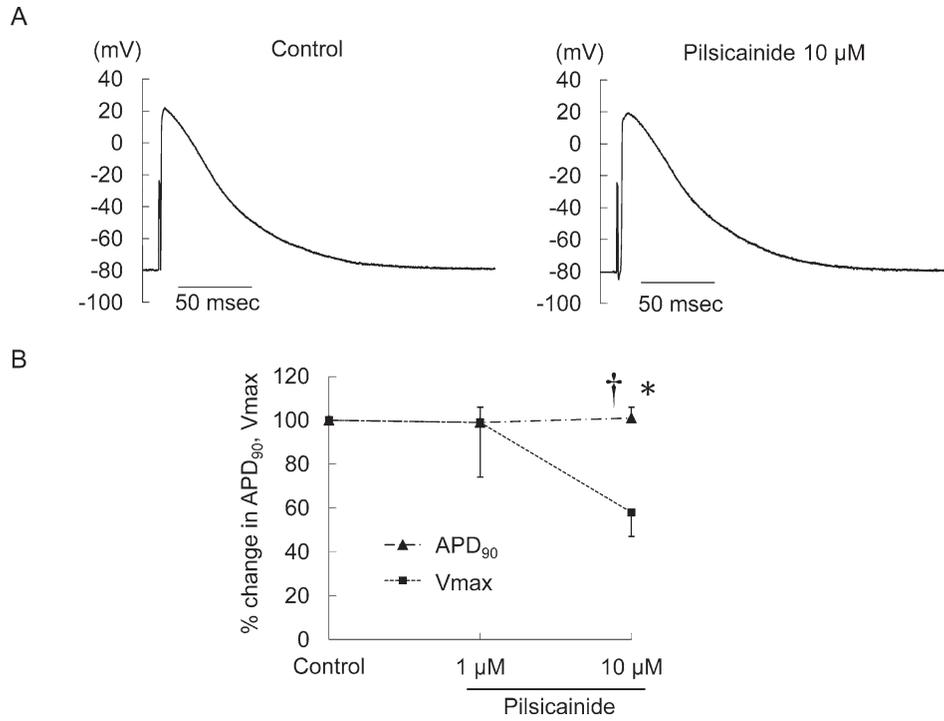


Fig. 2. Effects of pilsicainide on action potential (BCL 500 msec). (A) The representative tracings of action potentials in control condition and after pilsicainide administration. (B) Pilsicainide did not change the duration of action potential at 90% repolarization (APD₉₀) regardless of the remarkable decrease in maximum rate of rise of the action potential upstroke (Vmax) (*n* = 5). **P* < 0.01 vs. control, † *P* < 0.05 vs. pilsicainide 1 μM.

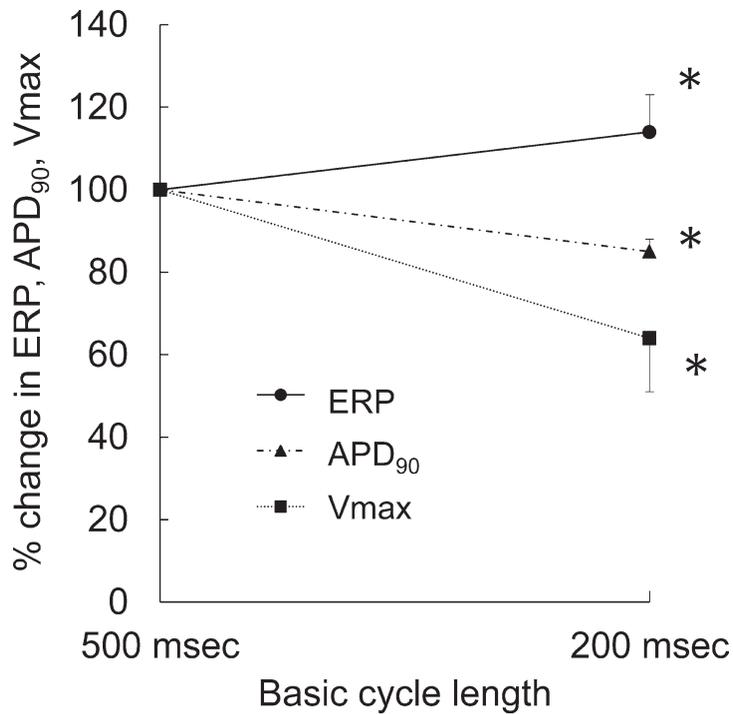


Fig. 3. Rate-dependent blocking effect of pilsicainide. % change in ERP was prolonged in the presence of pilsicainide after basic cycle length was shortened from 500 to 200 msec, whereas those in APD₉₀ and Vmax decreased (*n* = 4~5). **P* < 0.05 vs. basic cycle length 500 msec.

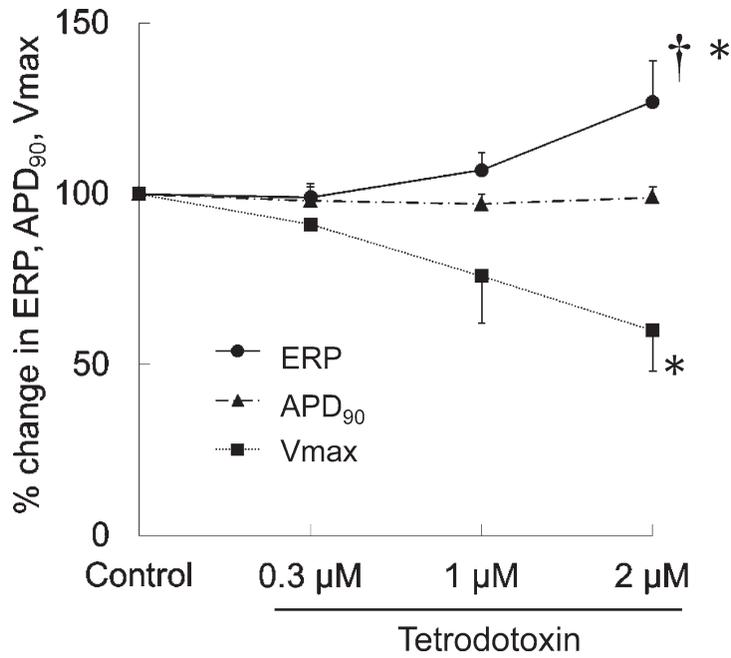


Fig. 4. Post-repolarization refractoriness in the presence of tetrodotoxin (BCL 500 msec).

Tetrodotoxin increased ERP in proportion to the reduction in Vmax in a dose-dependent manner, without affecting APD₉₀ ($n = 4-5$). See Figure 1 and 2 for abbreviations. * $P < 0.01$ vs. tetrodotoxin 0.3 μM , † $P < 0.05$ vs. tetrodotoxin 1 μM .

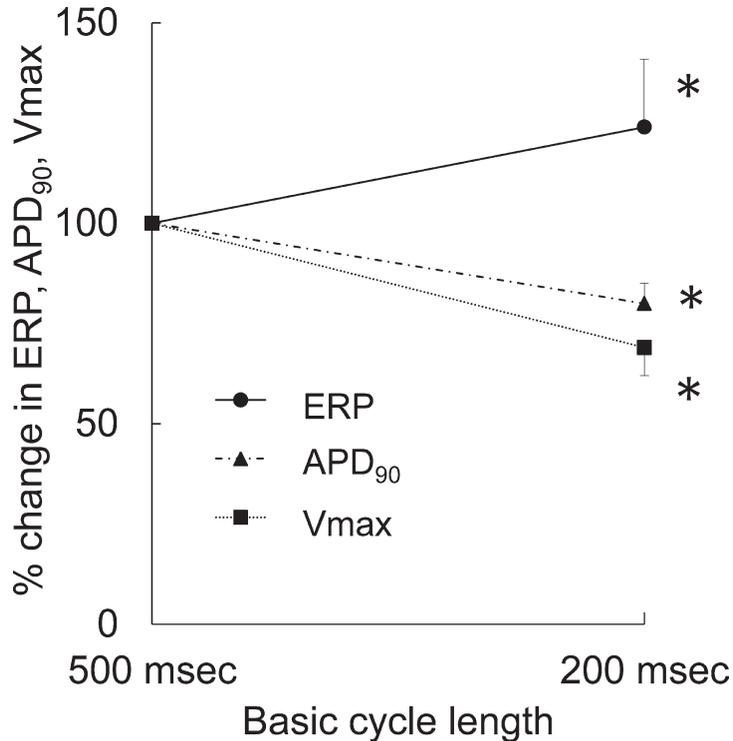


Fig. 5. Rate-dependent blocking effect of tetrodotoxin.

% change in ERP was prolonged in the presence of 1 μM tetrodotoxin when basic cycle length was shortened from 500 to 200 msec, whereas those in APD₉₀ and Vmax decreased ($n = 5$). * $P < 0.01$ vs. basic cycle length 500 msec.

inactivated state, and then recover to the closed state after repolarization of the membrane potential (Gettes and Reuter 1974). Effective refractory period (ERP) represents the maximal interval between the prior stimulation and the following stimulation that cannot develop the next action potential. ERP is usually dependent on action potential duration (APD) (Nademanee et al. 1990). In the previous human heart study, repetitive burst stimuli shortened both ERP and APD, and the encroachment of stimuli onto the preceding repolarization phase was associated with induction of tachyarrhythmia (Koller et al. 1995). Class I anti-arrhythmic agents, sodium channel blockers reduce an open probability of sodium channels and prolong ERP without increment of APD, so-called PRR. The enhancement of ERP through PRR could decrease vulnerability in cardiac tissue and prevent from intrusion of an early premature excitation and induction of tachyarrhythmias (Kirchhof et al. 1998).

The phenomena of post-repolarization refractoriness have been originally described in ventricular myocardium as a target of anti-arrhythmic agents. Nakaya et al. (1989) demonstrated post-repolarization refractoriness of guinea-pig papillary muscle by lidocaine and quinidine, and showed that these drugs caused depression of the excitability of second premature contractions more than first premature contractions. Inomata et al. (1987) reported that PRR in ventricular myocytes occurred by prolonging the time-dependent recovery of sodium channels from inactivation in addition to decreasing the excitable sodium channels with pilsicainide. This mechanism may also be applied to atrial PRR as demonstrated in the present study where atrial PRR was also accompanied by the inhibition of sodium current with pilsicainide. Shima et al. (1999) showed that procainamide enhanced atrial PRR in patients. Burashnikov et al. (2008) evaluated PRR in coronary-perfused atrial preparation from dogs chronically treated with amiodarone, showing that amiodarone increased ERP to a greater extent than APD_{90} , resulting in the development of PRR. These drugs inhibit not only sodium current but also potassium current that affects action potential duration. On the other hand, Kanki et al. (1998) revealed that a pure sodium channel blocker, pilsicainide, produced canine atrial PRR in which ERP was prolonged without increasing APD by using a monophasic action potential which facilitated accurate estimation of APD, but not to the evaluation of the precise quantitative sodium channel blockade. In the present study, we showed the enhancement of atrial PRR through the increment of ERP without the prolongation of APD in the presence of a pure sodium channel blockers, pilsicainide. Furthermore, we demonstrated atrial ERP increased in response to the decrement of the V_{max} , which represented the quantitative sodium channel availability, in a dose- and rate- dependent manner. It means that the prolongation of ERP by a substantial amount of sodium channel blockade correlates with atrial PRR. These phenomena were reconfirmed with tetrodotoxin, a highly specific

blocker of the sodium channel.

Local anesthetics including pilsicainide affect the voltage-dependent channel gating. Pilsicainide induces the frequency-dependent block and the shift of steady-state inactivation curve of the sodium channel (Inomata et al. 1987). On the other hand, the affinity of tetrodotoxin to sodium channel remains unchanged during maintained depolarization (tonic block), but it also affects the channel gating, especially frequent suprathreshold pulses produce extra use-dependent block beyond the tonic block and slow recovery from inactivation (Cohen C.J. et al. 1981). The repeat depolarization pacing protocol with tetrodotoxin in the present study can have a similar effect on sodium channel blockade as pilsicainide. So our data of tetrodotoxin support the relationship between the atrial PRR and substantial amount of sodium channel blockade seen in the pilsicainide experiments.

Here is other possible mechanism of atrial PRR. There have been some studies of the conduction block in the myocardium as a target of sodium channel blockers. Ohira et al. (1998) reported that procainamide (class Ia) and mexiletine (class Ib) increased the coupling interval of the rapid burst pacing to terminate monomorphic VT in a human study. Yoshizawa et al. (2001) reported the possibility of the association between conduction block by sodium channel blockade and PRR in their experimental isthmus model. They made the experimental isthmus on the right atrial surface parallel to the AV groove. Procainamide prolonged the coupling interval between basic pacing stimuli and the following single extra stimulus to induce the conduction block in the isthmus. They called it as block coupling interval (BCI). The BCI by procainamide was significantly longer than the prolongation of the local ERP. PRR might depend on BCI instead of local ERP prolongation in this situation. Thus the conduction block might work as an anti-arrhythmic target of sodium channel blockers as they claimed. On the other hand, a conduction slowing by sodium channel blockers instead of conduction block could lead to proarrhythmic effects (Kirchhof et al. 1998). The conduction block by sodium channel blocker is thought to be a considerable parameter in certain situations, but it should remain to be elucidated.

Potential anti-fibrillatory effects of pilsicainide

Anti-arrhythmic effects of pilsicainide on AF have been clinically recognized (Okishige et al. 2006; Horiuchi et al. 2009). The main mechanism of anti-fibrillation of sodium channel blockers is attributed to ERP prolongation through an enhancement of post-repolarization refractoriness (Kanki et al. 1998; Kirchhof et al. 2005). Sodium channel blockers shift the steady-state inactivation curve to more negative potentials. It requires more hyperpolarized potential to reach the critical degree of sodium channel recovery that produces next action potential. It follows the prolongation of repolarization process and refractory period. Pilsicainide, which belongs to class Ic, has a slow

recovery from inactivation, so repetitive depolarization of cardiac muscle increases the number of inactivated sodium channels, leading to the enhancement of ERP prolongation (rate-dependent manner) (Inomata et al. 1987).

Sodium channel blockade also leads to slowing conduction velocity with resultant decrease in re-entrant circuit size and stabilization of a leading circle reentry. These effects are counteractive effects on the wavelength. Shinagawa et al. reported that pilsicainide increased refractory period to a greater extent than the reduction in conduction velocity, resulting in prolongation of wavelength when AF terminated (Shinagawa et al. 2000). This means that anti-fibrillatory effects by pilsicainide are expected when enhancement of PRR is greater than decrease in conduction velocity. On the other hand, pilsicainide can terminate AF without prolongation of wavelength (Hayashi et al. 1998). Kawase et al. (2003) indicated that pilsicainide extended the excitable gap by conduction velocity delay and facilitates the excitation of the core of the mother rotor, leading to termination of AF.

Clinical implications

Recently, catheter ablation has prevailed as rhythm control therapy for paroxysmal AF (Wilber et al. 2010), and sodium channel blockers have been re-evaluated as the combination therapy with catheter ablation (Tojo et al. 2005; Kumagai et al. 2006). Class I sodium channel blockers increase ERP without affecting APD, or so-called PRR. This effect of ERP prolongation is unique as compared with Class III potassium channel blockers that increase ERP through APD prolongation with resultant calcium overload by QT prolongation and increased pro-arrhythmic risks (Jackman et al. 1988; Mykitysey et al. 2007). In addition, the effects of sodium channel blockers on PRR are enhanced in a rate-dependent manner, which is a key mechanism of prevention and termination of AF (Kanki et al. 1998; Shima et al. 1999). On the other hand, potassium channel blockers exhibit reverse rate-dependent prolongation of refractoriness (Jurkiewicz and Sanguinetti 1993; Yang et al. 1995). This means that sodium channel blockers might have superior effects on the prevention of AF initiation where atrial contraction rapidly increases compared with potassium channel blockers.

Study Limitations

Several limitations should be mentioned for the present study. First, in the present study, electrophysiological recordings were performed in isolated normal left atrium, where no modification factors of atrial ERP and APD (e.g. autonomic tones and hormones) are present. Second, we analyzed atrial PRR only in the left atrial appendage. So the effects of pilsicainide in this study might be different from those in other sites of atrium. Third, since the effectiveness of anti-arrhythmic agents may vary depending on pathophysiological conditions (Kumagai et al. 2006). The present findings with pilsicainide may not be readily appli-

cable to clinical conditions with paroxysmal and/or chronic AF.

Conflict of Interest

All authors do not have any conflict to declare.

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