Effects of Rapid Atrial Pacing on the Arrhythmogenic Activity of Single Cardiomyocytes From Pulmonary Veins

Implication in Initiation of Atrial Fibrillation

Yi-Jen Chen, MD; Shih-Ann Chen, MD; Yao-Chang Chen, MSc; Hung-I Yeh, MD, PhD; Paul Chan, MD; Mau-Song Chang, MD; Cheng-I Lin, PhD

Background—Pulmonary veins (PVs) are important sources of paroxysmal atrial fibrillation. Long-term rapid atrial pacing (RAP) changes atrial electrophysiology and facilitates the maintenance of atrial fibrillation. It is not clear whether RAP alters the arrhythmogenic activity of PVs. The purpose of this study was to isolate single PV cardiomyocytes from control and RAP dogs and evaluate their electrophysiological characteristics.

Methods and Results—The action potential and ionic currents were investigated in PV cardiomyocytes from control and long-term (6 to 8 weeks) RAP (780 bpm) dogs by use of the whole-cell clamp technique. Dissociation of PVs yielded rod-shaped single cardiomyocytes without (n=91, 60%) or with (n=60, 40%) pacemaker activity. Compared with the control group, the RAP dog PV cardiomyocytes had faster beating rates (0.86±0.28 versus 0.45±0.07 Hz, P<0.05) and shorter action potential duration. The RAP dog PV cardiomyocytes with pacemaker activity have a higher incidence of delayed (59% versus 7%, P<0.001) or early (24% versus 0%, P<0.005) afterdepolarization. The RAP dog PV cardiomyocytes with pacemaker activity had smaller slow inward and transient outward but larger transient inward (0.017±0.004 versus 0.009±0.002 pA/pF, P<0.05) and pacemaker (0.111±0.019 versus 0.028±0.008 pA/pF, P<0.001) currents. The RAP dog PV cardiomyocytes without pacemaker activity had only smaller slow inward and transient outward and larger pacemaker currents.

Conclusions—PVs contain multiple cardiomyocytes with distinct electrophysiological characteristics. RAP changes the electrophysiological characteristics and arrhythmogenic activity of PVs. (Circulation. 2001;104:2849-2854.)

Key Words: action potentials | fibrillation | ion channels | pacing | lung | veins

Pulmonary veins (PVs) have been demonstrated to be important sources of ectopic beats with the initiation of paroxysmal atrial fibrillation and focal atrial fibrillation.1,2 Previous anatomic and electrophysiological studies in isolated PV specimens have demonstrated that PVs contain a mixture of pacemaker cells and myocardial cells.3,4 Electrical recording in guinea pig PVs have shown that PVs have spontaneous activities or tachyarrhythmia after the infusion of ouabain.4 In canine PV strips, we have demonstrated that PVs have several types of cardiac muscle cells, which may induce atrial tachyarrhythmia through the enhancement of spontaneous activities or the high-frequency irregular rhythms.5

Long-term rapid atrial pacing (RAP) has been shown to be a useful model in the study of atrial fibrillation.6 After RAP or atrial fibrillation, the shortening of atrial refractoriness, the slower conduction velocity, and the increase of heterogeneity promote the maintenance of atrial fibrillation.6–8 In our previous study, RAP has been demonstrated to have a higher incidence of high-frequency irregular rhythms in PVs.9 These findings suggest that RAP has significant effects on the electrophysiological characteristics of PVs and may increase the arrhythmogenic activities of PVs. The purposes of the present studies were to investigate the electrophysiological characteristics of single canine PV cardiomyocytes and evaluate the effects of RAP on the arrhythmogenic activity of PV cardiomyocytes.

Methods

Animal Preparation

The procedures followed were in accordance with institutional guidelines. Fifteen normal dogs served as healthy controls. For the dogs subjected to long-term RAP (n=7), the animals (weight, 15 to 20 kg) were anesthetized with sodium pentobarbital (30 mg/kg IV). A bipolar pacing wire was positioned in the high right atrium as described recently.5 The pacing dogs received RAP (at a rate of 780 bpm) for 6 to 8 weeks in the conscious and freely
moving state. Two of these dogs developed sustained atrial fibrillation after long-term RAP.

Isolation of Single Cardiomyocytes

After the dogs were anesthetized, the hearts were rapidly removed through a thoracotomy and dissected at room temperature in normal Tyrode’s solution with a composition (in mmol/L) of NaCl 137, KCl 4, NaHCO3 15, NaH2PO4 0.5, MgCl2 0.5, CaCl2 2.7, and dextrose 11. Tyrode’s solution was equilibrated with a gas mixture of 97% O2/3% CO2, with a pH of ~7.4.

The PVs were separated from the left atrium 5 mm proximal to the junction of the PVs and left atrium. The veins were separated from the lung parenchyma 20 mm distal to the ending of the myocardial extension onto the PVs. The isolated PVs after the lumen was reversed were perfused from the distal end through a polyethylene tube, which was connected to a perfusion pump with a perfusion rate of 500 mL/h. The proximal end and side branches of the PVs were ligated with silk thread. The PVs were perfused with oxygenated Tyrode’s solution and then replaced with oxygenated Ca2+-free Tyrode’s solution containing 300 U/mL collagenase (Sigma, type I) and 0.5 U/mL protease (Sigma, type XIV). After the PVs had been softened, they were cut into fine pieces and gently shaken in 5 to 10 mL of Ca2+-free oxygenated Tyrode’s solution until single cardiomyocytes were obtained. The solution was then gradually changed to normal oxygenated Tyrode’s solution. Only cells showing cross-striations were used. Experiments were carried out at room temperature (22°C to 25°C). The cells were stabilized in the bath for ≥30 minutes before experiments.

The whole-cell patch-clamp technique was used by means of an Axopatch 1D amplifier (Axon Instruments). Borosilicate glass electrodes (outer diameter 1.8 mm) were used, with tip resistances of 3 to 5 MΩ. Before formation of the membrane-pipette seal, tip potentials were zeroed in Tyrode’s solution. Junction potentials (8 mV) were corrected for action potential (AP) recording. The pipette solution contained (in mmol/L) KCl 120, MgCl2 1, Na2ATP 5, HEPES 10, EGTA 0.5, and CaCl2 0.01, adjusted to pH 7.2 with 1N KOH. To visually identify whether the cells had pacemaker activity, we did not add ionic current blockers in the pipette solution. The APs were recorded in current-clamp mode and ionic currents in voltage-clamp mode as described previously. Normal Tyrode’s solution was used as bath solution for current and AP recordings. A small, hyperpolarizing step from a holding potential of −50 mV to a testing potential of −55 mV for 80 ms was delivered at the beginning of each experiment. The differences between the capacitive currents was divided by the applied voltage step to obtain the total cell capacitance. APs were elicited by pulses of 2 ms and suprathreshold voltage (range 50 to 90 mV). Voltage command pulses were generated by a 12-bit digital-to-analog converter controlled by pCLAMP software (Axon Instruments). AP measurements were begun 5 minutes after cell rupture, and the steady-state AP duration (APD) was measured at 50% (APD50) and 90% (APD90) of full repolarization. Recordings were low-pass-filtered at half the sampling frequency. Data were sampled at rates varying from 2 to 25 kHz. Early afterdepolarization (EAD) was defined as the cells generating oscillatory potentials at depolarized levels.

Depolarization-induced currents were elicited at clamped potentials from −40 to +60 mV in 10-mV steps for 1 second at a frequency of 0.1 Hz. A holding potential of −40 mV was used to inactivate the sodium channel. Hyperpolarization-activated currents were activated from −40 mV to test potentials ranging from −20 to −120 mV in 10-mV steps for 1 second at a frequency of 0.1 Hz. A slowly activated inward current that did not inactivate was measured as the pacemaker current, I1. Transient inward current (I2) was induced at clamped potentials from −40 to +40 mV for a duration of 3 seconds and then repolarized to −40 mV. The amplitude of I2 was measured as the difference between the peak of the transient current and the mean of current just before and after the transient current.10

Figure 1. Confocal images demonstrate morphologies of single PV cardiomyocytes. In general, although they vary in length, width, and number of branches, these cells are rod-shaped, with a centrally located nucleus. Cells stained with hematoxylin and eosin are scanned with setting for FITC, as described.24 Bar=15 µm.

Statistics

All quantitative data are expressed as mean±SEM. The differences between pacemaker and nonpacemaker or between control and RAP dogs were analyzed by 2-way ANOVA followed by a post hoc Tukey test or by unpaired t test for the continuous data; χ² test with Yates’ correction or Fisher’s exact test was used for the categorical data. A value of P<0.05 was considered to be statistically significant.

Results

Cell Morphologies of PV Cardiomyocytes

Dissociation of the canine PVs yielded single rod-shaped cells with striations, characteristic of cardiomyocytes (Figure 1). In the control dog PV cardiomyocytes, the majority (n=91, 60%) of these cells did not beat spontaneously and had a rapid upstroke of AP with a steady level of membrane potential and no diastolic depolarization (Figure 2A). The other PV cardiomyocytes (n=60, 40%) had pacemaker activity and beat spontaneously or with spontaneous depolarization (15±3 mV/s) (Figure 2B). The PV cardiomyocytes with pacemaker activity have a beating rate of 0.45±0.07 Hz (measured from consecutive beats in 10 seconds). The cell size and electrical capacitance were similar between the PV cardiomyocytes with and without pacemaker activity (Table).

There were also cardiomyocytes with (n=17, 25%) and without (n=50, 75%) pacemaker activity from the RAP dog PVs (P>0.05 compared with control dogs). The RAP dog PV cardiomyocytes with pacemaker activity had faster beating rates (0.86±0.28 Hz, P<0.05) than the control dogs. The cell width and electrical capacitance in the RAP dog cardiomyocytes with and without pacemaker activity were larger than those in the control dogs (Table).

AP Configurations of PV Cardiomyocytes

The maximum diastolic potential (−63±1 versus −65±1 mV, P>0.05) and AP amplitude (87±2 versus 91±2 mV,
Cell Size in Control and RAP Dog PV Cardiomyocytes

<table>
<thead>
<tr>
<th></th>
<th>Control Dog PV Cardiomyocytes</th>
<th>RAP Dog PV Cardiomyocytes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pacemaker</td>
<td>(n=60)</td>
<td>(n=50)</td>
</tr>
<tr>
<td>Non-pacemaker</td>
<td>(n=91)</td>
<td></td>
</tr>
<tr>
<td>Length, μm</td>
<td>120±4</td>
<td>122±12</td>
</tr>
<tr>
<td>Width, μm</td>
<td>14±1</td>
<td>17±1*</td>
</tr>
<tr>
<td>Capacitance, pF</td>
<td>123±8</td>
<td>183±24*</td>
</tr>
</tbody>
</table>

Values are mean±SEM.

*P<0.05, significantly different from the control dog PV cardiomyocytes.

P(<0.05) were not significantly different between control (n=43) and RAP (n=41) dog PV cardiomyocytes without pacemaker activity. The APD₅₀ and APD₉₀ in RAP dogs (103±11 and 274±18 ms), however, were significantly shorter than those of control dogs (170±11 and 483±24 ms, respectively, P(<0.05).

As shown in Figure 2A, in control PV cardiomyocytes without pacemaker activity, the APDs were significantly shortened during a faster stimulation rate. The ratios of APD₅₀ and APD₉₀ between electrical stimulation at 1 and 0.1 Hz were 113±4% (P(<0.005) and 79±5% (P(<0.005), respectively. Figure 2C shows an example of the AP in the RAP dog PV cardiomyocytes with pacemaker activity. In RAP PV cardiomyocytes without pacemaker activity, the APD was not significantly changed during different electrical stimulation rates. The ratios of APD₅₀ and APD₉₀ between electrical stimulation with 1 and 0.1 Hz were 113±4% (P(0.05) and 99±4% (P(0.05), respectively.

In normal Tyrode’s solution, 10 (59%) of the RAP dog PV cardiomyocytes with pacemaker activity had delayed afterdepolarization (DAD). Figure 2D shows an example of the occurrence of DAD in a RAP PV cardiomyocyte with pacemaker activity under electrical stimulation at a rate of 0.1 Hz. In contrast, only 4 (7%) of the control dog PV cardiomyocytes with pacemaker activity had DAD (P(<0.001). In addition, 4 (24%) of the RAP dog PV cardiomyocytes with pacemaker activity had EAD in normal Tyrode’s solution, as shown in Figure 2, E and F. In contrast, none of the control dog PV cardiomyocytes with pacemaker activity had EAD during normal Tyrode’s solution superfusion (P(<0.005). Neither the RAP nor control dog PV cardiomyocytes without pacemaker activity had EAD or DAD in normal Tyrode’s solution.

Ionic Currents of Cardiomyocytes in Control and RAP Dogs

Depolarization-Induced Currents

As shown in Figure 3, A and B, slow inward currents occurred from the holding potential of −40 mV, consistent with the behavior of L-type calcium current (I_{Ca,L})¹¹ in the control dog PV cardiomyocytes. The densities of the I_{Ca,L} were similar between the cardiomyocytes with (n=29) and without (n=24) pacemaker activity (Figure 3E). In addition, there were transient outward currents (I_{to})¹² with rapid activation kinetics and progressive increases in amplitude with increasing depolarization steps in PV cardiomyocytes. The densities of I_{to} (measured from the outward current peak to the quasi steady state at 200 ms from −40 to +60 mV) were similar between cardiomyocytes with (n=29) and without (n=24) pacemaker activity (Figure 3F). The long depolarizing steps also induced a slowly activating nonactivating outward current similar to the characteristics of delayed rectified outward current (I_{Ks}).¹³ The density of the peak I_{Ks} (measured from the outward current at the end of 1 second of depolarization from −40 to +60 mV) was similar between the cardiomyocytes with (n=43) and without (n=28) pacemaker activity (Figure 3G).

As shown in Figure 3, C and D, there were small I_{Ca,L}S in the RAP dog PV cardiomyocytes with or without pacemaker activity during depolarization. The densities of I_{Ca,L} in the RAP dog PV cardiomyocytes with (n=17) or without (n=35)

![Figure 2. AP configurations and afterpotentials in control and RAP dog PVs. A and B, APs of control dog PV cardiomyocytes without and with pacemaker activity. C, APs of RAP dog PV cardiomyocytes without pacemaker activity; D, DAD in RAP dog PV pacemaker cardiomyocytes during electrical stimulation at a rate of 0.1 Hz in normal Tyrode’s solution. E and F, EAD generates at depolarized levels during spontaneous beating. Electrical stimuli at 1 Hz (•) and 0.1 Hz (○). Arrows indicate EAD; *, DAD.]
pacemaker activity were significantly smaller than those in the control dogs (Figure 3E). The densities of the currents in the RAP dog PV cardiomyocytes with (n=17) or without (n=30) pacemaker activity were significantly smaller than those in the control dogs (Figure 3F). In contrast, the densities of the currents in the RAP dog PV cardiomyocytes with (n=17) or without (n=35) pacemaker activity were similar to those in the control dogs (Figure 3G).

Repolarization-Induced Currents

Figure 4 shows examples of the currents in control and RAP dog PV cardiomyocytes with pacemaker activity. In control dog PVs, the current identified in 18 (49%) of 37 cardiomyocytes with or 12 (44%) of 27 cardiomyocytes without pacemaker activity (P<0.05). As shown in Figure 5A, the density of the current was similar between the control dog PV cardiomyocytes with (n=44) or without (n=32) pacemaker activity. Compared with control, however, there was greater in the RAP dog PV cardiomyocytes with pacemaker activity (n=16) but similar in the RAP dog PV cardiomyocytes without pacemaker activity (n=32).

Hyperpolarization steps from the holding potential induced an instantaneous inward current with slow inactivation kinetics in PV cardiomyocytes similar to the properties of the cardiac inward rectified currents (I_K).

Figure 6, A and B, shows examples of the current in the PV cardiomyocytes with pacemaker activity. The density of the current was similar between PV cardiomyocytes with and without pacemaker activity (Figure 5B). The densities of the current in the RAP dog PV cardiomyocytes with (n=16) or without (n=35) pacemaker activity were similar to those in the control dogs (Figure 3G).

Effects of Isoproterenol

After the infusion of isoproterenol in the control dog PV cardiomyocytes with pacemaker activity, the spontaneous activities were increased from 0.5 ± 0.0 to 1.8 ± 0.3 Hz (n=9).

Figure 7A illustrates that, in a PV cardiomyocyte with pacemaker activity, 10 nmol/L isoproterenol increased the spontaneous activities. In addition, in 20 PV cardiomyocytes with pacemaker activity, isoproterenol induced the occurrence of DAD in 8 cardiomyocytes (40%) and EAD in 3 cardiomyocytes (15%). Figure 7, B and C, shows examples of isoproterenol-induced oscillatory afterpotentials (DAD and EAD) in PV pacemaker cardiomyocytes.
Discussion

Electrophysiological Characteristics of Canine PV Cardiomyocytes

Through perfusion methods, we isolated single cardiomyocytes with or without pacemaker activity from canine PVs, thus confirming the observations in embryonic heart, where PV was suggested to work as a subsidiary pacemaker. Pacemaker cells may play a role in the occurrence of atrial fibrillation, because new wavelets of atrial fibrillation may arise from these cells. Therefore, it is possible that PVs may induce atrial arrhythmia through the pacemaker activity. Moreover, isoproterenol-induced EAD and DAD in PV cardiomyocytes suggested that triggered activities play a role in the arrhythmogenic mechanisms of PVs. Previous clinical and in vitro studies also have indicated that triggered activity underlies PV arrhythmogenic activity.

The ionic experiments demonstrated that PV cardiomyocytes have ionic characteristics similar to the properties of heart cells, although we cannot completely dissect out the target currents without the administration of channel blockers. Unlike sinus nodal cells, however, only the part of canine PV cardiomyocytes with pacemaker activity were similar to the nature of pacemaker cells in sinus node. The small $I_{\text{K1}}$ in the PV cardiomyocytes with pacemaker activity were similar to the nature of pacemaker cells in sinus node. Unlike sinus nodal cells, however, only the part of canine PV cardiomyocytes with pacemaker activity were similar to the nature of pacemaker cells in sinus node.

Effects of RAP on the Arrhythmogenic Activities of Canine PV Cardiomyocytes

Rapid atrial rates change the cellular characteristics of atrial myocytes. Our study also found that RAP dog PV cardiomyocytes have significantly larger electrical capacitance and cell size. Moreover, RAP dog PV cardiomyocytes had shorter APD and maladaptation of APD to change in rates, which were similar to the effects of RAP on atrial myocytes. The electrophysiological changes after RAP would easily induce reentrant circuits, like the finding of high-frequency irregular rhythms in isolated PVs. In addition, RAP also increased the spontaneous beating rates of cardiomyocytes with pacemaker activity.
activity. The increasing occurrences of DAD or EAD in the RAP dog PV pacemaker cardiomyocytes also suggested that RAP enhanced the triggered activity of PVs. These findings suggested that RAP may facilitate the occurrence of atrial fibrillation through the enhancement of arrhythmogenic activity of PVs.

As in the previous studies in atrial myocytes, RAP would decrease $I_{\text{Ca,L}}$ and $I_{\text{f}}$ but did not have significant effects on the $I_{\text{K}}$ in PV cardiomyocytes. The decrease of $I_{\text{Ca,L}}$ may account for the shortening of APD in the RAP dog PV cardiomyocytes. This finding also suggests that PV cardiomyocytes have electrical remodeling after RAP. In addition, we found that PV cardiomyocytes have increased $I_{\text{f}}$ after RAP. Previous studies have demonstrated the increases of $I_{\text{f}}$ in failing ventricle, which were considered to contribute to the increasing arrhythmogenic activity of diseased hearts. The increase of $I_{\text{f}}$ may increase the arrhythmogenic activity and underlie the increased spontaneous activities of RAP dog PV pacemaker cells.

Coronary sinus myocytes have been demonstrated to have $I_{\text{Ca,S}}$, which may result in the triggered activity of coronary sinus. Knowledge about the $I_{\text{f}}$ in PV cardiomyocytes, however, was limited. In this study, we demonstrated the presence of $I_{\text{f}}$ in PV cardiomyocytes. In addition, RAP would increase $I_{\text{Ca,S}}$ in PV cardiomyocytes with pacemaker activity. It has been postulated that RAP may induce calcium overload in atrial myocytes. The increase of intracellular calcium may result in the increase of $I_{\text{Ca,L}}$ in our PV cardiomyocytes, which further enhances DAD in the RAP dog PV cardiomyocytes with pacemaker activity.

Conclusions

This study demonstrated that PVs contain a mixture of cardiomyocytes with distinct electrophysiological characteristics. RAP changes the electrophysiological characteristics and may increase the arrhythmogenic activity of PVs.

Acknowledgments

This study was supported by grants NSC89-2320-B016-095, NSC90-2314-B075-044, VTY 90-P5-44, and DOD-90-11, Taiwan, ROC.

References

Effects of Rapid Atrial Pacing on the Arrhythmogenic Activity of Single Cardiomyocytes From Pulmonary Veins: Implication in Initiation of Atrial Fibrillation
Yi-Jen Chen, Shih-Ann Chen, Yao-Chang Chen, Hung-I Yeh, Paul Chan, Mau-Song Chang and Cheng-I Lin

_Circulation_. 2001;104:2849-2854
doi: 10.1161/hc4801.099736

_Circulation_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2001 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circ.ahajournals.org/content/104/23/2849