

# Temperature Regulates the Arrhythmogenic Activity of Pulmonary Vein Cardiomyocytes

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## Key Words

Action potential · Atrial fibrillation · Triggered activity

## Abstract

Temperature plays an important role in the electrophysiology of cardiomyocytes. Pulmonary veins (PVs) are known to initiate paroxysmal atrial fibrillation. The effects of temperature on the arrhythmogenic activity of rabbit single PV and atrial cardiomyocytes were assessed using the whole-cell clamp technique. PV cardiomyocytes had different beating rates at low (22–25°C), normal (38–39°C) and high (40–41°C) temperatures ( $0.9 \pm 0.1$ ,  $3.2 \pm 0.4$ ,  $6.4 \pm 0.6$  Hz, respectively;  $p < 0.001$ ). There were different action potential durations and incidences of delayed afterdepolarization in PV cardiomyocytes with pacemaker activity (31, 59, 63%;  $p < 0.05$ ), PV cardiomyocytes without pacemaker activity (16, 47, 60%;  $p < 0.001$ ), and atrial myocytes (0, 0, 21%;  $p < 0.05$ ). However, oscillatory afterpotentials were only found in PV cardiomyocytes with pacemaker activity at normal (50%) or high (68%) temperatures, but not at low temperatures ( $p < 0.001$ ). Both PV and atrial cardiomyocytes had larger transient inward currents and inward rectified currents at high temperatures. Additionally, PV cardiomyocytes with and without pacemaker activity had larger pacemaker currents at higher temperatures. This study dem-

onstrated that PV cardiomyocytes have an increase in arrhythmogenic activity at high temperatures because of enhanced automaticity, induced triggered activity, or shortening of action potential duration.

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## Introduction

Temperature is known to play an important role in the electrophysiological characteristics of myocytes [1, 4, 12, 14, 15, 18, 24]. Previous studies have shown that changes in temperature alter action potential (AP) duration [1, 15] and increases in temperature facilitate the occurrence of triggered activity with the genesis of delayed afterdepolarization (DAD) [4, 18]. Several researchers have evaluated the effects of temperature on cardiac arrhythmia, but their results remain controversial. Changes in temperature alter the spontaneous activity of sinoatrial cells [2, 16]. Hypothermia has been shown to prevent the occurrence of ventricular arrhythmia [22], but it has also been shown to increase ventricular arrhythmia [25]. Additionally, studies on the effects of hyperthermia on the electrophysiological characteristics of myocytes are lacking and our knowledge about the roles of temperature on atrial rhythms or pacemaker activity is limited.

Pulmonary veins (PVs) are known to be important sources of ectopic beats (with the initiation of paroxysmal atrial fibrillation) or the foci of ectopic atrial tachycardia and focal atrial fibrillation [10, 13, 27]. Anatomical and electrophysiological studies on isolated PV specimens have demonstrated that PVs contain a mixture of pacemaker cells and working myocardium [17, 19, 23]. Our previous study demonstrated the presence of spontaneous activity or high-frequency irregular rhythms in isolated canine PVs [7]. These electrical activities may underlie the arrhythmogenic activity of PVs. After the isolation of single cells, PVs were found to have cardiomyocytes with or without pacemaker activity as well as distinct electrophysiological characteristics and arrhythmogenic potentials [6, 8, 9]. Moreover, studies of ionic currents indicated that transient inward or pacemaker currents ( $I_f$ ) may contribute to the arrhythmogenic activity of PVs [6, 8]. Clinical reports have shown that elevation of body temperature induced atrial tachyarrhythmia from PVs by an unknown mechanism [20]. These findings suggest that temperature has a significant effect on the electrophysiological characteristics and increases arrhythmogenic activity of PVs. Increased temperature has been shown to increase *L*-type calcium currents and potassium currents in ventricular myocytes [1, 15]. However, it is not clear whether temperature also changes the PV arrhythmogenic activity through its effects on  $I_f$  or transient inward currents. The purpose of the present study was to evaluate the effects of temperature on the electrical activity and membrane currents of PV cardiomyocytes.

## Materials and Methods

### *Isolation of PV Cardiomyocytes*

The investigation conformed with the institutional Guide for the Care and Use of Laboratory Animals. As previously described [6, 9], rabbits weighing 1–2 kg were anesthetized with an intraperitoneal injection of sodium pentobarbital (40 mg/kg). A mid-line thoracotomy was then performed and the heart and lungs were quickly removed. The PVs were perfused in a retrograde manner via polyethylene tubing cannulated through the aorta and left ventricle into the left atrium. The free end of the polyethylene tubing was connected to a Langendorff perfusion column for perfusion with oxygenated normal Tyrode's solution at 37°C (containing, in mM: NaCl 137, KCl 5.4, CaCl<sub>2</sub> 1.8, MgCl<sub>2</sub> 0.5, HEPES 10 and glucose 11; pH was adjusted to 7.4 by titrating with 1 *N* NaOH). The perfusate was replaced with oxygenated Ca<sup>2+</sup>-free Tyrode's solution containing 300 U/ml collagenase (Sigma, Type I) and 0.25 U/ml protease (Sigma, Type XIV) for 8–12 min. Afterwards the proximal PVs (8–12 mm) were cut away from the atrium and lung and placed in a dissection chamber containing Ca<sup>2+</sup>-free oxygenated Tyrode's solution. The piece of tissue was cut into fine pieces and gently shaken in

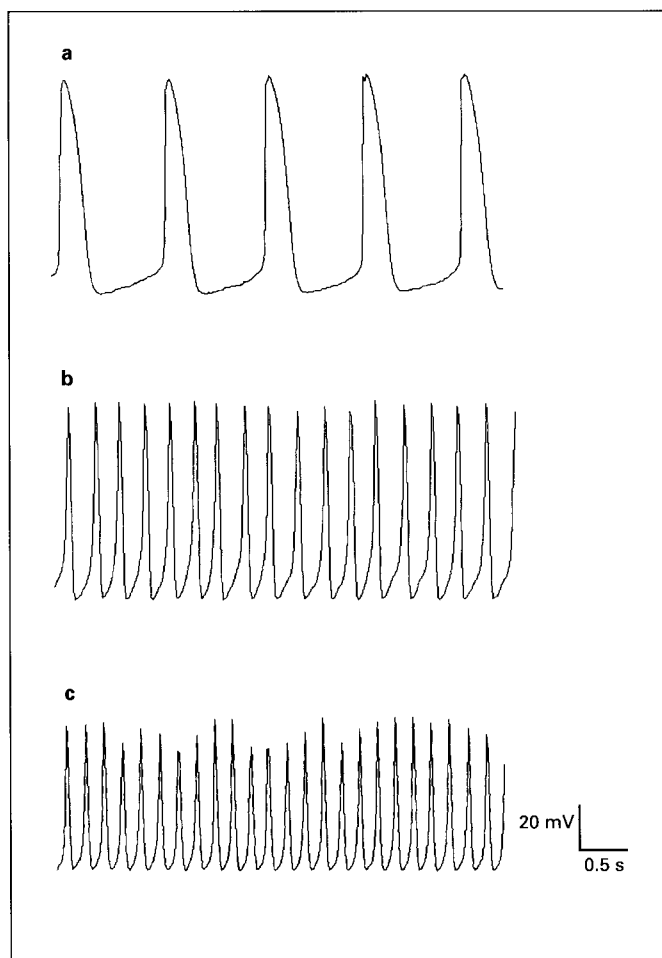
5–10 ml of Ca<sup>2+</sup>-free oxygenated Tyrode's solution until single cardiomyocytes were obtained. Only cells showing clear cross striations were used. In this retrograde perfusion method, atrial myocytes with rod-shaped morphologies and cross striation were also isolated from the left atrial appendage. Electrophysiological experiments were carried out at low (22–25°C), normal (38–39°C, the normal body temperature of rabbits) and high temperatures (40–41°C). Experiments with varying temperatures were performed on different cardiomyocytes. Therefore we eliminated the impact of varying temperatures repeatedly in a short period and had stable electrical recordings. The cells were allowed to stabilize in a bath for at least 30 min before experiments.

### *Electrophysiological Study*

The whole-cell patch-clamp technique was used by means of an Axopatch 1D amplifier (Axon Instruments, Calif., USA). Borosilicate glass electrodes (outer diameter 1.8 mm) were used, with tip resistances of 3–5 MΩ. Before formation of the membrane-pipette seal, tip potentials were zeroed in Tyrode solution. Junction potentials (9 mV) were corrected for AP recordings. The pipette solution contained (in mM) KCl 20, K aspartate 110, MgCl<sub>2</sub> 1, Mg<sub>2</sub>ATP 5, HEPES 10, EGTA 0.5, and LiGTP 0.1, Na<sub>2</sub> phosphocreatine 5 and was adjusted to pH 7.2 with 1 *N* KOH. The series resistances (6.4 ± 0.6 MΩ) were electrically compensated. The APs were recorded in current-clamp mode and ionic currents in voltage-clamp mode, as described previously [8, 9]. Normal Tyrode 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 was delivered at the beginning of each experiment. The area under the capacitive currents was divided by the applied voltage step to obtain the total cell capacitance. The cell capacitances at low, normal and high temperatures were 80 ± 5 pF (*n* = 60), 66 ± 6 pF (*n* = 44) and 71 ± 6 pF (*n* = 46) for PV cardiomyocytes and 68 ± 6 pF (*n* = 24), 60 ± 7 pF (*n* = 20) and 63 ± 8 pF (*n* = 21) for atrial myocytes, respectively.

APs were elicited by pulses of 2 ms and suprathreshold stimulation (50–90 mV) at a driven rate of 1 Hz. Voltage command pulses were generated using a 12-bit digital-to-analog converter controlled by pCLAMP software (Axon Instruments). AP measurements were begun 5 min after cell rupture and the steady-state AP duration was measured at 50% (APD<sub>50</sub>) and 90% (APD<sub>90</sub>) of full repolarization. Recordings were low pass filtered at half the sampling frequency. Data were sampled at rates varying from 2 to 25 kHz. DAD was defined as an AP developed after complete repolarization. Oscillatory afterpotentials were defined as APs generated at a depolarized level, and the AP failed to repolarize to the maximal diastolic potential [3].

Transient inward current was induced by clamped potentials from –40 to +40 mV for 2 s and then repolarized to –40 mV. The amplitude of the transient inward current was measured as the difference between the peak of the transient current and the mean of the current just before and after the transient current [26]. Hyperpolarization-activated currents were activated from –40 mV to test potentials ranging from –60 to –120 mV in 10-mV steps for 1 s at a frequency of 0.1 Hz. The induced instantaneous currents with slow inactivation kinetics were consistent with the properties of the cardiac inward rectifier currents,  $I_{K1}$  [21]. A progressively larger inward current developed with slow voltage-dependent kinetics and was not inactivated, which was suppressed by 5 mM cesium and measured as  $I_f$ .



**Fig. 1.** Different beating rates of PV cardiomyocytes at low (**a**), normal (**b**) and high (**c**) temperatures. Traces are spontaneous APs recorded from 3 cells at different temperatures.

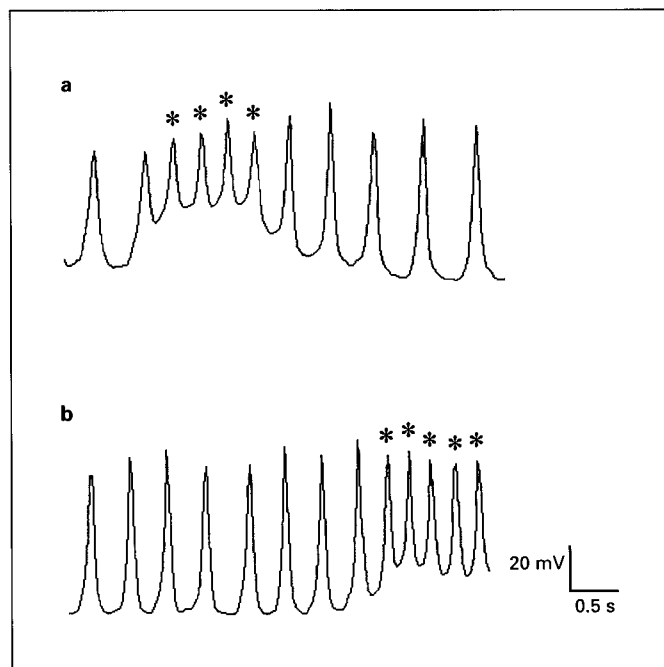
#### Statistics

Continuous variables are expressed as means  $\pm$  SE. Differences between PV and atrial cardiomyocytes at different temperatures were analyzed using two-way ANOVA. Multiple comparisons were analyzed with the Fisher LSD test. Nominal variables were compared using  $\chi^2$ -square analysis with Yates correction or Fisher's exact test. A  $p$  value  $< 0.05$  was considered to be statistically significant.

## Results

#### Effects of Temperature on APs of PV Cardiomyocytes

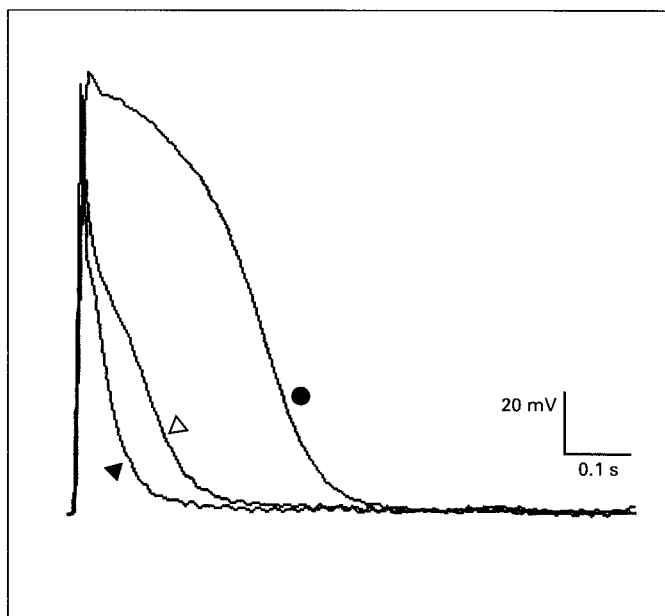
**PV Cardiomyocytes with Pacemaker Activity.** The beating rates of PV cardiomyocytes increased as the temperature increased (low temperature,  $n = 26$ ,  $0.9 \pm$



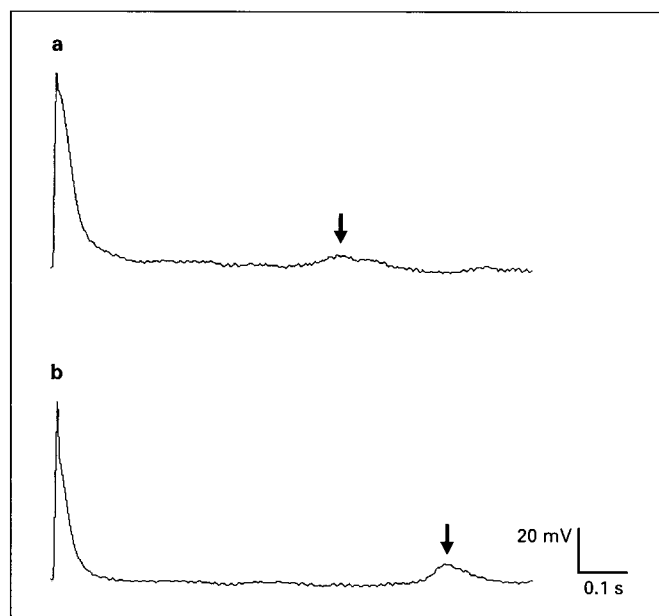
**Fig. 2.** Occurrence of oscillatory afterpotentials during spontaneous beating in 2 PV cardiomyocytes with pacemaker activity at normal (**a**) and high (**b**) temperature. Oscillatory afterpotentials developed before full repolarization of the AP. Asterisks indicate the onset of oscillatory afterpotentials following a previous spontaneous AP.

$0.1$  Hz; normal temperature,  $n = 17$ ,  $3.2 \pm 0.4$  Hz; high temperature,  $n = 19$ ,  $6.4 \pm 0.6$  Hz;  $p < 0.001$ ). Figure 1 shows examples of the different beating rates of PV cardiomyocytes at varying temperatures. In addition, as the examples in figure 2 show, there were bursting firings of oscillatory afterpotentials during spontaneous beating in PV cardiomyocytes at normal (fig. 2a) and high (fig. 2b) temperatures. PV cardiomyocytes with pacemaker activity at high (14/19, 68%) or normal (8/17, 47%) temperatures had higher incidences of oscillatory afterpotentials than those at low temperatures (0/26, 0%;  $p < 0.001$ ). Moreover, PV cardiomyocytes with pacemaker activity at low temperature had a lower incidence (8/26, 31%;  $p < 0.05$ ) of DAD than those at normal (10/17, 59%) or high temperatures (13/19, 63%).

**PV Cardiomyocytes without Pacemaker Activity.** Table 1 summarizes the AP configurations of PV cardiomyocytes without pacemaker activity at different temperatures. There was a significantly longer AP duration ( $APD_{50}$  and  $APD_{90}$ ) and larger amplitude of AP in PV cardiomyocytes at low temperatures. Figure 3 shows the



**Fig. 3.** Examples of APs of 3 PV cardiomyocytes without pacemaker activity at low (●), normal (□) and high (▼) temperatures, respectively. The AP duration at 50% repolarization (APD<sub>50</sub>) and 90% repolarization (APD<sub>90</sub>) was shortened as the temperature rose.



**Fig. 4.** Occurrence of delayed afterdepolarizations (DADs) in 2 PV cardiomyocytes without pacemaker activity at normal (a) and high (b) temperatures. Electrical stimulations were applied at a rate of 0.1 Hz. Arrows indicate the first DAD following the driven AP.

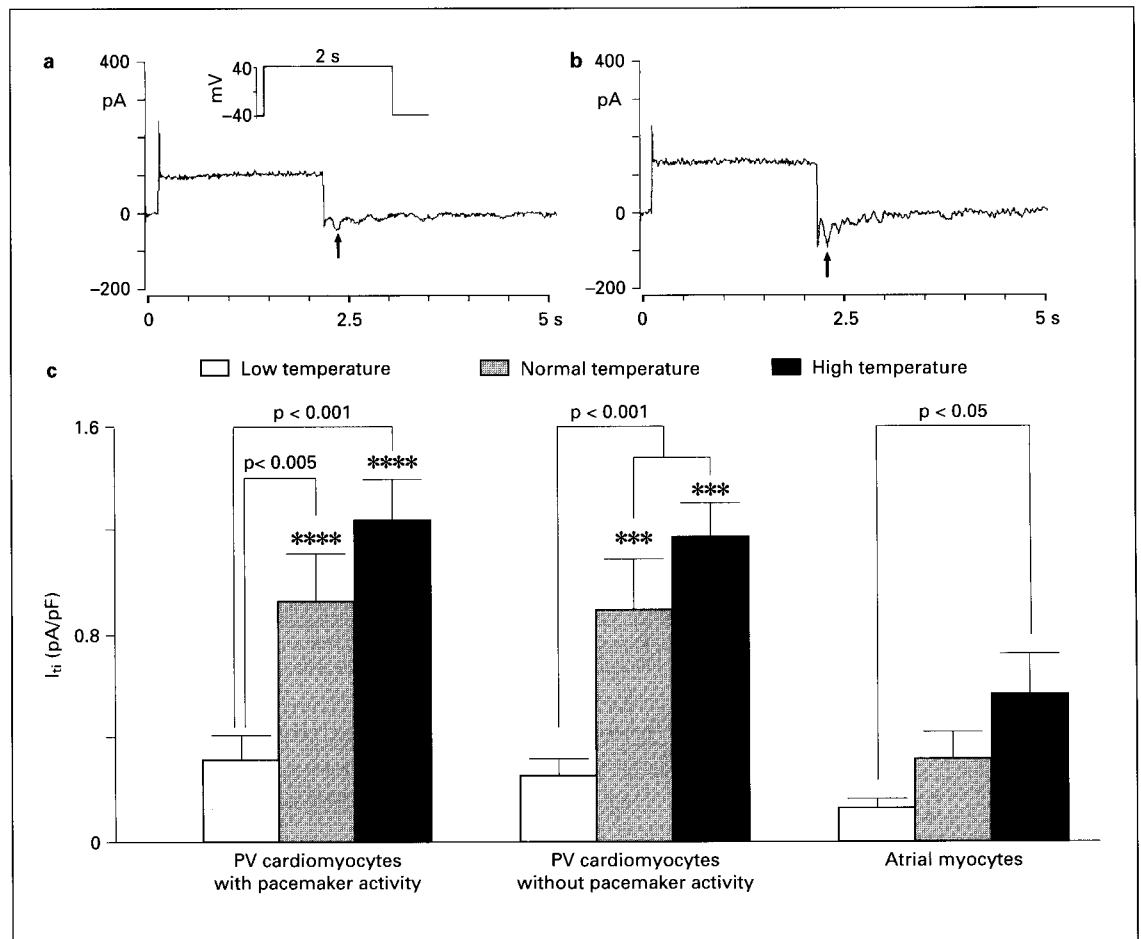
**Table 1.** The AP parameters of PV cardiomyocytes without pacemaker activity and atrial myocytes at different temperatures

	MDP, mV	APA, mV	APD <sub>50</sub> , ms	APD <sub>90</sub> , ms
<i>PV cardiomyocytes</i>				
Low temperature, n = 19	-65 ± 1	96 ± 3*	176 ± 19**	272 ± 20**
Normal temperature, n = 15	-69 ± 2	85 ± 3	33 ± 6	125 ± 12
High temperature, n = 10	-65 ± 3	81 ± 4	16 ± 2	79 ± 7
<i>Atrial myocytes</i>				
Low temperature, n = 14	-67 ± 1	103 ± 2*	150 ± 16**	243 ± 18**
Normal temperature, n = 16	-65 ± 2	93 ± 3	24 ± 2	98 ± 9
High temperature, n = 13	-65 ± 2	90 ± 3	23 ± 3	86 ± 9

The myocytes were driven electrically at a rate of 1 Hz. APD<sub>50</sub> and APD<sub>90</sub> = AP duration at 50% and 90% repolarization, respectively; APA = amplitude of AP; MDP = maximal diastolic potential. Values are means ± SE. \*  $p < 0.05$ , \*\*  $p < 0.001$  versus the same parameter of other groups analyzed.

APs of PV cardiomyocytes without pacemaker activity at different temperatures. AP duration decreased as the temperature rose. Moreover, during electrical stimulation, PV cardiomyocytes without pacemaker activity at high (6/10, 60%) or normal (7/15, 47%) temperatures had higher incidences of DAD than those at the low temperature (3/19, 16%;  $p < 0.05$ ). Figure 4 shows examples of the

genesis of DAD in PV cardiomyocytes without pacemaker activity at normal (fig. 4a) and high (fig. 4b) temperatures. In atrial myocytes, AP durations (APD<sub>50</sub> and APD<sub>90</sub>) at high or normal temperatures were shorter than those at low temperatures (table 1). During electrical stimulation, atrial myocytes at high temperatures had a higher incidence (4/13, 31%;  $p < 0.001$ ) of DAD than



**Fig. 5.** Effects of temperature on transient inward currents of PV and atrial cardiomyocytes. **a, b** Recordings of transient inward currents ( $I_{ti}$ ) of 2 PV cardiomyocytes with pacemaker activity at normal and high temperatures. Arrows indicate first  $I_{ti}$  on repolarization. The ionic currents were elicited on depolarization from  $-40$  to  $+40$  mV for 2 s and then repolarized to  $-40$  mV. **Inset** shows the clamp protocols. **c** Average current density of PV cardiomyocytes with or without pacemaker activity and atrial myocytes. \*\*\*  $p < 0.005$ , \*\*\*\*  $p < 0.001$  versus  $I_{ti}$  of atrial myocytes at the respective temperature.

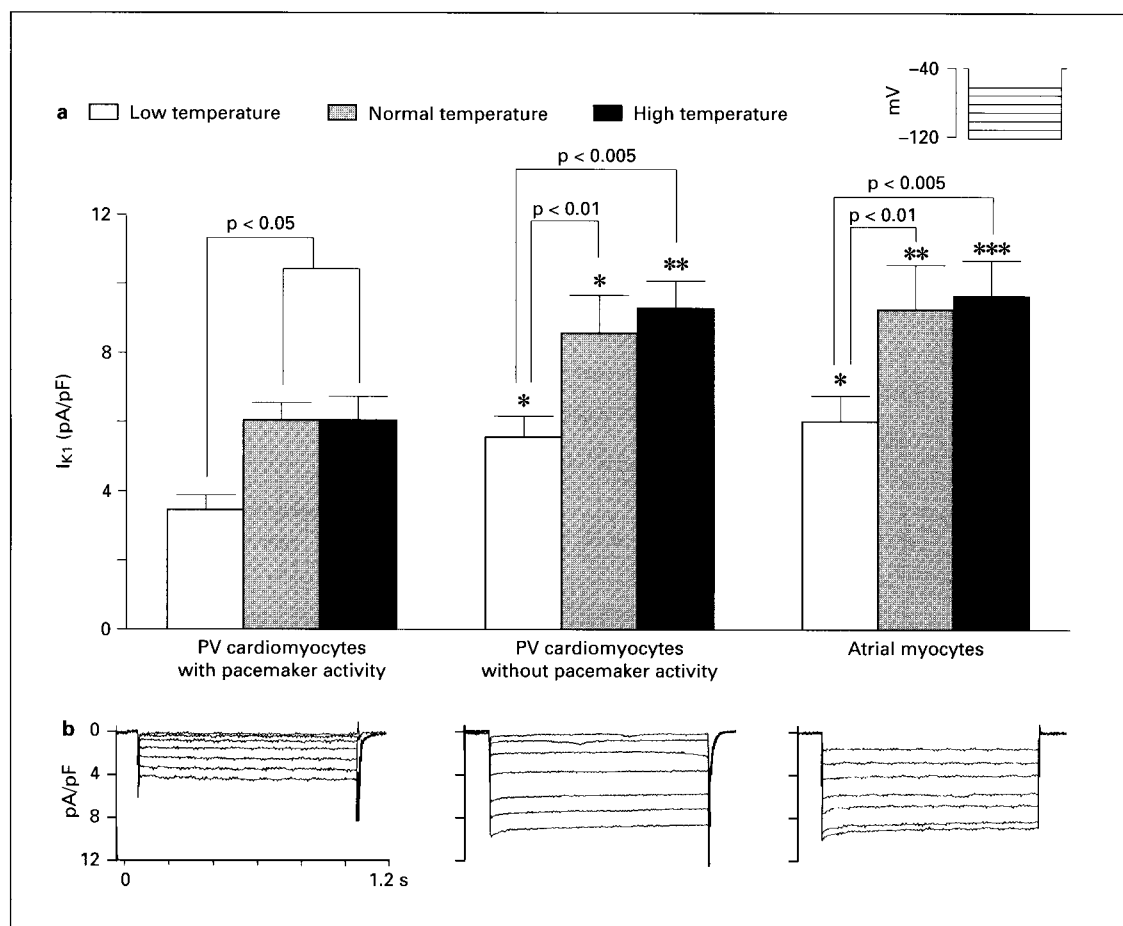
those at normal (0/16, 0%) or low temperatures (0/14, 0%).

Comparisons between PV and atrial cardiomyocytes showed that PV cardiomyocytes had higher incidences of DAD at normal temperatures than atrial cells ( $p < 0.001$ ).

#### *Effects of Temperature on Membrane Currents of PV Cardiomyocytes*

In PV cardiomyocytes with pacemaker activity, 83% of the 18 cells at low, 100% of the 15 cells at normal and 100% of the 23 cells at high temperatures had transient inward currents. Figures 5a, b show the examples of tran-

sient inward currents in PV cardiomyocytes with pacemaker activity at normal and high temperatures. The transient inward currents of PV cardiomyocytes with pacemaker activity at normal or high temperatures were larger than those at low temperatures (fig. 5c). In PV cardiomyocytes without pacemaker activity, 72% of the 29 PV cells at low, 85% of the 13 cells at normal and 100% of the 16 cells at high temperatures had transient inward currents. The transient inward currents of PV cardiomyocytes without pacemaker activity at normal or high temperatures were also larger than those at low temperatures (fig. 5c).

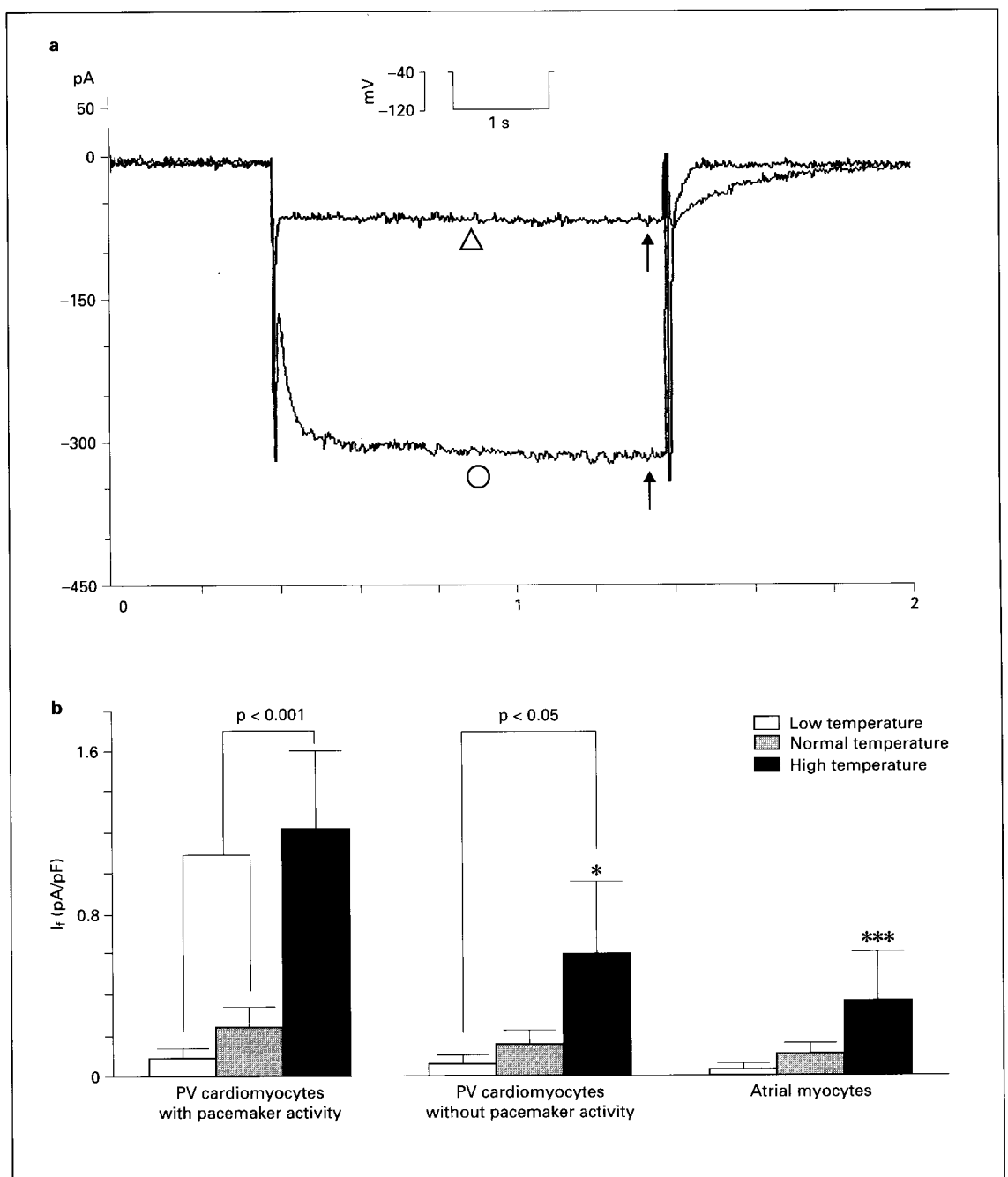


**Fig. 6.** Effects of temperature on inward rectified currents ( $I_{K1}$ ) of PV cardiomyocytes. **a** Average current densities of peak  $I_{K1}$  activated from  $-40$  mV to  $-120$  mV in PV and atrial cardiomyocytes at different temperatures. There was a significant difference in  $I_{K1}$  at different temperatures. **b** Recordings of  $I_{K1}$  in 3 PV cardiomyocytes without pacemaker activity at low (left panel), normal (middle panel) and high (right panel) temperatures. The ionic currents were elicited from a holding potential of  $-40$  mV to test potentials ranging from  $-60$  to  $-120$  mV. **Inset** shows the various clamp protocols. \*  $p < 0.05$ , \*\*  $p < 0.01$ , \*\*\*  $p < 0.005$  versus  $I_{K1}$  of PV cardiomyocytes with pacemaker activity at the respective temperature.

In atrial myocytes, transient inward currents were found in 47% of the 15 cells at low, 50% of the 14 cells at normal and 53% of the 17 cells at high temperatures ( $p > 0.05$ ). As shown in figure 5c, atrial myocytes had larger transient inward currents at high than at low temperatures. Comparisons between PV and atrial cardiomyocytes showed that atrial cells had smaller transient inward currents than PV cardiomyocytes at normal or high temperatures (fig. 5c).

Hyperpolarization activated  $I_{K1}$  in PV and atrial cardiomyocytes. As the results show (fig. 6a), the peak  $I_{K1}$  of PV cardiomyocytes with pacemaker activity at normal ( $n = 14$ ) or high ( $n = 15$ ) temperatures was larger than that

at low ( $n = 15$ ) temperatures. Similarly, the peak  $I_{K1}$  of PV cardiomyocytes without pacemaker activity at normal ( $n = 14$ ) or high ( $n = 11$ ) temperatures was larger than that at low ( $n = 22$ ) temperatures (figure 6a). Atrial myocytes at normal ( $n = 13$ ) or high ( $n = 15$ ) temperatures also had larger peak  $I_{K1}$  than atrial myocytes at low ( $n = 11$ ) temperatures (fig. 6a). Figure 6b shows the examples of  $I_{K1}$  in PV cardiomyocytes without pacemaker activity at different temperatures. Comparisons between PV and atrial cardiomyocytes showed that PV cardiomyocytes with pacemaker activity had smaller  $I_{K1}$  at different temperatures than atrial myocytes or PV cardiomyocytes without pacemaker activity (fig. 6a).



**Fig. 7.** Effects of temperatures on  $I_f$  of PV and atrial cardiomyocytes. **a** Recordings of  $I_f$  elicited from a holding potential of  $-40$  mV to  $-120$  mV in 2 PV cardiomyocytes with pacemaker activity at low ( $\Delta$ ) and high ( $\circ$ ) temperatures. The  $I_{fS}$  are indicated by upward arrows. There were similar cell capacitances between the two PV cardiomyocytes. **Inset** shows the clamp protocols. **b** Average current densities of peak  $I_f$  in PV cardiomyocytes or atrial myocytes. At varying temperatures, there were significant differences of peak  $I_f$  among PV cardiomyocytes, but there were similar peak  $I_f$  among atrial myocytes.  $*$   $p < 0.05$ ,  $***$   $p < 0.005$  versus  $I_f$  of PV cardiomyocytes with pacemaker activity at high temperatures.

In PV cardiomyocytes with pacemaker activity, 33% of the 15 cells at low, 43% of the 14 cells at normal and 73% of the 15 cells at high temperatures had  $I_f$  ( $p > 0.05$ ). As the examples in figure 7a illustrate, the peak  $I_f$  of PV cardiomyocytes with pacemaker activity was different at varying temperatures. The peak  $I_f$  of PV cardiomyocytes with pacemaker activity at high temperatures was larger than those at normal and low temperatures (fig. 7b). In PV cardiomyocytes without pacemaker activity, 10% of the 22 cells at low, 29% of the 14 cells at normal and 36% of the 11 cells at high temperatures had  $I_f$  ( $p > 0.05$ ). The peak  $I_f$  of PV cardiomyocytes without pacemaker activity at high temperatures was larger than that at low temperatures (fig. 7b). In atrial myocytes, 9% of the 11 cells at low, 23% of the 13 cells at normal and 27% of the 15 cells at high temperatures had  $I_f$  ( $p > 0.05$ ). The peak  $I_f$  density of atrial myocytes at varying temperatures was not significantly different (fig. 7b). Comparisons between PV and atrial cardiomyocytes at high temperatures showed that PV cardiomyocytes with pacemaker activity had larger peak  $I_f$  than atrial myocytes or PV cardiomyocytes without pacemaker activity (fig. 7b).

## Discussion

### *Effects of Temperature on AP of PV Cardiomyocytes*

In this study, temperature was shown to change the AP duration of PV cardiomyocytes and atrial myocytes. Previous studies have shown that a rise in temperature increased transient outward and delayed rectified potassium currents. These ionic alternations shortened the AP duration in PV and atrial myocytes at higher temperatures [15]. The results of our previous study showed that reentrant excitation may account for the occurrence of high-frequency irregular rhythms in isolated canine PV specimens [7]. Shortening of the AP duration decreased refractoriness and facilitated the genesis of reentrant circuits in PVs. This study also demonstrated that a rise in temperature would increase the spontaneous activity of PV cardiomyocytes with pacemaker activity, which was similar to the results of previous studies on sinoatrial cells [2, 16]. Moreover, this study demonstrated that rising temperatures induced the occurrence of oscillatory afterpotentials during spontaneous beating in PV cardiomyocytes. The findings suggest that enhancement of automaticity plays a role in the arrhythmogenesis of hyperthermic PVs.

Rising temperatures have been shown to induce the genesis of DAD in Purkinje fiber [4, 18]. Similar to these

studies, we demonstrated that a rise in temperature increases the occurrence of DAD in PV or atrial cardiomyocytes. At higher temperatures, the higher incidences of DAD in PV cardiomyocytes compared to atrial myocytes suggest that enhanced triggered activity may result in the high arrhythmogenic activity of PV during higher temperatures. Previous studies have also suggested that triggered activity played a role in the arrhythmogenic mechanism of PVs [6, 7, 9, 10].

### *Effects of Temperature on Membrane Currents of PV Cardiomyocytes*

This study showed that elevation of temperature increased  $I_{K1}$  in PV or atrial cardiomyocytes.  $I_{K1}$  affects the third phase of AP and also determines AP duration. The larger  $I_{K1}$  in cardiomyocytes at higher temperatures may play a role in the shortening of the AP duration of cardiomyocytes. However, reports in the literature have provided limited information about the effects of temperature on  $I_f$ . The results of our study show that temperature could alter the density of  $I_f$  in PV cardiomyocytes but not in atrial myocytes.  $I_f$  has been suggested to contribute to the automaticity of cardiomyocytes and play a role in the arrhythmogenic activity of diseased hearts [5, 11]. The increase of  $I_f$  may contribute to the faster beating rates of PV cardiomyocytes at higher temperatures. Additionally, the small effect of temperature on  $I_f$  of atrial myocytes indicates the presence of different electrophysiological characteristics between PV and atrial cardiomyocytes.

Transient inward currents have been shown to be associated with genesis of DAD [26]. In this study, similar to the findings in our previous studies [6, 8], transient inward currents were found in PV cardiomyocytes. The larger transient inward currents in PV cardiomyocytes at normal or high temperatures compared to atrial myocytes suggest the importance of transient inward currents in the arrhythmogenic activity of PVs. In addition, changes of transient inward currents during different temperatures indicate that temperature has a significant effect on the genesis of transient inward currents. These findings were similar to the effects of temperature on other cardiomyocytes [4, 18]. Although we did not investigate the effects of temperature on other currents in this study, it is still possible that other mechanisms, such as the changes of calcium and potassium currents or the oscillatory calcium release, may also contribute to the increase of arrhythmogenic activity at high temperatures. It is known that temperature has an effect on several ionic currents [4, 12, 14, 15, 24].

## Conclusions

Temperature changes the electrophysiological characteristics and arrhythmogenic activity of PV cardiomyocytes. Enhancement of automaticity, induction of triggered activity and shortening of the AP duration may underline the increasing arrhythmogenic activity of PV cardiomyocytes during increased temperatures.

## Acknowledgments

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