Endothelin-1 Modulates the Arrhythmogenic Activity of Pulmonary Veins

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Effect of Endothelin-1 on Pulmonary Veins. ***Objective:*** **Endothelin-1 has important cardiovascular effects and is activated during atrial fibrillation. Pulmonary veins (PVs) play a critical role in the pathophysiology of atrial fibrillation. The aim of this study was to evaluate whether endothelin-1 affects PV arrhythmogenic activity.**

Methods: **Conventional microelectrodes were used to record the action potentials (APs) and contractility in isolated rabbit PV tissue specimens before and after the administration of endothelin-1 (0.1, 1, 10 nM). The ionic currents of isolated PV cardiomyocytes were investigated before and after the administration of endothelin-1 (10 nM) through whole-cell patch clamps.**

Results: **In the tissue preparation, endothelin-1 (1, 10 nM) concentration dependently shortened the AP duration and decreased the PV firing rates. Endothelin-1 (10 nM) decreased the resting membrane potential. Endothelin-1 (0.1, 1, 10 nM) decreased the contractility and increased the resting diastolic tension. In single PV cardiomyocytes, endothelin-1 (10 nM) decreased the PV firing rates from 2.7 $\pm$ 1.0 Hz to 0.8 $\pm$ 0.5 Hz (n = 16). BQ-485 (100 μM, endothelin-1 type A receptor blocker) reversed and prevented the chrono-inhibitory effects of endothelin-1 (10 nM). Endothelin-1 (10 nM) reduced the L-type calcium currents, transient outward currents, delayed rectifier currents, transient inward currents, and sodium–calcium exchanger currents in the PV cardiomyocytes with and without pacemaker activity. Endothelin-1 (10 nM) increased the inward rectifier potassium current, hyperpolarization-induced pacemaker current, and the sustained outward potassium current in PV cardiomyocytes with and without pacemaker activity.**

Conclusion: **Endothelin-1 may have an antiarrhythmic potential through its direct electrophysiological effects on the PV cardiomyocytes and its action on multiple ionic currents.** *(J Cardiovasc Electrophysiol, Vol. 19, pp. 285-292, March 2008)*

atrial fibrillation, endothelin-1, pulmonary veins, ion currents

Introduction

Atrial fibrillation (AF) is the most common cardiac arrhythmia seen in clinical practice and induces cardiac dysfunction and strokes.[1,2] Activation of endothelin-1 is involved in the pathophysiology of AF.[3-5] Both acute and chronic stretch of the atria can cause the release of endothelin-1.[6] Endothelin-1 may affect acutely the electrical properties and ion channels in cardiomyocytes to modulate cardiac arrhythmias.[7,8] Endothelin-1 has been shown to aggravate ventricular arrhythmias due to vasoconstriction or its direct ionic effects;[8] however, its effects on the atria are not fully elucidated. Endothelin-1 may cause a calcium release from the intracellular stores and inhibit the delayed rectifier potassium current (I_K) in atrial mycoytes.[8,9] Endothelin-1 may have atrial proarrhythmic effects through the action of the intracellular Ca^{2+} via activation of inositol 1,4,5-triphosphate and shortening of the action potential (AP) duration.[9-11] Various studies have found increased levels of endothelin-1 in patients with heart failure with or without AF.[4-5] In contrast, Redpath *et al*. found that endothelin-1 has antiarrhythmic effects through its antiadrenergic action on human atria.[12] Endothelin-1 induced negative inotropic effects predominate in isolated and *in situ* hearts.[13,14]

The pulmonary veins (PV) are important sources of ectopic beats for the initiation of paroxysmal AF.[15,16] PVs contain cardiomyocytes with and without pacemaker activity and have a high arrhythmogenic activity.[17,18] However, the effects of endothelin-1 on PV cardiomyocytes are still to be elucidated. Endothelin-1 has also been implicated in the pathophysiology of pulmonary hypertension secondary to congenital heart disease and cardiopulmonary bypass surgery.[19] It is also suggested that PVs are involved in the pathogenesis of pulmonary hypertension.[20] Various studies have shown the beneficial effects of endothelin-1 receptor blockers in the

The present work was supported by the Topnotch Stroke Research Center Grant, Ministry of Education and grants NSC 94-2314-B-075-093, NSC 94-2314-B-010-056, NSC-94-2314-B-010-053, NSC 95-2314-B-016-015, NSC 95-2314-B-038-026,VGH 94-204, VGH-94-005, VGH-94-206, VGH-94-009, V95A-008, and SKH-TMU-95-04 from Shih Kong Wu Ho-Su Memorial Hospital.

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Manuscript received 18 June 2007; Revised manuscript received 2 October 2007; Accepted for publication 5 October 2007.

doi: 10.1111/j.1540-8167.2007.01033.x

treatment of pulmonary hypertension.[21] Therefore, it is reasonable to hypothesize that endothelin-1 may also have significant effects on the PVs. The purpose of our study was to investigate whether endothelin-1 can regulate the PV electrophysiological characteristics and arrhythmogenesis.

Methods

Rabbit PV Tissue Preparations

The investigation conformed to the institutional *Guide for the Care and Use of Laboratory Animals*. As previously described,[22,23] rabbits (weight: 1–2 kg) were anesthetized with an intravenous injection of sodium pentobarbital (40 mg/kg). The PVs were separated from the atrium at the level of the left atrial-PV junction and separated from the lungs at the ending of the PV myocardial sleeves in Tyrode's solution with a composition (in mM) of 137 NaCl, 4 KCl, 15 $NaHCO_3$, 0.5 NaH_2PO_4, 0.5 $MgCl_2$, 2.7 $CaCl_2$, and 11 dextrose. One end of the preparation, consisting of the PVs and atrial-PV junction, was pinned with needles to the bottom of a tissue bath. The other end was connected to a Grass FT03C force transducer with a silk thread. The adventitia of the PVs faced upward. The PV tissue strips ($\sim 10 \times 10 \times 0.5$ mm) were superfused with Tyrode's solution that was saturated with a 97% O_2–3% CO_2 gas mixture. The temperature was maintained constant at 37°C and the preparations were allowed to equilibrate for 1 hour before the electrophysiological study.

Electrophysiological and Pharmacological Studies

The transmembrane AP of the PVs was recorded using machine-pulled glass capillary microelectrodes filled with 3 M of KCl and the PV preparation was connected to a WPI model FD223 electrometer under tension with 150 mg. The electrical and mechanical events were displayed simultaneously on a Gould 4072 oscilloscope and Gould TA11 recorder. An electrical stimulus with a 10-ms duration and supra-threshold strength (30% above the threshold) was provided by a Grass S88 stimulator through a Grass SIU5B stimulus isolation unit. Different concentrations of endothelin-1 (0.1, 1, 10 nM) were sequentially superfused to test the pharmacological responses at least 30 minutes. The AP durations at 90% and 50% of the AP amplitude (APD_{90}, APD_{50}, respectively), AP amplitude (APA), diastolic resting tension, and contractile force were measured during 2-Hz electrical stimuli before and after the drug administration.[18,22,23]

Electropharmacological Study of Isolated PV Cardiomyocytes

The PV cardiomyocytes from rabbits were enzymatically dissociated, as previously described.[22,23] The PV cardiomyocytes with pacemaker activity were identified by the presence of constant spontaneous beating during perfusion with Tyrode's solution with a composition (in mM) of NaCl 137, KCl 5.4, HEPES 10, $MgCl_2$ 0.5, $CaCl_2$ 1.8, and glucose 10. The cells were allowed to stabilize in the bath for at least 30 minutes before the experiments.

A whole-cell patch clamp was performed in the PV cardiomyocytes with and without the administration of endothelin-1 (10 nM) or BQ-485 (100 μM, endothelin-1 type A receptor blocker) by an Axopatch 1D amplifier (Axon Instruments, Foster City, CA, USA) at 35 ± 1°C. 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 area under the capacitative currents was divided by the applied voltage step to obtain the total cell capacitance. The APs were elicited by pulses of 2 ms and 70 mV as a driven rate of 1 Hz. The APs were recorded in the current-clamp mode and ionic currents in the voltage-clamp mode. The delayed afterdepolarization (DAD) was defined as the presence of a spontaneous depolarization of the impulse after full repolarization had occurred. The amplitude of the DADs was measured during 1 Hz electrical stimuli. Micropipettes were filled with a solution containing (in mM) CsCl 130, $MgCl_2$ 1, Mg_2ATP 5, HEPES 10, EGTA 10, NaGTP 0.1, and Na_2 phosphocreatine 5, titrated to a pH of 7.2 with CsOH for the experiments on the L-type calcium current ($I_{Ca\text{-}L}$). The micropipettes were filled with a solution containing (in mM) NaCl 20, CsCl 110, $MgCl_2$ 0.4, $CaCl_2$ 1.75, tetraethylammonium 20, BAPTA 5, glucose 5, Mg_2ATP 5, and HEPES 10, titrated to a pH of 7.25 for the experiments on the sodium–calcium exchanger (NCX) current, and containing (in mM) KCl 20, K aspartate 110, $MgCl_2$ 1, Mg_2ATP 5, HEPES 10, EGTA 0.5, LiGTP 0.1, and Na_2 phosphocreatine 5, titrated to a pH of 7.2 with KOH for the experiments on the APs, potassium currents, and transient inward currents. Voltage command pulses were generated by a 12-bit digital-to-analog converter controlled by pCLAMP software (Axon Instruments). Recordings were low pass-filtered at half the sampling frequency.

The $I_{Ca\text{-}L}$ was measured as an inward current during depolarization from a holding potential of –50 mV to testing potentials ranging from –40 to +60 mV in 10-mV steps for 300 ms at a frequency of 0.1 Hz using a perforated patch-clamp with amphotericin B. The NaCl and KCl in the external solution were replaced by tetraethylammonium chloride and CsCl, respectively.[18,23]

The transient inward current (I_{ti}) 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 the transient inward current was measured as the difference between the peak of the transient current and mean of the current just before and after the transient current.[18,23]

The NCX current was elicited by depolarizing pulses between –100 to +100 mV from a holding potential of –40 mV for 300 ms at a frequency of 0.1 Hz. The amplitudes of the NCX current were measured as 10 mM nickel-sensitive currents. The external solution (in mM) consisted of NaCl 140, $CaCl_2$ 2, $MgCl_2$ 1, HEPES 5, and glucose 10 with a pH of 7.4 and contained strophanthidin (10 μM), nitrendipine (10 μM), and niflumic acid (100 μM).

The I_K was measured from the peak outward current at the end of 1 second and the depolarization from –40 to +60mV in 10-mV steps at a frequency of 0.1 Hz during the infusion of $CdCl_2$ (200 μM) and 4-aminopyridine (2 mM) in the bath solution.[19,24] The inward rectifier potassium current (I_{K1}) was 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 under the infusion of $CdCl_2$ (200 μM) and 4-aminopyridine (2 mM) in the bath solution. The amplitudes of the I_{K1} were measured as 1 mM barium-sensitive currents.[18,23] Under the infusion of 1 mM of barium to inhibit the I_{K1}, a progressive large inward current that developed with slow voltage-dependent kinetics was measured as the pacemaker

current (I_f) during hyperpolarization from a holding potential of −40 mV to a test potential of −120 mV for 1 second.

The transient outward current (I_{to}) was studied with a double-pulse protocol. A 30-ms prepulse from −80 to −40 mV was used to inactivate the sodium channels, followed by a 300-ms test pulse to +60 mV in 10-mV steps at a frequency of 0.1 Hz. $CdCl_2$ (200 μM) was added to the bath solution to inhibit the $I_{Ca\text{-}L}$. The I_{to} was measured as the difference between the peak outward current and steady-state current.[18,23] The sustained outward potassium currents (I_{Ksus}) were measured as the outward current density at the end of the steady state.

Statistics

All quantitative data are expressed as the mean ± SEM. A repeated-measures ANOVA with a Fisher LSD and paired *t*-test were used to compare the differences before and after the drug administration in the PV tissue specimens and cardiomyocytes. An unpaired *t*-test was used to compare the effects of endothelin-1 on the PV cardiomyocytes with and without pacemaker activity. A P-value of less than 0.05 was considered statistically significant.

Results

Effects of Endothelin-1 on the PV Tissue Preparations

As shown in Figure 1A, the superfusion of endothelin-1 (1, 10 nM) reduced the automatic rhythm significantly in the six spontaneously active PV tissue preparations in a concentration dependent manner (Fig. 1A). In the PVs without spontaneous activity, endothelin-1 (1, 10 nM) concentration dependently reduced the APD_{90}, APD_{50}, and APA significantly (Fig. 1B,C). Endothelin-1 (10 nM) decreased the resting membrane potential. Moreover, endothelin-1 concentration dependently reduced the PV contractile force but increased the PV resting diastolic tension (Fig. 1C).

Effects of Endothelin-1 on Isolated PV Cardiomyocytes

Similar to that in the tissue preparations, we also found that the endothelin-1 (10 nM) reduced the automatic rhythm significantly (Fig. 2A) in the PV cardiomyocytes with pacemaker activity from 2.7 ± 1.0 to 0.8 ± 0.5 Hz (n = 16, 65 ± 5% reduction, P < 0.05). The effect of endothelin-1 (10 nM) on the PV beating rates was more significant in the PV cardiomyocytes than in the tissue preparation (decreased by 35 ± 5%, P < 0.05). In the 8 PV cardiomyocytes with delayed after-depolarizations (DADs), endothelin-1 (10 nM) completely suppressed the amplitudes of the DADs (7.9 ± 4.2 mV, Fig. 2B).

Interaction between Endothelin-1 and Receptor Blockers

In the presence of BQ-485 (100 μM), endothelin-1 did not change the PV's pacemaker activity (2.3 ± 0.8 Hz vs 2.4 ± 0.7 Hz, n = 3, P > 0.05, Fig. 2C). Moreover, the administration of BQ-485 (100 μM) partially reversed the negative chronotropic effect of endothelin-1 on the PV cardiomyocytes from 0.7 ± 0.9 Hz to 1.9 ± 1.3 Hz (n = 4, P < 0.05, Fig. 2D)

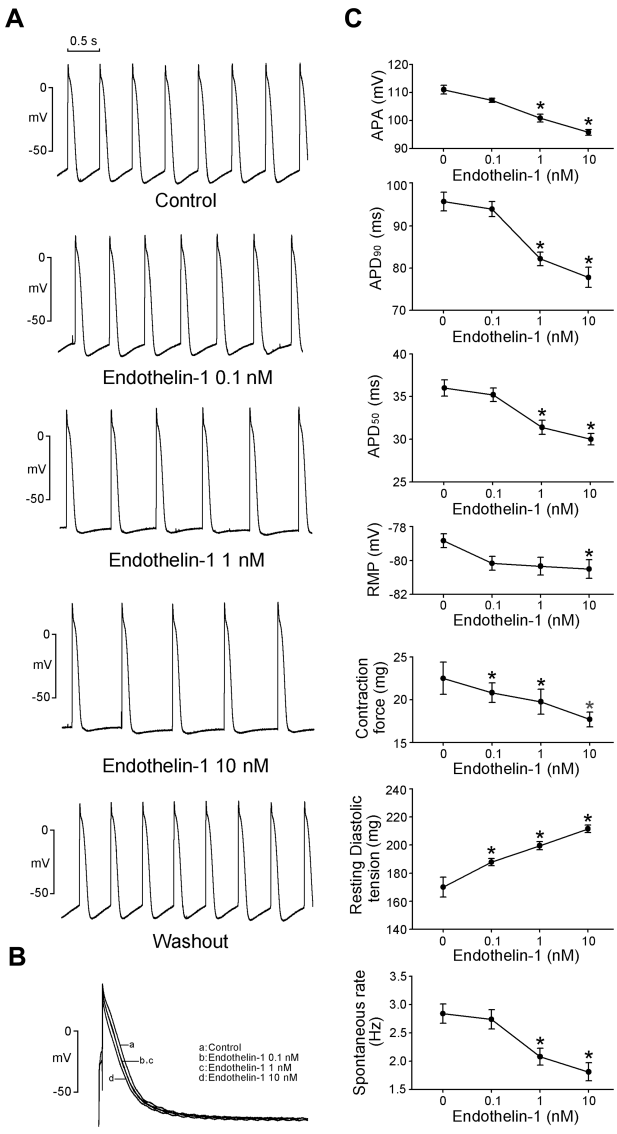

Figure 1. *The effects of endothelin-1 on the PV tissue preparations. Panel A shows the tracings of different concentrations (0.1, 1, 10 nM) of endothelin-1 on the spontaneous firing rates in the PVs. Increasing concentrations of endothelin-1 decreased the PV spontaneous rates (n = 6). Panel B shows the superimposed tracings of the different concentrations of endothelin-1 on the AP configuration in the PV tissue specimen without spontaneous activity. Panel C shows the concentration–response curve of the effects of endothelin-1 on the ADP_{90}, APD_{50}, APA, resting membrane potential, resting diastolic tension, and contractile force in whole tissue specimen (n = 6). *P < 0.05 versus control.*

Effect of Endothelin-1 on the Ionic Currents of the PV Cardiomyocytes

Figure 3 shows the tracings and I–V relationship of endothelin-1 on the $I_{Ca\text{-}L}$ in the PV cardiomyocytes. Endothelin-1 decreased the $I_{Ca\text{-}L}$ in the PV cardiomyocytes with and without pacemaker activity to a similar extent (Fig. 3). Figure 4 shows the effects of endothein-1 on the $I_{to,}$ I_{Ksus}, and I_K. Endothelin-1 decreased the I_{to} in the PV cardiomyocytes with and without pacemaker activity to a similar extent. However, endothein-1 did not have any significant effects on the I_{Ksus}. Moreover, endothelin-1 decreased the I_K in the PV cardiomyocytes with and without pacemaker activity

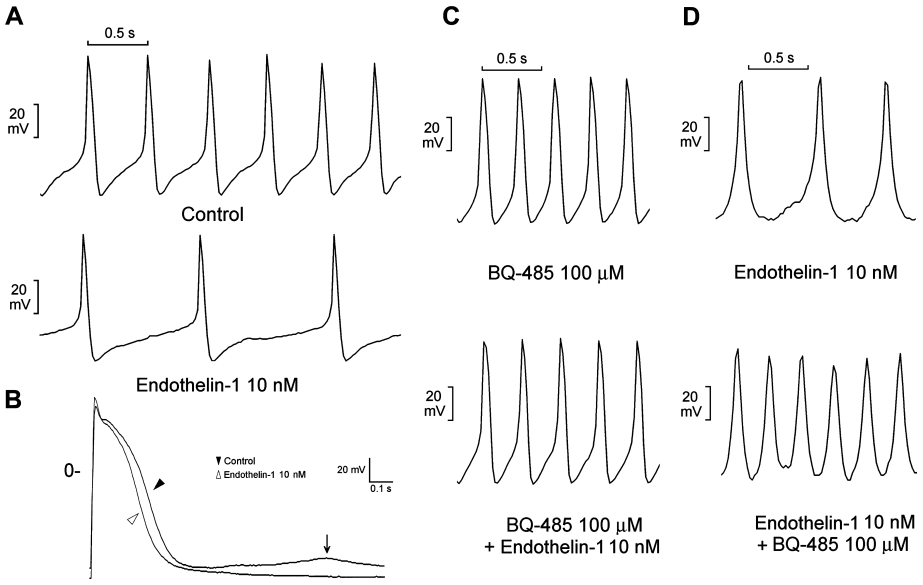

Figure 2. *The effects of endothelin-1 and interaction of the endothelin-1 and BQ-485 on isolated PV single cardiomyocytes. Panel A shows that the endothelin-1 (10 nM) decreased the PV spontaneous firing rates. Panel B shows that the endothelin-1 reduced the delayed after-depolarizations in the PV cardiomyocytes (↓). Panel C shows no significant effect of the endothelin-1 (10 nM) on the spontaneous activity of the PV cardiomyocytes in the presence of the endothelin-1 blocker BQ-485. Panel D shows that the administration of BQ-485 reversed the chrono-inhibitory effect caused by the endothelin-1 on the PV cardiomyocytes.*

to a similar extent (Fig. 4). Figure 5A shows the tracings and I–V relationship of endothelin-1 on the I_{K1} in the PV cardiomyocytes. Endothelin-1 increased the I_{K1} currents in the PV cardiomyocytes with and without pacemaker activity. The I_f was found in 8 of 43 (19%) PV cardiomyocytes with pacemaker activity and 7 of 24 (29%) PV cardiomyocytes without pacemaker activity. Endothelin-1 increased the I_f in the PV cardiomyocytes with and without pacemaker activity (Fig. 5B).

Figure 6 shows the tracings and I–V relationship of endothelin-1 on the NCX and I_{ti} in the PV cardiomyocytes. Endothelin-1 significantly decreased the outward and inward nickel-sensitive NCX currents in the PV cardiomyocytes with and without pacemaker activity to a similar extent. Moreover, the administration of endothelin-1 decreased the I_{ti} in the PV cardiomyocytes with pacemaker activity and also in those without pacemaker activity (Fig. 6).

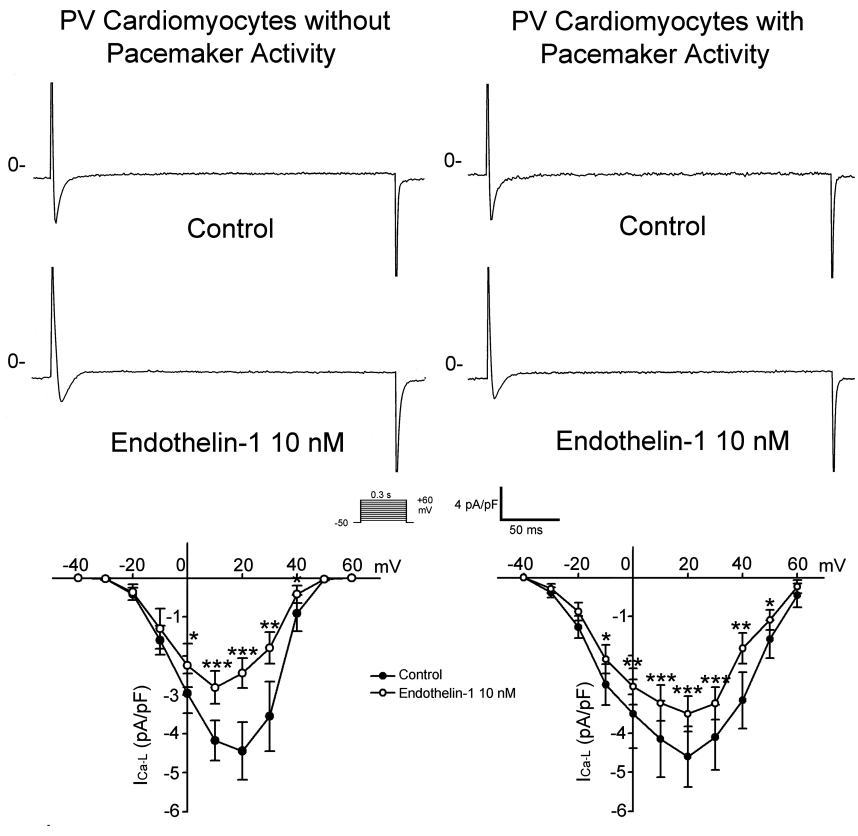

Figure 3. *Effect of endothelin-1 on the $I_{Ca\text{-}L}$ in the PV cardiomyocytes. The current traces (depolarization from –50 to + 10 mV) and I–V relationship of the $I_{Ca\text{-}L}$ before and after the administration of endothelin-1 (10 nM) in the PV cardiomyocytes with (n = 11) and without pacemaker activity (n = 6). *P < 0.05, **P < 0.01, ***P < 0.001 versus control. The insets in the current traces show the various clamp protocols.*

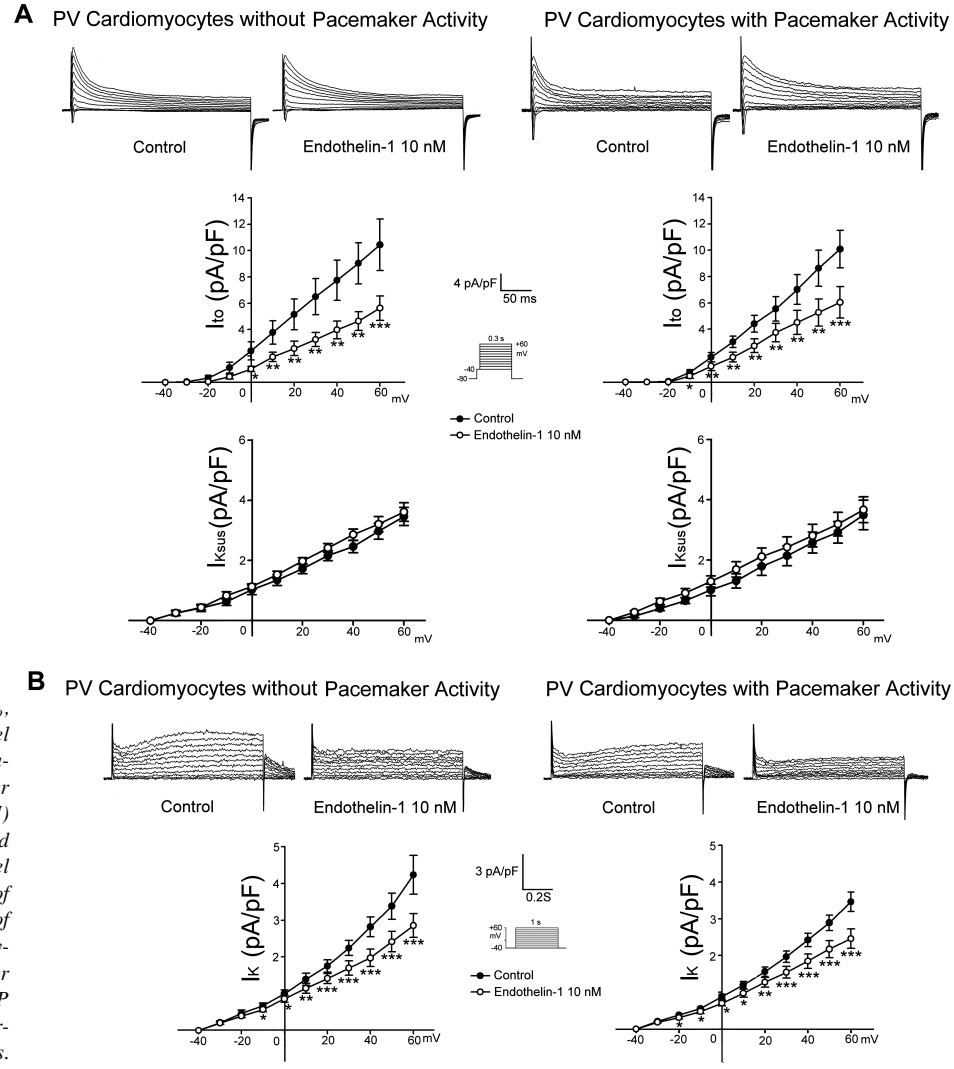

Figure 4. *Effect of endothelin-1 on the I_{to}, I_{Ksus}, and I_K in the PV cardiomyocytes. Panel A shows the current traces and the I–V relationship of the I_{to} and I_{Ksus} before and after the administration of endothelin-1 (10 nM) in the PV cardiomyocytes with ($n = 8$) and without pacemaker activity ($n = 6$). Panel B: the current traces and I–V relationship of the I_K before and after the administration of endothelin-1 (10 nM) in the PV cardiomyocytes with ($n = 9$) and without pacemaker activity ($n = 7$). $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ versus control. The insets in the current traces show the various clamp protocols.*

Discussion

Effect of Endothelin-1 on the PV Electrical Activity

Endothelin-1 has negative chronotropic effects on guinea pig atria[24] and also on rabbit sinoatrial nodal cells.[25] In tissue experiments, we found a negative chronotropic effect in the PVs. However, it is not clear which mechanisms are responsible for those results because endothelin-1 may have direct effects on the PV cardiomyocytes or indirect effects through mechano-electrical feedback in PVs. In this study, we found that that endothelin-1 also decreased the PV firing rates in single PV cardiomyocytes, but the negative chronotropic effect in the isolated cardiomyocytes was more profound than in the whole tissue preparations. The smaller chronotropic effect of endothelin-1 in the PV tissue preparation may have been caused by the vasoconstriction of the PVs, which is known to accelerate the PV electrical activity. Mechanoelectric feedback is important in PV electrical activity.[26] Chang *et al*. showed that the stretch increased the PV automaticity and triggered activity.[26] Taken together, these findings suggest that endothelin-1 in the absence of stretch and vasoconstriction may have a predominantly negative chronotropic effect. It is likely also that the diffusion of endothelin-1 would be more difficult in the whole tissue than the PV cardiomyocytes. This would also be responsible for the smaller chronotropic effect seen in the whole tissue preparations than in the PV cardiomyocytes. Increased automaticity and triggered activity has been suggested to play an important role in the PV arrhythmogenesis. The results of this study showed that endothelin-1 reduced the automaticity and number of DADs in the PV cardiomyocytes. DADs are caused by conditions leading to a calcium overload in the cells. Endothelin-1 suppressed the $I_{Ca\text{-}L,}$ NCX, and I_{ti} currents in our study that may have been responsible for the decrease in the DADs as well. In a recent study, endothelin-1 abolished the afterdepolarizations provoked by isoproterenol in human atria.[12]

Endothelin-1 has been shown to exert arrhythmogenic effects due to an AP duration shortening in atrial mycoytes.[27] Similarly, endothelin-1 significantly reduced the ADP_{90} and APD_{50} in the rabbit PV cardiomyocytes. Although the shortening of the AP duration may reduce the calcium influx reducing the PV automaticity and triggered activity,[22,23] the AP duration shortening in PV cardiomyocytes with pacemaker activity would be arrhythmogenic due to the facilitation of reentry circuits. However, in the PV cardiomyocytes with pacemaker activity, endothelin-1 was demonstrated to have an antiarrhythmic potential due to decreasing the PV beating rates and triggered activity. Similar to that in this study, endothelin-1 also has been shown to decrease the beating rates in rabbit sinoatrial pacemaker cells with the inhibition

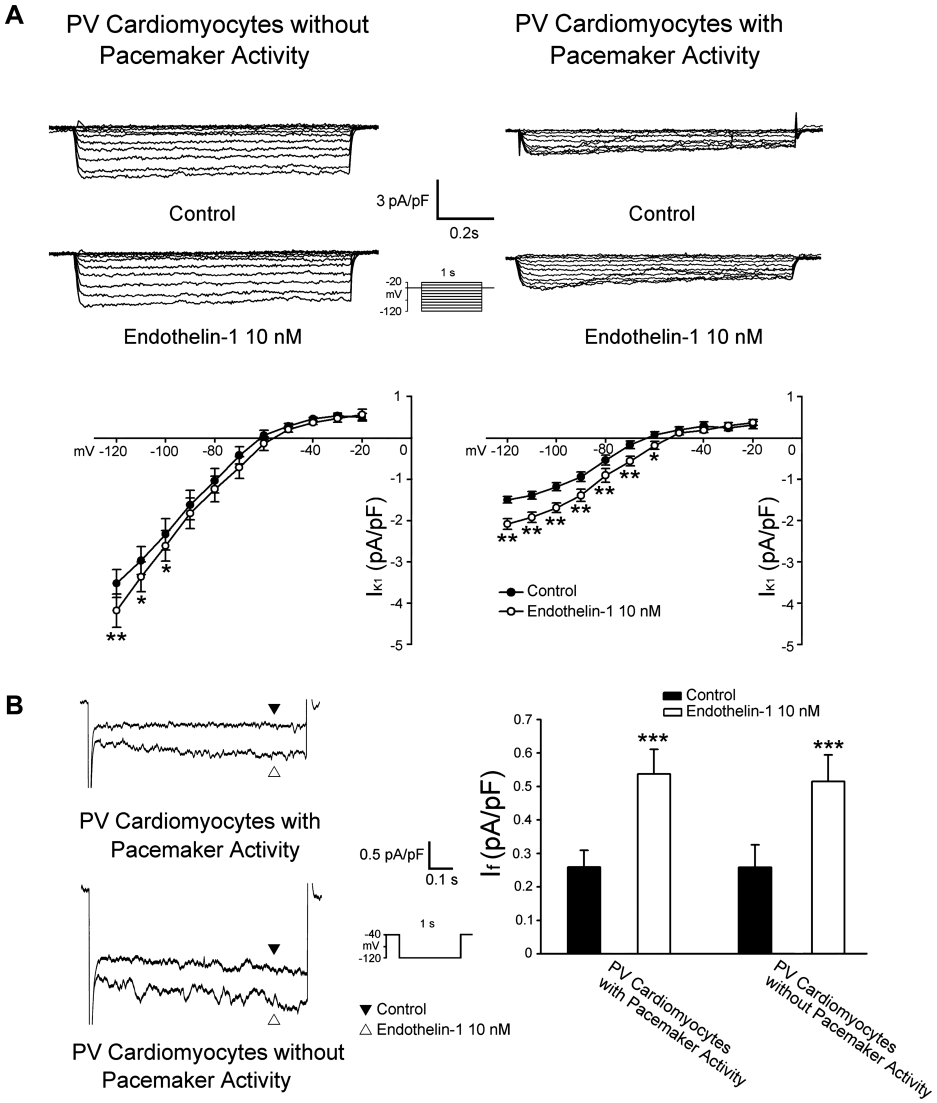

Figure 5. *Effect of endothelin-1 on the I_{K1} and I_f in the PV cardiomyocytes. Panel A: the current traces and I–V relationship of the I_{K1} before and after the administration of endothelin-1 (10 nM) in the PV cardiomyocytes with ($n = 7$) and without pacemaker activity ($n = 8$). *$P < 0.05$, **$P < 0.01$ versus control. The insets in the current traces show the various clamp protocols. Panel B: the current traces and the average data of the I_f before (▲) and after (△) the administration of endothelin-1 (10 nM) in the PV cardiomyocytes with ($n = 8$) and without pacemaker activity ($n = 7$). ***$P < 0.001$ versus control.*

of the $I_{Ca\text{-}L}$.[25] These results suggest the complex effects of endothelin-1 on PV cardiomyocytes with and without pacemaker activity. Previous studies have shown that the activation of endothelin-1 type A receptors will produce a vasoconstrictor response.[28] Theoretically, this effect may induce mechanoelectrical feedback to enhance the PV arrhythmogenesis. However, this study showed that endothelin-1 at a concentration of 10 nM still has a negative chronotropic effect on the PVs. Therefore, the clinical implication of endothelin-1 may be determined from the balance of its arrhythmogenic and antiarrhythmic effects. The selective endothelin-1 blocker, BQ-485 was able to partially revert the inhibitory effects of endothelin-1 on the automaticity and suggested that the effects of endothelin-1 on the PVs were mediated by endothelin-1 type A receptors.[25] However, as compared with the control, BQ-485 did not increase the triggered activity or automaticity in the PV cardiomyocytes and only partially reversed the PV firing rate in the presence of endothein-1. Thus, endothelin-1 receptor blockers may not be arrhythmogenic for PVs.

The effect of endothelin-1 on the cardiac contractility has not yet been fully clarified. Our study found a significant decrease in the contractility of the PV tissue. The reduction in the PV contractility caused by endothelin-1 may have been attributed to the decrease in the $I_{Ca\text{-}L}$ via the Gi protein/protein kinase G pathway.[28] In contrast, endothelin-1 increases the PV vascular tone, which may be explained by the known effects of the increase in the intracellular Ca^{2+} in vascular smooth muscle caused by endothelin-1.[28]

Effect of Endothelin-1 on the Ionic Currents of PV Cardiomyocytes

In this study, we found that endothelin-1 reduced the $I_{Ca\text{-}L}$, which was similar to the results of the other studies in guinea pig and rabbit atrial cells[27] and human atrial cells.[29] $I_{Ca\text{-}L}$ is the major ion channel responsible for the AP duration, including that in human atrial cells. The decrease in the $I_{Ca\text{-}L}$ channels as a result of endothelin-1 can explain in part the shortening of the AP duration observed in our study and also the reduction in the automaticity and incidence of DADs in the PV cardiomyocytes. Previous studies have shown that the NCX current plays a critical role in the PV arrhythmogenesis.[22,23] The activation of the NCX may increase the sarcoplasmic reticular Ca^{2+} content and leads to diastolic depolarization, diastolic Ca^{2+} release, and genesis of DADs. Therefore, the suppression of the NCX by endothelin-1 will reduce the sarcoplasmic reticular Ca^{2+} content and decrease

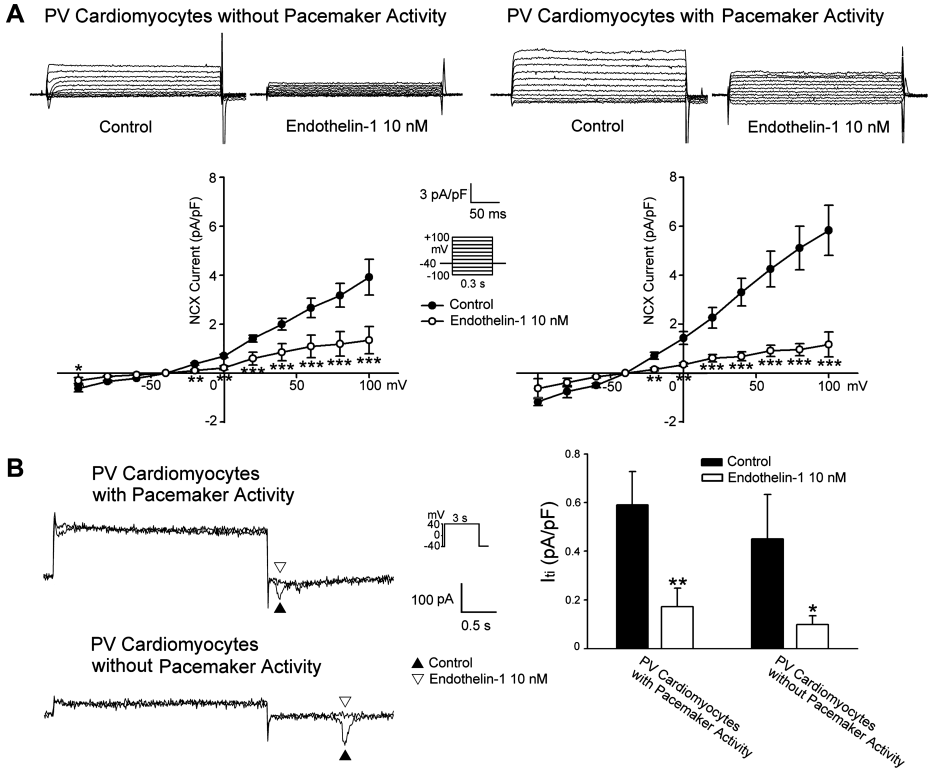

Figure 6. *Effect of the endothelin-1 on the NCX and I_{ti} in the PV cardiomyocytes. Panel A: the current traces and I–V relationship of the NCX before and after the administration of endothelin-1 (10 nM) in the PV cardiomyocytes with ($n = 7$) and without pacemaker activity ($n = 6$). *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ versus control. Panel B: the superimposed current traces and average data of the I_{ti} before (▲) and after (△) the administration of endothelin-1 (10 nM) in the PV cardiomyocytes with ($n = 14$) and without pacemaker activity ($n = 11$). *$P < 0.05$ versus control. The insets in the current traces show the various clamp protocols.*

the PV firing rates and DADs. The suppression of NCX also causes a decrease in the I_{ti} and contributes to the reduction in the automaticity in the cardiomyocytes. Similarly, our previous studies also have shown that the inhibition of the NCX may reduce the PV arrhythmogeneis.[22,23] Although I_f may play a role in the automaticity of pacemaker cells, endothelin-1's effects on the I_f may not play a significant role in the PV spontaneous activity because only some PV cardiomyocytes have an I_f and the current density of the I_f is relatively small.

Endothelin-1 also reduced the I_K in the PV cardiomyocytes with and without pacemaker activity, which was similar to that in the previous studies in rabbit and guinea pig atrial cells.[24,25] An inhibition of the I_K ions by 80% caused by endothelin-1 was observed in human atrial cells by Cheng *et al*.[29] For the first time, this study showed that endothelin-1 has inhibitory effects on the I_{to} in PV cardiomyocytes. I_{Kur} has a significant role in the AP morphology of atrial cardiomyocytes; however, it is not clear what the role of I_{Kur} is on the PV cardiomyocytes with or without pacemaker activity. In this study, we measured the I_{Ksus} because the current density and I–V relationship of I_{Ksus} and I_{Kur} are quite similar and both I_{Kur} and I_{Ksus} are expressed by the same Kv1.5α subunit.[30,31] We found that endothelin-1 did not have any significant effect on the I_{Ksus}.. Therefore, it is more likely that the decrease in the AP duration was due to the dominant effect of endothelin-1 on the $I_{Ca\text{-}L}$ currents than on the I_K currents. Previous studies also have found that a significant inhibition of the $I_{Ca\text{-}L}$ by endothelin-1 may cause a shortening of the AP duration in atrial mycoytes.[25,27] An increase in the I_{K1} as a result of the endothelin-1 has been observed in the atrial cells of rats, rabbits, and guinea pigs.[27,32] However, limited knowledge has been available on the effects of endothelin-1 on pacemaker cells. PV cardiomyocytes with pacemaker activity have been shown to have a smaller I_{K1} than those without pacemaker activity.[18] Our results show that endothelin-1 increases the activity of the I_{K1} current both in the PV cardiomyocytes with and without pacemaker activity. This increase in the I_{K1} may be responsible for the negative shift in the resting membrane potential and also may in part contribute to the shortening of the AP duration.

Conclusions

Endothelin-1 has significant electrophysiological and mechanical effects on PVs. The decrease in the automaticity and triggered activity caused by endothelin-1 suggests that the endothelin-1 may have an antiarrhythmic potential through its effects on the ion currents.

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