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Angiotensin 1-7 modulates electrophysiological characteristics and calcium homoeostasis in pulmonary veins cardiomyocytes via MAS/PI3K/eNOS signalling pathway

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Abstract

Background: Atrial fibrillation (AF) is the most common sustained arrhythmia, and pulmonary veins (PVs) play a critical role in triggering AF. Angiotensin (Ang)-(1-7) regulates calcium (Ca^{2+}) homoeostasis and also plays a critical role in cardiovascular pathophysiology. However, the role of Ang-(1-7) in PV arrhythmogenesis remains unclear.

Materials and methods: Conventional microelectrodes, whole-cell patch-clamp and the fluo-3 fluorimetric ratio technique were used to record ionic currents and intracellular Ca^{2+} in isolated rabbit PV preparations and in single isolated PV cardiomyocytes, before and after administration of Ang-(1-7).

Results: Ang (1-7) concentration dependently (0.1, 1, 10 and 100 nmol/L) decreased PV spontaneous electrical activity. Ang-(1-7) (100 nmol/L) decreased the late sodium (Na^+), L-type Ca^{2+} and Na^+-Ca^{2+} exchanger currents, but did not affect the voltage-dependent Na^+ current in PV cardiomyocytes. In addition, Ang-(1-7) decreased intracellular Ca^{2+} transient and sarcoplasmic reticulum Ca^{2+} content in PV cardiomyocytes. A779 (a Mas receptor blocker, 3 μmol/L), L-NAME (a NO synthesis inhibitor, 100 μmol/L) or wortmannin (a specific PI3K inhibitor, 10 nmol/L) attenuated the effects of Ang-(1-7) (100 nmol/L) on PV spontaneous electric activity.

Conclusion: Ang-(1-7) regulates PV electrophysiological characteristics and Ca^{2+} homoeostasis via Mas/PI3K/eNOS signalling pathway.

KEYWORDS
angiotensin-(1-7), calcium homoeostasis, electrophysiology, pulmonary vein

Yen-Yu Lu and Wen-Shiann Wu contributed equally to this manuscript.

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1 | INTRODUCTION

Atrial fibrillation (AF), the most common clinical arrhythmia, increases the risk of stroke or heart failure and is associated with cardiovascular morbidity and mortality.[1] The renin-angiotensin (Ang) system (RAS) plays a critical role in the pathophysiology of AF as well as in atrial remodelling characterised by interstitial fibrosis and contributes to the onset and maintenance of AF in paced animal models.[2-4] The formation of Ang II involves two main steps: the aspartyl protease renin cleaves angiotensinogen to form the decapeptide Ang I which is then converted by Ang-converting enzyme (ACE) to Ang II. The action of Ang II is mediated by Ang II receptor subtypes 1 and 2. In addition, Ang I is hydrolysed by ACE 2 to form Ang-(1-9), which is then converted by ACE to Ang-(1-7).[5] It has been reported that Ang-(1-7) opposes the molecular and cellular effects of angiotensin II and has a functional interaction between the Ang-(1-7) receptor Mas with AT_1 and AT_2 receptors.[6] A previous study has shown that modulation of RAS by ACE inhibitors or ARB may reduce the risk of AF,[2] which suggests that Ang-(1-7) may potentially regulate AF genesis. Ang-(1-7) has been found to prevent acute electrical remodelling in canines with acute atrial tachycardia.[7] Ang-(1-7) inhibits the growth of rat cardiac myocytes and reduces collagen synthesis and growth factor expression in fibroblasts [8] which may contribute to cardioprotective effects.[9] Furthermore, Ang-(1-7) has direct electrophysiological effect on cardiomyocytes and also attenuates the pacing-decreased L-type calcium (Ca^{2+}) current ($I_{Ca\text{-}L}$) and sodium (Na^{+}) current (I_{Na}) in atrial myocytes, which prevent tachycardia-induced ionic remodelling.[9-11]

Pulmonary veins (PVs) play a critical role in the genesis and maintenance of AF.[12] PVs are important sources of ectopic beats initiating paroxysmal AF and ectopic atrial tachycardia.[13,14] Extensions of the left atrium myocardium over the PVs, the so-called myocardial sleeves, contain cardiomyocytes with distinct electrophysiological characteristics that include spontaneous activity and triggers, which may contribute to PV arrhythmogenesis. Angiotensin II was shown to increase PV arrhythmogenesis, which is attenuated by losartan.[15] Furthermore, direct renin-inhibition may reduce PV arrhythmogenesis,[16] and these findings suggest the important role of RAS in PV arrhythmogenesis. Mas, the Ang (1-7) receptor, promotes beneficial cardiovascular outcome via activation of the PI3K pathway, leading to increased phosphorylation of endothelial nitric oxide synthase (eNOS),[17,18] a key to the generation of NO, which has cardiovascular protective effects such as vasodilation and antioxidation. Previous studies had shown that NO is critical to the regulation of PV electrical activity. Accordingly, Ang-(1-7) may regulate PV arrhythmogenesis and AF genesis. The purpose of this study was an exploration of the effects of Ang (1-7) on PV arrhythmogenesis and the potential mechanisms involved.

2 | MATERIALS AND METHODS

2.1 | Electromechanical and pharmacological studies of the PV preparations

The investigation was approved by a local ethics review board (IACUC-16-317) and conformed to the institutional Guide for the Care and Use of Laboratory Animals and the "Guide for the Care and Use of Laboratory Animals" published by the United States National Institutes of Health (8 ed. Washington DC, 2011). Male rabbits (1-2 kg) were intravenously injected with sodium pentobarbital (100 mg/kg). A midline thoracotomy was then performed, and the

heart and lungs were removed as described previously.[19] To dissect the PV, the PV was opened by an incision along the mitral valve annulus, extending from the coronary sinus to the septum, in Tyrode's solution with a composition (in mmol/L) of 137 NaCl, 4 KCl, 15 $NaHCO_3$, 0.5 NaH_2PO_4, 0.5 $MgCl_2$, 2.7 $CaCl_2$ and 11 dextrose. 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. One end of the preparations, consisting of the PVs and atrial-PV junction, was pinned with needles to the bottom of a tissue bath. The other end (distal PV) was connected to a Grass FT03C force transducer with a silk thread. The PV tissue strips were superfused at a constant rate (3 mL/min) with Tyrode's solution saturated with a 97% O_2-3% CO_2 gas mixture. The temperature was maintained at 37°C, and the preparations were allowed to equilibrate for 1 h before the electrophysiological assessment.

Transmembrane action potentials (APs) were recorded by machine-pulled glass capillary microelectrodes filled with 3 mol/L of KCl which were connected to a WPI Duo 773 electrometer under a tension of 1.47 mN (150 mg). Electrical and mechanical events (contractile force and diastolic tension) were simultaneously displayed on a Gould 4072 oscilloscope and a Gould TA11 recorder. Using a data acquisition system, signals were recorded with DC coupling and a 10-kHz low-pass filter cut-off frequency. Signals were recorded digitally with a 16-bit accuracy at a rate of 125 kHz. Electrical stimulation was provided using a Grass S88 stimulator through a Grass SIU5B stimulus isolation unit. PV preparations were perfused with different concentrations (0.1, 1, 10 and 100 nmol/L) of Ang-(1-7) (Sigma St Louis, MO, USA) for 30 minutes to investigate the dose-response relationship of Ang-(1-7) on PV spontaneous electrical activity. In the presence of A779 (a Mas receptor blocker, CPC Scientific, CA, USA, 3 μmol/L), L-NAME (NO synthesis inhibitor, Sigma, 100 μmol/L) or wortmannin (a specific PI3K inhibitor, Thermo Fisher, CA, USA, 10 nmol/L), Ang-(1-7) (100 nmol/L) superfused for 30 minutes to test the pharmacological responses.

2.2 | Isolation of PV cardiomyocytes and a whole-cell patch-clamp

Single PV cardiomyocyte was enzymatically dissociated through the same procedure as described previously.[20] A whole-cell perforated (for $I_{Ca\text{-}L}$) or ruptured (for others) patch-clamp was performed in single isolated cardiomyocyte before and after the administration of Ang (1-7) using an Axopatch 1D amplifier (Axon Instruments, Foster City, CA, USA) at 35 ± 1°C. Borosilicate glass electrodes (o.d., 1.8 mm) with tip resistances of 3~5 MΩ were used. Before the formation of the membrane-pipette seal, the tip potentials were zeroed in Tyrode's solution. The ionic currents were recorded in the voltage-clamp mode. A small hyperpolarising step from a holding potential of −50 mV to a test 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. Normally 60% ~80% series resistance (Rs) was electronically compensated for. Micropipettes were filled with a solution containing (in mmol/L) CsCl 130, $MgCl_2$ 1, MgATP 5, HEPES 10, EGTA 10, NaGTP 0.1 and Na_2 phosphocreatine 5 (pH 7.2 with CsOH) for the $I_{Ca\text{-}L}$; containing (in mmol/L) NaCl 20, CsCl 110, $MgCl_2$ 0.4, $CaCl_2$ 1.75, tetraethylammonium 20, 1,2-bis(2-aminophenoxy)ethane-N,N,N',N'-tetraacetic acid (BAPTA) 5, glucose 5, MgATP 5 and HEPES 10, titrated to a pH of 7.25 for the Na^+-Ca^{2+} exchanger (NCX) current; containing (in mmol/L) CsCl 133, NaCl 5, EGTA 10, MgATP 5, TEACl 20 and HEPES 5 (pH 7.3 with CsOH) for the I_{Na}; containing (in mmol/L) 10 NaCl, 130 CsCl, 5 EGTA, 5 HEPES, 5 glucose and 5 ATP-Mg for the late Na^+ current ($I_{Na\text{-}Late}$).

The I_{Na} was recorded during depolarisation from a holding potential of −120 mV to the test potentials which ranged −90 to +60 mV in 10-mV steps for 40 ms at a frequency of 3 Hz at room temperature (25 ± 1°C) with an external solution containing (in mmol/L) NaCl 5, CsCl 133, $MgCl_2$ 2, $CaCl_2$ 1.8, nifedipine 0.002, HEPES 5 and glucose 5 at pH 7.3.

The $I_{Na\text{-}Late}$ was recorded at room temperature with an external solution containing (in mmol/L) NaCl 130, CsCl 5, $MgCl_2$ 1, $CaCl_2$ 1, HEPES 10 and glucose 10 at a pH of 7.4 with NaOH by a step/ramp protocol (−100 mV step to +20 mV for 100 ms, then ramp back to −100 mV over 100 ms). An equilibration period (5-10 minutes) for dialysis was allowed for adequately clamping cell currents. $I_{Na\text{-}late}$ was measured as the tetrodotoxin (30 μmol/L)-sensitive portions of the current traces obtained during voltage ramp back to −100 mV as described previously.[21,22]

The $I_{Ca\text{-}L}$ was measured as an inward current during depolarisation from a holding potential of −50 mV to test potentials ranging −40 to +60 mV in 10-mV steps for 300 ms at a frequency of 0.1 Hz by means of a ruptured patch-clamp. The NaCl and KCl in the normal Tyrode's solution were replaced by TEACl and CsCl, respectively.

The NCX current was elicited by test potentials between −100 and +100 mV from a holding potential of −40 mV for 300 ms at a frequency of 0.1 Hz. Amplitudes of the NCX current were measured as 10-mmol/L nickel-sensitive currents. The external solution (in mmol/L) for the measurement of NCX contained NaCl 140, $CaCl_2$ 2, $MgCl_2$1, HEPES 5 and glucose 10 with a pH of 7.4 and also contained strophanthidin (10 μmol/L), nitrendipine (10 μmol/L) and niflumic acid (100 μmol/L).

2.3 | Measurement of Ca^{2+} transients and intracellular Ca^{2+}

Pulmonary veins cardiomyocytes were loaded with a fluorescent Ca^{2+} (10 μmol/L) fluo-3/AM for 30 minutes at room temperature. The excess extracellular dye was removed by changing the bath solution and allowing for the intracellular hydrolysis of the fluo-3/AM after 30 minutes. The fluo-3 fluorescence was excited with a 488-nm line of an argon ion laser. The emission was recorded at >515 nm. For line-scan imaging (8 bit), the cells were repetitively scanned at 2-ms intervals. Fluorescence imaging was performed with a laser scanning confocal microscope (Zeiss LSM 510) and an inverted microscope (Axiovert 100). The fluorescent signals were corrected for variation in dye concentration by normalising the fluorescence (F) against baseline fluorescence (F_0), to obtain reliable information about transient intracellular Ca^{2+} ($Ca^{2+}{}_i$) changes from the baseline values (F/F_0) and to exclude variations in the fluorescence intensity by different volumes of injected dye.[23] The $Ca^{2+}{}_i$ transient, peak systolic $Ca^{2+}{}_i$, diastolic $Ca^{2+}{}_i$ and decayed portion of the $Ca^{2+}{}_i$ transient were measured during a 2-Hz field stimulation with 10-ms twice-threshold strength square-wave pulses. The $Ca^{2+}{}_i$ was determined by the monoexponential least-squares fit. After achieving a steady-state Ca^{2+} transients with the repeated pulses from −40 to 0 mV (1 Hz for 5 seconds), the sarcoplasmic reticulum (SR) Ca^{2+} content was estimated by integrating the Na^+-Ca^{2+} exchanger (NCX) current following application of 20 mmol/L of caffeine within 0.5 seconds during rest with the membrane potential clamped to −40 mV to cause SR Ca^{2+} release.[24] The total SR Ca^{2+} content (expressed as mM of cytosol) was determined by use of the equation: SR Ca^{2+} content = $[(1 + 0.12)(Ccaff/F \times 1000)]/(Cm \times 8.31 \times 6.44)$, where Cm = membrane capacitance; F = Faraday's number; cell surface-to-volume ratio = 6.44 pF/pL.[25,26]

2.4 | Statistical analysis

All continuous variables are expressed as mean ± standard error of the mean (SEM). One-way repeated measures ANOVA followed by the Bonferroni analysis was used to compare the difference before and after drug administration on PVs. A $P < .05$ was considered statistically significant.

3 | RESULTS

3.1 | Effects of Ang (1-7) on PV Ca^{2+} handling and membrane currents

Compared to the baseline, Ang-(1-7) (100 nmol/L) reduced the amplitude of $Ca^{2+}{}_i$ transients (Figure 1A). In addition, Ang-(1-7) (100 nmol/L) reduced SR Ca^{2+} content measured by the integration of caffeine-induced NCX currents (Figure 1B).

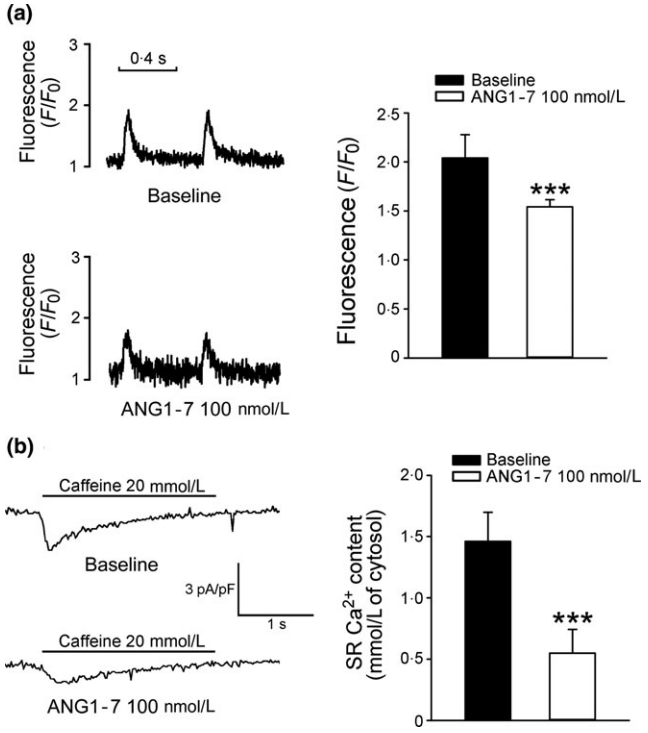


FIGURE 1 Effects of Ang-(1-7) on the intracellular calcium ($Ca^{2+}{}_i$) and sarcoplasmic reticulum Ca^{2+} content of the PV cardiomyocytes. A, The tracings and average data from the $Ca^{2+}{}_i$ transient before and after the administration of Ang-(1-7) (100 nmol L^{-1} in the PV cardiomyocytes (n = 11). B, The tracings of the caffeine-induced NCX currents and average data of SR Ca^{2+} content from integrating the NCX current before and after the administration of Ang-(1-7) (100 nmol/L) in the PV cardiomyocytes (n = 12). ***$P < .005$ vs before Ang-(1-7)

As shown in Figure 2A, the current density of the peak I_{Na} was similar in PV cardiomyocytes before and after Ang-(1-7) (100 nmol/L) treatment. However, the $I_{Na\text{-}Late}$ was reduced by 38% (Figure 2B). Ang-(1-7) (100 nmol/L) decreased the $I_{Ca\text{-}L}$ (Figure 2C) and decreased the reverse and forward NCX currents (Figure 2D).

3.2 | Effects of Ang-(1-7) on PV electrical activity

As shown in Figure 3, the effect of Ang (1-7) is concentration-dependent and decreased PV spontaneous electrical activity to an acute administration of 0.1, 1, 10 and 100 nmol/L. Compared to the baseline, Ang (1-7) significantly decreased PV spontaneous beating rate by 28 ± 10% at a concentration of 100 nmol/L.

In our assessment of the role of Ang-(1-7)-Mas axis in PV electrophysiological characteristics, we investigated the involvement of PI3K and eNOS in the modulation of PV electrical activity. We treated the PV preparations with

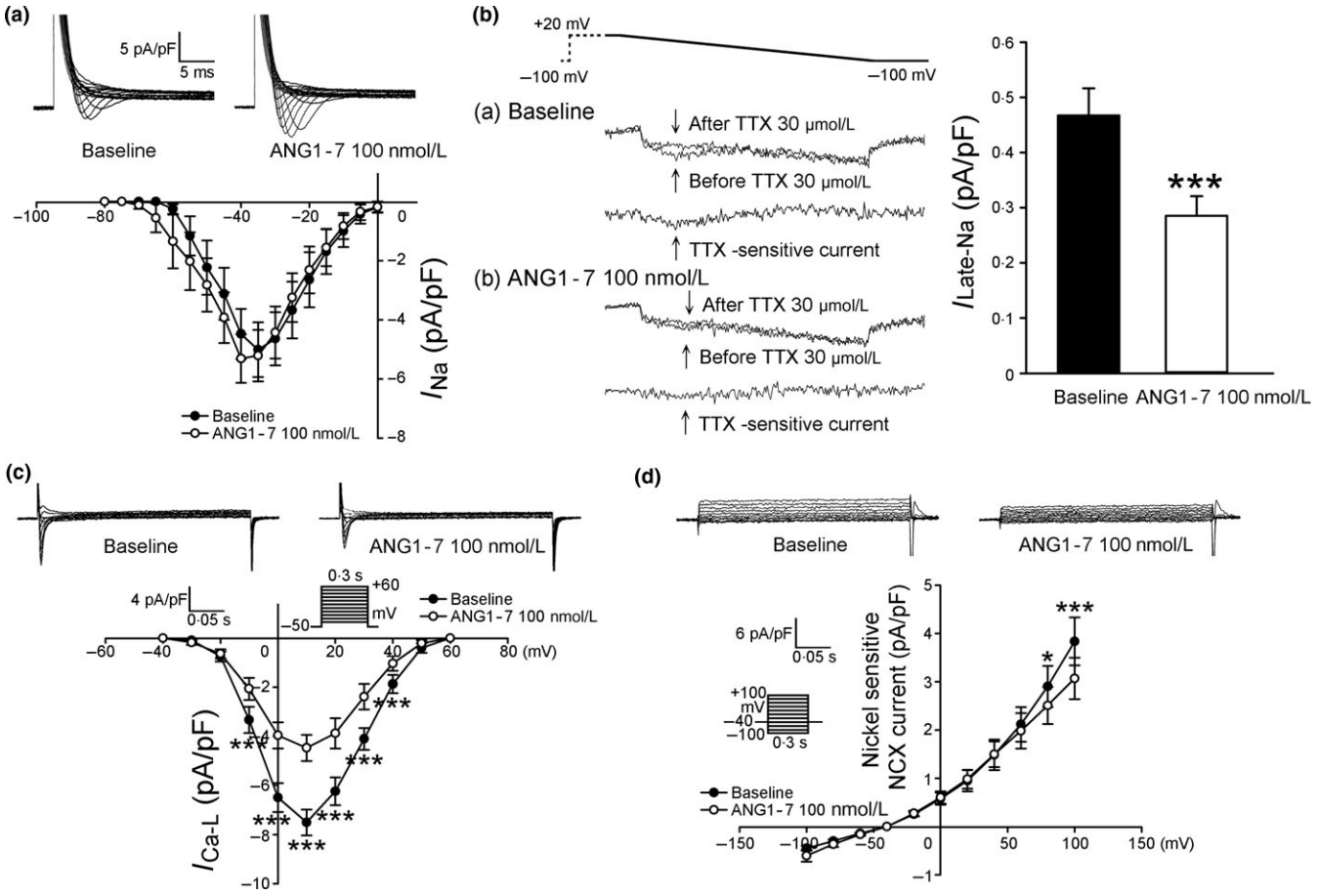


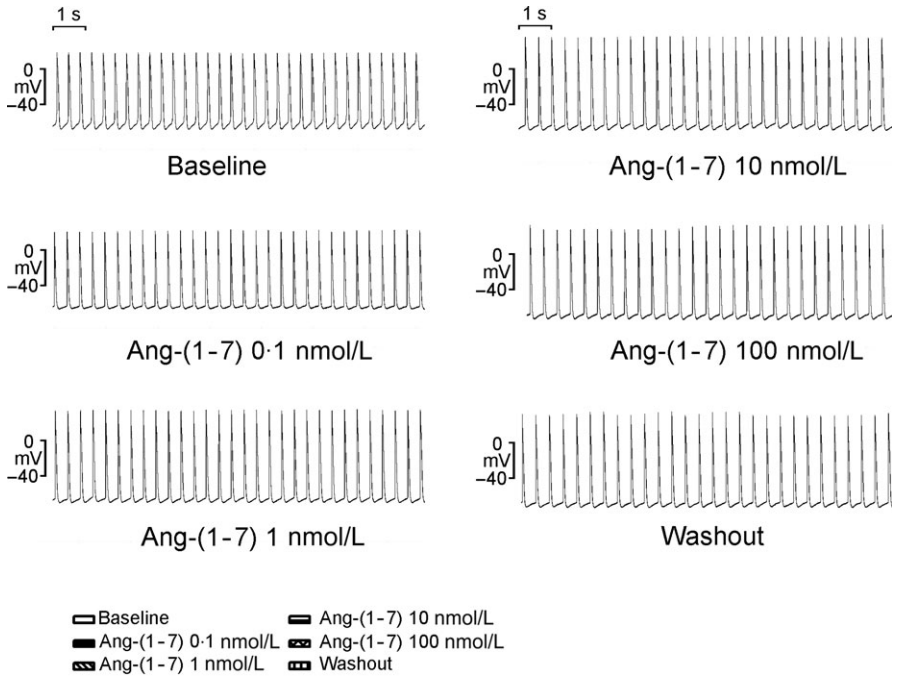
FIGURE 2 Effects of Ang-(1-7) on the sodium current (I_{Na}), the late sodium current ($I_{Na\text{-}Late}$), the L-type calcium channel ($I_{Ca\text{-}L}$) and the sodium-calcium exchanger (NCX) in pulmonary vein (PV) myocytes. A, Examples and the I-V relationship of the I_{Na} before and after the administration of Ang-(1-7) (100 nmol/L) in the PV cardiomyocytes (n = 13). B, Examples and the average data of the $I_{Na\text{-}Late}$ before and after the administration of Ang-(1-7) (100 nmol/L) in the PV cardiomyocytes (n = 9). C, The current traces and I-V relationship of $I_{Ca\text{-}L}$ before and after the administration of Ang-(1-7) (100 nmol/L) in the PV cardiomyocytes (n = 10). D, The current tracings and I-V relationship of the NCX currents before and after the administration of Ang-(1-7) (100 nmol/L) in the PV cardiomyocytes (n = 9). The insets in the current traces show the various clamp protocols. *$P < .05$; ***$P < .005$ vs before Ang-(1-7)

A779 (Mas receptor blocker, 3 μmol/L) and found A779 reduced PV spontaneous activity by 9 ± 3%. However, Ang-(1-7) (100 nmol/L) did not change spontaneous electrical activity of the PV preparations in the presence of A779. Wortmannin (a PI3K inhibitor, 10 nmol/L) reduced PV spontaneous activity to a similar extent (9 ± 2%), and Ang-(1-7) did not change PV spontaneous electrical activity in the presence of wortmannin. L-NAME (a NO synthesis inhibitor, 100 μmol/L) reduced PV spontaneous activity by 6 ± 2%. Again, Ang-(1-7) did not change spontaneous electrical activity of the PV preparations in the presence of L-NAME (100 μmol/L; Figure 4).

4 | DISCUSSION

Renin-angiotensin (Ang) system plays a vital role in AF and PV arrhythmogenesis. Previous studies have suggested that Ang-(1-7) is a potential target for future of antiarrhythmic therapy.[27] Experimental research has shown that Ang-(1-7) may modulate ion fluxes and limit ventricular and atrial arrhythmias.[10,11,17] This study has demonstrated, for the first time, that Ang-(1-7) reduces PV electrical activity and modulates Ca^{2+} homoeostasis with modulating Mas signalling pathway. As PV arrhythmogenesis critically contribute to the genesis of AF, this finding suggests the anti-AF potential of Ang-(1-7).

Changes in $Ca^{2+}{}_i$ are a major feature in heart failure-induced AF and PV cardiomyocyte pathology.[28,29] In previous studies, it was found that Ang-(1-7) had no significant effect on $I_{Ca\text{-}L}$ in normal ventricular myocytes, but $I_{Ca\text{-}L}$ and $Ca^{2+}{}_i$ were increased in heart failure ventricular myocytes.[30-32] We found that Ang-(1-7) decreased $I_{Ca\text{-}L}$, which might cause a reduction in $Ca^{2+}{}_i$ by inhibition of Ca^{2+}-induced Ca^{2+} release. In addition, the effects of Ang-(1-7) on $Ca^{2+}{}_i$ transients and SR Ca^{2+} content may cause a drop



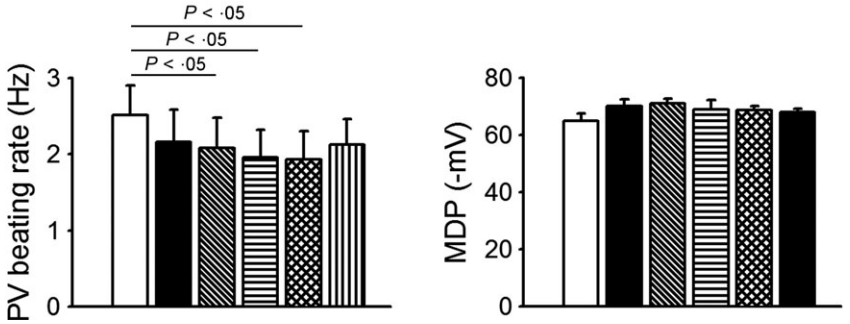


FIGURE 3 The concentration-dependent effect of Ang-(1-7) on pulmonary vein (PV) electrical activity. Representative recordings and average data of spontaneous electric activity in the PV preparations (N = 6) treated with or without Ang-(1-7) (0.1, 1, 10 and 100 nmol/L). Right lower panel illustrated the dose-response curve for a series of Ang-(1-7) that inhibits PV spontaneous electrical activity

in PV electrical activity through modulation of Ca^{2+} homoeostasis in PV cardiomyocytes. Similarly, a previous study showed ventricular cardiomyocytes from transgenic rats with chronic overproduction of Ang-(1-7) were protected from Ca^{2+} signalling dysfunction induced by Ang II.[33]

$I_{Na\text{-}Late}$ plays an important role in the genesis of AF and PV arrhythmogenic potentials.[22,34,35] In a previous study, we showed that an increase in $I_{Na\text{-}Late}$ can induce PV arrhythmogenesis with increased triggered activity,[34] and in this study, we have found Ang-(1-7) reduces $I_{Na\text{-}Late}$ in PV cardiomyocytes. The decrease in $I_{Na\text{-}Late}$ by Ang-(1-7) prevents $Ca^{2+}{}_i$ overload, resulting in reduced PV arrhythmogenesis.[36,37] In contrast, we have also found that Ang-(1-7) does not change the I_{Na} in PV cardiomyocytes, which is different from the increasing effects of Ang-(1-7) on I_{Na} in canine atrial myocytes under rapid atrial pacing.[10] NCX plays a critical role in atrial and PV arrhythmogenesis.[20,38] We found that Ang-(1-7) reduced NCX current in PV cardiomyocytes. The observed $Ca^{2+}{}_i$ transient and SR Ca^{2+} content reduction in Ang-(1-7)-treated PV cardiomyocytes may be caused by the suppression of NCX by Ang-(1-7). Decreased $I_{Ca\text{-}L}$, NCX and $I_{Na\text{-}Late}$ may reduce the PV spontaneous electric activity and triggered activity.[20,34]

The interaction between Ang-(1-7) and Mas is important for the maintenance of cardiac rhythm and prevention of atrial arrhythmias.[9,39] The downstream Mas/PI3K/eNOS signalling pathway may also reduce AF vulnerability.[7] We studied the molecular mechanisms underlying the effects of Ang-(1-7) on PV cardiomyocytes and found that Ang-(1-7) did not change PV spontaneous electrical activity in the presence of A779, wortmannin or L-NAME. These findings suggest that Ang-(1-7) interferes with PV electrical activity through the Mas/PI3K/eNOS signalling pathway. The PI3K pathway activated by Mas receptor induces post-translation eNOS activation by phosphorylation of Ser-1177.[18] The effects of Ang-(1-7) on PV electrical activity indicated that the Mas/PI3K/eNOS signalling pathway may play a pivotal role in PV arrhythmogenesis, and Ang-(1-7) may reduce AF inducibility and perpetuation.

In conclusion, the findings of this study demonstrate that Ang-(1-7) modulates PV electrophysiological characteristics and Ca^{2+} homoeostasis through activation of the

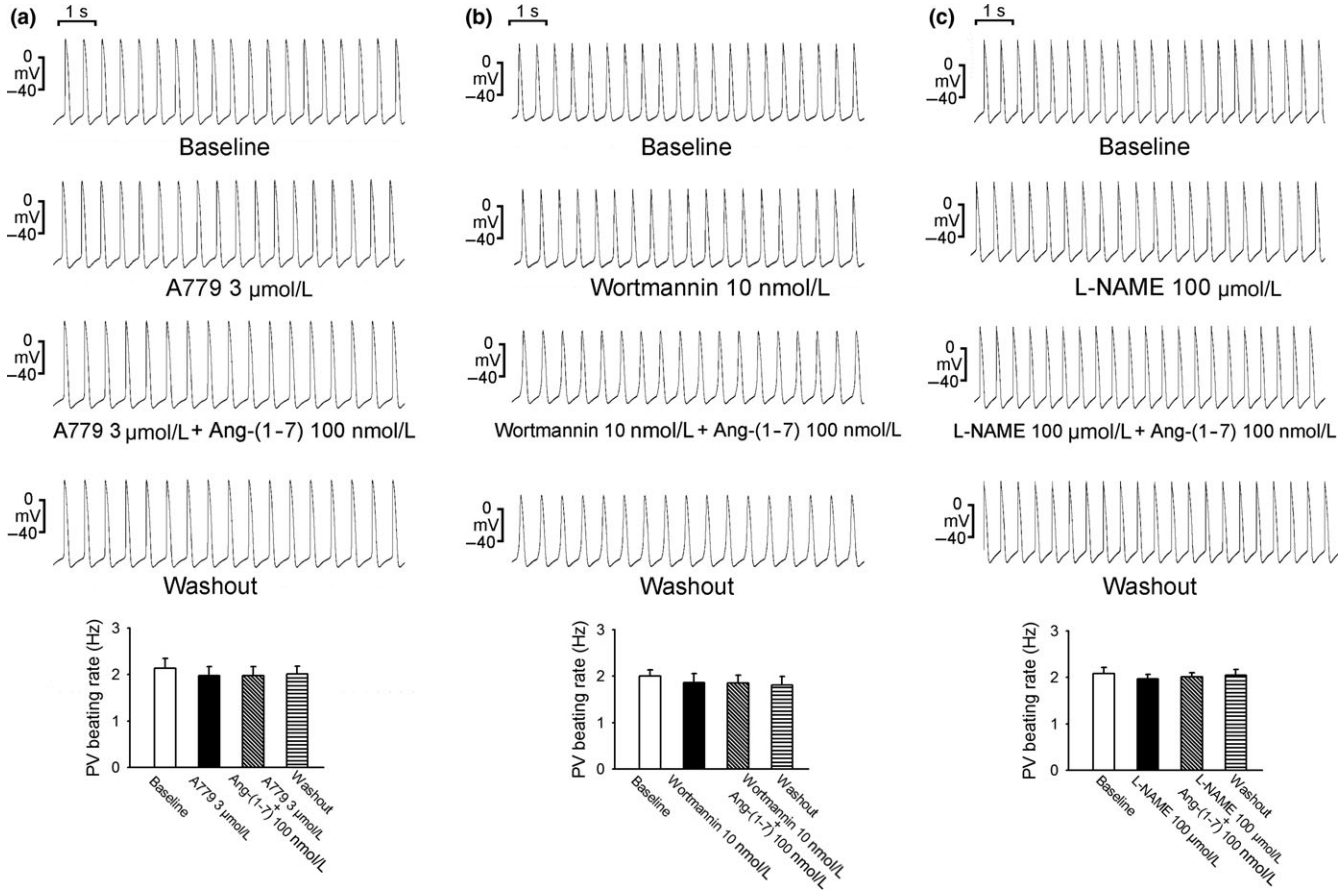


FIGURE 4 Interactions of Ang-(1-7) with A779, wortmannin or L-NAME on pulmonary vein (PV) electrical activity. Representative recordings and average data of spontaneous electric activity in the PV preparations treated with or without Ang-(1-7) (100 nmol/L), A779 (3 μmol/L, N = 6), wortmannin (10 nmol/L, N = 6) or L-NAME (100 μmol/L, N = 6). A779, wortmannin or L-NAME decreased PV spontaneous electrical activity, and Ang-(1-7) (100 nmol/L) had no effect on PV spontaneous electrical activity in the presence of A779, wortmannin or L-NAME

Ang-(1-7)/Mas axis and up-regulation of the PI3K/eNOS pathway.

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DISCLOSURES

No conflict of interest, financial or otherwise is declared by the authors.

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REFERENCES

1. Beyerbach DM, Zipes DP. Mortality as an endpoint in atrial fibrillation. *Heart Rhythm* 2004;1:B8-B18. discussion B18-9.
2. Ehrlich JR, Hohnloser SH, Nattel S. Role of angiotensin system and effects of its inhibition in atrial fibrillation: clinical and experimental evidence. *Eur Heart J*. 2006;27:512-518.
3. Pellman J, Lyon RC, Sheikh F. Extracellular matrix remodeling in atrial fibrosis: mechanisms and implications in atrial fibrillation. *J Mol Cell Cardiol*. 2010;48:461-467.

4. Schotten U, Verheule S, Kirchhof P, Goette A. Pathophysiological mechanisms of atrial fibrillation: a translational appraisal. *Physiol Rev*. 2011;91:265-325.
5. Keidar S, Kaplan M, Gamliel-Lazarovich A. ACE2 of the heart: from angiotensin I to angiotensin (1-7). *Cardiovasc Res*. 2007;73:463-469.
6. Castro CH, Santos RA, Ferreira AJ, Bader M, Alenina N, Almeida AP. Evidence for a functional interaction of the angiotensin-(1-7) receptor Mas with AT1 and AT2 receptors in the mouse heart. *Hypertension*. 2005;46:937-942.
7. Zhao J, Liu E, Li G, Qi L, Li J, Yang W. Effects of the angiotensin-(1-7)/Mas/PI3K/Akt/nitric oxide axis and the possible role of atrial natriuretic peptide in an acute atrial tachycardia canine model. *J Renin Angiotensin Aldosterone Syst*. 2015;16:1069-1077.
8. Tallant EA, Ferrario CM, Gallagher PE. Angiotensin-(1-7) inhibits growth of cardiac myocytes through activation of the mas receptor. *Am J Physiol Heart Circ Physiol*. 2005;289:H1560-H1566.
9. Zhao J, Liu T, Liu E, Li G, Qi L, Li J. The potential role of atrial natriuretic peptide in the effects of Angiotensin-(1-7) in a chronic atrial tachycardia canine model. *J Renin Angiotensin Aldosterone Syst*. 2016;17:1470320315627409.
10. Wang X, Li G. Angiotensin-(1-7) prevent atrial tachycardia induced sodium channel remodeling. *Pacing Clin Electrophysiol*. 2014;37:1349-1356.
11. Liu E, Xu Z, Li J, Yang S, Yang W, Li G. Enalapril, irbesartan, and angiotensin-(1-7) prevent atrial tachycardia-induced ionic remodeling. *Int J Cardiol*. 2011;146:364-370.
12. Pappone C, Oreto G, Rosanio S, et al. Atrial electroanatomic remodeling after circumferential radiofrequency pulmonary vein ablation: efficacy of an anatomic approach in a large cohort of patients with atrial fibrillation. *Circulation*. 2001;104:2539-2544.
13. Haissaguerre M, Jais P, Shah DC, et al. Spontaneous initiation of atrial fibrillation by ectopic beats originating in the pulmonary veins. *N Engl J Med*. 1998;339:659-666.
14. Chen YJ, Chen SA, Chang MS, Lin CI. Arrhythmogenic activity of cardiac muscle in pulmonary veins of the dog: implication for the genesis of atrial fibrillation. *Cardiovasc Res*. 2000;48:265-273.
15. Chen YJ, Chen YC, Tai CT, Yeh HI, Lin CI, Chen SA. Angiotensin II and angiotensin II receptor blocker modulate the arrhythmogenic activity of pulmonary veins. *Br J Pharmacol*. 2006;147:12-22.
16. Tai CF, Chen YC, Lin YK, Chen SA, Chen YJ. Electromechanical effects of the direct renin inhibitor (aliskiren) on the pulmonary vein and atrium. *Basic Res Cardiol*. 2011;106:979-993.
17. Ferreira AJ, Santos RA, Almeida AP. Angiotensin-(1-7): cardioprotective effect in myocardial ischemia/reperfusion. *Hypertension*. 2001;38:665-668.
18. Sampaio WO, Souza dos Santos RA, Faria-Silva R, da Mata Machado LT, Schiffrin EL, Touyz RM. Angiotensin-(1-7) through receptor Mas mediates endothelial nitric oxide synthase activation via Akt-dependent pathways. *Hypertension*. 2007;49:185-192.
19. Chang SL, Chen YC, Chen YJ, et al. Mechanoelectrical feedback regulates the arrhythmogenic activity of pulmonary veins. *Heart*. 2007;93:82-88.
20. Wongcharoen W, Chen YC, Chen YJ, et al. Effects of a Na^+/Ca^{2+} exchanger inhibitor on pulmonary vein electrical activity and ouabain-induced arrhythmogenicity. *Cardiovasc Res*. 2006;70:497-508.
21. Lin YK, Chen YC, Chen JH, Chen SA, Chen YJ. Adipocytes modulate the electrophysiology of atrial myocytes: implications in obesity-induced atrial fibrillation. *Basic Res Cardiol*. 2012;107:293.
22. Lu YY, Chen YC, Kao YH, Chen SA, Chen YJ. Extracellular matrix of collagen modulates arrhythmogenic activity of pulmonary veins through p38 MAPK activation. *J Mol Cell Cardiol*. 2013;59:159-166.
23. Chang SH, Chen YC, Chiang SJ, et al. Increased Ca^{2+} sparks and sarcoplasmic reticulum Ca^{2+} stores potentially determine the spontaneous activity of pulmonary vein cardiomyocytes. *Life Sci*. 2008;83:284-292.
24. Suenari K, Chen YC, Kao YH, et al. Discrepant electrophysiological characteristics and calcium homeostasis of left atrial anterior and posterior myocytes. *Basic Res Cardiol*. 2011;106:65-74.
25. Hove-Madsen L, Llach A, Bayes-Genis A, et al. Atrial fibrillation is associated with increased spontaneous calcium release from the sarcoplasmic reticulum in human atrial myocytes. *Circulation*. 2004;110:1358-1363.
26. Lu YY, Chen YC, Kao YH, et al. Colchicine modulates calcium homeostasis and electrical property of HL-1 cells. *J Cell Mol Med*. 2016;20:1182-1190.
27. Kucharewicz I, Pawlak R, Matys T, Chabielska E, Buczko W. Angiotensin-(1-7): an active member of the renin-angiotensin system. *J Physiol Pharmacol*. 2002;53:533-540.
28. Chen YJ, Chen YC, Wongcharoen W, Lin CI, Chen SA. Effect of K201, a novel antiarrhythmic drug on calcium handling and arrhythmogenic activity of pulmonary vein cardiomyocytes. *Br J Pharmacol*. 2008;153:915-925.
29. Chang SL, Chen YC, Yeh YH, et al. Heart failure enhanced pulmonary vein arrhythmogenesis and dysregulated sodium and calcium homeostasis with increased calcium sparks. *J Cardiovasc Electrophysiol*. 2011;22:1378-1386.
30. Zhou P, Cheng CP, Li T, Ferrario CM, Cheng HJ. Modulation of cardiac L-type Ca^{2+} current by angiotensin-(1-7): normal versus heart failure. *Ther Adv Cardiovasc Dis*. 2015;9:342-353.
31. Zhang X, Cheng HJ, Zhou P, et al. Cellular basis of angiotensin-(1-7)-induced augmentation of left ventricular functional performance in heart failure. *Int J Cardiol*. 2017;236:405-412.
32. de Almeida PW, de Freitas Lima R, de Morais Gomes ER, et al. Functional cross-talk between aldosterone and angiotensin-(1-7) in ventricular myocytes. *Hypertension*. 2013;61:425-430.
33. Gomes ER, Lara AA, Almeida PW, et al. Angiotensin-(1-7) prevents cardiomyocyte pathological remodeling through a nitric oxide/guanosine 3′,5′-cyclic monophosphate-dependent pathway. *Hypertension*. 2010;55:153-160.
34. Lu YY, Cheng CC, Chen YC, Chen SA, Chen YJ. ATX-II-induced pulmonary vein arrhythmogenesis related to atrial fibrillation and long QT syndrome. *Eur J Clin Invest*. 2012;42:823-831.
35. Lu YY, Lin YK, Wen ZH, Chen YC, Chen SA, Chen YJ. Latrunculin B modulates electrophysiological characteristics and arrhythmogenesis in pulmonary vein cardiomyocytes. *Clin Sci*. 2016;130:721-732.

36. Noble D, Noble PJ. Late sodium current in the pathophysiology of cardiovascular disease: consequences of sodium-calcium overload. *Heart* 2006;92(Suppl 4):iv1-iv5.
37. Bers DM, Bassani JW, Bassani RA. Na-Ca exchange and Ca fluxes during contraction and relaxation in mammalian ventricular muscle. *Ann N Y Acad Sci*. 1996;779:430-442.
38. Chang CJ, Cheng CC, Yang TF, et al. Selective and non-selective non-steroidal anti-inflammatory drugs differentially regulate pulmonary vein and atrial arrhythmogenesis. *Int J Cardiol*. 2015;184:559-567.
39. Santos RA, Castro CH, Gava E, et al. Impairment of in vitro and in vivo heart function in angiotensin-(1-7) receptor MAS knockout mice. *Hypertension*. 2006;47:996-1002.

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