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腎素抑制劑 Aliskiren 調控肺靜脈和心房心肌細胞內鈣離子流及細胞電氣生理特性作用之研究

研究成果報告(精簡版)

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Electromechanical effects of the direct renin inhibitor (aliskiren) on the pulmonary vein and atrium

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ORIGINAL CONTRIBUTION

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Abstract Activation of the atrial renin–angiotensin system plays an important role in the pathophysiology of atrial fibrillation (AF). The pulmonary vein (PV) and left atrium (LA) are important trigger and substrate for the genesis of AF. We investigate the effects of a direct renin inhibitor, aliskiren, on the PV and LA arrhythmogenic activity and the underlying electromechanical mechanisms. Conventional microelectrodes were used to record action potentials and contractility in isolated rabbit PVs and LA tissues before and after the administration of aliskiren (0.1, 1, 3 and 10 μM). By the whole-cell patch clamp and indo-1 fluorimetric ratio techniques, ionic currents and intracellular calcium transient were studied in isolated single PV and LA cardiomyocyte before and after the administration of aliskiren (3 μM). Aliskiren (0.1, 1, 3 and 10 μM) reduced PV firing rate in a concentration-dependent manner (6, 10, 14 and 17%) and decreased PV diastolic tension, which could be attenuated in the presence of 100 μM L-N^G-Nitroarginine Methyl Ester (L-NAME). Aliskiren induced PV automatic rhythm exit block causing slow and irregular PV activity with variable pauses. Aliskiren increased PV and LA contractility, which could be abolished by pre-treating with 0.1 μM ryanodine. Aliskiren (3 μM) decreased L-type calcium currents, but increased reverse-mode of Na^+/Ca^{2+} exchanger currents, intracellular calcium transients, and sarcoplasmic reticulum calcium content in PV and LA cardiomyocytes. Pretreatment with renin, losartan or angiotensin II did not alter the effect of aliskiren on sarcolemmal calcium flux. In conclusion, aliskiren reduces PV arrhythmogenic activity with a direct vasodilatory property and has a positive inotropic effect on cardiomyocytes. These findings may reveal the anti-arrhythmic and anti-heart failure potentials of aliskiren.

Keywords Aliskiren · Arrhythmia · Pulmonary vein · Calcium transient

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Introduction

Atrial fibrillation (AF) is the most common sustained arrhythmia in clinical medicine. Activation of the atrial renin–angiotensin system plays an important role in the genesis of AF by inducing a combination of electrical and structural remodeling with increasing vulnerability to AF induction [7, 16, 20, 21]. These studies indicate that angiotensin II may be involved in remodeling and have a direct electrophysiological action. The recent development of direct

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renin inhibitor (aliskiren) provides an alternative approach to blockade of the renin–angiotensin system at its most proximal rate-limiting enzyme for the formation of angiotensin II. By exerting a more complete blockade on the renin–angiotensin system, aliskiren may afford greater cardiovascular and renal protective benefits [30, 32, 38]. Fischer et al. [19] recently demonstrated that aliskiren improves electrical remodeling with anti-arrhythmic benefits in a transgenic rat high-renin hypertension model. However, information about the effect of aliskiren on the occurrence of AF is lacking.

Pulmonary veins (PVs) and left atrium (LA) are commonly identified sources for the initiation and maintenance of AF, and encircling ablations of these areas are proved effective in treating AF [5, 6, 31]. The PVs contain a mixture of pacemaker cells and working myocardium with a high arrhythmogenic potential [9, 10, 14, 29]. Our previous study showed that angiotensin II and angiotensin receptor blocker losartan directly modulate the PV electrical activity [11]. Nevertheless, whether aliskiren can directly regulate the PV arrhythmogenic activity remains elusive. Mechanoelectrical feedback plays an important role in the PV arrhythmogenesis [4, 22]. Imanishi et al. [23] demonstrated a beneficial effect of aliskiren on the endothelial function by improving nitric oxide release and bioactivity. Therefore, it is possible that aliskiren may modulate PV spontaneous activity by reducing vascular tension or directly changing the electrophysiological effects of PV cardiomyocytes. The purposes of the present study were to investigate the effects of aliskiren on the PV and LA arrhythmogenic activity and to evaluate the underlying mechanisms.

Methods

Rabbit PV and LA tissue preparations

The investigation conformed to the institutional Guide for the Care and Use of Laboratory Animals. The rabbits (weight: 1–2 kg) were anesthetized with an intraperitoneal injection of sodium pentobarbital (40 mg kg^{-1}). Hearts and lungs were removed after mid-line thoracotomy. For dissection of the PV, the LA was opened by an incision along the mitral valve annulus extending from the coronary sinus to the septum in normal 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. The PVs were separated from the atria at the LA-PV junction and separated from the lungs at the end of the PV myocardial sleeves. Sample preparation size was approximately 10 mm in length and 5 mm in width. One end of the preparation consisting of PV, atrium-PV junction, and atrial tissue (within 1 mm in length), was pinned 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 adventitia of the PVs faced upwards. For LA experiments, the LA appendage (approximately 10 × 5 × 0.5 mm) was isolated and prepared as described previously [4]. The PV and LA tissue strips were superfused at a constant rate (3 mL/min) with Tyrode's solution that was saturated with a 97% O_2–3% CO_2 gas mixture. Temperature was maintained constant at 37°C and preparations were allowed to equilibrate for 1 h before the electrophysiological study.

Electrophysiological and pharmacological studies

Transmembrane action potentials (APs) of PVs and LA were recorded by machine-pulled glass capillary microelectrodes filled with 3 mol/L of KCl and connected to a WPI Duo 773 electrometer under tension with 150 mg. Electrical and mechanical events (contractile force and diastolic vascular tension) were displayed simultaneously 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 cutoff frequency. Signals were recorded digitally with a 16-bit accuracy at a rate of 125 kHz. Electrical stimulation with 10 ms duration and suprathreshold strength (30% above the threshold) was provided using a Grass S88 stimulator through a Grass SIU5B stimulus isolation unit. Different concentrations of aliskiren (0.1, 1, 3, and 10 μM; Novartis) were sequentially superfused to test pharmacological responses. For each concentration, PV and LA preparations were treated with aliskiren for at least 30 min. The concentrations of aliskiren in this experiment were considered in parallel with clinically measured plasma levels [3]. In order to study the casual relationship between aliskiren and nitric oxide, a nitric oxide synthase inhibitor, L-N^G-Nitroarginine Methyl Ester (L-NAME, 100 μM), was given for at least 1 h before aliskiren administration. The PVs with spontaneous activity were identified by the presence of constantly occurring spontaneous APs without using any electrical stimuli. The AP duration at repolarization of 90 and 50% of the AP amplitude (APD_{90}, APD_{50}), membranous diastolic potential, and contractile force were measured during 2 Hz electrical stimuli before and after aliskiren administration in the LA and PVs without spontaneous activity.

Aliskiren on sarcolemmal calcium flux in single PV and LA cardiomyocytes

PV and LA cardiomyocytes were enzymatically dissociated through a previously described procedure [8, 10–12, 39, 43]. A whole-cell patch-clamp was performed on single PV or LA cardiomyocytes before and after the administration of aliskiren (3 μM) with or without the presence of renin (human recombinant renin 600 U/L; Sigma Chemical, St.

Louis, MO, USA), angiotensin II (100 nM), and angiotensin II type 1 receptor blocker losartan (10 μM) using an Axopatch 1D amplifier (Axon Instruments, CA, USA) at 35 ± 1°C [11, 18, 34]. Borosilicate glass electrodes (o.d. 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's solution. Ionic currents were measured in voltage-clamp mode. At the beginning of each experiment, a small hyperpolarizing step from a holding potential of −50 mV to a testing potential of −55 mV for 80 ms was delivered. The area under the capacitative currents was divided by the applied voltage step to obtain the total cell capacitance. Normally, 60–80% series resistance (R_s) was electronically compensated. The extracellular solution contained the basic composition (in mM) of NaCl 137, KCl 5.4, HEPES 10, $MgCl_2$ 0.5, $CaCl_2$ 1.8, and glucose 10. The solution was titrated to a pH of 7.4 with NaOH. 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. This solution was titrated to a pH of 7.2 with CsOH for the experiments on the L-type calcium current ($I_{Ca\text{-}L}$). Micropipettes were filled with a solution containing (in mM) NaCl 20, CsCl 110, $MgCl_2$ 0.4, $CaCl_2$ 1.75, tetraethylammonium chloride (TEACl) 20, BAPTA 5, glucose 5, Mg_2ATP 5, and HEPES 10. This solution was titrated to a pH of 7.25 with CsOH for the experiments on sodium-calcium exchanger (NCX) current. 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 test potentials ranging from −40 to +60 mV in 10-mV steps for 300 ms at a frequency of 0.1 Hz by means of perforated patch-clamp with amphotericin B. NaCl and KCl in the external solution were replaced with tetraethylammonium chloride 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 mM nickel-sensitive currents. The external solution (in mM) for the measurement of NCX contained NaCl 140, $CaCl_2$ 2, $MgCl_2$ 1, HEPES 5, glucose 10 with a pH of 7.4, strophanthidin (10 μM), nitrendipine (10 μM), and niflumic acid (100 μM).

Measurement of intracellular calcium concentration

The intracellular calcium transient ($[Ca^{2+}]_i$) was recorded by a fluorometric ratio technique through the same procedure as previously described [8, 12, 39, 43]. The fluorescent indicator, indo-1, was loaded by incubating cardiomyocytes at room temperature for 20–30 min with 25 μM of indo-1/AM (Sigma Chemical, St. Louis, MO, USA). PV and LA cardiomyocytes were then perfused with normal Tyrode's solution at 35 ± 1°C for at least 30 min to wash out the extracellular indicator and to allow for intracellular de-esterification of the indo-1. Using cells without indo-1 loading, the background and cell autofluorescence levels were canceled out by zeroing the output of the photomultiplier tubes. Experiments were performed during superfusion before and after aliskiren (3 μM). Ultraviolet light at strength of 360 nm from a monochromator was used to excite the indo-1 from a xenon arc lamp that was controlled by a microfluorometric system (OSP100-CA; Olympus, Tokyo, Japan). The excitation light beam was directed into an inverted microscope (IX-70, Olympus). Emitted fluorescence signals from indo-1/AM-loaded cardiomyocytes were digitized at 200 Hz. The ratio of fluorescence emissions at 410 and 485 nm was recorded. The $R_{410/485}$ value was used as an index of $[Ca^{2+}]_i$. This approach avoided uncertainties from the calibration of the fluorescent Ca^{2+} indicators. The $[Ca^{2+}]_i$ transient, peak systolic $[Ca^{2+}]_i$, diastolic $[Ca^{2+}]_i$, and decay portion of the Ca^{2+} transient (τ_{Ca}) were measured during 2-Hz field stimulation with 10-ms square-wave pulses at twice the diastolic threshold strength. The $[Ca^{2+}]_i$ transient was calculated from the difference between the peak systolic $[Ca^{2+}]_i$ and diastolic $[Ca^{2+}]_i$. The τ_{Ca} was determined by the mono-exponential least-squares fit. The sarcoplasmic reticulum (SR) Ca^{2+} content was measured by integrating the NCX current from rapidly adding 20 mM of caffeine to the cells during rest with the membrane potential clamped to −40 mV [8, 39]. The time integral of the NCX current was converted to amoles of Ca^{2+} released from the SR.

Statistical analysis

All quantitative data were expressed as the mean ± SEM. The paired Student's *t* test or one-way repeated analysis of variance (ANOVA) was used to compare the differences before and after drug administration in the PV or LA specimens. Multiple comparisons were analyzed with Fischer's least significant difference test. Statistical differences were considered significant if the *P* value was less than 0.05.

Results

Effects of aliskiren on the PVs with spontaneous activity

As the examples shown in Fig. 1A, aliskiren (0.1, 1, 3, and 10 μM) significantly reduced the PV beating rate in a concentration-dependent manner from 2.39 ± 0.45 to

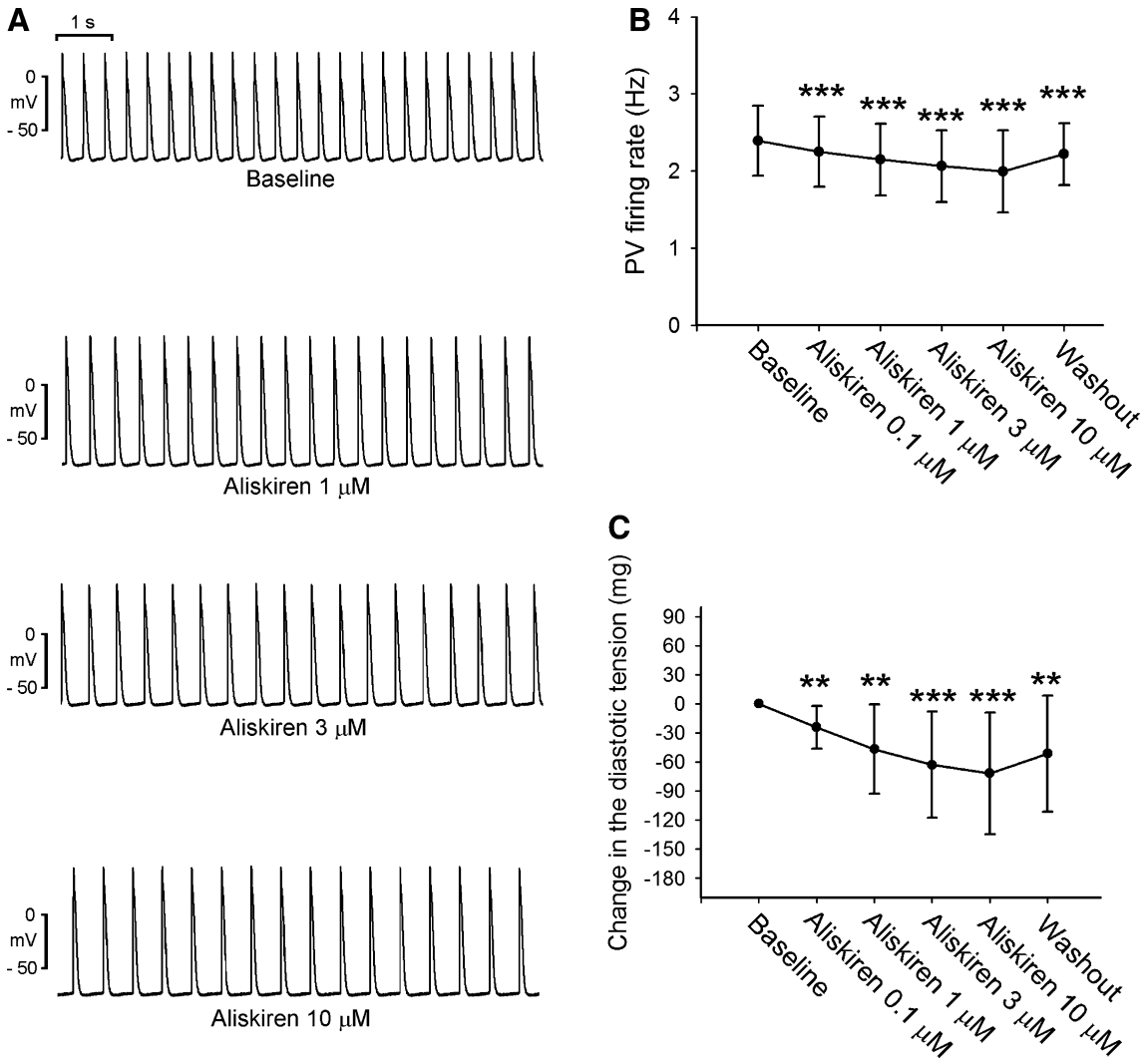


Fig. 1 Effects of aliskiren on the PV spontaneous activity. **a** The tracings show that aliskiren (1, 3, 10 μM) decreased the PV spontaneous activity. **b**, **c** Show the concentration–response of aliskiren's effects on the PV beating rates and diastolic tension ($n = 14$). $^{**}P < 0.01$, $^{***}P < 0.005$ versus before administration of aliskiren

2.25 ± 0.46, 2.15 ± 0.47, 2.06 ± 0.47, and 1.99 ± 0.53 Hz ($n = 14$, $p < 0.05$) in PVs with spontaneous activity (Fig. 1b). Compared with the baseline, aliskiren (0.1, 1, 3, and 10 μM) reduced PV spontaneous activity by 6 ± 1, 10 ± 2, 14 ± 2 and $17 \pm 3\%$, respectively. The effect was partially reversible after washout of aliskiren for 30 min. Aliskiren (0.1, 1, 3, and 10 μM) administration also significantly decreased PV diastolic vascular tension in a concentration-dependent manner (Fig. 1c). Moreover, in the presence of L-NAME (100 μM, $n = 7$), aliskiren (1 and 10 μM) also reduced PV beating rate (6 ± 2 and $10 \pm 3\%$) (Fig. 2a, b), and decreased PV diastolic tension (Fig. 2c). In addition, we also studied the effects of aliskiren on isolated rabbit sinus node and found that aliskiren (10 μM) did not significantly change the automaticity of sinus node (from 2.3 ± 0.2 to 2.3 ± 0.2 Hz, $n = 5$, $p > 0.05$) (Fig. 3).

As shown in the examples in Fig. 4, aliskiren (0.1, 1, 3, and 10 μM) can induce slow and irregular PV spontaneous activity with intermittent long pause in 57, 57, 65, and 72% of 14 rabbit PVs. The irregularity with variable pauses may be caused by exit block in the PV automatic rhythm rather than a direct effect on automatic activity. We recognized PV automaticity exit block through a demonstrated mathematical relationship between the longer and shorter PV activity cycles. Irregularly slow PV spontaneous activity is characterized by repetitive or intermittent failure of an impulse to emerge from the PV automatic focus, whereas repetitive 2:1 or 3:1 exit block (Fig. 4c, d) and intermittent high-grade exit block (Fig. 4e) cause a markedly decreased PV beating rate by aliskiren (0.1, 1, 3, and 10 μM) in 7, 29, 36, and 50% of PVs. Also as noted in Fig. 4c–e, small oscillations of the membrane potential with reference to PV automatic focus activity were noted at rest membrane

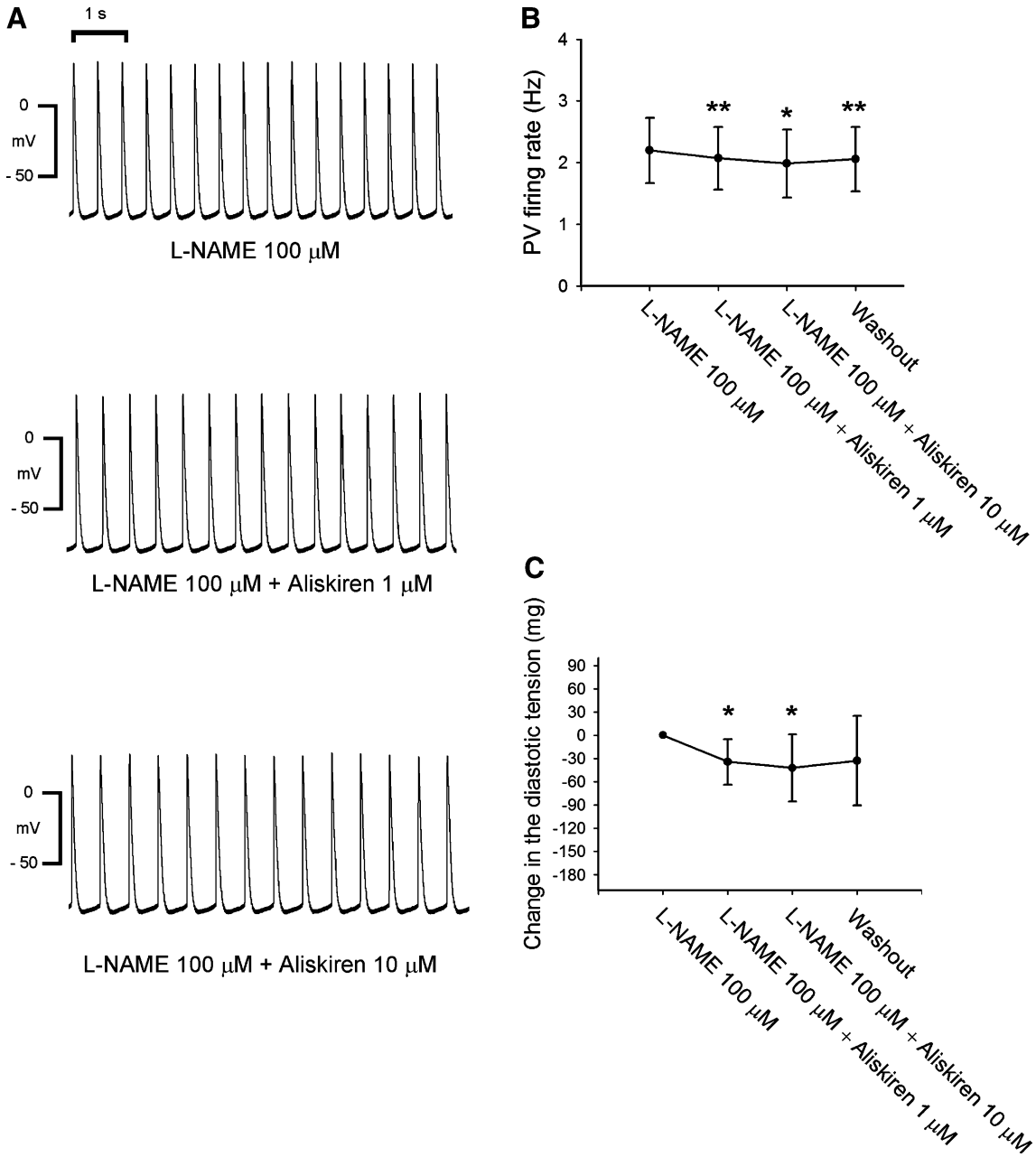


Fig. 2 Effects of aliskiren on the PV spontaneous activity in the presence of L-NAME. **a** The tracings show that aliskiren (1, 10 μM) mildly decreased the PV spontaneous activity in the presence of L-NAME (100 μM). **b**, **c** Show the concentration–response curve of aliskiren's effects on the PV beating rates and diastolic tension in the presence of L-NAME (100 μM) ($n = 7$). $^{*}P < 0.05$, $^{**}P < 0.01$ versus before administration of aliskiren

during PV pause period. In Fig. 4f, we recorded the actual pacemaker site with recordings of typical phase 4 depolarization at a rate of 2.4 Hz before drug administration. Irregular PV beating was observed after administration of 3 μM aliskiren. By moving the microelectrode to the neighboring peri-pacemaker site, we found that irregular PV pulsations were due to PV automaticity exit block presenting as varied periods of long PV pause with repetitive oscillations of the resting membrane potential at a frequency of 2.4 Hz (Fig. 4f). Validity of recordings with stable frequency excluded the possibility of motion artifacts.

Effects of aliskiren on the PV without spontaneous activity and LA

In PVs without spontaneous activity ($n = 10$) or LA tissues ($n = 12$), administration of aliskiren (0.1, 1, 3, and 10 μM)

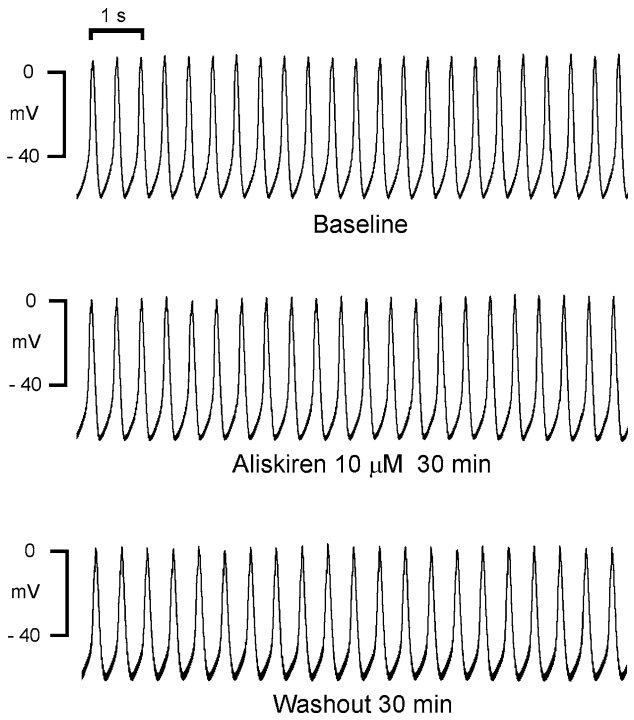


Fig. 3 Effects of aliskiren on the sinus node pacemaker activity. The tracings show that aliskiren (10 μM) did not significantly decrease the sinus node activity

did not significantly change the resting membrane potential, AP amplitude, APD_{50} or APD_{90} (Fig. 5a, b). However, in the two PVs with delayed afterdepolarizations (DADs), aliskiren reduced the amplitude of the DAD by 15 ± 4% (6.7 ± 1.4–5.7 ± 1.4 mV) at 3 μM, and by 32 ± 4% (6.7 ± 1.4–4.5 ± 1.0 mV) at 10 μM. Moreover, aliskiren (0.1, 1, 3, and 10 μM) significantly increased PV and LA contractile force in a concentration-dependent manner (Fig. 6a, b). In order to study the potential mechanism underlying aliskiren-induced myocardial contractility, aliskiren (1 and 10 μM) was administered in LA tissue in the presence of ryanodine (0.1 μM), which can decrease the opening probability of ryanodine receptors. Ryanodine (0.1 μM) markedly decreased atrial contractility, and thereafter administration of aliskiren (1 and 10 μM) did not increase LA contractility in the presence of ryanodine (Fig. 6c).

Effect of aliskiren on calcium homeostasis of LA and PV cardiomyocytes

Figure 7a shows the tracings and I–V relationship of the $I_{Ca\text{-}L}$ before and after 3 μM aliskiren in LA and PV cardiomyocytes, whereas aliskiren significantly decreased the current density of $I_{Ca\text{-}L}$. As compared with baseline, aliskiren (3 μM) decreased the peak $I_{Ca\text{-}L}$ (elicited from −50 to +10 mV) in LA and PV cardiomyocytes by 35 ± 5 and 44 ± 7%, respectively. As shown in Fig. 7b, the effects of aliskiren on $I_{Ca\text{-}L}$ in PV cardiomyocytes were not abolished by pre-treatment with renin, losartan, or angiotensin II. As compared with that before aliskiren administration, aliskiren (3 μM) significantly decreased the peak $I_{Ca\text{-}L}$ (elicited from −50 to +10 mV) in PV cardiomyocytes pretreated with renin (600 U/L), losartan (10 μM), and angiotensin II (100 nM) by 42 ± 6, 48 ± 5, and 46 ± 3%, respectively.

Figure 8a shows the effects of aliskiren on nickel-sensitive NCX currents in LA and PV cardiomyocytes. Aliskiren (3 μM) significantly increased the density of reverse mode but not the forward mode of NCX currents in both cardiomyocytes. As compared with baseline, aliskiren increased the peak reverse mode (elicited from −40 to +100 mV) of nickel-sensitive NCX currents in LA and PV cardiomyocytes by 64 ± 33 and 76 ± 19%, respectively. As shown in Fig. 8b, the effect of aliskiren on NCX in PV cardiomyocytes was not abolished by pre-treatment with renin, losartan, or angiotensin II. As compared with that before aliskiren administration, aliskiren (3 μM) significantly increased the peak reverse mode of nickel-sensitive NCX currents (elicited from −40 to +100 mV) of PV cardiomyocytes pretreated with renin (600 U/L), losartan (10 μM), and angiotensin II (100 nM) by 107 ± 44, 54 ± 20, and 29 ± 5%, respectively. Aliskiren (3 μM) did not significantly change the forward mode of NCX current in the presence of renin, losartan or angiotensin II.

As shown in Fig. 9, aliskiren (3 μM) significantly increased the systolic $[Ca^{2+}]_i$ and the amplitudes of the ratio of the $[Ca^{2+}]_i$ transients by 27 ± 2% in PV cardiomyocytes. However, the diastolic $[Ca^{2+}]_i$ remained unchanged prior to and after the administration of aliskiren (3 μM). The aliskiren-treated PV cardiomyocytes prolonged the decay portion of the $[Ca^{2+}]_i$ transient. Through the rapid infusion of caffeine (20 mM), aliskiren increased the SR Ca^{2+} content measured by integrating the NCX current in PV cardiomyocytes (Fig. 9c). Similarly, aliskiren (3 μM) significantly increased the amplitudes of the ratio of the $[Ca^{2+}]_i$ transients by 16 ± 2% (from 0.20 ± 0.06 to 0.24 ± 0.07, $p < 0.001$) and prolonged the decay portion of the $[Ca^{2+}]_i$ transient (from 23.9 ± 11.6 to 28.2 ± 15.9 ms, $p < 0.05$) in LA myocytes ($n = 8$).

Discussion

Effects of aliskiren on electrical activity of PV cardiomyocytes

This study demonstrated that aliskiren decreased the PV spontaneous beating rates and also reduced the PV resting vascular tension. However, aliskiren had no effect on the

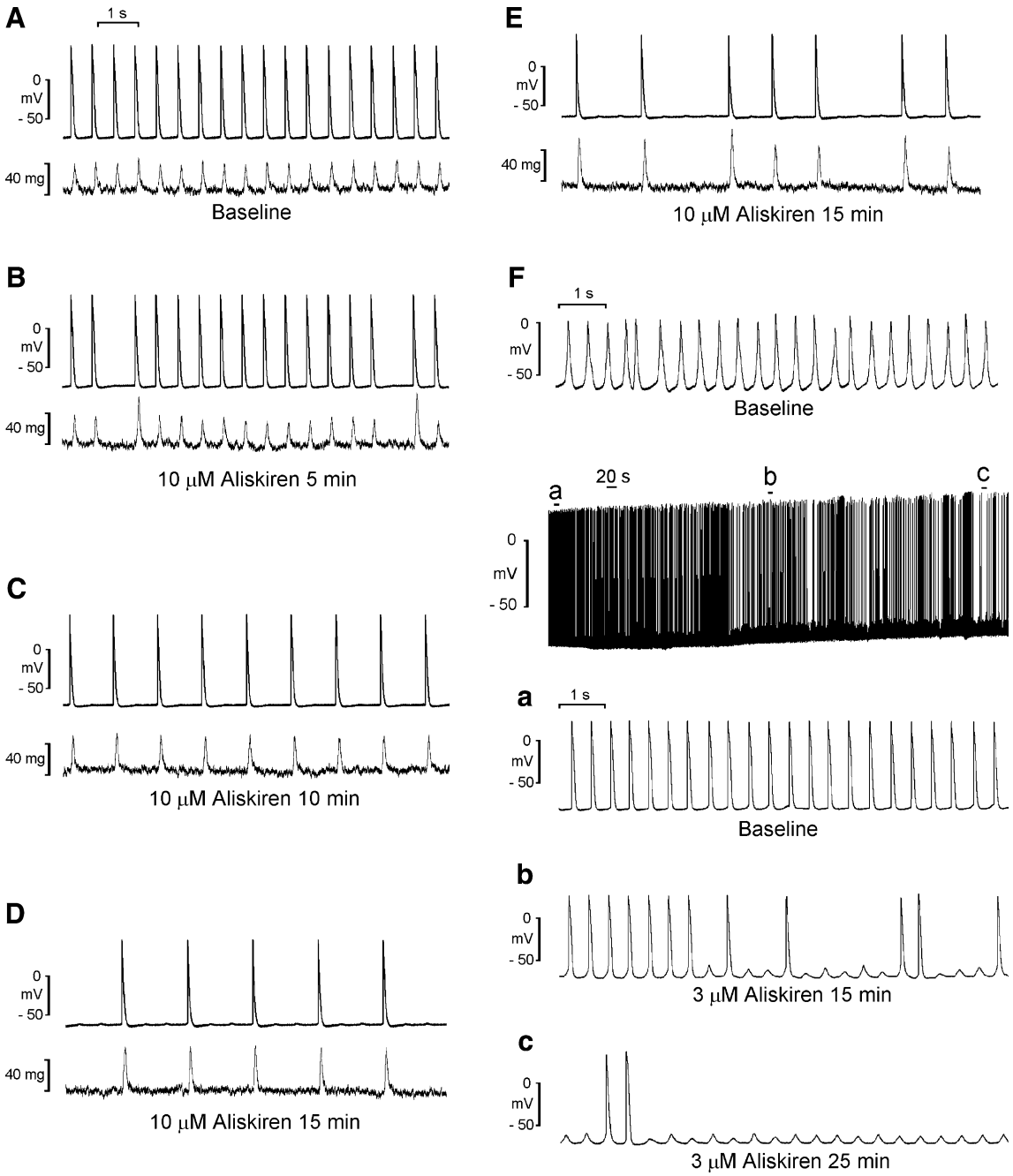


Fig. 4 PV electrical activity and contractile activity with repetitive or intermittent exit block from PV automatic rhythm. **a** Shows the PV activity (2.0 Hz) before aliskiren. **b** Shows intermittent 2:1 PV automatic activity exit block after aliskiren (10 μM). There is a pause in the PV automatic rhythm following the third and fifteenth cycles. In these pauses, the PV electrical and contractile activity interval was twice that of other cycles, indicating 2:1 PV automaticity exit block as depicted. **c**, **d** show the occurrence of repetitive 2:1 and 3:1 PV automaticity exit block, respectively. The pauses seen at **c** and **d** were approximately equal to twice and three times the observed PV firing cycle length in **a**. **e** Shows an intermittent high degree PV automaticity exit block (3:1 block after first cycle and 4:1 block after second and fifth cycle). The frequency (2.0 Hz) of PV firing was similar in **b**–**e**. **f** Shows that PV electrical activity decreased progressively followed by periods of various degrees of pause after the administration of aliskiren (3 μM). Tracings **b** and **c** are expanded section as point indicated and show intermittent and long PV pauses of varied duration. Repetitive oscillations of the membrane potential (transient diastolic membrane depolarizations) that fail to reach a threshold voltage for phase 0 depolarization were recorded at the same frequency (2.4 Hz) during these pauses

sinus node pacemaker activity and the AP characteristics of PV cardiomyocytes. It has been reported that the rising atrial pressure and the subsequent stretch of the atrium and PVs increase the PV arrhythmogenesis and vulnerability to induce AF [4, 24, 35]. Our previous study also showed that statins reduce the PV arrhythmogenesis by reducing PV

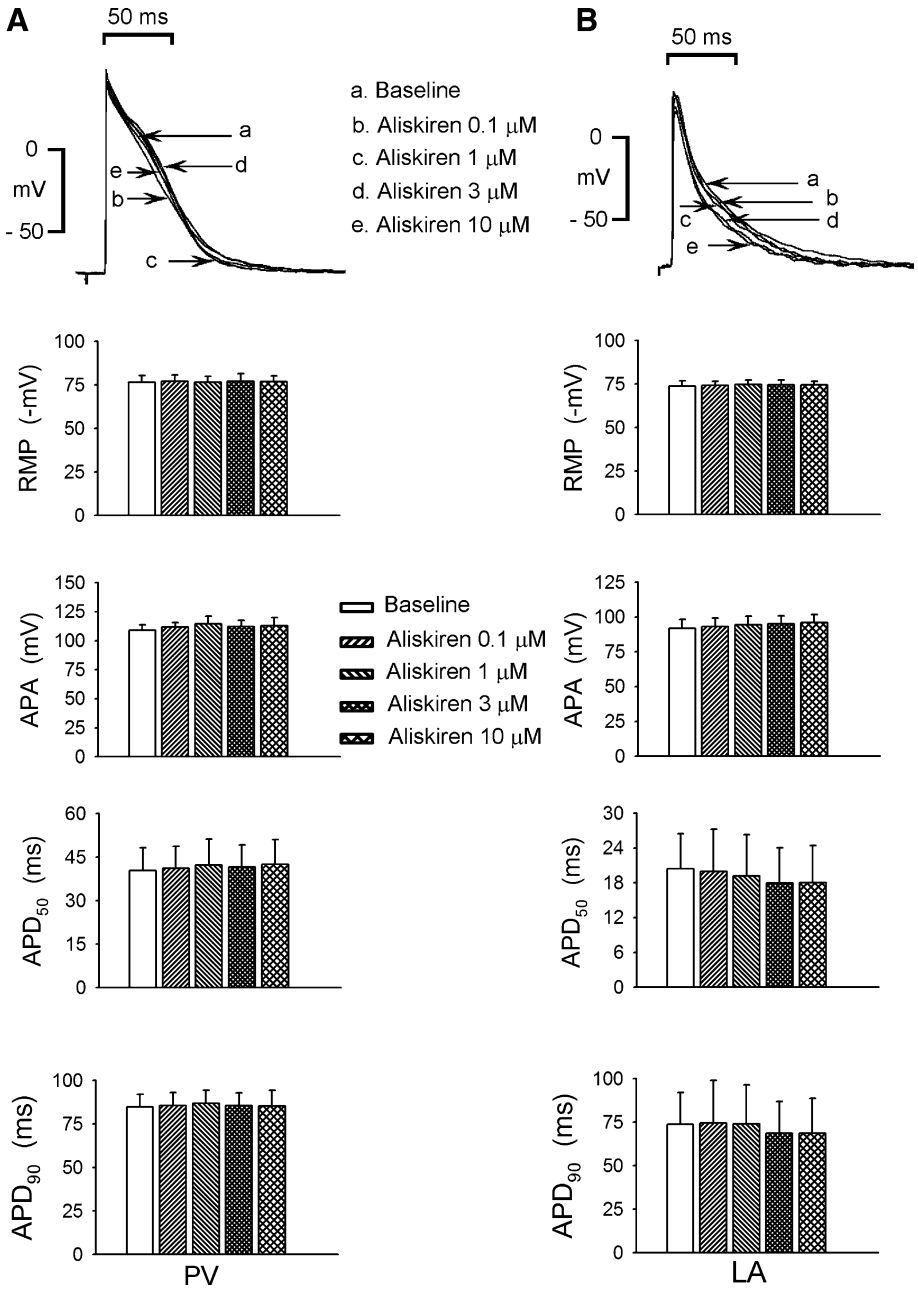


Fig. 5 Effects of aliskiren on the action potential in left atrium and PVs without spontaneous activity. **a** Superimposed tracings of the AP configuration and average data of the AP parameters in PVs ($n = 10$) before and after administration of different concentrations (0.1, 1, 3, 10 μM) of aliskiren. **b** Superimposed tracings of AP configuration and average data of the AP parameters in LA ($n = 12$) before and after administration of different concentrations (0.1, 1, 3, 10 μM) of aliskiren

vascular diastolic tension through nitric oxide pathway [22]. Recent studies have implied the beneficial hemodynamic effects of aliskiren by exerting a vasodilatory effect and/or increasing nitric oxide bioavailability [13, 23]. Therefore, aliskiren could relax the PV vascular tension to relieve vessel stretch, leading to a decrease in PV beating rates. However, pre-treatment with L-NAME (nitric oxide synthase inhibitor) did not completely suppress the effects of aliskiren on the PV electrical activity and vascular tension. This suggested that an alternative mechanism, other than nitric oxide pathways, may contribute to the vasodilatory effect of aliskiren. Aliskiren at a concentration of 3 μM can decrease the $I_{Ca\text{-}L}$ in PV cardiomyocytes. It seems reasonable to assume that decreased $I_{Ca\text{-}L}$ in vascular smooth myocytes may contribute to the vasodilatory effect of aliskiren.

Our results demonstrate that aliskiren may induce PV automaticity exit block and cause slow irregular PV electrical activity. Nevertheless, the exact mechanism how aliskiren could interfere with spontaneous PV activity is unclear. The recordings from the pacemaker and peripacemaker sites add further insight into the basis for the conduction block rather than a direct effect on automatic activity. Previous studies based on histological and high-density mapping experiments have shown that highly anisotropic and heterogeneous PV architecture could lead

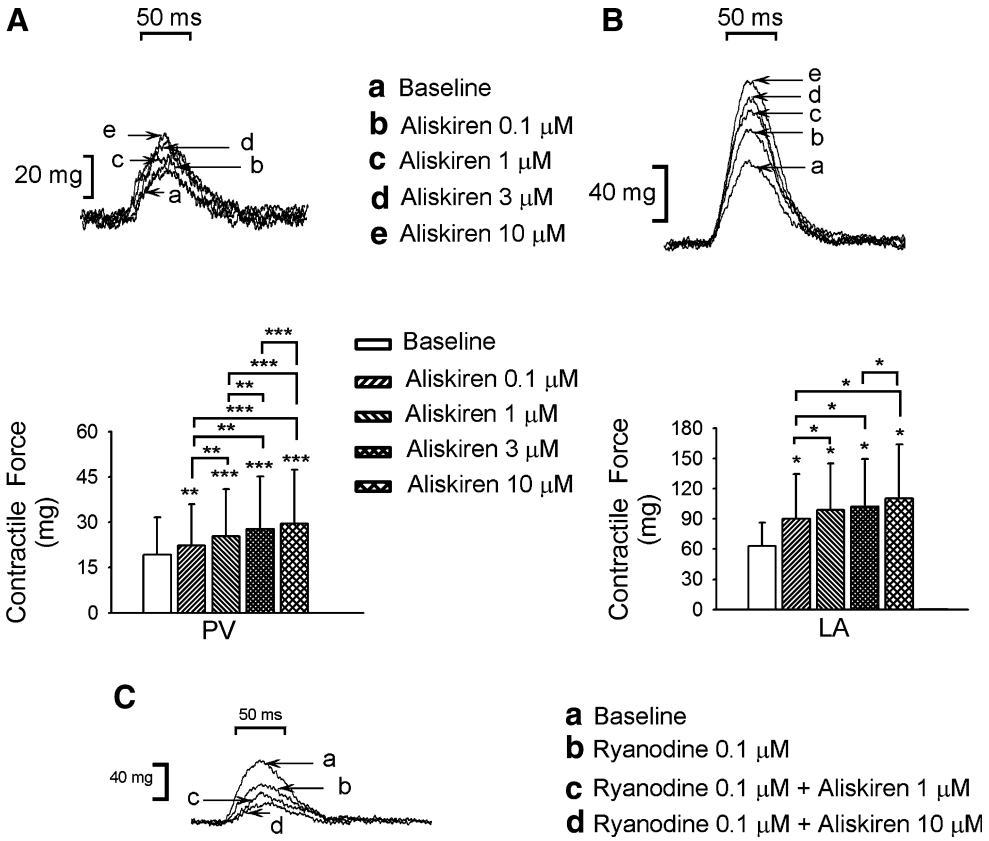


Fig. 6 Effects of aliskiren on the myocardial contractility in left atrium and PVs without spontaneous activity. **a** Superimposed tracings and average data of the contractile force in PVs ($n = 10$) before and after administration of aliskiren (0.1, 1, 3, 10 μM). **b** Superimposed tracings and average data of the contractile force in LA ($n = 12$) before and after administration of aliskiren (0.1, 1, 3, 10 μM). **c** Superimposed tracings of the LA contractility after pretreatment with ryanodine (0.1 μM) and aliskiren (1 and 10 μM). $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.005$ versus before administration of aliskiren

to intra-PV discontinuous propagation [1]. This study further delineated the functional status of PV automaticity propagating to exit block and their contribution to the irregular PV activity modulated by aliskiren. Structural and functional similarity to the peripheral sinoatrial node, PV automaticity exit block, appears to prevail [2, 42]. Fedorof et al. [17] recently identified narrow and discrete sinoatrial exit pathways in the canine sinoatrial node. Conduction failure in these sinoatrial exit pathways leads to sinoatrial node exit block. The exit block mechanism is invariably attributable to source-sink mismatch when excitation passes through the narrow exit pathway. Histologically, the PV myocardial sleeve is separated from the smooth muscle of pulmonary vein by a plane of fibro-fatty tissues [33]. Kohl et al. [26] proposed that mechanosensitive cardiac fibroblasts could depolarize upon stretch, which may contribute to the positive chronotropic response of coupled cardiomyocytes to stretch. Therefore, it may be speculative that mechanosensitive cardiac fibroblasts also participate in the PV automaticity exit block during aliskiren administration. The mechanism of exit block may also involve a short-term change in the properties of atrial tissue near the exit pathways under certain conditions. As shown in Fig. 4f, repetitive oscillations of the membrane potential that failed to reach a threshold voltage for triggering an action potential were recorded during the pauses. Those transient diastolic membrane depolarizations may arise from short-term electrophysiological changes of myocardial cells near the exit site after administration of aliskiren.

Effects of aliskiren on the intracellular calcium handling and contractility of cardiomyocytes

This study showed for the first time that aliskiren could significantly increase PV and LA contractility. We also demonstrated that aliskiren significantly increased systolic Ca^{2+} transient in the PV and LA cardiomyocytes. As expected, the increased contractile strength is consistently indicative of underlying increased cytosolic Ca^{2+} transient. Moreover, this inotropic effect can be abolished by ryanodine pretreatment, which suggests that enhanced SR Ca^{2+} release through ryanodine receptor plays a major role in aliskiren-induced positive inotropy. The gating of ryanodine receptors is sensitive to cytosolic Ca^{2+} concentration. Our study on the sarcolemmal Ca^{2+} flux showed that aliskiren decreased $I_{Ca\text{-}L}$ currents but increased reverse-mode NCX currents. The decrease in Ca^{2+} entry via $I_{Ca\text{-}L}$ is likely due to increased Ca^{2+}-dependent inactivation of the $I_{Ca\text{-}L}$ as a result of the larger systolic Ca^{2+} transient. Recent work on NCX knockout mice proves the idea that Ca^{2+} entry via reverse-mode NCX contributes to Ca^{2+}-induced Ca^{2+} release by acting synergistically with the opening of

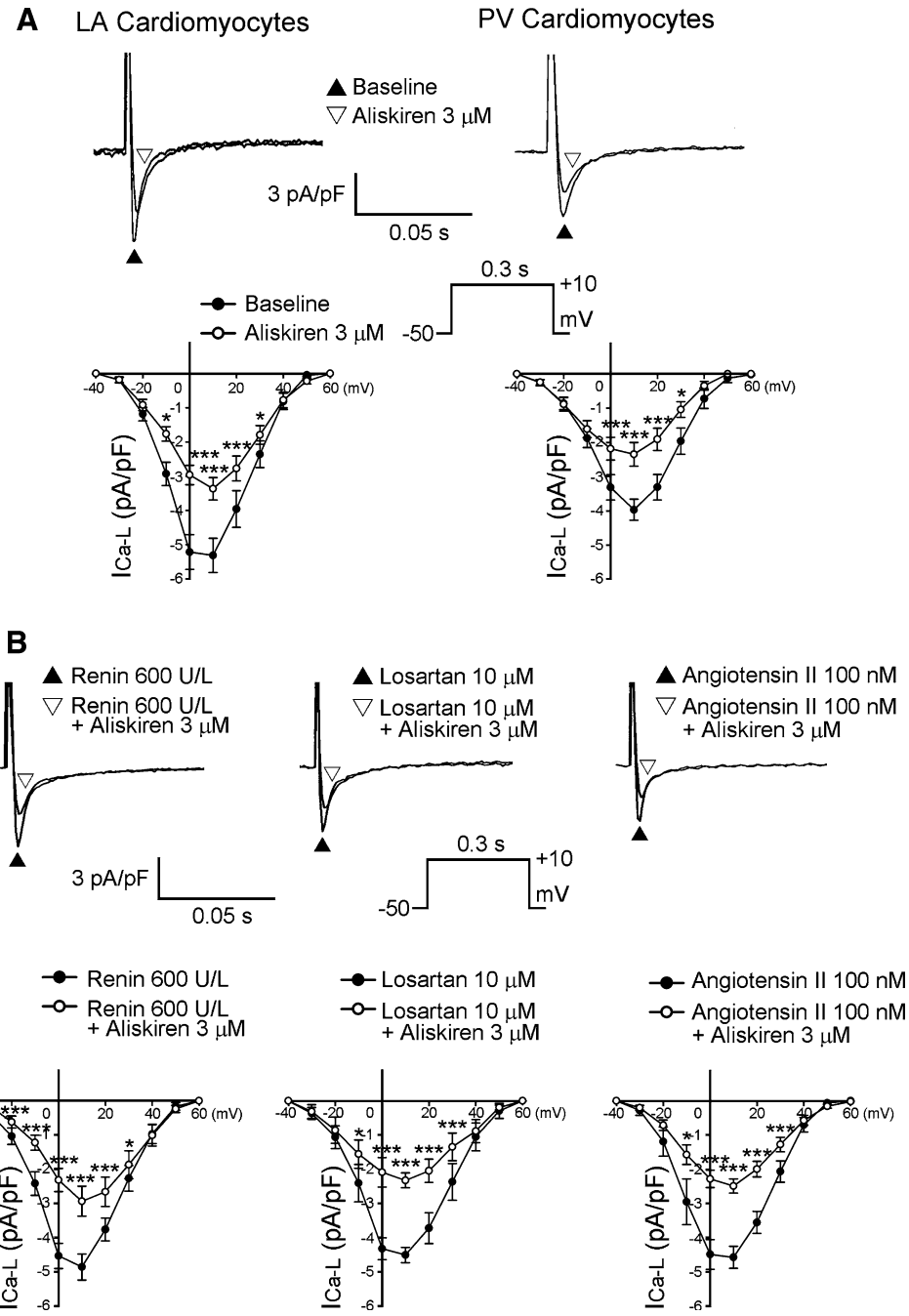
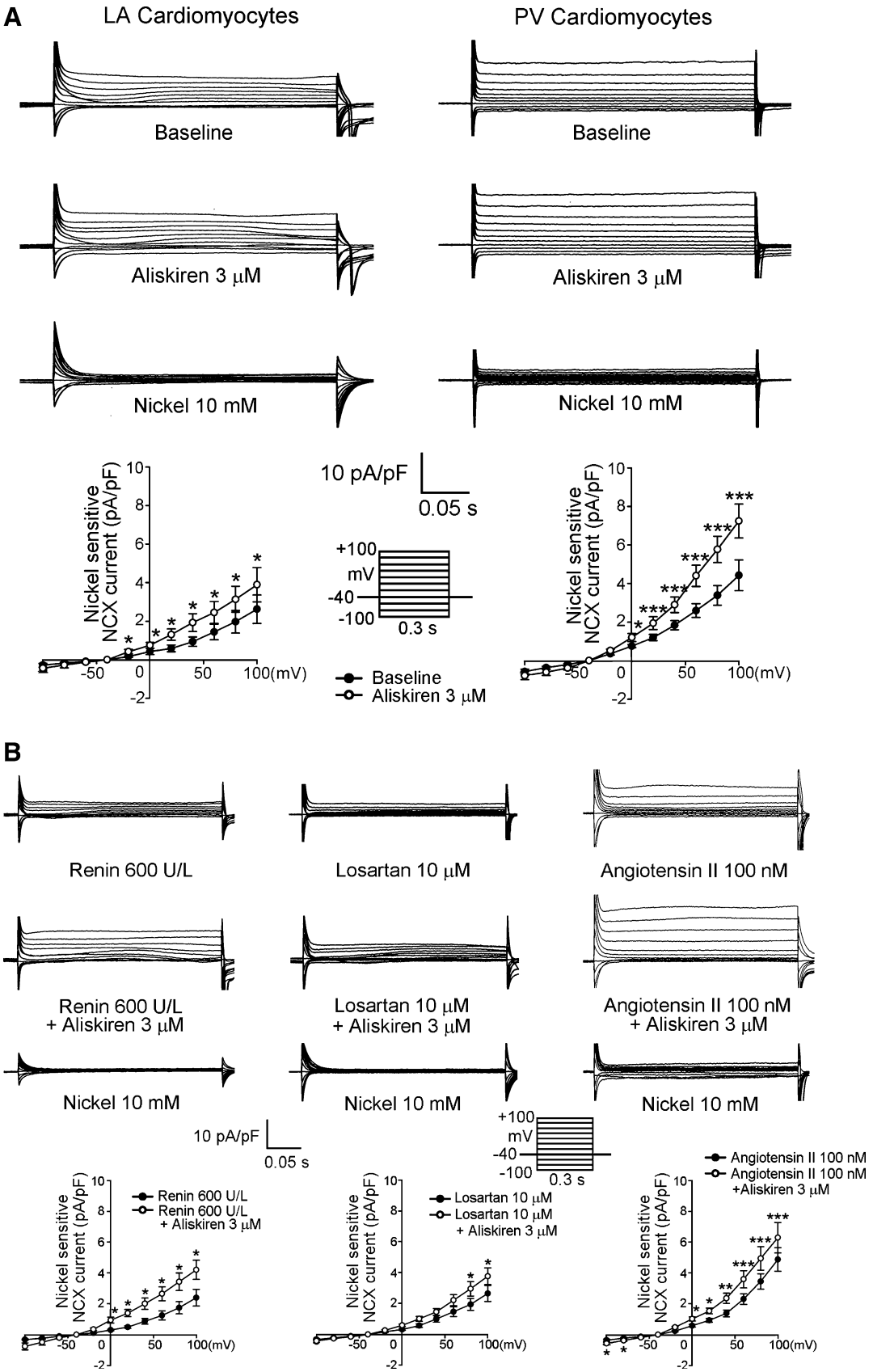


Fig. 7 Effect of aliskiren on the cardiomyocyte L-type calcium currents ($I_{Ca\text{-}L}$). **a** The examples of current traces and the I–V relationship of $I_{Ca\text{-}L}$ before and after administration of aliskiren (3 μM) in the LA ($n = 11$) and PV ($n = 14$) cardiomyocytes. **b** The examples of current traces and the I–V relationship of $I_{Ca\text{-}L}$ before and after administration of aliskiren (3 μM) in the PV cardiomyocytes pretreatment with renin (600 U/L) (*left panel*, $n = 8$) or losartan (10 μM) (*middle panel*, $n = 8$) or angiotensin II (100 nM) (*right panel*, $n = 8$). $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.005$ versus before administration of aliskiren

$I_{Ca\text{-}L}$ to trigger Ca^{2+} release from the SR [27, 28, 37]. Therefore, it is possible that reverse-mode NCX can activate Ca^{2+} release from the SR and increase contraction. It is plausible that aliskiren could directly modulate the property of the ryanodine receptor to enhance its open probability as an alternative mechanism. In atrial myocytes, it had been demonstrated that inositol-3-phosphate receptor (IP_3R)-dependent Ca^{2+} signaling facilitates SR Ca^{2+} release through ryanodine receptor release clusters

Fig. 8 Effect of aliskiren on cardiomyocytes Na^{+}–Ca^{2+} exchanger (*NCX*) currents. **a** The examples of current traces and the I–V relationship of nickel-sensitive NCX before and after administration of aliskiren (3 μM) in the LA ($n = 6$) and PV ($n = 7$) cardiomyocytes. **b** The examples of current traces and the I–V relationship of nickel sensitive NCX before and after administration of aliskiren (3 μM) in the PV cardiomyocytes pretreatment with renin (600 U/L) (*left panel*, $n = 6$) or losartan (10 μM) (*middle panel*, $n = 5$) or angiotensin II (100 nM) (*right panel*, $n = 7$). $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.005$ versus before administration of aliskiren



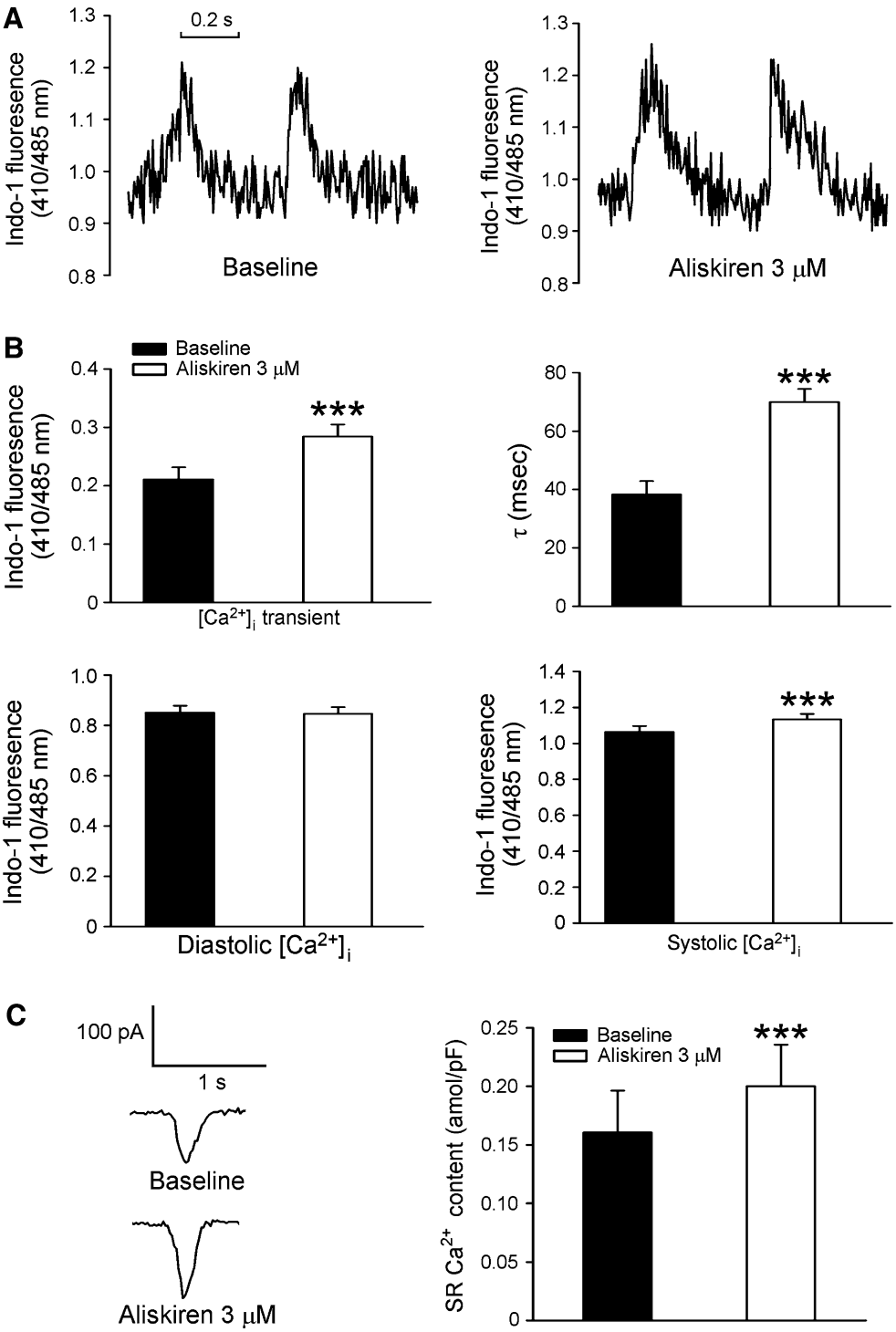


Fig. 9 Effect of aliskiren on the intracellular calcium concentration ($[Ca^{2+}]_i$) and sarcoplasmic reticulum (SR) Ca^{2+} content in PV cardiomyocytes. **a**, **b** show the tracings and average data ($n = 11$) of the $[Ca^{2+}]_i$ transient before and after administration of aliskiren (3 μM) in the PV cardiomyocytes. **c** 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 aliskiren (3 μM) in PV cardiomyocytes. $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.005$ versus before administration of aliskiren

and enhances the Ca^{2+} transient during excitation–contraction coupling [44]. Nonetheless, it remains unclear whether the effect of aliskiren on $[Ca^{2+}]_i$ is elicited through the possible protein kinase C or IP_3 signaling pathways [25, 44]. We also demonstrated aliskiren increased SR Ca^{2+} content in parallel with an increased $[Ca^{2+}]_i$. It has been reported that overexpression of the NCX in transgenic mice results in an activation of contraction and an increased SR Ca^{2+} load [40]. An increase of SR Ca^{2+} content increases the amplitude of the systolic Ca^{2+} transient. Our finding of an increase of SR Ca^{2+} content after aliskiren administration strongly suggests that the larger systolic Ca^{2+} transient is caused by an increased SR Ca^{2+} content. Therefore, a causative mechanism of the inotropic effect of aliskiren on atrial myocardium is likely due to an elevated SR Ca^{2+} load.

Additionally, the effects of aliskiren on contractility in cardiomyocytes may contribute to its known beneficial effects on cardiac function in clinical trials [30, 38].

The present results showed that the administration of aliskiren to the extracellular fluid decreases the $I_{Ca\text{-}L}$ density and increases reverse-mode NCX in PV cardiomyocytes, an effect independent of the activity of renin–angiotensin system. De Mello demonstrated that renin increased peak $I_{Ca\text{-}L}$ in a cardiomyopathic model, an effect dependent on angiotensin II formation [15]. In the present study, the effects of aliskiren on $I_{Ca\text{-}L}$ and NCX were not abolished in cells exposed to renin, losartan, and angiotensin II, indicating that this effect might be independent of renin or angiotensin II action. Furthermore, these findings were consistent with the idea that aliskiren has no effect on renin action of (pro)renin receptor-mediated and angiotensin II-independent intracellular signal cascade [18, 34].

Recent clinical studies showed that the renin–angiotensin system blockers may be useful for reducing AF incidence, particularly in patients with left ventricular hypertrophy or failure [16, 36]. Our study demonstrated that aliskiren could modulate the PV and atrial electrical activity, suggesting that aliskiren may have a potential benefit in treating patients with AF. However, without in vivo experiments, this study has not yet proved that these cellular phenomena caused by aliskiren truly contribute to its possible therapeutic effects on atrial arrhythmias. The effects in our study are found in the micromolar range, whereas aliskiren inhibits renin with an IC_{50} of 0.6 nM [3]. Thus, it is not too likely that these effects play a predominant role in vivo. Furthermore, inconsistent clinical results (e.g. GISSI-AF trial) clearly raised the issue that the renin–angiotensin system blockade may not offer the same benefit in all patients [41]. The therapeutic benefits of renin–angiotensin system blockers on preventing AF remain inconclusive pending further reports.

Conclusions

Aliskiren can modulate PV arrhythmogeneity through multiple mechanisms comprising possible mechanoelectrical feedback, and also induce PV automaticity exit block and long pause. Aliskiren may have therapeutic potential to reduce the risk of AF. Aliskiren alters sarcolemmal calcium flux independent of renin and angiotensin II action, and it increases atrial myocardial contractility by modulating intracellular calcium homeostasis with an increase of systolic Ca^{2+} transient and SR Ca^{2+} content. The increase in myocardial contractility with vasodilatory effects also implies that aliskiren will have potential therapeutic application in heart failure. These new effects of aliskirin are only seen on supra-therapeutic (μM range) concentrations, while the therapeutic renin–antagonistic effects of aliskiren are found in the nM range.

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Conflict of interest None to be declared.

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國科會補助計畫衍生研發成果推廣資料表

日期:2011/07/29

國科會補助計畫	計畫名稱: 腎素抑制劑Aliskiren調控肺靜脈和心房心肌細胞內鈣離子流及細胞電氣生理特性作用之研究
	計畫主持人: 蔡青峰
	計畫編號: 99-2314-B-040-017- 學門領域: 心胸內科

無研發成果推廣資料

99 年度專題研究計畫研究成果彙整表

計畫主持人：蔡青峰	計畫編號：99-2314-B-040-017-
計畫名稱：腎素抑制劑 Aliskiren 調控肺靜脈和心房心肌細胞內鈣離子流及細胞電氣生理特性作用之研究	

成果項目			量化			單位	備註（質化說明：如數個計畫共同成果、成果列為該期刊之封面故事...等）
			實際已達成數（被接受或已發表）	預期總達成數(含實際已達成數)	本計畫實際貢獻百分比		
國內	論文著作	期刊論文	0	0	100%	篇	
		研究報告/技術報告	0	0	100%		
		研討會論文	0	0	100%		
		專書	0	0	100%		
	專利	申請中件數	0	0	100%	件	
		已獲得件數	0	0	100%		
	技術移轉	件數	0	0	100%	件	
		權利金	0	0	100%	千元	
	參與計畫人力（本國籍）	碩士生	1	1	100%	人次	
		博士生	0	0	100%		
		博士後研究員	0	0	100%		
		專任助理	1	1	100%		
國外	論文著作	期刊論文	1	1	100%	篇	
		研究報告/技術報告	0	0	100%		
		研討會論文	1	1	100%		
		專書	0	0	100%	章/本	
	專利	申請中件數	0	0	100%	件	
		已獲得件數	0	0	100%		
	技術移轉	件數	0	0	100%	件	
		權利金	0	0	100%	千元	
	參與計畫人力（外國籍）	碩士生	0	0	100%	人次	
		博士生	0	0	100%		
		博士後研究員	0	0	100%		
		專任助理	0	0	100%		

其他成果 (無法以量化表達之成果如辦理學術活動、獲得獎項、重要國際合作、研究成果國際影響力及其他協助產業技術發展之具體效益事項等，請以文字敘述填列。)	無

	成果項目	量化	名稱或內容性質簡述
科教處計畫加填項目	測驗工具(含質性與量性)	0	
	課程/模組	0	
	電腦及網路系統或工具	0	
	教材	0	
	舉辦之活動/競賽	0	
	研討會/工作坊	0	
	電子報、網站	0	
	計畫成果推廣之參與（閱聽）人數	0	

國科會補助專題研究計畫成果報告自評表

請就研究內容與原計畫相符程度、達成預期目標情況、研究成果之學術或應用價值（簡要敘述成果所代表之意義、價值、影響或進一步發展之可能性）、是否適合在學術期刊發表或申請專利、主要發現或其他有關價值等，作一綜合評估。

1. 請就研究內容與原計畫相符程度、達成預期目標情況作一綜合評估

■達成目標

□未達成目標（請說明，以 100 字為限）

□實驗失敗

□因故實驗中斷

□其他原因

說明：

2. 研究成果在學術期刊發表或申請專利等情形：

論文：■已發表 □未發表之文稿 □撰寫中 □無

專利：□已獲得 □申請中 ■無

技轉：□已技轉 □洽談中 ■無

其他：(以 100 字為限)

3. 請依學術成就、技術創新、社會影響等方面，評估研究成果之學術或應用價值（簡要敘述成果所代表之意義、價值、影響或進一步發展之可能性）(以 500 字為限)

1. 研究結果有助於了解心房顫動的致病機轉，以及肺靜脈在心律不整所扮演的角色，可以擴展對心血管的細胞生理學的知識。

2. 可有助於開發新的治療心衰竭藥物，或新的治療方法。

3. 了解心衰竭鈣離子調控之機轉。

4. 可以擴展對 ryanodine 接受器、鈣離子亮點、鈣離子調控與電生理特性之關聯性心血管的細胞生理學的知識。

5. 可以了解新型高血壓藥物對於 ryanodine 接受器、鈣離子調控、鈣離子亮點、電生理特性以及離子流的知識。