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Research Article **· Open Access ·**

Effects of allocryptopine on outward potassium current and slow delayed rectifier potassium current in rabbit myocardium

Yi-Cheng FU, Yu ZHANG, Liu-Yang TIAN, Nan LI, Xi CHEN, Zhong-Qi CAI, Chao ZHU, Yang LI

Division of Cardiology, Chinese PLA General Hospital, Beijing, China

Abstract

Objective Allocryptopine (ALL) is an effective alkaloid of Corydalis decumbens (Thunb.) Pers. Papaveraceae and has proved to be anti-arrhythmic. The purpose of our study is to investigate the effects of ALL on transmural repolarizing ionic ingredients of outward potassium current (I_{to}) and slow delayed rectifier potassium current (I_{Ks}). **Methods** The monophasic action potential (MAP) technique was used to record the MAP duration of the epicardium (Epi), myocardium (M) and endocardium (Endo) of the rabbit heart and the whole cell patch clamp was used to record I_{to} and I_{Ks} in cardiomyocytes of Epi, M and Endo layers that were isolated from rabbit ventricles. **Results** The effects of ALL on MAP of Epi, M and Endo layers were disequilibrium. ALL could effectively reduce the transmural dispersion of repolarization (TDR) in rabbit transmural ventricular wall. ALL decreased the current densities of I_{to} and I_{Ks} in a voltage and concentration dependent way and narrowed the repolarizing differences among three layers. The analysis of gating kinetics showed ALL accelerated the channel activation of I_{to} in M layers and partly inhibit the channel openings of I_{to} in Epi, M and Endo cells. On the other hand, ALL mainly slowed channel deactivation of I_{Ks} channel in Epi and Endo layers without affecting its activation. **Conclusions** Our study gives partially explanation about the mechanisms of transmural inhibition of I_{to} and I_{Ks} channels by ALL in rabbit myocardium. These findings provide novel perspective regarding the anti-arrhythmogenesis application of ALL in clinical settings.

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1 Introduction

The enhancement of heterogeneity of ventricular action potential duration (APD) is thought to form the substrate of reentry arrhythmias in many pathologic conditions.[1] On the cellular level, it is well established that ventricular myocardium is comprised of at least three electrophysiologically and functionally distinct cell types: epicardium (Epi), mid-myocardium (M cells) and endocardium (Endo).[2] The typical electrophysiological change in the failing heart is the enhancement of transmural dispersion of repolarization (TDR) which usually manifests as the rising disparity between QT intervals of myocardium on the electrocardiogram. In isolated ventricular myocytes, Endo cells display longer action potential duration than Epi cells, and M cells show significant rate-dependency characteristics that their APDs are much longer when heart rate is slow. Besides, Epi and Endo layers were found to repolarize before the M cells, due to the significant population of M cells in the ventricular wall.[3] These transmural electrical gradients form the anatomic basis of electrical modeling of the heart, thus may provide as new targets for agents to treat cardiac arrhythmias in a diseased heart.[4]

The ionic mechanism of ventricular repolarization heterogeneity mainly differ with respect to phase 1 and phase 3 repolarization by the regional differences of certain repolarizing channels.[5] The relatively larger transient outward potassium currents (I_{to}) give phase 1 of APD of Epi and M layers a spike and dome shaped configuration that already been found in human and animal ventricular myocytes.[6] The rectifier outward potassium current I_{Kr} (rapid) and I_{Ks} (slow) play a dominant role in the phase 3 of repolarization. M Cells with reduced I_{Ks} usually display long APD and steep dependence of APD on rate whereas I_{Kr} densities were similar among three layers,[7] I_{Ks} blockade contributes importantly to drug-induced long QT syndrome,[8] and serves as an important compensator of when repolarization reserve

Correspondence to: Yang LI, MD, PhD, Division of Cardiology, Chinese PLA General Hospital, 28 Fuxing Road, Haidian District, Beijing 100853, China. E-mail: liyangbsh@163.com

Telephone: +86-10-66936762 **Fax:** +86-10-66936762

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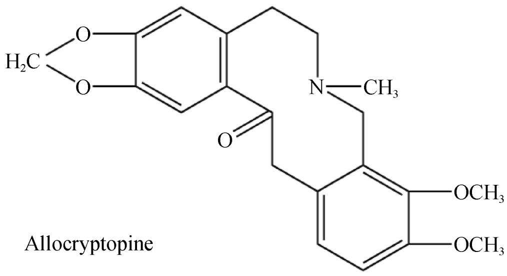
is reduced by pathogenic factors.[9] Thus, exploring the relationships between and intrinsic repolarization characteristics and electronic influences of cardiovascular drugs in ventricular myocardium is central to our understanding of pharmacotherapeutics.

Alpha-allocryptopine (ALL) is a derivative of tetrahydropalmatine, which is extracted from the Corydalis decumbens (Thunb.) Pers. Papaveraceae (Figure 1).[10] It is speculated to have potential anti-arrhythmic effects due to its isoquinoline structure which has already been found in many anti-arrhythmic drugs. Previous studies have demonstrated that ALL could restore myocardial electrophysiological characteristics by blocking certain ionic channel components. ALL has been proved to inhibit Nav1.5 and hERG channels in HEK-293 cells,[11] and suppressed delayed after depolarization (DAD)- and early after depolarization (EAD)- related triggered arrhythmia by reducing transient inward current (I_{ti}) and L-type calcium current ($I_{Ca,L}$) in mouse ventricular myocytes.[12] However, limited information can be available on what mechanism of ALL to modulate other repolarizing currents such as I_{to} and I_{Ks} in cardiac myocardium. Therefore, our study will examine the relative contribution of ALL to these repolarization components and focus on its electrophysiological effects, aim to provide comprehensive evidence of use of ALL for prevent electrophysiological abnormalities.



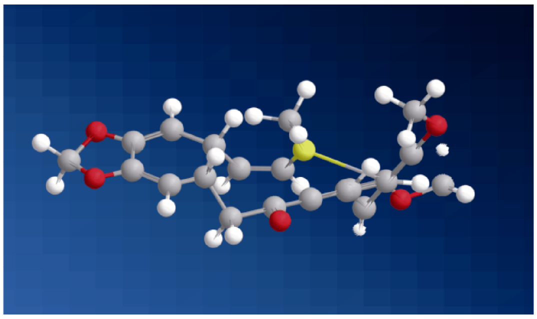


Figure 1. The chemical and three-dimensional molecular structure of allocryptopine.

2 Methods

2.1 Animals, reagents and solutions

All experimental procedures and protocols were approved by the Animal Experimental Committee of Chinese PLA General Hospital and conformed to the ‘Guide for the Care and Use of Laboratory Animals’ published by the National Institutes of Health (publication No.85–23, revised 1996). Twelve Male adult rabbits weighing 2.0–2.5 kg were provided by the Experimental Animal Center of the PLA general Hospital (Certificate No. 2011B106).

The allcryptopine (molecular weight 365, melting point 168°C, a white crystal powder, purity > 99.0%) was supplied by the Pharmaceutical Department of Lanzhou University (Lanzhou, China) and dissolved with Ca^{2+}-free Tyrode solution at concentrations of 30 µmol/L, according to previous study.[13] Bovine serum albumin, type I collagenase, protease E, taurine, K-aspartate, L-glutamic acid, N-methyl-Dglutamine, Ethylene Glycol Tetraacetic acid (EGTA), 4-(2-hydroxy ethyl)-1-piperazineethanesulfonic (HEPES) were purchased from Sigma Chemical (Sigma-Aldrich Biochemical Co. St Louis, USA). NaCl, KCl, $MgCl_2$, NaH_2PO_4, $MgCl_2$, Na-pyruvate, K_2ATP, egtazic acid, glucose were purchased from Beijing Chemical Reagent Co. (Beijing, China).

The solutions used for myocytes preparations were confected as follows: Ca^{2+} free Tyrode’s solution (mmol/L): NaCl 137, KCl 5.4, $MgCl_2$ 1.0, NaH_2PO_4 0.33, HEPES 10, and glucose 10 (pH 7.35, adjusted with NaOH).

The Krebs buffer (KB) solution for cell storage (mmol/L): KCl 40, KH_2PO_4 20, $MgCl_2$ 3.0, KOH 70, L-glutamic acid 50, HEPES 10, taurine 20, glucose 10, and EGTA 0.5 (pH 7.35, adjusted with KOH).

Cell dissociation solution: 0.33 mg/mL type I collagenase, 0.025 mg/mL protease E and 1.25 mg/mL bovine serum albumin melted in 10 mL Ca^{2+} free Tyrode’s solution.

For the I_{Ks} recordings, the pipette internal solution contained (mmol/L): K-aspartate 85, KCl 45, Na-pyruvate 5, K_2ATP 3, $MgCl_2$ 4, egtazic acid 10, HEPES 10, glucose 11 (pH 7.3, adjusted with KOH). The extracellular solution for I_{Ks} currents measurements contained (mmol/L): N-methyl-Dglutamine 149, $MgCl_2$ 5, HEPES 5 (pH 7.4, adjusted with HCl). 1 µmol/L dofetilide was used to block I_{Kr} and 2 mmol/L 4-aminopyridine (4-AP) was used to block I_{to} during current recording.

For the I_{to} recordings, the pipette solution contained (mmol/L): K aspartate 85, KCl 45, Na pyruvate 5, K_2ATP 3, $MgCl_2$ 4, egtazic acid 10, HEPES 10 and glucose 11 and was adjusted to pH 7.4 by KOH. The extracellular solution for I_{to} currents measurements contained (mmol/L): NaCl 30, choline chloride 110, KCl 5.4, $MgCl_2$ 1.0, NaH_2PO_4 0.33, HEPES 10, glucose 10 and CdCl 0.3, adjusted to pH 7.35 with NaOH. 1 µmol/L dofetilide was used to block I_{Kr}, 10 µmol/L

Chromanol 293B was used to block I_{Ks} and 50 μmol/L $BaCl_2$ was used to block I_{K1} during current recording.

2.2 Monophasic action potential duration measurements

Rabbits were anesthetized by sodium pentobarbital (30 mg/kg) intravenously and delivered with heparin (1000 U/kg, *i.v.*). The heart were rapidly excised after thoracotomy and mounted on Langendorff retrograde perfusion apparatus. The heart was perfused with warm Tyrode's solution (gassed with 100% O_2 at 37 °C) at a pressure of 70 cm H_2O. The coronary perfusion pressure was adjusted to 50 mmHg. For MAP recordings, a reference silver electrode was placed on the aortic root and the contact Ag-AgCl electrode was positioned on the epicardium close to the septum on the anterior wall. The MAP signals were amplified and simultaneously recorded from the Epi, M and Endo layers with an RM-6240 Biological Signal Acquisition System (Taimeng Technology Co., Ltd., Chengdu, China). Isolated rabbit hearts were subjected to retrograde aortic perfusion of ALL solution (30 μmol/L) followed by rapid washing out with modified Tyrode's solution when the contraction curve recovered to baseline. The infusion velocity was 0.05 mL/s. The stimulation electrodes were inserted in the atrial appendage of the right atrium. The pacing protocols were provided by RM-6240 system. The time constant was maintained at 2 min and APD were obtained at the frequency range of 4.5–7.5 Hz. The main parameters analyzed were the monophasic action potential durations (MAPD) at 90% repolarizations ($MAPD_{90}$), and TDR as the difference between the longest and the shortest APD_{90} of the three layers at the same point.

2.3 Cell preparation

Rabbits were anesthetized with sodium pentobarbital (30 mg/kg), and killed by cervical dislocation. Heart was quickly moved to Langendorff perfusion apparatus and perfused with Tyrode's solution which contained (mmol/L) NaCl 137, KCl 5.4, $MgCl_2$ 1.0, NaH_2PO_4 0.33, HEPES 10, and glucose 10 for 37 °C 4 min, and equilibrated with 100% O_2 continuously. The Ca^{2+} free Tyrode's solution was immediately perfused for the next 5 min when blood was totally washed out of the coronary arteries. Then the cell dissociation solution which contained 0.33 mg/mL type I collagenase, 0.025 mg/mL protease E and 1.25 mg/mL bovine serum albumin was melted in Ca^{2+} free Tyrode's solution and the perfusion speed of Langendorff apparatus was adjusted to 50 drops per min. Myocardial cells were isolated by enzyme digestion with retrograde perfusion for 25 min, the temperature was maintained at 37 °C. The tissue slices of Epi, M and Endo of the left ventricular free wall were dissected with surgical blade and were put in three separate culture dishes. The tissue was then minced and blew gently in 37 °C KB solution which contained KCl 40, KH_2PO_4 20, $MgCl_2$ 3.0, KOH 70, L-glutamic acid 50, HEPES 10, taurine 20, glucose 10, and EGTA 0.5 in order to obtain single ventricular myocytes of each myocardium. The supernatant was collected every 5 min and filtered by nylon mesh. Cells from three layers were collected and stored in normal KB solution at 4 °C for later use.

2.4 Electrophysiologic recording

The Recordings were performed at room temperature. Single cells were transferred to 30 mm Pet3ri dish mounted on the stage of an inverted microscope. The cells were perfused at 2 mL/min with Ca^{2+} free Tyrode's solution. The quiescent, rod shaped ventricular myocytes that had smooth surface and clearly cross striations were selected for electrophysiological measurements. The current was measured using the whole-cell technique and Multi-Clamp 700B amplifier (Axon Instruments Inc. Foster City, USA). Micropipettes (resistance = 3–5 MΩ) were made by pp-830 puller (Narishige International Inc., Tokyo, Japan) from capillary tubes and had resistance of 1–3 MΩ. The average junction potential was limited to 5 mV. The current signal was filtered at 3 kHz, through a 16 bit A/D digital converter Digi-data 1322A (Axon Instruments Inc., Foster City, USA) and filtered at 3 kHz. Trace acquisition and analysis were performed by pClamp 9.2 software (Axon Instruments Inc. Foster City, USA). A routine series resistance compensation was performed for value of > 80% and the uncompensated Rseries was < 2 MΩ. The membrane capacitance was measured on each of the cells and was compensated by 80%–90% of their initial value and calculated using the manual whole-cell capacitance controls on the Axopatch amplifier (Axon Instruments Inc. Foster City, USA). Trace acquisition and analysis were performed by pClamp 9.2 software (Axon Instruments Inc. Foster City, USA)

2.5 Statistical analysis

The data were expressed as the mean ± SD and *n* represents the number of cells. pCLAMP version 9.2 (Axon Instruments) and Origin (Microcal Software) were used for the data analysis. Continuous variables from two groups were compared by Student's *t*-test. One-way analysis of variance (ANOVA) was used when comparing multiple groups, and the significance between any two groups was evaluated by ANOVA followed by a Student–Newman–Keuls (S–N–K) post-hoc test. All data were analyzed using SPSS 19.0 (IBM Co., USA). Statistical significance was considered to be $P < 0.05$.

3 Results

3.1 Effects of ALL on MAPs in Langendorff-perfused rabbit hearts

After perfused with 30 μmol/L ALL, the phase 3 of MAPs in the Epi, M and Endo cells were all prolonged (Figure 2A and B) and the $MAPD_{90}$ of Epi, M and Endo layers were respectively increased from 180.5 ± 7.0 ms, 186.0 ± 12.0 ms and 157.2 ± 9.0 ms to 207.0 ± 8.2 ms, 216.1 ± 10.0 ms and 200 ± 8.2 ms compared to the Ctrl group ($P < 0.05$, $n = 12$ per group as shown in Figure 2C). The TDR of three layers were significantly decreased, from 29.5 ± 3.4 ms to 16.2 ± 5.8 ms ($P < 0.01$, $n = 12$ per group), as shown in Figure 2D.

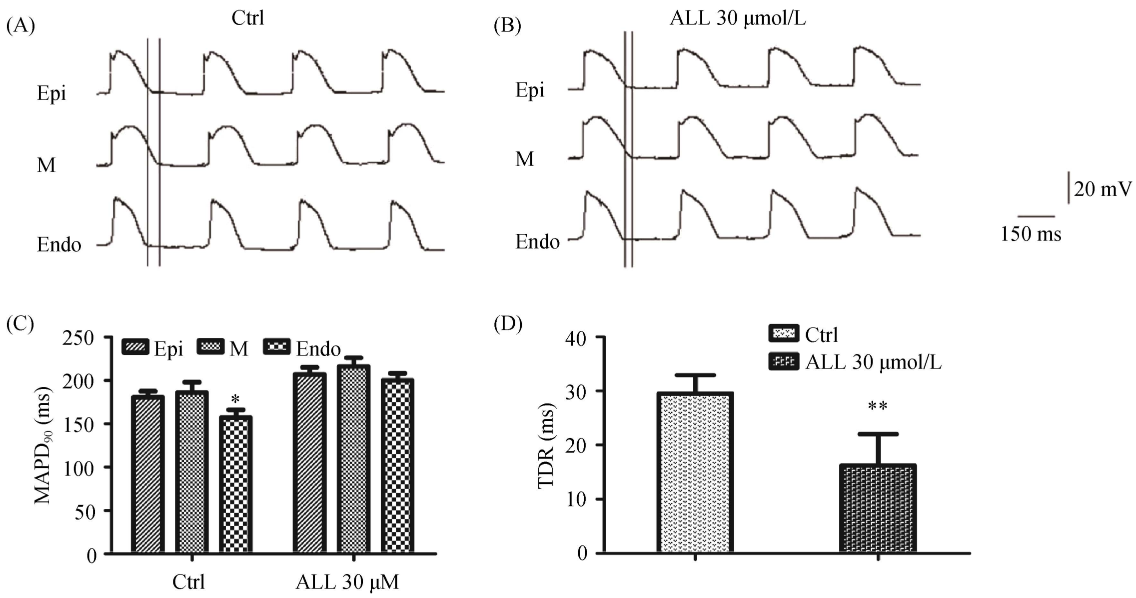


Figure 2. The effect of ALL on monophasic action potential morphology (A & B), $MAPD_{90}$ (C), and TDR (D) of rabbit heart. Vertical lines through symbols indicate the standard error. *$P < 0.05$ compared with the M layer; **$P < 0.01$ compared with the Ctrl group. ALL: allocryptopine; Ctrl: control group; Endo: endocardium; Epi: epicardium; M: midcardium; MAPD: monophasic action potential duration; TDR: transmural dispersion of repolarization.

3.2 Effects of ALL on APs in rabbit myocytes

Under the current clamp circumstance, the action potentials (APs) of Epi, M and Endo layers were elicited by applying 1500 pA with 5 ms duration stimuli at frequency of 1.0 Hz. Figure 3A shows the changes of APs in rabbit cardiomyocytes after intervention with 30 μmol/L ALL. As observed, ALL could effectively prolong the APD_{90} of all myocardial layers; the extent of AP prolongation in the Epi and Endo layers was relatively longer than that of M cells. The concentration response relationship curve of the effects of ALL on rabbit cardiomyocytes were shown in Figure 3B. The fraction of the maximum inhibition was calculated after exposure to various concentrations of ALL. The results suggested that ALL enhanced the APs in a concentration-dependent manner. The half maximum effective concentration value (EC_{50}) was 24.3 μmol/L, and the Hill coefficient was 1.04. The APD_{90} of Epi, M and Endo layers, which were measured at 90% of repolarization, increased from 233.0 ± 11.0 ms, 253.2 ± 16.5 ms, 207.1 ± 10.2 ms to 260.0 ± 12.5 ms, 274.4 ± 12.8 ms and 258.1 ± 8.2 ms, respectively after adding drugs ($P < 0.01$, $n = 12$, as shown in the Figure 3C). The intrinsic transmural heterogeneity from Epi to Endo was significantly improved by the pharmacological effects of ALL, which made the TDR decrease from 46.2 ± 7.0 ms to 22.2 ± 4.8 ms ($P < 0.01$), as shown in Figure 3D.

3.3 Transmural gradient block effects of ALL on I_{to} currents

I_{to} was elicited by 300 ms step depolarizing pulses from a holding potential of –80 mV to a testing potential of –40 to +70 mV with increases of 10 mV and a conditioning test of –40 mV for 50 ms to eliminate the sodium current. Before administering 30 μmol/L ALL, the properties of the I_{to} current of the Epi, M and Endo layers were distinct from each other in a normal rabbit heart, and the peak current amplitude of I_{to} in the M layers was the highest, then the Epi layers, and then the Endo layers with the smallest amplitude, as shown in Figure 4A. As a result, the current amplitudes of the three layers were all decreased, with the I_{to} current of M cells descending most

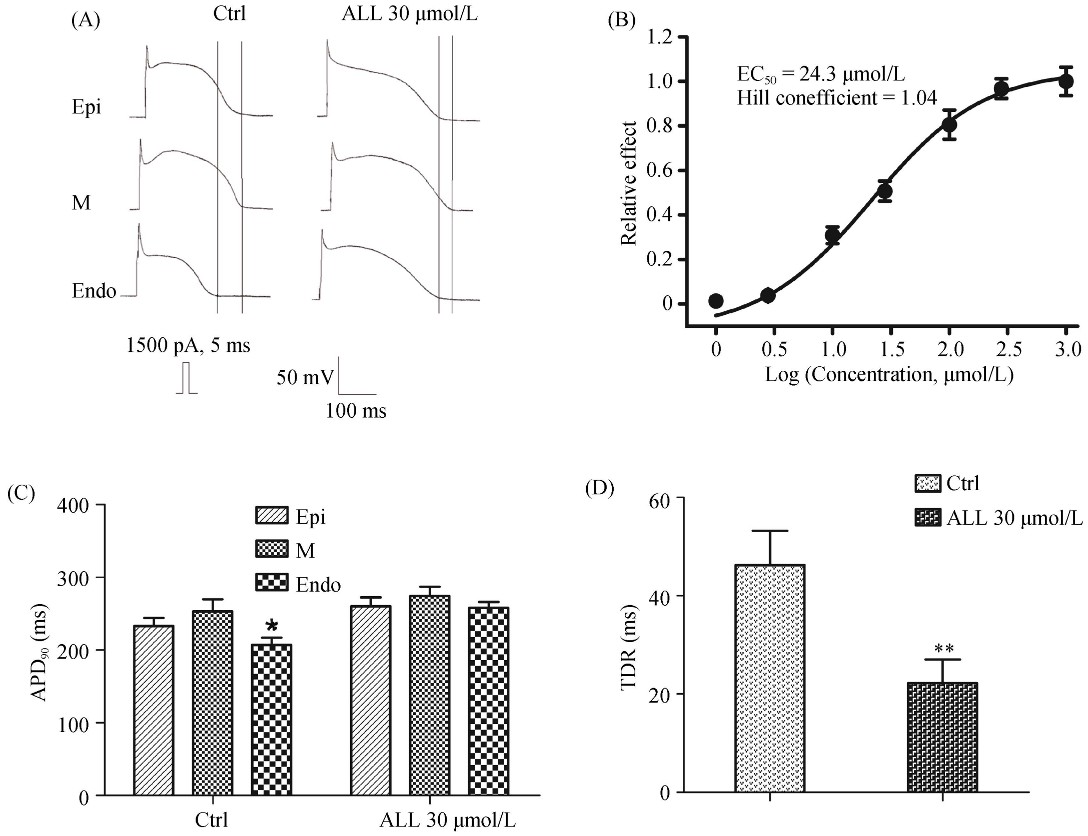


Figure 3. The effect of ALL on action potentials (A & B), APD_{90} (c), and TDR (D) of rabbit cardiomyocytes. **P* < 0.05 compared to M cells; ***P* < 0.01 compared to Control group. ALL: allocryptopine; APD_{90}: monophasic action potential duration measured at 90% of repolarization. Ctrl: control group; EC_{50}: half maximum effective concentration value; Endo: endocardium; Epi: epicardium; M: midcardium; TDR: the transmural dispersion of repolarization.

quickly and falling to just over half, which reduced the differences of Ito among three layers and could partly contribute to the TDR reduction that previously described. Figure 4B showed the concentration-response relationship of ALL on Ito currents. The results suggested the effects of ALL on Ito currents of Epi, M and Endo layers were concentration-dependent. The half maximal inhibitory concentration (IC_{50}) of ALL was 18.6 μmol/L, and the Hill conefficient was 1.21. At the depolarized pulse of +50 mV, the peak current densities of I_{to} in Epi, M and Endo cells were respectively reduced from 23.3 ± 2.4 pA/pF to 15.9 ± 1.5 pA/pF, 35.3 ± 2.6 pA/pF to 15.9 ± 1.5 pA/pF, and 17.5 ± 0.9 pA/pF to 11.3 ± 0.8 pA/pF with the administration of 30 μmol/L ALL ($P < 0.01$, n =10 per group), as shown in Figure 4C. The results indicated that ALL could effectively alter the voltage dependence of I_{to} channels in rabbit cardiomyocytes, especially in the M cells.

3.4 Transmural block effect of ALL on gating kinetics of I_{to} channels

The steady-state activation kinetics of I_{to} in Epi, M and Endo layers were recorded by using the same protocol when determining the current-voltage relationships and were evaluated in the presence of 30 μmol/L ALL. The activation data of I_{to} were best fit by the Boltzmann equation: $G/Gmax = 1/(1 + \exp[(V_m - V_{1/2})/k]$ ($V_{1/2}$: half activation voltage, k: the activation curve slope) and were shown in Figure 5A. Because of the inhibition effect of ALL, the activation curve of I_{to} in M layers was significantly shifted to the right, whereas the activation curves of I_{to} in the Epi and Endo layers were slightly changed. The $V_{1/2,act}$ of I_{to} of M cells was remarkably reduced from –62.18 ± 6.52 mV to –32.57 ± 3.26 mV ($P < 0.05$, n = 11). The changes in the $V_{1/2,act}$ value of I_{to} in the Epi and Endo layers were not obvious with ALL, which were reduced from –65.47 ± 3.52 mV and –65.56 ± 6.52 mV to –62.57 ± 5.26 mV and –62.45 ± 8.26 mV, respectively ($P > 0.05$, n = 11). The voltage dependence of the I_{to} inactivation was determined with a protocol of 1000 ms conditioning pulses between –70 and +20 mV, followed by a test pulse of +50 mV for 300 ms. The mean data of inactivation were best fit by the Boltzmann equation: $I/I_{max} = 1/(1+\exp[(V_{1/2} - V_m)/k])$. The inactivation curves of three layers were mildly affected after the interven-

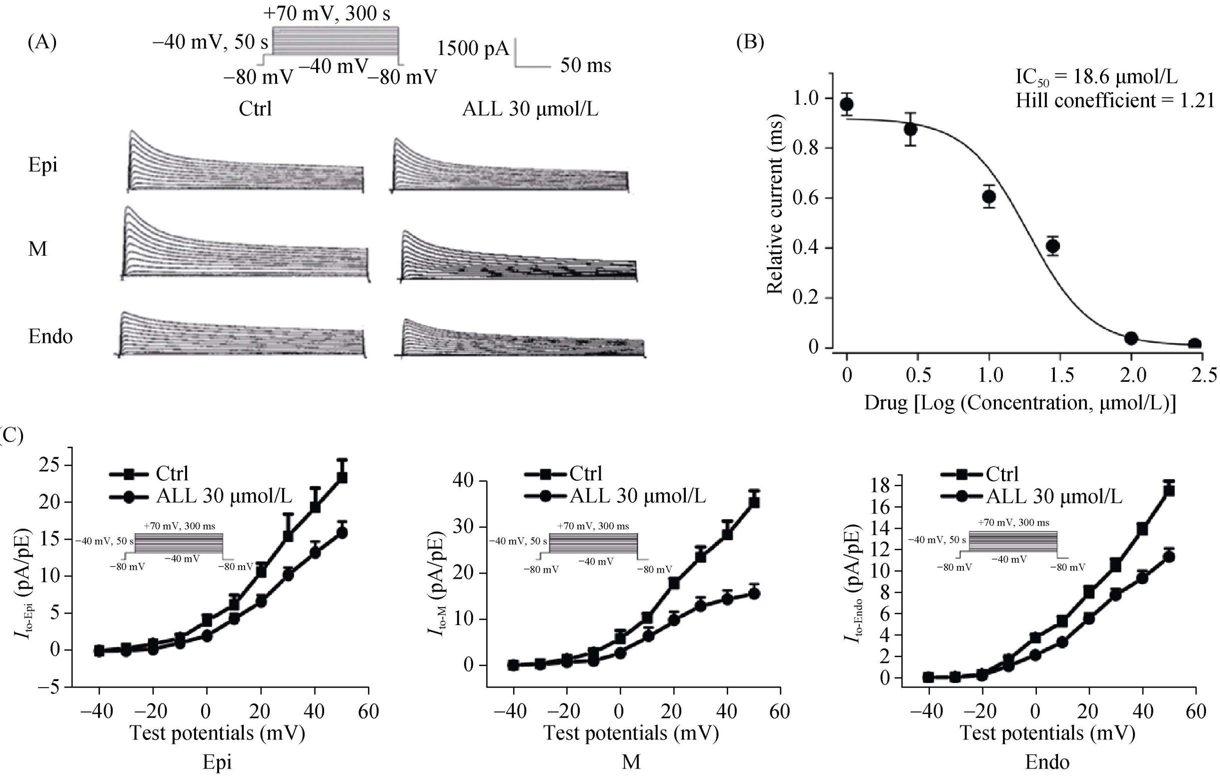


Figure 4. Effects of ALL on I_{to} currents (A), concentration dependency (B), and I-V relationships in Epi, M and Endo cells (C). $^{**}P < 0.01$, $n = 10$. ALL: allocryptopine; Ctrl: control group; Endo: endocardium; Epi: epicardium; I_{to} : transient outward potassium current; IC_{50}: half maximal inhibitory concentration value; M: midcardium.

tion of the 30 μmol/L ALL, as shown in Figure 5B. Both results indicated that ALL minimized the I_{to} current densities of different myocardial layers through lessening its channel activation kinetics.

The voltage-dependence of the time course of recovery from inactivation of I_{to} was evaluated with a paired-pulse protocol. Holding the potential at –80 mV, pre-stimulation was given –40 mV, 20 ms, and a conditioning pulse was applied at +40 mV for 1000 ms from the holding potential of –80 mV, following test potentials of +40 mV for 300 ms during different time intervals of 20 ms, 40 ms, 80 ms, 160 ms, 320 ms, 640 ms, 960 ms, 1280 ms and 2560 ms. The time course of recovery from fast inactivation was fitted by a single-exponential function. The results are shown in Figure 5C. After administration of 30 μmol/L ALL, the recovery curve from inactivation of I_{to} in the M layers was significantly decreased ($P < 0.05$, $n = 13$), but the drug had no effect on the other two layers. The time constant of the closed-state inactivation of I_{to} was induced by following a depolarization pulse of +50 mV for 300 ms, returning to –100 mV, and depolarizing to –70 mV during different time pluses of 10 ms, 20 ms, 50 ms, 100 ms, 200 ms, 500 ms, 1000 ms, 2000 ms, 2500 ms, 4000 ms and 5000 ms. Before ALL administration, the closed-state inactivation of I_{to} in the Epi, M and Endo layers was relatively small; there was approximately 90% channel opening until the time pulses of 5000 ms. After administration of 30 μmol/L ALL, the closed-state inactivation velocities of I_{to} in the three layers were all significantly increased, and the closed-state time constants of I_{to} were also shortened, which resulted in the reduction of channel opening in each myocardial layer. The I_{to} channel openings of three layers were reduced to 57%, 40% and 85%, respectively; the M and Epi layers were remarkably affected by ALL ($P < 0.01$, $n = 12$), as shown in Figure 5D.

3.5 Transmural gradient block effects of ALL on I_{Ks} currents

I_{Ks} and $I_{Ks,tail}$ were recorded by applying various voltage pulses ranging from –120 mV to +80 mV for both 4000 ms and 3000 ms from the holding potential of –40 mV. Figure 6A shows the current traces of I_{Ks} before and after the intervention of 30 μmol/L ALL. The intrinsic I_{Ks} current of M cells was relatively small compared to that of Epi and Endo cells. After exposure to ALL, the I_{Ks} currents of the three layers were all decreased, especially in the Epi and Endo cells. The minor blockade effect of ALL on I_{Ks} was suggested to be one of the main reasons for APD prolongation of M cells. Besides, the effects of ALL on I_{Ks} currents of three layers were concentration dependent. The half maximal inhibitory concentration (IC_{50}) of ALL was 28.8 μmol/L, and the Hill

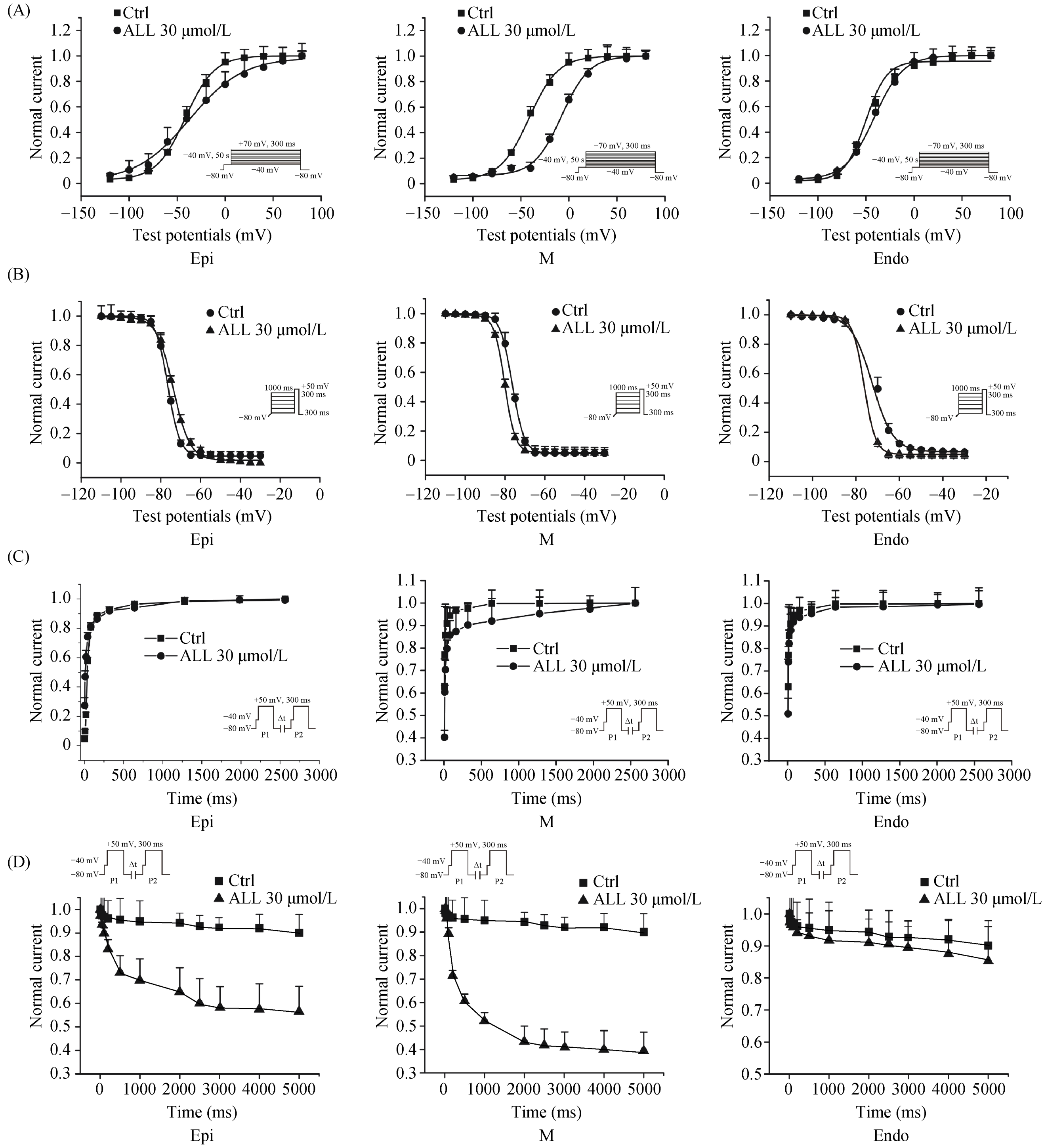


Figure 5. Effect of ALL on gating kinetics of I_{to} in Epi, M and Endo cells of rabbit. (A): The steady-state activation; (B): the steady-state inactivation; (C): the time course of recovery; (D): the closed-state inactivation. ALL: allocryptopine; Ctrl: control group; Endo: endocardium; Epi: epicardium; I_{to} : transient outward potassium current; M: midcardium.

conefficient was 0.97, as shown in Figure 6B. The current-voltage relationships of I_{Ks} in three myocardial cells are shown in Figure 6C. At a depolarizing pulse of +80 mV, the tail current density of I_{Ks} in Epi, M and Endo cells were, respectively, decreased from 12.3 ± 0.7 pA/pF, 5.8 ± 0.9 pA/pF, and 10.3 ± 0.5 pA/pF to 4.9 ± 0.3 pA/pF, 4.2 ± 0.7 pA/pF and 4.3 ± 0.4 pA/pF after exposure to 30 μmol/L ALL ($P < 0.01$, $n = 9$). The inhibition effects of ALL in the three myocardium layers were voltage dependent but did not change the outward rectifier characteristics of I_{Ks} channels.

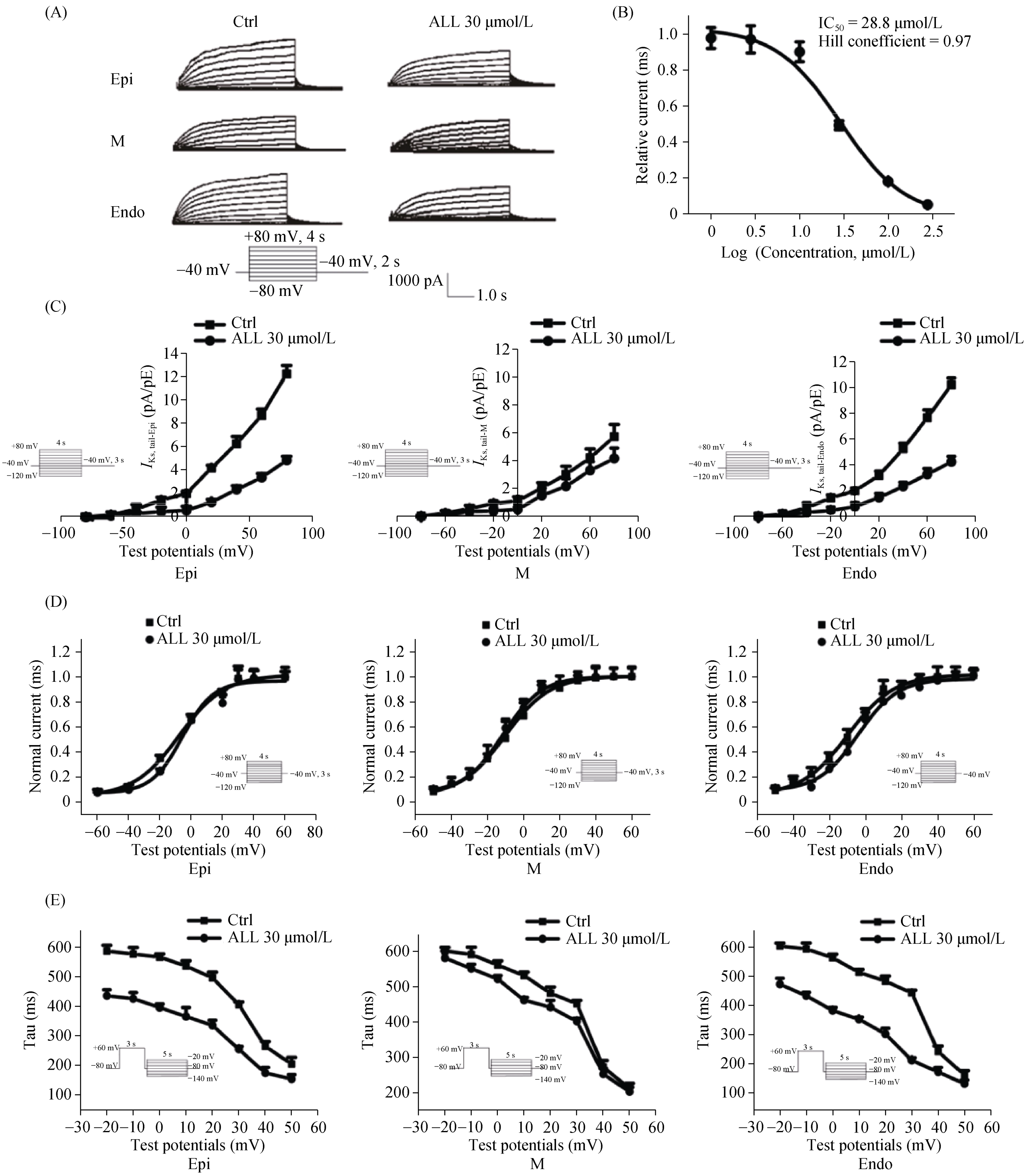


Figure 6. Effect of ALL on concentration dependency, I-V relationshops and gating kinetics of I_{Ks} currents in Epi, M and Endo cells of rabbit. (A): The I_{Ks} current; (B): the concentration dependency; (C): the I-V relationship; (D): the steady-state activation; (E): the deactivation kinetics. ALL: allocryptopine; Ctrl: control group; Endo: endocardium; Epi: epicardium; IC_{50}: the half maximal inhibitory concentration value; I_{Ks}: slow delayed rectifier potassium current; M: midcardium.

3.6 Transmural block effect of ALL on gating kinetics of I_{Ks} channels

The cardiomyocytes were depolarized to potentials in the range of −120 mV to +80 mV for 4000 ms, and the tail current was recorded at –40 mV for 3000 ms. The steady-state activation of I_{Ks} was fitted to the Boltzmann equation: G/G_{max}

= 1/(1 + exp[(V_m – $V_{1/2}$)/*k*]). As shown in Figure 6D, 30 μM/L ALL did not affect the activation kinetics of I_{Ks} in Epi, M and Endo layers either before or after drug intervention. The half-inactivated voltages ($V_{1/2,inact}$) and the activated curve slopes (*k)* of the activation curves of three layers had barely changed (P > 0.05, data not shown).

At a holding potential of –40 mV, the deactivation kinetics of I_{Ks} in the three types of cells were determined by providing pre-stimulation of +60 mV for 3 s and applying various voltage pulses that ranged from –140 mV to –20 mV in 20 mV increments for 5 s. The deactivation procedure of the I_{Ks} were fitted with a bi-exponential equation [$I = A_1exp(-t/\tau_1) + A_2exp(- t/\tau_2) + C$]. As shown in Figure 6E, after exposure to 30 μM/L ALL, The Tau value of deactivation of I_{Ks} in Epi and Endo cells were decreased from 200 ± 22.7 ms and 160 ± 14.6 ms to 150 ± 14.6 ms and 130 ± 12.7 ms (P < 0.05, n = 8), whereas a reduction in the I_{Ks} deactivation was not observed in M layers.

4 Discussion

In this study, we tested cardiac transmural electrical heterogeneity of transient I_{to} and I_{Ks} ALL rabbit ventricular myocardium. The results of the present study suggested that: (1) ALL could effectively narrowed the repolarization differences among myocardium and further reduce TDR of rabbit heart; and (2) The transmural dispersion existed inherently in the myocardial distribution of I_{to} and I_{Ks} channels. ALL affected the current densities as well as gating kinetics of I_{to} and I_{Ks} channels of each myocardium in various degrees, the final effects might be benefit for reducing cardiac repolarization heterogeneity.

As an anti-arrhythmic agent, ALL has combined effect similar as amiodarone that preventing cardiomyocytes and providing superior antiarrhythmic efficiency by prolong action potential duration. The MAP recording technique allowed us to directly and simultaneously measure MAPD in *in vivo* rabbit heart. $MAPD_{90}$ were measured as the interval between the fast MAP upstroke to the next 90% repolarization level. Our results found that normal MAPD of Epi and M cells showed a prominent phase 1 which had a spike and notched configuration, MAPD of Endo cells had a different morphology with no distinct phase 1 notch. M cells are characterized by prolonged MAPD compared with the others, this finding support the idea put forward by Antzelevitch.[14] Administration of ALL narrowed the regional differences among three myocardial layers, the notches of phase 1 of MAP in Epi and M layers were unapparent, The $MAPD_{90}$ of Epi and M layers were prolonged which made the MAPD of three myocardium keep consistent. As a result, TDR was decreased from 29.5 ± 3.4 ms to 16.2 ± 5.8 ms (P < 0.01). We dissected the myocytes of the Epi, M and Endo layers from rabbit left ventricular free wall and went into more detail on the mechanism of the multi-blocking effects of ALL. We found that APD_{90} of Epi and Endo cells were prolonged by ALL in a concentration dependent manner. This fully proves the selectively inhibiting effect of ALL on different myocardium.

Cardiac repolarization is initiated and controlled by a number of potassium channel currents. Among them the I_K and I_{to} play the most important roles in regulating action potential duration. The I_{to} channels are expressed in most mammalian cardiomyocytes and contribute importantly to the early phase of the action potential durations and plateau phases. The higher expression of I_{to} channels in Epi myocytes of other species was once considered to be a major cause of AP heterogeneity.[15] Increasing of I_{to} during early phase 1 may affect repolarization reserve of phase 2 and 3 and facilitate EADs. In diseases such as long QT syndrome, Brugada or heart failure, this amplification of transmural heterogeneities might lead to development of malignant arrhythmias.[16] Thus targeting I_{to} has been proposed as an anti-arrhythmic therapy.[17] Our data clearly demonstrated that there exists strong transmural Ito electrical heterogeneity in rabbit ventricular wall. The prominent I_{to} mediated the notch of action potential in Epi and M cells, create transmural voltage gradients, which may be responsible for the ST-segment elevation observed in the Brugada syndrome or serve as the trigger for VT/VF.[18] The voltage and concentration-dependently blocking effect of ALL on I_{to} currents of the Epi, M and Endo layers were disequilibrium. The decrease in the Epi and M notch size of APs was accompanied by an accentuation of Ito currents. The maximum inhibitions were observed in M cells, due to the delayed channel activation by ALL. Besides, the opening proportion of I_{to} in the Epi and Endo layers were recorded an average of 40%–50% less than those in M groups (85%), which means it would further result in a general reduction of I_{to} currents by limiting the channel opening of each myocardial layer.

In our study, the distribution of innate I_{Ks} channels were most in Epi, Endo layers and least in M layers, the APD of M cells tended to be longer than the other two layers. Moreover, I_{Ks} blockade were found to mainly influence $MAPD_{90}$, which is primarily the sum of the plateau and the fast repolarization phase of the action potential duration.[19] Unlike I_{Kr} channels, which is homogeneity expressed in all three layers, I_{Ks} expression is reduced in M cells, partially contribute to the prolonged APD of M cells and providing the electrical basis for TDR.[20] We found ALL prominently suppressed I_{Ks} in Epi and Endo layers, thus markedly decreased the anti-frequency dependency of I_{Ks} that caused by high distribution selectivity, which would further lead to the reduction of the TDR. Our results also found that allocryptopine's blocking effect of I_{Ks} repolarizing currents was mainly depended on current-voltage relationships as well as channel deactivations. The deactivation curves of I_{Ks} were slowed respectively in epicardium and endocardium by ALL intervention. The slowed deactivation process will lead to the I_{Ks} channels be constitutively open when channels are activated by fast heart rates.

Because of the restriction of time and the ability, there are some unsettled issues in our study. Although we found the reduction of I_{to} and I_{Ks} currents in three myocardium, the combination influences of ALL in channel protein synthesis or channel redistribution in myocardium, or down regulation of channel gene expression are still unclear. Thus the pharmacological function of ALL demands more accurate understanding of biological processes at molecular level, which will be our striving direction of research in the future.

In conclusion, we demonstrated that the transmural heterogeneities of I_{to} and I_{Ks} currents were innately existed in rabbit myocardium and formed the ionic basis for dispersion of repolarization. ALL is an anti-arrhythmic alkaloid and our findings provide the first evidence that its effects on I_{to} and I_{Ks} currents were distinct. It could selectively reduce the current densities of I_{to} and I_{Ks} in myocardium, and affect the channel gating kinetics in various ways. This feature will contribute to the stabilization of cardiac repolarization process and lowers the risk for generating arrhythmias, thus provide an anti-arrhythmic mechanism in the therapeutic perspective.

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