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Artemisinin blocks activating and slowly activating K^+ current in guinea pig ventricular myocytes

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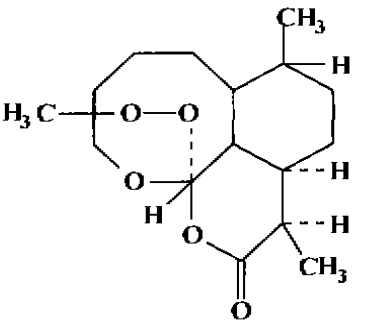
KEY WORDS artemisinin; anti-arrhythmia agents; potassium channels; myocardium; cells; electrophysiology

AIM: To study the effect of artemisinin (Art) on outward rectifier potassium current in ventricular myocytes. **METHODS:** In isolated guinea pig ventricular myocytes, the effects of Art on the two components of delayed outward rectifier K^+ current (I_K), the rapidly activating inward K^+ current (I_{Kr}), and the slowly rectifying outward K^+ current (I_{Ks}) were observed by the whole cell patch-clamp technique. **RESULTS:** Art decreased I_K in a concentration-dependent manner. The I_{Kstep} and I_{Ktail} were reduced from 387 ± 46 pA to 240 ± 48 pA and from 299 ± 30 pA to 130 ± 38 pA, respectively at holding potential of + 40 mV by Art 50 $\mu mol \cdot L^{-1}$. The envelope of tail analysis suggested that both I_{Kr} and I_{Ks} were inhibited. **CONCLUTION:** Art blocked the two components of delayed outward rectifier K^+ current (I_{Kr} and I_{Ks}) in guinea pig ventricular cells.

In most cases the efficacy of anti-arrhythmic agents which selectively prolong APD and ERP has been attributed to their ability to block the delayed rectifier potassium current I_K[1,2]. The rectifier potassium current (I_K) in guinea pig cardiomyocytes is composed of two distinct components, the rapidly activating inward K^+ current (I_{Kr}) and the slowly rectifying outward K^+ current (I_{Ks}), which are distinguished based on their different kinetics, pharmacology, voltage dependence, and rectification properties[3,4]. I_{Kr} is a rapidly activating, inwardly rectifying, lanthanum-sensitive component, whereas I_{Ks} is activated slowly and shows slight outward rectification at more positive potentials. During long voltage-clamp steps to plateau potentials, I_{Ks} is much larger than I_{Kr}, but during relatively brief pulses that approximate APD, both components have similar magnitudes[5].

Artemisinin (Art) is an antimalarial drug which is also effective on some animal arrthymias induced by aconitine, coronary artery ligation, and electric stimulation[6]. This study was to evaluate the effects of Art on I_K (I_{Kr} and I_{Ks}) in guinea pig ventricular myocytes.



Artemisinin

MATERIALS AND METHODS

Guinea pigs of either sex, weighing 320 ± 20 g, were purchased from Experimental Animal Center, Harbin Medical University.

Solutions The standard Tyrode's solution contained: NaCl 126, KCl 5.4, $MgCl_2$ 1, $CaCl_2$ 1, NaH_2PO_4 0.33, glucose 10, and HEPES 10 $mmol \cdot L^{-1}$, pH ajusted to 7.4 with NaOH. Storage medium contained: KCl 20, KH_2PO_4 10, glucose 10, potassium glutamate 70, β-hydroxy-butyric acid 10, taurine 10, egtazic acid 0.5, and 1 % albumin $mmol \cdot L^{-1}$, pH adjusted to 7.3 with KOH. The pipet solution contained: KCl 20, potassium aspartate 110, $MgCl_2$ 1, HEPES 10, egtazic acid 5, GTP 0.1, and Mg-ATP 5 $mmol \cdot L^{-1}$, pH adjusted to 7.2 with KOH. Stock solution of Art (from Shenyang Pharmaceutical University) was freshly prepared to 10 $mmol \cdot L^{-1}$ with distilled water. $BaCl_2$ and $CdCl_2$ stock solutions were prepared as 0.2 mol

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$\cdot L^{-1}$, which were used to block inwardly rectifying potassium channel current (I_{KI}) and/or I_{Ca}.

Preparation of ventricular myocytes Ventricular cells were isolated from hearts[7]. Briefly, guinea pig hearts were perfused with Ca^{2+} Tyrode's solution at 36 ℃ for 5 – 10 min. After the heart was perfused with Ca^{2+}-free Tyrode's solution for 8 – 10 min, it was enzymatically digested for 15 – 20 min with Type Ⅱ collagenase 100 – 150 kU · L^{-1} (CLS Worthington Biochemical, Fredhold, USA). The myocytes were incubated in the storage medium at room temperature.

A 0.1 mL solution containing the isolated cells was placed in an open perfusion chamber (1 mL) mounted on an inverted microscope. Myocytes were allowed to adhere to the bottom of the dish for 5 – 10 min and then superfused at 2 – 3 mL · min^{-1} with Tyrode's solution at 36 ± 0.5 ℃. Only quiescent rod-shaped cells showing clear cross striations were studied.

Electrophysiologic recording The whole cell patch-clamp was employed to record ionic currents. The resistance of the borosilicate glass electrodes used was 2 – 4 MΩ, and were connected to a patch-clamp amplifier (Axopatch 1-D, Axon Instruments, Foster City, USA). Command pulses were generated by a 12-bit digital-to-analog converter controlled by pCLAMP software (Axon Instruments). Recordings were filtered at 1 kHz and data were acquired by analog-to-digital conversion at the maximum rate of 100 kHz (Model TM 125, Scientific Solution, Solon, USA) and stored on the harddisk of an IBM-compatible computer. Pipet tip potentials (2 – 10 mV) were corrected before the pipet touched the cell. After a tight pipet-membrane had been obtained (seal resistance > 10 GΩ), the membrane was ruptured with gentle suction to obtain the whole cell voltage-clamp configuration. Liquid junction potentials between pipet solution and perfusion solution (10 – 11 mV) was not corrected after membrane rupture. In all of the cells studied, the series resistance was electrically compensated to minimize the duration of the capacitive transient.

I_K was determined in guinea pig ventricular myocytes with 3-s voltage steps between – 40 to + 60 mV from a holding potential of – 60 mV. I_{Ca} was blocked by Ca^{2+} 0.2 mmol · L^{-1}, and I_{KI} was blocked by Ba^{2+} 0.1 mmol · L^{-1}. After cell membrane rupture, 20 – 30 min period was needed to observe stable I_K for pharmacological study. The cells that showed any I_K run-down were rejected in our experiments. Envelop test was performed as following: the pulses were applied from a holding potential of – 60 to + 50 mV for durations ranging from 60 to 3000 ms. I_{tail}/I_K was calculated in the absence and the presence of Art 5 μmol · L^{-1}. In cells perfused with Cd^{2+} 200 μmol · L^{-1}, Ba^{2+} 100 μmol · L^{-1} and *dofetilide*-free solution, I_{tail}/I_K was dependent on the duration of the pulse.

Curve fitting was performed with a Marcquardt algorithm and Table Curve software (Jandel Scientific). Results are presented as $\bar{x} \pm s$ and compared with paired t test.

HEPES, potassium glutamate, potassium aspartate, β-hydroxybutyric acid, taurine, egtazic acid, albumin, and $CdCl_2$ were purchased from Sigma.

RESULTS

Art 5 and 50 μmol · L^{-1} decreased I_K (Fig 1B, 1C), especially on I_{Ktail}. The effect was partially reversed by 30-min washout (Fig 1D).

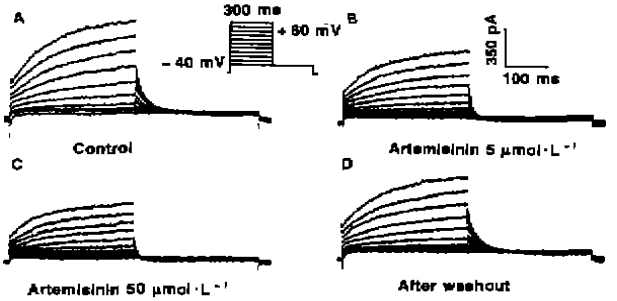

Fig 1. Effects of Art on I_K in guinea pig ventricular myocytes. I_K was elicited by 300 ms depolarizing pulse at 0.1 Hz to potentials ranging from – 40 to + 60 mV, from a holding potential of – 60 mV. Ca^{2+} and I_{KI} currents were abolished with Cd^{2+} (0.2 mmol · L^{-1}) and Ba^{2+} (0.1 mmol · L^{-1}).

I_{Kstep} was defined as time-dependent component to exclude the contamination of nonspecific currents, which was measured from initial activation to the current at the end of steps. Art 5 and 50 μmol · L^{-1} decreased I_{Kstep} and I_{Ktail} at some test potentials, and the effects were reversible after 30-min washout (Fig 2).

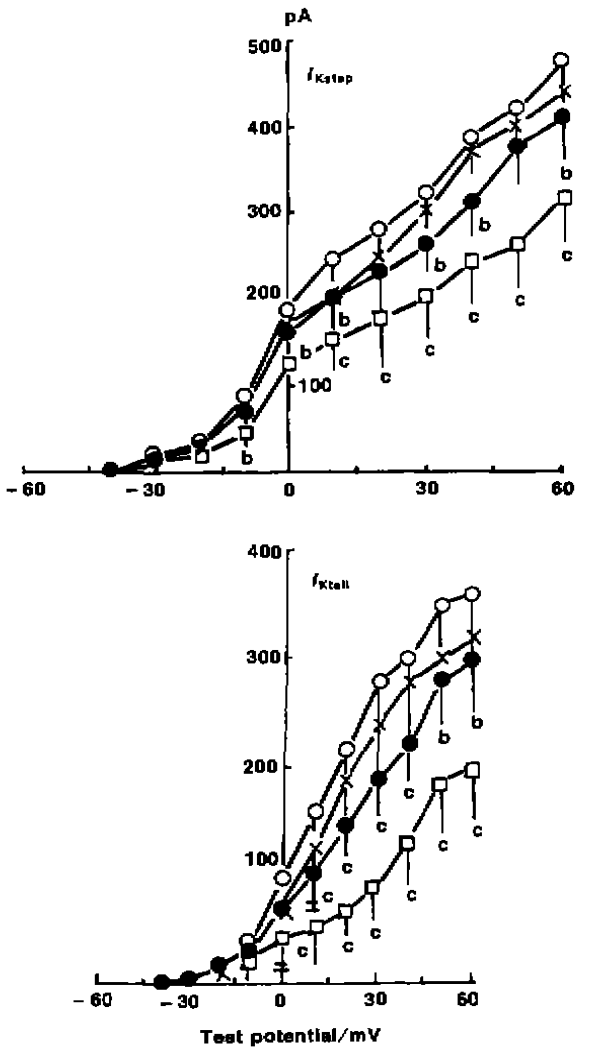

Fig 2. Effects of Art on $I-V$ relationship of I_{Kstep} and I_{Ktail} in guinea pig ventricular myocytes. (○) control, (●) Art 5 μmol·L^{-1}, (□) Art 50 μmol·L^{-1}, (×) washout. $\bar{x} \pm s$ from 6 guinea pigs, $^bP<0.05$, $^cP<0.01$ *vs* control.

At +40 mV, I_{Kstep} was decreased from 387 ± 46 pA to 310 ± 39 pA ($n=6$, $P<0.01$). After 30-min washout, the current was recovered to 370 ± 31 pA. Art 100 μmol·L^{-1} inhibited I_{Ktail} by 78 ± 40 pA, while I_{Kstep} by 109 ± 37 pA. Clearly, maximal effect of Art on I_{Ktail} was stronger than I_{Kstep} ($P<0.01$).

Art inhibited I_{Kstep} and I_{Ktail} in a concentration-dependent manner. To determine whether this compound had selective effect on I_{Kr} and/or I_{Ks}, we conducted envelop of tails test which showed that I_K consisted of more than one component, whereas after exposure to Art 5 μmol·L^{-1}, I_{Kr} and I_{Ks} were blocked (Fig 3). Since I_{tail}/I_K was not constant at 1.9 ± 0.3 in 180 ms of test pulse duration at 50 mV. I_{tail}/I_K was 1.02 ± 0.06 at 2100 ms of test pulse duration at 50 mV. The results indicated that Art inhibited either I_{Kr} or I_{Ks} in guinea pig ventricular myocytes without selectivity.

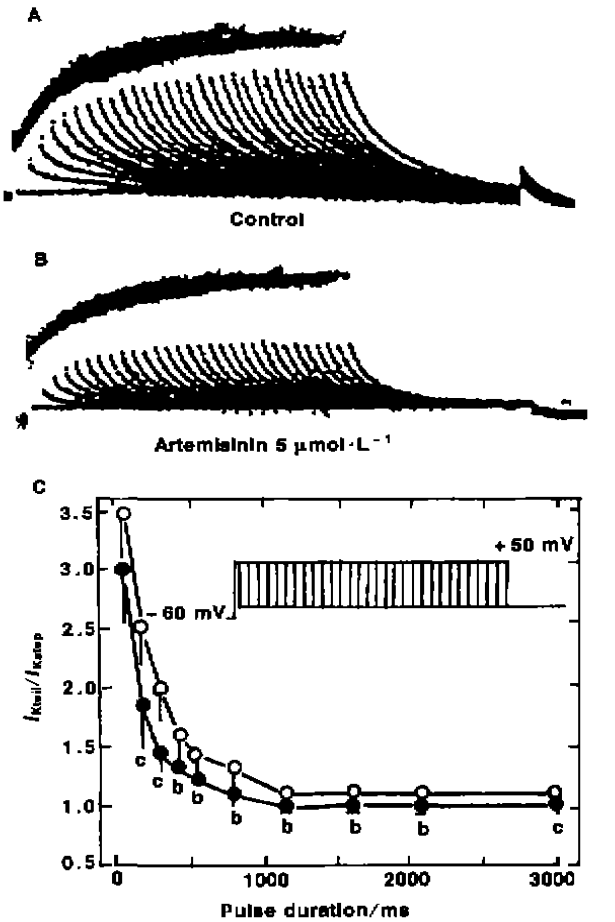

Fig 3. Envelope-of-tails test for the delayed rectifier K$^+$ current. (A) Control, (B) Art 5 μmol·L^{-1}, (C) Ratio of tail current to step current (I_{Ktail}/I_{Kstep}) is plotted as a function of pulse duration. (○) control, (●) Art 5 μmol·L^{-1}. $n=6$, $^bP<0.05$, $^cP<0.01$ *vs* control.

DISCUSSION

Activation of I_K during short depolarizing pulses (< 0.5 s) is characterized by tail currents that are equal to or larger than the time-dependent outward currents during the depolarizing pulse. However, as the duration of the test pulse was lengthened, the magnitude of

I_{Ktail} became less than that of I_K. This demonstrated the existence of two different components of I_K in guinea pig ventricular myocyte as result of the activation of two different types of K^+ channels: a rapidly activating K^+ channel, Kr, and a slowly activating one, Ks[8]. The results of the present study indicated that Art inhibited markedly I_{Kr} and I_{Ks} without selectivity and the effect of Art on I_{Ktail} was potent than that on I_{Kstep} in isolated guinea pig ventricular myocytes. Drug actions in the absence and in the presence of dofetilide indicated that Art agent inhibited unselectively at least two components of this time-dependent I_K.

These results more closly resembled the actions of aminodarone, a class Ⅲ anti-arrhythmic agent. Because of no blocking selectivity for I_{Kr}[9, 10], class Ⅲ agents possess fewer risks of producing reverse use-dependent actions on repolarization and proarrhythmic reactions due to excessive delays of repolarization at slow heart rates than new class Ⅲ drugs, for instance, *d*-sotalol[11]. Evidence for Art non-selective block was demonstrated in some ways: the block of tail current was very constant for longer depolarization between 3 and 5 s and became less with short depolarization. In conclusion Art significantly inhibited I_{Kr} and I_{Ks} without selectivity, and the I_K, which were the major mechanism of anti-arrhythmic actions of Art.

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青蒿素阻断豚鼠心室肌细胞活化和缓慢活化的钾电流

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关键词 青蒿素；抗心律失常药；钾通道；心肌；细胞；电生理学

目的：研究青蒿素(Art)对豚鼠心室肌细胞外向整流钾电流的作用. **方法**：以全细胞膜片箝技术观察 Art 对快速延迟整流钾电流(I_{Kr})和缓慢延迟整流钾电流(I_{Ks})的作用. **结果**：Art 剂量依赖性抑制时间依赖性外向钾电流(I_{Kstep})和 I_{Ktail}. Art 50 $\mu mol \cdot L^{-1}$在 +40 mV 时，使 I_{Kstep}从 387 ± 46 pA 减少到 240 ± 48 pA，I_{Ktail}从 299 ± 30 pA 减少到 130 ± 38 pA. **结论**：Art 抑制外向钾电流的两种成分 I_{Ks} 和 I_{Kr}.