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Effects of changrolin on potassium currents in guinea pig and rabbit single heart cells

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KEY WORDS changrolin; anti-arrhythmia agents; potassium; patch-clamp techniques; myocardium; cultured cells; electrophysiology; nifedipine; 4-aminopyridine

ABSTRACT

AIM: To elucidate whether or not changrolin (CRL) modifies the potassium currents (I_{TO}, I_K, and I_{KI}) in myocardial cells. **METHODS**: A tight seal whole-cell patch-clamp technique was used to record I_{TO}, I_K, and I_{KI} in single cells isolated from guinea pig and rabbit hearts. **RESULTS**: At a clinically relevant concentration, CRL 50 μmol · L^{-1} inhibited the transient outward current (I_{TO}) by 17.7 % ± 2.4 % (n = 8) in rabbit atrial cells. The voltage-dependence of steady-state inactivation of I_{TO} was not affected by CRL. This concentration of CRL did not influence the time-independent inward rectifier or the delayed rectifier K^+ currents (I_{KI} and I_K, respectively) in rabbit and guinea pig ventricular cells. **CONCLUSION**: CRL inhibited I_{TO}, but not I_K nor I_{KI}.

INTRODUCTION

Changrolin (CRL), 4-[3′, 5′-bis[(*N*-pyrrolidinyl)-methyl]-4′-hydroxyanilino]-quinazoline, was first synthesized in China. Subsequent clinical and experimental trials suggested that this agent exerts electrophysiologic effects on the heart. Therefore, CRL has been used as an anti-arrhythmic drug in China[1]. CRL inhibited the fast Na^+ current (I_{Na}) in a frequency-dependent manner[2,3]. CRL either prolonged[3] or shortened the action potential duration[4], probably depending on the species used. In the present study, we investigated the effects of CRL on the transient outward current (I_{TO}), the delayed rectifier K^+ current (I_K), and the time-independent inward rectifier (I_{KI}).

MATERIALS AND METHODS

Cell isolation Guinea pigs weighing 200 – 300 g (n = 15) and rabbits weighing 1.5 – 2.5 kg (n = 20) were killed. The excised hearts were mounted on a Langendorff apparatus and perfused for 5 – 10 min with a Ca^{2+}-free solution containing: NaCl 142, KCl 5.4, $MgCl_2$ 10, NaH_2PO_4 0.33, Na_2HPO_4 2.24, and glucose 10 mmol · L^{-1} (pH 7.4). Collagenase (Yakult, Tokyo, Japan) 0.01 g · L^{-1} and protease (Type 13, Sigma Chemical Co, USA) 0.01 g · L^{-1} were added for the subsequent 10 min. The heart was cut into small pieces, and were then stirred in the second enzyme solution containing: NaCl 142, KCl 5.4, NaH_2PO_4 1, $MgCl_2$ 10, HEPES 5, and glucose 10 mmol · L^{-1} (pH 7.4 adjusted by NaOH), collagenase (Type H, Sigma) 0.6 g · L^{-1}. Of the supernatant, 200 – 400 mL was collected every 3 – 5 min and diluted in 10 mL of 'Kraftbrühe' (KB) solution, which contained: K-glutamate 90, oxalate 10, KCl 25, KH_2PO_4 10, $MgSO_4$ 1, taurine 10, egtazic acid 0.5, HEPES 5, and glucose 10 mmol · L^{-1} (pH 7.2 adjusted by KOH). The isolated cells were collected by centrifugation at 70 × g for 3 min, then stocked in KB solution containing 0.1 % bovine serum albumin (Fraction 5, Sigma). The methods conformed to the Guide for the Care and Use of Laboratory Animals (US National Institutes of Health, 1985). The experiments were carried out on cells which showed a striated, rod-shaped appearance during perfusion with HEPES-buffered Tyrode solution containing Ca^{2+} 1.8 mmol · L^{-1} at 37 ℃.

Solution and materials The extracellular

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solution for I_{TO} measurements contained: KCl 5.4, NaCl 142, NaH_2PO_4 1, $CaCl_2$ 1.8, $MgCl_2$ 1, HEPES 5, and glucose 10 mmol·L^{-1} (pH 7.4 adjusted by NaOH). The pipette solution contained: K-aspartate 80, KCl 20, egtazic acid 10, $CaCl_2$ 1, $MgCl_2$ 1, K_2-ATP 5, Na_2-phosphocreatine 5, and HEPES 5 mmol·L^{-1} (pH 7.2 adjusted by KOH). The I_{K1} and I_K were measured using the same solution, except that the extracellular K^+ concentration was 2 mmol·L^{-1} for I_K to obtain a larger amplitude of I_K. CRL was provided by Shanghai Institute of Materia Medica, Chinese Academy of Sciences.

Electric measurements Voltage-clamp experiments were carried out by the single-pipette whole-cell clamp method. The amplifier was TM-1000 (ACT ME, Tokyo, Japan).

The current signals were filtered at 2.5 kHz for K^+ currents. These data were stored on a digital cassette recorder (TEAC RD101-T, Tokyo, Japan) and were subsequently analyzed on an NEC 98 computer (Tokyo, Japan). The data are presented as $\bar{x} \pm s$ and compared with t-test.

RESULTS

Transient outward current (I_{TO}) The I_{TO} of rabbit atrial myocytes was elicited by a 300-ms depolarization from −80 to +30 mV in the presence of $CdCl_2$ 100 μmol·L^{-1}. CRL reversibly inhibited the I_{TO} in a concentration-dependent manner in the rabbit atrial cells. That is, CRL 50 and 300 μmol·L^{-1} blocked the I_{TO} by 17.7 % ± 2.4 % (n = 8) and 39.8 % ± 3.5 % (n = 8), respectively (Fig 1).

The voltage-dependence of steady-state inactivation of I_{TO} was evaluated using a conventional double pulse protocol. After the application of 1-s prepulse (P_1) ranging from −100 to −20 mV, a depolarizing test pulse (P_2) to +30 mV was applied for 300 ms. By normalizing these I_{TO} amplitudes, the channel availability curves in the absence and presence of CRL were obtained. CRL 300 μmol·L^{-1} did not affect the voltage dependence of stead-state inactivation curve of I_{TO} at all (n = 5, Fig 2).

Delayed rectifier K^+ current (I_K) I_K was activated by a depolarization for 1 s from −40 to +30 mV in the presence of nifedipine 5 μmol·L^{-1} to abolish the contaminant I_{Ca}. CRL 50 μmol·L^{-1} did not induce any detectable change in the I_K in guinea-pig ventricular cells (n = 5) at all (Fig 3).

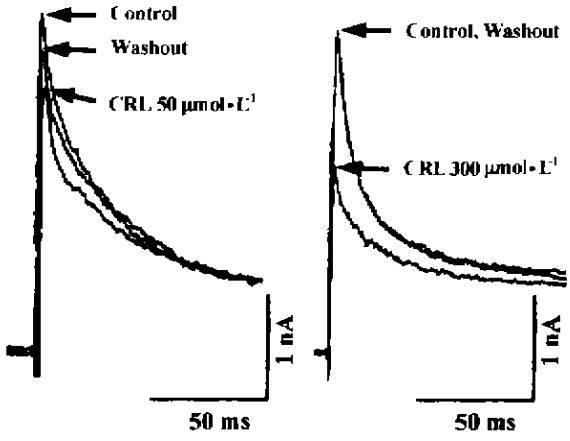

Fig 1. Effect of changrolin (CRL) perfusions on I_{TO}.

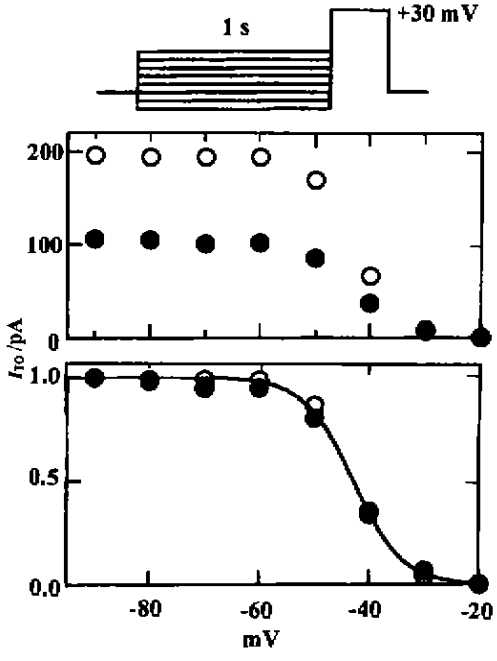

Fig 2. Absence of CRL effect on the voltage-dependence of stead-state inactivation curve of I_{TO} in rabbit atrial cell. (○) Control, (●) CRL. $P > 0.05$.

Time-independent inward rectifier K^+ current (I_{K1}) The time-independent inward rectifier (I_{K1}) in the rabbit ventricular cells was measured by 100 ms test pulses applied to various potentials from a holding potential of −40 mV in the presence of nifedipine 5 μmol·L^{-1} and 4-aminopyridine 5 mmol·L^{-1}.

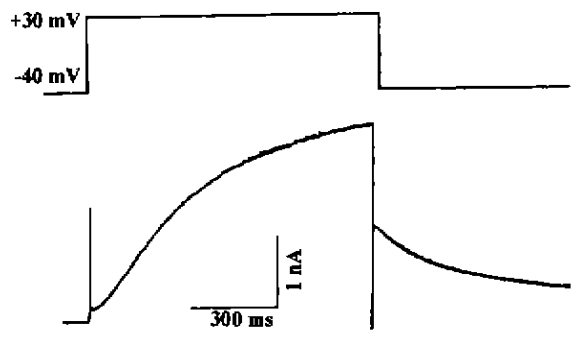

Fig 3. Effect of changrolin on I_K. Control or CRL.

CRL 300 μmol·L^{-1} did not significantly affect the I_{K1}. Note the superimposable tracings during the control and CRL perfusion (Fig 4).

DISCUSSION

These voltage-clamp measurements have identified an important new effect of CRL: a quite selective and potent inhibition of a transient outward current, which controls the early repolarization of the action potential. This effect occurs at therapeutic dose[5]. At a concentration of 50 μmol·L^{-1}, CRL reversible inhibited I_{TO} by 17.7 % ± 2.4 %. CRL inhibited the I_{TO} in a dose-dependent manner in the rabbit atrial cells. But we did not observe any distinctive effect of CRL on I_K or I_{K1} in guinea pig and rabbit single ventricular cells.

It is well known that ionic currents responsible for the repolarization of the cardiac action potential show important species-dependence. For example, although frog and guinea pig isolated myocytes exhibit a large, maintained time- and voltage-dependent I_K[6,7], this current is relatively small in atrial and ventricular myocytes isolated from rat, dog, rabbit, or human hearts[6-10]. In contrast, in these latter four species a large transient outward current, I_{TO}, is responsible to a major extent for the repolarization of the action potential[10]. This I_{TO} is much smaller in the ventricle than in the atrium[11]. Therefore, I_K and I_{TO} predominantly regulate the action potential duration in the guinea pig and rabbit heart, respectively. An intravenous application of CRL reportedly prolongs the QT interval on the electrocardiogram in dogs[12], Since I_{TO} plays an important role in the repolarization of the

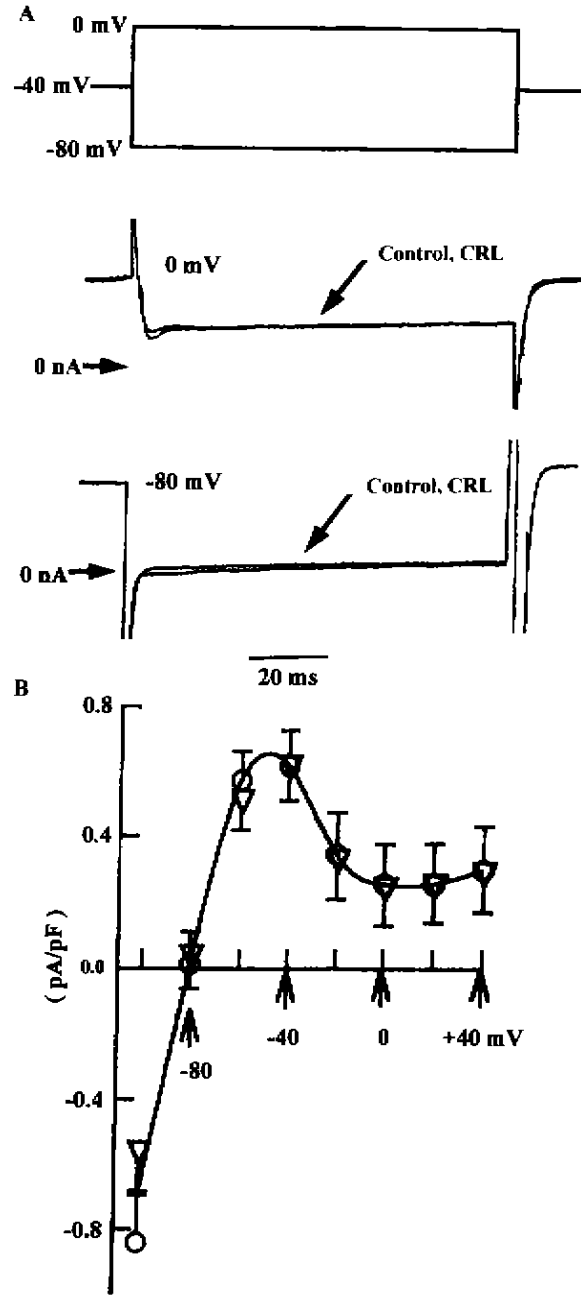

Fig 4. Effect of changrolin on I_{K1}. A) 100 ms pulses were applied from a holding potential of −40 mV to various potentials in 20 mV steps. B) The current-voltage relationship. Control (○); (△) CRL, $n = 5$.

action potential in the dog myocardium[13]. In addition, in rabbit ventricle, a relatively large time-independent inward rectifier potassium current (I_{K1}) is present at potentials near the resting potential[11]. Thus, in this study, we selected different cardiac preparations from different animal hearts. In conclu-

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sion, the results obtained in this paper demonstrate that in rabbit isolated atrial cells, CRL inhibits the I_{TO} current and this effect may be partly responsible for the effectiveness of CRL in supraventricular and ventricular arrhythmia described in both animal and human models[14,15], since the prolongation of the action potential has been recognized as an anti-arrhythmic mechanism. These results provide a new insight for the anti-arrhythmic effect of CRL on those cardiac tissues in which the I_{TO} is important in determining the action potential repolarization process.

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常咯啉对豚鼠和家兔单个心肌细胞钾电流的影响

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R972.2

关键词 常咯啉; 抗心律失常药; 钾; 膜片箝技术; 心肌; 培养的细胞; 电生理学; 硝苯地平; 4-氨基吡啶

目的: 研究常咯啉是否对心肌细胞的钾电流有影响作用. **方法**: 采用高阻抗密封膜片箝全细胞技术, 记录分离的豚鼠和家兔心肌单个细胞的钾电流. **结果**: 在临床用量常咯啉 50 $\mu mol \cdot L^{-1}$抑制家兔心房肌细胞的瞬间外向钾电流(I_{TO}) 17.7 % ± 2.4 % ($n=8$). 但并不影响电压依赖性通道. 同一剂量的常咯啉对单个家兔心室肌细胞的内向整流钾电流(I_{K1})和单个豚鼠心室肌细胞的延迟整流钾电流(I_K)并不产生任何作用. **结论**: 提示常咯啉具有阻制(I_{TO})的作用, 而对(I_K)和(I_{K1})无任何作用.

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