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KN-93 inhibits I_{Kr} in mammalian cardiomyocytes

Bence Hegyi [a], Ye Chen-Izu [a,b,c], Zhong Jian [a], Rafael Shimkunas [a,b], Leighton T. Izu [a], Tamas Banyasz [d,*]

[a] Department of Pharmacology, University of California, Davis, CA, USA
[b] Department of Biomedical Engineering, University of California, Davis, CA USA
[c] Department of Internal Medicine (Cardiology), University of California, Davis, CA USA
[d] Department of Physiology, Faculty of Medicine, University of Debrecen, Debrecen, Hungary

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ABSTRACT

Calcium/calmodulin-dependent protein kinase II (CaMKII) inhibitor KN-93 is widely used in multiple fields of cardiac research especially for studying the mechanisms of cardiomyopathy and cardiac arrhythmias. Whereas KN-93 is a potent inhibitor of CaMKII, several off-target effects have also been found in expression cell systems and smooth muscle cells, but there is no information on the KN93 side effects in mammalian ventricular myocytes. In this study we explore the effect of KN-93 on the rapid component of delayed rectifier potassium current (I_{Kr}) in the ventricular myocytes from rabbit and guinea pig hearts. Our data indicate that KN-93 exerts direct inhibitory effect on I_{Kr} that is not mediated via CaMKII. This off-target effect of KN93 should be taken into account when interpreting the data from using KN93 to investigate the role of CaMKII in cardiac function.

1. Introduction

Calcium/calmodulin-dependent protein kinase II (CaMKII) is in the focus of research because it is known to modulate various cell functions. Pathologic activation of this enzyme is implicated in different forms of cardiac arrhythmias and CaMKII inhibition is reported to prevent or alleviate development of irregular heartbeats [1–5]. KN-93 (2-[N-(2-hydroxyethyl)]-N-(4-methoxybenzenesulfonyl)]amino-N-(4-chlorocinnamyl)-N-methylbenzylamine) is widely used as a pharmacological tool to inhibit CaMKII in several studies [1,2,6]. However, besides its primary effect, KN-93 has been found to have off-target effects including an open-channel blockade of some voltage-gated potassium channels [7,8]. Until now the side effects of KN-93 have been explored on several voltage-dependent potassium channels in smooth muscle cells, atrial myocytes and expression cell systems, but no data are available for ventricular myocytes. Furthermore, those studies were performed at room temperature and the KN-93 concentrations used were equal or higher than that needed for complete inhibition of CaMKII [7–9].

In the present work we explore the inhibitory effect of KN-93 on the rapid component of delayed rectifier potassium current (I_{Kr}) in freshly isolated rabbit and guinea pig ventricular myocytes at body temperature. We used both the traditional voltage clamp and action potential voltage clamp (APC) methods [6,10] to study the effect of KN-93 on altering the amplitude and the time course of I_{Kr} during AP and to determine the dose–response of I_{Kr} to KN93 treatment.

2. Methods

All animal handling and laboratory procedures conform to the approved protocols of the local Institutional Animal Care and Use Committee confirming to the Guide for the Care and Use of Laboratory Animals published by the US National Institute of Health (8th edition, 2011). Chemicals and reagents were purchased from Sigma-Aldrich if not specified otherwise. E-4031 and HMR-1556 were from Tocris. All experiments were conducted at 36 ± 0.1 °C.

2.1. Cell isolation

Ventricular myocytes were isolated from 3 to 4 month old New Zealand White rabbits and 4–6 month old Hartley guinea pigs by a standard enzymatic technique [6] using collagenase type II (Worthington, USA) and protease type XIV (Sigma, USA).

2.2. Electrophysiology

Cells were transferred to a temperature-controlled Plexiglas chamber (Cell Microsystems, USA) and continuously superfused with a bicarbonated Tyrode (BTY) solution containing (in mmol/L):

* Corresponding author at: University of Debrecen, Faculty of Medicine, Department of Physiology, H-4012 Debrecen, Nagyerdei krt. 98., PO Box 22, Hungary.
E-mail address: banyasz.tamas@med.unideb.hu (T. Banyasz).

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NaCl 125, $NaHCO_3$ 25, KCl 4, $CaCl_2$ 1.2, $MgCl_2$ 1, HEPES 10, and Glucose 10; pH was set to 7.4 with NaOH. Electrodes were fabricated from borosilicate glass (World Precision Instruments, USA) with tip resistances of 2–2.5 MΩ when filled with internal solution. In experiments aimed to preserve the physiological Ca^{2+} cycling during AP, the internal solution contained (in mmol/L): K-Aspartate 108, KCl 25, NaCl 5, Mg-ATP 3, HEPES 10, cAMP 0.002, phosphocreatine dipotassium salt 10, and EGTA 0.01; pH was set to 7.3 with KOH. To study the effect of KN-93 cytosolic calcium concentration below diastolic level, $[Ca^{2+}]_i$ was buffered to nominally zero by using an internal solution containing (in mmol/L): K-Aspartate 100, KCl 25, NaCl 5, Mg-ATP 3, HEPES 10, cAMP 0.002, phosphocreatine dipotassium salt 10, and BAPTA 10, with pH = 7.3. The cells were stimulated in current-clamp experiments with supra-threshold depolarizing pulses (2 ms duration) delivered via the patch pipette at 2 Hz pacing rate. By switching the amplifier to voltage-clamp, APC experiments were conducted as described previously [10]. In conventional voltage-clamp experiments, measuring I_{Kr}, $[Ca^{2+}]_i$ was buffered and BTY was supplemented with 1 μM HMR-1556 plus 10 μM nifedipine. I_{Kr} was measured as the tail current amplitude (which was calculated as the difference between the peak current and the pedestal current after repolarization).

2.3. Statistical analysis

Data are reported as Mean ± SEM. Statistical significance of differences was evaluated using Student's t-test followed by ANOVA. Difference is deemed significant if $p < 0.05$. ANOVA is used for group-wise comparison. Student's t-test is used for pair-wise comparison. The difference in the mean values is deemed significant if $p < 0.05$.

3. Results

3.1. KN-93 abolishes I_{Kr} recorded with action potential clamp method

First, we recorded I_{Kr} as the E-4031 sensitive current under AP-clamp condition when intracellular Ca^{2+} cycling was preserved (no exogenous Ca^{2+} buffer was used in the pipette solution).

In the first group of experiments E-4031 sensitive current was recorded with the same, pre-recorded (canonical) AP without calcium chelator in the pipette solution (Ca^{2+} cycling). As seen in Fig. 1A, I_{Kr} builds up in accelerating manner during AP plateau and reaches a maximum (0.839 ± 0.066 A/F, n = 18) during phase 3. Then the current declines rapidly and reduces to zero when resting membrane potential is reached. 1 μM KN-93 pre-treatment abolished E-4031 sensitive current (Fig. 1B). No attempt was made to determine current maximum under these circumstances, because of the lack of a distinguishable peak during the AP. We determined and compared the net charge carried by I_{Kr} (Q_{Kr}) by integrating the current between the AP peak and APD_{95} (AP duration measured at 95% repolarization). The average value of Q_{Kr} under control conditions and in the presence of 1 μM KN-93 was 0.0384 ± 0.0046 C/F and 0.0024 ± 0.0011 C/F respectively when Ca^{2+} was not buffered. The inhibitory effect of KN93 on I_{Kr} was also observed with pipette solution containing 10 mM BAPTA to chelate Ca^{2+}. Hence, cytosolic calcium had no influence on the Q_{Kr}, and I_{Kr} was abolished by 1 μM KN-93 regardless of the Ca^{2+} concentration. Moreover, KN-92, a sister molecule of KN-93 known as "kinase inactive control drug" reduced Q_{Kr} by 45.28% when applied in 1 μM concentration, indicating a non-CaMKII mediated effect on I_{Kr} (Fig. 1D). To visualize the KN93 effect on the ionic currents during AP, we also recorded KN-93 sensitive current under AP-clamp. The KN-93 sensitive current is a composite current displaying multiple negative peaks along the plateau and

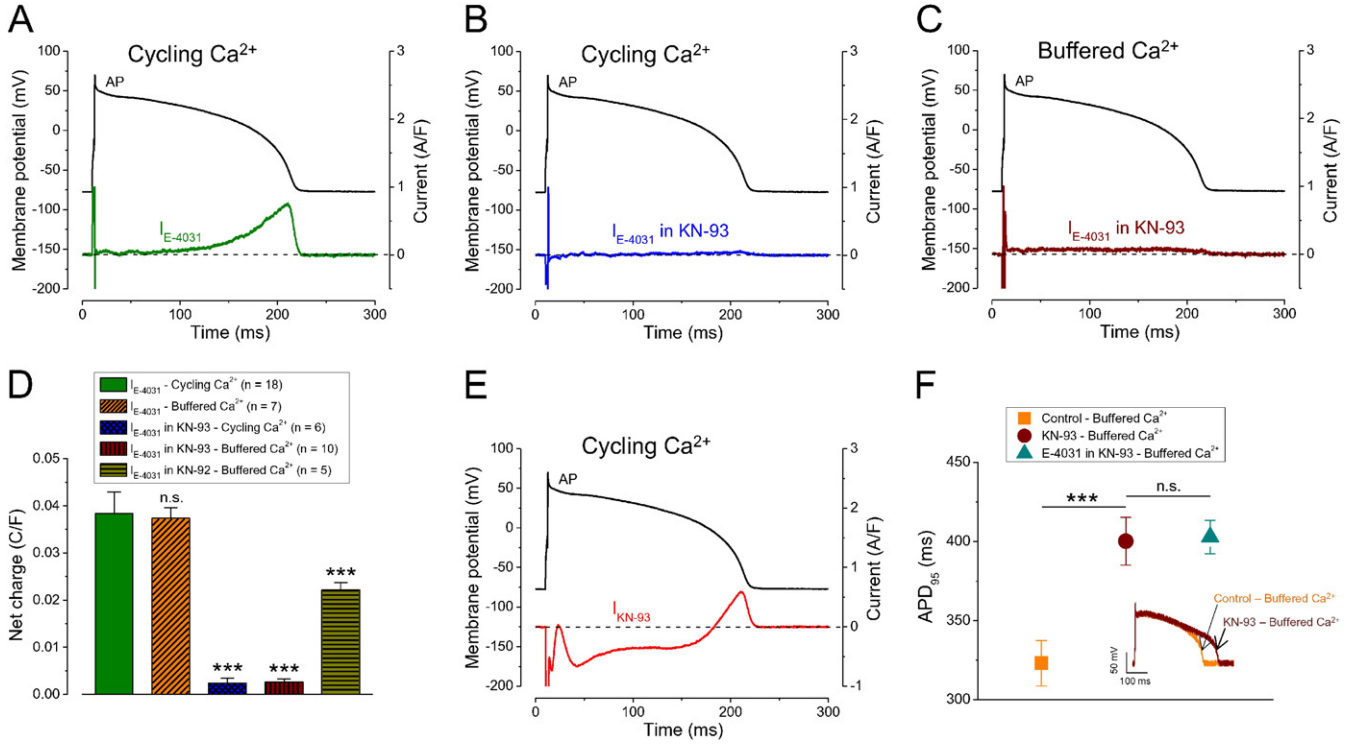

Fig. 1. I_{Kr} is abolished by 1 μM KN-93 in rabbit ventricular myocyte. Panels A and B show representative traces of I_{Kr} as E-4031 sensitive currents recorded under AP clamp method in the absence (A) and presence (B) of 1 μM KN-93 without buffering cytosolic calcium. E-4031 sensitive current was abolished by 1 μM KN-93 when cytosolic Ca^{2+} was buffered by 10 mM BABTA in pipette solution too (C). Panel D displays the net charge carried by E-4031 sensitive current, calculated by integration of the current profile during the AP. 1 μM KN-93 reduced net charge profoundly regardless of the presence or absence of cytosolic calcium buffer. Panel E displays representative traces of KN-93 sensitive current measured under AP clamp condition. The positive hump present during phase 3 of AP resembles the late phase of E-4031 sensitive current seen on Panel A. Panel F shows the AP prolonging effect of 1 μM KN-93 with buffered cytosolic calcium. When 1 μM E-4031 was added upon KN-93, no further prolongation was observed. Asterisks indicate significant differences from control (*: $p < 0.05$, **: $p < 0.02$, ***: $p < 0.001$).