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Effects of calcium channel blockers on calcium release-activated calcium currents in rat hepatocytes[1]

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KEY WORDS calcium channels; calcium channel blockers; patch-clamp techniques; liver; verapamil; diltiazem; nifedipine; cultured cells

ABSTRACT

AIM: To study the influences of calcium channel blockers on calcium release-activated calcium currents (I_{CRAC}) in rat hepatocytes. **METHODS**: Whole-cell patch-clamp technique was used. **RESULTS**: The peak amplitude of I_{CRAC} was -0.41 nA $\pm$ 0.09 nA (n = 15), its reversal potential was about 0 mV. Verapamil (Ver), diltiazem (Dil), and nifedipine (Nif) decreased I_{CRAC} strikingly, without affecting its reversal potential. The inhibitory rate of Ver 5 μmol·L^{-1} was 40 % $\pm$ 12 % (n = 3), Ver 50 μmol·L^{-1} reduced the peak amplitude of I_{CRAC} from -0.49 nA $\pm$ 0.12 nA to -0.20 nA $\pm$ 0.09 nA ($P < 0.01$ *vs* control, $n = 5$). The inhibitory rate was 57 % $\pm$ 15 %. Dil 50 μmol·L^{-1} and Nif reduced I_{CRAC} from -0.43 nA $\pm$ 0.10 nA to -0.29 nA $\pm$ 0.07 nA ($P < 0.01$ *vs* control, $n = 5$), from -0.32 nA $\pm$ 0.08 nA to -0.27 nA $\pm$ 0.08 nA ($P < 0.01$ *vs* control, $n = 5$). The inhibitory rate was 31 % $\pm$ 11 %, 19 % $\pm$ 7 %, respectively. The amplitude of I_{CRAC} was dependent on extracellular Ca^{2+} concentration. The peak amplitude of I_{CRAC} was -0.21 nA $\pm$ 0.08 nA (n = 3) in Tyrode's solution with Ca^{2+} 1.8 mmol·L^{-1} ($P < 0.01$ *vs* the peak amplitude of I_{CRAC} in external solution with Ca^{2+} 10 mmol·L^{-1}). **CONCLUSION**: The three calcium antagonists inhibited I_{CRAC} effectively and protected hepatocytes from calcium overload via the inhibition of I_{CRAC}.

INTRODUCTION

The drastic increase of calcium in hepatocytes caused by the hepatotoxic substance diamidinothionaphthene (98/202) killed the cell. Verapamil (Ver), nifedipine (Nif), and diltiazem (Dil) were effective in preventing the cell death caused by substance 98/202[1]. Mora NP had a transplantation experiment of liver, which had been protected hypothermally with Ver for 24 h and Ver had a protecting function[2].

The Ca^{2+} influx was mainly mediated by voltage-dependent Ca^{2+} channels and receptor-mediated Ca^{2+} channels. Most scholars held that store depletion-dependent Ca^{2+} channel mainly acted to regulate Ca^{2+} influx in nonexcitable cells, namely, by the calcium release-activated calcium current, which was called receptor-mediated Ca^{2+} channels generally[3].

Hepatocytes as the nonexcitable cells were short of the voltage-dependent Ca^{2+} channels[4]. Calcium influx in isolated hepatocytes was studied by indirect methods and it mainly depended on receptor-mediated Ca^{2+} entry[4]. Calcium antagonists Ver, Nif, and Dil principally affected the voltage-dependent Ca^{2+} channels. Then how did calcium antagonists decrease the cytosolic free Ca^{2+} concentration and protect hepatocytes? The purpose of this paper was to observe the effects of calcium antagonists on calcium release-activated calcium current (I_{CRAC}) in isolated rat hepatocytes and probe into initially the mechanism of protection from cytosolic calcium overload.

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MATERIALS AND METHODS

Isolation of single hepatocytes Hepatocytes were isolated[6]. Briefly, adult Wistar rats of either sex ($n = 43$, Grade Ⅱ, Certificate № 034, weighing 175 g ± 25 g) were anesthetized by inhalation of ether and ip pentobarbital sodium 30 mg·kg^{-1}. The portal vein was cannulated and perfused with oxygenated Ca^{2+}-free Hanks' solution 25 mL·min^{-1} at 37 ℃ for 4 – 5 min followed by perfusion with Ca^{2+}-free Hanks' solution containing collagenase (Type Ⅳ, Sigma) (0.5 g·L^{-1}) for 10 – 15 min. The liver was chopped in Ca^{2+}-free Hanks' solution 10 mL. The cell suspension was filtered through gauze to remove fibrous tissues. Cells were incubated in KB (Krebs-Henseleit) medium for 2 – 3 h and preserved in DMEM (Dulbecco's modified Eagle's medium) at 4 ℃.

Drugs and solutions Ca^{2+}-free Hanks' solution was prepared without Ca^{2+} and Mg^{2+}. The KB solution was composed of glutamic acid 70, turine 15, KCl 130, KH_2PO_4 10, HEPES 10, glucose 11, edetic acid 0.5 mmol·L^{-1}. The external solution contained NaCl 140, KCl 2.8, $CaCl_2$ 10, $MgCl_2$ 0.5, glucose 11, HEPES 10 mmol·L^{-1}. The internal solution contained potassium-glutamate 145, NaCl 8, $MgCl_2$ 1, Mg-ATP 0.5, egtazic acid 10, HEPES 10 mmol·L^{-1}). The Tyrode's solution was composed of NaCl 144, KCl 4.0, $CaCl_2$ 1.8, $MgCl_2$ 0.53, NaH_2PO_4 0.33, glucose 5.5, and HEPES 5 mmol·L^{-1}, pH 7.2. Ver, Nif (Beijing Tianfeng Pharmaceutical Factory), Dil (Sigma).

Electrophysiologic recording The recording chamber (1.5 mL) was perfused with external solution 2 – 3 mL·min^{-1} at 22 ± 2 ℃. Membrane ionic currents were measured in a standard whole-cell patch clamp configuration with an Axopatch-1D amplifier (Axon Instruments, USA). The pipettes were pulled in two stages from hard glass capillaries using a vertical microelectrode puller (Narishige, Japan). Electrode had a resistance of 3 – 5 MΩ for whole-cell recording. Membrane potential and current signal were monitored with a dual beam memory oscilloscope (VC-10, Nihon Kohden) and stored in a computer. For the current measurement, the holding potential was kept at 0 mV, the command potential was −100 mV, and the duration was 200 ms. Data were presented as $\bar{x} \pm s$ and compared by t test.

RESULTS

Voltage pulses were applied every 5 s at holding potential of 0 mV, command potential of −100 to +80 mV, with 20 mV increments. The peak amplitude of I_{CRAC} was −0.41 nA ± 0.09 nA ($n = 15$), its reversal potential was about 0 mV (Fig 1). The current was steady and without rundown within 5 min.

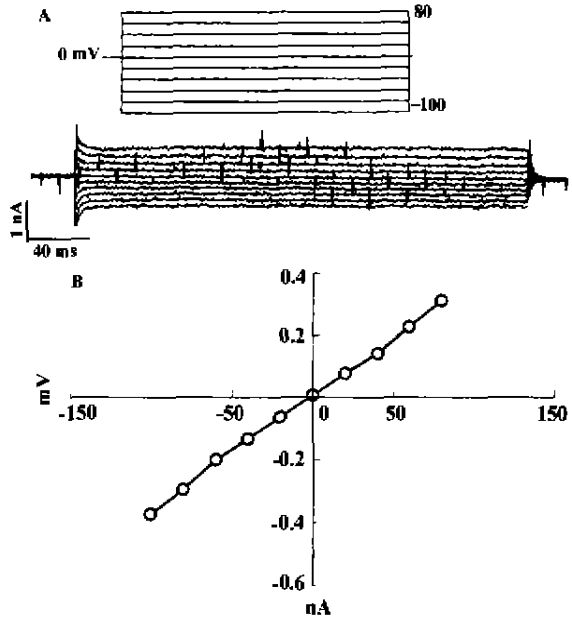

Fig 1. Current of calcium release-activated calcium current (I_{CRAC}) in rat hepatocytes. A) I_{CRAC}: voltage pulses were applied every 5 s at holding potential of 0 mV, command potential of −100 to +80 mV, in 20 mV increments. B) Current-voltage relationship from the data in A. Measured at the beginning point of the pulse.

Ver, Dil, and Nif decreased I_{CRAC}, and did not affect its reversal potential. The inhibitory rate of Ver 5 μmol·L^{-1} was 40 % ± 12 % ($n = 4$), Ver 50 μmol·L^{-1} reduced the peak amplitude of I_{CRAC} from −0.49 nA ± 0.12 nA to −0.20 ± 0.09 nA ($P < 0.01$ *vs* control, $n = 5$), the inhibitory rate was 57 % ± 15 % (Fig 2).

Either Dil or Nif 50 μmol·L^{-1} reduced I_{CRAC} from −0.43 nA ± 0.10 nA to −0.29 nA ± 0.07 nA ($P < 0.01$ *vs* control, $n = 5$) and from −0.32 nA ± 0.08 nA to −0.27 nA ± 0.08 nA ($P < 0.01$ *vs* control,

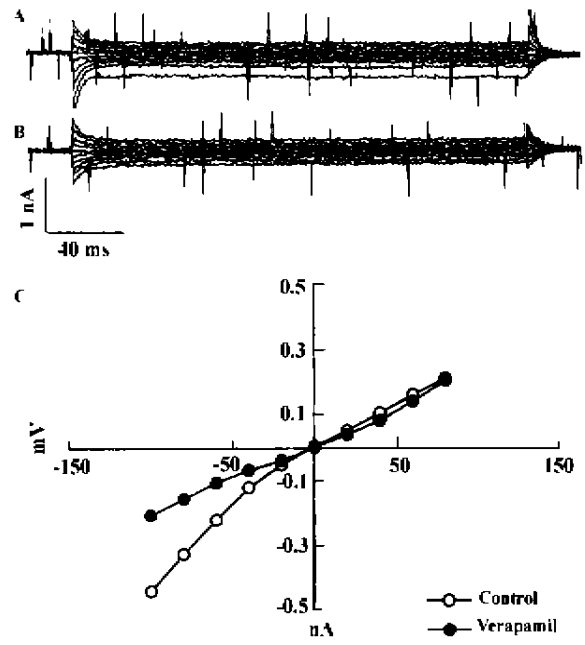

Fig 2. I_{CRAC} **in isolated rat hepatocytes before (A) and after (B) Ver 50 μmol·L^{-1}.**

$n = 5$), respectively. The inhibitory rate was 31 % ± 11 %, 19 % ± 7 %, respectively (Fig 3).

The amplitude of I_{CRAC} was dependent on extracellular Ca^{2+} concentration. The peak amplitude of I_{CRAC} was -0.21 nA ± 0.08 nA ($n = 3$) in Tyrode' solution with Ca^{2+} 1.8 mmol·L^{-1} ($P < 0.01$ *vs* the peak amplitude of I_{CRAC} in external solution with Ca^{2+} 10 mmol·L^{-1}). Ver 5 μmol·L^{-1} decreased I_{CRAC} from -0.21 nA ± 0.08 nA to -0.14 nA ± 0.05 nA ($n = 3$).

DISCUSSION

Calcium influx depended on the activation of Ca^{2+} currents caused by the depletion of intracellular stores in nonexcitable cells[7], which had been named I_{CRAC} The existence of I_{CRAC}-type currents has been recently demonstrated in many different cells.

The experiments had been carried out in high extracellular Ca^{2+} concentration in order to observe the effect of drugs on I_{CRAC}. The results showed that the reversal potential of I_{CRAC} in rat hepatocytes was about 0 mV and the holding potential was 0 mV appeared, although the gating of I_{CRAC} was independent of membrane voltage, there was, nevertheless, a strong dependence of Ca^{2+} influx on the driving force exerted by the membrane potention, ie. the influx rate increased with hyperpolarization and decreased with depolarization[3]. Our results were the same as

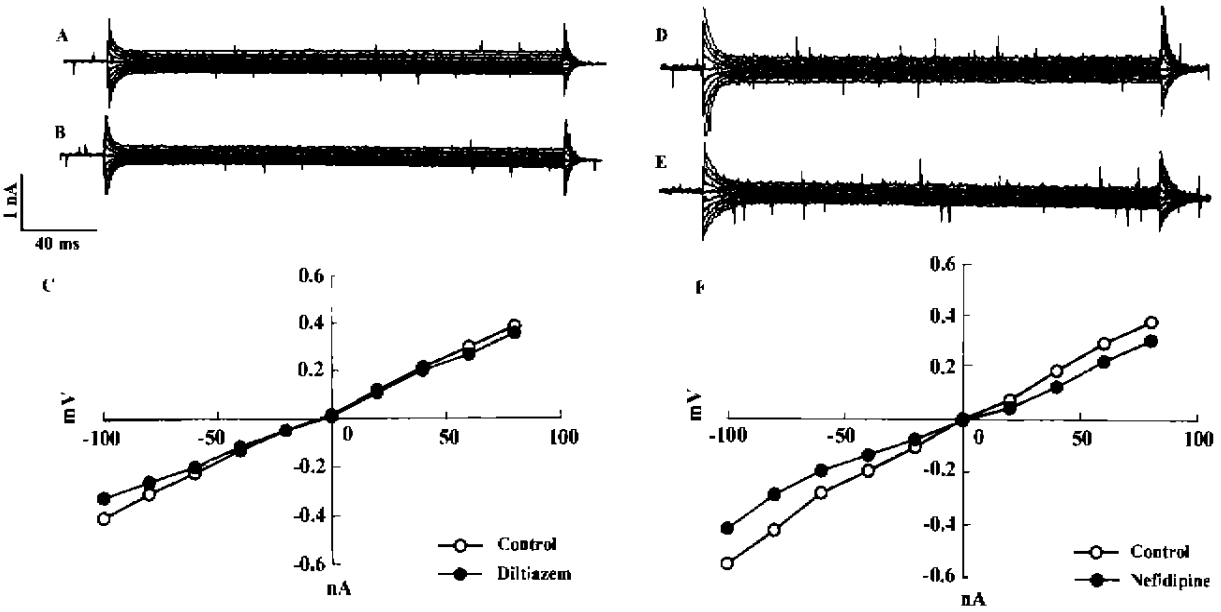

Fig 3. I_{CRAC} **in rat hepatocytes. A, B) control and Dil 50 μmol·L^{-1}; C) current-voltage relationship of I_{CRAC} obtained from A and B; D, E) control and Nif 50 μmol·L^{-1}; F) current-voltage relationship of I_{CRAC} obtained from currents of D and E.**

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documents. The amplitude of I_{CRAC} depended on $[Ca^{2+}]_o$ in rat hepatocytes. The amplitude of I_{CRAC} was strikingly lower in Tyrode's solution ($CaCl_2$ 1.8 mmol · L^{-1}) than that in high external Ca^{2+} concentration ($CaCl_2$ 10 mmol·L^{-1})[8].

Calcium antagonists were mainly used in cardiovascular system, which was widely applied in clinic as antihypertension, antiangina pectoris, antiarrhythmic drugs and organ transplantation for its protection of histocyte by inhibiting calcium overload. Calcium antagonists inhibited calcium overload by decreasing calcium influx, which blocked voltage-operated calcium channels (VOC) in cardiac muscle and smooth muscle[9]. Three calcium antagonists Ver, Dil, and Nif decreased the amplitude of I_{CRAC} at 50 μmol·L^{-1} in the experiments, the effect of Ver was the most distinct and that of Dil and Nif the second place among them. As the nonexcitable cell rat hepatocytes is without VOC, the experiment results indicated that the 3 calcium antagonists could protect hepatocytes by inhibiting I_{CRAC} to decrease intracellular Ca^{2+} concentration.

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钙通道阻滞剂对大鼠肝细胞钙释放激活的钙电流的影响[1]

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关键词 钙通道; 钙通道阻滞剂; 膜片箝技术; 肝; 维拉帕米; 地尔硫䓬; 硝苯地平; 培养的细胞

目的: 研究钙通道拮抗剂对大鼠肝细胞钙释放激活的钙电流(I_{CRAC})的影响. **方法**: 应用全细胞膜片箝技术. **结果**: I_{CRAC}的电流峰值是(-0.41±0.09) nA (n=15), 反转电位约为 0 mV. 维拉帕米(Ver), 地尔硫䓬(Dil)和硝苯地平(Nif)显著降低I_{CRAC}, 不影响它的反转电位. Ver 5 μmol·L^{-1}的抑制率是 40 %±12 % (n=3), Ver 50 μmol·L^{-1}使 I_{CRAC}的幅值从(-0.49±0.12) nA 降到(-0.20±0.09) nA (n=5, P<0.01), 抑制率为 57 %±15 %. Dil 和 Nif 50 μmol·L^{-1}分别从(-0.43±0.10) nA (n=5), (-0.32±0.08) nA (n=5)降到(-0.29±0.07) nA (P<0.01), (-0.27±0.08) nA (P<0.01). 抑制率分别为 31 %±11 %, 19 %±7 %. I_{CRAC}的幅值大小依赖细胞外钙浓度. I_{CRAC}在含台氏液 1.8 mmol·L^{-1}中电流幅值为(-0.21±0.08) nA (n=3, P<0.01). **结论**: 这三种钙通道拮抗剂有效抑制 I_{CRAC}并且通过抑制I_{CRAC}减少肝细胞钙超载.

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