BIBLID: ISSN 0253-9756 Acta Pharmacologica Sinica 中国药理学报 1998 Jan; 19 (1): 24 – 26

Effects of *N*-methyl berbamine on delayed outward potassium current in isolated rat hepatocytes[1]

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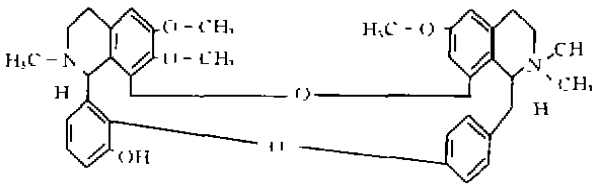
KEY WORDS *N*-methyl berbamine; patch-clamp techniques; ion channels; potassium channels; liver

AIM: To study the efftcts of *N*-methyl berbamine (NMB) on the delayed outward potassium currents (I_k) in isolated rat hepatocytes. **METHODS**: With patch-clamp techniques and whole-cell recording method, holding potential −50 mV, command potential +30 to +140 mV, duration 900 ms. **RESULTS**: NMB reducded I_k in a concentration-dependent manner. When the concentrations of NMB were 20, 50, 400 nmol·L^{-1} and 50 μmol·L^{-1}, the amplitude values of I_k were decreased to 3.6 ± 0.4 ($P > 0.05$), 2.1 ± 1.6 ($P > 0.05$), 3.7 ± 1.6 ($P < 0.05$), 2.3 ± 1.3 nA ($P < 0.01$) from 4.4 ± 1.0 ($n = 4$), 2.5 ± 1.8 ($n = 4$), 5.8 ± 2.1 ($n = 5$), 4.6 ± 1.3 ($n = 6$) nA of control, respectively. The inhibitory rates were 10 %, 15 %, 37 %, and 51 %, respectively. **CONCLUSION**: NMB was a K^+ channel inhibitor.

N-methyl berbamine (NMB) is a dibezyliso-quinoline alkaloid isolated from a Chinese plant, *Berberis poireii* Schneid. The previous studies showed that NMB could block calcium channel and had inhibition effect on outward K^+ current[1]. The purpose of this study was to observe the effect of *N*-methyl-berbamine (NMB) on delayed outward K^+ current in isolated rat hepatocytes with patch-clamp techniques in whole cell configuration.



***N*-Methyl berbamine (NMB)**

MATERIALS AND METHODS

Isolation of single hepatocytes Hepatocytes were isolated using the method[2] but modified. Briefly, ♂ adult Wister rats (205 ± 22 g) were anesthetized by inhalation of ether and ip of pentobarbital sodium 30 mg·kg^{-1}. The portal vein was cannulated and the inferior vena cava was cut off. Oxygenated Ca^{2+}-free Hanks' solution at 37 ℃ was perfused 25 mL·min^{-1} 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 10 mL of Ca^{2+}-free Hanks' solution. The cell suspension was filtered through gauze to remove fibrous tissues. Cells were incubated in KB medium for 2 – 3 h.

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, taurine 15, KCl 130, KH_2PO_4 10, *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulphonic acid 10, glucose 11, edetic acid 0.5 (mmol·L^{-1}). The bath solution contained NaCl 144, KCl 4.0, $CaCl_2$ 1.8, $MgCl_2$ 0.53, NaH_2PO_4 0.33, glucose 5.5, HEPES 5 (mmol·L^{-1}). The intraelectrode solution contained KCl 130, K_2ATP 5, creatine phosphate 5 and HEPES 5 (mmol·L^{-1}), pH 7.4. NMB (white powder, purity >98.2 %) was provided from Department of Plant Chemistry in the Forestry and Pedology Institute in Shenyang city.

Electrophysiologic recording The recording chamber (1.5 mL) was perfused with bath solution 2 – 3 mL·min^{-1} at room temperature (23 ± 2 ℃). Membrane ionic currents were measured in a standard whole-cell patch-clamp configuration with an Axopatch-1D amplifier (Axon Instruments, USA)[3]. 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 on a computer. For the current measurement, the holding potential was kept at −50 mV, the command potential was +140 mV and the duration was 900 ms. Data were represented as $x \pm s$.

[1] Project supported by the National Natural Science Foundation of China. No 39400138
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Received 1996-08-03 Accepted 1997-07-02

RESULTS

Depolarizing voltage pulses were applied every 5 s from command potential of +30 to +140 mV, in 10 mV increments. The current was steady and without rundown (Fig 1).

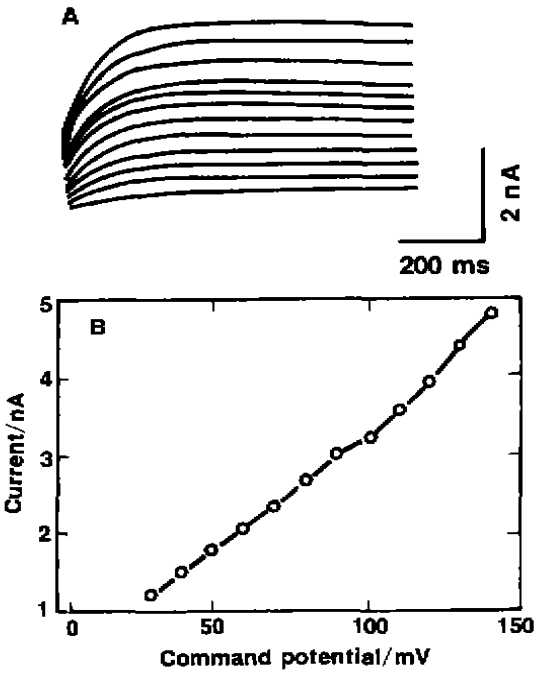

Fig 1. *Outward K^+ current in isolated rat hepatocytes.* A) Outward currents at 23 ± 2 ℃. B) Current-voltage relationship from the data in A. Measured at the end point of the pulse.

When the bath solution was changed to tetraethylammoniumchlorid (TEA) 4 mmol·L^{-1}, the K^+ current was inhibited by TEA. The inhibition recovered almost completely after 5 min of washing with the bath solution (Fig 2).

The peak amplitudes of K^+ current (I_k) were decreased by NMB concentration-dependently. When cells were kept at −50 mV and command potential was +140 mV, I_k reached the peak. At 20, 50, 400 nmol·L^{-1}, and 50 μmol·L^{-1}, it was decreased from 4.4 ± 1.0 (n = 4), 2.5 ± 1.8 (n = 4), 5.8 ± 2.1 (n = 5), and 4.6 ± 1.3 (n = 6) nA of control to 3.6 ± 0.4 (P > 0.05), 2.1 ± 1.6 (P > 0.05), 3.7 ± 1.6 (P < 0.05), and 2.3 ± 1.3 (P < 0.01) nA, respectively. The inhibitory rates were 10 %, 15 %, 37 %, and 51 %, respectively (Fig 1).

The effect of NMB was partly canceled after

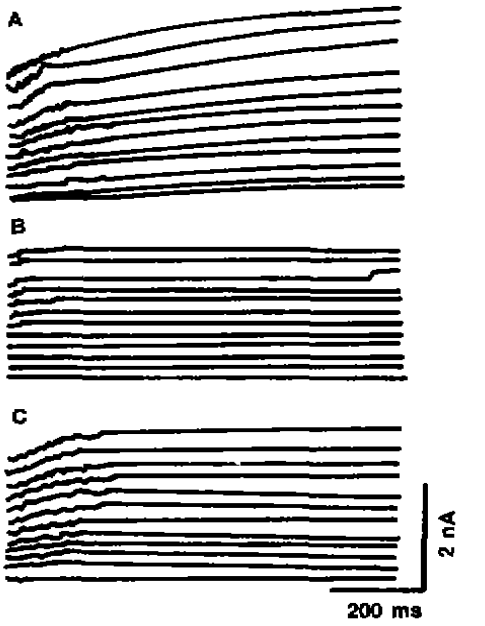

Fig 2. Effects of TEA on delayed outward K^+ current A) control; B) TEA 4 mmol·L^{-1}; C) washout.

washout of NMB. The current-voltage relationships of control and exposure to NMB 400 nmol·L^{-1} were shown in Fig 3.

The concentration-effect curve of NMB was shown in Fig 4.

DISCUSSION

NMB decreased the I_k distinctly and concentration-dependently. K^+ is significant of the metabolism of liver. It was reported that K^+ was associated with the water equilibrium of hepatocytes[4]. Hepatocellular hydration state was one important determinant of protein turnover, an increase in the hepatocellular hydration state apparently acted as an anabolic signal by inhibiting proteolysis and stimulating protein synthesis. Conversely, cellular shrinkage acted like a protein catabolic signal[5]. Transcellular bile acid transport was integrated in the regulation of intracellular pH, K^+ homeostasis and the membrane potential. K^+-depletion resulted in inhibition of bile acid secretion despite rising intracellular concentration[6].

During ischemia and hypoxia caused by injury and other factors, hepatocyte volume and K^+ conductance were increased. It was reported that the extracellur

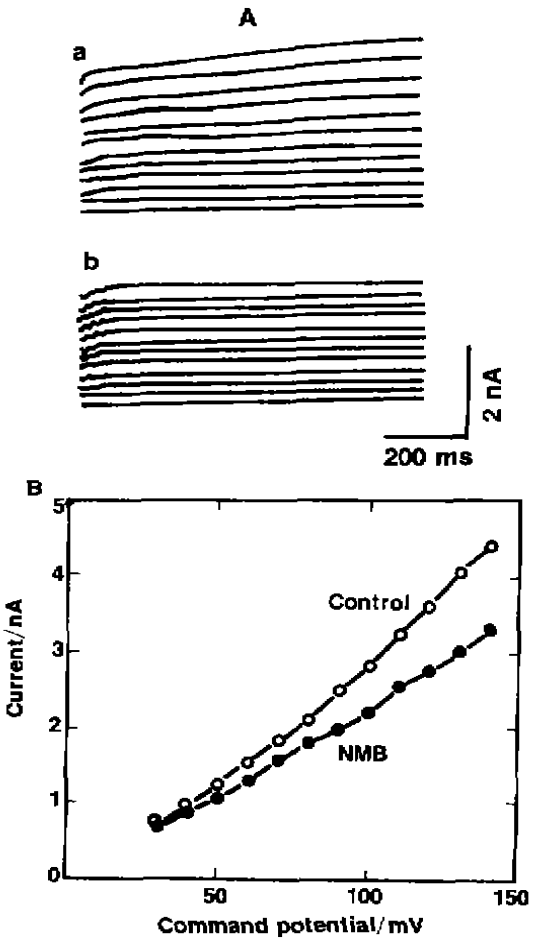

Fig 3. Effect of NMB on I_k of isolated rat hepatocytes. Holding potential −50 mV; Command potentials from +30 to +140 mV; stimulating duration 900 ms. A: a) Control, b) NMB. B: Current-voltage relationship of I_k in NMB.

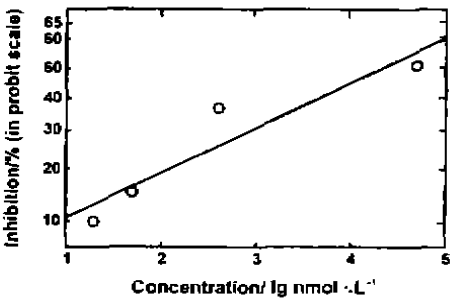

Fig 4. Concentration-response curve of NMB on I_k in isolated rat hepatocytes.

K^+ increase would result in hyperpolarization and hyperexcitability of cells. This would lead to a delayed cell death[7]. We found that the outward K^+ current was increased during hypothermia in isolated rat hepatocytes which could support above ideas (not issued). The result suggested that NMB protected liver because it could block K^+ channel and decrease the extracellular K^+.

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N-甲基小檗胺对离体大鼠肝细胞外向钾电流的影响[1]

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关键词 *N*-甲基小檗胺；膜片箝技术；离子通道；钾通道；肝

目的：研究 *N*-甲基小檗胺（NMB）对大鼠肝细胞外向钾电流的影响。**方法**：应用膜片箝技术和全细胞记录方法，箝制电位 −50 mV，指令电位 +30 至 +140 mV，持续时间 900 ms。**结果**：NMB 以浓度依赖方式降低外向钾电流。电流幅值在 20，50，400 nmol·L^{-1} 和 50 μmol·L^{-1} 时分别从 4.4±1.0（n=4），2.5±1.8（n=4），5.8±2.1（n=5），4.6±1.3（n=6）nA 降到 3.6±0.4（P>0.05），2.1±1.6（P>0.05），3.7±1.6（P<0.05），2.3±1.3（P<0.01）nA。抑制率分别为 10 %，15 %，37 %，51 %。**结论**：NMB 是一种钾通道阻断剂，