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Bepridil inhibition of sodium current in rat hippocampal CA1 neurons[1]

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KEY WORDS hippocampus; patch-clamp techniques; bepridil; sodium channels; electrophysiology

ABSTRACT

AIM: To study the effects of bepridil on sodium current in rat hippocampal neurons. **METHODS**: All experiments were performed on acutely isolated hippocampal pyramidal neurons by means of whole-cell patch-clamp techniques. Recording media contained ion channel blockers to allow the selective activation of voltage-dependent sodium currents. **RESULTS**: Bepridil reduced the amplitudes of sodium current in time- and concentration-dependent manners. The half-blocking time was about 10 min in bepridil 10 $\mu mol \cdot L^{-1}$, and IC_{50} was 2.6 (2.3 - 2.9) $\mu mol \cdot L^{-1}$. Bepridil 10 $\mu mol \cdot L^{-1}$ shifted the maximal activation of sodium current from - 50 mV to - 40 mV, and the characteristic voltage of inactivation from - 71 mV to - 89 mV without changing the slope factor. **CONCLUSION**: Bepridil blocked voltage-dependent sodium current of hippocampal CA1 neurons and might have therapeutic actions for ischemia-induced brain damage.

INTRODUCTION

Bepridil is an antianginal agent with multiple therapeutic actions[1-2], and has been used for the treatment of supraventricular and ventricular arrythmias[3]. Bepridil decreased the upstroke velocity of depolarization of the action potential (V_{max}) and suppressed idiopathic ventricular fibrillation[4,5]. Patch-clamp studies showed that bepridil inhibited calcium and sodium currents in guinea pig ventricular myocytes[6-8], potassium currents in rat hippocampal CA1 neurons[9], and blocked NMDA channels in rat hippocampal neurons[10].

Recently, we have reported that bepridil prevented ischemic brain damage induced by carotid ligation[11]. Ischemia is imbalance between energy supply and demand. Down-modulation of voltage-gated sodium channels is an effective way of reducing energy demand[12]. The effects of bepridil on sodium current in CNS neurons, however, are uncertain. In the present study, we examined the effects of bepridil on sodium currents in hippocampal neurons.

MATERIALS AND METHODS

Chemicals Bepridil obtained from Changzhou Fourth Pharmaceutical Factory was dissolved in the extracellular solution and bath applied. Tetraethylammonium (TEA) and tetrodotoxin (TTX) were purchased from Sigma Co.

Rats Sprague-Dawley rats (n = 60, ♀ ♂, 12 - 16 d old, Grade Ⅱ, Certificate № 003) were obtained from the Experimental Animal Center, Institute of Zoology, Chinese Academy of Sciences.

Cell preparation and solutions All experiments were performed on hippocampal CA1 pyramidal cells, which were acutely isolated from 12 - 16 d old SD rats. Hippocampal slices were treated enzymatically with subsequent mechanical isolation of single cells as previously described[9]. The cell suspension was transferred into a 35-mm culture dish, filled with

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extracellular solution 2 mL containing NaCl 150, KCl 5, $MgCl_2$ 1.1, $CaCl_2$ 2.6, HEPES 10, glucose 10 $mmol \cdot L^{-1}$, adjusted to pH 7.4, and was ready for experiments after 20 min. Cells with a pyramidally shaped soma and short dendrites and axon (20 - 40 μm) were chosen for study and used within 3 h after dissociation.

Sodium current recording technique Whole-cell currents were recorded with an EPC-7 patch-clamp amplifier (List, Germany), filtered at 3 kHz, digitized at 5 kHz, and stored in a PC 486 computer using a Labmaster TL-1 interface (Axon Instrument, USA) and pCLAMP version 5.5.1 software. Patch electrodes were pulled in two steps from borosilicate glass capillaries 1.5 mm in outer diameter and had tip resistance of 2 - 5 MΩ when filled with intracellular solution CsCl 140, TEA-Cl 2, $MgCl_2$ 2, HEPES 20, egtazic acid 10, Mg-ATP 3 $mmol \cdot L^{-1}$, adjusted to pH 7.2 with Tris. Cells were held at holding potential (HP) - 100 mV and Na^+ currents were elicited by a series of 80 ms command depolarizing steps from - 100 mV to + 30 mV (10 mV increment each step) applied at a rate of 1 Hz. All experiments were conducted at room temperature (21 - 24 ℃).

Data analysis All data were analyzed by the use of pCLAMP CLAMPFIT procedures (Axon Instrument) and Sigmaplot (Jandel Scientific) software, and were given as $\bar{x} \pm s$. Significant differences between groups were evaluated by F test.

RESULTS

Effects of bepridil on sodium current In voltage clamp mode, when internal KCl was replaced by CsCl, and internal solution was Ca^{2+}-free, a series of depolarization steps to 0 mV from - 100 mV activated inward currents, which could be blocked by tetrodotoxin (TTX, 1 $\mu mol \cdot L^{-1}$), therefore referred to as sodium current (I_{Na}).

In control experiments, there was a little decrease in the amplitude of I_{Na} time-dependently. The peak current recorded by the end of 15 min was decreased by 9.1 % ± 5.2 % (n = 10). Upon the administration of bepridil (10 $\mu mol \cdot L^{-1}$), there was a substantial decrease in amplitude of I_{Na}. As shown in Fig 1A, this blocking action progressed time-dependently.

The onset of action was at 1 - 2 min after addition of bepridil 10 $\mu mol \cdot L^{-1}$, there was a significant decrease in the I_{Na} at 3 min. Blocking action reached a steady state about 15 min after application of bepridil, the half-blocking time for I_{Na} was (10 ± 3) min

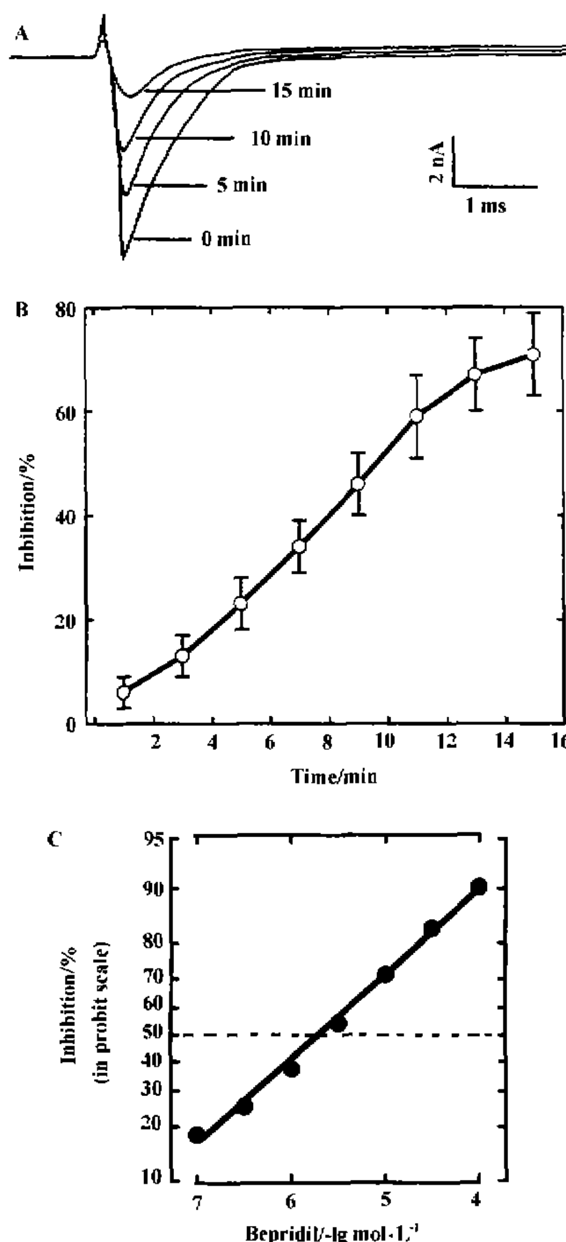

Fig 1. Effect of bepridil 10 $\mu mol \cdot L^{-1}$ on I_{Na} in a pyramidal neuron. A) The maximal I_{Na} were evoked from - 100 mV (HP) to - 50 mV (at 0 and 5 min) and - 40 mV (at 10 and 15 min). B) and C) Blocking action of bepridil was determined according to $(1 - I_{bep}/I_c) \times 100$ %, where I_c and I_{bep} were the maximal I_{Na} before and after bepridil, respectively. n = 5 - 6 cells. $\bar{x} \pm s$.

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($n=5$). The maximal suppressive rate of bepridil 10 $\mu mol \cdot L^{-1}$ on I_{Na} was 73 % ± 7 % (Fig 1B). After a 15-min drug exposure and washing the cell for 10 min, amplitudes of I_{Na} were difficult to be reversed.

Bepridil also produced a concentration-dependent blockade of I_{Na}. This blocking action progressed with increment in concentrations from 0.1 to 100 $\mu mol \cdot L^{-1}$ at a 15-min drug exposure. The IC_{50} value for blockade of I_{Na} was 2.6 (2.3 – 2.9) $\mu mol \cdot L^{-1}$, with the maximal suppression of I_{Na} up to 90 % ± 10 % at 100 $\mu mol \cdot L^{-1}$ (Fig 1C).

Effects of bepridil on the current-voltage relationship Current-voltage (I-V) curves of I_{Na} were obtained by depolarization steps from a holding potential of −100 mV to 30 mV. The maximal activation of I_{Na} occurred at the −50 mV or −40 mV, and then followed by a progressive decrease in I_{Na}. After a 15-min exposure of the cell to each drug concentration, bepridil elevated the I-V curves in a concentration-dependent manner, and delayed maximal activation of I_{Na} by 10 mV at 10 $\mu mol \cdot L^{-1}$ (Fig 2).

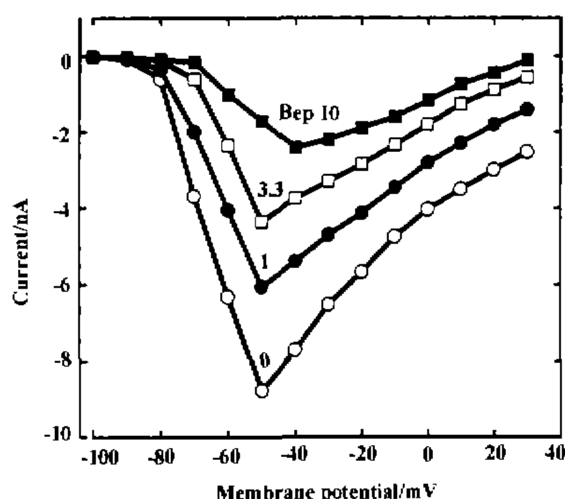

Fig 2. Influence of bepridil 1 – 10 μmol·L⁻¹ on the peak current amplitudes of I_{Na}, evoked by the step pulses from −100 mV to +30 mV at 15 min. $n=6$ cells.

Effect of bepridil on activation and inactivation kinetics of I_{Na} The peak amplitudes for I_{Na} evoked by a series of step pulses from −100 mV to −30 mV were converted into conductance by use of the equation $G = I/(V - V_k)$, where V = membrane potential, V_k = reversal potential. The normalized conductance was fitted well with a Boltzmann equation: $G/G_{max} = 1/\{1 + \exp[(V - V_h)/k]\}$, with V_h being the membrane potential at half-activation and k being the slope factor. The values of V_h for activation of I_{Na} in the absence and the presence of bepridil 10 $\mu mol \cdot L^{-1}$ at 15 min were −65 mV ± 7 mV ($n=5$) and −54 mV ± 7 mV ($n=5$, $P<0.05$), respectively, with a slope factor k of −6.2 mV ± 1.4 mV ($n=5$) and −6.5 mV ± 1.3 mV ($n=5$, $P>0.05$). Bepridil shifted activation of curve towards more positive potential without change the slope factor (Fig 3).

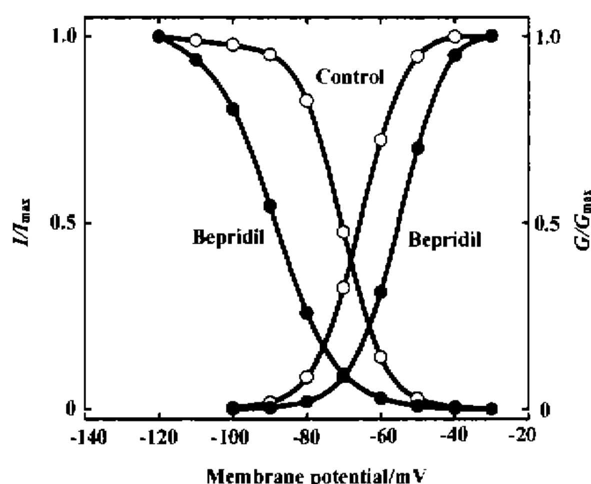

Fig 3. Effects of bepridil on steady state activation and inactivation of I_{Na} before and 15 min after bepridil 10 μmol·L⁻¹. $n=6$ cells

The inactivation curve of I_{Na} was assessed at selected membrane potentials using the standard two-pulse protocol. A 1000-ms prepulse to the designated level of membrane potential was followed by a 1-ms interpulse interval then by a 30-ms test pulse to −30 mV. The curves drawn through the data point were described by a Boltzmann equation. Under control conditions, V_h was −71 mV ± 4 mV ($n=6$), with a slope factor k of 6.0 ± 2.1 ($n=6$); and in the presence of bepridil 10 $\mu mol \cdot L^{-1}$ at 15 min, V_h was −89 mV ± 12 mV ($n=5$, $P<0.05$), with a slope factor k of 6.6 ± 1.4 ($n=5$, $P>0.05$). The results showed that bepridil substantially shifted inactivation curve of I_{Na} towards more negative potential without change in the slope factor (Fig 3).

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DISCUSSION

The results of the present study indicated that bepridil inhibited the I_{Na} of rat hippocampal neurons in a concentration- and time-dependent fashion with change of the activation and inactivation kinetics.

Brain ischemia occurs when a reduction of cerebral blood flow induced oxygen and glucose deprivation. Brain tissue damage subsequent to ischemia results from multifactoral and interrelated processes. But cation imbalance of neurons is also an important factor. Ischemia or severe anoxia induced sustained Na^+ influx and caused intracellular Na^+ overload, which reversed the action of the Na^+/K^+-ATPase and stimulated ATP turnover, facilitated energy expenditure[13]. In addition, Na^+ loading also reversed Na^+/Ca^{2+} exchange, resulted in an increase of intracellular Ca^{2+}[14,15]. Activity of Ca^{2+} ATPase was decreased because of energy deprivation, Ca^{2+} efflux was reduced, which caused Ca^{2+} overload, triggering a cascade of harmful events via activation of proteases, phospholipases and endonucleases[16]. Sodium channel blockers delayed depolarization and reduced Na^+ influx with subsequent energy preservation. Many studies have demonstrated that sodium channel blockers such as lidocaine, lifarizine, and BW1003C87 prevent against ischemic brain damage[12]. Our previous findings showed that bepridil was effective in protection against cerebral ischemia[9]. Blockade of sodium channels is considered to be a target for cerebral protection[17]. The result that bepridil blocked Na^+ current on hippocampal CA1 neurons may afford its neuroprotective properties.

In conclusion, bepridil potently inhibited the inward sodium current that may be an interpretation for its prevention against ischemic brain damage.

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苄普地尔抑制大鼠海马 CA1 区锥体细胞钠电流[1]

胡慧娟，阳冬梅[2]，戴德哉[3]，吴才宏[2]，周培爱[2]（中国药科大学中药药理教研室，[3]药理研究室，南京 210009；[2]北京大学生物膜与膜生物工程国家重点实验室，北京 100871，中国）

关键词 海马；膜片箝技术；苄普地尔；钠通道；电生理学

目的：研究苄普地尔对大鼠海马 CA1 区锥体细胞钠电流的影响．**方法**：膜片箝全细胞记录技术．**结果**：苄普地尔显著降低大鼠海马 CA1 区锥体细胞钠电流，作用呈时间及浓度依赖关系．苄普地尔 10 μmol·L^{-1}的半阻断时间约为 10 min．IC_{50}为 2.6 (2.3－2.9) μmol·L^{-1}．苄普地尔 10 μmol·L^{-1}右移最大电流的激活电位 10 mV，左移半失活膜电位 18 mV，表明其电压依赖地影响钠通道的激活和失活过程．**结论**：苄普地尔阻断大鼠海马 CA1 区锥体细胞钠电流，可能是其抗脑缺血机制之一．

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