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Effects of excitatory amino acids and nimodipine on calcium currents in cultured rat cortical neurons[1]

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KEY WORDS calcium channels; neurons; patch-clamp techniques; nimodipine; cerebral cortex; excitatory amino acids; Bay-K-8644; glutamate; aspartic acid

ABSTRACT

AIM: To study the effect of excitatory amino acid (EAA) and calcium channel blocker on neuronal calcium channels. **METHODS**: With patch-clamp technique (whole-cell recording), the effects of Bay-K-8644, cesium glutamate, potassium aspartate, and nimodipine (Nim) on calcium currents (I_{Ca}) in cultured cortical neurons of neonatal rats were studied. **RESULTS**: I_{Ca} was raised obviously by Bay-K-8644 and glutamate. I_{Ca} was raised concentration-dependently by aspartate (0.5, 5, 50 mmol·L^{-1}), with increasing rates 15 % ± 3 %, 37 % ± 3 %, and 53 % ± 6 %, respectively. The inhibition of I_{Ca} was obvious while adding Nim in the bath solution. With Nim 10 μmol·L^{-1}, the inhibitory rate was 46 % ± 4 %. **CONCLUSION**: EAA had increasing effects on neuronal calcium currents and Nim inhibited Ca^{2+} influx in neurons.

INTRODUCTION

In recent years, Ca^{2+} overload in neurons was reported during traumatic brain injury or ischemic brain damage. The reasons of neuronal Ca^{2+} overload were involved in many ways. The effect of excitatory amino acids (EAA) was one of the most important reasons of it[1]. However, the effect of EAA on neuronal calcium channels was not clear. The method to study Ca^{2+} overload in neurons was usually used in biochemical methods[1,2]. The purpose of the present study was to investigate the possibility of EAA inducing the opening of neuronal calcium channels, and to clarify the inhibitory effect of nimodipine (Nim) on neuronal calcium channels.

MATERIALS AND METHODS

Cell culture Cortical cells were obtained from newborn (1 - 2 d) Wistar rats (n = 40, Grade Ⅱ, Certificate № 96A033) with an enzymatic (0.25 % trypsin, 37 ℃, 30 min) and mechanical dissociation method. The density of the cells was about 1 × 10^9 cells·L^{-1}. The cells were grown on glass cover slips (18 mm × 18 mm), collagen treated in a culture dish (35 mm in diameter, 2 mL/dish). The cells were cultured for 3 - 7 d in 80 % basal Eagles' medium supplemented with 10 % heat-inactivated horse serum, 10 % calf serum, glucose 6 g·L^{-1}, benzylpenicillin 100 kU·L^{-1}, and streptomycin 100 mg·L^{-1}. Arabinosylcytosine (10 μmol·L^{-1}) was added from 24 h to 72 h to prevent the growth of the non-neuronal cells. The typical neurons (Fig 1) that had a big nucleus and long dendrites were used for patch-clamp recording.

Electrophysiology The whole-cell configuration of the patch-clamp technique was used to record the whole-cell I_{Ca} with an Axopatch 1D voltage-clamp amplifier (Axon Instruments). The micropipettes were pulled by two-stage from borasilicate glass capillaries and heat-polished at the tip. Electrode resistances were 5 - 10 MΩ after filling with pipette solution. The liquid

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junction was nullified with an offset circuit before the formation of gigaseals. Currents were lowpass filtered at 1 kHz. The data were analyzed using an IBM computer with the clamp software.

Fig 1. Cultured cortical neurons (←).

Solution for electrophysiologic studies The cultures were constantly perfused with a bath solution containing tetraethlaminonium chloride (TEA-Cl) 130, CsCl 5, $BaCl_2$ 10, $MgCl_2$ 1, HEPES 10, glucose 10 mmol·L^{-1}, and tetrodotoxin (TTX) 0.5 μmmol·L^{-1} (titrated to pH 7.4 by CsOH). For experiments in the whole-cell configuration, the micropipettes for electric recording were filled with a solution containing CsCl 130, TEA-Cl 10, HEPES 10, $MgCl_2$ 2, K_2-ATP 2, egtazic acid (EGTA) 10 mmol·L^{-1}. All experiments were done at 23 ℃ ± 2 ℃. Na^+ and K^+ currents were inhibited by TTX and Cs^+/TEA, respectively. Ba^{2+} was used as the charge carrier of the current through Ca^{2+} channels to reduce Ca^{2+}-dependent run-down. When aspartate was added to the bath solution, osmotic pressure was adjusted by reducing TEA-Cl and pH was adjusted to 7.4 by CsOH.

RESULTS

When holding potential was −90 mV, with 10 mV depolarizing from −110 mV to −10 mV, the command potential that caused a maximal inward calcium current (I_{Ca}) was −50 mV (Fig 2).

I_{Ca} was raised obviously from (142 ± 34) pA to (582 ± 115) pA by Bay-K-8644 and to (340 ± 92) pA by glutamate ($P < 0.01$, $n = 8$).

When holding potential was −40 mV, the

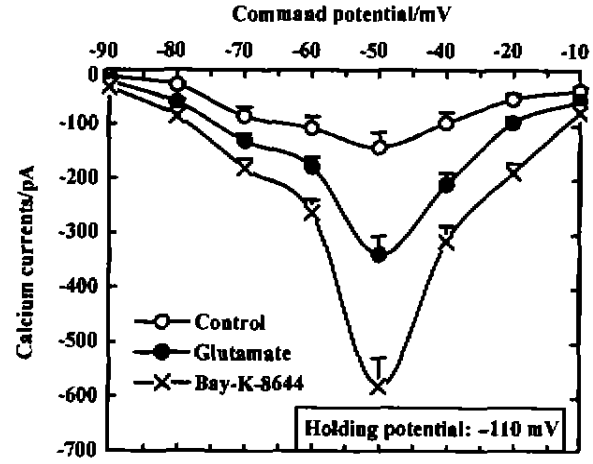

Fig 2. Effects of glutamate and Bay-K-8644 on calcium currents in cultured neurons. Current-voltage curves for peak calcium currents in control (○), glutamate (●), and Bay-K-8644 (×).

command potential that caused a maximal inward calcium current was 0 mV (Fig 3).

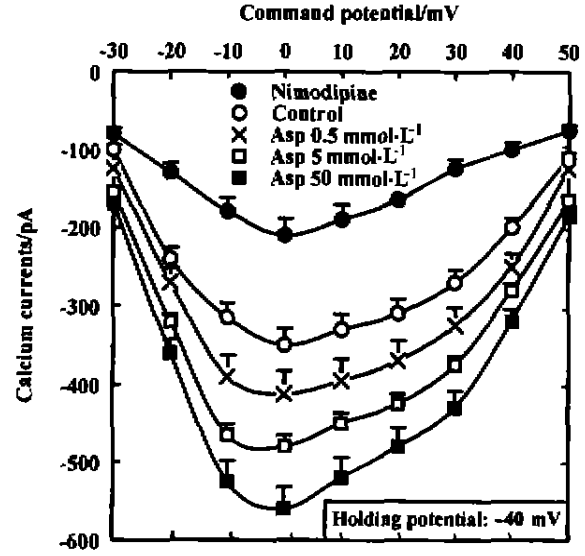

Fig 3. Effects of aspartate and Nim on calcium currents in cultured neurons. Current-voltage curves for peak calcium currents in control (○), Nim 10 μmol·L^{-1} (●), aspartate 0.5 mmol·L^{-1} (×), aspartate 5 mmol·L^{-1} (□), and aspartate 50 mmol·L^{-1} (■).

I_{Ca} was raised concentration-dependently by aspartate and reduced by Nim (Tab 1). The

Tab 1. Effects of aspartate and Nim on calcium currents in cultured neurons. $n = 8$ Cells. $\bar{x} \pm s$. $^bP < 0.05$, $^cP < 0.01$ *vs* Control.

Groups	Concentration	Calcium currents (pA)
Control		350 ± 45
Aspartate:	0.5 mmol·L^{-1}	412 ± 43^b
	5.0 mmol·L^{-1}	480 ± 49^c
	50 mmol·L^{-1}	537 ± 57^c
Nimodipine	10 μmol·L^{-1}	210 ± 40^c

decreasing rate was 46.2 % by Nim 10 μmol·L^{-1}.

DISCUSSION

Secondary central nervous system damage was always associated with massive Ca^{2+} influx[3,4]. Ca^{2+} influx may be caused by EAA, such as glutamate, aspartate, and glycine[1,2,5]. But the mechanism of EAA to cause secondary neuronal death is rarely known.

The present studies showed that aspartate raised I_{Ca} obviously and concentration-dependently under the condition where the potassium channels were blocked with Cs^+ and TEA, sodium channels were inhibited by tetrodotoxin. I_{Ca} was also raised obviously by glutamate and Bay-K-8644. The increase of I_{Ca} was involved in two ways. One was that aspartate and glutamate activated *N*-methyl-*D*-aspartate (NMDA) receptor-channel to admit Ca^{2+} directly into the cell; another was that aspartate and glutamate mediated a membarane potential change through the NMDA receptor which secondarily activated voltage-dependent calcium channels (VDCC)[4,5].

How to inhibit or block the sequence of secondary neuronal damage is the key to cure ischemic or traumatic brain injury. It was known that Ca^{2+} overload was the main reasons of secondary cell death[1-3]. It has been also showed that Nim, a calcium channel blocker, could reduce cerebral edema and improve the condition of neuronal damage[6,7]. However it was rarely clarified that whether or not Nim could inhibit Ca^{2+} influx when aspartate or glutamate induced Ca^{2+} influx through opening of VDCC and receptor-operated Ca^{2+} channels. The present study proved that Nim 10 μmol·L^{-1} could reduce about 46.2 % of these calcium currents. The result suggested that Nim could reduce secondary neuronal damage through inhibiting Ca^{2+} entering neurons. That was part of the reasons why Nim could improve neuronal function following ischemic or traumatic brain injury. In conclusion, EAA, such as glutamate or aspartate, had an effect on the increase of calcium currents. On the other hand, it was clarified in the present study that Nim could inhibit Ca^{2+} influx in neurons.

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兴奋性氨基酸及尼莫地平对培养大鼠脑皮层神经元钙电流的影响[1]

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关键词 钙通道；神经元；膜片箝技术；尼莫地平；大脑皮质；兴奋性氨基酸类；Bay-K-8644；谷氨酸盐；天门冬氨酸

目的：探讨兴奋性氨基酸（EAA）及钙通道阻断剂对神经元钙通道的影响。**方法**：用全细胞膜片箝技术研究 Bay-K-8644、谷氨酸（Glu）、天门冬氨酸（Asp）及尼莫地平（Nim）对培养大鼠皮层神经元钙电流（I_{Ca}）的作用。**结果**：Bay-K-8644 及 Glu 明显增加 I_{Ca}。Asp 0.5、5 及 50 mmol·L^{-1}浓度依赖性地增加 I_{Ca}（分别为 14.8 %、37.1 % 和 53.1 %）。细胞浴液中加入 Nim 10 μmol·L^{-1}，I_{Ca} 被抑制 46.2 %。**结论**：EAA 使神经元钙电流增加，Nim 抑制神经元的 Ca^{2+} 内流。

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