Short Communication

Protective Effect of Gabapentin on N-Methyl-D-aspartate–Induced Excitotoxicity in Rat Hippocampal CA1 Neurons

Young-Sick Kim¹, Hyun-Kyung Chang¹,²,⁵, Jin-Woo Lee¹, Yun-Hee Sung¹, Sung-Eun Kim¹, Mal-Soon Shin¹, Jae-Woo Yi¹, Je-Hoon Park¹, Hong Kim⁴, and Chang-Ju Kim¹,*

¹Department of Physiology, College of Medicine, Kyung Hee University, #1 Hoigi-dong, Dongdaemoon-gu, Seoul 130-701, South Korea
²Department of Anesthesiology, East-West Neo Medical Center, Kyung Hee University, #149 Sanggil-dong, Gangdong-gu, Seoul 134-890, South Korea
³Department of Surgery, Ilsan Paik Hospital, Inje University College of Medicine, #2240 Daehwa-dong, Ilsanseo-gu, Goyang-si, Gyeonggi-do 411-706, South Korea
⁴Department of Oriental Sports Medicine, College of Health & Therapy, Daegu Haany University, #290 Yugok-dong, Gyeongsan-si, Gyeongsangbuk-do 712-715, South Korea
⁵Graduate School of Medicine, Busan National University, #30, Jangjeong-dong, Geumjeong-gu, Busan 609-735, South Korea

Received March 10, 2008; Accepted November 17, 2008

Abstract. Gabapentin was developed as an anticonvulsant, but has also been used to alleviate hyperalgesia in neuropathic pain. In this study, the protective effect of gabapentin against N-methyl-D-aspartate (NMDA)-induced excitotoxicity in rat hippocampal CA1 neurons was investigated. Pre-treatment with gabapentin reduced the degree of neuronal damage induced by NMDA exposure in cultured hippocampal slices. Patch-clamp studies revealed that gabapentin significantly inhibited the NMDA receptor–activated ion current in dissociated hippocampal CA1 neurons, resulting in suppression of glutamate-induced neuronal injury. These results show that gabapentin may exert protective effects against glutamate-induced neuronal injury at least in part by inhibiting the NMDA receptor–activated ion current.

Keywords: gabapentin, N-methyl-D-aspartate, neuroprotection

Glutamate is the major excitatory neurotransmitter in the central nervous system. The ionotropic glutamate receptors are further classified according to their preferred agonists as the N-methyl-D-aspartate (NMDA), α-amino-3-hydroxy-5-methyl-4-isoxazoleproponic acid (AMPA), and kainate receptors. The ionotropic glutamate receptors mediate intercellular communications in normal cells and cell death in injured cells (1).

In ischemic attacks, depletion of oxygen and glucose brings about rapid losses in ATP, which depolarizes the membrane and leads to an increase in the synaptic release of glutamate (2). The increased extracellular concentration of glutamate over-stimulates the NMDA receptor, leading to an increase in Ca²⁺ influx, which in turn disables mitochondrial functions, rapidly increases the concentration of cytoplasmic reactive oxygen species (ROS), and ultimately causes neuronal cell death (3, 4). Because the NMDA receptor plays a crucial role in glutamate-induced acute neuronal damage, NMDA-receptor antagonists are thought to reduce neuronal cell death during and following ischemic attacks (5).

Gabapentin was designed as an anti-epileptic drug with structural analogy to γ-aminobutylic acid (GABA). The use of gabapentin has recently been broadened to include the treatment of neuropathic and post-operative pain (6). Gabapentin blocks α₂δ-Ca²⁺ channels (7) and modulates mitochondrial ATP-sensitive K⁺ channels (8). The action of gabapentin is known to be associated with protein kinase C (6).

Glutamate plays an important role in various physiological functions; however, abnormally high glutamate exposure induces neurotoxicity known as excitotoxicity (9). In the present study, we investigated the effect of gabapentin on the excitotoxicity induced by NMDA in a...
hippocampal slice culture, as well as the effect of gabapentin on the ion currents induced by glutamate and other agonists of glutamate-subtype receptors in hippocampal CA1 neurons.

The experimental procedures were performed in accordance with the animal care guidelines of the National Institutes of Health (NIH) and the Korean Academy of Medical Sciences. Organotypic hippocampal slice cultures were prepared as previously described (10). The hippocampi of Sprague-Dawley rats (postnatal day 7) were isolated and cut transversely at a thickness of 350 μm with a McILWAIN tissue chopper (Mickle Laboratory Engineering Co., Surrey, UK). The slices were placed on Millicell-CM inserts (Millipore) in 6-well plates containing 1 ml of culturing medium composed of 50% minimum essential medium (MEM), 25% Hank’s balanced salts solution (HBSS), and 25% horse serum. The slices were cultured for 14 days at 36°C in a 5% CO₂ incubator. The slice cultures were divided into 6 groups: the control group, the 10⁻⁴ M NMDA-treated group, the 10⁻⁷ M gabapentin pre-treated and 10⁻⁴ M NMDA–treated group, the 10⁻⁶ M gabapentin–pre-treated and 10⁻⁴ M NMDA–treated group, the 10⁻⁵ M gabapentin–pre-treated and 10⁻⁴ M NMDA–treated group, and the 10⁻⁴ M gabapentin–pre-treated and 10⁻⁴ M NMDA–treated group. Slices were pre-treated with gabapentin for 1 h before NMDA treatment for 36 h. Propidium iodide (PI, 5 mg/ml) was added to each well and PI-stained images were captured using an inverted fluorescence microscope.

For the patch-clamp study, the ionic composition of the incubation solution was 124 mM NaCl, 5 mM KCl, 1.2 mM KH₂PO₄, 1.3 mM MgSO₄, 2.4 mM CaCl₂, 10 mM glucose, and 24 mM NaHCO₃. The composition of the standard external solution was 150 mM NaCl, 5 mM KCl, 1 mM MgCl₂, 2 mM CaCl₂, 10 mM glucose, and 10 mM N-2-hydroxyethyl)piperazine-N’-2-ethanesulphonic acid (HEPES). A Mg²⁺-free standard solution with 2 x 10⁻⁴ M glycine was used to study the NMDA-activated current. The hippocampal CA1 neurons were freshly dissociated as previously described (10, 11). In brief, 10–15-day-old Sprague–Dawley rats were decapitated under Zoletil 50™ anesthesia (50 mg/kg, i.m.). The brain was removed and transverse slices (400-μm thickness) were prepared with a microslicer (DTK-1000; DSK, Tokyo). The slices were treated with 1 mg/ml pronase for 40 min followed by treatment with 1 mg/ml thermolysin for 20 min at 32°C. The hippocampal CA1 region was micropunched out from the slices with an electrolytically polished injection needle. The micropunched hippocampal CA1 regions were mechanically dissociated.

Electrical recordings were performed in the nystatin-perforated patch-recording mode under voltage-clamp conditions (10, 11). The patch pipettes were prepared from glass capillaries with an outer diameter of 1.5 mm on a 2-stage puller (PB-7; Narishige, Tokyo). Electrical stimulation, current recordings, and current filtration (at 2.0 kHz) were obtained with an EPC-7 patch-clamp amplifier (List-Electronic, Darmstadt, Germany).

The results were presented as the mean ± S.E.M. Statistical analysis was conducted using one-way ANOVA and Duncan’s multiple comparisons. Differences were considered statistically significant at $P<0.05$.

PI staining was used to visualize NMDA-induced neuronal damage in the pyramidal layer of the hippocampal CA1 region. The PI uptake in the control group was 9.3 ± 5.5%. After 36 h of exposure to 10⁻⁴ M NMDA, PI uptake was markedly increased to 62.0 ± 4.0%. The PI uptake in the groups pre-treated with gabapentin at 10⁻⁷, 10⁻⁶, 10⁻⁵, and 10⁻⁴ M was 59.0 ± 2.9%, 61.2 ± 6.1%, 37.5 ± 5.7%, and 36.2 ± 7.1%, respectively. These results showed that 10⁻⁵ and 10⁻⁴ M gabapentin suppressed NMDA-induced neuronal cell death (Fig. 1).

In the patch-clamp experiment, application of 10⁻⁵ and 10⁻⁴ M gabapentin alone to CA1 neurons did not elicit any ion currents. Glutamate (10⁻⁵ M) was applied every 2 min, and the magnitude of the resulting current was used as the control current. The magnitude of the glutamate-induced ion current was decreased to 100.4 ± 1.6%, 96.9 ± 1.7%, 97.1 ± 1.5%, 96.2 ± 3.4%, 95.0 ± 3.3%, and 94.6 ± 1.2% of the control value at 2, 4, 6, 8, 10, and 12 min, respectively, after 10⁻⁴ M gabapentin application for 2 min. The magnitude of the glutamate-induced ion current was decreased to 82.0 ± 3.2%, 94.6 ± 1.1%, 96.2 ± 1.5%, 99.6 ± 1.5%, and 97.3 ± 1.4% of the control value at 2, 4, 6, 8, 10, and 12 min, respectively, after 10⁻⁴ M gabapentin application for 2 min. Pre-treatment with 10⁻⁵ M gabapentin slightly inhibited the glutamate-activated ion current; however, this inhibition was not statistically significant. Pre-treatment with 10⁻⁴ M gabapentin reversibly inhibited glutamate-activated ion current (Fig. 2).

NMDA at 10⁻⁴ M was applied every 2 min, and the magnitude of the resulting current was used as the control current. The NMDA-induced ion current was decreased to 84.0 ± 2.4%, 96.5 ± 3.9%, 100.6 ± 3.7%, 98.0 ± 1.5%, 99.4 ± 1.6%, and 97.1 ± 1.4% of the control value at 2, 4, 6, 8, 10, and 12 min, respectively, after 10⁻⁴ M gabapentin application for 2 min. The NMDA-induced ion current was decreased to 82.5 ± 3.0%, 82.1 ± 3.6%, 80.6 ± 5.0%, 75.9 ± 2.3%, 74.6 ± 1.7%, and 82.3 ± 2.1% of the control value at 2, 4, 6, 8, 10, and 12 min, respectively, after 10⁻⁴ M gabapentin application for 2 min. Pre-treatment with 10⁻⁷ M
gabapentin reversibly inhibited the NMDA-induced ion current, and pre-treatment with $10^{-4}$ M gabapentin continuously inhibited the NMDA-induced ion current (Fig. 3).

AMPA at $10^{-5}$ or $10^{-4}$ M was applied every 2 min, and the magnitude of the resulting current was used as the control current. The magnitude of the AMPA-induced ion current was not significantly affected by $10^{-5}$ or $10^{-4}$ M gabapentin pre-treatment.

Kainate at $10^{-5}$ or $10^{-4}$ M was applied every 2 min, and the magnitude of the resulting current was used as the control current. The magnitude of the kainate-induced ion current was not significantly affected by $10^{-5}$ or $10^{-4}$ M gabapentin pre-treatment.

NMDA is associated with the anti-nociceptive effect of gabapentin, which blocks the excitation of pain-transmitting nerves at the spinal cord level (12). Gabapentin-lactam was also reported to protect against both oxygen and glucose deficiencies in acute retinal ischemia by inhibiting the release of glutamate (13). Furthermore, glutamate-receptor antagonists are known to exert neuroprotective effects (14). Among the various subtypes of glutamate receptors, the NMDA receptor is closely implicated in ischemia-induced neuronal toxicity (5, 9). The present slice culture experiments showed that NMDA caused neuronal injury in hippocampal slice cultures and that $10^{-4}$ and $10^{-5}$ M gabapentin significantly decreased the neuronal damage caused by NMDA.

It is widely hypothesized that NMDA-receptor antagonists salvage neurons by inhibiting Ca$^{2+}$ entry through NMDA receptors following the release of glutamate from presynaptic neurons. Ca$^{2+}$-dependent nitric oxide synthase is thus not activated and injurious superoxides are not generated (15). The hippocampal
CA1 region is known to be highly vulnerable to ischemic brain damage by excitotoxicity. The present patch-clamp experiments revealed that \(10^{-4}\) and \(10^{-5}\) M gabapentin significantly suppressed the NMDA receptor–activated ion current in acutely dissociated hippocampal CA1 neurons, thus demonstrating that gabapentin inhibits NMDA receptor–activated channels. The effect of gabapentin on NMDA-induced current was not on the amplitude, but rather on the recovery time; in patch-clamp studies, gabapentin dose-dependently delayed the recovery time.

In the present study, we showed that gabapentin exerts an inhibitory effect against glutamate-induced neuronal injury by inhibiting the NMDA receptor–activated ion current. The inhibitory effect of gabapentin on NMDA-induced current may be a mechanism for the neuroprotective effect of gabapentin against glutamate toxicity.