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NEUROPROTECTION BY MK-801 FOLLOWING CEREBRAL ISCHEMIA IN MONGOLIAN GERBILS

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Abstract — Global cerebral ischemia in Mongolian gerbils is an established model in experimental research on cerebral ischemia, which is characterized morphologically by selective neuronal damage in the hippocampus, striatum, and cortex. Elevated glutamate levels are thought to be a primary cause of neuronal death after global cerebral ischemia. The purpose of this study was to investigate the potential neuroprotective effects of dizocilpine malate (MK-801), a non-competitive glutamate antagonist, in the model of 10-min gerbil cerebral ischemia. Gerbils were given MK-801 (3 mg/kg i.p.) or saline immediately after the occlusion. On day 4 after reperfusion, neuronal damage was examined in the hippocampus (30 μm) and striatum slices (5 μm) stained with hematoxylin/eosin, fluorescent Nissl staining and membrane tracer DiI. The striatum and C3 regions of the hippocampus were analyzed by confocal microscopy. Neuroprotection was determined by quantifying the degree of cell loss, reduction of morphologically damaged cells, and the degree of preservation of recognizable neuroanatomical pathways after the ischemic insult. Our results demonstrate that the neuronal damage induced by sustained ischemia is related to abnormalities in glutamatergic function associated with NMDA receptors. MK-801 significantly prevented neuronal loss in the tested brain structures. All of this contributes to a better understanding of the given pathophysiological process causing ischemic neuronal damage.

Key words: Cerebral ischemia, gerbils, MK-801, neuroprotection, hippocampal cell loss, striatum, confocal microscopy

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INTRODUCTION

Stroke and cerebral ischemia are the leading causes of death and permanent disability, with no effective treatment currently known. Transient global cerebral ischemia occurs during cardiac arrest, cardiopulmonary bypass surgery, and other situations that deprive the brain of oxygen and glucose for short periods (Block, 1999). In both humans and animals, ischemia damages neurons in vulnerable regions of the brain, including the hippocampus, striatum, cerebral cortex, and cerebellum.

Models of transient global cerebral ischemia in gerbils (Catania et al., 2002; Iqbal et al., 2002a, 2002b) and rats (Chaulk et al., 2003; Khan et al., 2003) have been extensively used in investigating the cellular mechanisms of ischemic cell death, as well as in studies on the pharmacology of cerebral ischemia. In gerbils, global cerebral ischemia is easily induced by simple bilateral common carotid artery occlusion due to their lack of posterior communicating arteries, which normally connect the posterior circulation of the brain from the vertebral arteries with the anterior circulation from the carotid arteries in the circle of Willis (Olsson et al., 2003).

Elevated glutamate levels are thought to be a primary cause of neuronal death after ischemia. Excessive glutamate release during ischemia activates NMDA receptors to trigger long-term potentiation of synaptic transmissions. As a consequence, even normal levels of glutamate release may activate neural networks with a higher efficiency after ischemia. The ionotropic glutamate receptors NMDA and AMPA/KA open a cationic channel that allows the passage of Na^+, K^+, and Ca^{2+}. Neocortical AMPA and KA receptors show little permeability

to Ca^{2+}, except for a subpopulation of interneurons (Janssens et al., 2001). The NMDA receptor, in addition to allowing the passage of Na^{+} and K^{+}, is the major Ca^{2+} ionophore of the cerebral cortex. This receptor differs from the other glutamate receptors by being both ligand-gated and voltage sensitive (Kaczmarek et al., 1997). Regional distribution of NMDA and AMPA/KA receptors of the rodent brain was found to be highest in deep layers (layer 5) of the forebrain cortex, the cerebellar granule cell layer, and the caudate putamen (Carroll et al., 1998; Bailey et al., 2001).

Considerable evidence supports a link between Ca^{2+} influx and glutamate receptor-mediated neurodegeneration (Varju et al., 2001; Kosenko et al., 2003). Brief periods of highly Ca^{2+}-permeable NMDA channel activation can result in substantial intracellular Ca^{2+} accumulation and widespread neuronal injury (Lu et al., 1996; Hyrc et al., 1997; Tseng et al., 2003). Mitochondria can buffer these large Ca^{2+} loads, but they do so at the expense of triggering injurious reactive oxygen species production (Peng et al., 1998). However, the final and definitive pathway to neuronal death has not yet been elucidated.

The ability of the non-competitive NMDA receptor antagonist MK-801 to prevent neuronal loss in the gerbil striatum and hippocampus caused by cerebral ischemia is examined in this study.

MATERIALS AND METHODS

Animals

Adult male Mongolian gerbils (*Meriones unguiculatus,* 60 - 75 g) were submitted to different duration of cerebral ischemia. Groups of four gerbils per cage (Erath, FRG) were housed in standard conditions [23±2°C, 55±10% humidity, light for 12 h a day (from 07.00 to 19.00 h), and commercial food and tap water *ad libitum*]. Animals used for procedures were treated in strict accordance with the NIH Guide for Care and Use of Laboratory Animals (1985) and with approval of the Ethical Committee of the Serbian Laboratory Animal Science Association (SLASA, reg. No. 210-1342/2-2005-06).

Occlusion of common carotid arteries

Because mature gerbils lack collateral communications between carotid and vertebrobasilar circulations, occlusion of both common carotid arteries results, reproducibly, in global forebrain ischemia. The Mongolian gerbils were anesthetized by diethyl ether and placed in the dorsal position. The neck area was shaved, and both common carotid arteries were exposed carefully by blunt dissection and then clamped for 10 minutes with microaneurysm clips. After the clips were removed, reperfusion was confirmed visually, and the skin was sutured by 3 - 4 loose silk stitches. For sham-operated animals, both common carotid arteries were exposed, but not occluded. Rectal temperature was controlled carefully and maintained close to 37°C during ischemia using a heating lamp and a heating pad. Gerbils were allowed to recover in their home cages for 2 h and then returned to the animal quarters.

Histology

The gerbils were killed by decapitation on the forth day after reperfusion. Brains were removed from the skull and fixed in 4% paraformaldehyde for at least 24 h before dehydration through a range of alcohols and embedded in paraffin wax. Five-micrometer-thick striatum (dorsolateral of the caudate nucleus, at 0.90 mm rostral to the bregma) and 30-μm-thick hippocampus (medial, intermediate, and lateral sectors of CA3 hippocampus sections at 1.9 - 2.0 mm posterior to the bregma) brain sections were cut in a microtome, thaw-mounted into gelatine-coated glass slides, and stained with hematoxylin and eosin. The extent of cell damage in the striatal region was analyzed under a light microscope (Zeiss Axioscop 2).

Confocal microscopy

To reveal its cytoarchitecture, hippocampal CA3 brain structure was subjected to Nissl staining with Neurotrace fluorescence and fluorescent membrane tracer DiI (Molecular Probes, Germany) and viewed using a confocal laser microscope (Zeiss Axiovert 100 M) equipped for laser scanning microscopy. An argon laser (488 nm) and helium-neon laser (543 nm) were utilized for excitation of Nissl staining and

DiI, retrospectively. Following acquisition, images were processed using the Zeiss LSM 510 Basic software package, v. 3.2.

RESULTS

Comparison of the morphological appearance of neurons within the coronal sections of intact gerbils and sham-operated animals shows the effect of mechanical stress caused by the experimental procedure - the surgical intervention. There was no significant difference between the morphological appearance of brain neurons in these two groups. This means that the surgical intervention alone is not sufficient to trigger oxidative stress and/or excitotoxicity and cause neuron damage. We therefore considered sham-operated animals as controls (data not presented).

Neuronal damage was examined in striatal slices four days after 10-min ischemia (Fig. 1A-C). Neuronal death was characterized morphologically by cell shrinkage, cytoplasmic hypereosinophilia, and moderate nuclear pyknosis with later chromatin dispersal and disintegration. In some neurons, the nuclear profile became less rounded and more triangular (Fig. 1E). MK-801 significantly prevented this neuronal loss (Fig. 1F).

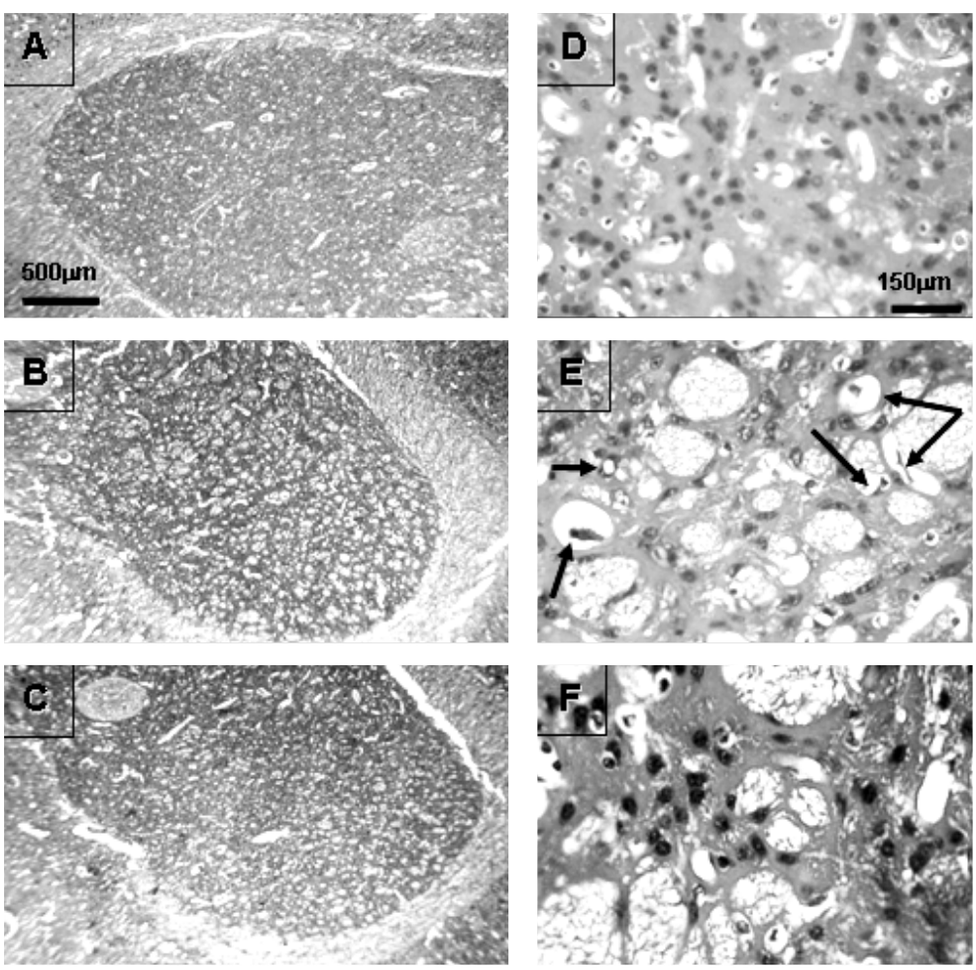

Fig. 1. Morphological appearance of neurons within the gerbil striatum four days after 10-min cerebral ischemia stained with hematoxylin/eosin viewed using a light microscope. A, D– sham; B, E – 10-min ischemia; C, F – ischemia plus MK-801.

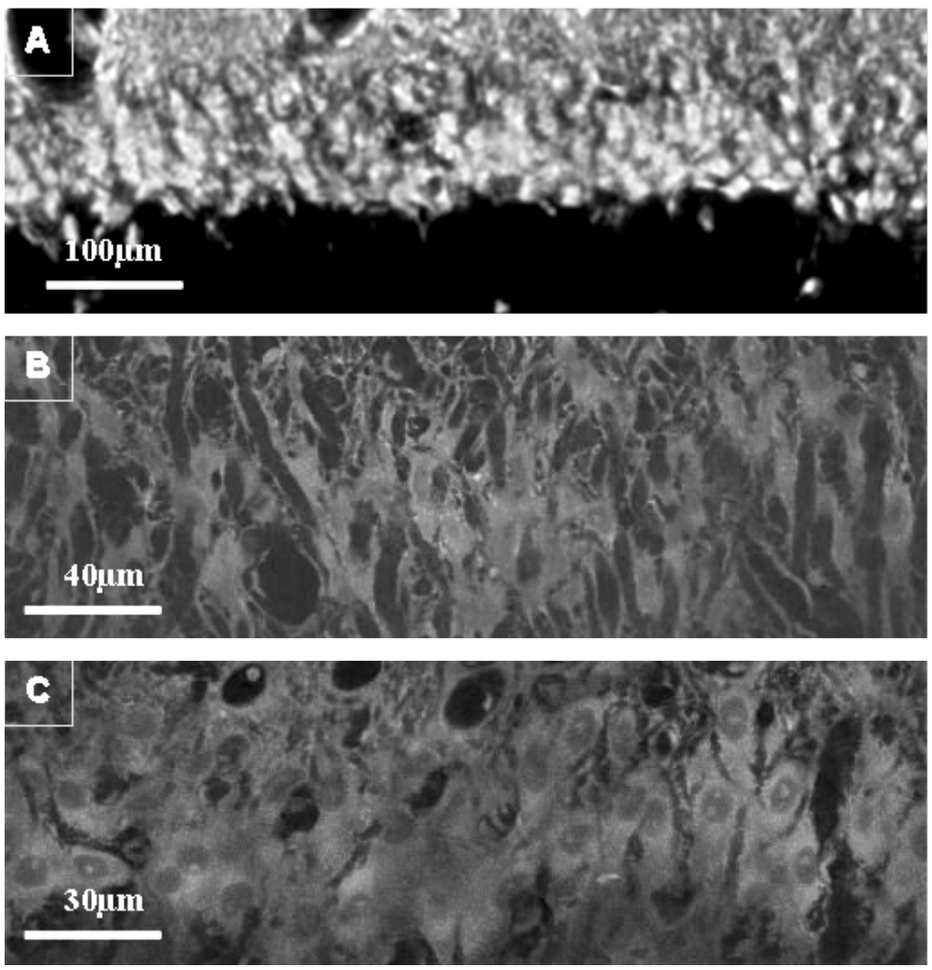

Fig. 2. Effect of MK-801 on neuronal damage in the CA3 region of the gerbil hippocampus four days after 10-min cerebral ischemia stained by Nissl staining with Neurotrace fluorescence (green) and fluorescent membrane tracer DiI (red) viewed using a confocal laser microscope. A – sham; B – 10-min ischemia; C – ischemia plus MK-801.

Upon examination of three portions of area CA3 (medial, intermediate, and lateral) four days after ischemia, obvious cell damage was observed (Fig. 2B). When MK-801 was administrated soon after cerebral ischemia, neuronal damage was partially prevented (Fig. 2C).

DISCUSSION

A number of synthetic glutamate receptor antagonists, especially drugs interfering with NMDA receptors, have been identified as promising neuroprotective agents, but they failed in clinical trials because of undesirable side effects or lack of efficacy (Wong et al., 1986). That is the pharmacological difference between glutamate and its receptor agonists. Unlike its receptor agonists, glutamate can activate all glutamate receptors and can be taken up by many different cells, including neurons and glia. The glutamate transport system may play a role in the difference between glutamate and its receptor agonists in the observed excitotoxic effects. These glutamate transporters can be inhibited by peroxynitrite, which is formed by a combination of superoxide and nitric oxide and is sensitive to the cell's redox state (Urenjak et al., 2000). Reduced glutamate uptake will prolong glutamate receptor activation by extracellular glutamate, which will

exacerbate neuronal injury (Varju et al., 2001).

Glutamatergic pathways originating from the sensomotor cortex and the subthalamic nucleus are the major route of excitatory input to the corpus striatum. They play a crucial role in cognition and motor coordination and participate in the pathogenesis of neurodegenerative diseases (Garcia et al., 1995). In our study, 10-min duration of cerebral ischemia resulted in a progressive loss of striatal neurons. As the main component of basal ganglia, the striatum receives glutamatergic inputs from the cortex and thalamus, and considerable attention has therefore been devoted to the role of excitotoxicity in striatal disorders. The striatum receives significant glutamatergic input from cortical areas and dense dopaminergic input from the substantia nigra and ventral tegmental area. Striatal neuronal damage is mainly located in the dorsolateral part and affects medium-seized spiny striatal projection neurons. MK-801 significantly prevented this neuronal loss.

Transient global cerebral ischemia in rodents induces selective degeneration affecting hippocampal CA1 and CA4 neurons while relatively sparing CA3 neurons and dentate granule cells (Wang et al., 2002). We demonstrated CA3 neuronal damage after 10-min duration of ischemic insult. Four days after reperfusion, neuronal damage was detected in the striatum and CA3 hippocampus.

Our histological results showed that MK-801 reduced neuronal damage in the striatum and hippocampus when administered soon after ischemia. MK-801, per se, did not affect the morphology of striatal and hippocampal neurons (data not shown). Moreover, MK-801 reduced the mortality associated with 15 minutes of ischemia.

Treatment with glutamate antagonists, calcium channel blockers, or radical scavengers resulted in reduced neuronal loss. All of these lower neuronal damage following global ischemia, which supports the hypothesis that these pathophysiological processes contribute to ischemic neuronal damage.

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НЕУРОПРОТЕКТИВНИ ЕФЕКАТ МК-801 ПОСЛЕ ЦЕРБЕРАЛНЕ ИСХЕМИЈЕ КОД МОНГОЛСКИХ ГЕРБИЛА

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Испитиван је неуропротективни ефекат МК-801, некомпетитивног NMDA глутаматског антагониста у моделу церебралне исхемије, четири дана после десетоминутне исхемије код џербила – пустињских мишева. Степен оштећења неурона у стријатуму и CA3 региону хипокампуса је процењиван применом конфокалне микроскопије (двоструко бојење). Апликација МК-801 непосредно после исхемије значајно смањује оштећење и смрт неурона у тестираним можданим структурама.