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Research report

Temporal patterns of motor behavioural improvements by MK-801 in Mongolian gerbils submitted to different duration of global cerebral ischemia

Branka Janać[a,*], Vesna Selaković[b], Lidija Radenović[c]

[a] Department of Neurophysiology, Institute for Biological Research, Bulevar Despota Stefana 142, 11000 Belgrade, Serbia
[b] Institute for Medical Research, MMA, Serbia
[c] Center for Laser Microscopy, Department of Physiology and Biochemistry, Faculty of Biology, University of Belgrade, Serbia

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ABSTRACT

The purpose of this study was to investigate the temporal pattern of NMDA receptors antagonist—MK-801 on motor behaviour parameters in gerbils submitted to different duration of global cerebral ischemia. The common carotid arteries of gerbils were occluded for 5, 10 or 15 min. Gerbils were given MK-801 (3 mg/kg i.p.) or saline immediately after the occlusion in normothermic conditions prior to testing. Motor activity was registered 1, 2, 4, 7, 14, 21 and 28 days after reperfusion during 60 min by open field test. At the same time, the effect of NMDA receptor blockade was followed *in vivo* by monitoring the neurological status of whole animals or at the cellular level by standard light and confocal microscopy on brain slices. Post-ischemic gerbils quickly developed hypermotor response with the most intensity in animals submitted to 15 min ischemia. MK-801 administrated immediately after ischemia significantly decreased this hyperactivity. In all ischemic-treated animals, behavioural suppression by MK-801 was observed already 1 day after occlusion and was lasting as far as observed ischemia-dependent hypermotor responses. Beneficial effect of MK-801 was also confirmed by morphological and neurological status data. These findings suggest that sustained ischemia-induced hyperactivity is related to abnormalities in NMDA glutamatergic function, as well as its manifestation could be completely abolished by NMDA receptor blockade immediately after ischemic insult.

1. Introduction

Global cerebral ischemia damages neurons in vulnerable regions of the brain, including hippocampus, striatum, cerebral cortex and cerebellum. Considering the very important role of these brain structures in control of different types of motor behaviour it is expected that, besides the well-known morphological changes, global cerebral ischemia also leads to functional changes which can be assessed by behavioural studies [5].

In an experiment on transient ischemic attack using Mongolian gerbils, many transmitters are released during and immediately after this treatment [41,46]. Excessive glutamate release during ischemia activates NMDA receptors to trigger long-term potentiation of synaptic transmissions [42]. As a consequence, even normal levels of glutamate release may activate neural networks with a higher efficiency after ischemia. This may be further cause abnormal excitation, which leads to the greatly enhanced energy demands and intracellular Ca^{2+} accumulation, and eventually to neuronal death. If this is the case, the toxicity of glutamate and Ca^{2+} does not result from Ca^{2+} overload during ischemia, but from the widespread and persistent strengthening of neural transmission after ischemia. The mechanism of neuronal loss in non-ischemic areas remote from ischemic core may be explained by this hypothesis.

Paradoxically, pharmacological evidence for the involvement of glutamate antagonists in global cerebral ischemia protection is unclear [1,22,24]. MK-801 is a potent, selective, non-competitive antagonist of NMDA receptors that readily blocks the neurotoxic effects of NMDA-induced depolarization [38]. Its neuroprotective effect is also established *in vivo* in different experimental models of hypoxia and focal cerebral ischemia [4,25,31], while the effects in global cerebral ischemia are still controversial. Irrespective of pre-ischemic [19,20] or post-ischemic [18,30] administration of MK-801 to normothermic or non-normothermic animals, the experimental results regarding neuroprotection are either beneficial [11,15,17,28] or detrimental [13,40]. It is also considered that the outcome depends on the severity of the global ischemia [32].

The present study was undertaken to examine character and temporal pattern of MK-801 modulatory effect administrated immediately after ischemia on hypermotor response and striatal

* Corresponding author. Tel.: +381 11 2078 339; fax: +381 11 2761 433.
E-mail address: janac@ibiss.bg.ac.yu (B. Janać).

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neuronal damage in gerbil model of global cerebral ischemia. Considering our previously results [26], attention was directed to influence of MK-801 on ischemia-induced disturbance of locomotion, stereotypy and resting time, since the other measured parameters of motor activity (rearing and rotations) did not show significant changes under this treatment. Striatum is less vulnerable brain structure in ischemia (as opposed to hippocampus CA1 region), however, it is still particularly important for the decay of motor activity in stroke. This study also emphasizes the temporal follow-up of the neurological outcome for the rarely observed long time periods (up to 1 month) after the ischemic insult.

2. Materials and methods

2.1. 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 an air-conditioned room, at temperature of 23 ± 2 °C, with 55 ± 10% humidity, and with lights on 12 h/day (07:00–19:00). The gerbils were given commercial food and tap water *ad libitum*. All experimental manipulations were performed during the light phase, between 9:00 and 15:00 under identical conditions. Animals used for procedures were treated in strict accordance with the NIH Guide for Care and Use of Laboratory Animals (1985) and European Communities Council Directive (86/609/EEC), as well as with approval of the local Ethical Committee (SLASA, reg. no. 210-1342/2-2005-06). All efforts were made to minimize animal suffering and to reduce the number of animals used.

2.2. Experimental procedure—occlusion of common carotid arteries

Because mature gerbils lack of posterior communicating arteries, that normally connect the posterior circulation of the brain from the vertebral arteries with the anterior circulation from the carotid arteries within the circle of Willis [19], 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, both common carotid arteries were exposed carefully by blunt dissection and then clamped for 5, 10 or 15 min with microaneurysm clips. After the clips were removed, reperfusion was confirmed visually, and the skin was sutured by three to four loose silk stitches. The gerbils were randomly divided into three basic groups and further depend of drug treatment in two subgroups (Fig. 1). Control groups were intact and sham-operated (n = 12 animals per group) while the treatment group was submitted to 5, 10 and 15 min ischemia (n = 12 animals per ischemia). According to the drug treatment, gerbils were given MK-801 ((+)-5-methyl-10,11-dihydro-5*H*-dibenzo(*a*,*d*)-cycloheptan-5,10-imine malate, Sigma Chemical Co., USA, 3 mg/kg i.p. dissolved in 0.1 M saline, pH 7.2; 200 μl total volume) or saline immediately after the occlusion. Intact gerbils were not submitted to any type of surgical procedure, while sham-operated gerbils were exposed to the same surgical intervention as ischemic gerbils but without occlusion of both common carotid arteries. Post-ischemic temperature was carefully monitored due to the fact that the gerbil model typically shows an intrinsic hyperthermic response during the initial hours of recirculation [29]. However, MK-801 is known to induce a hypothermic effect [8], albeit at higher doses (5–10 mg/kg). Since the changes in body temperatures are known to have impact on the consequences of global ischemia [12], it was maintained at 37 ± 0.3 °C throughout the surgical procedure by means of a feedback-controlled heating pad (TR-100, PS-100, Fine Science Tool, North Vancouver, Canada). Gerbils were allowed to recover in their home cages for 2 h under a Xenon heating lamp (the rectal temperature of animals was carefully monitored and maintained at 38 °C by an external heating lamp) and then returned to animal quarters. Thus, the possible MK-801 neuroprotective effect due to its hypothermic effect was ruled out (in spite of the fact that lower doses −3 mg/kg were applied). The gerbil rectal temperature was further controlled in 6 h—intervals within 48 h of monitoring. Survival of experimental animals was estimated 2 days after occlusion on a separate group of animals (n = 120) in 6 treatment subgroups of 20 gerbils.

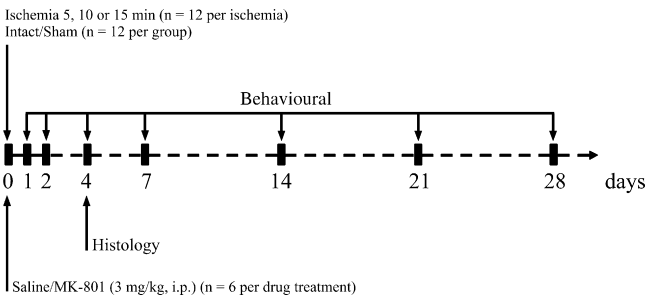

Fig. 1. Experimental protocol: time line of surgical procedure, drug treatment and behaviour assays.

2.3. Motor activity measurement system

The motor activity of Mongolian gerbils was monitored in the open field by an automatic device Columbus Auto-Track System (Version 3.0 A, Columbus Institute, OH, USA). Each monitoring instrument (Opto-Varimex) consisted of a Plexiglas cage (44.2 cm × 43.2 cm × 20 cm) connected to the Auto-Track interface and intercrossed by horizontal and vertical infrared beams. Interruption of a beam generated an electrical impulse, which was subsequently processed and sent to a computer linked to the Auto-Track interface. The Auto-Track system detected 11 behavioural parameters, including locomotor activity (distance traveled in cm), stereotypic activity (such as sniffing, self-grooming, licking and head weaving), rearing (lifting both forepaws off the floor), clockwise rotation (heading changes in the right hand direction), counter clockwise rotation (heading changes in the left hand direction) and resting time (time period without any type of motor activity). The type of activity, characterized by the animal's movements, was determined by a user-defined box size (in this experiment, set to three beams). The described parameters were defined in accordance with Auto-Track system. To eliminate any interaction of gerbils with the environment during the experimental sessions, Opto-Varimexes were placed into the light- and sound-attenuated chambers, with artificially regulated ventilation and illumination. After registration, floor of each cage was washed and dried to neutralize smells of previously animal.

All behavioural experiments were conducted during the light cycle (lights on 7:00/off 19:00) and for each test the gerbils were transported to the testing room 20 min prior to the start of the experiment. Motor activity of all gerbil groups was measured 1, 2, 4, 7, 14, 21 and 28 days after reperfusion, at the same time in the day, during 60 min (Fig. 1). Gerbils were not exposed to the open field prior to ischemia.

2.4. Histology

In order to examine ischemia-induced neuronal damage, we performed histological analysis of brain sections at the level of dorsal striatum obtained 4 days after reperfusion (Fig. 1). Animals were fixed by transcardiac perfusion with 4% paraformaldehyde under deep anesthesia. Brains were removed from the skull and fixed in paraformaldehyde (4%) for at least 24 h before dehydration through a range of alcohol concentrations and finally embedded in paraffin wax. Five-micrometer thick striatal sections were cut on a microtome, thaw-mounted into gelatin-coated glass slides, and stained with haematoxylin and eosin. The quantification of ischemic brain damage was done by counting the basophilic cells seen in striatum (dorsolateral to the caudate nucleus, at 0.90 mm rostral to bregma) for each hemisphere. Extend of cell damage in striatal region was quantified under Zeiss Axioscop 2 as the mean number of the persisting neurons in the coronal sections. Three defined 300 μm^2 fields of striatal region were recorded with camera MC 100095 (Carl Zeiss Jena) and counted for persisting neurons in a computer-assisted image analysis system (KS 300, Carl Zeiss Jena).

2.5. Confocal microscopy

In order to obtain better cell recognition and to reveal the cytoarchitecture of the striatum (30 μm thick) brain sections were stained with fluorescent Nissl stain (Neurotrace, Molecular Probes) to visualize the cytosol of neurons and with fluorescent membrane tracer DiI (Molecular Probes). Nissl stain (Neurotrace) marked neurons (visualizes both nuclear DNA and ribosomal RNA throughout the cytosol) while DiI stained cell membranes. This double staining was used for better morphological assessment of living *vs.* deteriorating cells. Imaging was done using a confocal laser scanning microscope (Zeiss LSM 510) with an argon laser (488 nm) and a helium–neon laser (543 nm) utilized for the excitation of Nissl staining and DiI, respectively. Following acquisition, images were processed using the Zeiss LSM 510 Basic software package v. 3.2.

2.6. Statistical analysis

Survival of experimental animals was statistically analyzed using Z-test. Statistical significance of differences between groups regarding to extent of neuronal damage in striatum was evaluated using one-way ANOVA test followed by Dunnett's test.

The results of behavioural experiments were analyzed by two-way repeated ANOVA with treatment as the between subject factor and time after reperfusion as the within subject repeated measure. When appropriate, subsequent statistical comparisons were performed by LSD test or planned comparison. The data which did not have normal distribution, assessed by Kolmogorov–Smirnov test, were log transformed before analysis.

In all cases, $P < 0.05$ was considered as statistically significant difference.