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Research article

Neuroprotective effects of MK-801 against cerebral ischemia reperfusion

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ABSTRACT

Introduction: & Objective: Cerebral ischemia/reperfusion (I/R) injury, the second cause of death globally, involves increased NMDA receptor activity leading to neuronal damage due to excessive sodium and calcium ion entry. Therefore, targeting NMDA receptor may potentially reduce cell death induced by brain injury. Our study aimed to investigate the role of NMDA receptors in hippocampal neuronal activity induced by I/R.

Methods: In this study, Wistar rats were divided into four groups: sham, I/R, I/R + MK801, and I/R + NMDA. Cerebral I/R injury was induced by temporarily occluding the common and vertebral carotid arteries, followed by reperfusion. MK801 or NMDA was administered to the rats after a specific reperfusion time. Neuronal density and cell morphology in the hippocampal CA1 region were assessed using Nissl and H&E staining. The expression of BDNF, *p*-CREB, and c-fos was evaluated through Western blot analysis. Additionally, neuronal activity in CA1 pyramidal neurons were examined using single unit recording technique.

Results: Our results showed that cerebral I/R injury caused significant damage to CA1 pyramidal neurons compared to the sham group. However, treatment with MK-801 improved hippocampal cell survival compared to the I/R group. Furthermore, MK-801 administration in I/R rats increased BDNF, c-fos, and *p*-CREB levels while decreasing cleaved caspase-3 activity compared to the I/R group. Additionally, electrophysiological data showed that MK-801 increased firing rates of CA1 pyramidal neurons during the reperfusion phase.

Conclusion: MK-801 shows promise as a therapeutic agent for cerebral I/R injury by enhancing cell survival, upregulating neuroplasticity factors, and increasing firing rates of CA1 pyramidal neurons. It exerts a specific protective effect against cerebral I/R injury.

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1. Introduction

Stroke is widely recognized as a major cause of long-term disability in adults and ranks as the second leading global cause of death [1]. It occurs when a blood clot forms in a cerebral artery, leading to cerebral infarction and neurological impairment [2]. Reperfusion, which restores blood flow to the affected area, can have both beneficial and detrimental effects. It enhances blood flow but also triggers oxidative stress and inflammation, worsening neuronal cell death [3]. This phenomenon is commonly known as ischemia/reperfusion (I/R) damage or injury. However, the primary therapy for stroke patients in clinical settings involves mechanical or pharmacological interventions to restore blood circulation [4]. Unfortunately, this approach often increases neuronal death [5], leads to significant brain infarction, and impairs cognitive abilities [6]. Therefore, prioritizing the restoration of cerebral blood circulation and protecting brain tissue appears more advantageous in managing ischemic stroke. This highlights the importance of utilizing a combination of thrombolytic medications and neuroprotective therapies [7].

Ischemia reduces brain blood flow, depriving neurons of oxygen and nutrients needed for normal function and ion balance [8]. Disruption of the cell's ionic gradient leads to the release of excitatory neurotransmitters like glutamate into the synaptic region, activating N-Methyl-D-Aspartate (NMDA) glutamate receptors in postsynaptic neurons. Excessive glutamate accumulation can be toxic and cause neuronal damage [9,10]. In pathological situations, overactivation of NMDA receptors can lead to neuronal death through excitotoxicity and apoptosis. Given the crucial role of NMDA receptors in initiating excitotoxic signaling during cerebral ischemia, they represent promising targets for stroke therapeutic interventions [11,12]. NMDA receptor antagonists have been identified as a potential treatment target for memory loss in Alzheimer's disease [13]. MK-801, a non-competitive NMDA receptor antagonist, can block NMDA ion channels [12]. It has been demonstrated to enhance neural plasticity-related cytokine expression, reduce glutamate synthesis, and decrease cell death [14].

Three key factors contribute to the pathophysiology of ischemia events: excessive release of glutamate, activation of NMDA-type glutamate receptors, and influx of calcium into neurons, likely through NMDA receptor-linked calcium channels [15]. After injury, the brain can potentially adapt through the formation of new connections between unaffected neurons and functional modifications [16]. Structural alterations play a role in supporting neuronal plasticity and functional recovery [17]. After a stroke, brain-derived neurotrophic factor (BDNF) plays a crucial role in brain plasticity, regulating activity-dependent synaptic plasticity [18,19]. BDNF is essential for pre-synaptic vesicle cycling in cultured neocortical neurons, as observed in BDNF-knockout mice [20]. Disruptions in BDNF's signaling pathways contribute to neurological conditions like depression, Alzheimer's disease, and Huntington's disease [21]. Enhancing post-ischemia production of BDNF holds promise for therapeutic strategies [22]. Phosphorylation of cAMP response element binding protein (*p*-CREB) is vital for memory acquisition and consolidation. CREB activates gene transcription, including BDNF induction [23]. *p*-CREB initiates long-term memory formation involving the early activated transcription factor c-fos [24]. CREB and c-fos transcription protect synaptic components, promoting action potential and neuron plasticity [25]. In stroke, CREB activates genes influencing neuronal excitability. Enhancing CREB signaling post-stroke can improve cellular excitability and motor function recovery [26].

Considering the hippocampus vulnerability to I/R injury [27], this study aimed to investigate the role of NMDA receptors in an in-vivo model of cerebral I/R injury. Additionally, we assessed the levels of BDNF, c-fos, P-CREB, cleaved caspase-3 activity, electrophysiological changes, and neuronal death in the CA1 region of the hippocampus.

2. Material and methods

2.1. Animals

Male Wistar rats, weighing 270–320 g, were housed in a controlled environment with a 12-h light/12-h dark cycle and given unrestricted food and water access. All experiments were conducted in accordance with the international Care and Use of Laboratory Animals guidelines (NIH Publications No. 80–23, revised, 1996) and were approved by the Ethics Committee of Tehran University of Medical Sciences.

2.2. Induction of cerebral ischemia/reperfusion injury and experimental design

To induce the cerebral ischemia/reperfusion (I/R) model, the animals were first anesthetized by intraperitoneal injection of urethane at a dose of 1.7 mg/kg. After the anesthesia, the animals were placed in the stereotaxic apparatus, and both vertebral arteries were blocked by electrocauterization. The common carotid arteries (CCA) of the animals were identified and separated from the vagus nerves. Subsequently, suture number 0–4 was placed around the arteries, and they were then closed with a clamp for 15 min. Afterward, the clamp was removed, and reperfusion started. After 30 min of reperfusion [28], the animals were treated with normal saline, MK-801, or NMDA via the tail vein based on their respective groups. After 6 h, the animals were euthanized, and their brains were removed for molecular and histological tests. To maintain unobstructed airflow during the recording process, a surgical tracheostomy was performed on all rats.

The animals were randomly assigned to four groups: (a) sham group (n = 8): The vertebral arteries and the CCA were both exposed without occlusion, and normal saline was injected intravenously, (b) I/R group (n = 8), (c) I/R group + NMDA receptor antagonist (n = 15): the animals underwent the I/R surgery and received an intravenous injection of MK-801 (0.5 mg/kg) [29–31], and (d) I/R group + NMDA receptor agonist (n = 15): the rats underwent I/R surgery and received an intravenous injection of 0.5 mg/kg of NMDA

after a 30-min reperfusion period. The dose of NMDA was established based on previous study [32] and our preliminary study. In groups c and d, 8 rats were considered for molecular assessments and 7 rats for single unit recording of CA1 region.

2.3. Laser Doppler flowmetry technique

Cortical cerebral blood flow was measured using a laser doppler flowmetry (LDF). On the day prior to the initiation of I/R, the rats were given anesthesia and a guide was placed on the skull's surface using stereotaxic coordinates: 5 mm laterally and 1 mm posteriorly to the bregma. The next day, the laser Doppler probe was inserted using the guide, and the recording process began. Once a consistent recording was obtained, the ischemia was initiated. The blood flow was continuously monitored from before the onset of ischemia and throughout the 30-min ischemic period. The data on cerebral blood flow were utilized to determine which animals should be excluded from the study based on the following criteria: (1) rats that did not exhibit a perfusion rate lower than 65 % of the baseline after inserting the occlusion filament were excluded, as this indicated that ischemia was not effectively induced; and (2) rats that experienced surgical complications, such as subarachnoid hemorrhage, were eliminated from the study.

2.4. Single unit recording

Experiments were conducted on anesthetized rats using a stereotaxic device. The animals were anesthetized with intraperitoneal injection of chloral hydrate (250 mg/kg) and Ketoprofen (2 mg/kg). A parylene-coated tungsten microelectrode (A-M systems), with a shaft diameter of 127 μm, tip exposure ranging from 1 to 3 μm, and a tip impedance of 5 MΩ, was stereotactically inserted into the CA1 region of the hippocampus. The insertion depth was 2.4 mm below the skull surface. The microelectrode was lowered through the pyramidal cell layer using a manual Microdrive until distinct spike activity, with a signal-to-noise ratio above 2, was isolated from the background noise. The recorded signals from the electrode were amplified by a factor of 10,000 using an eLab recording system (ScienceBeam, Iran). A band-pass filter was applied to refine the signals, allowing frequencies between 0.3 and 10 kHz. The signals were digitized at a sampling rate of 50 kHz and a voltage resolution of 12 bits.

Hippocampal pyramidal neurons were identified based on a firing rate of 8 spikes per second or lower [33]. All recorded neurons exhibited spike lengths above 1.3 ms, consistent with typical durations of extracellularly recorded pyramidal cells. Once a stable pyramidal cell with constant firing frequency, spike amplitude, and waveforms was identified, a baseline recording of approximately 10 min was conducted. Then, MK-801 or NMDA was administered, and the recording continued for a total duration of 60 min. The recorded spike data were stored on a computer for further offline analysis. Immediately after the recording, a negative direct current of 50 μA was applied for 15 s via the recording electrode to create a reference point at the recording site for histological verifications.

Spike analysis was performed using Offline Sorter (Plexon) as explained elsewhere [34]. Briefly, after applying a low-cut filter at 300 Hz, spikes were detected by adjusting an amplitude threshold. Feature extraction was done using principal components 1–3. Spikes were clustered by applying valley-seeking algorithm. The units were then exported to NeuroExplorer software (NEX technologies) for rate analysis and constructing time histograms.

2.5. Caspase-3 activity assay

The activity of caspase-3 in the hippocampal tissue was evaluated by employing the Caspase-3 Assay kit (Sigma-Aldrich) according to the manufacturer's instructions. In summary, the tissues were lysed with cold lysis buffer to extract their contents. After removing any remaining debris through centrifugation, the protein levels in the lysates were quantified using the Bradford assay. Next, 50 μg of protein were combined with the DEVD-pNA substrate. The release of the chromophore *p*-nitroanilide (pNA) from the substrate, resulting from its cleavage, was measured using a micro plate ELISA reader at a wavelength of 405 nm. The absorbance of the color (pNA) generated in the tissues of treated animals was compared to that of control animals in order to determine the percentage change in caspase-3 activity.

2.6. Western blotting

Hippocampus tissues were lysed using a cold RIPA buffer to extract proteins. After centrifugation of the tissue lysates, the resulting supernatants were collected. The protein concentration in the supernatant was assessed using the Bradford assay. Following that, 30 μg of protein samples were loaded onto a 12.5 % SDS-PAGE gel to facilitate separation. Following electrophoresis, the proteins underwent transfer to PVDF membranes and were subsequently blocked with 5 % skimmed milk in TBST for 1 h at room temperature. The blocked membranes were then subjected to overnight incubation at 4 °C with primary antibodies, including BDNF (1:1000), P-CREB (1:1000), CREB (1:1000), c-fos (1:1000), and β-actin (1:2500). After undergoing five washes with TBST, the blots were exposed to HRP-conjugated secondary antibody (1:5000) for a duration of 1 h at room temperature. Subsequently, the membranes underwent five additional washes with TBST, and the immunoreactivity was detected using an enhanced chemiluminescent substrate. Band intensity was quantitatively analyzed using Image J software, with intensities normalized to β-actin.

2.7. Nissl Staining

Nissl Staining technique was employed investigate the density and quantity of dark neurons within the hippocampus region. Following the fixation of samples, a paraffin mold was created. Subsequently, coronal sections with a thickness of 5 μm were generated

using a rotary microtome apparatus and these sections were then placed onto albumin-coated slides. Following clarification and hydration procedures, the slides were subjected to staining using a 1 % cresyl violet solution. Finally, coverslips were applied to the sections. The slides from each experimental group were examined under a light microscope, and three CA1 regions were captured using a digital camera. Utilizing ImageJ software, the number of dark neurons within the CA1 region was quantified for each experimental group.

2.8. Hematoxylin and eosin staining

To evaluate neuronal damage and cell death, the CA1 region of the hippocampus was specifically selected in the coronal sections, and the number of viable neurons within this region was quantified. Initially, the entire brain underwent perfusion using a 9 % normal saline solution for 5 min, followed by perfusion with a 4 % paraformaldehyde solution for 10 min. The subsequent procedure involved preparing paraffin blocks using the fixed tissue. Slices, measuring 4 μm in thickness, were obtained from brain blocks and then underwent deparaffinization using xylene. They were subsequently dehydrated by sequential immersion in alcohol solutions of increasing concentrations (70 %, 80 %, 90 %, and 100 %). Finally, the slices were rinsed with a saline buffer. Following this, the slices were incubated at room temperature in a saline solution for 15 min. After the incubation, the slices were stained using the hematoxylin and eosin method. The slides from each group were examined under a light microscope, and three CA1 areas were captured from each slide using a digital camera. The ImageJ program was utilized to quantify the population of viable cells within the CA1 area for each experimental group.

2.9. Statistical analysis

Statistical analysis was performed using GraphPad Prism 8 software. One-way Analysis of Variance (ANOVA) approach was used to compare several groups, while Tukey's post-hoc analysis was utilized to ascertain the differences between these groups. The Brown-

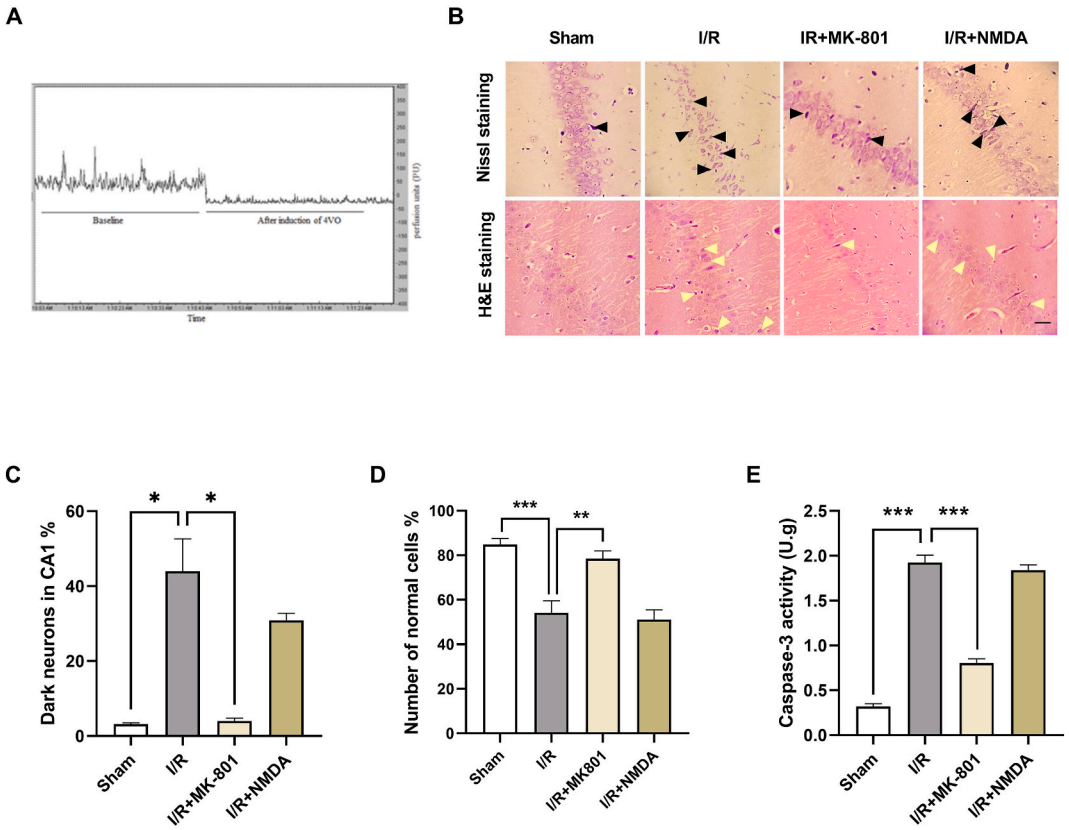

Fig. 1. MK-801 Attenuates Pathological Damage and Apoptosis in Hippocampal Tissue
Representation of cerebral blood flow (perfusion unit) measurement by LDF before and after 4-vessel occlusion (4-VO) in rat (A). Histological change of hippocampus CA1 area cells in experimental groups Nissl and H&E staining (scale bar = 20 μm). Black arrow heads showed dark neurons in Nissl staining and yellow arrow heads represented damaged cells in H&E staining after cerebral ischemia (B). Analysis of dark neurons by Nissl staining (C) and dead cells by H&E staining (D). Measurement of caspase-3 activity in the hippocampus of experimental groups (E). Data are presented as Mean ± SEM. *P < 0.05, **P < 0.01, ***P < 0.001, (n = 4).

Forsythe & Welch test was also used in cases where the variances of the groups were not close to each other (in Nissl staining). T-test was used to determine the difference in electrophysiology groups. The data are shown as mean ± SEM. The experiments were accomplished three times. p values < 0.05 was considered statistically significant.

3. Results

3.1. MK-801 Attenuates Pathological Damage and apoptosis in hippocampal tissue

To confirm the occurrence of cerebral hypoperfusion, the cerebral blood flow was measured before the closure of both vertebral arteries and CCA and immediately after the ligation of the carotid artery using LDF. Our results revealed that the cerebral blood flow has decreased compared to baseline (Fig. 1A).

Nissl staining and H&E staining were employed to assess the protective impact of MK-801 on the hippocampal CA1 region of I/R rats. Nissl staining results showed an increase in the number of dark neurons in the I/R group compared to the sham group, indicating that cerebral ischemia resulted in more neuronal damage ($p < 0.05$). In the sham group, the neurons in the brain tissue exhibited organized and compact arrangements, and the nuclei appeared clear and resembling vacuoles. However, in the I/R group, the neurons suffered significant damage, displaying irregular arrangements and atrophy. Furthermore, the Nissl bodies showed lighter staining, a loss of certain neurons was observed, and there was an increase in the intercellular space ($p < 0.05$). However, in the I/R + MK-801group, there was a noticeable improvement in the morphological alterations of neurons, with a more compact arrangement. Moreover, the I/R + MK-801 group exhibited a significant reduction in the number of dark neurons compared to the I/R group ($p < 0.05$). In addition, there is no significant difference in I/R + NMDA group compared to I/R group ($p < 0.05$) (Fig. 1B and C).

H&E staining analysis demonstrated that the hippocampal neurons in the sham group exhibited regular morphology, abundant cytoplasm, clear nucleoli, and a closely arranged structure. However, in the I/R group, evident damage to hippocampal neurons was observed, characterized by extensive softening, disrupted arrangement of neurons, and the disappearance of neuronal nuclei. The number of normal neuronal cells in the hippocampus decreased significantly compared to the sham group ($P < 0.001$). Treatment with

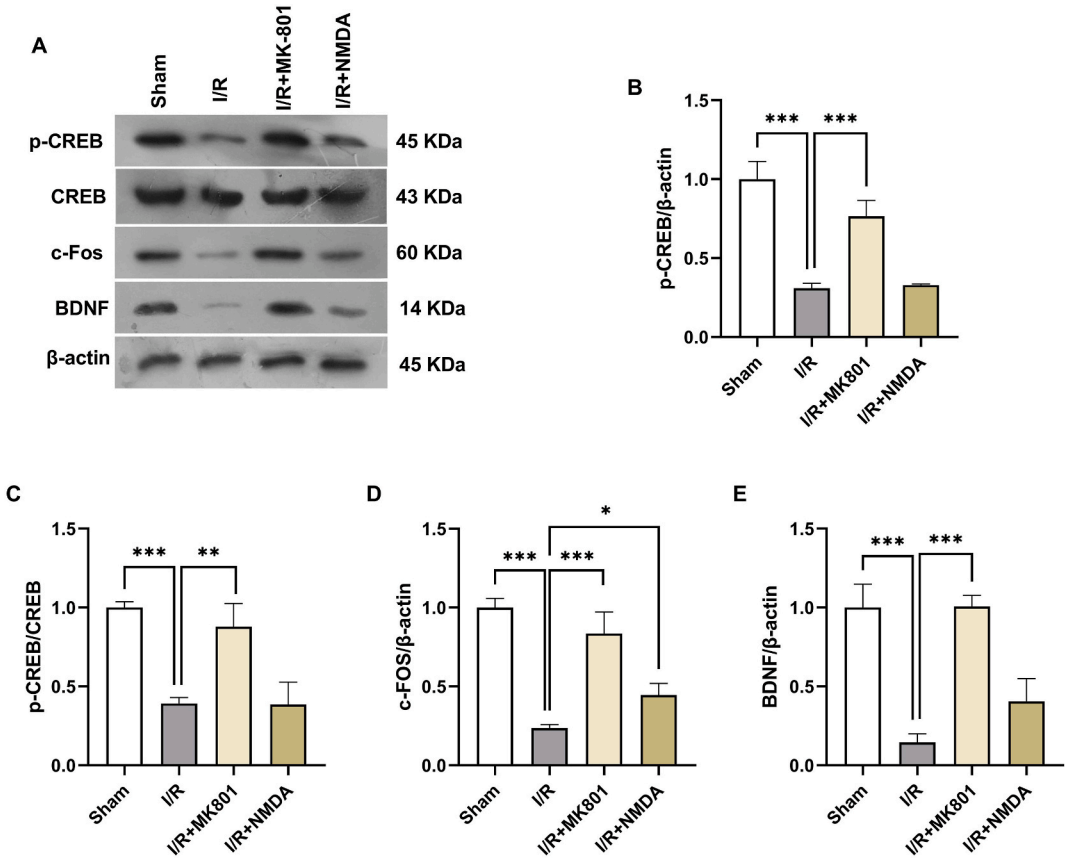

Fig. 2. MK-801 Upregulate BDNF, c-fos, and *p*-CREB Levels in the Hippocampus Tissues Represented blots of *p*-CREB, CREB, BDNF, c-fos, and β-actin in the hippocampus of experimental groups (A). The density analysis of *p*-CREB, CREB, BDNF, c-fos proteins band to β-actin was measured in the hippocampus (B–E). Data are presented as Mean ± SEM. *$P < 0.05$, **$P < 0.01$ and ***$P < 0.001$, ($n = 4$). The uncropped versions of Fig. 2A are presented as a supplementary file.

MK-801 showed varying degrees of improvement in pathological damage. Most nerve cells returned to a normal state, with only a few experiencing shrinkages, and the overall number of viable cells increased. However, no significant difference was observed between the I/R + NMDA group and the I/R group ($p < 0.05$), (Fig. 1B and D). Taken together, these findings indicate that MK-801 has the ability to effectively mitigate neuronal damage and cell death in the hippocampi of rats undergoing I/R.

As shown in Fig. 1E, the activity of cleaved caspase-3 was increased in the injured brain region. In the I/R + MK-801 group, the administration of MK-801 significantly reduced cleaved caspase-3 activity compared to the I/R group ($P < 0.001$). Nevertheless, the injection of NMDA to the rats subjected to I/R did not yield any notable alterations in the expression levels of these proteins when compared to the I/R group ($P > 0.05$), (Fig. 1E).

3.2. MK-801 upregulate BDNF, c-fos, and p-CREB levels in the hippocampus tissues

To assess the impact of MK-801 on neural protection, we analyzed the protein levels of BDNF, c-fos, and *p*-CREB in rat hippocampus tissues. Our findings revealed that I/R significantly decreased the expression of BDNF, c-fos, and *p*-CREB compared to the sham group ($P < 0.001$). Conversely, treatment with MK-801 effectively restored the downregulated expression levels of these proteins induced by I/R ($P < 0.001$). However, the administration of NMDA to the I/R rats did not result in any significant changes in the expression levels

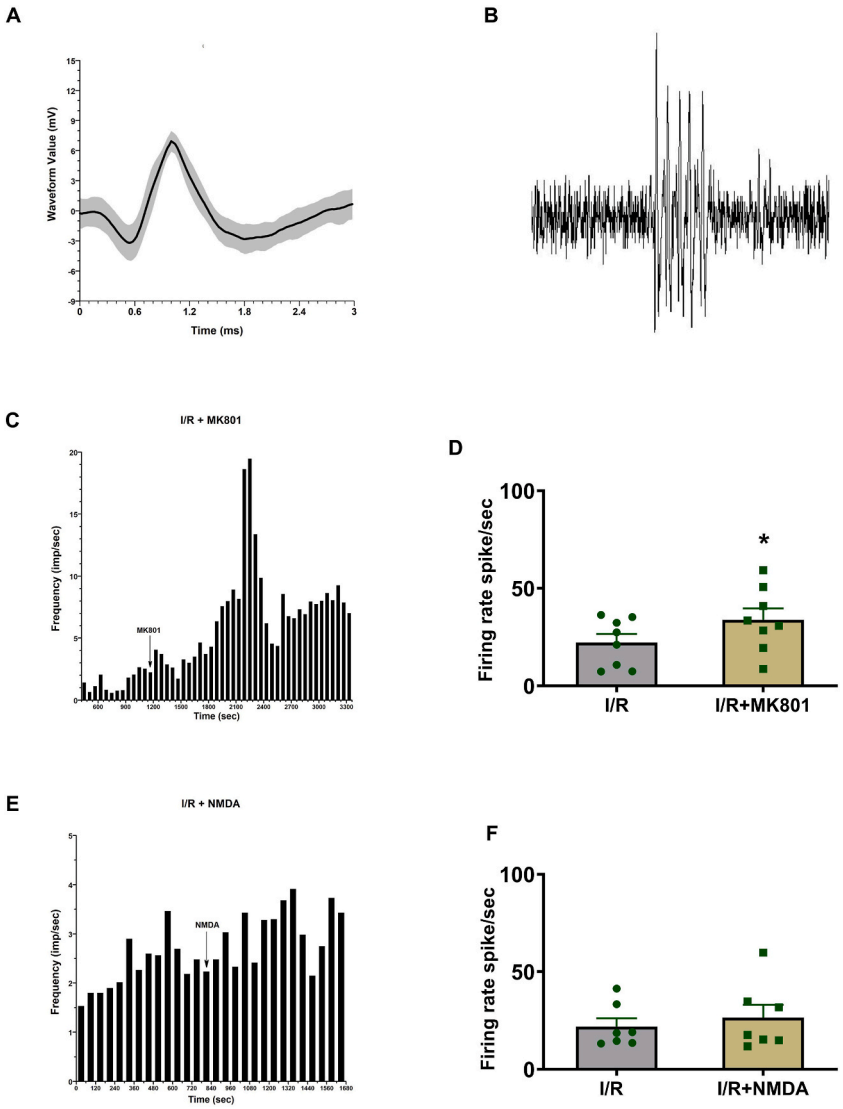

Fig. 3. MK-801 Enhances Firing Rate of Pyramidal Neuron Representation of a single unit recording of hippocampal pyramidal neurons (A). Summation of hippocampal pyramidal neurons spikes in 100 ms (B). Histogram of hippocampal pyramidal neurons firing rate by injection of MK801 (C) and NMDA (E). Firing rate changes of pyramidal neuron in response to injection of MK801 (D) and NMDA compared to the reperfusion phase (F). Data are presented as Mean ± SEM. *$P < 0.05$, ($n = 7$).

of these proteins compared to the I/R group ($P > 0.05$), (Fig. 2A–E). These findings highlight the potential therapeutic benefits of MK-801 in neural protection.

3.3. MK-801 enhances firing rate of pyramidal neuron

In order to examine the impact of the NMDA receptor on the electrical activity of pyramidal neurons in the CA1 region of the hippocampus, the firing rate of neurons was compared to their initial baseline firing rate during the reperfusion phase. Fig. 3A and B represented a single unit recording of hippocampal pyramidal neurons and summation of hippocampal pyramidal neurons spikes in 100 ms, respectively. Data analysis revealed a notable rise in the firing rate of pyramidal neurons after the administration of MK-801, in comparison to their baseline activity ($P < 0.05$), (Fig. 3C and D). However, no significant variation was observed in the firing rate during NMDA treatment compared to its baseline activity ($P > 0.05$), (Fig. 3E and F).

4. Discussion

The pathophysiology of stroke is a complex and intricate process. Excitotoxicity plays a significant role and is considered a primary contributor to tissue injury after a stroke. Excitotoxicity can cause immediate cellular death through necrosis and can also trigger subsequent molecular processes associated with delayed cellular death, including apoptosis. Therefore, the management of excitotoxicity is a crucial objective in the therapeutic approach to stroke. The current therapeutic efficacy of treatments that aim to inhibit excitotoxicity in both laboratory and in vivo settings is strongly associated with the suppression of glutamate receptors [11,12]. Multiple studies have consistently indicated that rats exposed to transient global cerebral ischemia display a distinctive loss of neurons in the CA1 region of the hippocampus [35]. Based on studies, the CA1 region is an appropriate region to investigate the effects of NMDA signaling during cerebral ischemia [36]. One of the primary mechanisms underlying this selective neuronal injury is the excessive release of excitatory amino acids. The excitotoxic effects can be attributed to the activation of a specific ionophoric receptor known as the NMDA receptor by glutamate [9].

Studies have shown that the 6-h time point is critical for evaluating early changes in gene expression, protein levels, and cellular responses in the context of cerebral ischemia/reperfusion injury [29,37,38]. Based on these evidences, we assessed molecular parameters 6 h following reperfusion for gaining a comprehensive understanding of the acute effects of MK-801 and NMDA in this experimental model. Moreover, Data have demonstrated that NMDA exhibits a dual effect in modulating neuronal toxicity, as evidenced by previous research. Despite these findings, the precise role of NMDA in the in vivo cerebral ischemia model remains elusive [39]. The intricate interplay of NMDA receptors in the context of cerebral ischemia underscores the complexity of their function, highlighting the need for further investigation to elucidate the specific mechanisms through which NMDA exerts its dual effects in neuronal responses to ischemic insults. Understanding the nuanced role of NMDA in vivo could provide valuable insights into potential therapeutic targets and strategies for mitigating neuronal damage in ischemic conditions.

NMDA receptors have a pivotal role in the neuronal cell death induced by ischemic stroke known as excitotoxicity. Several preclinical studies have demonstrated the neuroprotective effects of NMDA receptor antagonists in reducing ischemic neuronal death [9]. MK801, a non-competitive antagonist of NMDA receptors, has demonstrated neuroprotective properties in different brain injury models, such as ischemic stroke. In our study, we investigated the effects of MK-801 on hippocampal neuronal damage in rats that underwent I/R. We found that MK-801 had a positive impact on reducing such damage. Specifically, it increased the levels of important proteins such as BDNF, *p*-CREB, and c-fos, which are known to promote neuronal survival and function. Moreover, MK-801 was found to enhance the activity of caspase-3, an enzyme involved in cell death processes. This suggests that MK-801 may have a protective role in preventing excessive cell death in the hippocampus after I/R injury. Furthermore, we found that MK-801 had the additional effect of boosting the firing rate of pyramidal neurons in the hippocampus, which could contribute to improved cognitive function.

In our study, we demonstrated that 0.5 mg/kg of MK-801 reduced cell death and neuronal destruction in I/R rats. Consistent with our results, previous studies have demonstrated that the NMDA receptor antagonist effectively reduce cell death and neuronal destruction across a range of experimental studies. The use of MK-801 has been shown to reduce brain lesion volume and the degree of cerebral edema in various injury models, providing a good neuroprotective effect [40]. For instance, MK-801 has been showed neuroprotective effects in various animal models of cerebral ischemia, supporting its potential role in reducing ischemia-induced neuronal degeneration [41]. Additionally, another study demonstrated that MK-801 can reduce the expression of TNF-α in DRG neurons, indicating its neuroprotective effects [42]. Furthermore, Qian et al. (2016) conducted a study demonstrating the neuroprotective effects of administering the MK-801 to rats with focal ischemia induced by middle cerebral artery ligation shock [43]. These findings suggest that MK-801 may have a neuroprotective role in reducing cell death and neuronal destruction, and highlight the therapeutic potential of MK-801 in preventing or reversing the detrimental effects of ischemic insults on neuronal function and survival.

The activation of NMDA receptors has been associated with the induction of apoptosis and the increase in caspase-3 activity [44,45]. Prolonged stimulation of NMDA receptors causes neuronal apoptosis by facilitating the entry of sodium and calcium ions into the cellular environment. This process also reduces the activation of ERK, CREB, and BDNF [11,46,47]. Furthermore, our results demonstrated an increase in caspase-3 activity in rats subjected to I/R, while MK-801 significantly reduced this overactivity.

Previous study has shown that the overstimulation of NMDA receptors by glutamate can lead to the induction of apoptosis in cultured cerebellar granule neurons [48]. Additionally, the activation of NMDA receptors can transiently activate caspase-3 via the mitochondrial pathway without causing cell death [49]. On the other hand, a study showed that inhibiting NMDA receptors resulted in

apoptotic cell death in rat cultured cortical neurons, accompanied by the activation of caspase-3 [45].The activation of NMDA receptors plays a dual role in the regulation of apoptosis. It is important to note that the role of NMDA receptor activation in apoptosis is complex, as it involves the differential contribution of NMDA receptor subtypes. Specifically, the activation of NR2A-containing NMDA receptors has been linked to survival signaling through anti-apoptotic mechanisms, while the activation of NR2B-containing NMDA receptors has been associated with promoting apoptosis [44,46]. Therefore, the effect of NMDA receptor activation on apoptosis is context-dependent and involves a delicate balance between pro-survival and pro-apoptotic signaling pathways [50].

BDNF, c-fos, and *p*-CREB are all proteins that play important roles in neuronal survival, synaptic plasticity, and learning and memory. The reduction of these proteins can lead to neuronal damage and cognitive impairment [21,51,52]. In our study, we investigated the effects of an I/R event on the levels of *p*-CREB, BDNF, and c-fos expressions in the CA1 hippocampal regions. We observed a significant decrease in the levels of these important proteins 6 h after the I/R event, indicating a disruption in the cellular signaling and neuroplasticity processes. However, we also examined the impact of administering MK-801 on this phenomenon. Interestingly, we found that the administration of MK-801 effectively restored the downregulation of *p*-CREB, BDNF, and c-fos levels. This suggests that MK-801 has the potential to preserve the signaling pathways crucial for neuroprotection and synaptic plasticity in the CA1 hippocampal regions following an I/R event.

Phosphorylation of *p*-CREB is crucial for memory acquisition and consolidation and has been implicated in brain recovery and cognition [53]. The induction of CREB accelerates the reorganization of impaired cortical sensory maps following a stroke, aligning with accelerated functional recovery. This effect is mediated through activation of specific genes that enhance neuronal excitability and potentially contribute to motor recovery. Enhancing CREB signaling post-stroke increases cellular excitability and improves motor function, suggesting that promoting CREB expression may be a strategy for brain repair in stroke [26,54]. BDNF, another important player in brain recovery, preserves neurons after a stroke. Increased BDNF levels in penumbra regions correlate with improved brain function, while decreased levels impair neuroplasticity and hinder cognitive enhancement [55,56]. Augmenting BDNF production with pharmacological interventions after ischemic events shows promise [57]. Inhibition of NMDA receptors, like MK-801, offers neuroprotective effects in ischemia, brain injury, and other insults [43,58]. In a study by Cassol Jr. et al., the administration of a modest dosage of MK-801 in sepsis survivor rats prevented memory loss [59]. Additionally, Yu et al. demonstrated in a 2015 study using primary astrocyte cultures that MK-801 increased the expression of BDNF and its receptor in the cell culture medium [60]. Furthermore, Jussi Väisänen and colleagues found that MK-801 induced the expression of c-fos in rats with schizophrenia [61]. These findings collectively highlight the potential of targeting *p*-CREB, BDNF, c-fos to promote neuroprotection, enhance neuroplasticity, and facilitate recovery in various neurological conditions, including stroke and other brain insults.

Following a stroke, the electrical activity of pyramidal neurons is diminished due to the heightened vulnerability of hippocampal neurons to ischemic conditions [62,63]. During the reperfusion phase, there have been recorded instances of disruptions in the electrical activity of neurons within the CA1 area [64]. Studies have demonstrated that during stroke, increased intracellular calcium levels and the activation of glutamate receptors play a significant role in neuronal damage, cell death, and heightened neuronal excitability [11]. Therefore, it is crucial to prevent excessive upregulation of NMDA receptors. Recent research has shown that administering MK-801 results in a significant increase in the electrical activity of rat pyramidal neurons in the medial prefrontal cortex. Additionally, MK-801 has been found to effectively reduce the excitatory response to NMDA ion currents [65]. Furthermore, our results demonstrated that the administration of MK-801 leads to a significant augmentation in the firing rate of CA1 pyramidal neurons compared to the reperfusion phase. This finding suggests that MK-801 has the capacity to notably enhance neuronal excitability and amplify the activity of CA1 pyramidal neurons beyond what naturally occurs during the reperfusion phase. Inconsistent with our results, MK-801 has been found to enhance the firing rates of neurons and the amplitude of gamma-frequency oscillations in field potentials of putative pyramidal neurons in freely moving rats [66].

In contrast to our findings, a separate investigation presented evidence that the administration of MK-801 led to the suppression of neuronal electrical activity specifically in the CA1 region under normal physiological conditions [67]. It is important to acknowledge that the presence of NMDA receptors is essential for the generation of electrical activity in CA1 neurons under normal physiological circumstances. Therefore, the expected outcome of consuming MK-801 would be the inhibition of neuronal activity. The aforementioned investigation utilized an NMDA receptor antagonist in the context of pathological conditions associated with ischemia. Furthermore, the intravenous infusion of NMDA did not yield a statistically significant effect on the firing rate of neurons in the hippocampus, as observed in our study. In another study, memantine, which is also an NMDA receptor antagonist, was found to significantly decrease the electrical activity of CA1 pyramidal neurons in an Alzheimer's rat model. This study also demonstrated that memantine had different effects on the firing rate of CA1 pyramidal neurons in intact and lesioned rats, with some neurons being unaffected, excited, or inhibited [68]. Another study indicated that reduced NMDA receptor function has contrasting impacts on prefrontal cortex interneurons and pyramidal neurons. Inhibition of NMDA receptors primarily leads to decreased activity in potential GABA interneurons, while it results in an increased firing rate of pyramidal neurons, albeit with a delayed effect. Therefore, the activity of cortical inhibitory interneurons is primarily influenced by NMDA receptors, indicating that inhibiting NMDA receptors leads to cortical excitation through the disinhibition of pyramidal neurons [69].

However, it is important to note that the dual roles of NMDA receptors in pro-survival and pro-death signaling in neurons may lead to potential adverse effects, and the translation of these findings to clinical practice requires further research and development [9]. Therefore, while the use of NMDA receptor antagonists for neuroprotection in the context of ischemia shows promise, additional studies are needed to fully understand their potential benefits and limitations.

5. Conclusion

The findings of this study suggest that MK-801 may have a protective effect on the brain by restoring the expression levels of BDNF, c-fos, and *p*-CREB proteins. However, further research is needed to fully understand the mechanisms underlying this effect and determine the potential clinical applications of MK801 in the treatment of brain injuries. In conclusion, this statement emphasizes the neural protection and potential therapeutic benefits of MK-801 in restoring hippocampal neuronal activity after I/R injury.

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Ethical approval

This study was approved by the Research Ethics Committee of Tehran University of Medical Sciences [Ethical number: IR.TUMS.AEC.1401.200].

Data availability statement

The data of this research are available from the corresponding author upon request.

CRediT authorship contribution statement

Zahra Yaghoobi: Writing – original draft, Project administration, Formal analysis. **Saeid Ataei:** Writing – original draft, Formal analysis. **Esmail Riahi:** Writing – original draft, Formal analysis. **Mohsen Parviz:** Writing – original draft, Formal analysis. **Fardin Sehati:** Writing – original draft, Formal analysis. **Meysam Zare:** Writing – original draft, Formal analysis. **Razieh Angizeh:** Project administration. **Ghorbangol Ashabi:** Writing – review & editing, Supervision, Methodology, Conceptualization. **Saereh Hosseindoost:** Writing – review & editing, Writing – original draft, Supervision, Methodology, Conceptualization.

Declaration of competing interest

The authors declare that they have no conflicts of interest with respect to the research, authorship, and/or publication of this article.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e33821.

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