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Traumatic Brain Injury Decreases NR2B GluR Levels in the Cerebral Cortex Neurons of C57BL/6 Mice

Abstract

Traumatic brain injury (TBI) is one of the major medical problems in societies and a major cause of death and disability in developed and developing countries. One of the pathophysiological factors in TBI is the change in glutamate receptor levels in the brain following trauma. In the present study, it was hypothesized that a change in glutamate receptor levels in the cerebral cortex neurons of C57BL/6 mice was associated with traumatic brain injury (TBI). 32 C57BL/6 mice were divided into four groups of 24 hr, 48 hr, 72 hr, and 1 week with the same number (n = 8) in each group. Weight dropping model was used to cause brain injury. NR2B GluR levels in rat cortex tissue were measured by Western blotting. The results of Western blotting analysis showed a significant decrease in the NR2B GluR levels in 1 week after traumatic brain injury (TBI) compared to the control (ANOVA, $p < 0.05$) (Cortex sham: 100±47, FP 93.8±4.8). There was no significant difference in other groups compared to the control group. The results of this study showed that Traumatic brain injury decreases the NR2B GluR levels.Traumatic brain injury decreases the NR2B GluR levels.

Keywords: *Traumatic brain injury, Glutamate receptor, NR2B GluR, Cerebral cortex neurons*

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Introduction

Traumatic brain injury is one of the major medical problems in societies and a major cause of death and disability in developed and developing countries (1-7). Traumatic brain injury (TBI) is one of the most well-known environmental risk factors for chronic traumatic encephalopathy and neurodegenerative diseases such as Alzheimer's disease (8). The pathophysiological mechanisms resulting in neurological injury in TBI include primary physical injury to brain tissue and the vascular system following TBI (9). Secondary injury resulting from brain trauma can be caused by increased glutamate levels, inflammation, oxidative stress, and neuronal dysfunction (10-14). More than two decades of a considerable number of studies in the area of TBI have shown that increasing extracellular concentrations of glutamate play a major role in brain pathology (15). It has been proven that traumatic brain injury increases glutamate and increases the activity of its receptors, leading to the death of nerve cells (16). Activation of the glutamate N-methyl-D-aspartate (NMDAR) receptor, which is a ligand-valued ion channel (calcium and sodium) results in channel activation and the entry of ions into the cell.These processes mediate delayed excitatory toxic neuronal death after trauma and ischemic brain injury. Thus, glutamate receptors are involved in the pathophysiology of traumatic brain injury (17), so that disorder in the regulation of glutamate receptors is known as one of the causes of neurological disorders in brain trauma (18). Evidence suggests that different subtypes of NMDA receptors are involved in a variety of brain disorders, for example, NR2B is involved in pain and NR3A is involved in the injury of the white matter of the brain (19).

Excitatory neurotransmitter in the central nervous system involved in many physiological conditions such as brain development, synaptic plasticity, memory, and learning. Like other neurotransmitters, glutamate is stored in synaptic vesicles and is released in a calcium-dependent manner by exocytosis. Most glutamate is stored in nerve endings, about 100 mM of it is stored in the synaptic vesicles and 10 mM of it is stored in the cytoplasm. Extracellular concentration of glutamate is very small amounts and varies from 0.5 to 4 µM, and in cerebrospinal fluid, it varies from 1 to 10 µM. Its concentration in the synapse space is between 2 and 1000-2000 based on neuronal activity. Glutamate is removed by astrocytes and is returned to nerve cells by specific transmitters after being converted to glutamine. Glutamate can cross the blood-brain barrier and thus, it is made in the central nervous system mainly from glucose by the Krebs cycle in the mitochondria of nerve cells or from glutamine by glial cells and is provided to nerve cells (20-24).

Glutamate exerts its effects through two types of receptors, including ionotropic and metabotropic receptors. Based on pharmacological and electrophysiological properties, ionotropic glutamate receptors are classified into three categories, including N-methyl-D-aspartate (NMDARs) receptors, α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPARs) receptors, and kainic acid receptors (KARs). The binding of glutamate to its ionotropic receptors opens cationic channels in the nerve cell membrane. Metabotropic glutamate receptors include a large group of G protein-coupled receptors (GPCRs) that play a major role in regulating the normal activity of central nervous system neurons. These receptors do not directly activate ion channels, but through G-proteins, they activate secondary messaging systems in neurons. Eight types of metabotropic glutamate receptors

(mGluR) have been identified so far, which are classified into three main categoriesI, II, and III BasedBased on amino acid sequence, message transmission mechanisms; and pharmacological properties. These receptors are widely distributed at different synapses and sites outside of synapsis in glial cells and neurons of the central nervous system and regulate the release of glutamate and other neurotransmitters (25.26).

Group I receptors include the mGluR5 and mGluR1 subunits, which are located mainly in the postsynaptic membrane and regulate neuronal excitability. Group I receptors activate phospholipase C (PLC) by activating G-proteins, which catalyzes the production of inositol 1, 4, and 5 triphosphate (IP3) and diacylglycerol (DAG)IP3 molecule stimulates the release of Ca^{2+} from intracellular sources, and DAG activates protein kinase C (PKC). Group II receptors are composed of mGluR3 and mGluR2 subunits, and group III is composed of mGluR8, mGluR7, mGluR6, and mGluR4 subunits. Receptors II and III are often located in the presynaptic membrane and regulate the release of neurotransmitters. Receptors II and III bind to G proteins and negatively regulate adenylate cyclase activity (27.28). Ionotropic glutamate receptors in the form of heterotetramer include two fixed subunits of GluN1 and two subunits of GluN2 (GluN2A-D) or GluN3 (29).

During excitatory neurotransmission, glutamate is released from the presynaptic membrane and produces a potential for excitatory postsynaptic action (EPSP) by binding to its receptors on the postsynaptic membrane and activating them. Activation of glutamate receptors opens an ion channel that is selective for cations, resulting in the entry of Na^{+} and Ca^{2+} ions and the departure of K^{+} ions (30). In mammals, two types of glutamate receptors, including the ionotropic glutamatergic receptors of NMDA and AMPA, mediate rapid neurotransmission in the central nervous system. NMDA receptors play a vital role in brain development, neuropathology, and synaptic plasticity (31). They also have a high affinity for excitatory mediator L-glutamate (32), so among excitatory amino acids, L-glutamate is the most potent NMDA agonist (EC_{50} 2.3 µM) (33). AMPA and NMDA glutamate receptors are permeable to Ca^{2+} and are expressed in areas of the brain responsible for cognitive functions, such as the neocortex and hippocampus (34).

Materials and Methods:

In the present study, 32 adult male C57BL/6 mice (6-8 weeks) weighing 32-35 g were selected and randomly assigned to four equal groups of 24 hr, 48 hr, 72 hr,, and 1 week after brain injury (8 in each group). In each group, four mice were tested and four mice were controlled. Weight weightweight-dropping model was used to cause brain injury in the experimental group of mice. Accordingly, the mice were hit by a free-falling metal bullet weighing 75 g from a height of 20 cm to the middle of the skull (35). They were anesthetized by intraperitoneal injection of a mixture of ketamine (60 mg/kg) and xylazine (5 mg/kg). To remove the mice's brains, their heads were first amputated with a guillotine and then the brain tissue was gently removed from the skull bone. The isolated tissues were placed in PBS (Phosphate buffered saline) (1X, pH 7.4) (Betacell) and washed several times.

The tissues were cut into smaller pieces. RIPA (Radioimmunoprecipitation assay buffer 1X) (CMGRIPA) was added to each tissue in a ratio of 10: 1 (10 ml RIPA lysis buffer per gram of tissue) and was homogenized separately with a high-speed ultrasonic homogenizer for several minutes so that no pieces remained. The homogenized tissues were centrifuged at 10000 g for 10 minutes and the supernatant was transferred to new tubes for further examination. Bradford method was used to determine the protein concentration of the samples and the protein concentration of each sample was calculated using a standard curve.

Electrophoresis:

To prepare the sample, a commercial sample buffer volume was added to 4 protein sample volumes. All proteins were isolated based on molecular by using electrophoresis on 30% polyacrylamide gel in the presence of SDS (SDS-PAGE). The gel was placed in the apparatus and it was transferred to the tank after preparation. The reservoirs of the tank and the apparatus were filled with electrode buffer (reservoir) and sample buffer, respectively, at the appropriate height. To prepare the electrode buffer, 3g of Tris base, 14.4 g of glycine, and 1 g of SDS were dissolved in 1 liter of distilled water (the final pH of this solution is 8.3). By using a sampler, 10-20 µl of the prepared sample was poured into a well. Electrophoresis was performed with a current intensity of 20-30 mA for 1.5 to 2 hours.

Western blotting:

The NR2B GluR levels were measured by the Western blotting technique. Transfer of proteins isolated from acrylamide gel to polyvinylidene fluoride (PVDF) membrane was performed using electric current and using trice-glycine transfer buffer (containing 25 mM Tris, 192 mM glycine, and 15% methanol). Transfer of protein to the membrane was performed by electrophoresis with a current intensity of 300 amps for 4 hours. Blocking was performed using Skim milk 5%. Tween-20 was used as a detergent. After the blocking step, the membrane was washed 3 times, 5 minutes at each time, in a salt buffer containing Tween-0.05% (TBS-T). The membrane was placed in the primary antibody in the Rabbit anti-phospho-NMDA receptor NR2B subunit (Boster) for 1-2 hours at a

dilution of 1: 1000. The membrane was washed 4 times each time for 5 minutes in TBS-T. The membrane was placed in a secondary antibody (Peroxidase conjugated goat anti-rabbit IgG) (Boster) at a dilution of 1: 4000 for 1-2 hours. Protein bands appeared after adding a sufficient amount of electrochemical luminescence (ECL) substrate for 5-15 minutes by using radiography. After the bands appeared, the membrane was washed with a large amount of distilled water. The membrane was dried and placed in a dark place.

Statistical analysis:

Statistical analysis of data was performed in SPSS-19 software. Continuous variables were expressed as mean ± SD. Analysis of variance (ANOVA) was used to examine significant differences between groups. Data were analyzed using ImageJ software to quantify protein levels in Western blotting. Quantification was performed using the ratio of the bond of each protein to the beta-actin bond as an internal control.

Results:

The results of Western blotting analysis showed a significant decrease in the NR2B GluR levels in 1 week after traumatic brain injury (TBI) compared to the control (ANOVA, $p < 0.05$) (Cortex sham: 100±47, FP 93.8±4.8).There was no significant difference in other groups compared to the control group (Figure 1). Western blotting analysis of NR2B GluR levels in the supernatant samples of homogenized cortex tissues (sham, 24 hr, 48 hr, 72 hr, and 1 week) after TBI showed in Figure 2.

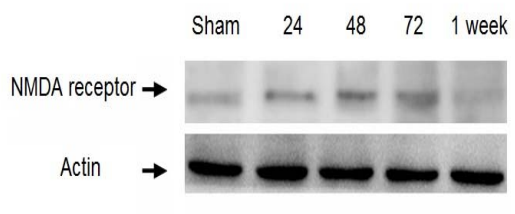

Figure 1: Western blotting of NR2B GluR levels in the supernatant samples of homogenized cortex tissues (sham, 24 hr, 48 hr, 72 hr, and 1 week) after TBI.

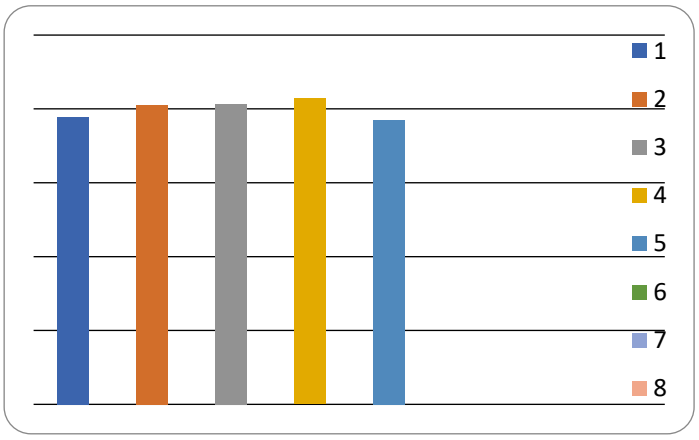

Figure 2: Western blotting analysis of NR2B GluR levels in the supernatant samples of homogenized cortex tissues (sham, 24 hr, 48 hr, 72 hr, and 1 week) after TBI.

Discussion:

Excitotoxicity caused by increased concentrations of glutamate in the synaptic space and extracellular fluid following brain trauma may lead to injury and death of nerve cells (36). Nerve cell death due to excitotoxicity is caused by the long-term activity of glutamate receptors (37). It may lead to the accumulation of calcium ions inside nerve cells and the spread of neuron death due to glutamate. However, an increase in Ca^{2+} through voltage-dependent calcium channels does not lead to excitotoxicity (38). Several studies suggest that improper regulation of Ca^{2+} entry through NMDA receptors contributes to neuronal death in acute brain injury, including stroke and ischemia, as well as neurodegenerative diseases such as Alzheimer's disease (AD) and Huntington's disease (HD) (39-42). Since NMDA receptors are involved in neuroplasticity, any change in the activity of these receptors may alter neuroplasticity (43). The relative early reduction in NMDA receptor expression immediately after brain injury may be due to the rapid death of neurons. Significant reduction in NMDA receptor expression occurred within one week after brain injury, which may indicate neuronal death or down-regulation of these receptors in brain injury. Traumatic brain injury (TBI) can reduce the level of the NR2B GluR levels, thereby reducing the activity of these receptors in brain neurons. Given what was stated, it is possible to state to what extent the complications and neurological disorders caused by TBI can be related to the expression and activity of NMDA receptors. Also, changes in the activity of NMDA receptors may be due to changes in the composition of different subunits of these receptors. Given the significant change in the activity of NMDA receptors in traumatic brain injury (TBI), it is recommended to examine the changes in the composition of different subunits of NMDA receptors in TBI.

Conclusion:

Given what was stated, it is possible to state to what extent the complications and neurological disorders caused by TBI can be related to the expression and activity of NMDA receptors. Also, changes in the activity of NMDA receptors may be due to changes in the composition of different subunits of these receptors. Given the significant change in the activity of NMDA receptors in traumatic brain injury (TBI), it is recommended to examine the changes in the composition of different subunits of NMDA receptors in TBI.

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Ethics statement:

The ethical guidelines of the study are based on the list of working with laboratory animals.

References:

1. Capizzi A et. al.,. Traumatic brain injury: AnAn overview of epidemiology, pathophysiology, and medical management. Medical Clinics. 2020 Mar 1;104(2):213-38.

2. Van der Naalt J et. al.,. Highlights mild traumatic brain injury 2021. Current opinion in anaesthesiology. 2022 Oct 1;35(5):577-82.

3. Kochanek PM et. al.,. Management of pediatric severe traumatic brain injury: 2019 consensus and guidelines-based algorithm for first and second tier therapies. Pediatric Critical Care Medicine. 2019 Mar 1;20(3):269-79.

4. Sussman ES et. al.,. Mild traumatic brain injury and concussion: terminology and classification. Handbook of clinical neurology. 2018 Jan 1;158:21-4.

5. Picetti E et. al.,. Guidelines for the management of severe traumatic brain injury fourth edition. Neurosurgery. 2017 Jul 1;81(1):E2.

6. Galgano M et. al.,. Traumatic brain injury: current treatment strategies and future endeavors. Cell transplantation. 2017 Jul;26(7):1118-30.

7. Mahran DG et. al.,. Pattern and trend of injuries among trauma unit attendants in upper Egypt. Trauma monthly. 2016 May;21(2).

8. Monsour M et. al.,. A review of the pathology and treatment of TBI and PTSD. Experimental neurology. 2022 May 1;351:114009.

9. Kaur P et.al.,. Recent advances in pathophysiology of traumatic brain injury. Current neuropharmacology. 2018 Oct 1;16(8):1224-38.

10. Brett BL et. al.,. Traumatic brain injury and risk of neurodegenerative disorder. Biological psychiatry. 2022 Mar 1;91(5):498-507.

11. Islam MB et. al.,. Differential neuropathology and functional outcome after equivalent traumatic brain injury in aged versus young adult mice. Experimental neurology. 2021 Jul 1;341:113714.

12. Ng SY et. al.,. Traumatic brain injuries: pathophysiology and potential therapeutic targets. Frontiers in cellular neuroscience. 2019 Nov 27;13:528.

13. DeKosky ST et. al.,. Injury cascades in TBI-related neurodegeneration. Brain injury. 2017 Jul 29;31(9):1177-82.

14. Mckee AC et. al.,. The neuropathology of traumatic brain injury. Handbook of clinical neurology. 2015 Jan 1;127:45-66.

15. Gabrieli D et. al.,. NMDA receptor alterations after mild traumatic brain injury induce deficits in memory acquisition and recall. Neural computation. 2021 Jan 1;33(1):67-95.

16. Luo P et.al.,. Preso regulates NMDA receptor-mediated excitotoxicity via modulating nitric oxide and calcium responses after traumatic brain injury. Cell Death & Disease. 2019 Jun 24;10(7):496.

17. Vieira M et. al.,. Regulation of NMDA glutamate receptor functions by the GluN2 subunits. Journal of neurochemistry. 2020 Jul;154(2):121-43.

18. Estrada-Rojo F et. al.,. Diurnal variation of NMDA receptor expression in the rat cerebral cortex is associated with traumatic brain injury damage. BMC Research Notes. 2018 Dec;11(1):1-7.

19. Yuan H et. al.,. Context-dependent GluN2B-selective inhibitors of NMDA receptor function are neuroprotective with minimal side effects. Neuron. 2015 Mar 18;85(6):1305-18.

20. Niswender CM et. al.,. Metabotropic glutamate receptors: physiology, pharmacology, and disease. Annual review of pharmacology and toxicology. 2010 Feb 10;50:295-322.

21. Niswender CM et. al.,. Metabotropic glutamate receptors: physiology, pharmacology, and disease. Annual review of pharmacology and toxicology. 2010 Feb 10;50:295-322.

22. Blanke ML et. al.,. 13 Activation Mechanisms of the NMDA Receptor. Biology of the NMDA Receptor. 2008 Oct 29:283.

23. Farooqui AA et. al.,. Excitatory Amino Acid Receptors in Brain. InNeurochemical Aspects of Excitotoxicity 2008 (pp. 21-35). Springer, New York, NY.

24. Bolshakov AP. Glutamate neurotoxicity: Perturbations of ionic homeostasis, mitochondrial dysfunction, and changes in cell functioning. Neurochemical Journal. 2008 Sep 1;2(3):135-45.

25. Reybrouck M et. al.,.Music and brain plasticity: how sounds trigger neurogenerative adaptations. Neuroplasticity Insights of Neural Reorganization. 2018 Jun 6;85.

26. Saraf J et. al.,.A friend or foe: calcineurin across the gamut of neurological disorders. ACS CentralCentral SScience. 2018 Jun 27;4(7):805-19.

27. Zhu S et. al.,. Mechanism of NMDA receptor inhibition and activation. Cell. 2016 Apr 21;165(3):704-14.

28. Carvajal FJ et. al.,. Regulation of phosphorylated state of NMDA receptor by STEP61 phosphatase after mild- traumatic brain injury: role of oxidative stress. Antioxidants. 2021 Oct 5;10(10):1575.

29. Yi F et. al.,. Properties of triheteromeric NMDA receptors containing two distinct GluN1 isoforms. Molecular pharmacology. 2018 Jan 1:mol-117.

30. Zhao Z et. al.,. Dexmedetomidine inhibits the PSD95-NMDA receptor interaction to promote functional recovery following traumatic brain injury. Experimental and Therapeutic Medicine. 2021 Jan 1;21(1):1-.

31. Hanson JE et. al.,. Therapeutic potential of N-methyl-D-aspartate receptor modulators in psychiatry. Neuropsychopharmacology. 2023 Jun 27:1-6.

32. López-Picón F et. al.,. Ex vivo tracing of NMDA and GABA-A receptors in rat brain after traumatic brain injury using 18F-GE-179 and 18F-GE-194 autoradiography. Journal of Nuclear Medicine. 2016 Sep 1;57(9):1442-7.

33. Moojen VK et. al.,. NMDA preconditioning prevents object recognition memory impairment and increases brain viability in mice exposed to traumatic brain injury. Brain research. 2012 Jul 23;1466:82-90.

34. Villéga F et. al.,. Cognitive and psychiatric features of anti-NMDA receptor encephalitis. The Lancet Neurology. 2022 Oct 1;21(10):861-2.

35. Kahriman A et. al.,. Mouse closed head traumatic brain injury replicates the histological tau pathology pattern of human disease: characterization of a novel model and systematic review of the literature. Acta Neuropathologica Communications. 2021 Jun 29;9(1):118.

36. Baracaldo-Santamaría D et. al.,. Revisiting excitotoxicity in traumatic brain injury: From bench to bedside. Pharmaceutics. 2022 Jan 8;14(1):152.

37. Simões AP et. al.,. Glutamate-induced and NMDA receptor-mediated neurodegeneration entails P2Y1 receptor activation. Cell Death & Disease. 2018 Feb 20;9(3):297.

38. Verma M et. al.,. Excitotoxicity, calcium,, and mitochondria: A triad in synaptic neurodegeneration. Translational neurodegeneration. 2022 Jan 25;11(1):3.

39. Baev AY et. al.,. Interaction of mitochondrial calcium and ROS in neurodegeneration. Cells. 2022 Feb 17;11(4):706.

40. Armada-Moreira A et. al.,. Going the extra (synaptic) mile: excitotoxicity as the road toward neurodegenerative diseases. Frontiers in cellular neuroscience. 2020 Apr 24;14:90.

41. Belov Kirdajova D et. al.,. Ischemia-triggered glutamate excitotoxicity from the perspective of glial cells. Frontiers in Cellular Neuroscience. 2020 Mar 19;14:51.

42. El-Sayes J et. al.,. Exercise-induced neuroplasticity: a mechanistic model and prospects for promoting plasticity. The Neuroscientist. 2019 Feb;25(1):65-85.

43. Kourosh-Arami M et. al.,. Brain is modulated by neuronal plasticity during postnatal development. The Journal of Physiological Sciences. 2021 Dec;71:1-6.