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TAURINE ATTENUATES HIPPOCAMPAL AND CORPUS CALLOSUM DAMAGE, AND ENHANCES NEUROLOGICAL RECOVERY AFTER CLOSED HEAD INJURY IN RATS

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Abstract—The protective effects of taurine against closed head injury (CHI) have been reported. This study was designed to investigate whether taurine reduced white matter damage and hippocampal neuronal death through suppressing calpain activation after CHI in rats. Taurine (50 mg/kg) was administered intravenously 30 min and 4 h again after CHI. It was found that taurine lessened the corpus callosum damage, attenuated the neuronal cell death in hippocampal CA1 and CA3 subfields and improved the neurological functions 7 days after CHI. Moreover, it suppressed the over-activation of calpain, enhanced the levels of calpastatin, and reduced the degradation of neurofilament heavy protein, myelin basic protein and αII-spectrin in traumatic tissue 24 h after CHI. These data confirm the protective effects of taurine against gray and white matter damage due to CHI, and suggest that down-regulating calpain activation could be one of the protective mechanisms of taurine against CHI.

Key words: taurine, closed head injury, calpain, corpus callosum, hippocampus.

INTRODUCTION

Traumatic brain injury (TBI) can result in neurological impairment because of immediate tissue disruption in the central nervous system (primary injury), but, additionally, surviving cells may be secondarily damaged by complex mechanisms triggered by the primary event, leading to further damage and disability. The critical mechanisms of secondary injury after brain trauma include inflammation, oxidative stress, ionic imbalance, increased vascular permeability, mitochondrial dysfunction, and excitotoxic damage. This combination of cellular and physiologic disturbances causes increased neuronal cell death, lesion enlargement, and the impairment of neurological behavior, motor, and cognition (Saatman et al., 2010; Sande and West, 2010; McAllister, 2011).

Taurine (2-aminoethanesulfonic acid) is the major intracellular free β-amino acid present in most mammalian tissues. It is not involved in primary metabolism, and neither is incorporated into proteins. It possesses a number of cytoprotective properties through its actions as a neurotransmitter, neuromodulator, osmoregulator, modulator of intracellular calcium homeostasis, antioxidant, membrane stabilizer, and anti-inflammation factor, and is reported to protect against a variety of pathological conditions including hypoxia, glutamate-induced neurotoxicity, and inflammation (Huxtable, 1992; Schuller-Levis and Park, 2004; Louzada et al., 2004). Under cell-damaging conditions, the release of taurine is increased; meanwhile, the uptake of taurine is inhibited. The increase in the extracellular levels of taurine in cell-damaging conditions may be an important endogenous protective mechanism (Saransaari and Oja, 2000). These reports suggest that taurine may act as an endogenous neuroprotectant to block multiple targets of detrimental cascade after TBI.

Marmarou's weight drop model is one of the most frequently used constrained rodent models of acceleration closed head injury (CHI) as it is inexpensive and easy to perform, although the biomechanics of the impact produced by this model is not fully and strictly controlled. Although this model cannot produce supratentorial focal brain lesion, it induces widespread damage of the neurons, axons, dendrites, and microvasculature. It is noteworthy that this model causes massive diffuse axonal injury, particularly in the corpus callosum, internal capsule, optic tracts, cerebral and cerebellar peduncles, and the long tracts in the

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Abbreviations: CHI, closed head injury; EAAs, excitatory amino acids; EDTA, ethylenediaminetetraacetic acid; EGTA, ethylene glycol tetraacetic acid; HE, hematoxylin eosin; HEPES, *N*-2-hydroxyethylpiperazine-*N*′-2′-ethanesulfonic acid; HPFs, high-power fields; IODs, integrated optical densities; LFB–PAS, Luxol fast blue–periodic acid Schiff; MBP, myelin basic protein; NF-H, neurofilament heavy protein; NSS, Neurological Severity Score; ODs, optical densities; SDS–PAGE, sodium dodecyl sulfate–polyacrylamide gel electrophoresis; TBI, traumatic brain injury.

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brainstem (Marmarou et al., 1994; Cernak, 2004). In addition, the neurons in hippocampal CA1 and CA3 subfields are more vulnerable to TBI (McCarthy, 2003), and the hippocampal neuronal damage due to CHI has been reported (Griesemer and Mautes, 2007; Chao et al., 2012). In a previous study, we have reported protective effects of taurine against brain trauma in the rat model induced by CHI, and found that taurine dose-dependently lowered the brain edema and blood–brain barrier permeability, enhanced the activity of superoxide dismutase and the level of glutathione, and reduced the levels of malondialdehyde in traumatic tissue, improved the neurological functions and attenuated the neuronal cell death in hippocampal CA1 and CA3 subfields (Sun et al., 2014). Therefore, in this study, we primarily investigated the protective effects of taurine on the white and gray matter damage through down-regulating calpain activity by using a rat model of CHI modified from Marmarou's weight drop model.

EXPERIMENTAL PROCEDURES

Ethical statement

The current study was reviewed and approved by Animal Ethics Committee of Beijing Neurosurgical Institute.

CHI

The experimental designs and all procedures were in accordance with the ARRIVE Guidelines (NC3Rs Reporting Guidelines Working Group, 2010). Every effort was made to minimize the number of animals used and their suffering. Male adult Sprague-Dawley rats (3 months old, specific pathogen free, weighing 290–330 g, Beijing Vital River experimental animals Technology Ltd., Beijing, China) were kept under controlled light conditions with a 12-h/12-h light/dark cycle. Food and water were provided *ad libitum*. All the animal experiments were performed in SPF laboratory. With the rats under chloral hydrate anesthesia (400 mg/kg, i.p.), experimental CHI was induced using a weight drop device described previously (Sun et al., 2014). Briefly, the skull of the rat was exposed by a longitudinal incision of the skin. A metal disk of 0.45 cm in diameter and 2 mm in thickness was firmly fixed by quick adhesive to the right skull vault of the rat 1 mm lateral to the midline, just in front of the coronal suture. The rat was placed on a foam bed in the prone position right under a 25-cm-tall Plexiglas tube. A 200-g weight inside the tube was allowed to precisely strike the disk cemented to the skull face. The foam bed together with the rat was then moved away from underneath the tube immediately after the impact to insure a single hit. The rat was placed on the operating table for close observation to determine whether the skull vault was fractured. The scalp was then sutured and the rat was allowed to recover from anesthesia. Rats that died on impact and those with skull fractures were excluded. In sham-operated rats, the surgical procedure was prepared for impact in the same way as above, but the animals were not subjected to head trauma. Rectal temperature was continuously monitored and maintained at 37 ± 0.5 °C by a negative-feedback-controlled heating pad during the whole experiment.

Experimental protocols

Referring to the doses of taurine used in experimental TBI and stroke, 50 mg/kg of taurine was used in this study (Sun and Xu, 2008; Sun et al., 2014). According to the latin square design, rats were randomly allocated to three groups treated with taurine or vehicle: (1) Taurine (Nanjing Pharmaceutical Factory Co., Ltd., Jiangsu, China), 50 mg/kg; (2) vehicle, normal saline (2 ml/kg), and (3) sham, normal saline (2 ml/kg). Taurine was administered intravenously twice, in a volume of 2 ml/kg, 30 min and again 4 h after induction of CHI. Traumatic control and sham animals were given vehicle (normal saline). Neurological Severity Score (NSS) was evaluated at 24 h, 48 h, and 7 days after CHI (n = 10 per group). The activities of calpain were determined, and the levels of calpastatin, neurofilament heavy protein (NF-H), myelin basic protein (MBP) and αII-spectrin were measured by Western blot 24 h after CHI (n = 7 per group). The histopathology of corpus callosum and hippocampus was observed 7 days after CHI (n = 10 per group).

Neurobehavioral evaluation

In all animals, a battery of neurobehavioral tests was performed before CHI and at 24 h, 48 h, and 7 days after CHI by an investigator blinded to the experimental groups. Neurological function was measured in terms of the NSS, an 18-point scale that assesses functional neurological status based on the presence of certain reflexes and the ability to perform motor and behavioral tasks such as beam walking, beam balance, and spontaneous locomotion (Chen et al., 2001). All the animals were subjected to neurological scoring, and histology was done on the same animals as the neurological scoring. The NSS results of animals for calpain assay and Western blot analysis were recorded but not presented in Fig. 1.

Sample collection and preparation

The tissues of right hemisphere were dissected according to the experimental protocols at 4 °C, and samples were prepared as described previously (Sun et al., 2011).

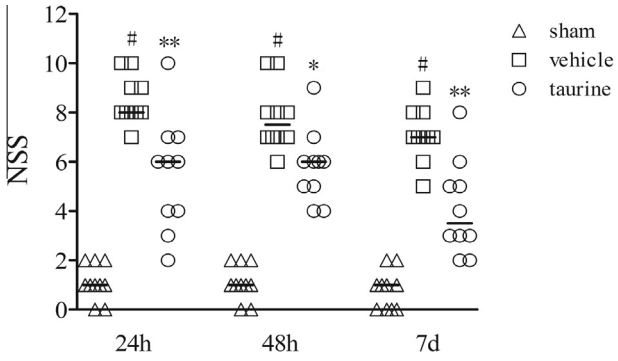

Fig. 1. Effects of taurine on the neurological deficits after closed head injury. Vehicle or taurine was administered by intravenous injection over 1 min, twice 30 min and again 4 h after induction of closed head injury. Data were presented as scatterplots, with bar as the median. n = 10. $^{\#}P < 0.001$ *vs.* sham-operated rats. $^{*}P < 0.05$ and $^{**}P < 0.01$ *vs.* vehicle-treated rats.