NUCLEAR FACTOR KAPPA B REGULATES SYMPATHETIC NERVE EXCITABILITY FOLLOWING A HEAD INJURY BY INFLUENCING THE EXPRESSION OF TUMOR NECROSIS FACTOR ALPHA

Kai Wang[#], Li Wang[#], Shuang Wu, and Lei Zhang[*]
Department of Neurosurgery, Xijing Hospital, Air Force Medical University, Xi'an, 710032, China
[#]*These authors contributed equally to this work as co-first authors*

ABSTRACT

Objective: *We sought to analyze whether nuclear transcription factor kappa B (NF-κB) regulates sympathetic nerve excitability after brain injury by adjusting the expression of tumor necrosis factor alpha (TNF-α).*

Methods: *Sixty clean-grade healthy male Sprague–Dawley rats were randomly selected to establish a rat model of sympathetic nerve excitability following a head injury. Thirty rats were randomly selected and stratified into a sham operation group and model group, respectively, with five rats in the sham operation group and 25 rats in the model group. At three, nine, 24, 48, and 72 hours after modeling (where rats were divided into five subgroups at each time point, with five rats in each group), changes in the mean arterial pressure, heart rate, plasma norepinephrine level, and expression level of TNF-α in the paraventricular nucleus of the hypothalamus were measured. The rat brain tissue was fetched and pathological changes in the rat brain tissue in each group were measured. Fifteen rats were randomly selected and divided into a sham operation group, a model group, and a TNF-α–inhibition group, respectively, with five rats included in each group. Among them, rats in the TNF-α–inhibition group were given the TNF-α inhibitor pentoxifylline at 40 μL/h; then, the expression level of TNF-α in paraventricular nucleus tissue from the hypothalamus and the change in plasma norepinephrine level in each group were determined. The remaining 15 rats were randomly divided into a sham operation group, a model group, and an NF-κB–inhibition group, with five rats included in each group; rats in the NF-κB inhibition group were given the NF-κB inhibitor pyrrolidine dithiocarbamate at 150 mg/d. The expression levels of NF-κB and TNF-α in the paraventricular nucleus of the hypothalamus were determined in each group.*

Results: *The average arterial pressure, heart rate, plasma norepinephrine level, and TNF-α expression level in hypothalamic paraventricular nucleus tissue from rats in the sham operation group were not statistically different at each time point (P>0.05). In the model group, compared with at three hours, the mean arterial pressure, plasma norepinephrine level, and TNF-α expression in paraventricular nucleus tissue from the hypothalamus were increased significantly at nine hours, while the heart rate was decreased significantly (P<0.05); further, starting after 24 hours, as more time elapsed, the levels of the above indicators gradually increased. The brain tissue of rats in the sham operation group showed regular shape, clear structure, and neatly arranged nerve fibers; in contrast, the brain tissues of rats in the model group were more traumatized, neurons were disintegrated and degenerated, and edema to a certain degree was observed. Compared with in the sham operation group, the expression levels of TNF-α, NF-κB, and plasma norepinephrine in hypothalamic paraventricular nucleus tissue from the model group were significantly increased (P<0.05); separately, compared with in the model group, the expression levels of TNF-α, NF-κB, and plasma norepinephrine in paraventricular nucleus tissue from the hypothalamus in the TNF-α–inhibition group and the NF-κB inhibition group were significantly reduced (P<0.05).*

Conclusion: *Craniocerebral injury can cause sympathetic nerve excitement, and there is an obvious inflammatory response; NF-κB may regulate the sympathetic nerve excitability by influencing the level of TNF-α.*

Keywords: *NF-κB, TNF-α, brain injury, sympathetic excitability.*

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Introduction

Craniocerebral injury is one of the most common traumas addressed with medical treatment, and it is also a medical problem that has consistently plagued the world, carrying a high fatality rate and high disability rate. According to statistics, more than 60 million patients are affected by this condition worldwide. The treatment effects, complications, and clinical prognosis of patients with craniocerebral injury often depend on the severity of the disease. In the treatment of patients with severe craniocerebral injury, more complex complications often occur, leading more frequently to a poor clinical prognosis[(1)].

Morbidity and mortality following a head injury are often caused by non-neurological consequences due to brain injury. Studies have found that, even if there is no direct extracranial organ injury, more than 85% of patients with craniocerebral injury will show obvious extracranial organ dysfunction, which can affect multiple systems[2]. The high-level nerve center is located in the hypothalamus.

The paraventricular nucleus of the hypothalamus is a multifunctional nucleus composed of a variety of neurons and is considered to be a key part of the central nervous system regulating sympathetic nerve activity that can directly or indirectly affect sympathetic nerve function[3]. It has been reported that direct excitement of the paraventricular nucleus of the hypothalamus or blocking its inhibitory pathways by drugs can increase the level of deep sympathetic nerve excitement, thereby enhancing sympathetic nerve activities such as blood pressure and heart rate[4]. When a brain injury occurs, the immune system in the brain is activated to produce a large number of pro-inflammatory cytokines, such as tumor necrosis factor alpha (TNF-α). TNF-α may play an important role in sympathetic nerve activity[5]. Nuclear factor kappa B (NF-ϰB) is a family of inducible transcription factors that play an important role in the immune system, which can participate in cell growth, apoptosis, and carcinogenesis and are particularly important in cell inflammation response and oxidative stress[6].

However, it is not clear whether NF-ϰB can regulate the excitability of the sympathetic nervous system after craniocerebral injury by regulating the expression of TNF-α. In order to explore the mechanism of action, the present study was designed.

Materials and methods

Experimental animals

Sixty clean-grade healthy male Sprague–Dawley rats (SCXK 2016-0011; Beijing Weitong Lihua Laboratory Animal Technology Co., Ltd., Beijing, China) aged eight weeks and weighing 213±17 g were randomly selected.

They were raised in the laboratory with a temperature of 23°C±2°C, a humidity of 51%±11%, and a 1:1 ratio of day to night and were allowed to eat freely. The "3R" principle was followed during all experiments.

Trial equipment and reagents

Hematoxylin and eosin staining (Beijing Borsi Technology Co., Ltd., Beijing, China); immunohistochemical staining kit (Changzhou Beiyuanxin Biotechnology Co., Ltd., Changzhou, China); anti-NF-ϰB antibody (Nanjing Saihongrui Biotechnology Co., Ltd., Nanjing, China); anti-TNF-α antibody (Beijing Qunxiao Keyuan Biotechnology Co., Ltd., Beijing, China); animal ventilator (model 687; Beijing Youcheng Shengda Biotechnology Co., Ltd., Beijing, China); electronic balance (model YP6001; Dongguan Pubiao Experimental Equipment Technology Co., Ltd., Dongguan, China); paraffin slicer (model ASONE; Shanghai Fuze Trading Co., Ltd., Shanghai, China); low-temperature, high-speed centrifuge (model MC-15; Beijing Qianming Gene Technology Co., Ltd., Beijing, China); and constant temperature water bath (model DWB20-P; Shanghai Yihui Biological Technology Co., Ltd., Shanghai, China).

Establishing a rat model of sympathetic nerve excitability after a head injury

The rats were anesthetized, the supine position was established, the tracheal intubation was inserted, each rat was placed into the center of the disk of the experimental device that can synchronously generate instantaneous line acceleration and angular acceleration pain, and the fixation device with the card posts was linked on both sides.

When the rat changed its posture due to discomfort, the pressure was released, and the cylinder controlled by the switch and the percussion machine were run to force the rat's head and body to swing laterally by 75° from the coronal plane for a moment while rotating in the horizontal direction along a rotation line in the same direction.

Observation indicators

Thirty rats were randomly selected and divided into a sham operation group and model group, respectively, with five rats in the sham operation group and 25 rats in the model group. Sham-operated rats were only subjected to the experimental steps before the head strike, without injury.

At three, nine, 24, 48, and 72 hours after modeling (with five subgroups assigned per time point, with each group containing five rats), the changes in average arterial pressure and heart rate of rats in each group were measured; 3 mL of tail vein blood was also drawn per rat at each time point, and the change in plasma norepinephrine level in each group was assessed by enzyme-linked immunosorbent assay. At each time point, the rat hypothalamic paraventricular

nucleus tissue was taken, crushed, and centrifuged to obtain the supernatant, and the optical density values of rats in each group at 450 nm were detected by a microplate reader to calculate the TNF-α expression level in hypothalamic paraventricular nucleus tissue.

Rat brain tissues were collected and paraffin sections were routinely created, and the pathological changes in the rat brain tissues in each group were determined by hematoxylin and eosin staining. Fifteen rats were randomly selected and divided into a sham operation group, a model group, and a TNF-α–inhibition group, with five rats included in each group.

Among them, rats in the TNF-α–inhibition group were given the TNF-α inhibitor pentoxifylline at 40 μL/h. The expression level of TNF-α in the paraventricular nucleus of the hypothalamus and changes in the plasma norepinephrine level were measured by enzyme-linked immunosorbent assay at 72 hours after the attack.

The remaining 15 rats were randomly divided into a sham operation group, a model group, and an NF-ϰB inhibition group, with five rats included in each group. Rats in the NF-ϰB–inhibition group were given the NF-ϰB inhibitor dithiocarbamate at 150 mg/d. At 72 hours after the attack, hypothalamic paraventricular nucleus tissue was collected from each group, and paraffin sections were routinely created; then, the expression level of NF-ϰB in hypothalamic paraventricular nucleus tissue samples from each group was determined by immunohistochemistry and western blotting.

The expression level of TNF-α in the paraventricular nucleus of the hypothalamus in each group was measured by enzyme-linked immunosorbent assay. All rats were killed after the experiment.

Statistical methods

In this study, the chi-squared test was used for a comparison of enumeration data, which were expressed as [n (%)].

Measurement data were compared among multiple groups using a single-factor multisample means approach, while an independent-samples t-test was used to compare data between the two groups, with the results of both expressed as $\bar{x}\pm s$.

In this study, the Statistical Package for the Social Sciences version 20.0 software program (IBM Corporation, Armonk, NY, USA) was used for statistical data analysis, and the statistical result $P<0.05$ was regarded as statistically significant.

Results

Comparison of changes in mean arterial pressure, heart rate, plasma norepinephrine level, and TNF-α expression level in the paraventricular nucleus of the hypothalamus in each group of rats

The average arterial pressure, heart rate, plasma norepinephrine level, and TNF-α expression level in hypothalamic paraventricular nucleus tissue from rats in the sham operation group were not statistically different at each time point ($P>0.05$).

Meanwhile, in the model group, the mean arterial pressure, plasma norepinephrine level, and TNF-α expression level in paraventricular nucleus tissue from the hypothalamus increased significantly from three hours to nine hours, while heart rate was reduced significantly ($P<0.05$); then, starting after 24 hours, as more time elapsed, the levels of the above indicators gradually increased (Table 1).

Group		n	Mean arterial pressure (mmHg)	Heart rate (time/min)	Norepinephrine (ng/mL)	TNF-α
The sham operation group	3 h	5	105.13 ± 8.65	372.11 ± 13.28	50.03 ± 4.37	3.97 ± 0.69
	9 h	5	110.14 ± 7.38	378.48 ± 11.47	52.73 ± 5.61	3.98 ± 0.65
	24 h	5	108.49 ± 6.27	383.59 ± 11.67	54.26 ± 3.73	4.00 ± 0.58
	48 h	5	109.63 ± 6.39	389.37 ± 14.58	55.49 ± 3.48	4.01 ± 0.54
	72 h	5	111.28 ± 5.72	390.67 ± 14.64	56.52 ± 4.57	4.02 ± 0.63
The model group	3 h	5	110.53 ± 6.83	415.53 ± 17.42	52.49 ± 3.56	4.07 ± 0.65
	9 h	5	136.36 ± 8.74[a]	375.83 ± 10.26[a]	65.76 ± 5.47[a]	4.98 ± 0.57[a]
	24 h	5	122.54 ± 6.85	381.22 ± 8.45	61.36 ± 4.29	4.84 ± 0.55
	48 h	5	149.29 ± 5.94	389.46 ± 13.87	68.39 ± 5.52	5.24 ± 0.76
	72 h	5	160.48 ± 6.37	391.97 ± 10.25	75.95 ± 4.58	6.12 ± 0.68

Table 1: Comparison of changes in mean arterial pressure, heart rate, plasma norepinephrine and TNF-α expression levels in the paraventricular nucleus of the hypothalamus in each group of rats ($\bar{x}\pm s$).
Note: Compared with three hours, [a]P<0.05.

Pathological changes in rat brain tissue from each group

The brain tissue of rats in the sham operation group showed a regular shape, clear structure, and neatly arranged nerve fibers; in contrast, brain tissue samples taken from rats in the model group appeared more traumatized, with disintegrated and degenerated neurons and some amount of edema (Figure 1).

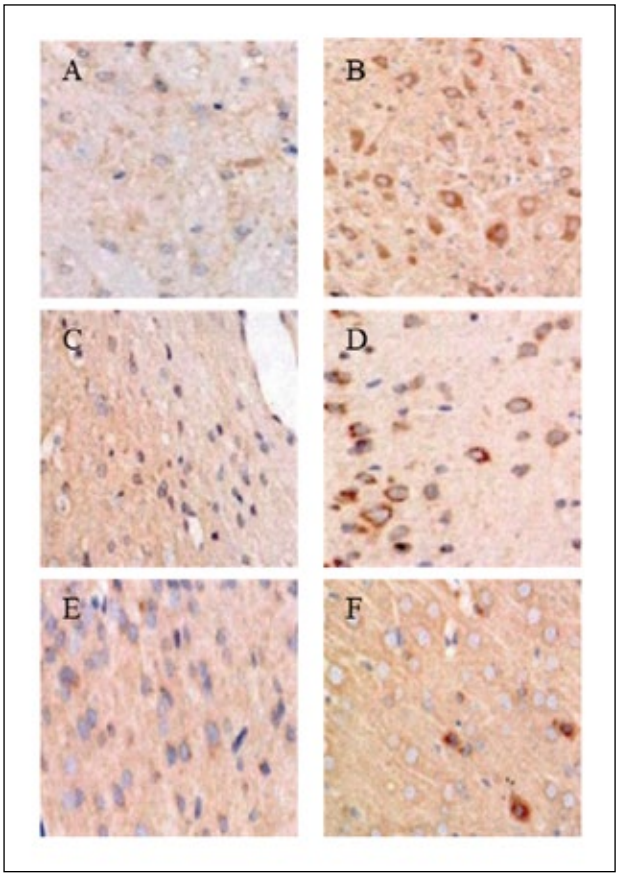

Figure 1: Pathological changes in rat brain tissue in each group.
A: Brainstem in the sham operation group. B: Brainstem in the model group. C: corpus callosum in the sham operation group. D: corpus callosum in the model group. E: hippocampus in the sham operation group. F: hippocampus in the model group.

Comparison of TNF-α expression levels and plasma norepinephrine levels in the hypothalamic paraventricular nuclei of rats in each group

Compared with in the sham operation group, the expression levels of TNF-α and plasma norepinephrine in hypothalamic paraventricular nucleus tissue from the model group were significantly increased (P<0.05); separately, compared with in the model group, the expression levels of TNF-α and plasma norepinephrine levels in paraventricular nucleus tissue from the hypothalamus in the TNF-α–inhibition group were significantly reduced (P<0.05) (Table 2).

Comparison of the expression levels of NF-ϰB and TNF-α in hypothalamic paraventricular nucleus tissue from rats in each group

Compared with in the sham operation group, the expression levels of NF-ϰB and TNF-α in hypothalamic paraventricular nucleus tissue from the model group were increased significantly (P<0.05). Separately, the expression levels of NF-ϰB and TNF-α in hypothalamic paraventricular nucleus tissue from the NF-ϰB–inhibition group were significantly reduced relative to in the model group (P<0.05) (Figures 2 and 3 and Table 3).

Group	n	TNF-α	Norepinephrine (ng/mL)
The sham operation group	5	3.98 ± 0.18	53.73 ± 2.48
The model group	5	6.29 ± 0.98	68.49 ± 3.57
TNF-α–inhibition group	5	4.32 ± 1.93	61.36 ± 2.94
F		4.95	29.68
P		0.027	< 0.001

Table 2: Comparison of TNF-α expression levels and plasma norepinephrine levels in the hypothalamic paraventricular nuclei of rats in each group ($\bar{x}$±s).

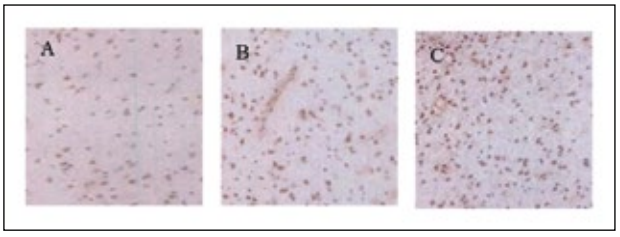

Figure 2: The expression of NF-ϰB in paraventricular nucleus tissue from the hypothalami of rats in each group. *A: The sham operation group. B: The model group. C: The NF-ϰB–inhibition group.*

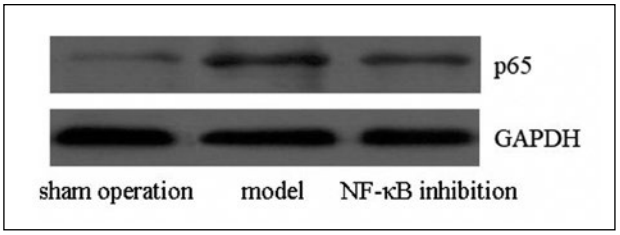

Figure 3: The expression of NF-ϰB in paraventricular nucleus tissue from the hypothalami of rats in each group.

Group	n	TNF-α
The sham operation group	5	3.92 ± 0.68
The model group	5	6.12 ± 1.02
NF-ϰB inhibition group	5	4.26 ± 0.52
F		11.86
P		0.001

Table 3: Comparison of the expression levels of TNF-α in hypothalamic paraventricular nucleus tissue from rats in each group ($\bar{x}$±s).

Discussion

Craniocerebral injury is a common disease in neurosurgery, and its fatality and disability rates rank first among those of various traumatic diseases. According to published statistics, more than 600,000 people suffer from head injury in China each

year, and more than 100,000 of these individuals die from the event annually. The pathogenesis of craniocerebral injury is more complicated, with various causes, and it is difficult to treat and resolve this condition. Once a craniocerebral injury occurs, the patient's condition often deteriorates rapidly, and death or serious complications can occur within a short period of time[7]. With continuous advancements in science and technology, however, the diagnostic level of craniocerebral injury has been significantly improved, yet its mortality and disability rates remain high. Therefore, it is important to strengthen the study exploration of the pathogenesis of craniocerebral injury, identify any changes in its occurrence and development, improve its cure rate, and reduce its death and disability rates.

Studies have found that patients with craniocerebral injury often have some symptoms similar to sympathetic excitement, especially in those with severe craniocerebral injuries involving deep brain structures, where the proportion of such symptoms is greater[8]. The emergence of sympathetic excitement after head injury often makes the treatment of this type of injury more difficult. The hypothalamus, the high-level center of the sympathetic nervous system, is small in size and located in the center of the brain, where it maintains the normal physiological functions of the brain[9]. The paraventricular nucleus in the hypothalamus and the rostral ventrolateral medulla nucleus in the brainstem, respectively, are the key nuclei for sympathetic excitement; these structures are connected to one another and compose the excitement center of the sympathetic nervous system. The cell composition of the paraventricular nucleus of the hypothalamus determines its functions in regulating the cardiovascular system, neurohumoral balance, and sympathetic nerve excitement, and is an important source of sympathetic excitement[10].

It is reported that different excitatory and inhibitory neurotransmitters and neuromodulators, such as angiotensin II, γ-aminobutyric acid, and norepinephrine, in the paraventricular nucleus of the hypothalamus may regulate the tonic activity of neurons[11]. Norepinephrine is the main excitatory afferent transmitter in the paraventricular nucleus of the hypothalamus, which can activate postsynaptic neurons and further excite sympathetic neurons; In addition, craniocerebral injury itself includes a series of inflammatory reactions. When the brain structure is damaged, a large number of inflammatory factors are released, upsetting the balance in the expression of excitatory and inhibitory neurotransmitters, thereby causing sympathetic nerve excitement[12, 13]. TNF-α is one of the most common inflammatory factors. Studies have confirmed that TNF-α can activate the hypothalamic–pituitary–adrenal axis, facilitate adequate levels of norepinephrine and glucocorticoids, and induce upregulation of sympathetic nerve activity[14].

The results of this study found that craniocerebral trauma may cause sympathetic nerve excitement, and the degree of excitement increases with time; in addition, the levels of inflammatory factors in the paraventricular nucleus of the hypothalamus were increased significantly after head injury and are closely correlated with sympathetic excitement.

NF-κB is a transcription factor widely expressed in organisms; it plays an important role in the body's immune system and can also regulate various inflammatory responses. Studies have found that there is a complex regulatory relationship between TNF-α and NF-κB, and TNF-α can activate NF-κB, thereby playing a role[15]. Some scholars in the area of heart failure found that inhibiting NF-κB activation can significantly improve heart failure in rats and inhibit sympathetic nerve activity[16]. Separately, the results of this study suggested that NF-κB in the paraventricular nucleus of the hypothalamus is able to regulate the expression of TNF-α, thereby influencing sympathetic excitement.

In conclusion, craniocerebral injury can cause sympathetic nerve excitement, and there is an obvious inflammatory response; NF-κB may regulate the sympathetic nerve excitability by regulating the level of TNF-α.

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Corresponding Author:
Lei Zhang
Email: phg35t@163.com
(China)