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EFFECTS OF LOCAL HYPOTHERMIA ON SPINAL CORD INJURY IN RATS

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Abstract

The aim of this study was to evaluate possible neuroprotective effects of local hypothermia on the functional state of the spinal motor centers of the calf muscles after a contusion injury of the spinal cord (SCI) in rats. It was assumed that local hypothermia can decrease the secondary damage of SCI and can be considered as a way to treat SCI. The experiments were carried out on 49 adult Wistar rats with the weight of 250–300 g. All procedures were performed in accordance with the bioethics rules. SCI was performed in the region of Th8–Th9 vertebrae. The analysis of the motor functions assessed in the open field test and of the parameters of the motor evoked potentials of hindlimb muscles induced by epidural stimulation of the spinal cord was carried out during a month period after the injury. It was shown that the locomotor ability of rats in the open field test did not differ between the group with SCI and the group with local hypothermia. The amplitude of motor evoked potentials of the calf muscles significantly decreased and the thresholds of inducing these potentials increased in the group with hypothermia. Thus, the results suggest that local hypothermia after SCI can delay the development of functional excitation of the neuro-motor pathways induced by SCI. The obtained data can be useful for developing new therapeutic approaches, which are necessary to delay the pathophysiological processes associated with the secondary damage, as well as to limit the expression of neurological dysfunction induced by SCI.

Keywords: spinal cord injury, motor evoked potentials, epidural stimulation, locomotor activity, local hypothermia

Introduction

Spinal cord injury is accompanied by compression, which leads to nervous tissue damage and loss of motor functions below the injury site [1]. The experiments with animal SCI models confirm that decompression surgery is most effective during the first 1–3 h after the injury [2, 3]. In clinical conditions, decompression is performed in 10–24 h after the injury (including transportation, diagnosis, and preparation for surgery) [4–6]. Secondary spinal cord damage is initiated by hemorrhage and expansion of the edema. This, along with neuronal metabolism disruption and apoptosis, aggravates the spinal cord damage and weakens the nerve signal transfer [7–10].

Therefore, there is high need for a therapy to prevent the neurological deterioration of spinal cord before decompression surgery. One possible preventing treatment that has shown an encouraging result over the past few years is therapeutic hypothermia. Therapeutic hypothermia approaches can be either local or systemic. Systemic hypo-

thermia results from whole-body temperature lowering, while local hypothermia is associated with cooling only the area of injury or spinal cord. Both approaches presumably inhibit any secondary damage of SCI and delay subsequent inflammatory and metabolic changes [11–15].

However, it has been shown that systemic hypothermia can be associated with unwanted effects, such as bradycardia, impaired phagocytic function of leukocytes, and violation of thermoregulation leading to pneumonia and uncontrolled tremors [16]. Thus, local hypothermia can be a safer alternative [17, 18]

The aim of the present work was to evaluate possible neuroprotective effects of local hypothermia on the motor function and functional state of the spinal motor centers of the calf muscles in rats subjected to a contusion injury of the spinal cord.

Materials and Methods

The experiments were carried out on adult Wistar rats (weight 250–300 g; $n = 49$). All procedures were performed in accordance with the bioethics rules: the keeping, feeding, caring for animals and their elimination from the experiment were in line with the requirements of the Order of the Ministry of High and Secondary Specialized Education of the USSR no. 742 of November 13, 1984, which approved the "Rules for using experimental animals" and is valid until the present time; the Directive of the European Parliament and of the Council of September 22, 2010 on the protection of animals used for scientific purposes (no. 2010/63/UE). The research protocol was approved by the Ethics Committee of the Kazan Federal University.

Surgical procedures. All surgical procedures were performed under general anesthesia by intramuscular injection of 1 mg/kg of Zoletil 50 and 0.05–0.10 mL/kg of Xylovet injection.

Electrode implantation. Partial laminectomy at the level of two vertebrae, L2 and L4, was performed under general anesthesia to implant the stimulating electrode. The stainless-steel wire with a Teflon coating (793500 PFA-Coated Stainless Steel, A-M Systems) was passed under the spinous process over the dura mater of the remaining vertebrae between the laminectomized areas. When a small part (~ 1 mm) of the Teflon coating was removed, the electrodes were aligned along the midline of the S1 segment of the spinal cord. The ground electrode was placed subcutaneously in the shoulder region. Intramuscular EMG electrodes were sutured bilaterally to the middle of the muscles [19]. Implantation of electrodes was carried out immediately before electrophysiological testing.

Model of spinal cord injury. Standard contusion spinal cord injury (SCI) was performed by the modified Allen's methods (1911) [20]. The weight of 10 g vertically fell from the height of 2.5 cm on the spinal cord in the Th8-Th9 level of the opened vertebrae. Reflex stretching of the hindlimbs was observed as an indicator of successful contusion injury. The weight was immediately removed from the spinal cord after the impact. The operated animals were subcutaneously injected with 5 mL of a 0.9% solution of sodium chloride. During the first day, additional heating was provided. After all the procedures, the animals were placed in individual cells with free

access to water and food. In the postoperative period, the bladders of the contusion rats were expressed manually three times per day for the first two days after the surgery. The maximum period of clinical observation of the animals was 30 days.

Local hypothermia procedure. Local hypothermia was applied immediately after SCI. The prepared refrigerant was placed in the decompression window of the laminectomized vertebra on the dura mater of the injured spinal cord for 20 min. All procedures were carried out according to R.F. Tumakaev's methodology [21]. A solution of 0.9% sodium chloride was used as a refrigerant.

Evaluation of locomotor activity. The locomotor ability of the experimental animals was accessed by the open field test according to the Basso–Beattie–Breshnan (BBB) scale [22]. The animals were placed in an open field area and their motor ability was assessed during 4 min. The early stage (0–7) of BBB scores corresponded to complete or partial paralysis of hindlimbs, intermediate stage (8–13) corresponded to partial recovery of movement coordination, and late stage (14–21) corresponded to complete recovery of fore-limb and hindlimb coordination. Open field testing was carried out on days 1, 3, 7, 14, 21, and 30 after SCI.

Electrophysiological registration. Motor evoked potentials of the rat calf muscles induced by the stimulation of the S1 segment of spinal cord were registered using the A-M Systems equipment and Datawave software. The intensity of stimulation varied from 0, 1 to 10 V, the stimulus duration was 0.5 ms.

A single stimulus applied to the S1 spinal-cord segment induced a motor evoked potential, consisted from the early component (ER), middle component (MR), and late polysynaptic component (LR) [23]. The latency, threshold, and maximum amplitude of the response components were determined. All electrophysiological records from the muscles were analyzed in the interval of 14 ms from the stimulus and divided into three windows by latency: 1.5–6.5 ms for ER, 6.5–10.5 ms for MR, and 10.5–13.5 ms for LR. The peak amplitude from peak to peak of each response was calculated as the average of seven responses and is presented as a percentage of the reference value.

Experimental design. The experimental animals were divided into 2 groups: animals of the first group (SCI) did not receive any treatment after SCI ($n = 20$); animals of the second group (SCI + Hypothermia) were subjected to local hypothermia during 20 min after SCI ($n = 22$).

The motor evoked potentials of m. soleus and m. tibialis anterior registered from intact animals were control ($n = 7$).

The electrophysiological recordings were carried out on days 1, 3, 7, 14, 21, and 30 after SCI.

Statistical processing of experimental results. Statistical processing of the experimental results was made with the help of the SigmaPlot software. The results were shown as mean ± SEM. The one-way ANOVA was used for comparison of the experimental groups. The statistical significance criterion was $p < 0.05$.

Results

Evaluation of the effects of local hypothermia on the motor ability of rats in the open field test after SCI. The obtained data show that before the injury animals had normal locomotor ability (21 scores) (Fig. 1). The movement of hindlimbs in both experimental groups drastically reduced in 24 h after SCI. Thus, the first (n = 17) and second (n = 16) groups had 2.7 ± 0.6 and 1.96 ± 0.6 scores on the BBB scale, respectively. On the third day after the injury, there was an increase in the motor ability. The BBB score increased up to 8.6 ± 1.6 in the first group of animals, as compared with 3.9 ± 0.6 ($p < 0.05$) in the second group of animals with local hypothermia after the spinal cord injury.

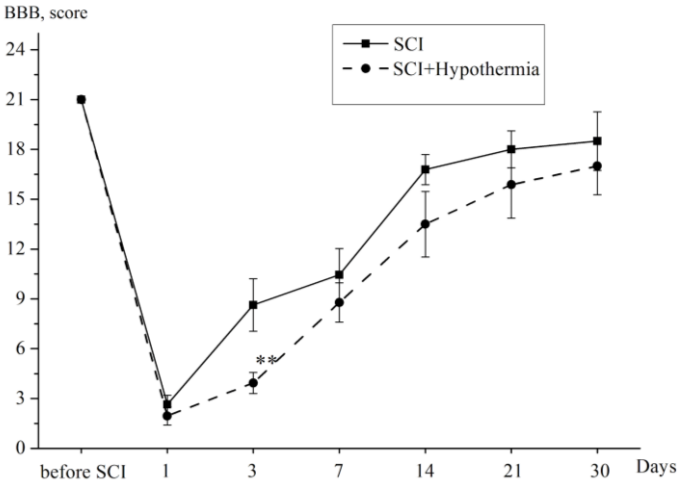

Fig. 1. Changes in the locomotor ability of the experimental group in open field test according to the BBB scale. SCI – the first group of animals with SCI; SCI + Hypothermia – the second group of animals with local hypothermia treatment. Vertical axis shows the values of locomotor ability in scores of the BBB scale. Horizontal axis shows the days after SCI. ** – $p < 0.05$, differences between the second and first experimental groups

Changes in the maximum amplitude of ER and MR components of the motor evoked potentials of m. soleus and m. tibialis anterior in a month after SCI. The recordings of the motor evoked potentials of m. soleus in rats of the first group showed that the amplitude of the early component (ER) significantly increased on day 1 after SCI, by 149 ± 14% ($p < 0.05$, difference from the intact animals, n = 12), and significantly decreased on day 7, by 67.9 ± 16% (n = 8). In the second group of animals, the maximum amplitude of ER from m. soleus significantly decreased on day 1 after the injury, 47 ± 9% ($p < 0.05$, n = 8), and this trend continued in the subsequent seven days after SCI. On day 30 after SCI, the ER amplitude slightly decreased in the first group of animals and increased in the second group (Fig. 2A).

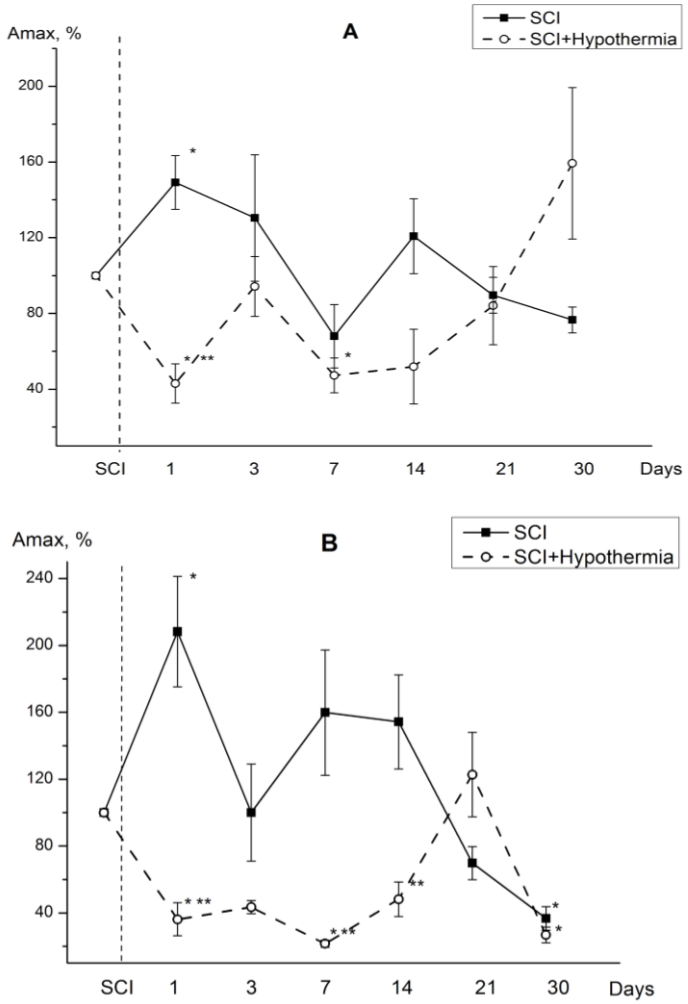

Fig. 2. Changes in the maximum amplitude of ER of the motor evoked potentials of m. soleus (A) and of m. tibialis anterior (B) in rats from both experimental groups. SCI – the first group of animals with SCI; SCI + Hypothermia – the second group of animals with local hypothermia treatment. Vertical axis shows the values of maximum ER amplitude in percentage from the control value. Horizontal axis shows the days after SCI. * – $p < 0.05$ differences between the control values and the values of the experimental groups, ** – $p < 0.05$, differences between the second and first experimental groups

The recordings of the motor evoked potentials of m. tibialis anterior showed another dynamics of changes after the injury. The ER amplitude of m. tibialis anterior in the first group significantly increased on day 1 after SCI, by 208 ± 33% ($p < 0.05$, $n = 10$), and significantly decreased only on day 30 after the injury, by 36 ± 7% ($p < 0.05$, $n = 8$). In the second group of animals, the ER amplitude decreased during the first week after SCI and reached 21 ± 2% ($p < 0.05$) on day 7 after SCI. On day 30, the ER amplitude of m. tibialis anterior decreased by 26 ± 4% ($p < 0.05$, $n = 6$) (Fig. 2B).

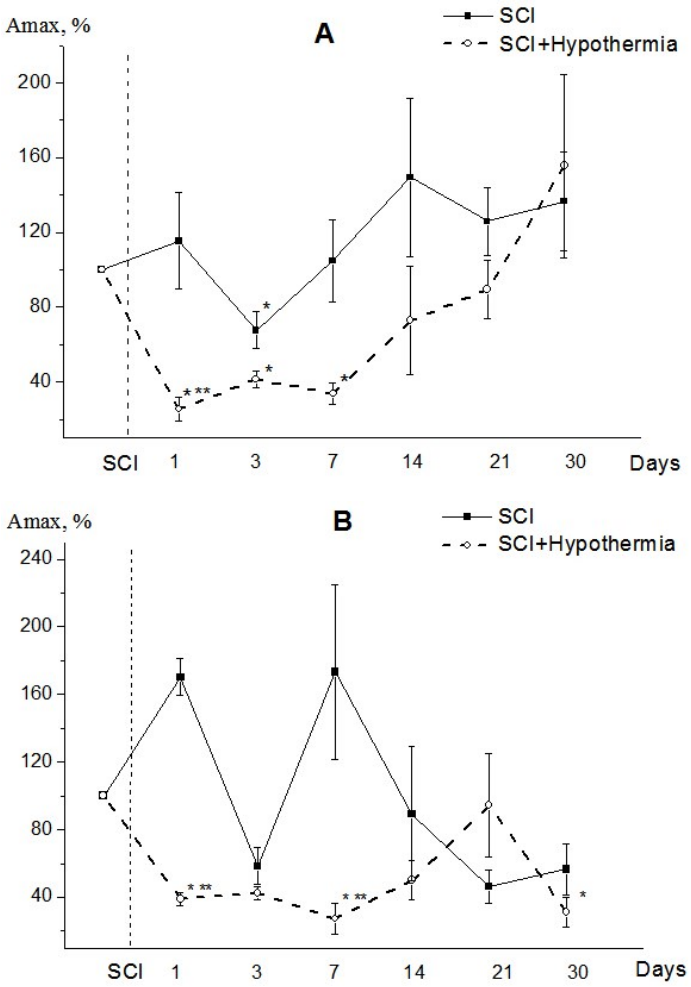

Fig. 3. Changes in the maximum amplitude of MR of the motor evoked potentials of m. soleus (A) and of m. tibialis anterior (B) in rats from both experimental groups. SCI – the first group of animals with SCI; SCI + Hypothermia – the second group of animals with local hypothermia treatment. Vertical axis shows the values of maximum MR amplitude in percentage from the control value. Horizontal axis shows the days after SCI. * – $p < 0.05$, differences between the control values and the values of the experimental groups, ** – $p < 0.05$, differences between the second and first experimental groups

The analysis of the maximum amplitude of the MR of m. soleus showed that the MR amplitude did not significantly change during the month in the first group of animals. However, the MR amplitude of m. soleus in the second group decreased to 25.6 ± 6.5% ($p < 0.05$) on day 1 after SCI and was up to 33 ± 5% ($p < 0.05$) on day 7 after SCI (Fig. 3A). The MR amplitude of m. tibialis anterior in the first group of animals did not significantly increase on day 1 and had no significant decrease on day 21 (Fig. 3B). In the second group, the MR amplitude decreased from day 1 after SCI, 39 ± 4% ($p < 0.05$), to day 30, 31.9 ± 9% ($p < 0.05$) (Fig. 3B).

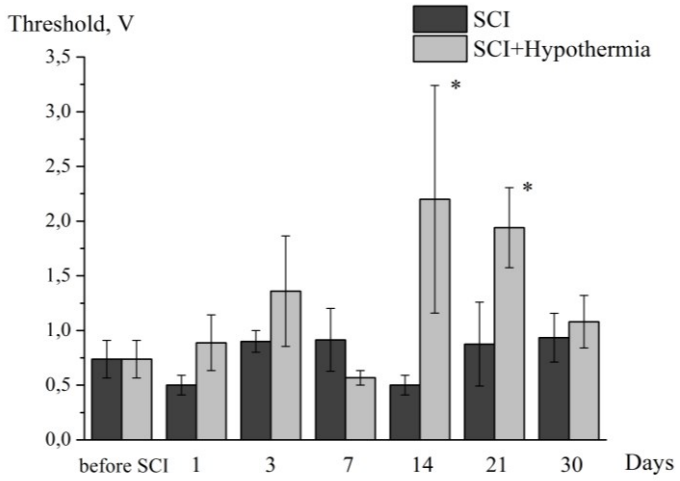

Fig. 4. Changes in the threshold of inducing of the ER component of the motor evoked potentials of m. tibialis in rats from both experimental groups. SCI – the first group of animals with SCI; SCI + Hypothermia – the second group of animals with local hypothermia treatment. Vertical axis shows the absolute values of the threshold of inducing of the ER component of the motor evoked potentials. Horizontal axis shows the days after SCI. * – $p < 0.05$, differences between the control values and the values of the experimental groups

Changes in the thresholds of inducing of the motor evoked potentials of m. soleus and m. tibialis anterior during a month after SCI. The threshold of inducing of the ER of the motor evoked potentials of m. soleus did not change significantly in either the first or second groups of animals. Nevertheless, the threshold of the ER of the motor evoked potentials of m. tibialis anterior significantly increased in the second group of animals, up to 2.2 ± 1.04 V, on day 14 and up to 1.94 ± 0.4 V on day 21 after SCI, but did not significantly change in the first group of animals (Fig. 4).

The threshold of inducing of the MR of the motor evoked potentials of m. soleus did not significantly change in the first group of animals, but increased to 1.8 ± 0.2 V on day 21 after SCI in the second group of animals (Fig 5A). The threshold of the MR of the motor evoked potentials of m. tibialis anterior did not significantly change in the first group of animals as well, and increased to 2 ± 0.3 V on day 21 day after SCI in the second group (Fig. 5B).

Discussion

SCI treatment has been limited to methylprednisolone, surgical interventions, and rehabilitation. However, these clinical approaches are not sufficient to provide recovery of the motor functions after SCI. Currently, new therapeutic approaches have been developed to delay the pathophysiological processes associated with the secondary damage, to limit the expression of neurological dysfunction induced by SCI. One of these promising approaches is hypothermia [24].

However, the role of hypothermia in curing spinal cord injuries with neurological deficit is still uncertain. Hypothermia can reduce the area of spinal cord injury, limit the secondary damage of SCI, and improve the neurological recovery [25–27].

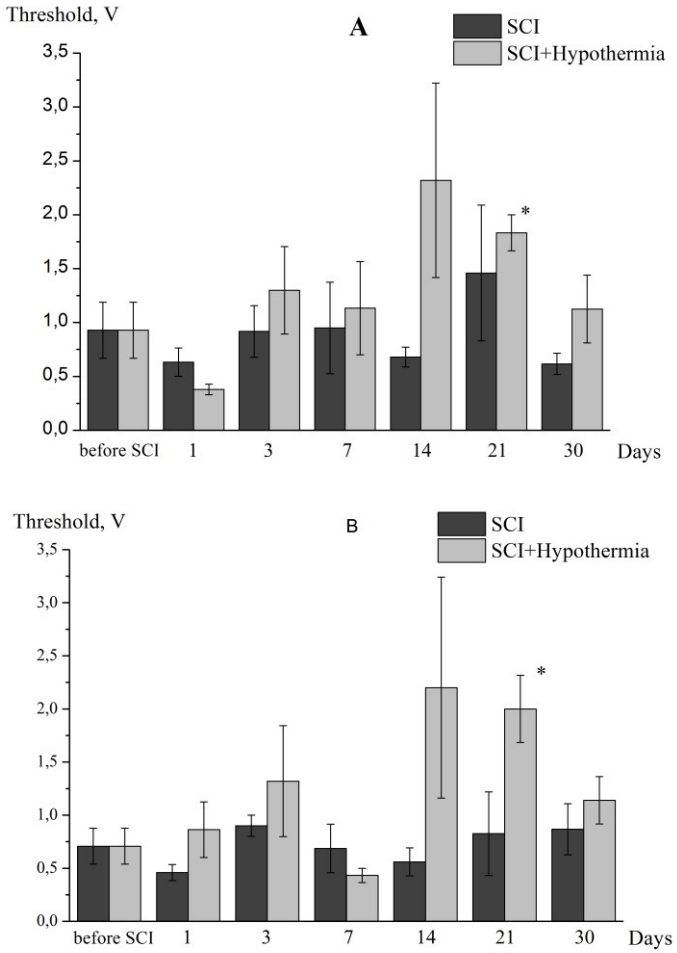

Fig. 5. Changes in the threshold of inducing of the MR component of the motor evoked potentials of m. soleus (A) and m. tibialis anterior (B) in rats from both experimental groups. SCI – the first group of animals with SCI; SCI + Hypothermia – the second group of animals with local hypothermia treatment. Vertical axis shows the absolute values of the threshold of inducing of the MR component of the motor evoked potentials. Horizontal axis shows the days after SCI. *– $p < 0.05$, differences between the control values and the values of the experimental groups

C.E. Casas et al. [28] reported that epidural hypothermia with physiological infusion during the 3 h after the injury had no neuroprotective effect on the recovery of rats after 6 weeks of estimating following the injury. The BBB score of locomotor ability did not differ between the experimental groups. Moreover, the authors pointed out that hypothermia can potentially intensify the secondary damage by decreasing the blood flow to the damaged area of the spinal cord. In our investigation, we did not found significantly differences in the functional testing between the first and the second experimental groups. The main reason was probably that the methods of hypothermia application were not optimal and need further improvement. We applied hypothermia immediately after the injury during 20 min to prevent development of the secondary damage. We also used intensive hypothermia on the epidural space

with the refrigerant temperature from 0 °C to 4 °C and it was impossible to control the temperature of the surrounding tissue.

The duration and depth of cooling, and the rate of rewarming to normothermia as well, are the important factors influencing the results. It was reported that the longer hypothermia was more efficient [29], but no optimal timing for hypothermia application was defined. Probably, in our experiments higher intensity of temperature application inhibited and aggravated the secondary damage that had a negative impact on the results of the functional tests. On the other hand, R. Morochovic et al. [30] did not find any functional improving after the hypothermia applied subcutaneously in the model of compression injury in rats.

J.R. Dimar et al. [31] showed that the motor activity of injured rats in the open field test significantly improved after applying the moderate injury, but did not improved in case of heavy injury. It was shown that deep epidural hypothermia after the use of the moderate model of injury, such as contusion injury, did not reproduce the effects that are normally observed in the model of spinal cord ischemia.

The inhibition of the direct first response after SCI by hypothermia was reported earlier in the clinical research on human patients with paraplegia [32] and in the experiments on rats with SCI [33]. This adaptation can be explained by the reversible changes of the axonal properties after the injury [34, 35], which correlated with the inhibition of the M-wave in patients after SCI [32].

The increasing of monosynaptic reflexes (correspond to MR in our experiments) after SCI was earlier described in the experiments on the rat SCI model [36, 37], on the cats model [38], and in clinical research [39, 32]. The reflex facilitation after SCI can be related with morphofunctional changes of motoneurons and/or changes at the interneurons level, appearing as the results of disinhibition after the abolition of supraspinal control. The primary reason of the changes must have been the hyperactivity of α-motoneuros initiated by the changes in the intrinsic properties of motoneurons [40]. The second reason can be the reduction of presynaptic inhibition [41, 42] or decrease of the postactivation depression of Ia fibers [43].

In addition, SCI can induce changes in the relationship between motoneurons and the surrounding glial cells or in the ionic balance inside the intercellular space, thereby affecting the biochemical and biophysical properties of motoneurons. At the second stage of SCI, the absence of inhibitory inputs from the destroyed descending pathways can lead to an uncontrolled change in the properties of motoneurons, causing hyperreflexia and increasing the muscle tone.

The use of local hypothermia in our study led to opposite changes in the parameters of the motor-evoked muscle responses. The mechanisms underlying this inhibitory effect included a decrease in the release of excitatory amino acids, inhibition of calcium influx, inhibition of the inflammatory response, suppression of edema formation, and decrease in the rate of oxygen metabolism and anesthesia [44–45].

The hemodynamic consequences of cooling the spinal cord also have a bad effect on the recovery process, because lowering the rate of blood flow to critical levels disrupt tissue retention and, therefore, changes the functional outcome [46-48]. The activity of neurotransmitters depends on temperature, but inhibiting this activity, in contrast to the consequences of the secondary activation, can play a less important role in the recovery process [49, 50].

Thus, the results of our study suggested that local hypothermia after contusion spinal cord injury can delay the development of functional excitation of neuro-motor pathways induced by the spinal cord injury in the short-time period after the application.

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Эффекты локальной гипотермии при травме спинного мозга у крыс

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Аннотация

В статье анализируются возможные нейропротекторные эффекты локальной гипотермии на двигательную функцию и функциональное состояние спинальных двигательных центров мышц голени крысы после контузионной травмы спинного мозга. Мы обследовали 49 половозрелых крыс линии Wistar обоего пола с массой тела 250–300 г. Все эксперименты были выполнены с соблюдением действующих биоэтических норм. В ходе экспериментов воспроизводили стандартную модель контузионной травмы спинного мозга средней степени тяжести. Оценку двигательной активности в тесте «открытое поле» и параметров вызванных моторных потенциалов мышц задних конечностей крысы при эпидуральной стимуляции спинного мозга производили до 30 сут после травмы. Полученные результаты не выявили существенной разницы между животными двух групп в функциональных тестах. Амплитуда вызванных ответов мышц голени при эпидуральной стимуляции в группе с локальной гипотермией снижалась, пороги увеличивались. Таким образом, мы можем сделать вывод, что локальная гипотермия после контузионной травмы спинного мозга может задерживать развитие функционального возбуждения нейромоторного аппарата у крыс непосредственно при воздействии гипотермии.

Ключевые слова: травма спинного мозга, крыса, электрофизиология, эпидуральная стимуляция, локальная гипотермия, двигательная активность

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