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Excitability of the Nociceptive System in Rats after Blast-Induced Traumatic Brain Injury

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In a group of healthy adult male albino rats (n=6), we measured pain thresholds under conditions of electrocutaneous stimulation of the limbs (in a chamber with an electrified floor). The animals were subjected to the action of a modeled baroacoustic wave (excess pressure 26–36 kPa) leading to mild blast-induced traumatic brain injury (bTBI). It was found that such a trauma resulted in a long-lasting (up to four weeks) decrease in the above threshold (i.e., in an increase in the sensitivity of the nociceptive system) estimated according to the minimum intensity (μA) of 50-Hz alternating stimulation current evoking a pain-related behavioral response (vocalization). The pain threshold was measured at repeated stimulations of the increasing intensity of animals under light inhalation (halothane) anesthesia. There were reasons to believe that such an effect included two phases, an early (up to three days) and a later more long-lasting phase. The dynamics of the pain threshold in the bTBI group of rats were compared with those in the groups of fully intact rats (intact group) and rats subjected to the procedures of inhalation anesthesia and soft fixation but with no action of the baroacoustic wave (sham group). It is concluded that even mild blast-related trauma leads to significant long-lasting changes in the functioning of the nociceptive and antinociceptive brain neuronal systems, especially in their opioid-mediated components. These shifts develop due to energy deficiency, oxidative stress, and the accompanying mitochondrial damage. Such findings confirm suppositions that blast trauma-related changes in the cerebral opioid systems play a considerable role in the disorders of brain cognitive functions disturbed because of a blast-induced brain injury.

Keywords: modeled baroacoustic wave, blast-induced traumatic brain injury (bTBI), pain threshold, electrocutaneous stimulation, nociceptive and antinociceptive cerebral systems.

INTRODUCTION

Studies of various links to the pathogenesis of brain damage resulting from the action of a blast wave, the main pathogenetic factor of an explosion, are at present very urgently needed due to widespread use of various types of explosives in the course of military conflicts, including the Russian large-scale war against Ukraine, which is continuing to this day [1]. Data from clinical observations and experimental studies demonstrated that even a mild blast-induced traumatic brain injury (bTBI) is accompanied by the development of anxiety, depression, and noticeable memory impairments [2, 3]. Also, many patients suffering from the results of the action of explosions and traumatic brain injuries (TBIs) from other geneses complain of intense headache, a type of central pain that significantly negatively affects the patient's life quality [4]. Studies of functional disorders of pain perception of TBI allowed researchers to conclude that subdivisions of the nervous system responsible for pain perception and also those responsible for the conduction of impulses from the brain to efferent components of the nervous system are noticeably disordered due to the formation of a central pain focus. There are also important reasons to believe that there are significant pathological events in the central nociceptive and antinociceptive systems [5]. At the same time, it is necessary to recognize that specificities of the brain response to pain-evoking stimulation have not been convincingly identified in the case of bTBI [6].

It should be taken into consideration that the perception of the main-inducing irritation and corresponding responses to the action of pain significantly affect the processes of increased nervous activity [7]. Pain is one of the evolutionarily oldest nervous phenomena in mammals and, at

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the same time, a process whose mechanisms of initiation, transmission, and perception in the case of external and/or internal damages are rather difficult to investigate [8]. Additionally, it should be noted that there is no single specific pain center in the brain; a number of different structures of the CNS are assembled into the nociceptive system reacting to any painful stimuli [9]. In addition to the nociception system, there is also a central antinociceptive system providing analgesic effects, which includes opioid and nonopioid subsystems that significantly influence the perception of a pain-evoking stimulation [10, 11]. For these reasons, we undertook an experimental study in which we measured pain thresholds with respect to electrocutaneous stimulation in rats subjected to the action of a modeled blast wave evoking a mild bTBI.

METHODS

Traumatic brain injury was induced in experimental animals using a device capable of adequate modeling of a blast wave (see the following). It should be taken into consideration that results of the measurements of the pain threshold are significantly affected by the actions of stressogenic factors, pain-inducing stimulations in all three experimental groups repeated within the entire experimental period (28 days), and blast-induced TBI in the respective main experimental group before the beginning of the preceding measurements. We tried to limit the influence of the blast wave-induced stress in group bTBI by subjecting animals of this group to inhalation anesthesia. Taking into account that such anesthesia should inevitably affect values of the pain thresholds, we created a sham group in which rats were not subjected to blast TBI but were anesthetized and fixed in the experimental setup. The third group of completely intact animals served as the main control group.

The study was carried out on 18 healthy mature male Wistar albino rats (age 6 to 7 months, body mass 220–270 g). The animals were kept under standard vivarium conditions at the 12/12 h light/dark cycle; food and water were provided *ad libitum*.

All experimental rats were randomly divided into three groups. Animals of the main experimental group (bTBI; n=6) were subjected to mild blast-induced brain injury. Preliminarily, rats of this group were anesthetized with halothane, softly fixed in a horizontal position on the abdomen, and positioned with their muzzle at a distance of 5 cm in front of the opening of the device for modeling the blast wave. For short-term (2–3 min) anesthesia, rats were placed in a 3-liter desiccator, and 3 ml of halothane was injected with a syringe into the latter. The anesthesia depth in rats of the bTBI group was assessed by general myorelaxation and absence of the corneal reflex [12]. The blast wave was modeled by the instantaneous (using an electromagnetic valve) opening of a reservoir filled with compressed air (15 atm, i.e., ≈1520 kPa), which under the conditions of our experiments, generated a baroacoustic wave with an excess pressure of 26–36 kPa, on average [13].

Six animals of the intact (Int) group serving as the control were not subjected to any experimental procedures (anesthesia, fixation, and impact of an explosive wave). Rats of the sham (Sh) group (n=6) were subjected only to inhalation anesthesia with halothane (Halothan Hoechst AG, Germany) and fixation.

Measurements of the pain threshold under conditions of electrocutaneous stimulation of the rats' limbs were carried out in a rectangular chamber (40×40×40 cm) provided with an electrified floor. Each rat was placed in the center of the chamber and exposed to stimulation with alternating current (50 Hz, stimulus duration 0.2 sec). The intensity of stimulation started from a 20 μA value and gradually increased by 5-μA steps. Stimuli were applied at 20-sec-long intervals until the intensity provided initiation of a vocalization response; the respective current intensity (μA) was considered corresponding to the pain threshold with respect to electrocutaneous stimulation of the limbs. After 60-sec-long rests, the rat was again subjected to the second and third stimulation series identical to the first one; in each series, the threshold of the pain-related reaction (vocalization) was measured in each case.

The average value of the pain threshold was calculated for each animal in the three-stimulation series, and the mean value of this index was calculated for each experimental group. Estimation of the pain threshold was begun on the next day (day 1) after modeling the blast-related trauma in group bTBI. The analogous measurements were carried out in all three groups synchronously, on days 1, 3, 7, 14, 21, and 28 [14, 15].

The numerical results were subjected to standard statistical processing using STATISTICA

6.1 software (StatSoftInc., serial No. AGAR909E415822FA). Means and s.e.m. values were calculated. Intergroup differences were estimated using the Mann–Whitney *U*-test and considered statistically significant at $P \leq 0.01$ or $P \leq 0.05$.

RESULTS

Dynamics of the pain threshold for animals of all three experimental groups within the 28-day-long experimental period are shown in Fig. 1. As can be seen, the mean pain threshold estimated by the behavioral event (vocalization) in rats of group bTBI on the next day after inducing mild bTBI (day 1) was comparatively low (46.5±8.0 μA, i.e., only 66%, as compared with the respective value on day 28). This was indicative of the significantly increased sensibility of the nociceptive system of the animal with respect to electrocutaneous stimulation. On day 3, this index demonstrated a clear (but statistically insignificant, $P > 0.05$) trend toward increase (to 77% of the day-28 value). On day 7, however, the pain threshold again dropped to a value smaller than that on day 1 (62% of that on day 28). Values of the pain threshold within the subsequent period (days 14 and 21) demonstrated a clear increase. However, this index (56.7±4.6 and 57.0±3.4 μA, respectively) remained moderately but statistically significantly lower than that on day 28 where the pain threshold reached the value of 70.3±3.3 μA. Thus, the period of noticeably increased sensitivity of the nociceptive system in group bTBI was rather long (exceeding three weeks).

Dynamics of the analyzed index in the control group Int demonstrated significant differences in comparison with that already described for group bTBI. The mean pain threshold in intact animals on day 1 of the experimental period was dramatically low (only 43.0±2.0 μA); however, this index, increased relatively rapidly, exceeding 68 μA from day 14. On day 28, the mean pain threshold was equal to 71.2±4.0 μA. It should be emphasized that the mean values of this index within days 14–28 did not statistically differ significantly ($P < 0.05$) from each other. Thus, the observed mean pain thresholds in the control group Int on days 14–28 could be considered the reference values.

The dynamics of the mean pain threshold in the Sh group were, however, quite different. On day 1, this index (63.0±6.0 μA) was significantly higher ($P < 0.05$) than the analogous values in both the Int and bTBI groups. On days 3 and 7, the mean pain threshold was nearly the same as that on day 1 ($P > 0.05$). Later on, on days 14–28, the values demonstrated a clear similarity to the respective values in the control group Int. These indices varied from 68.7 to 72.5 μA and demonstrated no statistically significant differences from each other ($P > 0.05$).

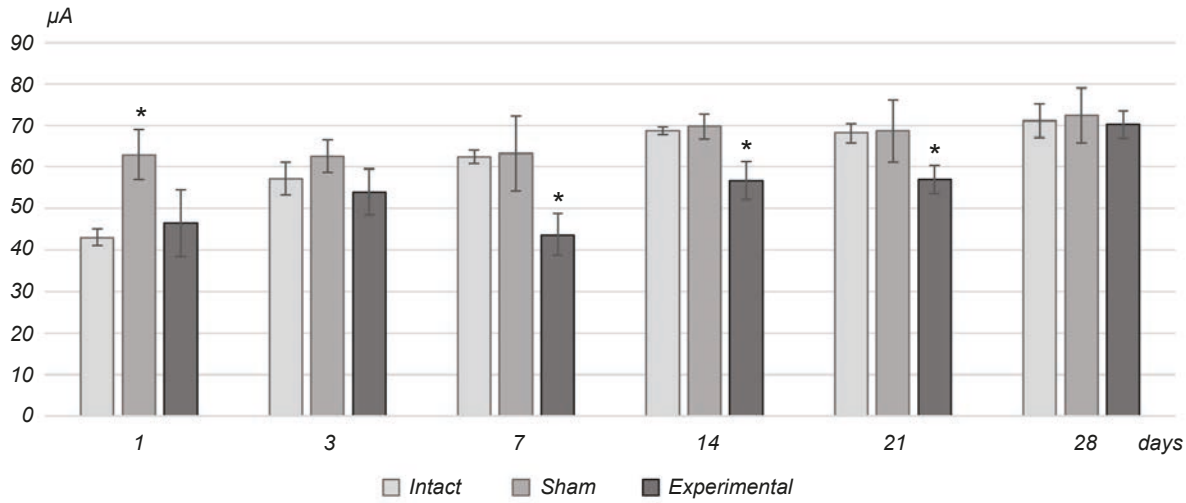

Fig. 1. Pain thresholds (μA) for experimental rats under conditions of electrocutaneous stimulation of the limbs (in a chamber with an electrified floor) during the experimental period. Means ± s.e.m. are shown. Experimental groups (n=6 in each): intact (Int), sham (Sh), and animals subjected to blast-related traumatic brain injury (bTBI). Terms of the threshold measurements after the induction of bTBI in the respective group, days, are shown below. *Difference is significant in comparison with the Int group at the respective experimental day. Results of comparison within the groups at different experimental days are described in the text.

DISCUSSION

The previously described results showed that traumatic injuries resulting in our experiments from the action of the baroacoustic wave in group bTBI should be qualified as mild. No experimental animals in this group died because of the action of such a wave, and pathological changes in the measured index (drops in the pain threshold under conditions of electrocutaneous stimulation) practically disappeared within four weeks after the aforementioned trauma.

Mild bTBI results in a diffuse many-sided injury of the entire organism mostly due to the action of a blast wave. The main result of our study confirmed a conclusion that such TBI exerts considerable effects on the functioning of the nociceptive and antinociceptive subdivisions of the nervous system of the experimental animals, and such shifts represent an important aspect of the pathological effects of such injuries. It should be recognized that the respective pathophysiological influences affect a number of subcortical structures and cortical areas [16].

As an index characterizing the sensitivity of the nociceptive system of experimental rats, we, similarly to many other experimenters, took into account the threshold for initiation of a behavioral pain-related phenomenon (vocalization of the experimental animals) under conditions of electrocutaneous stimulation of the rats' limbs in a chamber with the electrified floor. This threshold was determined according to a minimum value of the stimulation current evoking the aforementioned pain-related vocalization. This index was measured in the course of tests repeatedly carried out over 28 days after subjecting the animals of the respective (bTBI) group to the bast-related trauma.

When we try to interpret the obtained results, the following important circumstances should be taken into account. In the preceding experimental arrangement, electrostimulation of the limbs carried out in all three experimental groups until reaching the pain threshold and the subsequent identification of a precise value of the minimum "nociceptive" stimulus should be considered a clear stress-inducing procedure. In group bTBI, the action of the modeled "blast" wave on the respective experimental animals is surely also a powerful stress-inducing factor. The action of pain-evoking stimulation on rats of all three groups is repeated within the entire experimental period. At the same time, only animals of the bTBI group were subjected to the induction of the blast-related brain trauma. We tried to limit the effectiveness of the stressogenic action of this factor using short-lasting inhalation narcotization of animals of this group by halothane. Considering that such anesthesia and a procedure of fixation of the rats may appear noticeable factors affecting values of the pain threshold, we determined the latter parameter in the sham group (Sh).

In the bTBI group, induction of the brain trauma was accompanied by a rather long-lasting period of a clear increase in the sensitivity of the nociceptive system (not less than three weeks). Within this period, two phases of such an effect could possibly be distinguished. The first phase was manifested immediately after the action of blast-induced trauma, followed by the observation, on day 3, of a slight increase in the pain threshold (partial normalization?). This, however, was followed by the late longer-lasting phase of increased pain sensitivity visible even on day 21.

Analysis of the changes in the pain threshold in group Sh demonstrated that halothane anesthesia applied on the initial day of the experimental series significantly smoothed the stress-inducing effect of the measurement of the above threshold. As can be seen, halothane anesthesia provided a much higher value of the mean pain threshold on day 1, as compared with the analogous value in another control group, group Int.

Thus, a dramatic increase in the pain sensitivity of rats of the bTBI group on day 1 of the experimental period should be considered as mostly related to the effect of the baroacoustic trauma in this group. The same can be said regarding the subsequent long-lasting (observed during at least three weeks) analogous shifts in the aforementioned sensitivity.

On the 3rd day of the posttraumatic period, the electric current strength that led to vocalization as a response to stimulation in the experimental rats increased compared to the 1st day of the same group, thus indicating the activation of compensatory mechanisms after bTBI. However, this was lower compared to the Sham and Intact groups. This indicates that the pain threshold was still low in the rats of the experimental group. In the Sham group, the analgesic effect of halothane was still observed, as evidenced by higher electric current strengths compared to the experimental and intact groups. Our previous studies of behavioral and cognitive brain functions in rats with the same grouping and corresponding manipulations showed the presence

of the halothane effect on days 1 and 3, despite that the effect of anesthesia was supposed to be short-term [17].

Within a week (on 7th day) after the blast wave exposure, we observed a normalization of the response to electro-painful stimulation in the Sham group, which corresponded to the Intact group. At the same time, a decrease in pain sensitivity was observed in the experimental animals compared with both the Sham and Intact groups, as well as with the data of the experimental group on day 1. It was thought that this result was indicative of the depletion of compensatory mechanisms in rats with bTBI.

On the 14th and 21st days, the electric current strength for the experimental rats' vocalization was lower compared to the Sham and Intact groups, but gradually increased and by the 28th day and was almost equal in comparison to both groups. In all rats, a gradual adaptation to electro-pain stimulation was observed, as evidenced by an increase in electric current strength on the 28th day compared with the 1st day of the posttraumatic period. In general, we observed manifestations of adaptation to electro-pain stimulation in all rats, as evidenced by a gradual increase in the strength of the electric current throughout the study period.

Modern researchers point out the analgesic effect of halothane is provided mostly by activation of the endogenous opioid mediator system [18]. Therefore, it may be assumed that halothane anesthesia within the first days of the experimental period leads to activation of the cerebral opioid system and intensified synthesis of endorphins and enkephalins [19]. This, in turn, increases the sensitivity of opioid receptors and provides significant pain relief and a relative increase in the threshold value for producing a pain-related vocal response. However, such an effect cannot be observed in rats of the bTBI group. Thus, a long-lasting period of increased pain sensitivity observed in the latter group should be considered exclusively a consequence of the blast-related trauma.

After analyzing our results and data from previous studies, we suggest that possible reasons for the deficiency of pain relief in rats with mild bTBI are structural and metabolic disorders leading to suppression of the cerebral antinociceptive system due to impaired synthesis of endogenous opioids (endorphins and enkephalins) [20]. Disorders caused by the action of the blast wave include increased permeability of the blood-brain barrier and diffuse massive damage of the axons and neurons based on pathological changes in their membranes and disruption of the ion channels. Probably, damage at the level of cell organelles, including mitochondria, plays a significant role [17]. These changes lead to considerable impairment of neuronal excitability and disorders regarding transmission of the nerve impulses [21].

As a consequence of such primary injuries, secondary pathophysiological mechanisms are triggered. These include disorders in the production of anti-inflammatory mediators, energy deficiency, the development of oxidative stress, and disorders in the functions of voltage-dependent calcium channels [22–24].

It was found that, during the stress of the different genesis (immobilization, intense emotions, and/or pain) or significant trauma (e.g., related to experimental laparotomy), the synthesis of endogenous opioids is usually intensified [25]. However, in our study we observed a certain insufficiency of this system throughout the posttraumatic period in group bTBI, which probably led to a deficiency in the formation of compensatory mechanisms under such experimental conditions. We believe that, in this case, the mitochondria are impaired and a significant energy deficit developed (especially in the brain). Probably, these factors are mostly important regarding the development of a deficiency in the production of sufficient amounts of mediators in the antinociceptive system. Naturally, such shifts are complicated by the development of oxidative stress [26]. The most intense suppression of the antinociceptive system was observed in group bTBI on day 7 of the posttraumatic period.

As already reported, people subjected even to mild bTBI frequently demonstrate noticeable cognitive impairment combined with intensified anxiety, depression, and disorders of various manifestations of memory phenomena [27]. There are indications that the system of endogenous opioids is considerably involved in the realization of cognitive functions of the CNS, and normalization of synthesis of these agents or use of the respective exogenous substances may lead to the improvement in cognitive processes [17, 27]. Therefore, our assumption that the opioid system is seriously impaired in animals with mild bTBI is in agreement with the respective general concept, and disorders of this system are some of the main links in the pathogenesis of TBI-related negative shifts in CNS functioning; disorders of the pain perception are an

important aspect of such shifts.

Measurements of the pain threshold under conditions of electrocutaneous stimulation of experimental rats, thus demonstrated that mild blast-induced traumatic brain injury induces significant long-lasting disorders in the pain perceptivity of such animals. It seems that two phases can be differentiated in the dynamics of the above parameter affected by such trauma. An initial significant increase in pain sensitivity within the first days of the experimental period is accompanied by a secondary phase lasting from day 7 and up to four weeks after the action of the blast wave. This phase is probably mostly related to negative pathobiochemical shifts in the brain and the organism in general.

Observations of the effects of halothane anesthesia in the main experimental (bTBI) and sham groups allowed us to assume that a blast-related deficiency of the functions of the endogenous opioid antinociceptive systems plays an important role in the pathogenesis of blast-induced traumatic brain injury-related disorders of the CNS functions.

All studies were conducted according to a previously developed and approved plan by the authors. The procedures followed the recommendations of the NIH Guide for the Care and Use of Laboratory Animals. All effort was made to keep both the suffering and the number of the animals to a minimum, this was evidenced by an excerpt from the minutes of the meeting of the Commission on Biomedical Ethics DSMU №. 3, 02.11.2021.

The authors, Yu. V. Kozlova and O.M. Demchenko, confirm the absence of any conflicts over commercial or financial relations and relations with organizations or individuals that could in any way be related to the study.

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