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EXPERIMENTAL PAPERS

Exploring the Molecular and Genetic Mechanisms of Action of the α2-Adrenergic Agonist Mafedine in Experimental Traumatic Brain Injury in Rats

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Abstract—Neurological impairments due to traumatic, vascular, or neurodegenerative brain diseases have a high prevalence worldwide. Among them are motor, cognitive, and mental disorders, which have a serious negative impact on the working and social activities of the patients. This calls for the search and development of novel effective neuroprotective agents. Previous studies have shown the pyrimidine-derived α2-adrenergic agonist mafedine to be highly effective for the amelioration of neurological deficits in experimental traumatic brain injury (TBI) in rats. Despite the results of the previous works favouring the major role of the α2 adrenergic receptor activation in the mechanism of action of mafedine, the search for additional molecular targets is an important part of the development of any drug to be used in clinical practice. In this work, we evaluated the effects of 7 day-long course administration of mafedine (2.5 mg/kg b.w.) on the expression of brain-derived neurotrophic factor (BDNF), the proinflammatory cytokines interleukin (IL)-1β, -6, tumour necrosis factor (TNF)-α, and the $\alpha2_A$, $\alpha2_B$, and $\alpha2_C$ α2-adrenergic receptor subtypes in the brain cortex of rats subjected to TBI, using the reverse-transcription real-time polymerase chain reaction method. TBI was modelled by the controlled cortical impact technique in an open area of sensorimotor cortex of the left brain hemisphere. Behavioural alterations in the injured animals were assessed in the Open field test, and the fore- and hindlimb motor function, in the Limb placing, Cylinder, and Beam walking tests. Our experiments show that TBI causes severe motor impairments as well as decreases exploration in rats. Besides, at post-TBI day 7, a reduction in the expression of all analyzed genes is seen, which is the most pronounced in the contralateral (uninjured) hemisphere. Course administration of mafedine (2,5 mg/kg b.w.) resulted in moderate stimulation of the injured rats' behaviour, increased exploratory activity compared to controls, and improved sensorimotor deficit as assessed by the Beam walking test. Gene expression analysis results indicated that mafedine decreased $\alpha2_B$-adrenergic receptor, TNF-α, and IL-6 expression in the injured hemisphere. At the same time, compared to rats with TBI having received no treatment, mafedine-treated animals exhibited higher $\alpha2_B$-adrenergic receptor and IL-1β expression in the injured rather than the intact hemisphere. These results confirm the previously observed

neuroprotective activity of mafedine and imply that it may exert its effects via suppression of $\alpha 2_B$-adrenergic receptor and proinflammatory cytokine expression in the injured brain hemisphere, at the same time increasing their expression in the intact one.

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INTRODUCTION

Traumatic, vascular and neurodegenerative diseases of the brain affect millions of people annually, being one of the main causes of disability worldwide [1–3]. As a result of these diseases, patients have reduced social and labor activity, which imposes material encumbrance on their relatives and on society as a whole [4]. Effective treatment of such patients requires neuroprotective drugs that can act on various parts of pathogenesis of these diseases, thereby preventing neuronal death and accelerating functional recovery of motor, cognitive, and mental functions. Despite the large variety of proposed neuroprotective drugs that have shown efficacy in animal experiments, only some of them are being clinically tested, and even fewer are effective in clinical practice [5]. It is assumed that the low translational potential of such studies is due to the fact that the studied agents act in isolation on one or another mechanism of disease pathogenesis, without affecting the parallel pathological processes [6]. For this reason, neuroprotective drugs with different molecular targets in the mechanism of action seem to be the most promising for study and further development.

One such group, for which different mechanisms of neuroprotective action have been shown, are α_2-adrenergic receptor agonists. In the early 1970s, this group was actively used as antihypertensive agents. Later, their use in this capacity cooled down, but the prospects for their use as sedative, anti-anxiety and antiaddictive agents expanded. In the early 90's there were studies that demonstrated positive effects of clonidine on ischemic stroke models in rats [7, 8]. It is especially important that its neuroprotective effects were blocked when rats were injected with the α_2-adrenergic receptor agonist atipamezole, which allowed us to assume that the activation of these receptors is the cause of the observed effects. Subsequent studies in various models of ischemic stroke in rodents have shown neuroprotective activity for other α_2-adrenergic receptor agonists, such as tizanidine and dexmedetomidine [9]. The latter drug is currently the object of detailed study by many researchers, and it has been repeatedly shown that in traumatic and vascular brain injuries it can reduce the severity of inflammatory processes and oxidative stress, inhibit apoptosis of neurons and glial cells, normalize the barrier function of the blood-brain barrier, maintain the balance of procoagulant and anticoagulant systems, and prevent the development of cerebral vasospasm [10]. Of particular importance is the fact that dexmedetomidine has shown efficacy in clinical practice, for example, in ischemic stroke, as evidenced by the results of a metaanalysis involving 879 patients [11]. In addition, the neuroprotective effect of this drug has been demonstrated in glioma removal [12] and in the surgical treatment of temporal lobe epilepsy [13].

Another α_2-adrenergic receptor agonist mafedine synthesized at the St. Petersburg Chemical-Pharmaceutical University showed the ability to reduce the severity of neurological deficit in rats that underwent traumatic brain injury (TBI). Its course 7-day administration at a dose of 2.5 mg/kg increased the overall motor activity of injured animals, positively influenced the motor function of the fore and hind limbs, reduced the volume of brain damage, and reduced the severity of inflammatory processes in the trauma focus [14]. In subsequent experiments, the neuroprotective activity of mafedine in a model of a TBI in rats was confirmed using electrophysiological methods (electrocorticography and analysis of visual and somatosensory evoked potentials). It was found that administration of this compound led to normalization of the interhemispheric connections of the brain regions distant from the injury area as well as the intrahemispheric connections of the healthy hemisphere by day 7 after injury. In addi-

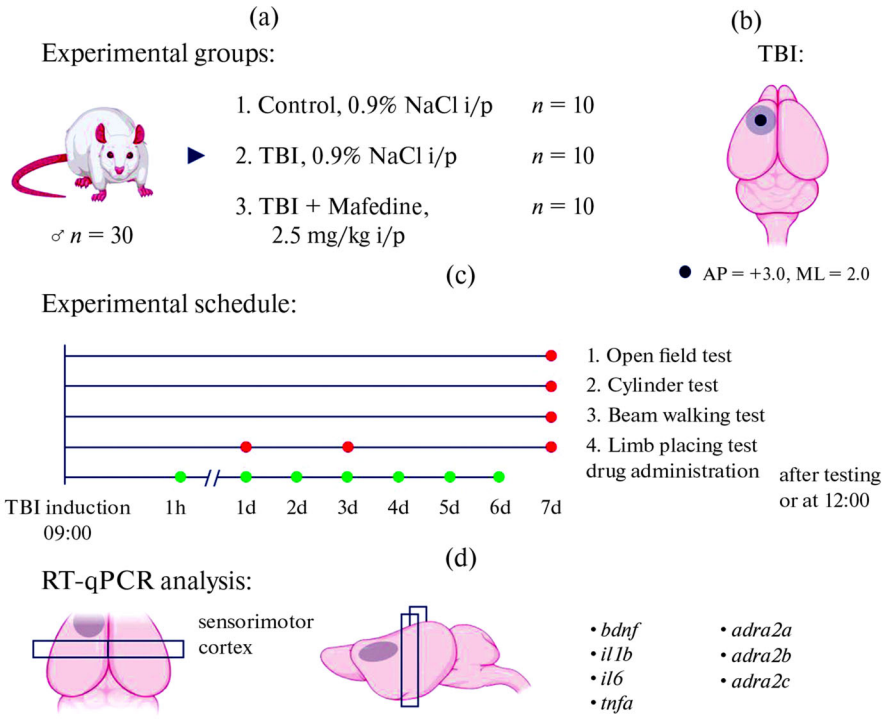

Fig. 1. (a) Experimental groups, (b) coordinates of traumatic brain injury, (c) schedule of experiments and procedures, (d) regions of material collection for molecular analysis. TBI—Traumatic brain injury, i/p—intraperitoneal, AP—anterior-posterior, ML—mediolateral, RT-qPCR—real-time quantitative reverse-transcription polymerase chain reaction.

tion, positive changes in the cortical responses to photo- and somatosensory stimulation were observed in the animals injected with mafedine [15].

Since yohimbine administration in equimolar quantities canceled most of the positive effects of mafedine, we can conclude that the neuroprotective effect of the latter is realized by activation of α2-adrenoreceptors [14]. However, to create a drug that can be further used in clinical practice, it is necessary to study its molecular mechanisms of action. Therefore, the aim of the present work was to study the molecular and genetic mechanisms of mafedine action in the model of rat TBI using quantitative reverse-transcription real-time polymerase chain reaction (RRT-qPCR). We selected the brain-derived neurotrophic factor BDNF, inflammatory mediators interleukin IL-1β, IL-6 and tumor necrosis factor TNF-α, as well as α_2-adrenergic receptor subtypes: $\alpha2_A$, $\alpha2_B$ and $\alpha2_C$ as potential molecular targets of the compound under study.

MATERIALS AND METHODS

The study was performed on 30 male Wistar rats aged 3 months and weighing 250–300 g obtained from the [Rappolovo] laboratory animal supplier (Leningrad Region, Russia). Rats were kept 5 individuals per cage, at an indoor temperature of 20–22°C and a light regime of 12 h of light 750 lx/12 h of darkness. All animals received a standard chow (dry full-rate pelleted extruded compound feed recipe PK-120, LLC "LABORATORKORM", Russia) and had access to food and water *ad libitum*. Rats of all experimental groups were taken from the same batch and quarantined for 14 days. Each animal was assigned an identification number and divided into 3 groups—control, TBI and TBI+mafedine, by random number randomization. All experimental groups had 10 animals each (Fig. 1a).

The TBI was simulated by applying a controlled impact to the sensorimotor cortex area using a traumatizer (RWD Life Science Inc., USA). The

localization of the sensorimotor cortex area was determined using the atlas of stereotactic coordinates by Paxinos and Watson [16]. Before surgery the animals were anesthetized with tiletamine/zolazepam solution (Zoletil 50®, Virbac, France; 30 mg/kg, intramuscularly), after which trepanation was performed in the left frontal part of the skull over the sensorimotor cortex area. The center of the trepanation hole was 3.0 mm rostral and 2.0 mm lateral to the bregma (Fig. 1b). Thereafter, a movable steel piston 4 mm in diameter with a stroke of 5 mm was placed in the trepanation hole, on which a 60 g weight sliding in a steel tube was struck from a height of 22 cm. The drilled plate was returned to its place and the skin incision was sutured.

After coming out of anesthesia, the rats were returned to their home cages with free access to water and food for the entire study period. The condition of the animals was monitored daily in the morning and evening; if necessary, sutures were treated with 10% betadine solution. Antibiotics, analgesics, and anti-inflammatory drugs were not used in the present study due to the fact that most of them can affect the course of pathological processes in traumatic brain injury to some degree, thus distorting the study results. Due to the absence of signs of pain, distress, and the development of local or systemic inflammatory reactions, none of the animals were prematurely removed from the experiments.

Mafedine (6-oxo-1-phenyl-2-(phenylamino)-1,6-dihydropyrimidine-4-olate sodium) (2.5 mg/kg intraperitoneally (i./p.)) [14, 15] or physiological solution (0.5 mL i./p.) were administered to the respective groups of animals 1 h after injury and then every day according to the schedule (Fig. 1c) for 6 days. Behavioral and functional tests in experimental animals were performed on the 1st, 3rd, and 7th days after surgery. Group masking ("blinding" of the operators) was used to objectify the results obtained. After testing on the 7th day, the rats were euthanized by decapitation and a section of the cerebral cortex located caudal to the injury site (sensorimotor cortex area) was taken for further study (Fig. 1d).

On the 1st, 3rd, and 7th days after injury, the severity of neurological deficit was assessed in the Limb placing test. The test consisted in evaluating the response of the hind and forelimbs to tactile and proprioceptive stimulation. The testing process consisted of 7 different tests; the results were expressed as a sum of points. The following scoring system was used to evaluate limb impairment: 2 points, the rat performed the test completely; 1 point, the rat performed the test with a delay of more than 2 s and/or not completely; 0 points, the rat did not respond to limb stimulation. The maximum possible total number of points was 14. The result was expressed as a sum of points [14].

On the 7th day we evaluated general motor and exploratory activity in the Open Field (OF) test, recording animal movements in the unit (Open Science Ltd., Russia) using a video camera (Canyon, ASBIS, Cyprus) for 3 min. The obtained video recordings were analyzed using VideoMot2 3.0.1 software (TSE Systems, Germany). We estimated the distance traveled (cm), the average speed of movement (cm/s), the number of field sections visited, the number of freezings, the total time of freezings (s), the time spent in the center of the field (s), the number of grooming, rears, and peeking into the holes [17].

In the "Cylinder" test, the asymmetry of the animal's use of the forelimbs during the examination of the cylinder walls was evaluated. The rat was placed in a transparent plastic cylinder ("NPK Open Science", Russia) and its movements were videorecorded. The testing time was not limited, achieving at least 10 touches of the cylinder walls. Viewing of the video recording was conducted in the frame-by-frame mode, counting the number of acts of independent use of the ipsi- and contralateral to the injured limb during the examination of the cylinder wall after rear, as well as simultaneous use of both limbs. The results were presented as the percentage of use of the contralateral limb from the total number of behavioral acts (CL, %) and were calculated according to the formula [17]: $CL = (Col + 0.5*St) / (Ipl + Col + St) \times 100$, where Col is the contralateral limb to the injured place; St is simultaneous use of both forelimbs; Ipl is the ipsilateral limb to the injured place.

The walking beam test (WB) allows estimating the severity of sensorimotor deficit of the fore and hind limbs. The device (OOO "NPK Open Science", Russia) represents two smoothly tapering

paths 165 cm long, located directly under each other. The lower track fulfills the function of a platform, on which the animal's limbs are located during sliding from the upper track. At the end of the tracks there is a black box, which is the final goal of the animal. Before simulating a TBI, rats were trained to cross the WB for 3 consecutive days. During testing, animal movements were recorded on a video camera. Then, the number of placements of the limb contralateral to the injury site on the lower track (errors), the number of slips from the upper track to the lower track, and the total number of steps were counted in frame-by-frame view. The number of errors, the number of slips, and the total number of steps for the anterior and posterior contralateral limbs were counted separately. The data obtained as a result of three attempts were averaged, and the degree of sensorimotor deficit (SD, %) was calculated according to the formula and expressed as a percentage: SD = Errors + 0.5 × Slips / Total number of steps × 100 [17].

After performing all behavioral and sensorimotor tests on the 7th day after injury, animals previously anesthetized with tiletamine/zolazepam were decapitated and the sensorimotor cortex areas of the left and right cerebral hemispheres were extracted. The choice of these cortical areas for further analysis was due to the fact that mafedine had been studied in previous studies and had shown positive effects to a greater extent in sensorimotor tests. The obtained material was stored at −80°C, after which the tissues were homogenized using a Precellys Evolution 3D rotor-type homogenizer (Bertin Technologies, France) for subsequent isolation of nucleic acids from the homogenized material and PCR. A BioMaster LRU-100-50 kit (Biolabmix, Russia) was used to isolate ribonucleic acid (RNA). RNA was isolated according to the protocol specified by the manufacturer. After that, RNA concentration was measured using a NanoPhotometer NP80 spectrophotometer (Implen, Germany), adjusted to 0.1 ng/μL, and then complementary deoxyribonucleic acid DNA (cDNA) was synthesized by reverse transcription using the BioMaster OT M-MuLV-RH reagent kit (Biolabmix, Russia). The cDNA concentration after measurement was adjusted to 50 ng/μL, then the relative degree of expression of *bdnf* (BDNF), *il1b* (IL-1β), *il6* (IL-6), *tnfa* (TNF-α), *adra2a*, *adra2b*, and *adra2c* ($\alpha2_A$, $\alpha2_B$, and $\alpha2_C$-adrenoreceptor, respectively) genes was determined by RRT-qPCR (CFX 96 amplifier, Bio-Rad, USA) using BioMaster Hs-qPCR (2x) premixed reagents (Biolabmix LLC, Russia). The level of gene expression was assessed relative to the expression of the housekeeping gene glyceraldehyde-3-phosphate dehydrogenase (GAPDH). The synthesis of primers (Table 1) was performed by Beagle (Moscow). The specificity of the amplification products was monitored by melting curves.

The data were statistically processed using GraphPad Prism 9.0.0 software package. To test for normality of data distribution, we used the Shapiro–Wilk *W*-test; significance of differences between groups with normal distribution was assessed using one-factor ANOVA with Tukey *post hoc* test; with nonnormal distribution, we used nonparametric Kruskal–Wallis test with Dunn *post hoc* test. Wilcoxon–Mann–Whitney test was used to compare the relative level of gene expression between hemispheres within the same group. Differences were considered statistically significant at $p < 0.05$. Statistical power analysis was not performed.

RESULTS

Traumatic lesions of the sensorimotor cortex and the underlying structures of the left hemisphere resulted in pronounced behavioral and motor disorders in the rats, which were registered throughout the study period. On the first day after the trauma, one animal each from the TBI and TBI+Mafedine groups died; therefore, 9 rats in each of these groups participated in the experiments.

In the OF test on the 7th day after surgery, animals in the TBI group were less likely to perform rears ($p < 0.05$) and peek-a-boo ($p < 0.05$) compared to the control group (Fig. 2). In the limb stimulation test (Fig. 3a), the sum of scores in the injured animals was lower ($p < 0.01$) on all days of testing (days 1, 3, and 7 after surgery) compared to the healthy group. It is worth mentioning that in this test, spontaneous recovery of motor function in fore and hind limbs was observed in ani-

Table 1. Nucleotide sequences of the primers used

Gene	Primers	Reference
bdnf	FW: 5'-CCGGTATCCAAAGGCCAACT-3' RV: 5'-CTGCAGCCTTCCTTGGTGTA-3'	[18]
il1b	FW: 5'-GTTCTGTCCATTGAGGTG-3' RV: 5'-ATTGTGGCTGTGGAGAAG-3'	[19]
il6	FW: 5'-TACTTCACAAGTCCGGAG-3' RV: 5'-TCCAGAAGACCAGAGCAG-3'	[19]
tnfa	FW: 5'-CACGCTCTTCTGTCACTGA-3' RV: 5'-GGACTCCGTGATGTCTAAGT-3'	[20]
adra2a	FW: 5'-GGTAAGGTGTGGTGGGAGAT-3' RV: 5'-CAGCGCCCTTCTTCTCTATG-3'	[21]
adra2b	FW: 5'-GCACCACACAAAACCTGTTCCT-3' RV: 5'-TTGTAGATGAGGGGCGGTAG-3'	[21]
adra2c	FW: 5'-TACTGTGCTGGTTCCCCTTC-3' RV: 5'-CAGAGGCCCAGTTGTCTCTC-3'	[21]

mals with TBI ($p < 0.01$ for the 1st day compared to the 3rd day), but on the 7th day it was still incomplete, as evidenced by the fact that the results of this group differed significantly from those of the control group. In the "Cylinder" test (Fig. 3b), the injured rats were almost 2 times less likely ($p < 0.05$) to use the contralateral (right) paw while exploring the wall of the cylinder compared with animals without injury. In addition, the pronounced motor impairments in the TBI group were confirmed in the WB test (Fig. 3c), in which the degree of their sensorimotor deficit was higher for both the anterior ($p < 0.05$) and posterior ($p < 0.01$) contralateral limbs compared with the control group.

Analysis of gene expression by RRT-qPCR (Fig. 4) showed that traumatic lesions in the left hemisphere resulted in a decrease in the relative expression levels of all the genes analyzed (*bdnf*, *il1b*, *il6*, *tnfa*, *adra2a*, *adra2b*, and *adra2c*) in both the left (injured) and right (healthy) hemispheres on the 7th day after injury. A common pattern was also that for all genes, the degree of expression was lower in the contralateral hemisphere than in the ipsilateral hemisphere ($p < 0.05$ for *tnfa* and $p < 0.01$ for *il6* and *adra2b*). It is important to note that in healthy rats we observed a pronounced interhemispheric asymmetry in the level of expression of one or another gene, but there were no significant differences between hemispheres when averaging the values.

A course of 7 days of mafedine administration increased the mean walking speed ($p < 0.01$) and the number of hole pokes ($p < 0.05$) in the OF test in the injured rats compared to the untreated animals. In addition, this group performed a greater number of transitions ($p < 0.01$) and had a higher mean speed ($p < 0.01$) compared to the control group. There was no significant positive effect of mafedine in the limb placing and Cylinder tests, but in the latter there was a tendency to increase the frequency of use of the contralateral limb in the treated group. The degree of sensorimotor deficit of the posterior contralateral limb in the WB test was lower ($p < 0.05$) in rats administered mafedine compared to the TBI group.

Similar to the group of traumatized animals without treatment, rats injected with mafedine after trauma had a pronounced decrease in the expression level of all the analyzed genes (Fig. 4) in both hemispheres compared to the control group. However, in contrast to the TBI group, in animals injected with mafedine, the expression level for most genes (except for *il6*) was higher in

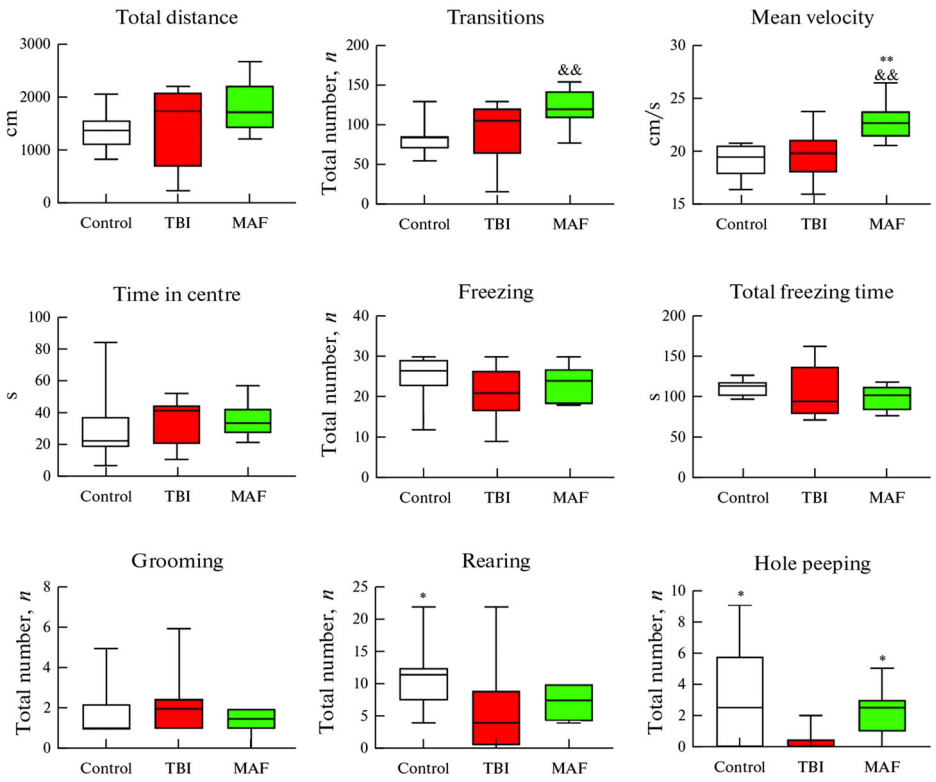

Fig. 2. Behavioural parameters of the Control (Control, $n = 10$), TBI (TBI, $n = 9$), and TBI+mafedine (MAF, $n = 9$) group animals in the Open field test. Data are presented as median (min; max). For the number of line crossings ($F_{2,25} = 3.972$, $p < 0.05$) and mean velocity ($F_{2,25} = 7.453$, $p < 0.01$), one-way ANOVA and *post hoc* Tukey's test were used; for the number of rears ($H = 7.105$, $p < 0.05$) and hole pokes ($H = 8.184$, $p < 0.05$), the Kruskal–Wallis and *post hoc* Dunn's tests were used. * $p < 0.05$, ** $p < 0.01$, statistically significant differences from the TBI group; && $p < 0.01$, statistically significant differences from the Control group.

the right, healthy hemisphere than in the left, injured one ($p < 0.01$ for *adra2b* and *il1b*). When comparing index values between groups, the expression levels of *adra2b*, *tnfa*, and *il6* in the left hemisphere were significantly lower ($p < 0.05$ in all cases) in the mafedine group compared to the untreated group.

DISCUSSION

In the present study, it was found that a course of 7-day administration of mafedine to rats that had suffered a TBI increased the locomotor and search-exploration activity of the latter in the OF test on the 7th day after surgery. These data are consistent with the results obtained in two previous series of experiments [14], in the first of which the effective dose of mafedine was selected and compared with the activity of another α_2-adrenergic receptor agonist clonidine, and in the second, the effects of mafedine against the administration of α_2-adrenergic receptor agonist yohimbine. In both cases, mafedine at a dose of 2.5 mg/kg increased total motor activity (calculated as the sum of squares crossed, rears, hole poking, and grooming) of rats with TBI in the OF test compared with the control on the 3rd day after surgery (Fig. 5).

It is worth noting that in this study, the behavior of the rats with TBI differed little from that of the control animals, and significant differences between the groups were obtained only by two indices: the number of rears and hole pokes. In past series of experiments [14], the decrease in total motor activity in injured rats on the 3rd day after surgery was always statistically significant. Similar changes in the behavior of injured animals were also obtained when studying other molecules [17, 21]. This is probably due to the fact that in the present series, we tested the rats in the OF on the

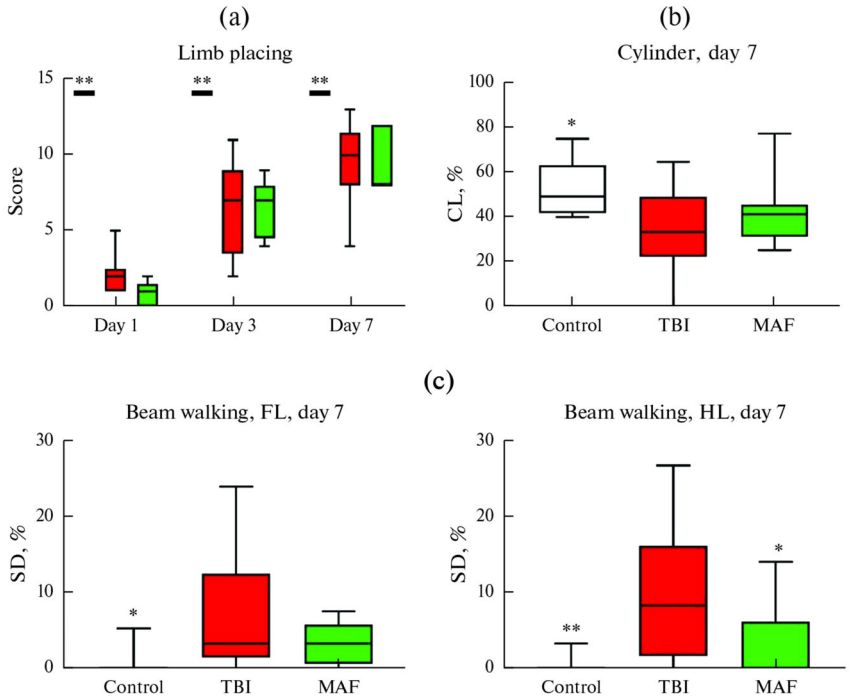

Fig. 3. Contralateral fore- and hindlimb function of the Control (Control, $n = 10$), TBI (TBI, $n = 9$), and TBI+mafedine (MAF, $n = 9$) group animals as assessed by the Limb placing (a), Cylinder (b), and Beam walking (c) tests. Data are presented as median (min; max). CL, contralateral (left) forelimb use rate, FL, forelimb, HL, hindlimb, SD, sensorimotor deficit severity (%); * $p < 0.05$, ** $p < 0.01$, statistically significant differences from the TBI group (the Kruskal–Wallis and *post hoc* Dunn's tests). (a): $H = 22.34$, $p < 0.01$, $H = 19.67$, $p < 0.01$, and $H = 20{,}19$, $p < 0.01$ for days 1, 3, and 7, respectively; (b): $H = 7.158$, $p < 0.05$; (c): $H = 8.964$, $p < 0.05$ and $H = 12.1$, $p < 0.01$ for the fore and hind limbs, respectively.

7th rather than the 3rd day after injury, and the spontaneous recovery of locomotor activity, characterized by such indices as the distance covered, the number of crossed squares, average speed, as well as the number of freezings and their total duration, had already occurred [23].

In addition, the present work had two additional differences in the experimental conditions from the past series. First, the injectable drug chloral hydrate used was replaced by the less toxic tiletamine/zolazepam [24, 25], and, second, a new device was used to deliver a controlled impact to the sensorimotor cortex area (Table 2). When comparing the parameters of trauma application, we can conclude that in the present study, the TBI was more severe and, therefore, the severity of neurological deficit in all tests should have been higher than in the past experiments. Although the degree of sensorimotor deficit in rats with TBI in the limb placing test in the present study was similar to that in the previous series (the test results were used as a criterion for the success of the TBI simulation), in the WB test this index was several times lower for both paws, which indicates a more rapid spontaneous recovery of sensorimotor functions. We can conclude that in the present study, there was greater damage to the left hemisphere, but by day 7, the degree of recovery of sensorimotor functions of the anterior and posterior contralateral paws was higher. The most probable explanation for this feature is the neuroprotective effect of tiletamine and zolazepam used to anesthetize the rats before surgery. In spite of the fact that both chloral hydrate and tiletamine/zolazepam have neuroprotective effects [26, 27], when comparing their effectiveness in the model of ischemia-reperfusion by middle cerebral artery occlusion in rats, it was shown that the second agent is more active [27]. In animals injected with tiletamine/zolazepam at a dose of 40 mg/kg 24 h before modeling ischemia, there was a more rapid recovery of motor functions in the Limb placing test, as well as less infarct volume and severity of brain edema. Thus, the less pronounced neuro-

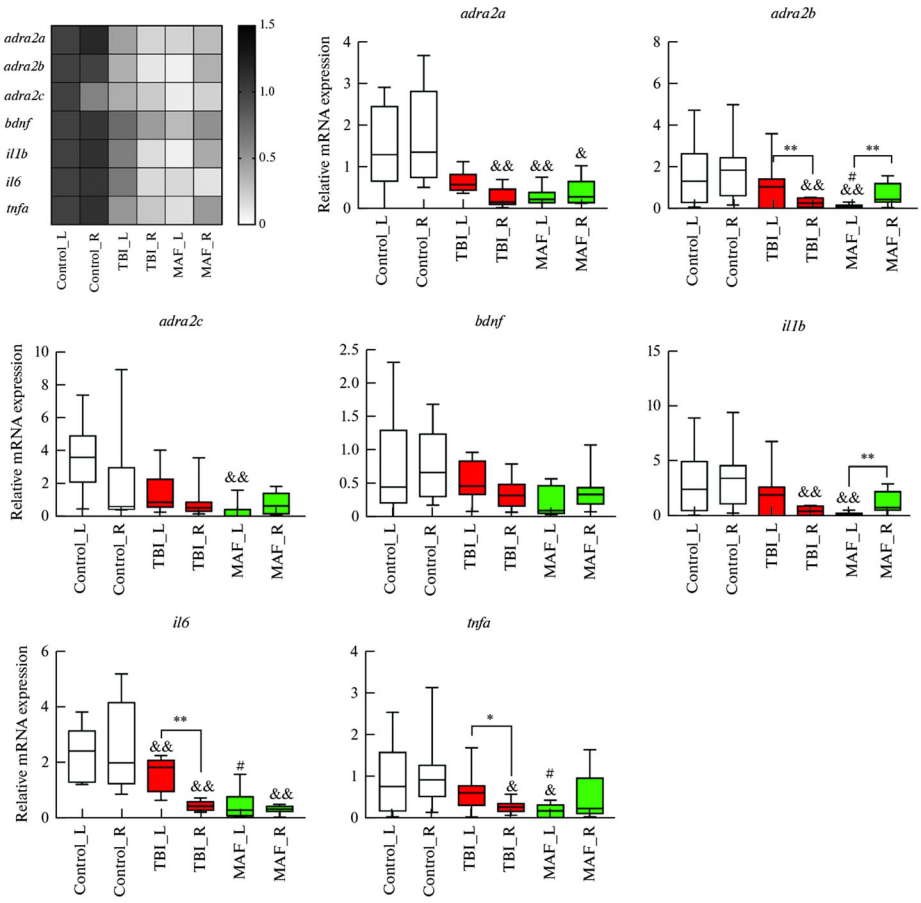

Fig. 4. Relative expression of the *adra2a*, *adra2b*, *adra2c*, *bdnf*, *il1b*, *il6*, *tnfa* genes in the left (L) and right hemispheres in the experimental animals of the Control (Control, $n = 10$), TBI (TBI, $n = 9$), and TBI+mafedine (MAF, $n = 9$) groups. Left panel represents a heatmap of gene expression; 100% corresponds to the respective intact values (left hemisphere). Data are presented as median (min; max). * $p < 0.05$; ** $p < 0.01$, significant differences between the two hemispheres (the Kruskal–Wallis and *post hoc* Dunn's tests); & $p < 0.05$, && $p < 0.01$, significant differences from respective intact values (respective hemisphere; the Kruskal–Wallis and *post hoc* Dunn's tests); # $p < 0.05$, significant difference from respective TBI values (respective hemisphere; the Kruskal–Wallis and *post hoc* Dunn's tests). For comparison of relative gene expression levels between the two hemispheres within a group, the Wilcoxon–Mann–Whitney test was used. *adra2a:* H = 12.35, $p < 0.01$ and H = 14.37, $p < 0.01$ for Control_L vs TBI_L vs MAF_L and Control_R vs TBI_R vs MAF_R; *adra2b:* H = 18.83, $p < 0.01$ and H = 10.97, $p < 0.01$ for Control_L vs TBI_L vs MAF_L and Control_R vs TBI_R vs MAF_R; *adra2c:* H = 16.44, $p < 0.01$ and H = 2.361, $p = 0.3071$ for Control_L vs TBI_L vs MAF_L and Control_R vs TBI_R vs MAF_R; *bdnf:* H = 4.25, $p = 0.1194$ and H = 4.816, $p = 0.0900$ for Control_L vs TBI_L vs MAF_L and Control_R vs TBI_R vs MAF_R; *il1b:* H = 8.352, $p < 0.05$ and H = 10.71, $p < 0.01$ for Control_L vs TBI_L vs MAF_L and Control_R vs TBI_R vs MAF_R; *il6:* H = 16.47, $p < 0.01$ and H = 19.22, $p < 0.01$ for Control_L vs TBI_L vs MAF_L and Control_R vs TBI_R vs MAF_R; *tnfa:* H = 8.998, $p < 0.05$ and H = 7.551, $p < 0.05$ for Control_L vs TBI_L vs MAF_L and Control_R vs TBI_R vs MAF_R.

protective effect of mafedine in the present series can be attributed to the fact that, first, the testing of injured rats in the OF test was performed at a later period after the TBI, and, second, the anesthesia used could mask the effects of the compound under study due to its own neuroprotective effect.

The effect of α_2-adrenergic receptor agonists on the course of pathological processes during ischemic or traumatic brain damage has been repeatedly shown in experiments on rodents [7–9, 28]. Initially, the following were considered as the supposed mechanisms of neuroprotective action of this group of drugs: reduction of excessive release of excitatory mediators by action on presynaptic α_2-adrenergic receptor, blockade of potential-dependent calcium channels, activation of G-protein-bound K^+ channels of internal rec-

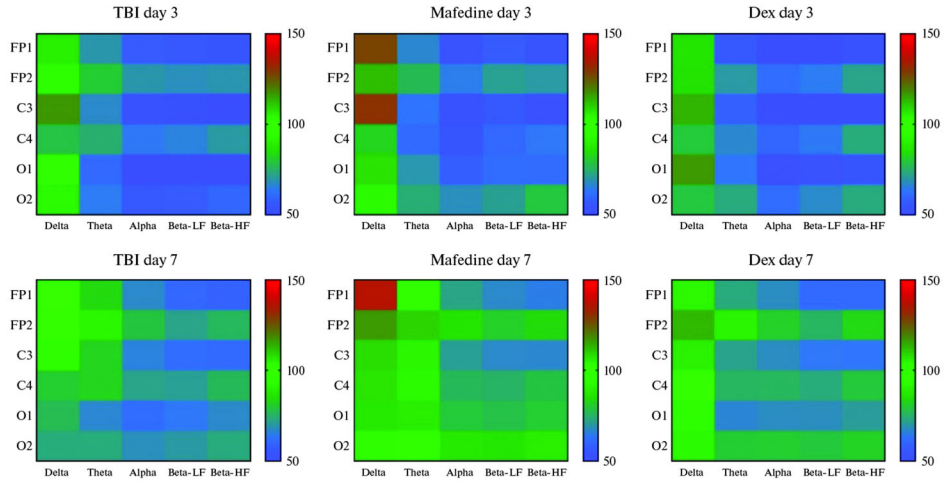

Fig. 5. A heatmap of mean δ-, θ-, α-, low and high-frequency β-wave amplitudes in channels FP1, FP2, C3, C4, O1, and O2 at post-traumatic brain injury days 3 and 7. TBI—rats with traumatic brain injury and no treatment, Mafedine—rats with traumatic brain injury having received mafedine at 2.5 mg/kg b.w. q.d. for 7 d, Dex—rats with traumatic brain injury having received the α2-adrenergic agonist dexmedetomidine (reference drug) at 25 μg/kg b.w. q.d. for 7 d. 100% coresponds to the respective intact values. Figure is taken from our previous work [22].

Table 2. Parameter comparison of the devices used to induce traumatic brain injury in the previous [14, 15, 17, 22] and current work

Parameter	Previous works	Current work
Impacting surface diameter, mm	3	4
Impacting surface stroke length, mm	4	5
Weight fall height, cm	10	22
Weight, g	50	60
Impacting surface shape	cylinder-like	spheric

tification, inhibition of adenylate and guanylate cyclase or development of systemic hypothermia [9, 29]. Later, numerous works demonstrated the ability of dexmedetomidine to reduce the intensity of inflammatory processes in the injury area, normalize the function of the blood-brain barrier, reduce cerebral edema, as well as inhibit the process of neuronal apoptosis and prevent autophagy [10]. Particular attention of experimental studies was paid to the effect of the drug on the TLR4/MyD88/NF-κB signaling pathway, which produces cytokines that can cause damage to the nervous system, such as IL-1β, IL-6, and TNF-α [30, 31]. Therefore, these inflammatory mediators were chosen as potential targets of mafedine action in the present study.

In contrast to this work, most other studies using a model of TBI in rats have examined the expression of IL-1β, IL-6 and TNF-α within the first 3 days after injury. This is due to the fact that the release of proinflammatory cytokines occurs in the acute period after a TBI, which was clearly demonstrated by Dalgard et al. [33]. This study also showed that the increase in proinflammatory cytokines occurs to a greater extent in the injured hemisphere. Similarly, in experiments on C57BL/6 mice [33], TBI caused an increase in IL-1β, IL-6 levels (both gene and protein expression) in the injured hemisphere from days 1 to 7 after injury, but this rise was higher in the ipsilateral hemisphere compared to the contralateral one. In addition, a slight decrease in IL-1β mRNA levels by day 20 postinjury was observed in the latter work.

The unexpected decrease in the expression level of proinflammatory cytokines in rats with TBI compared to the control group in the present work may be related to the neuroprotective effect of the anesthetic agent used, which was mentioned earlier. Dalgard et al. [33] and Lagraoui et al. [34] used in their work a mixture of 2% isoflurane and 98% oxygen, which probably has no pronounced effect on proinflammatory cytokine expression. Nevertheless, despite some differences in the present work in changes of IL-1β, IL-6 and TNF-α expression in rats after injury from the results of other authors' works, the effects of mafedine are pronounced and in most cases statistically significant. The compound under study suppressed the levels of IL-1β, IL-6 and TNF-α in the injured hemisphere in rats as compared to untreated animals, while the expression of IL-1β in the contralateral hemisphere increased as compared to the ipsilateral hemisphere, this feature of mafedine action certainly cannot be unequivocally interpreted as a positive or negative effect. Nevertheless, the data obtained suggest that the compound studied may have an effect on the inflammatory processes in the cerebral cortex in rats after injury.

Neurotrophic factors, especially brain-derived neurotrophic factor BDNF, play an important role in the recovery processes after TBI, ensuring neuronal survival, axonal sprouting, and synaptogenesis [34]. Due to this fact, this trophic factor has become the object of close study of researchers engaged in neuroprotection. Experimental works on rodents have revealed a positive correlation between the induction (protein or mRNA) of BDNF in brain tissues, as well as the reduction of neurological deficit degree in animals in behavioral or functional tests (Neurological severity score, Morris water maze, Walking beam test, etc.) [34]. It has also been shown that in humans, BDNF gene polymorphism can influence the outcome of a traumatic brain injury [35, 36], but the mechanisms of this influence remain unstudied. Therefore, an increase in BDNF expression under therapeutic or pharmacological influence is usually considered as a manifestation of neuroprotective effect. It is worth noting that BDNF expression can vary over time after injury, as well as depending on the analyzed brain region [34]. For example, it has been shown that in the first few days after a TBI (acute period), it increases in the cortical and hippocampal regions located ipsilateral to the injury site. On the 7th day (chronic period), the BDNF level in these areas decreases, which was also demonstrated in this study. In spite of the fact that there were no significant differences between the experimental groups and hemispheres in the present study, the diagrams show that TBI leads to the decrease of BDNF expression in both hemispheres, and this decrease is more pronounced in the contralateral hemisphere of rats with untreated trauma. Administration of mafedine to rats after TBI reverses this pattern and makes the pattern of changes in BDNF expression similar to that of IL-1β, IL-6, and TNF-α, which can be considered as a consequence of the effect of the compound under study also on BDNF-dependent neuroplastic processes in the brain in rats after TBI.

It is now known that different subtypes of α_2-adrenergic receptors take part in the regulation of arterial pressure, transmission of pain impulses at different levels, implementation of the sedative effect of α_2-adrenergic receptor agonists and in some other physiological functions [37]. From the point of view of the central nervous system, behavior, and cognitive functions, α_{2C}-adrenergic receptors are of the greatest interest, since they have been shown to be involved in the regulation of locomotor activity [38], sensory information processing [39], and spatial and nonspatial memory [40]. It has also been shown that mice with the α_{2C}-adrenergic receptor gene knockout have an enhanced startle reflex, reduced prepulse inhibition, and greater isolation-induced aggression [41]. In view of the above, it is most likely that the neuroprotective effects of α_2-adrenergic receptor agonist can be realized due to the activation of the 2_C-subtype. However, if we consider the blockade of glutamate excitotoxicity to be the basis of the neuroprotective effect of these drugs, then not only 2_C-receptors but also 2_A-, since both subtypes are presynaptically located autoreceptors, become the assumed targets [42]. Nevertheless, in the present study, the expression pattern of all three α_2-adrenergic receptor subtypes in the brain of rats after the TBI was similar and, moreover, similar to that of IL-1β, IL-6, TNF-α, and BDNF. This similarity is probably

due to the fact that α_2-adrenergic receptors, inflammatory mediators, and BDNF are pathogenetically related, and the induction of their expression occurs sequentially within a common pathological cascade. Moreover, based on the data obtained, all 3 subtypes are almost equally involved in this cascade. The effect of mafedine was statistically significant on the expression level of the 2_B-subtype, nevertheless, the general pattern was also observed for the other subtypes.

In an experimental series devoted to the effect of mafedine on brain bioelectrical activity in rats that underwent a TBI [15], there was a pattern in which the rats that received daily mafedine administration at a dose of 2.5 mg/kg for 7 days had higher mean amplitude δ-rhythms in the injured area (channels FP1 and C3). At the same time, by the 7th day after surgery in the remote areas from injury (channels FP2, C4, O1, and O2), the values of the recorded rhythms were close to those of healthy rats. A marked rise in δ-rhythm activity is an indicator of organic lesions (e.g., TBI or stroke) of the brain in rats [14, 43]. Thus, the data obtained showed that mafedine, on the one hand, improves the condition of brain regions remote from the place of injury, and, on the other hand, probably aggravates the course of pathological processes in the area of injury. This assumption is consistent with the changes in the expression of the analyzed genes presented in the present study. It seems plausible to conclude that mafedine is capable of worsening the state of the injured brain region in rats during a TBI while activating compensatory mechanisms in areas distant from the injury site, such as caudal cortical regions of the injured hemisphere or the opposite, non-injured hemisphere. The possibilities of functional reorganization and axonal sprouting of healthy, preserved areas of the rodent brain in organic lesions have been repeatedly shown, for example, in models of ischemic stroke [44]. However, undoubtedly, additional studies using immunohistochemical methods of investigation and application of special methods of staining and labeling of new synaptic connections are required to prove the ability of mafedine to influence neuroplastic processes in the brain after injury.

Thus, our studies have established that mafedine when administered at a dose of 2.5 mg/kg for 7 days after a traumatic injury in rats reduces the degree of neurological deficit in injured animals, manifested by a decrease in exploratory activity as well as impaired motor function of the fore and hind limbs. When analyzing the expression levels of IL-1β, IL-6, TNF-α, BDNF, and α_2-adrenergic receptor subtypes in the cortex of the injured and healthy cerebral hemispheres, it was found that the compound under study suppresses the expression of all the analyzed genes in the injured hemisphere. At the same time, the expression of these genes increases in the opposite healthy hemisphere, which is probably associated with the development of compensatory mechanisms of neuroplasticity, which to some extent agrees with the previously obtained data on the effect of mafedine on the bioelectrical activity of the brain in rats after TBI. The results obtained suggest that further study of the molecular mechanisms of mafedine action should be aimed at studying the ability of the compound to influence the number and functional activity of the proteins encoded by the genes studied in this work in rats with traumatic brain injury. It is of particular interest to study the ability of mafedine to initiate neuroplastic changes in the brain after TBI, leading to a positive functional outcome in traumatized animals.

AUTHORS' CONTRIBUTION

Idea and planning of experiment (Yu.I.S., S.V.O.), conducting experiments and data processing (Yu.I.S., M.V.Sh., V.A.P., D.D.Sh., M.M.P.), preparation of illustrations (Yu.I.S., V.A.P.), preparation and editing the manuscript (Yu.I.S., V.A.P., S.V.O.)

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COMPLIANCE WITH ETHICAL STANDARDS

Experiments using laboratory animals were performed in accordance with the requirements of Directive 2010/63/EU of the European Parliament and Council of September 22, 2010 and "Rules of Laboratory Practice", approved by Order of the Ministry of Health and Social Development of the Russian Federation no. 708n of 23.08.2010. The protocol of the experiment was approved by the bioethical committee of SPCPU of the Russian Ministry of Health (protocol-application R-MAF-SA-21 of 10.01.2021). All measures were taken to reduce the number of animals used and minimize their suffering.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

REFERENCES

1. Faul M, Coronado V (2015) Epidemiology of traumatic brain injury. Handb Clin Neurol 127: 3–13. https://doi.org/10.1016/B978-0-444-52892-6.00001-5
2. Saini V, Guada L, Yavagal DR (2021) Global Epidemiology of Stroke and Access to Acute Ischemic Stroke Interventions. Neurology 97(20 Suppl 2): S6–S16. https://doi.org/10.1212/WNL.0000000000012781
3. Gao Z, Pang Z, Chen Y, Lei G, Zhu S, Li G, Shen Y, Xu W (2022) Restoring After Central Nervous System Injuries: Neural Mechanisms and Translational Applications of Motor Recovery. Neurosci Bull 38(12): 1569–1589. https://doi.org/10.1007/s12264-022-00959-x
4. Rochmah TN, Rahmawati IT, Dahlui M, Budiarto W, Bilqis N (2021) Economic Burden of Stroke Disease: A Systematic Review. Int J Environ Res Public Health 18(14): 7552. https://doi.org/10.3390/ijerph18147552
5. Xu SY, Pan SY (2013) The failure of animal models of neuroprotection in acute ischemic stroke to translate to clinical efficacy. Med Sci Monit Basic Res 19: 37–45. https://doi.org/10.12659/msmbr.883750
6. Marklund N, Hillered L (2011) Animal modelling of traumatic brain injury in preclinical drug development: where do we go from here? Br J Pharmacol 164(4): 1207–1229. https://doi.org/10.1111/j.1476-5381.2010.01163.x
7. Hoffman WE, Cheng MA, Thomas C, Baughman VL, Albrecht RF (1991) Clonidine decrease plasma catecholamines and improves outcome from incomplete ischemia in the rat. Anesth Analg 73: 460–464. https://doi.org/10.1213/00000539-199110000-00016
8. Hoffman WE, Kochs E, Werner C, Thomas C, Albrecht RF (1991) Dexmedetomidine improves neurologic outcome from incomplete ischemia in the rat: Reversal by the alpha-2—adrenergic antagonist atipamezole. Anesthesiology 75: 328–332. https://doi.org/10.1097/00000542-199108000-00022
9. Zhang Y, Kimelberg HK (2005) Neuroprotection by alpha 2-adrenergic agonists in cerebral ischemia. Curr Neuropharmacol 3(4): 317–323. https://doi.org/10.2174/157015905774322534
10. Hu Y, Zhou H, Zhang H, Sui Y, Zhang Z, Zou Y, Li K, Zhao Y, Xie J, Zhang L (2022) The neuroprotective effect of dexmedetomidine and its mechanism. Front Pharmacol 13: 965661. https://doi.org/10.3389/fphar.2022.965661
11. Jiang L, Hu M, Lu Y, Cao Y, Chang Y, Dai Z (2017) The protective effects of dexmedetomidine on ischemic brain injury: A meta-analysis. J Clin Anesth 40: 25–32. https://doi.org/10.1016/j.jclinane.2017.04.003
12. Luo X, Zheng X, Huang H (2016) Protective effects of dexmedetomidine on brain function of glioma patients undergoing craniotomy resection and its underlying mechanism. Clin Neurol Neurosurg 146: 105–108. https://doi.org/10.1016/j.clineuro.2016.05.004
13. Bindra A, Kaushal A, Prabhakar H, Chaturvedi A, Chandra PS, Tripathi M, Subbiah V, Sathianathan S, Banerjee J, Prakash C (2019) Neuroprotective role of dexmedetomidine in epilepsy surgery: A preliminary study. Neurol India 67(1): 163–168. https://doi.org/10.4103/0028-3886.253616
14. Sysoev YuI, Dagaev SG, Kubarskaja LG, Gaikova ON, Uzuegbunam BC, Modise K, Makwana TL, Okovitiy SV (2019) Study of the neuroprotective activity of mafedine, an alpha-2 adrenergic receptor agonist, by modeling a traumatic brain injury in rats. J Biomed 15(1): 62–77. (In Russ). https://doi.org/10.33647/2074-5982-15-1-62-77
15. Sysoev YI, Prikhodko VA, Chernyakov RT, Idiyatullin RD, Musienko PE, Okovityi SV (2021)

Effects of Alpha-2 Adrenergic Agonist Mafedine on Brain Electrical Activity in Rats after Traumatic Brain Injury. Brain Sci 11(8): 981. https://doi.org/10.3390/brainsci11080981
16. Paxinos G, Watson C (2013) The Rat Brain in Stereotaxic Coordinates; 7th ed. Acad Press, Cambridge; MA; USA.
17. Prikhodko VA, Kan AV, Sysoev YuI, Titovich IA, Anisimova NA, Okovityi SV (2021) Evaluation of the neuroprotective activity of a new allylmorpholine derivative in a rat model of traumatic brain injury. Drug development and registrat 10(4–1): 179–187. (In Russ). https://doi.org/10.33380/2305-2066-2021-10-4(1)-179-187
18. Rouhani F, Khodarahmi P, Naseh V (2019) NGF, BDNF and Arc mRNA Expression in the Hippocampus of Rats After Administration of Morphine. Neurochem Res 44(9): 2139–2146. https://doi.org/10.1007/s11064-019-02851-z
19. Lu H, Zhou J (2008) HBV X gene transfection upregulates IL-1beta and IL-6 gene expression and induces rat glomerular mesangial cell proliferation. J Huazhong Univ Sci Technolog Med Sci 28(3): 247–250. https://doi.org/10.1007/s11596-008-0304-5
20. Wang AL, Yu AC, He QH, Zhu X, Tso MO (2007) AGEs mediated expression and secretion of TNF alpha in rat retinal microglia. Exp Eye Res 84(5): 905–913. https://doi.org/10.1016/j.exer.2007.01.011
21. Zhang Y, Kolli T, Hivley R, Jaber L, Zhao FI, Yan J, Herness S (2010) Characterization of the expression pattern of adrenergic receptors in rat taste buds. Neuroscience 169(3): 1421–1437. https://doi.org/10.1016/j.neuroscience.2010.05.021
22. Sysoev YI, Uzuegbunam BC, Okovityi SV (2019) Attenuation of neurological deficit by a novel ethanolamine derivative in rats after brain trauma. J Exp Pharmacol 11: 53–63. https://doi.org/10.2147/JEP.S199464
23. Niskanen JP, Airaksinen AM, Sierra A, Huttunen JK, Nissinen J, Karjalainen PA, Pitkänen A, Gröhn OH (2013) Monitoring functional impairment and recovery after traumatic brain injury in rats by FMRI. J Neurotrauma 30(7): 546–556. https://doi.org/10.1089/neu.2012.2416
24. Fleischman RW, McCracken D, Forbes W (1977) Adynamic ileus in the rat induced by chloral hydrate. Lab Anim Sci 27: 238–243.
25. Ogino K, Hobara T, Kobayashi H, Iwamoto S (1990) Gastric mucosal injury induced by chloral hydrate. Toxicol Lett 52: 129–133. https://doi.org/10.1016/0378-4274(90)90146-d
26. Liu JH, Feng D, Zhang YF, Shang Y, Wu Y, Li XF, Pei L (2015) Chloral Hydrate Preconditioning Protects Against Ischemic Stroke via Upregulating Annexin A1. CNS Neurosci Ther 21(9): 718–726. https://doi.org/10.1111/cns.12435
27. Silachev DN, Usatikova EA, Pevzner IB, Zorova LD, Babenko VA, Gulyaev MV, Pirogov YA, Plotnikov EY, Zorov DB (2017) Impact of anesthetics on efficiency of remote ischemic preconditioning. Biochemistry (Moscow) 82(9): 1296–1308. (In Russ).
28. Liu H, Li J, Jiang L, He J, Zhang H, Wang K (2022) Dexmedetomidine pretreatment alleviates cerebral ischemia/reperfusion injury by inhibiting neuroinflammation through the JAK2/STAT3 pathway. Braz J Med Biol Res 55: e12145. https://doi.org/10.1590/1414-431X2022e12145
29. Ma D, Rajakumaraswamy N, Maze M (2005) Alpha2-Adrenoceptor agonists: shedding light on neuroprotection? Br Med Bull 71: 77–92. https://doi.org/10.1093/bmb/ldh036
30. Wang D, Xu X, Wu YG, Lyu L, Zhou ZW, Zhang JN (2018) Dexmedetomidine attenuates traumatic brain injury: Action pathway and mechanisms. Neural Regen Res 13(5): 819–826. https://doi.org/10.4103/1673-5374.232529
31. Huang GR, Hao FG (2021) Dexmedetomidine inhibits inflammation to alleviate early neuronal injury via TLR4/NF-κB pathway in rats with traumatic brain injury. Crit Rev Eukaryot Gene Expr 31(1): 41–47. https://doi.org/10.1615/CritRevEukaryotGeneExpr.2021037390
32. Dalgard CL, Cole JT, Kean WS, Lucky JJ, Sukumar G, McMullen DC, Pollard HB, Watson WD (2012) The cytokine temporal profile in rat cortex after controlled cortical impact. Front Mol Neurosci 5: 6. https://doi.org/10.3389/fnmol.2012.00006
33. Lagraoui M, Latoche JR, Cartwright NG, Sukumar G, Dalgard CL, Schaefer BC (2012) Controlled cortical impact and craniotomy induce strikingly similar profiles of inflammatory gene expression, but with distinct kinetics. Front Neurol 3: 155. https://doi.org/10.3389/fneur.2012.00155
34. Gustafsson D, Klang A, Thams S, Rostami E (2021) The Role of BDNF in Experimental and Clinical Traumatic Brain Injury. Int J Mol Sci 22(7): 3582. https://doi.org/10.3390/ijms22073582

35. Wang YJ, Chen KY, Kuo LN, Wang WC, Hsu YW, Wong HS, Lin CM, Liao KH, Zhang YF, Chiang YH, Chang WC (2018) The association between BDNF Val66Met polymorphism and emotional symptoms after mild traumatic brain injury. BMC Med Genet 19(1): 13. https://doi.org/10.1186/s12881-017-0518-0
36. Treble-Barna A, Wade SL, Pilipenko V, Martin LJ, Yeates KO, Taylor HG, Kurowski BG (2022) Brain-derived neurotrophic factor Val66Met and neuropsychological functioning after early childhood traumatic brain injury. J Int Neuropsychol Soc 25: 1–11. https://doi.org/10.1017/S1355617722000194
37. Philipp M, Brede M, Hein L (2002) Physiological significance of alpha(2)-adrenergic receptor subtype diversity: one receptor is not enough. Am J Physiol Regul Integr Comp Physiol 283(2): R287–R295. https://doi.org/10.1152/ajpregu.00123.2002
38. Sallinen J, Haapalinna A, Viitamaa T, Kobilka BK, Scheinin M (1998) D-Amphetamine and L-5-hydroxytryptophan-induced behaviours in mice with genetically-altered expression of the alpha2C-adrenergic receptor subtype. Neuroscience 86: 959–965. https://doi.org/10.1016/s0306-4522(98)00100-6
39. Scheinin M, Sallinen J, Haapalinna A (2001) Evaluation of the 2C-adrenoceptor as a neuropsychiatric drug target studies in transgenic mouse models. Life Sci 68: 2277–2285. https://doi.org/10.1016/s0024-3205(01)01016-5
40. Bjorklund M, Sirvio J, Puolivali J, Sallinen J, Jakala P, Scheinin M, Kobilka BK, Riekkinen P Jr (1998) Alpha2C-Adrenoceptor-overexpressing mice are impaired in executing nonspatial and spatial escape strategies. Mol Pharmacol 54: 569–576. https://doi.org/10.1124/mol.54.3.569
41. Sallinen J, Haapalinna A, Viitamaa T, Kobilka BK, Scheinin M (1998) Adrenergic Alpha2C-receptors modulate the acoustic startle reflex, prepulse inhibition, and aggression in mice. J Neurosci 18: 3035–3042. https://doi.org/10.1523/JNEUROSCI.18-08-03035.1998
42. Hein L, Altman JD, Kobilka BK (1999) Two functionally distinct alpha2-adrenergic receptors regulate sympathetic neurotransmission. Nature 402: 181–184. https://doi.org/10.1038/46040
43. Sysoev YI, Prikhodko VA, Kan AV, Titovich IA, Karev VE, Okovityi SV (2022) Changes in Brain Electrical Activity after Transient Middle Cerebral Artery Occlusion in Rats. Neurol Int 14(3): 547–560. https://doi.org/10.3390/neurolint14030044
44. Okabe N, Shiromoto T, Himi N, Lu F, Maruyama-Nakamura E, Narita K, Iwachidou N, Yagita Y, Miyamoto O (2016) Neural network remodeling underlying motor map reorganization induced by rehabilitative training after ischemic stroke. Neuroscience 339: 338–362. https://doi.org/10.1016/j.neuroscience.2016.10.008

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