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Effects of Modifications of the Functional State of the Central Cholinergic System on Neurological Deficiency Related to Experimental Traumatic Brain Injury

S. V. Zyablitsev, S. A. Khudoley, Yu. L. Sudilovskaya, and Yu. I. Strel'chenko

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Using a 100-point Todd scale, we examined the level of neurological deficiency in male albino rats subjected to dosed experimental traumatic brain injury (TBI). In one of the three experimental groups with TBI, animals were not subjected to additional influences; in another group, the central cholinergic system (CChS) was activated by introduction of a central-action cholinomimetic, choline alfoscerate (Gliatilin); in the third group, the activity of the above system was suppressed by a cholinoblocker, biperiden (Akineton). In rats with TBI, the level of neurological deficiency progressively increased; in this group, the estimates at 3 and 72 h after TBI induction corresponded to 474 and 950% of the respective values in intact animals. Blocking of the cholinergic system additionally aggravated manifestations of neurological deficiency, especially within earlier time intervals after TBI (3 and 24 h). Pharmacological activation of the CChS significantly smoothed manifestations of neurological disorders (several times, as compared with those in the TBI group with no additional influences).

Keywords: central cholinergic system, traumatic brain injury (craniocerebral trauma), neurological deficiency.

INTRODUCTION

At present, cases of traumatic brain injury (TBI) account for about 40% of the total number of traumas in Ukraine. Traumatic brain injury is one of the most important causes of mortality and disability among young and medium-age individuals. It is generally known that TBIs, as a rule, are accompanied by cognitive disorders of one level of severity or another. A functional insufficiency of the central cholinergic system (CChS) is an important pathogenetic factor leading to the development of cognitive disorders. Such disorders are determined, to a significant extent, by suppression of acetylcholine synthesis in the CNS structures, imbalance of the enzymes providing functioning of the cholinergic system, and necrotic death of a considerable proportion of cerebral cholinergic neurons. Information on the phenomenology and mechanisms of CChS insufficiency remains rather limited [1, 2].

Considering the above, we estimated the levels of neurological deficiency in male albino rats subjected to dosed experimental TBI. These estimates were recorded in the dynamics upon the action of isolated TBI and also after pharmacological activation and pharmacological blocking of CChS in such animals.

METHODS

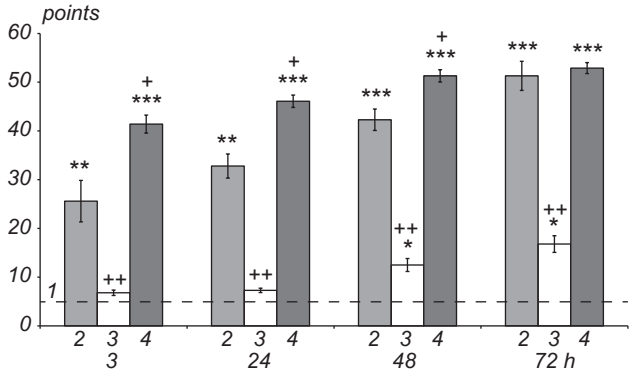
Experiments were carried out on 106 four-month-old male mongrel albino rats (body mass 180 to 200 g). The animals were divided onto four groups. The first group (n = 10) included intact animals. Rats of groups 2, 3, and 4 were subjected to dosed craniocerebral trauma. The head of the animal was fixed; the trauma was induced by the free fall of a metallic load (mass 98 g) on the dorsal head surface; the impact energy was 0.627 J. Such experimental arrangement allowed us to induce a standardized medium/severe TBI. Animals of the second group (with dosed TBI) were subjected to no additional influence except i.p. injection of 0.5 ml of Ringer solution. Animals of group 3 (n = 32) were preliminarily i.p. injected with a central-action cholinomimetic, choline alfoscerate (Gliatilin, 0.6 mg, diluted in 0.5 ml of standard Ringer solution). Animals of group 4 (n = 32) also subjected to craniocerebral trauma were i.p. injected with a central-action cholinoblocker, biperiden (Akineton,

[1] Donetsk National Medical University, Ministry of Public Health, Donetsk, Ukraine.
Correspondence should be addressed to S. V. Zyablitsev (e-mail: zsv@medic.donetsk.ua)

0.06 mg, in the same volume of Ringer solution). The above drugs were introduced according to special schemes considering their pharmacokinetics; this allowed us to provide their maximum effects on the CChS at the moment of inducing TBI.

The levels of neurological deficiency were estimated in the animals at 3, 24, 48, and 72 h after the induction of TBI using a 100-point Todd scale [3]. The above scale included estimates of the consciousness level (0–20 points), state of the reflex sphere (0–28 points), parameters of respiration (0–12 points), characteristics of the motor sphere (in particular, disorders of locomotion, 0–25 points), and characteristics of a few behavioral reactions (0–15 points).

The numerical results obtained were processed using standard methods of variational statistics. Numerical values are mentioned below as means ± ± s.e.m.



F i g. 1. Dynamics of the level of neurological deficiency in rats after experimental craniocerebral trauma resulting in traumatic brain injury (TBI, estimation according to a 100-point system). Time intervals after induction of TBI, hours, are shown below the horizontal axis. Dashed line indicates the mean level of neurological deficiency in intact animals (group 1); 2) level of neurological deficiency in the respective group of rats subjected to TBI with no additional pharmacological influences; 3 and 4) levels in the groups with TBI and preliminary activation or blocking of the central cholinergic system, correspondingly. One, two, and three asterisks show cases of significant differences from the values in group 1 ($P < 0.05$, 0.01, and 0.001, respectively); one, two, and three crosses show cases of the analogous intergroup differences, as compared with the values in group 2.

RESULTS AND DISCUSSION

Within the observation period (3 days), eight animals (25%) died in group 2 (rats with TBI only); in group 4 (rats with TBI + preliminary injection of the central cholinoblocker), the mortality was 12.5% (four animals). In groups 1 (intact rats) and 3 (rats with TBI injected with the cholinomimetic), all animals were alive.

The estimate of the level of neurological deficiency in rats of group 1 varied somewhat within the observation period; the mean value of this estimate was equal to 5.4 ± 0.75 point. Nonzero values of this index are probably determined by a natural circumstance, the presence of some animals with insignificant manifestations of one neurological disorder or another in the population of intact (supposedly healthy) rats.

In group 2 (rats with TBI and with no additional influences), the mean value of the level of neurological deficiency at 3 h after trauma was equal to 25.6 ± 4.25 points, i.e., to 474%, as compared with the respective value in group 1. Later on, the manifestations of deficiency in group 2 progressively increased and reached, at 72 h, 51.3 ± 2.98 points. In other words, this index was greater, as compared with the analogous value in the group of intact animals, by nearly an order of magnitude (Fig. 1, 2). The level of neurological deficiency increased mostly due to intensification of disorders in the reflex and motor spheres. Animals of the above group can be arbitrarily divided into two subgroups nearly equal in the number of animals manifesting moderate and dramatic impairment of the neurological sphere.

In group 3 (rats subjected to TBI and preliminarily injected with the CChS activator), the mean estimates of the level of neurological deficiency at 3 and 24 h after trauma exceeded the corresponding indices in group 1 (intact animals) only by 26 and 35%, respectively (intergroup differences were insignificant). At 48 and 72 h, the above index in group 3 was equal to 231 and 311% of the analogous values in the intact group (Fig. 1, 3). It is obvious that the respective increments were much smaller than those in groups 2 and 4. The above-mentioned intensification of neurological deficiency in group 3 was related to a few disorders in the motor sphere in some animals; suppression of the photoreaction was observed in all rats of this group.

Rats with TBI and preliminary blocking of the CChS (group 4) demonstrated a rather high index of neurological deficiency at the 3rd hour after trauma (41.4 ± 1.85 points). This value was equal to 767% of the control value in group 1; this level was by 62% higher than that in group 2 where rats were subjected to craniocerebral trauma without additional influences. Later on, the intensity of neurological deficiency of group 4 progressively

increased, but the relative difference between estimates of this deficiency in groups 2 and 4 became smaller. At 72 h after the induction of TBI, the mean estimate of neurological deficiency in rats with injections of the cholinoblocker corresponded to 980% of the respective value in intact animals. An overwhelming majority of the rats of group 4 were in the stupor state. In most animals, we observed mydriasis, exophthalmos, and the absence of the photoreaction. In rats of this group, grooming was practically absent, the frequency of respiratory movement dropped (bradypnea), and manifestations of hypoxia (cyanosis of the limbs) were obvious. Animals of group 4 refused food and drink, and their body mass dropped by 20-25 g.

Thus, dosed medium-intensity or severe TBI resulted, within three days after trauma, in the progressive development of neurological deficiency. Pharmacological activation of CChS by introduction of the cholinomimetic choline alfoscerate significantly smoothed down increases in the level of neurological deficiency. At the same time, blocking of the cholinergic system induced by introduction of biperiden aggravated the development of this deficiency, especially within the initial time interval (two days) after trauma. Therefore, disorders of CChS activity related to TBI should be considered a significant factor in the pathogenesis of the above neurological impairments. According to modern concepts, these disorders are related, to a considerable extent, to the imbalance between functions of the cholinergic (excitatory) and dopaminergic (inhibitory) cerebral systems, which are the most important central systems involved in the control of motor functions [4]. Active synthesis of acetylcholine in the CNS is realized in the loci characterized by a high density of cholinoreceptors, namely the hippocampus, striatum, *nucl. caudatus*, frontal cortex, basal forebrain regions, and pontine nuclei. This is why we, in our study, used agents that influence significantly the above CNS structures.

Our findings confirm that further studies of the effects of central cholinomimetics under conditions of TBI are clearly expedient. The respective pharmacotherapy can significantly suppress manifestations of neurological deficiency. Data on the effects of pharmacological modulation of the CChS activity will allow researchers to evaluate the significance of such effects on the clinical course and prognosis of the consequences of craniocerebral trauma.

The study was carried out in accordance with the international and Ukrainian standards for use of animals in experiments (the Council of Europe Convention from 18.03.1986 and the Law of Ukraine from 21.02.2006, No. 3447-IV).

The authors of this commynication, S. V. Zyablitsev, S. A. Khudoley, Yu. L. Sudilovskaya, and Yu. I. Strel'chenko, confirm that, in the course of the study, they have no conflict of interest pertinent to commercial or financial relations and relations with organizations or persons somehow or other related to the study, as well as to research group interaction.

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