Neurological Deficit and Disturbances in Higher Nervous Activity during Modeling of Perinatal Hypoxic-Ischemic Damage to the Central Nervous System in Rat Pups

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Seven-day-old Wistar rat pups were subjected to unilateral occlusion of the common cerebral artery and maintained in oxygen-low atmosphere. Neurological and behavioral changes were monitored for 12 weeks. The survival rate of treated animals was 90%. Body weight gain in these rats was lower than in the control. Neurological deficit was maximum 1 week after treatment and slightly regressed by the 12th week. Locomotor activity in treated rats was higher than in controls. Administration of ketamine in subanesthetic doses caused permanent ipsilateral rotational asymmetry in animals. Spatial disorientation and cognitive deficit in rats with hypoxic-ischemic damage to the central nervous system were revealed in passive avoidance, Y-maze, and rotarod tests. The total area of the hemisphere decreased, while the area of the lateral cerebral ventricle increased at the side of occlusion over the first 4-5 weeks of postnatal development. The size of the ipsilateral hemisphere remained low in adult animals.

Key Words: *model of hypoxic-ischemic brain damage; neurological deficit; higher nervous activity*

Perinatal hypoxic-ischemic brain damage (PHID) is one of the major causes of neonatal death and severe disorders of the central nervous system (CNS) [3-6]. Despite extensive studies in this field, there are no reliable methods for the therapy of PHID consequences. Additional researches on experimental models of PHID are required to develop new methods for the therapy and rehabilitation of patients with this disease. The recovery of impaired functions in CNS should be objectively recorded over a relatively long period until sexual maturation [2].

Modeling of PHID is a new line in experimental medicine. Occlusion of the common carotid artery (CCA) and hypoxic hypoxia in 7-day-old rat pups were proposed as a model of PHID by J. Rice *et al.* in 1981 [10]. Modified methods of cerebral hypoxia and ischemia in newborn rat pups, including prenatal hypoxic-ischemic injury, were used to produce PHID [5,6]. Monitoring was usually performed for 7 days after birth [3,8-10]. Only in some studies the animals were examined in delayed periods (30-90 days). It should be emphasized that the state of animals was evaluated by activity of CNS without considering age-related changes [2,4]. However, traditional methods for studying motor and neurological disorders in rat pups are primarily subjective. Morphological criteria reflecting the state of brain tissue in rats with experimental PHID are poorly elaborated [2].

In the present study neurological deficit and locomotor and behavioral disorders in rats were evaluated by means of objective tests over 3 months after mo-

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deling of PHID. A morphometric study of changes in the size of hemispheres and lateral cerebral ventricles was performed in this period.

MATERIALS AND METHODS

Experiments were performed on 118 adult male and female Wistar rat pups from 10 litters. PHID was produced by the method of J. Rice on day 7 of life [10]. Surgery was performed under inhalation narcosis (3-min exposure in a 0.3-liter desiccator containing cotton tampon moistened with 5.0 ml ether). The trunk of CCA was prepared through the median incision in the neck and ligated with a silk ligature. Termination of blood flow in CCA was controlled visually. Treated rat pups were kept in an incubator at 37°C for 1 h and placed in a 2.5-liter flow chamber (3.5 h). Wet gaseous mixture containing nitrogen and oxygen (8% O_2) passed at a flow rate of 5-6 liter/min and 37°C. Then the animals were returned to cages with feeding mothers. Control rats were subjected to similar manipulations except CCA ligation and modeling of hypoxic hypoxia. On days 25-28 of life rat pups were isolated from mothers and kept in individual cages (5 animal in each cage) under standard conditions and *ad libitum* water and food supply.

The relative number of males and females in the experimental (n=79) and control groups (n=39) was similar. Rat pups were examined until the 13th week of life. Body weight, neurological status, and locomotor activity were evaluated weekly immediately after modeling of brain injury. To exclude the effects of changes in nutrition and hormonal status during sexual maturation, motor and cognitive functions of CNS were recorded starting from the 4th week after injury at 2-week intervals. The tests were performed on different days.

Neurological status was estimated by the scale of Menzies: no deficit (0 points), tonic flexion of the forelimb contralateral to the side of CCA occlusion during tail suspension (1 point), low resistance of the contralateral forelimb during tail stretch (2 points), contralateral movement of the rat during tail pinch (3 points), and spontaneous contralateral rotation of the rat (4 points) [7].

Locomotor activity was monitored on a Coulbourn device. The absolute number of movements (including small and large movements) was estimated for 30 min.

For evaluation of locomotor and coordination disturbances the ability of rats to stay on a rotating rod (diameter 70 mm, length 200 mm) positioned at a distance of 80 cm from the floor was evaluated [4]. The animals were placed 3 times on a rotating rod (rotation rate 7 rpm) for 5 min before the experiment. The time on the rod rotating at the rate of 21 rpm was recorded. The test was terminated when the time exceeded 120 sec.

For evaluation of rotational asymmetry the rats were intraperitoneally injected with 5% ketamine (50 mg/kg) and placed in a hemispherical automatic rotameter for 30 min. The mean difference between the number of leftward and rightward rotations was calculated over 1 min (K-test) [1].

Passive avoidance behavior (conditional inhibition of moving downward from a platform) was studied in a wooden chamber (35×20×30 cm) with a metal-grid floor. A wooden platform (8×5 cm) was positioned in one corner of the box at a height of 7 cm. The rats were placed on a platform 1 day before the experiment (training session). When the animals came down, electric current (0.2 mA) was delivered through a metal grid until the rat returned to the platform. The course included 3 training sessions. The rats nearly immediately learned to stay on the platform. The latency (time from placing on a platform to the moment when animals touched the floor by 4 limbs in sec) was measured. The rats were removed from the chamber when they remained on the platform for 60 sec.

Before the Y-maze test the rats were deprived of water for 2 days. During training the animals were placed 3 times in the start arm of the maze. Drinking bowl with water was placed in one of the other arms. The rats were returned to the initial position when they found water and started to drink. The test was performed on day 3 of deprivation. The latency was the period from placing in the start arm to the start of drinking (sec). The rats not drinking for 60 sec were removed from the maze (even when they sat near the bowl).

For morphological study the brain was taken from 47 rats at various stages of the experiment. The animals were intraperitoneally narcotized with 120 mg/kg ketamine and consecutively perfused with 200 ml phosphate buffered saline (PBS) and 400 ml 4% neutral paraformaldehyde in PBS through the ascending aorta. Then the brain was removed and placed in paraformaldehyde of the same concentration. Brain samples were kept in 20% sucrose for at least 1 day, and serial sections were prepared on a frozen microtome. Eight coronary sections (40 μ) at a level of the paraventricular nucleus of the thalamus, posterior paraventricular nucleus of the thalamus (n=4), and triangular nucleus of the septum (n=4) were prepared from each brain. The sections were stained with cresyl violet (Merck) by the method of Nissl and scanned in the visible range on an Epson scanner (1600 dpi). Contours of hemispheres and lateral cerebral ventricles were visualized using Adobe Photoshop 5.5 software. The area of brain structures was expressed in pixels. The results were analyzed by Student's t test and Fischer's U test.

RESULTS

The survival rate of rats over 3 months after modeling of PHID was 90% (*vs*. 100% in the control). Starting from the 1st week after damage body weight gain in treated rats was lower than in control animals. As differentiated from control rats, animals with PHID failed to gain weight from the 10th week after surgery. The average body weights differed between control and treated rats starting from the 6th week after surgery and were 237±12 and 320±21 g, respectively, by the end of observations ($p<0.01$).

Total locomotor activity in treated rats surpassed that in control animals from the 5th week after surgery. Over the first 3 weeks the number of small movements in treated rats was higher than in control animals than more by 2-3 times. The differences were significant 2 (632.5±37.9 and 184.0±15.9, respectively, $p<0.01$) and 3 weeks after surgery (721±25 and 327±38, respectively, $p<0.01$, Fig. 1). The number of large movements in treated and control rats differed most significantly after 5 weeks (2818±151 and 1648±98, respectively, $p<0.01$).

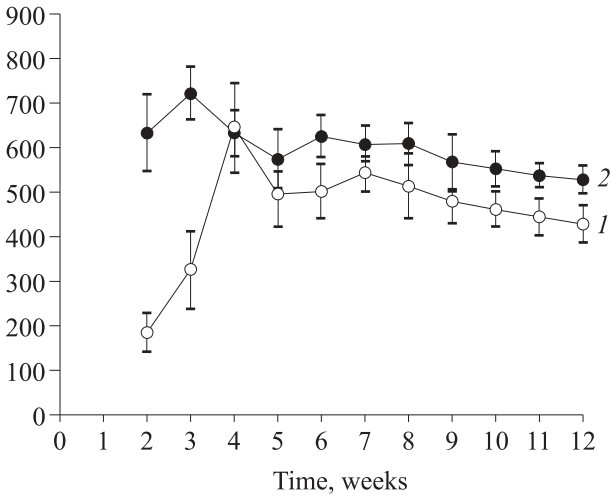

Fig. 1. Locomotor activity of control rats (*1*) and animals with perinatal hypoxic-ischemic brain damage (*2*). Abscissa: time after damage. Ordinate: absolute number of movements.

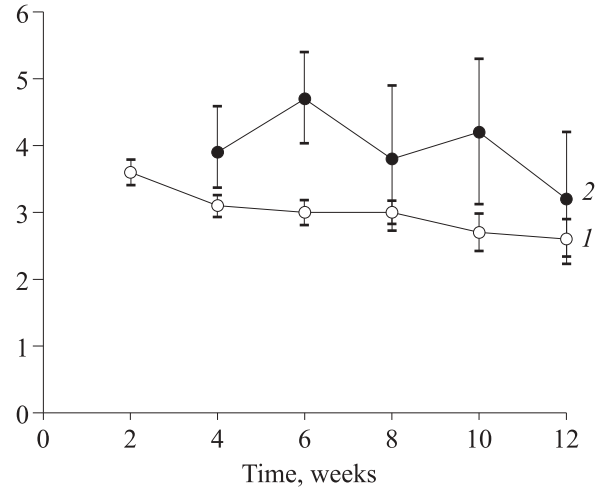

Fig. 2. Neurological deficit (points, *1*) and ketamine-induced rotational asymmetry (rpm, *2*) in rats with perinatal hypoxic-ischemic brain damage. Here and in Fig. 3: abscissa, time after damage.

Apart from hyperreactivity, treated rats were characterized by pronounced ipsilateral rotational asymmetry (K-test). In the initial period and by the end of observations the degree of asymmetry was 4.7±0.3 and 3.2±0.4 rpm, respectively (Fig. 2). Rotational locomotor asymmetry was not characteristic of control animals (0.23±0.20 rpm, $p<0.01$).

Neurological deficit was maximum 1 week after damage (3.6±0.1 points), then progressively regressed, and corresponded to 2.6±0.1 points on day 90 (Fig. 2). Neurological disturbances were not revealed in control animals.

The rotarod performance was impaired compared to the control. The observed differences were most pronounced after 8 and 10 weeks.

The rats with PHID were characterized by cognitive deficit. In various periods the absolute latency of passive avoidance of the electric stimulus decreased, while the time of searching for the drinking bowl after water deprivation increased. However, the average values did not differ between control and treated animals.

Morphometry of the brain showed that the size of hemispheres and ventricles in intact rats differed no more than by 4%. This value served as the criterion for changes in treated animals compared to the control. The size of cerebral hemispheres and lateral ventricles in intact rats was taken as 100%. Changes in treated animals were determined relative to this parameter. The size of the left cerebral hemisphere at the side of CCA occlusion significantly decreased over the 1st month after surgery, but then remained within physiological norma (Fig. 3). The size of the left lateral cerebral ventricle changed more significantly. It decreased 1 week postoperation due to brain edema, sharply increased over the next 3 weeks, decreased again after 2 and 3 months, but remained high by the end of observations (Fig. 3).

Monitoring of locomotor, neurologic, and cognitive disorders over the first 3 months of postnatal development showed that functional activity of CNS in rats with PHID [10] was impaired for a long time until sexual maturation. Although neurological deficit in treated rats progressively regressed, it remained high by the end of observations. Similar changes were observed in locomotor activity of animals. The rats with perinatal brain injury were characterized by permanent hyperreactivity (Fig. 1) and rotational asymmetry (Fig. 2). Locomotor function underwent wave-

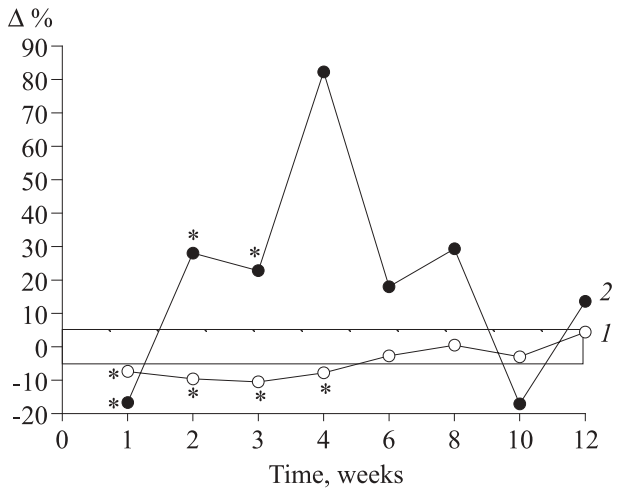

Fig. 3. Size of cerebral hemispheres and lateral ventricles at the side of common carotid artery occlusion in rats with perinatal hypoxic-ischemic brain damage. D%, differences in the size of left and right cerebral hemispheres and lateral ventricles in coronary brain sections. Shaded zone: differences in the size of left and right cerebral hemispheres and lateral ventricles in control rats. Hemisphere (*1*) and lateral ventricles (*2*). **p*<0.05 compared to the intact hemisphere or lateral ventricle.

like changes. For example, we observed a simultaneous increase in the degree of rotational asymmetry and locomotor activity on the 6th and 10th weeks of postnatal development.

Previous studies showed that rats with PHID are characterized by hyperreactivity [4]. Locomotor activity of animals was studied only in the early period after damage (2 weeks). No differences were revealed in the latency of passive avoidance behavior in control and treated rats 9 weeks after treatment. In our experiments cognitive deficit persisted in some rats with PHID even 3 months after modeling of ischemia (Y-maze and passive avoidance test). Passive avoidance test showed that the ratio of treated animals with memory disorders remained high 12 weeks after damage (35%). These changes were not observed in control rats.

Our experiment showed that changes in body weight are a reliable criterion for the state of animals. The rats with PHID failed to gain weight over the last 3 weeks of observations, while neurological and locomotor disorders were partially compensated at this term. A decrease in the area of the cerebral hemisphere and increase in the area of the lateral ventricle at the side of CCA occlusion were observed over the 1st month after damage and were probably related to necrosis and apoptosis. Some treated rats were characterized by CNS dysfunction and morphometric changes at the side of CCA occlusion by the end of the 3rd month. Our results indicate that this model of PHID simulates neuronal degeneration typical of perinatal hypoxic-ischemic encephalopathy in humans [2]. The complex of objective tests can be used to model perinatal damage to CNS and develop new methods for experimental therapy of this disorder.

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