Early and Delayed Effects of Hypoxia during the Infantile Period on Behavioral and Hormonal Reactions of Rats

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We studied the early and delayed effects of hypoxia during the infantile period on the behavioral reactions and corticosterone concentration in male rats. The elevation of corticosterone concentration, decrease in the immobility time (forced swimming test), and increase in the nociceptive response (formalin test) were observed in 7-day-old rats immediately after hypoxia. Adult animals exposed to hypoxia at the age of 7 days exhibited elevated basal corticosterone level and lengthened immobility time. Hypoxia had the same effect on plasma corticosterone concentration in 7-day-old and adult rats. Changes in corticosterone concentration after forced swimming were shown to differ in hypoxic animals and non-hypoxic specimens. Studying the dynamics of age-related variations in the test parameters will contribute to the understanding of pathogenetic mechanisms and development of new methods for pharmacological correction of postnatal changes in CNS after hypoxia during early ontogeny.

Key Words: *hypoxia; ontogeny; pain; depression; corticosterone*

Studying the response to stress exposures during early postnatal ontogeny and, particularly, in the early puerperal period is an urgent problem of developmental physiology. This period is characterized by a "sensory attack" and adaptation of newborns to new environmental conditions. Congenital defects and adverse consequences of prematurity manifest during this period. Hypoxia is a common neonatal stress that causes severe acute distress and long-term complications (*e.g.*, brain injury). This state is followed by locomotor disturbances, seizures, mental disorders, and other signs of cerebral deficiency [9]. Adaptation to neonatal hypoxia involves a variety of systems. The hypothalamic-pituitary-adrenal system (HPAS) plays a key role in this process [12]. Studying the effect of early hypoxia [3,15] on the behavioral and hormonal response during ontogeny will contribute to the development of new methods for the diagnostics, therapy, and prevention of serious disorders in newborns.

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MATERIALS AND METHODS

Experiments were performed on male Wistar rats aging 7 days (junior age group) and 110 days (senior age group). The day of birth was designated as day 0. Seven-day-old rats were separated from the mother and exposed to 3-h hypoxia in a high-altitude flow chamber at 160-180 mm Hg and 24°C. This level corresponded to the ascent at 11,000 m. The animals separated from the mother and maintained in an altitude chamber at normal atmospheric pressure for 3 h served as the control. Some animals were examined immediately after hypoxia. The remaining animals were kept in a vivarium up to the 110th day of life. The immobility time (depressive symptom) of 7-day-old rats was measured in the Porsolt forced swim test 30 min after hypoxia. The total number of

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flexions+shakes in the formalin test [4] was estimated 24 h after hypoxia served as a criterion of the nociceptive response. Long-term effects of hypoxia in animals of the senior age group were also studied in the forced swim test and formalin test.

Plasma corticosterone concentration in 7-day-old and 110-day-old rats was measured during the following periods: basal level (adult animals and rat pups maintained with the mother in a laboratory vivarium and not exposed to any treatment; 10.00); immediately after hypoxia and under control conditions (non-hypoxic specimens); and immediately after the forced swim test (hypoxic and non-hypoxic groups). The animals were decapitated. The blood was collected. Plasma corticosterone concentration was measured by the micromethod on a spectrofluorometer [1].

The duration of forced swimming for 7-day-old and adult rats was 45 sec and 5 min, respectively. The total time of immobility (suspension in water without swimming movements) serves a criterion of depression [4]. The long-term behavioral nociceptive response to inflammation was induced by a subcutaneous injection of 2.5% formalin (10 and 50 μl to 7-day-old and 110-day-old rats, respectively) [2,4]. The total number of flexions+shakes of the treated limb was evaluated for 1 h.

Each group consisted of 6-8 specimens. Our study was performed in accordance with the Helsinki declaration on the welfare of animals. The protocols of experiments were approved by the Ethics Committee (I. P. Pavlov Institute of Physiology).

The results were analyzed by Student's *t* test and Mann–Whitney *U* test. The differences were significant at $p<0.05$.

RESULTS

Severe hypoxia in 7-day-old rats did not cause death of animals. The early effect of hypoxia in 7-day-old rats manifested in the increase in plasma corticosterone concentration as compared to that in non-hypoxic animals (6.25±0.59 and 0.98±0.15 μg%, respectively, $p<0.001$) and basal level (1.63±0.33 μg%, $p<0.001$; Fig. 1, *a*). Corticosterone concentration tended to decrease in 7-day-old rats not exposed to hypoxia (0.98±0.15 *vs*. 1.63±0.33 μg% under basal conditions; Fig. 1, *a*).

Hypoxia was followed by a decrease in the immobility time, which serves as a depressive symptom in the forced swim test (0.38±0.18 *vs*. 14.3±1.8 sec in 7-day-old rats of the non-hypoxic group, $p<0.001$; Fig. 1, *b*).

Forced swimming was accompanied by the decrease in corticosterone concentration in 7-day-old rats of the hypoxic group (3.20±0.44 *vs*. 6.25±0.59 μg% in animals exposed to hypoxia and not subjected to the Porsolt test, $p<0.001$; Fig. 1, *a*). Corticosterone concentration tended to increase in 7-day-old rats of the hypoxic group that were subjected to the Porsolt test or remained untested (1.40±0.22 and 0.98±0.15 μg%, respectively; Fig. 1, *a*). The concentration of hormones in 7-day-old rats was elevated immediately after hypoxia as compared to animals of the non-hypoxic group (3.20±0.44 and 1.40±0.22 μg%, respectively, $p=0.03$; Fig. 1, *a*).

The total number of flexions+shakes (criterion of the nociceptive response during inflammation) in 7-day-old rats exposed to hypoxia was higher than in animals of the non-hypoxic group (370.9±58.9 and 164.1±23.2, respectively, $p<0.05$; Fig. 1, *c*).

We studied the delayed effect of hypoxia on plasma corticosterone concentration in 110-day-old rats exposed to hypoxia at the age of 7 days. No differences were found between animals of the hypoxic and non-hypoxic groups (20.5±4.8 and 15.7±3.7 μg%, respectively). However, the concentration of corticosterone in hypoxic rats differed significantly from the basal level of this hormone in animals that were not exposed to any treatment at the age of 7 days (20.5±4.8 and 10.1±2.5 μg%, respectively; Fig. 2, *a*).

Hypoxia was followed by an increase in the immobility time of animals in the forced swim test. These changes were observed in rats of the hypoxic and non-hypoxic groups (149.4±17.5 and 100.3±10.9 sec, respectively, $p=0.03$; Fig. 2, *b*).

Forced swimming did not significantly change corticosterone concentration in rats exposed to hypoxia at the age of 7 days (24.67±0.90 *vs*. 20.5±4.8 μg% in animals of the hypoxic group not examined in the Porsolt test; Fig. 2, *a*). The exposure of non-hypoxic rats to the Porsolt test was followed by an increase in corticosterone concentration (30.15±2.80 *vs*. 15.7±3.7 μg% in non-hypoxic animals that remained untested, $p=0.012$; Fig. 2, *a*).

Immediately after forced swimming, the concentration of corticosterone did not differ in rats of the hypoxic and non-hypoxic groups (30.15±2.80 and 24.67±0.90 μg%, respectively; Fig. 2, *a*).

The tonic nociceptive response in the formalin test practically did not differ between rats of the hypoxic and non-hypoxic groups (456.0±112.1 and 490.37± 69.50, respectively; Fig. 2, *c*).

Our results indicate that the early effect of severe hypoxia in 7-day-old rats manifested in an increase in plasma corticosterone concentration, which serves as a stress hormone. However, the concentration of this hormone in animals decreases after forced swimming. The immobility time of rats was shown to decrease in the forced swim test. The nociceptive response was elevated during inflammation in the formalin test. The delayed effect of hypoxic exposure at the age of 7

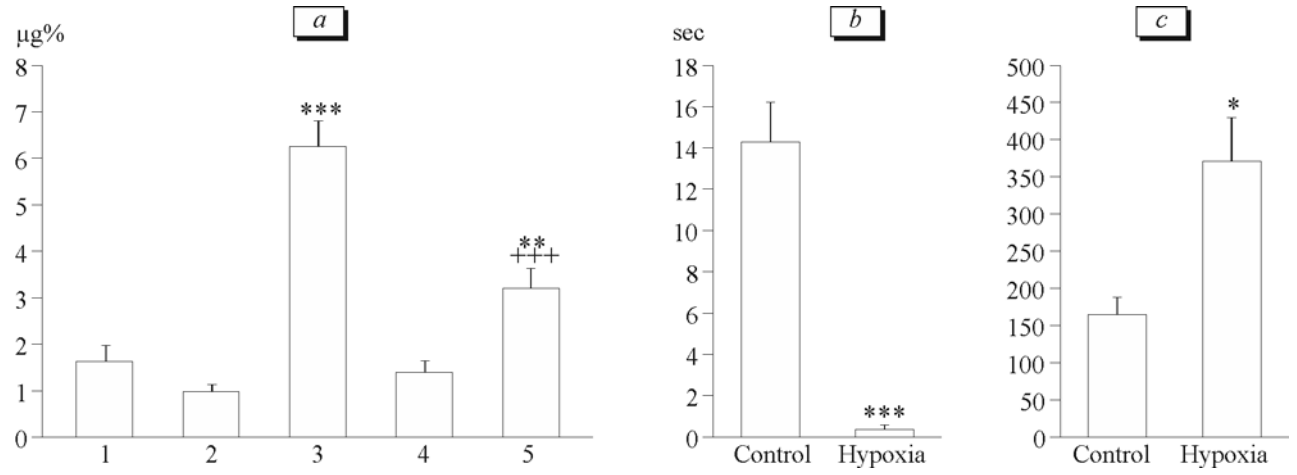

Fig. 1. Effect of hypoxia on plasma corticosterone concentration and variations in corticosterone level in the forced swim test with rats of the hypoxic and non-hypoxic groups (*a*). Action of hypoxia on behavioral parameters of 7-day-old rats (*b*, *c*). Here and in Fig. 2: corticosterone concentration (*a*): basal corticosterone level (*1*); corticosterone concentration in rats of the non-hypoxic group after removal from the altitude chamber (*2*); corticosterone concentration in rats of the hypoxic group after removal from the altitude chamber (*3*); corticosterone concentration in rats of the non-hypoxic group after the Porsolt test (*4*); corticosterone concentration in rats of the hypoxic group after the Porsolt test (*5*). Immobility time in the Porsolt test (*b*). Number of flexions+shakes in the formalin test (*c*). (*a*) ***p<0.001 compared to *2*; **p<0.01 compared to *4*; ^{+++}p<0.001 compared to *3*. (*b*, *c*) *p<0.05 and ***p<0.001 compared to the control.

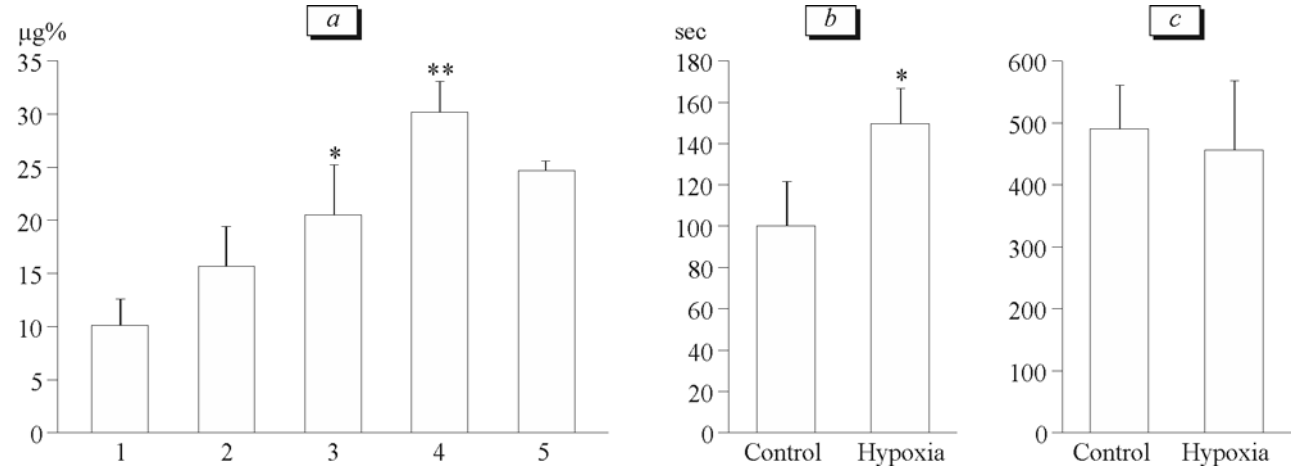

Fig. 2. Delayed effects of hypoxia at the age of 7 days on plasma corticosterone concentration and variations in corticosterone level in the forced swim test with rats of the hypoxic and non-hypoxic groups (*a*). Action of hypoxia on behavioral parameters of 110-day-old rats (*b*, *c*). (*a*) *p<0.05 compared to *1* and *2*; **p<0.01 compared to *2* and *3*. (*b*, *c*) *p<0.05 compared to the control.

days manifested in an increase in the immobility time of adult rats.

We conclude that HPAS of 7-day-old rats responds to 3-h severe hypoxia by a significant increase in corticosterone concentration. Previous studies showed that the exposure of rats to hypoxia on days 2-14 of the postnatal development is not followed by changes in corticosterone concentration [6]. This period corresponds to hyporeactivity of HPAS [14]. It was hypothesized that activity of HPAS is low during this period. It is manifested in the reduced concentration of hypothalamic hormone+x adenohypophyseal hormones, and corticosterone in blood plasma and no activation of the adrenal glands in response to stress [14]. Recent experiments revealed that the negative feedback mechanism of HPAS is well developed in rats exposed to hypoxia at the age of 7 days [12]. The response of ACTH to acute stress is abolished due to the sympathoadrenal system-induced ACTH-independent release of corticosterone (but not due to age-related hypoactivity of the hypothalamic-pituitary complex).

Forced swimming is severe physical and emotional stress. The decrease in the immobility time of hypoxic rats in the forced swim test (as compared to animals of the non-hypoxic group) is related to changes in activity of HPAS [11] and monoaminergic sys-

tems [5]. High concentration of corticosterone in adult animals correlates with an increase in the number of immobility episodes and decrease in the ratio of active behavioral reactions under stress conditions. Published data show that the immobility time in the forced swim test depends on the secretion of glucocorticoids [11]. It could be anticipated that high concentration of corticosterone contributes to longer time of immobility in rats of the hypoxic group (immediately after exposure in the altitude chamber, before the Porsot test) as compared to non-hypoxic animals. However, these rats were characterized by a shorter immobility period. These features are probably associated with age-related characteristics of animals, activation of the sympathoadrenal system, and increase in brain serotonin concentration in 7-day-old rats [10]. The increase in monoaminergic neurotransmission enhances behavioral activity of animals in the forced swim test [5]. Published data show that behavioral hyperactivity may be related to an imbalance between the GABAergic and glutamatergic systems [13]. Moreover, glutamate receptors play a role in hypoxic injury of brain structures in 7-day-old rats [7] and spinal nociceptive response of animals in the formalin test [8]. These factors probably play a role in the development of changes in animals that were exposed to hypoxia during early postnatal ontogeny.

The early and delayed effects of hypoxia were compared in studying the influence of a pathogenic factor on the tonic nociceptive response during inflammation. The basal corticosterone level is elevated, depressive symptoms persist, and tonic pain remains unchanged in adult rats exposed to hypoxia at the age of 7 days. Hypoxia has the same effect on plasma corticosterone concentration in 7-day-old and adult rats. Changes in corticosterone concentration after forced swimming were shown to differ in hypoxic and non-hypoxic animals. In the first case, the concentration of corticosterone decreases in 7-day-old rats, but remains unchanged in adult animals. In the second case the concentration of corticosterone remains unchanged in 7-day-old rats, but increases in adult animals.

Studying the cause of ontogenetic differences in these systems and performance of experiments on animals of various age groups on various models are required to evaluate the pathogenesis and to develop new methods for the correction of postnatal complications in CNS after hypoxia during early ontogeny.

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