Peptidergic Correction of Behavior in Adult Progeny of Albino Rats Exposed to Acute Hypoxia during Early Organogenesis

A. V. Graf, A. S. Maklakova, Ya. V. Krushinskaya, and N. A. Sokolova

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Physical development, spontaneous behavior, and training capacity were evaluated in adult progeny of albino rats exposed to acute hypobaric hypoxia on days 9-10 of gestation, corresponding to the early organogenesis period. Prenatal hypoxia caused delayed behavioral disorders, which were more pronounced in females born from mothers with low resistance to hypoxia. Therapeutic intranasal administration of Pro-Gly-Pro peptide in a dose of 1 mg/kg to rat pups on days 13-15 of life completely prevented the negative consequences of acute prenatal hypoxia in adult females and leveled virtually all negative consequences, except delayed physical development, in males.

Key Words: *prenatal hypoxia; early ontogenesis; Pro-Gly-Pro*

The problem of health deterioration in pregnant women and the resultant high incidence of perinatal losses and subsequent developmental pathologies in the progeny is a pressing problem of modern medicine. Of all stress exposures of the embryo in utero, hypoxia is the most important, prevalent, and clinically significant conditions, leading to embryogenesis disturbances and various developmental diseases. Children with a history of antenatal hypoxia are small for date, they develop imbalance in the visceral systems and biogenic amines, morphofunctional disorders in the brain, heart, and lungs [4,7,8]. The effects of prenatal hypoxia on the newborn and its aftereffects during the postnatal period largely depend on the gestation term. Two critical periods are distinguished in human and animal embryogenesis: preimplantation (days 1-5 after fertilization) and intensive organogenesis (days 13-17 in rats, weeks 3-8 in humans). The effects of hypoxic exposure during these periods on the development of newborns were studied not once, while the effects of acute hypoxic stress during the early organogenesis period (days 9-10 in rats, weeks 1-3 in humans) remain virtually not studied.

A possible approach to the correction of negative aftereffects of hypoxia is use of regulatory peptides playing the key role in the regulation and realization of various body functions not only in health, but also in disease. Regulatory peptides are characterized by multiple functions and cascade pattern of action leading to prolongation of the peptidergic effects [1]. One of these regulators is Pro-Gly-Pro (PGP) tripeptide, a member of the glyproline family. The antihypoxic effect and high neuroprotective activity of PGP were shown previously, as well as its manifest protective effect under conditions of disease in rats with behavioral disorders, exposed to stress: PGP prevented significant increase in anxiety and inhibition of orientation and exploratory activity [2].

We studied the effect of acute hypoxia during the early organogenesis period on the development of progeny and the possibility of correction of hypoxia-induced disorders by PGP therapy.

Department of Physiology of Man and Animals, Biological Faculty, M. V. Lomonosov Moscow State University

MATERIALS AND METHODS

The study was carried out on the progeny of outbred albino rats (194 females and 164 males) from 67 adult females. Adult females were mated with males (2:1). Pregnancy term was determined by the presence of spermatozoa in vaginal smears after mating; the day of detection of spermatozoa in vaginal smears was considered as pregnancy day 1 [5].

Females were exposed to acute hypobaric hypoxia (AHH) on days 9-10 of pregnancy. AHH was modeled in a pressure chamber at 145 mm Hg (corresponding to an altitude of 11,500 m above sea level). "Elevation" to this "altitude" was carried out within 1 min. A group of females with low resistance to AHH was detected by the life span under hypoxic conditions (LS; time from the end of "elevation" until respiratory arrest or the first agonal inspiration; LS<5min). After the progeny of these females reached the postpubertal age (day 57 of postnatal development), their behavioral patterns were studied in standard tests (hole chamber, elevated plus-maze, passive avoidance conditioning) and in parallel, the morphometric parameters were registered by Ketle's index (KI=m/l^2). The progeny of rats not exposed to AHH served as the control.

On days 13, 14, and 15 of life the pups of experimental and control groups received intranasally saline (1 μl/g) or PGP tripeptide (synthe-

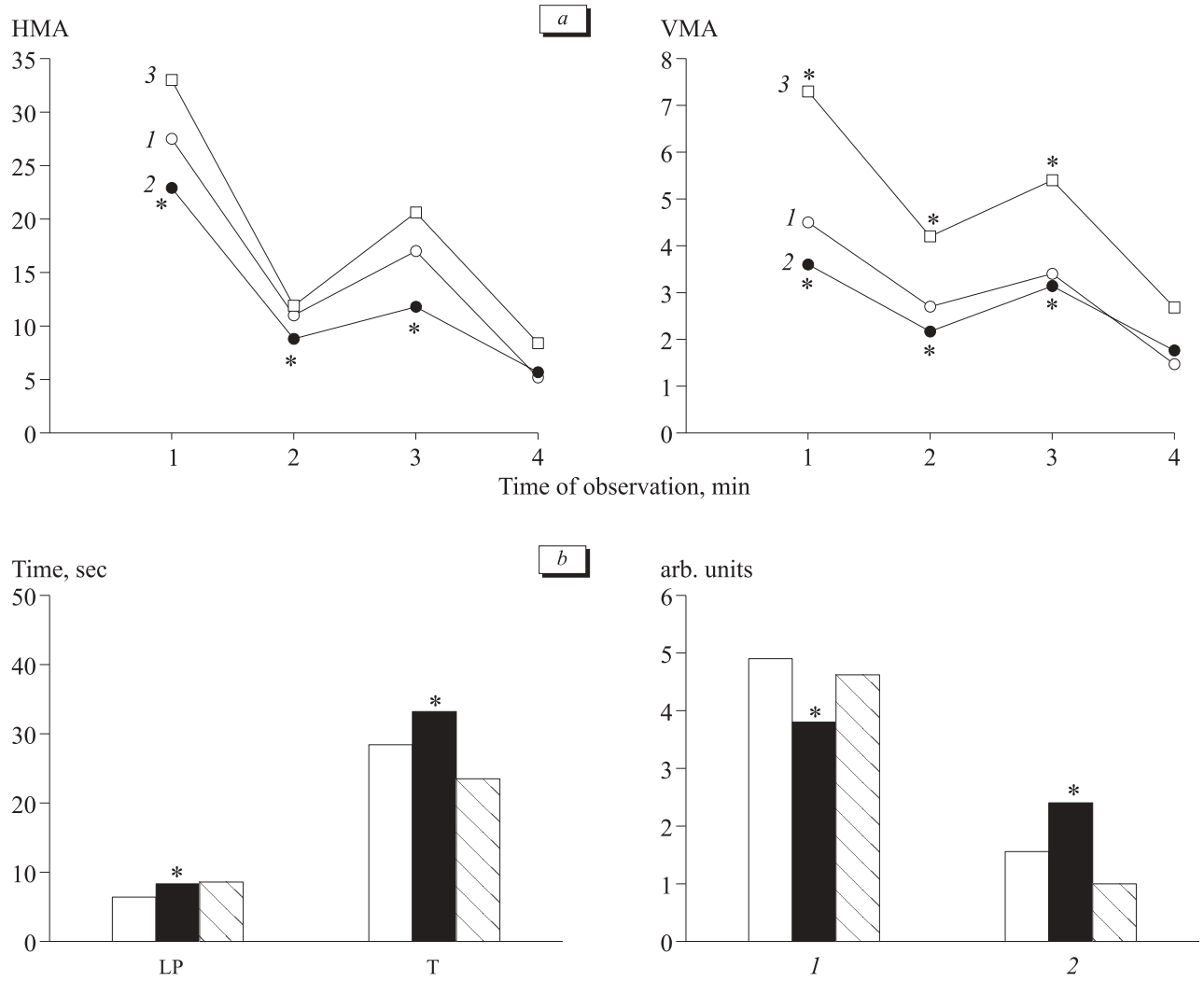

Fig. 1. Effect of PGP treatment on aftereffects of prenatal hypoxia during early organogenesis stage: behavior of females born from mothers with low resistance of acute hypobaric hypoxia (AHH) on day 57 of life in the hole chamber (*a*) and plus-maze tests (*b*). VMA: vertical motor activity; HMA: horizontal motor activity. *1*) control; *2*) progeny of low-resistant females exposed to AHH; *3*) PGP-treated pups of low-resistant females exposed to AHH (AHH+PGP). *1*) anxiety coefficient (grooming, peeping out, and defecation acts); *2*) risk coefficient (excursions to light arms of the maze, rearings in light arms, hanging-out acts). LP: latent period of the first excursion into the dark arm; T: total time spent in light arms of the maze. Here and in Figs. 2, 3: light bars: control; dark bars: AHH; cross-hatched bars: AHH+PGP. *p<0.05 compared to the control.

sized at Laboratory of Regulatory Peptides, Institute of Molecular Genetics, Russian Academy of Sciences) in the same volume of saline (1 mg/kg) [2]. The agent was chosen due to its high neuroprotective activity [6] and high stability: its half-life period in the brain tissues is much longer than 24 h [3]. This term of the peptide treatment was chosen because the histological structure of the brain of a 12-13-day-old rats corresponds to the brain structure of a full-term newborn (weeks 38-40 of gestation) [11], while intranasal route of administration was chosen due to high permeability of blood and lymph capillaries of the nasal mucosa for regulatory peptides and the possibility of their direct effect on the CNS due to slight blood-brain barrier between the nasopharyngeal lymph vessels and blood vessels of the basis cerebri [9,10].

The data were statistically processed using Excel and Statistica 6.0 software.

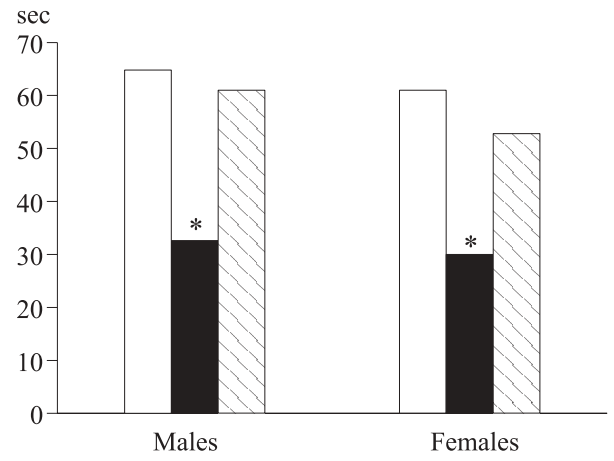

Fig. 2. Effect of PGP therapy on aftereffects of prenatal hypoxia (early organogenesis stage): passive avoidance training of 57-day-old progeny of females with low hypoxic resistance (latency of excursion into the dark section of the chamber) on days 1 and 2 of testing.

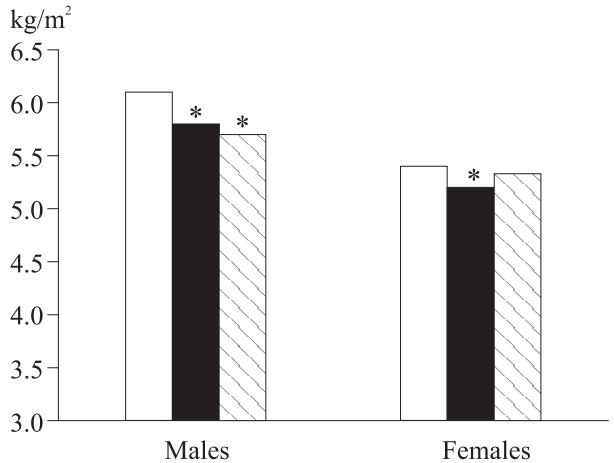

Fig. 3. Effect of PGP therapy on aftereffects of prenatal hypoxia (early organogenesis stage): physical development (Ketle index) of the progeny of females with low hypoxic resistance on day 57 of life.

RESULTS

The effects of AHH during the early organogenesis period detected on day 57 of postnatal development manifested by significant deviations in behavioral patterns. These changes were more pronounced in females born from mothers with low hypoxic resistance. These animals were characterized by reduced horizontal and vertical motor activities in hole chamber and reduced anxiety and increased risk behavior in an elevated plus-maze (Fig. 1). In male progeny, the effect of prenatal hypoxia on behavior manifested only in intensification of risk behavior. Adult progeny of both sexes with a history of prenatal hypoxia demonstrated significantly empained learning compared to control animals (Fig. 2). Behavioral disorders in females and males developed in the presence of significant delay in physical development (Fig. 3).

Intranasal administration of PGP to rat pups of nursing age completely eliminated the negative consequences of acute prenatal hypoxia in adult females and virtually leveled all negative effects except small-for-date size in males (Figs. 1-3).

Hence, the beginning of organogenesis should be referred to the "risk zone", because exposure to negative factors during this period negatively affects the postnatal development until the postpubertal age. The effect of PGP was detected 6-7 weeks after intranasal therapy. Pronounced therapeutic effect of PGP was most likely due to favorable impact of this tripeptide on impaired homeostasis by correcting the cascade reactions pathologically modified under the effect of AHH during the early stage of organogenesis.

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