Peptidergic Correction of the Effect of Acute Hypobaric Hypoxia in Pregnant Rats on Progeny

M. V. Maslova, K. S. Zemlyanskii, M. V. Shkol'nikova, A. S. Maklakova, Ya. V. Krushinskaya, N. A. Sokolova, and I. P. Ashmarin

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Acute hypobaric hypoxia of pregnant rats led to a significant delay in body weight gain, growth and time of eye opening in newborn rat pups which was paralleled by behavioral changes. Preventive intranasal instillations of peptide mixture (semax and β-casomorphine-7) to pregnant females prevented the effect of hypoxia on the progeny.

Key Words: *pregnant rats; hypoxia; effect on progeny; semax; β-casomorphine*

Acute hypoxia during pregnancy had a negative impact on progeny and in grave cases causes mental retardation and motor disorders [8,9]. In newborns, intrauterine hypoxia is responsible for early and delayed behavioral disorders [7], the most prevalent of them is attention-deficit hyperactivity disorder in children [8]. It is obvious that prevention or therapy of hypoxic injuries should be carried out *in utero*, when the brain is most sensitive to oxygen deficiency [6,7]. An attempt was undertaken to correct hypoxic injuries to the fetus at the early stage of intrauterine development by intranasal administration of regulatory peptides (RP) to pregnant females. For this purpose semax (Met-Glu-His-Phe-Pro-Gly-Pro) possessing antihypoxic activity [1,3] and β-casomorphine-7 (Tyr-Pro-Phe-Pro-Gly-Pro-Ile) reducing anxiety and defense motivation in animals [5] were used. We hypothesized that combined use of these peptides will extend the spectrum of their effects.

MATERIALS AND METHODS

The study was carried out on 168 rat pups of both sexes. Random-bred albino females were exposed to hypoxia on day 3 of pregnancy. Acute hypobaric hypoxia was induced in a 3.3-liter pressure chamber at a height of 11,500 m above sea level (elevation rate 200 m/sec) [4].

Fifteen minutes before hypoxia, experimental pregnant females were intranasally administered a mixture of semax and β-casomorphine-7 (total volume 40 μl). The peptides were synthesized at the Institute of Molecular Genetics, Russian Academy of Sciences. Semax dose (0.1 mg/kg) corresponded to the dose exerting an antihypoxic effect in adult rats [3], and β-casomorphine-7 dose (0.1 mg/kg) corresponded to the dose reducing anxiety in adult animals during stress [5]. Controls received the same volume of normal saline.

Behavioral characteristics of rat pups were recorded in a hole chamber for 4 consecutive days (days 15-18 or 22-25 of life). The animal was put into the center of the cage and its horizontal (HMA, crossed squares) and vertical motor activities (VMA, rearings), grooming reactions (touching muzzle with paws during washing) and the number of explored holes (complete submerging of the muzzle into the hole) were recorded during 2 min.

On day 22 of life, animal anxiety was evaluated in an elevated plus-maze. The animal was put into the center of the maze, with muzzle to the illuminated arm, and the latency of entering the dark arm (3 min), the time spent in illuminated arm, number of enters to illuminated and dark arms, peepings from dark arms, hang-

Department of Human and Animal Morphology, Biological Faculty, M. V. Lomonosov Moscow State University. ***Address for correspondence:*** a_maklakova@mail.ru. Maklakova A. S.

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TABLE 1. Elevated Plus-Maze Behavior of Newborn Rat Pups on Day 22 of Life

Parameter	Intact (*n*=12)	Acute hypobaric hypoxia	
		no correction (*n*=9)	correction (*n*=8)
Latency, sec	42.1	19.4	51.9
Time spent in light arms, sec	48.4	41.6	51.9^{+}
Visits to light arms	0.9	1.8*	0.3^{+}
Visits to dark arms	1.1	2.1*	0.8^{+}
Number of peepings out	0.5	0.6	0.1
Number of hangings off	1.5	2.3	1.6
Vertical motor activity, arb. units	0.8	1.5	0.0*$^{+}$
Number of grooming reactions	0.8	3.1*	2.5*

Note. $p<0.05$: **vs.* intact rats, $^{+}$compared to group without correction.

ings from illuminated arms, VMA, and number of grooming acts were recorded.

Morphometric parameters (body weight and length and time of eye opening) were recorded every other day from day 2 to 36.

The results were processed using nonparametrical Wilcoxon—Mann—Whitney test and precise Fisher test.

RESULTS

Rat pups from control females were characterized by slow weight gain starting from day 10 and slow growth starting from day 8 of life (Fig. 1, $p<0.05$). This retardation persisted to day 36, but was completely prevented by pretreatment of pregnant females with RP (Fig. 1, $p<0.05$). Moreover, rat pups exposed to intrauterine hypoxia opened their eyes later. By the age of 18 days 92.9% intact rat pups opened their eyes (*vs.* 76.3% in the control, $p<0.05$); however, treatment of pregnant females with RP did not prevented this symptom: only 79.5% rat pups opened their eyes by this term in the experimental group.

Deceleration of the postnatal development in some cases led to essential changes in behavior. In 15-18-day-old control pups HMA and VMA were decreased (Fig. 2).

Pretreatment with RP did not prevented this effect of hypoxia on the progeny: moreover, it led to an opposite reaction, which was most pronounced for HMA (Fig. 2).

It is noteworthy that antenatal acute hypobaric hypoxia did not affect exploratory activity (holes) and the degree of emotional stress (grooming) in 15-18-day-old

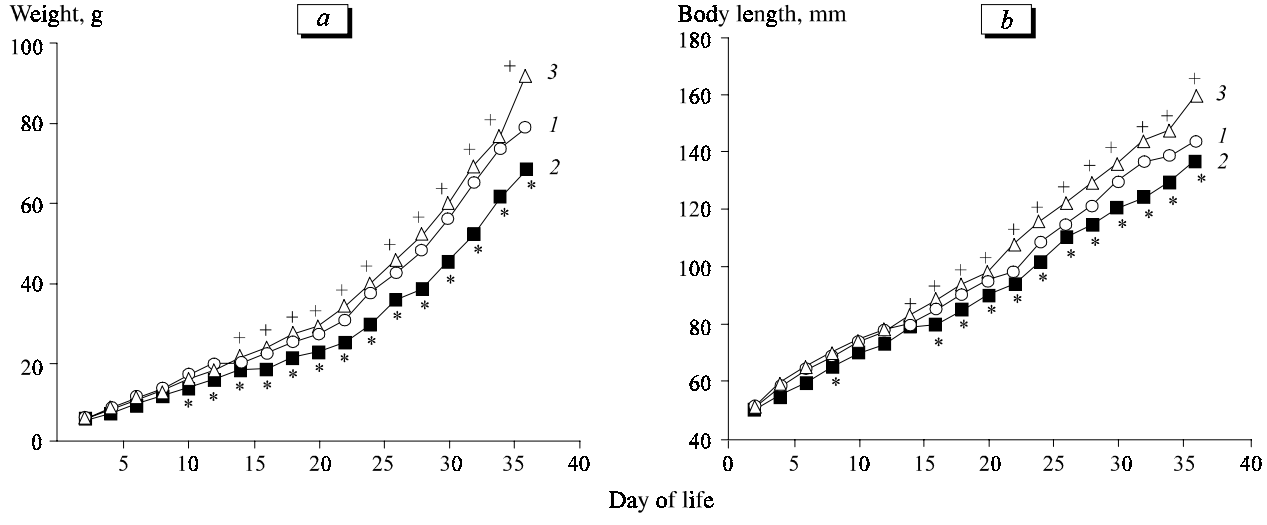

Fig. 1. Body weight (*a*) and length (*b*) in newborn rat pups exposed to intrauterine hypoxia. Here and in Figs. 2 and 3: *1*) intact rats; *2*) acute hypobaric hypoxia; *3*) acute hypobaric hypoxia+mixture of regulatory peptides. *$p<0.05$ *vs.* intact rats, $^{+}p<0.01$, $^{++}p<0.05$ *vs.* group without correction.

rat pups. This can be explained by immaturity of these functions during the early postnatal period [1].

More pronounced oppositely directed effects of intrauterine hypoxia were observed on days 22-25 in the control group. HMA was significantly increased on day 24, VMA on days 22 and 24, and the number of explored holes on day 24 of life. Moreover, the level of grooming reactions was increased in rat pups with a history of intrauterine hypoxia during the entire period of testing ($p<0.05$, Fig. 3), which indicates high emotional stress [1].

In experimental rat pups hypoxia-induced changes in behavioral characteristics were not observed ($p<0.05$, Fig. 3). An opposite reaction was observed in some cases: hypoactivity instead of hyperactivity, developing as a result of hypoxia ($p<0.05$, Fig. 3).

Testing of 22-day-old rat pups in an elevated plus-maze confirmed hyperactivity and emotional strain in the control (Table 1). There was a trend to a shorter latency before entering dark arms ($p=0.08$), a significantly greater number of enters to the dark and illuminated arms, hangings off the illuminated arms, and increased VMA in these animals in comparison with the control; their grooming values were also higher ($p<0.05$). In experimental animals all these effects, except the effect on grooming, were abolished ($p<0.05$).

Hence, acute hypobaric hypoxia of pregnant females during the first third of pregnancy damaged the embryo. This manifests in delayed physical development and behavioral abnormalities. This may be due to impairment of the cognitive functions of the brain and to changes in the hormonal status, resultant from intrauterine hypoxia [6]. Opposite changes in behavioral characteristics in early and later age can be associated with a different phase patterns of oxygen deficiency [2] and a definite period of maturing of this or that physiological function [1].

Preventive treatment with RP almost always removed the effect of hypoxia, but the mixture can exert

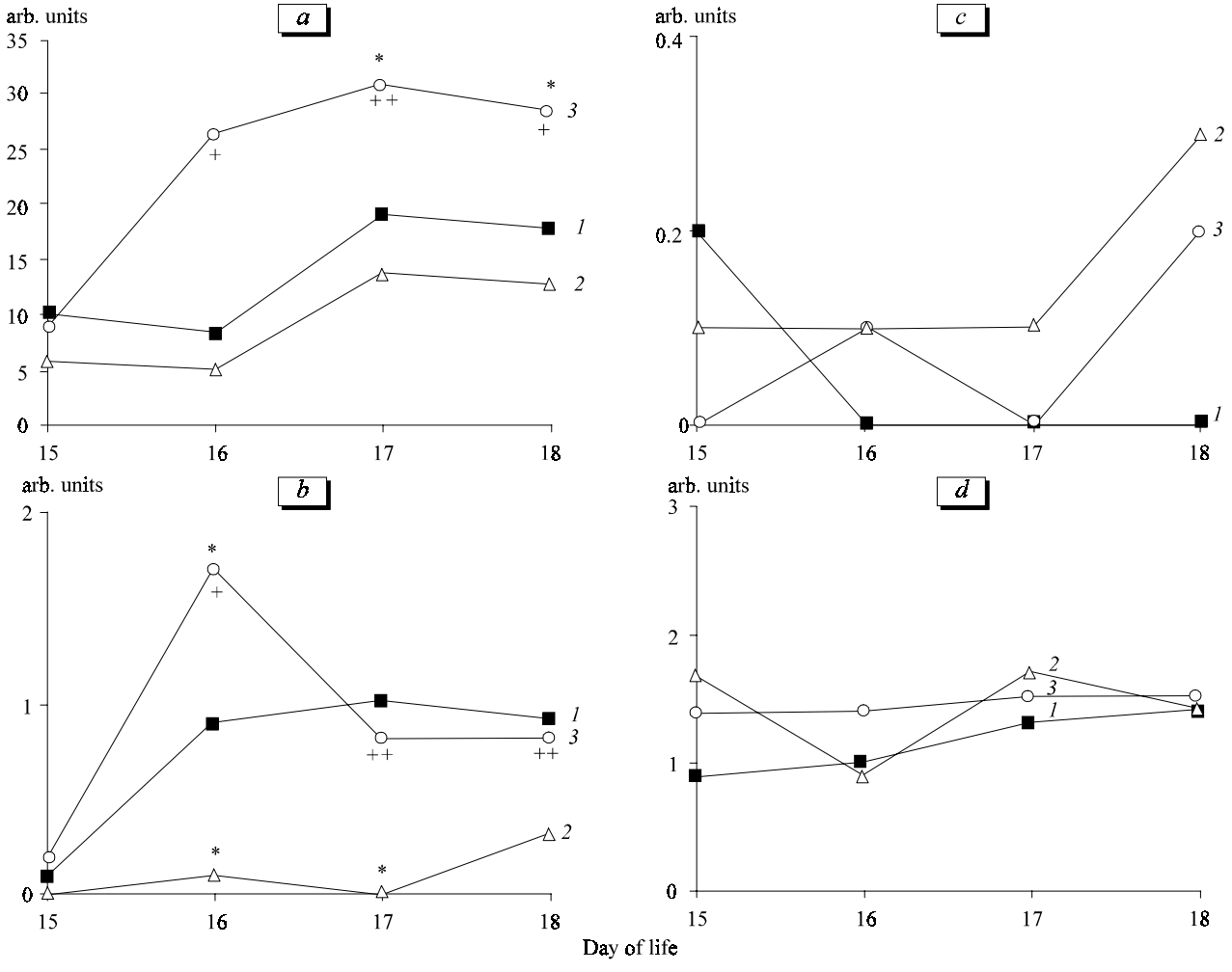

Fig. 2. Changes in behavioral characteristics of rat pups in hole chamber on days 15-18 of life. Here and in Fig. 3: horizontal (*a*) and vertical (*b*) activity, number of explored holes (*c*) and grooming reactions (*d*).

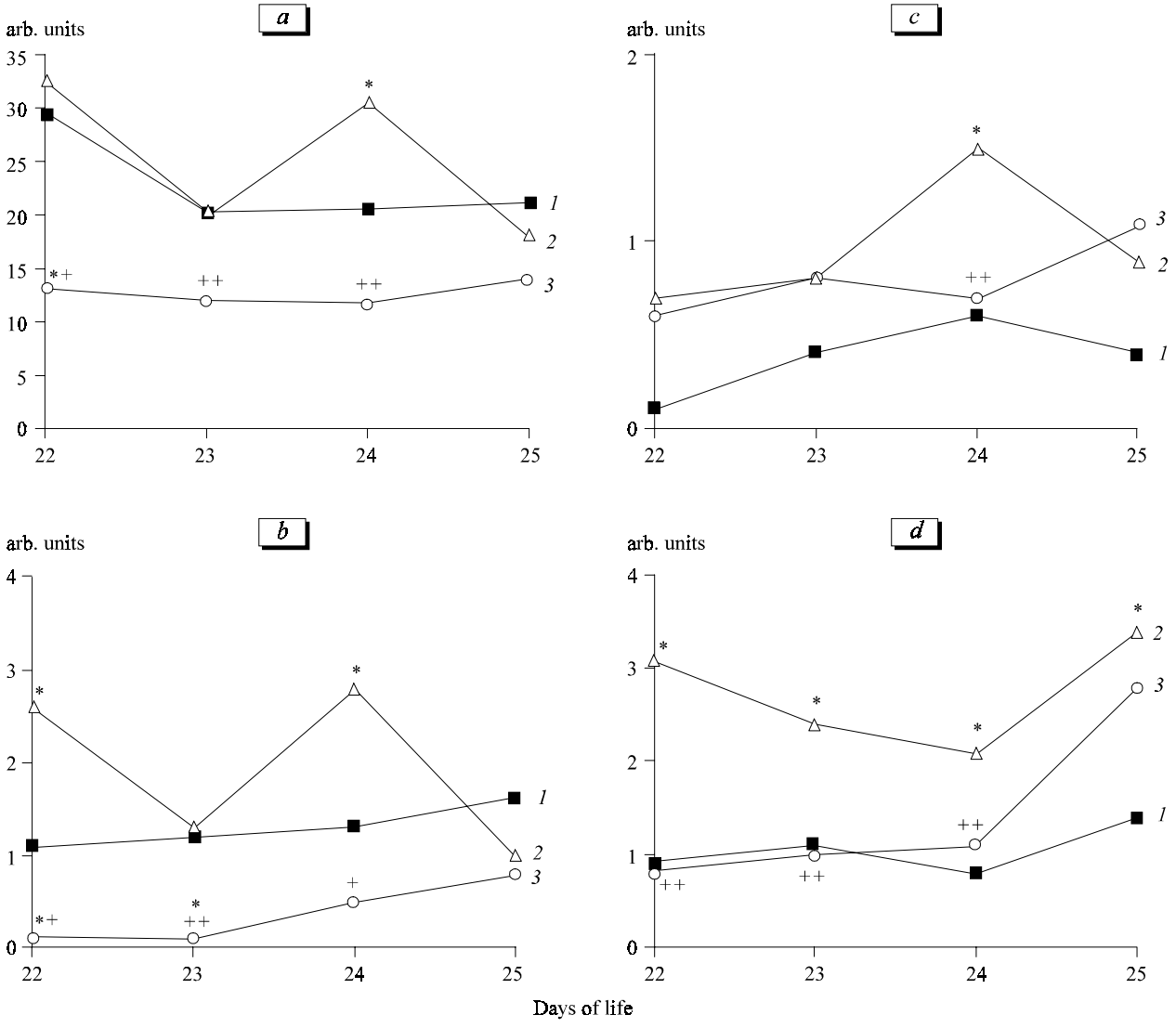

Fig. 3. Changes in behavioral characteristics of rat pups on days 22-25 of life in hole chamber.

an effect of its own on hypoxic embryos, which is to be investigated in detail.

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