Synthetic Derivative of Tuftsin Recovers Cognitive Functions Disturbed by Antenatal Hypoxia

T. P. Semenova, M. M. Kozlovskaya, N. I. Medvinskaya, I. I. Kozlovskii

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 125, No. 3, pp. 289-292, March, 1998
Original article submitted January 21, 1997

Intraperitoneal injection of synthetic derivate of tuftsin in a dose 300 μg/kg to animals exposed to antenatal hypoxia recovers directed attention and processes of learning and normalizes emotional behavior and the balance between the activities of brain monoaminergic systems. The peptide can be used for compensation of behavioral disorders caused by antenatal hypoxia.

Key Words: *antenatal hypoxia; cognitive process; emotions; monoamines; peptides*

According to current views, disorders in the communication between the peptidergic and monoaminergic (MA) systems in the brain are the basis for a number of cognitive and psychoemotional disturbances caused by antenatal hypoxia. In connection with this, an important significance gains the investigation aimed at evaluating the role of interaction of natural peptides and its synthetic derivates with MA-systems in the mechanisms of accurate and adequate correction of psychopatological reactions [2,3,13]. Previously, we showed that injection of tuftsin or its synthetic derivative Thr-Lys-Pro-Arg-Gly-Pro to animals improves cognitive functions [5,8], produces anxiolytic effect and normalizes the imbalance caused by neonatal injection of 5,7-dihydroxytryptamine between serotonin (5-HT) and norepinephrine (NE) levels in the brain [9].

During ontogenesisthe MA-systems are formed among the first mediator systems [11] that promote formation and development of integrative functions in the central nervous system [7]. Imbalance of these systems during early ontogenesis caused by prenatal hypoxia is accompanied by selective increase in the brain NE level and specific disturbances of explorative and emotional behavior [4].

Institute of Cell Biophysics, Russian Academy of Sciences, Pushchino; Institute of Pharmacology, Russian Academy of Medical Sciences, Moscow

Investigation of neurochemical mechanisms of behavior in such animals is not only of practical but also of theoretical significance because it opens new prospects in the search for new methods of correcting behavioral disorders with psychopharmacological preparations which modify the monoamine metabolism. Our aim was to explore the possibility of correcting cognitive processes, emotional behavior, and monoamine levels in different parts of the brain in animals exposed to hypoxia using the heptapeptide as a tool.

MATERIALS AND METHODS

Sixty-nine male Wistar rats were used. Some animals were subjected to a short-term antenatal hypoxia. To this end, 14-16-days pregnant rats were conditioned for 2 h in an altitude chamber at a height of 8000 m (220 mm Hg). The animals were kept on 12-h light/dark cycle and fed a standart granular fodder [4]. On postnatal day 120, the rats were tested for directed attention to different modality stimuli by Marshall's method in our modification [8] and for the learning ability and reaction of discrimination of emotionally different influences [8,9]. The animals were divided into three groups: control (n=28), exposed to hypoxia rats (n=26), and exposed to hypoxia after heptapeptide administration (n=15) in a dose of 300 μg/kg 15 min before experiment. The drug was synthesized at the Institute of Molecular

0007-4888/98/0003-0255$20.00

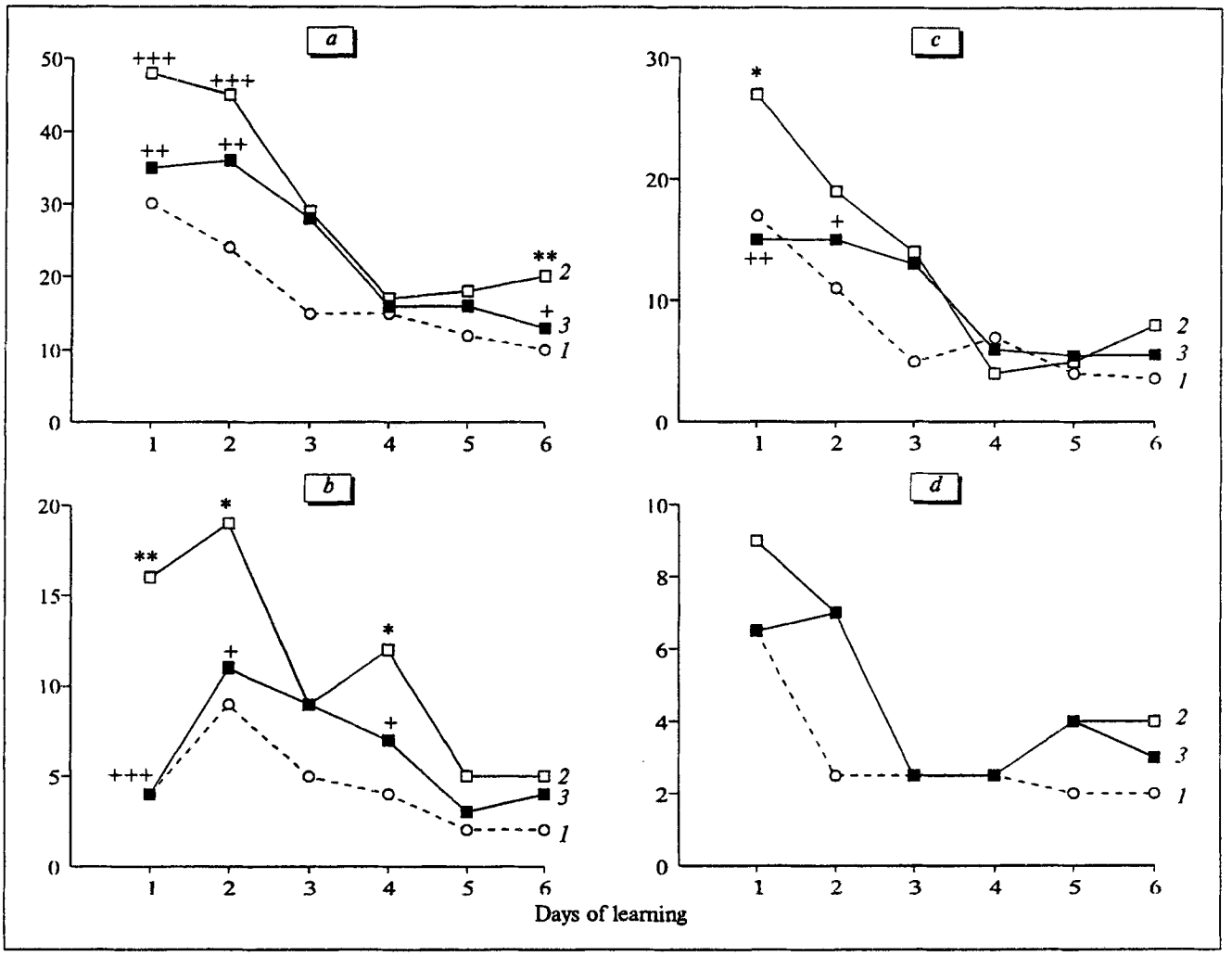

Fig. 1. Dynamics of alteration of total time of conditioned reflex reaction (*a*) and its components: time of departure from start chamber (*b*), time of moving through central (*c*) and targed section (*d*) in intact (*1*), hypoxia-affected (*2*), and hypoxia-affected heptapeptide-treated (*3*) rats. Ordinate: time, sec; *$p<0.01$, **$p<0.001$ compared with control; +$p<0.05$, ++$p<0.01$, +++$p<0.001$ compared with hypoxia-affected rats.

Genetics (Russian Academy of Sciences) by Dr. V. N. Nazarenko.

The levels of 5-HT, NE, and dopamine in the frontal neocortex area, hypothalamus and caudal portion of brainstem were measured by the spectrofluorometric method [12]. The data were analyzed using Student's *t* test.

RESULTS

The dynamic of learning in hypoxia-affected rats differed considerably in comparison with the control. The differences were the greatest for the initiac stage (Fig. 1). On the first day, the total time of conditioned reaction was 28.4 sec and 47.3 sec in control and hypoxia-affected rats, respectively ($p<0.001$). A more detailed analysis of separate conditioned reflectory reaction components showed the effect of hypoxia on the time of departure from the start chamber (Fig. 1, *b*) and the time of moving through central section of cage (Fig. 1, *c*). According to the theory of functional systems, such reactions are the

Table 1. Effect of Heptapeptide on Reaction to Various Modality Sensory Stimuli in Hypoxia-Affected Rats (*M±m*)

Group	Number of rats	Attention to sensory stimuli	
		somato-sensory	visual
Control	28	1.62±0.38	1.67±0.39
Hypoxia	26	0.75±0.26*	0.56±0.19**
Hypoxia+heptapeptide	15	1.49±0.19+	1.68±0.24+

Note. *$p<0.01$; **$p<0.001$ compared with control; +$p<0.01$ compared with hypoxia-affected rats.

consequence of disturbances in the processes of afferent synthesis and decision making [1].

Injection of the heptapeptide 15 min before learning facilitated the formation of purposeful conditioned reaction even on the first day (Fig. 1, *a*). Positive effect was more pronounced during two stages of conditioned reaction: time of departure from the start section in heptapeptide-treated rats decreased 3.5-fold ($p<0.001$) compared with hypoxia-affected rats and time of moving though central section of the cage decreased 1.7-fold ($p<0.001$).

Pronounced sensory attention disorders to somatosensory and visual stimuli were observed in hypoxia-affected rats (Table 1). Injection of heptapeptide led to recovery of these disorders.

In rats affected by antenatal hypoxia, the process of discrimination between emotionally different influences was disturbed. These animals responded to emotionally positive influences 4.6 times stronger ($p<0.001$), while the reaction to emotionally negative stimuli was weaker (Fig. 2). After administration of the heptapeptide, the discrimination coefficients of emotionally different influences increased almost to the control level (Fig. 2).

Our findings suggest that the heptapeptide normalizes cognitive processes and recovers the reaction of discrimination of emotionally different influences in rats exposed to hypoxia.

Analysis of biochemical data shows significant differences in 5-HT, NE, and dopamine metabolism. In rats subjected to antenatal hypoxia we observed a decrease in NE and dopamine levels, and an increase in 5-HT level in the frontal cortex, hypothalamus, and brainstem (Fig. 3, *a*).

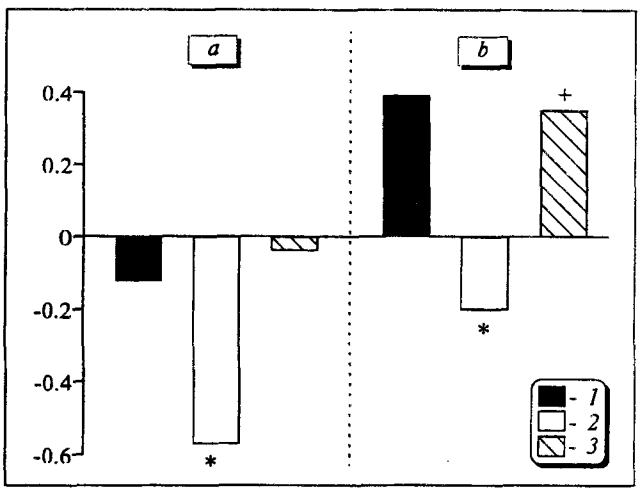

Fig. 2. Characteristic features of discrimination of emotionally positive (*a*) and emotionally negative (*b*) influences in hypoxia-affected rats after administration of heptapeptide. Ordinate: the coefficient of discrimination. *1)* intact; *2)* hypoxia-affected; *3)* heptapeptide-treated rats. $p<0.01$: *compared with control, **compared with hypoxia-affected rats.

Injection of the heptapeptide to hypoxia-affected animals elevated NE content in the cortex and brainstem (Fig. 3, *b*). Dopamine level increased in all studied structures. The level of 5-HT in hypoxia-affected rats treated by heptapeptide declined to the control in all investigated structures.

Comparison of monoamine levels in the brain of investigated animals shows that injection of heptapeptide increases the level of catecholamines (particularly of dopamine) and decreases the level of 5-HT, which are altered due to antenatal hypoxia, and recovers the ratio of these transmitters to the control.

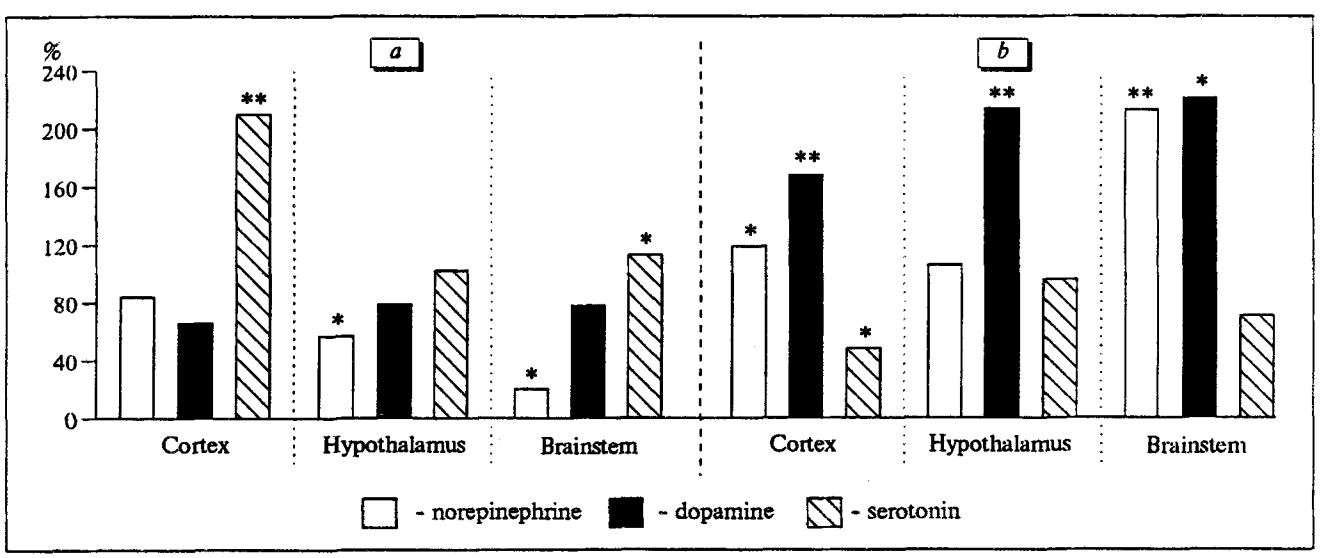

Fig. 3. Relative values of norepinephrine, dopamine, and serotonin levels in cortex, hypothalamus, and brainstem in rats exposed to antenatal hypoxia (*a*), and treated with heptapeptide (*b*). The level of monoamines in intact (*a*) and hypoxia-affected rats (*b*) was taken as 100%. *$p<0.5$, **$p<0.01$ compared with the control.

Analysis of behavior and levels of brain monoamines in hypoxia-affected rats reveals disturbances of cognitive functions, emotional behavior, and balance of activity in brain MA-systems. These changes are similar to those caused by the neurotoxin 6-hydroxydopamine in newborn animals [4,8], which modifies the ratio between the activities of 5 HT- and NEergic brain systems. These effects are similar to those observed in retarded children [6]. Our previous and present results together with the literature data suggest that the imbalance of brain MA-systems during the early stages of ontogenesis, i.e., during the crucial period of differentiation of 5-HT- and NEergic neurons, is the basis for pathologies of higher brain functions, in particular delay of mental development in children [7,10,11].

Thus, the recovery of balance in the activities in brain MA-systems and the possibility of correcting pathologies of higher brain function in hypoxia-affected animals by the heptapeptide indicates its prospectiveness for restoration of cognitive functions disordered by antenatal hypoxia.

This study was supported by the Russian Foundation for Basic Research (Grant No. 95-04-11911).

REFERENCES

1. P. K. Anokhin, *Biology and Neurophysiology of the Conditioned Reflex* [in Russian], Moscow (1968).
2. A. V. Val'dman, M. M. Kozlovskaya, *Path. Physiol.*, No. 3, 60-67 (1984).
3. N. L. Gritskevich, E. A. Koneva, *Byull. Eksp. Biol. Med.*, **116**, No. 10, 381-384 (1993).
4. E. A. Gromova, A. E. Fast, Yu. A. Katkov, *Ibid.*, **106**, No. 7, 14-17 (1988).
5. M. M. Kozlovskaya, in: *Gidazepam* [in Russian], Kiev (1992), pp. 63-75.
6. V. M. Lupandin, L. I. Lando, E. A. Gromova, et al., *Zh. Nevropatol. Psikhiatr.*, **78**, 1538-1544 (1978).
7. V. V. Raevskii, *Ontogenezis of Mediator Systems* [in Russian], Moscow (1991).
8. T. P. Sem'onova, *Mechanisms of Optimization of Learning and Memory Processes* [in Russian], Pushchino (1992).
9. S. B. Seredenin, T. P. Semyonova, M. M. Kozlovskaya, et al., *Eksp. Klin. Farmakol.*, No. 6, 3-6 (1995).
10. E. A. Gromova, A. R. Chubakov, T. P. Sem'onova, in: *Signal Molecules and Mechanisms of Animal Bihaviour*, W. Winslow, O. Vinogradova, D. Sakharov (Eds), Manchester - New York (1991), pp. 231-240.
11. I. M. Lauder, F. R. Bloom, *J. Comp. Neurol.*, **155**, 469-481 (1974).
12. M. Schlumpf, W. Liehtonsteiger, H. Laugemann, and P. G. Waser, *Biochem. Pharmacol.*, **23**, 2437-2445 (1974).
13. J. H. Woods, J. H. Katz, and G. Winger, *Pharmacol. Rev.*, **39**, 251-413 (1987).