Use of Selank to Correct Measures of Integrative Brain Activity and Biogenic Amine Levels in Adult Rats Resulting from Antenatal Hypoxia

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Translated from Rossiiskii Fiziologicheskii Zhurnal imeni I. M. Sechenova, Vol. 92, No. 11, pp. 1332–1338, November, 2006. Original article submitted April 24, 2006, revised version received July 17, 2006.

The effects of Selank, the active component of which is the heptapeptide Thr-Lys-Pro-Arg-Pro-Gly-Pro, which includes the tetrapeptide tuftsin and three natural levorotatory amino acids, on behavior and brain serotonin and noradrenaline levels were studied in adult rats subjected to antenatal hypoxia on days 14–16 of gestation. Administration of Selank (300 μg/kg, i.p.) improved sensory attention levels by factors of 2–3 ($p < 0.01$), facilitated the learning process by a factor of 1.5 ($p < 0.01$), normalized the level of investigative activity in the open field and hole board, and restored the balance of activity between the serotoninergic and noradrenergic systems of the brain. The data obtained here provide evidence that Selank can be used to compensate for the long-term negative effects of antenatal hypoxia on brain integrative activity and the activity of monoaminergic transmitter systems.

KEY WORDS: Selank, antenatal hypoxia, behavior, serotonin, noradrenaline.

It is known from clinical observations that the hypoxic state of the fetal CNS plays a significant role in disturbances in intrauterine development and is a leading factor in perinatal morbidity. The depth of lesions to CNS functioning in animals subjected to transient antenatal hypoxia is to a significant extent dependent on when it occurs. Fetal hypoxia at days 14–16 of gestation produces more profound changes in polyphosphoinositide levels in the brains of animals aged 15 and 90 days as compared with similar treatments applied on days 18–20 [14]. These animals also show profound structural damage of hippocampal and neocortical neurons developing predominantly by apoptosis [11] and inducing more profound functional disturbances. Previous studies have shown that intrauterine hypoxia applied on days 14–16 of gestation is accompanied by selective decreases in noradrenaline levels in brain structures [16] and increases in convulsive readiness in response to threshold doses of convulsive agents, with impairments in learning and memory processes [3, 8]. The sequelae of hypoxia in human fetuses can appear not only as severe impairments in neonates, requiring pharmacological correction, but also significantly later in life, as functional pathology of the CNS, including deficits in emotional behavior and impairments to cognitive processes [13, 15]. The incidence of delayed posthypoxic functional impairments to the developing brain confers great relevance to the search for prophylactic and therapeutic means, particularly pharmacological corrective agents.

Compounds of the family of biologically active peptides are accepted as having great significance in regulating various types of behavioral pathology. Peptide compounds within the large family of the immunotropic peptide tuftsin are of interest because of their wide spectrum of biological

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TABLE 1. Effects of Selank on the Time (sec) Taken to Perform the Conditioned Reflex Reaction in Adult Rats Subjected to Antenatal Hypoxia

Group	n	Study day					
		1	2	3	4	5	6
Control	6	30 ± 2	25 ± 1	16 ± 2	16 ± 1	12 ± 1	9 ± 1
Hypoxia	6	48 ± 3**	45 ± 2**	36 ± 2**	26 ± 2*	27 ± 2**	22 ± 2*
Hypoxia + Selank	6	34 ± 2++	36 ± 1++	26 ± 1+	20 ± 2	19 ± 1+	14 ± 1+

Notes (here and Tables 2–5). *n* is the number of animals in the group. Significant differences between control and hypoxia-exposed animals, Student's *t* test: $^*p < 0.05$, $^{**}p < 0.01$. Significant differences between hypoxia-exposed animals and Selank-treated animals, Student's *t* test: $^+p < 0.05$, $^{++}p < 0.01$.

TABLE 2. Effects of Selank on Discrimination of Emotionally Different Stimuli in Adult Rats Subjected to Antenatal Hypoxia

Group	n	Coefficient of discrimination, U	
		Emotionally positive stimuli	Emotionally negative stimuli
Control	6	–0.14 ± 0.03	+0.34 ± 0.06
Hypoxia	6	–0.62 ± 0.08**	–0.29 ± 0.03**
Hypoxia + Selank	6	–0.24 ± 0.03+	+0.14 ± 0.02+

activity. The founding agent of this group, the tetrapeptide tuftsin, has an activatory influence on behavior [1, 2, 19]. Studies at the Institute of Molecular Genetics, Russian Academy of Sciences, have used the endogenous tuftsin molecule as the basis for producing a new synthetic derivative – a heptapeptide containing the tetrapeptide tuftsin (Thr-Pro-Lys-Arg) and three natural levorotatory amino acids (Pro-Gly-Pro), which is the active agent in the new neurotropic agent Selank [5, 6, 12, 19]. Selank has been shown to have anxiolytic and psychostimulatory actions [5, 6, 11, 12]. Clinical observations at the State Science Research Institute of Pharmacology, Russian Academy of Medical Sciences, have supported the view that Selank has psychotropic activity consisting of a combination of anxiolytic and stimulatory actions, with no side effects [7].

The aim of the present work was to study the possibility of using Selank to correct cognitive processes, emotional behavior, and monoamine contents in various brain structures in adult animals subjected to antenatal hypoxia.

METHODS

Experiments were performed on 65 adult male Wistar rats subjected to transient antenatal hypoxia [3]. These animals were obtained by placing 14 females in barochamber in which an altitude of 8000 m (220 mmHg) was simulated for 2 h on days 14–16 of pregnancy. Behavioral measures were studied at age 120 days. Hypoxia-treated rats ($n = 18$) received i.p. Selank at a dose of 300 μg/kg (therapeutic form, Solutio Selank 0.15%, nipogin 0.1%, in 3-ml dropper flasks for intranasal use). Controls consisted of hypoxia-treated ($n = 28$) and intact ($n = 19$) rats given equivalent volumes of physiological saline.

Training was performed in a chamber of size 150 × × 16 × 23 cm divided into three sectors: start, central, and target sectors. Animals placed in the start sector acquired a conditioned reflex reaction in response to the opening of a door, the response consisting of running to the target sector and pressing a tray with the forelimbs. Training was performed over a period of six days, with five training sessions per day; the reinforcement consisted of a tablet of bread weighing 50 mg [11]. The total time taken to perform the reaction was measured (sec), along with the times taken for its individual components. Emotionally positive influences were provided using a ten-fold in the amount of food reinforcement, while emotionally negative influences were provided using a ten-fold reduction. Emotional reactions were characterized quantitatively using a coefficient of discrimination (K_d), which was the relative change in the speed of performing the reaction when the size of the reinforcement changed: $K_d = (T_2 - T_1)/T_1$, where T_1 is the time taken to perform the reaction before changing the size of the reinforcement and T_2 is the time taken for the reaction after the change [11].

Sensory attention or sensitivity to sensory stimuli was assessed by the Marshall method [18] as modified by ourselves [11]. Orientational reactions to a somatosensory stimulus were tested by applying von Frey fibers to area 18 of the animal's body with a pressure of 2 g/cm^2. Responses to visual stimuli were tested by alternately placing squares (5 × 5 cm) of contrasting colors in the visual fields of each eye at a distance of 4 cm from the head, without touching the whiskers. Responses to olfactory stimuli were tested by

TABLE 3. Effects of Selank on Sensitivity to Sensory Stimuli and Investigative Behavior in Adult Rats Subjected to Antenatal Hypoxia

Behavioral measure		Group		
		Control (n = 13)	Hypoxia (n = 23)	Hypoxia + Selank (n = 12)
Sensitivity to stimuli, U	Somatosensory	1.9 ± 0.1	0.9 ± 0.06*	1.6 ± 0.09$^+$
	Visual	1.7 ± 0.1	0.4 ± 0.04*	1.1 ± 0.09$^+$
	Olfactory	1.7 ± 0.1	0.8 ± 0.04*	1.4 ± 0.06$^+$
Open field	Number of squares	59.4 ± 1.6	24.4 ± 0.40**	51.4 ± 1.10$^+$
	Number of rearings	4.9 ± 0.1	1.2 ± 0.11**	2.8 ± 0.12$^+$
Hole board	Number of hole reactions	13.3 ± 0.8	7.2 ± 0.13*	10.2 ± 0.48$^+$
	Number of rearings	12.4 ± 0.5	5.3 ± 0.13*	9.4 ± 0.48$^+$

TABLE 4. Effects of Antenatal Hypoxia on Monoamine Contents in Brain Structures in Adult Rats (% in relation to levels in control animals)

Transmitter	Control (n = 9)	Hypoxia (n = 8)		
		Neocortex	Hypothalamus	Brainstem
Noradrenaline	100 ± 7.5	80 ± 5.6	48 ± 3.3*	18 ± 2.9**
Dopamine	100 ± 5.4	64 ± 4.1	72 ± 3.7	82 ± 5.6
Serotonin	100 ± 6.8	211 ± 11.5**	95 ± 4.8	130 ± 8.2*

sequentially presenting small pieces of fresh chocolate to each side of the animal's head, without touching the whiskers. The degree of orientation to the stimuli was evaluated in arbitrary units (U) on a four-point scale [11, 18].

Investigative activity was assessed in the rats using the open field and hole board methods. An open field of size 100 × 100 cm was divided into 100 squares and brightly illuminated with a 200-W bulb positioned 1 m from the center. The numbers of square crossings and vertical rearings in 3-min tests were counted in conditions of bright illumination. A hole board of size 40 × 40× 40 cm was divided into 16 squares, each having an opening of diameter 2.5 cm at its center. The numbers of hole responses and rearings were counted during 5-min tests.

Biochemical analyses of monoamine contents (serotonin, dopamine, noradrenaline) in the cortex, hypothalamus, and brainstem were performed as described in [4]. Data were processed statistically using Student's t test.

RESULTS

The results showed that hypoxia during the antenatal period produced impairments in the learning process in adults (Table 1). The total time taken to perform the conditioned reflex reaction in control animals on training day 1 was 30 ± 5 sec, compared with 48 ± 6 sec in animals exposed to hypoxia ($p < 0.01$). Periods were 15 ± 5 and 36 ± 4 sec (p < 0.01) on day 3 and 9 ± 2 and 22 ± 5 sec ($p < 0.05$) on day 6. More detailed analysis of the formation of the individual components of the conditioned reflex reaction revealed significant degradation in rats exposed to hypoxia in terms of the time taken to leave the start sector, demonstrating impairment of afferent synthesis and decision-taking processes. Administration of Selank to animals exposed to hypoxia facilitated the learning process. The total time taken to perform the conditioned reflex reaction in Selank-treated animals was significantly decreased, by a factor 1.5, compared with animals exposed to hypoxia (Table 1).

Table 2 presents data providing evidence that rats subjected to antenatal hypoxia had impairments in the process of discriminating emotionally different conditions. Animals exposed to hypoxia showed stronger (by a factor of 4.5) reactions to emotionally positive stimuli, while the reactions to emotionally negative stimuli were weakened ($p < 0.01$). After treatment with Selank, the coefficient of discrimination (K_d) for emotionally different situations in hypoxia-exposed animals increased, approaching values in intact animals (Table 2).

The data presented in Table 3 provide evidence of sharp impairments in animals exposed to hypoxia in the level of direct attention to sensory stimuli and measures of investigative activity. Adult rats subjected to antenatal hypoxia showed significant reductions in the level of attention to somatosensory, visual, and olfactory stimuli, by factors of 2.1, 4.2, and 2.1, respectively ($p < 0.05$). Treatment with Selank restored these values (Table 3). Animals sub-

TABLE 5. Effects of Selank on Monoamine Contents in Brain Structures of Adult Rats Subjected to Antenatal Hypoxia (% in relation to levels in hypoxia-exposed animals)

Transmitter	Hypoxia (n = 8)	Hypoxia + Selank (n = 7)		
		Neocortex	Hypothalamus	Brainstem
Noradrenaline	100 ± 5.2	134 ± 5.2**	141 ± 7.6*	168 ± 11.2**
Dopamine	100 ± 9.3	161 ± 12.4**	195 ± 12.4**	215 ± 18.0**
Serotonin	100 ± 7.8	40 ± 4.4**	92 ± 6.4	67 ± 8.4*

jected to antenatal hypoxia also showed sharp decreases in the level of investigative activity (Table 3). In the open field, the number of squares crossed by hypoxia-exposed rats decreased by a factor of 2.5 ($p < 0.01$), while the number of vertical rearings decreased by a factor of 4 ($p < 0.01$). In the hole board test, the number of rearings decreased 2.5-fold ($p < 0.05$) and the number of hole reactions decreased 2-fold ($p < 0.05$). After Selank, hypoxia-exposed animals showed significant recovery by 30–50% ($p < 0.05$) in all measures of activity in both the open field and hole board tests (Table 3).

Biochemical analysis of the brains of experimental animals showed that antenatal hypoxia produced prolonged (persisting for more than four months) changes in brain monoamine levels (Table 4). Hypoxia-exposed animals had 20% reductions in cortical noradrenaline levels, with reductions by 52% in the hypothalamus ($p < 0.05$) and 82% in the brainstem ($p < 0.01$). There were tendencies to reduced dopamine levels in all these structures. Conversely, serotonin levels increased by 111% ($p < 0.01$) in the neocortex and by 30% ($p < 0.05$) in the brainstem. Treatment of hypoxia-exposed rats with Selank produced significant increases in noradrenaline and dopamine levels in all structures. Serotonin levels in hypoxia-exposed animals treated with Selank decreased to normal in all the structures studied. Overall, comparison of monoamine levels in the brains of experimental animals showed that treatment returned the ratios of neurotransmitter levels to normal (Table 5).

DISCUSSION

Analysis of behavior and brain monoamine levels in adult animals subjected to antenatal hypoxia revealed impairments in cognitive functions, emotional behavior, and the balance of brain monoaminergic system activity, these changes persisting for at least four months after birth. These changes were similar to impairments seen after administration of 6-hydroxydopamine to neonatal animals, which also caused derangements in the normal ratios of activity of the brain serotoninergic and noradrenergic systems [16]. It is important to emphasize that in our experiments, hypoxia was produced on days 14–16 of pregnancy, i.e., during the critical period when the embryonic brain undergoes cell differentiation of monoaminergic neurons, monoamines at this stage of embryogenesis having trophic influences on the formation of innervation target structures. In animals and humans, the serotoninergic and noradrenergic nuclei in the brain are laid down at very early stages of embryogenesis and form one of the first neurotransmitter systems in ontogenesis [17, 20], facilitating the establishment and development of CNS integrative functions [9]. The differentiation of cellular monoamines in these nuclei starts 1–3 days earlier than in their target structures, i.e., the neocortex, hippocampus, and hypothalamus [17, 20].

Biochemical studies established that single doses of Selank produced significant changes in levels of noradrenaline, dopamine, serotonin, and their metabolites in the brains of Wistar rats, the durations of these changes being comparable with the durations of the action on the animals' behavior [11]. Considering the involvement of biogenic amines in controlling adaptive processes, it can be suggested that the effects of Selank on integrative brain activity in hypoxia-exposed animals are mediated by its influences on brain monoamine processes. Gromova et al. formulated the concept of the reciprocal nature of the regulatory influences of the serotoninergic and noradrenergic systems on the functional activity of the brain, whereby compensation of impairments in the behavior of animals with imbalance between the activities of the brain monoaminergic systems could be obtained either by activation of one system or by suppression of the activity of another [11, 16]. Selank, with its differently directed influences on noradrenaline and serotonin levels in the brains of hypoxia-exposed rats, thus facilitates restoration of the normal balance of monoamine systems, which is accompanied by compensation of behavioral impairments resulting from the imbalance of monoaminergic systems.

Thus, the results obtained here show that treatment with the peptide agent Selank, which has known psychotropic activity, is accompanied by normalization of impairments in the balance of activity of the monoaminergic systems, this persisting for more than four months after hypoxia. This preparation, which contains amino acids which are natural to the body [5, 6, 12], is safe for the body over a very wide range of doses, and has potential in compensating for long-term functional deficits of the CNS due to antenatal hypoxia.

Considering the widespread nature of functional CNS impairments due to exogenous and endogenous pathogenetic factors during the exclusively important period of fetal nervous system development, the search for means preventing or compensating for these impairments is an important current task for medical-biological investigations.

This study was supported by the Russian Foundation for Basic Research (Grant No. 04-04-49098).

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