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Effect of Selank on Cognitive Processes after Damage Inflicted to the Cerebral Catecholamine System during Early Ontogeny

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Effects of selank on learning, memory, and attention to sensory stimuli of different modality were studied in adult Wistar rats treated with 6-hydroxydopamine (neurotoxin selectively damaging catecholaminergic neurons and their terminals) during the first 3 days of life. Selank (300 μg/kg) restored cognitive processes disordered by chronic artificial inhibition of the cerebral catecholaminergic system.

Key Words: *selank; cognitive processes; 6-hydroxydopamine; chronic inhibition of cerebral catecholaminergic system*

The taftsin group heptapeptide (Thr-Lys-Pro-Arg-Pro-Gly-Pro), whose pharmacological characteristics were studied at V. V. Zakusov Institute of Pharmacology, and selank, a psychotropic drug created on the basis of this heptapeptide at Institute of Molecular Genetics, Russian Academy of Sciences, normalize animal behavior and restore normal proportions of norepinephrine, dopamine, and serotonin in brain structures of adult rats exposed to antenatal hypoxia [1,3,4].

We analyzed psychotropic activity of selank in Wistar rats neonatally injected with neurotoxin 6-hydroxydopamine (6-OHDA) selectively destructing catecholaminergic (CA) neurons and their terminals, which is paralleled by inhibition of cerebral integrative activity, increased anxiety, and disorders in the cognitive processes [5-7]. Chronic artificial suppression of CA activity in animals can serve as a good model for studies of the mechanisms of pharmacological effects on the course of diseases associated with hypoactivity of the brain CA systems caused by hyperproduction of endogenous 6-OHDA in brain structures.

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MATERIALS AND METHODS

The study was carried out on 52 male Wistar rats (180-200 g). Chronic artificial inhibition of CA activity was induced in rat pups by subcutaneous injections of 6-OHDA (Sigma) in a dose of 100 mg/kg during the first 3 days of life as described previously [7].

The animals were trained during one experimental session consisting of 30 training rounds (30 combinations of conditioned (door opening) and unconditioned (food) signals) in a 150×16×23 cm box divided into 3 sections: starting, central, and target. Photoelements were fixed in the walls between the sections for recording the time spent by

TABLE 1. Effect of Selank on Learning and Memory of Intact Animals and Animals Neonatally Treated with 6-OHDA

Group		Mean duration of reaction, sec	Duration of reaction, sec		
			after 24 h	after 7 days	after 1 month
Control	intact (n=8)	36±5	38±5	44±6	51±10
Experiment	selank (n=8)	24±3* (-33%)	19±5* (-50%)	26±3*** (-41%)	32±4*** (-38%)
Control	6-OHDA (n=7)	58±6^{++} [+61%]	54±8^{++} [+92%]	72±9^{++} [+75%]	75±9^{++} [+47%]
Experiment	6-OHDA+selank (n=9)	27±4** (-54%)	25±6** (-54%)	32±5** (-56%)	30±6*** (-60%)

Note. Here and in Tables 2, 3: *p<0.05, **p<0.01, ***p<0.001 compared to the control; ^{+}p<0.05, ^{++}p<0.01, ^{+++}p<0.001 compared to intact animals. Changes in the parameter in comparison with the control are shown in parentheses; in comparison with intact animals in brackets.

the animal in each section. Conditioned reflex motor alimentary reaction (CMAR) was formed in animals placed into the starting section. The reaction developed in response to a conditioned signal and included running to the target section, pressing the shelf with the fore paw, which was reinforced with food (unconditioned signal: a 50-mg bread tablet was offered). The maximum duration of the reaction during training was 90 sec. If the animal failed to perform it during this period, the round was considered erroneous. The level of motivation corresponded to 15% loss of the initial weight as a result of limited diet for 7 days.

The habit retention was tested after 24 h, 7 days, and 1 month: the animals were allowed to perform 10 running rounds no longer than 30 sec. Analysis of learning and memory was based on the CMAR duration, mean for the 5 last running rounds.

Sensory attention was evaluated as described previously [3]. The orientation reaction to somatosensory stimulation was tested by applying Frey hairs to 18 body areas at a pressure of 2 g/cm^2. Reaction to visual stimuli was evaluated by moving a contrast-colored 5×5 cm square in the visual field of each eye alternatively. Reaction to olfactory stimuli was evaluated by presenting a sweet-scented stimulus (fresh chocolate). The degree of orientation to sensory stimuli was evaluated using a 4-point scale: 0) no reaction; 1) very slight turn towards the stimulus; 2) half-turn towards the stimulus; and 3) precise orientation towards the stimulus.

Selank was injected in a dose of 300 μg/kg intraperitoneally. Selank solution (0.15%) with 0.1% nipagine in 3-ml dropper flasks for intranasal use (Selank dosage form) was used. The drug was injected in a single dose during the training session after the first 10 running rounds. The training was resumed 15 min after the injection. Control rats were injected with the same volume of saline.

Exploratory activity of rats was tested in an open field (100×100 cm) divided into 100 squares and brightly illuminated with a 200 W lamp hanging at the height of 1 m above the center of the open field. The number of crossed squares and rearing episodes were counted over 3 min.

The significance of differences between the control and experimental groups was evaluated by Student's t test.

RESULTS

Injection of selank in a dose of 300 μg/kg to rats at the early stage of learning facilitated this process (Table 1). This was seen from more rapid in comparison with the control (by 33%; p<0.05) CMAR at the final stage of learning, during performance of the final 5 rounds. Testing of the reaction rate after 24 h, 7 days, and 1 month after learning showed positive effect of selank on memory processes. CMAR

TABLE 2. Effect of Selank on Orientation to Sensory Stimuli of Different Modality in Intact Animals and Animals Neonatally Treated with 6-OHDA

Group		Orientation to stimuli, points		
		somatosensory	visual	olfactory
Control	intact (n=10)	1.5±0.1	1.6±0.1	1.8±0.2
Experiment	selank (n=10)	2.1±02* (+40%)	2.2±0.4* (+40%)	2.2±0.2 (+22%)
Control	6-OHDA (n=10)	0.7±01^{+} [-53%]	0.7±0.1^{+} [-56%]	1.1±0.2 [-39%]
Experiment	6-OHDA+selank (n=6)	1.7±0.3** (+143%)	1.8±0.3** (+157%)	1.9±0.3* (+73%)

TABLE 3. Selank Effect on Orientation and Exploratory Behavior of Intact Animals and Animals Neonatally Treated with 6-OHDA

Group		Activity in the open field	
		number of crossed squares	number of rearing episodes
Control	intact (n=13)	61±5	4.7±0.2
Experiment	selank (n=10)	97±8** (+59%)	6.6±0.4* (+40%)
Control	6-OHDA (n=19)	29±2^{+++} [-52%]	1.50±0.05^{+++} [-68%]
Experiment	6-OHDA+selank (n=10)	45±6** (+55%)	4.3±0.3** (+187%)

performance was significantly reduced in control animals after 7 and 30 days. Animals treated with selank exhibited better retention of the habit, which was seen from more rapid performance of CMAR in comparison with the respective control. Selank treatment of rats with normal CA activity resulted in improvement of attention to the sensory stimuli of different modality (Table 2). Fifteen minutes after drug injection the level of attention to somatosensory and visual stimuli increased by 40% (p<0.05) in comparison with the control. Selank stimulated orientation and exploratory behavior of rats with normal CA activity in the open field test: the number of crossed squares increased by 59% (p<0.01) and rearing episodes by 40% (p<0.05; Table 3).

Learning, memory, and attention processes were significantly disturbed in animals neonatally injected with 6-OHDA in comparison with controls (Table 1). Rats with damaged CA system needed more time for conditioning at the stage of CMAR formation (by 61%), and so they did during testing the memory trace after 24 h (by 92%), after 7 days (by 75%), and after 1 month (by 47% in comparison with control animals; Table 1). Injection of selank to animals with low CA activity improved consolidation of memory trace. This was seen from acceleration of CMAR formation and higher level of its retention shown by testing after 24 h, 7 days, and 1 month (Table 1).

Chronic suppression of CA system activity caused a significant reduction of attention to somatosensory, visual, and olfactory stimuli (by 53, 56, and 39%, respectively; p<0.05). Injection of selank to these animals produced a pronounced stimulatory effect (Table 2). Selank injection to animals neonatally treated with 6-OHDA increased attention to the somatosensory (by 143%; p<0.01), visual (by 157%; p<0.01), and olfactory stimuli (by 73%; p<0.05). The normalizing effect of selank on the level of attention in animals with imbalanced activities of the cerebral monoaminergic systems was more pronounced than in intact rats. In adult animals neonatally injected with 6-OHDA, selank treatment significantly increased horizontal (number of crossed squares) and vertical (rearing episodes) orientation and exploratory activities, which approached the values typical of intact animals (Table 3).

These data indicate that selank characterized by nootropic and psychostimulatory effects normalized learning, memory, focused attention to stimuli of different modality, and the level of exploratory activity disturbed by chronic damage to the cerebral CA system. We previously showed that injection of selank in a wide dose range, including 500 μg/kg, was inessential for the level of animal locomotor activity [1]. It is known that the CA system plays an important role in the mechanisms of brain development in humans and animals [2,5,6]. Violation of its normal functioning in the ontogeny modifies the consolidation of individual programs of adaptive behavior and delayss mental development [5,8]. Studies aimed at the development of drugs for correction of pathological conditions caused by endogenous formation of neurotoxins, selectively damaging CA neurons in brain structures are therefore an important task [6,7].

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