Neuroscience and Behavioral Physiology, Vol. 33, No. 7, 2003

The Optimizing Action of the Synthetic Peptide Selank on a Conditioned Active Avoidance Reflex in Rats

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UDC 612.821.6

Translated from Zhurnal Vysshei Nervnoi Deyatel'nosti, Vol. 52, No. 5, pp. 579–584, September–October, 2002. Original article submitted August 29, 2001, accepted October 24, 2001.

The actions of the synthetic heptapeptide preparation Selank on learning and memory processes in rats with initially low levels of learning ability were compared with those in normal rats, using a method based on acquisition of a conditioned active avoidance reflex, with repeated administration of peptide 15 min before the start of training sessions for four days. The effects of Selank (300 μg/kg) were compared with the effects of the nootrope piracetam (400 mg/kg). These experiments showed that Selank significantly activated the learning process in rats with initially poor learning ability, with effects apparent after first dose on training day 1. The effect progressively increased on repeated administration of Selank: the total number of correct solutions increased and the number of errors decreased ($p < 0.05$). The maximum optimizing activity of Selank on learning in normal rats was seen on day 3 of repeated administration and training, i.e., after formation of the initial consolidation phase. The dynamic features of the development of the activating action of Selank and piracetam were described. Comparison of the results obtained here with data on the anti-anxiety actions of Selank suggested potential for its use in optimizing mnestic functions in conditions of elevated emotional tension.

KEY WORDS: conditioned active avoidance reflex, neuropeptides, learning, memory.

Studies of the factors and features of peptide regulation of learning and memory processes are one of the most important areas in contemporary neurophysiology. Previous concepts of the important roles of neuropeptides in organizing integrative brain processes associated with learning and memory have found irrefutable support in recent years [2, 3, 10, 14, 16, 18, 19]. Adrenocorticotropic hormone and its fragments vasopressin and thyroliberin, as well as short peptides of the tuftsin family are of particular importance in regulating these processes [1, 5–7, 20–21, 23–25]. Despite the increasing number of reports on the peptide regulation of mnestic functions, the aim of optimizing and correcting learning processes using endogenous peptide bioregulators requires further deep study.

Our previous experiments demonstrated that tuftsin and a number of its derivatives affect learning and memory processes to different extents in rats with normal learning ability [3, 11, 13]. The maximum positive effects on learning in rats using a conditioned passive avoidance reflex method were obtained with the synthetic peptide preparation Selank, whose active agent is the novel heptapeptide Thr-Lys-Pro-Arg-Pro-Gly-Pro, a tuftsin derivative [8, 9]. The peptide preparation Selank was synthesized at the Institute of Molecular Genetics, Russian Academy of Sciences and has been studied at the Science Research Institute of Pharmacology, Russian Academy of Medical Sciences. The first phase of clinical trials of Selank as an anti-anxiety treatment has been completed. From the point of view of extending concepts of the peptidergic mechanisms of learning and practical value of the ability of the peptide preparation Selank to optimize learning in normal rats using a passive avoidance conditioned reflex (CR) method, it is important to study its effects on learning and memory processes not only in normal animals, but also in animals with initially reduced learning ability.

The aim of the present work was to compare the actions of the peptide preparation Selank on the learning and memory processes using an active avoidance CR in rats with functionally decreased learning ability and to compare the results with those obtained in normal animals; animals of both groups received repeated doses during training.

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0097-0549/03/3307-0639$25.00

Results were also compared with results obtained using the standard nootropic agent piracetam.

METHODS

Experiments were performed using 54 male Wistar rats weighing 200–220 g. Two groups of animals were used.

Group A (n = 30) was divided into three subgroups, each of 10 animals: 1) a control group with training in conditions of administration of physiological saline; 2) an experimental group with training combined with treatment with Selank; and 3) an experimental group with training combined with treatment with piracetam. Training to the active avoidance CR in animals of this group was performed in a Ugo Basile (Italy) shuttle box consisting of two identical sectors with an electric floor, separated by a barrier with an opening. The unconditioned electrocutaneous aversive stimulus was presented to the animal's paws after previous isolated exposure to the conditioned signal (a light and a sound, 4 sec). Stimulus parameters were: 50 Hz and 0.5–0.8 mA (depending on individual sensitivity, measured in terms of the strength of the response). Intervals between presentations of the conditioned stimuli and the electrocutaneous stimulation were 2 sec.; the maximum duration of each electrocutaneous stimulus was 4 sec; intervals between presentations of the conditioned signals were 20 sec. The active avoidance CR consisted of transfer to the neighboring sector of the chamber within 6 sec of the end of the conditioned stimulus. The active avoidance CR was developed over four days, with 25 stimulus combinations per day.

Group B (n = 24) consisted of rats subjected to preliminary testing for learning ability. Three subgroups were formed, each of which included eight animals: 1) a control group trained after administration of physiological saline; 2) an experimental group trained in association with Selank; and 3) an experimental group trained during treatment with piracetam. Group B included animals with relatively low learning ability ("poor students"). Rats with poor learning ability were identified by testing in an Automatic Reflex Conditioner apparatus (USA) using the Two-Way Activity Avoidance method. The conditioned signal was a sound (5 dB) and a light (80 W); the unconditioned signal was an electric shock (0.3 mA). Isolated exposure to the conditioned signal (light, sound) lasted 4 sec, and simultaneous exposure to the conditioned and unconditioned stimuli lasted a further 4 sec. Training was performed over five days at a rate of 30 combinations per day. Learning parameters were recorded automatically by the experimental apparatus and were expressed in arbitrary units (2550 units = 8 sec). Rats which made more than 15 inadequate or incomplete responses when tested after training day 5 were classed as poorly trained, i.e., animals with decreased learning ability. These animals were used for group B ("poor students"). Training in group B was restarted after 15 days in conditions of treatment with Selank, piracetam, and physiological saline (controls), and lasted four days.

All experimental groups receiving Selank were treated with doses of 300 μg/kg, and those receiving piracetam were given doses of 400 mg/kg, in each case for four days. Agents and physiological saline (for controls) were given i.p. in volumes of 0.2 ml. The dynamics of active avoidance CR formation were tested daily, 15 min after dosage. Habit retention was tested five days after the last session. Results were analyzed statistically using standard statistical programs for assessment of Student's t test.

RESULTS

The results of studies of the dynamics of active avoidance CR acquisition in group A (rats with normal learning ability) involved measurement of the following basic behavioral parameters characterizing the learning process: the number of avoidances and short-latency avoidance responses to electrocutaneous stimulation (less than 1 sec), the number of "incomplete" responses, and the number of intersignal responses (Table 1). The optimizing action of Selank on the rate of acquisition and the fixity of the active avoidance CR was significant on treatment days 3–4. The number of avoidance responses in the group increased 1.7–fold (4.7 compared with 2.5 in controls, $p < 0.05$). The number of short-latency avoidance responses increased 2.4–fold (10.0 compared with 4.2 in controls, $p < 0.05$). Attention is drawn to the fact that the number of intersignal responses, which reflects the degree of emotional tension of the animals during acquisition of the conditioned reflex, decreased significantly from training day 1, anticipating the other learning parameters recorded. Testing of the performance of the acquired habit five days after the last dose of the peptide Selank showed a significant level of retention of this type of conditioned reflex response: the number of short-latency avoidance responses was 1.9 times that in controls (12.0 compared with 7.2 in controls, $p < 0.05$), the decreased number of intersignal responses persisted (0.1 compared with 3.0 in controls, $p < 0.05$), as did the number of incomplete responses (7.0 compared with 12.0 in controls, $p < 0.05$). Piracetam, unlike Selank, significantly increased the number of conditioned avoidances on training day 2. However, the difference between the experimental and control groups disappeared by 3–4 days of training, after which the optimizing action of Selank was greater than that of piracetam.

Selank significantly increased the rate of learning in rats of group B, with initially reduced learning ability (Table 2). The total number of responses performed (to the conditioned and unconditioned stimuli) increased after the first dose of peptide, i.e., on training day 1 (13.2 ± 0.2

TABLE 1. The Effects of Selank on Acquisition of the Active Avoidance Conditioned Reflex

Parameter	Control				Selank (300 μg/kg)			
	Learning day							
	1	2	3	4	1	2	3	4
Number of avoidances	1.0	2.0	2.5	3.5	1.7	1.0	4.7*	6.8*
Number of short-latency avoidances	4.2	5.2	4.2	7.2	5.9	6.8	10.0*	9.8*
Number of intersignal responses	1.2	2.2	1.3	1.2	1.0	0.2*	0.2*	0.3
Incomplete responses	11.0	9.0	11.5	8.9	9.0	8.5	7.0*	6.0*

Notes. Controls received distilled water; the number of animals in each group was $n = 10$ (total number of animals = 30); *$p < 0.05$.

TABLE 2. The Effects of Selank (200 μg/kg) on Acquisition of the Active Avoidance Conditioned Reflex in Rats with Initially Decreased Learning Ability

Parameter	Substance	Observation day				
		1	2	3	4	5
Number of avoidances to conditioned signals (light, sound)	Control	0	0.4 ± 0.3	0.4 ± 0.3	0	0.5 ± 0.3
	Selank	1.0 ± 0.5	1.2 ± 0.5	1.6 ± 0.4*	5.0 ± 0.8*	9.0 ± 1.0*
	Piracetam	0.9 ± 0.4	2.0 ± 0.3	3.7 ± 0.8*	4.8 ± 0.8*	5.5 ± 1.0*
Total number of avoidance responses performed	Control	3.0 ± 2.5	4.0 ± 2.0	4.2 ± 2.0	3.2 ± 1.8	3.0 ± 2.1
	Selank	13.2 ± 2.0*	14.0 ± 1.8*	12.0 ± 2.5*	12.2 ± 2.5*	10.5 ± 1.8*
	Piracetam	17.0 ± 2.1*	15.5 ± 2.0*	18.0 ± 2.6	18.5 ± 2.6	13.2 ± 2.3
Latent period of avoidance performances in arbitrary counter units transformed into seconds (numbers in parentheses)	Control	2250 ± 125 (7.1 ± 0.4)	2500 ± 125 (7.8 ± 0.4)	2450 ± 120 (7.7 ± 0.4)	2520 ± 122 (7.9 ± 0.4)	2530 ± 115 (7.9 ± 0.4)
	Selank	2010 ± 123 (6.3 ± 0.4)	1995 ± 60 (6.3 ± 0.2)	2010 ± 130 (6.3 ± 0.4)	1520 ± 128* (4.8 ± 0.4)	1750 ± 125* (5.5 ± 0.4)
	Piracetam	2250 ± 125 (7.1 ± 0.4)	2500 ± 110 (7.8 ± 0.4)	2500 ± 60 (7.8 ± 0.2)	2510 ± 62 (7.9 ± 0.2)	2510 ± 55 (7.9 ± 0.2)
Number of inadequate responses (errors)	Control	27.0 ± 2.3	26.0 ± 3.5	27.0 ± 1.6	27.0 ± 1.5	26.5 ± 1.9
	Selank	15.0 ± 2.1	26.0 ± 2.2	16.0 ± 1.5*	12.0 ± 1.5*	12.0 ± 2.0*
	Piracetam	13.0 ± 2.5	13.0 ± 1.5	8.0 ± 2.7*	7.5 ± 1.7*	7.5 ± 2.0*

Notes. Controls received distilled water; the number of animals in each group was $n = 8$ (total number of animals = 24); *$p < 0.05$.

compared with 3.0 ± 2.5 in controls, $p < 0.05$). The total number of avoidances by the end of the training period (on days 4–5) was also greater than that in controls (Table 2). A tendency to an increase in the number of avoidance responses to the conditioned signal was seen from training day 1 and this continued to increase progressively, becoming significant compared with controls on training day 3. It should be noted that the latent period of performing avoidances decreased slightly, reaching significance on training day 4. The number of errors (incorrect responses), initially very high in the poor-learning rats, progressively decreased during treatment with Selank to day 4–5 (to12.0 ± 1.8, compared with 27.0 ± 1.7 in controls, $p < 0.05$). Piracetam had a similar effect. However, the number of avoidances to the conditioned signal on training day 5 in animals treated with Selank was 1.6 times greater than that in animals given piracetam.

DISCUSSION

The results obtained in experiments performed on rats with normal and functionally decreased learning abilities (“poor students”) using the active avoidance CR method showed that the peptide compound Selank, at a dose of 300 μg/kg, significantly improved learning parameters in both groups of animals. In general, the nature of the effects of Selank on measures of acquisition of the active avoidance CR was comparable with that of the typical nootrope piracetam, though the two agents had their own features. The greatest efficacy of Selank in normal animals was seen on training day 3, i.e., at the stage at which consolidation had started and was continuing to form, and increased progressively with further peptide doses. The optimizing effect of Selank on the process of active avoidance CR formation in normal rats receiving repeated doses was characterized

not only by a general increase in the number of animals correctly performing the conditioned avoidance response, but also by an increase in the number of short-latency avoidance responses, which appears to be evidence of activation of readiness to perform the response. The significant increase in the number of correctly performed responses on training day 3–4, as well as the early decrease in the number of intersignal responses, may result not only from the direct influence of Selank on the learning process, but also from its ability to decrease the levels of anxiety and fear accompanying and hindering the process of acquiring the conditioned reflex response to the aversive stimulus [15]. Piracetam had positive influences on the active avoidance CR at earlier stages of learning. However, by day 3–4 of piracetam treatment, the levels of learning in control and experimental animals were not different, while the activating effect of Selank continued to increase throughout the learning period.

It is extremely important that the activating effect of Selank on the learning process was also seen clearly when given to rats with initially decreased learning ability. In these conditions, the activating effect of the peptide was seen (unlike the situation in rats with normal learning ability) after a single dose, i.e., on training day 1, and progressively increased during repeated doses during the whole of the training period, significantly exceeding the effect of the nootrope piracetam.

Overall, the results obtained in the present experiments revealed the functional significance of the involvement of the peptide Selank in integrative brain activity associated with the formation of the learning process in normal conditions and in animals with initially decreased learning ability. Selank was shown to have an optimizing effect both on the rate of acquisition and on the level of fixation (reinforcement) of the active avoidance conditioned reflex. It would appear that training in conditions of Selank treatment leads to increases in the stability of the system of interneuronal connections formed during the training period. Many authors believe that this latter improves memory properties such as consolidation and "recollection" or, in the case of conditioned reflex behavior, performance of the behavioral act, in our experiments directed to avoiding the aversive stimulus [7, 12, 22, 23]. This is in agreement with data presented by T. P. Semenova showing that single doses of the native heptapeptide improved food-related conditioned reflex behavior in rats when given at the consolidation stage. Considering published data on the role of the hypothalamus and hypothalamic-hippocampal interactions in forming conditioned reflex behavior and memory processes, as well as our own data on the marked effects of Selank on behavioral responses induced by direct stimulation of the ventromedial hypothalamus, leads to the suggestion that that Selank probably has a functional involvement in forming normal and activating decreased learning and memory processes at this neurotransmitter level [4, 17, 20]. The property of the peptide preparation Selank presented here, i.e., its ability to optimize the learning process in rats using an active avoidance CR method, like the passive avoidance CR method [9], along with its anti-anxiety action [7, 14], reveals potential for its possible use in optimizing mnestic functions in conditions of increased emotional tension. The experimental results obtained here show that Selank has the property of improving learning ability in animals with initially decreased learning ability. The optimizing activity of Selank of learning measures in animals using the method employed here was greater than that of piracetam.

CONCLUSIONS

1. Repeated doses (4 days) of the synthetic peptide compound presented as the preparation Selank significantly facilitated the acquisition and reproduction of an active avoidance conditioned reflex in rats with normal and initially low learning abilities.

2. The optimizing action of Selank on the learning process in the active avoidance conditioned reflex method was most marked when given to animals which had passed the initial stage of forming the conditioned avoidance habit, at the initial stages of consolidation.

3. There were differences in the optimizing actions of the synthetic peptide preparation Selank and the nootrope piracetam on the process of acquiring the active avoidance conditioned reflex: a) Selank was active at a dose of 300 μg/kg, while the effective dose of piracetam was three orders of magnitude larger (400 mg/kg); b) the effect of Selank was apparent at the stage at which consolidation had started and was increasing, and was greater than the effect of piracetam from training day 3–4, while the effect of piracetam was evident from training day 1.

This study was supported by the Russian Fund for Basic Research (Grant No. 99-04-48557).

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