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Effect of Peptide Geroprotectors on Navigation Learning in Rats of Different Ages and Caspase-3 Systems in Their Brain Structures

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Abstract—The effects of peptide geroprotectors, such as cortexin and pinealon, on the training of rats of different ages and the caspase-3 system in their brain structures were studied in the experimental model of acute hypoxic hypoxy.[1] Regional changes were identified in the activity and the content of caspase-3 in the cerebral cortex and brainstem structures of young and old rats under the influence of peptide preparations. It is suggested that the functional state of the caspase-3 system in the brain is one of the reasons for the ability of animals to learn. Compared with cortexin, pinealon has a greater positive effect on the learning of both young and old animals in the Morris labyrinth.

Keywords: peptide geroprotectors, rats of different ages, training, caspase-3

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INTRODUCTION

It is known that signs of neurodegeneration, including violation of learning and memory, appear during aging [13, 23]. The age-related changes associated with programmed cell death and plasticity processes are the neurobiological basis of neurodegeneration. Mechanisms of functional and structural plasticity, including apoptosis, underlie the adaptation processes and the compensation of nerve functions disturbed because of extreme or pathological conditions [4].

The plasticity processes in the nervous tissue are provided by several mechanisms. First of all is the expression of the genome, which activates a number of cascade processes, e.g., the system of proteolytic enzymes, including caspases, the main function of which is to run apoptotic processes. In particular, changes in the activity of the caspase family (which are traditionally considered as a factor in cell death) can be observed in a normally functioning neuron, which is associated with plasticity processes [2, 20, 26].

Since not only apoptotic functions of caspase-3 are currently identified [11, 18], it is urgent to study in the brain pathology the state of the proteolytic systems in the processes of skill consolidation. Taking into account that the adaptive capacity of the nervous system reduces with age, especially in pathological conditions, the study of these processes is promising in animals of different age groups. Some preparations are developed to correct these changes. However, they do not solve all the problems associated with cognitive disabilities in elderly and geriatric patients [1, 7].

The goal of this work was a comparative analysis of the effect of peptide geroprotectors, such as cortexin and pinealon, on the latent learning and functional state of caspase-3 in the brain of rats of different ages.

MATERIALS AND METHODS

All animal experiments were carried out in accordance with the ethical principles and regulations recommended by the European Science Foundation (ESF) and the Declaration on the humane treatment of animals. The experiment was carried out on albino male rats aged 3–4 months with a body mass of 180–250 g (young rats, $n = 54$) and aged 18 months with a body mass of 350–450 g (old rats, $n = 54$). Rats of each age group were divided into six groups, one of which (1) was the control. The second group (2) was subjected to acute hypoxic hypoxia (AHH), the third group (3) was treated with cortexin at a dose of 1 mg/kg, the fourth group (4) was treated with cortexin at a dose of 1 mg/kg before AHH modelling, the fifth group (5) was treated with pinealon at a dose of 10 μg/kg, and the sixth group (6) was treated with pinealon at a dose of 10 μg/kg before AHH modelling.

Acute hypoxic hypoxia, which causes cerebral ischemia as a model of age-related pathology, was created by placing the rats in a supply and exhaust pres-

[1] This term means that the animal's state is directly caused by hypoxia when an animal is placed in an oxygen deficient chamber. Another type of hypoxia, i.e., hypobaric hypoxia, is the state when an animal is placed in a chamber with reduced pressure, which also causes hypoxia. The ischemic type of hypoxia occurs, for example, after carotid artery clamping, etc.

Table 1. Effects of acute hypoxic hypoxia (AHH) and peptide geroprotectors on cognitive functions in young rats in Morris water labyrinth (c, $M \pm m$)

Group	Sum of four attempts		
	first day of testing	second day of testing	third day of testing
First, control ($n = 72$)	28.53 ± 1.09	11.63 ± 0.27	8.49 ± 0.31
Second, AHH ($n = 28$)	$37.52 \pm 1.49^{2)*}$	$24.54 \pm 0.10^{1)*}$	$17.83 \pm 0.65^{1)*}$
Third, cortexin ($n = 32$)	$26.63 \pm 0.93^{3)*}$	$18.49 \pm 0.67^{1)*, 2)*, 3)*}$	$12.26 \pm 0.40^{1)*, 2)*, 3)*}$
Fourth, cortexin + AHH ($n = 60$)	31.83 ± 1.26	$25.38 \pm 0.18^{1)*, 2)*}$	$11.52 \pm 0.37^{1)*, 2)*, 3)*}$
Fifth, pinealon ($n = 32$)	$23.51 \pm 1.08^{3)*}$	$9.33 \pm 0.42^{1)*, 2)*, 3)*}$	$5.81 \pm 0.21^{1)*, 2)*, 3)*}$
Pinealon + AHH ($n = 80$)	$26.39 \pm 1.24^{3)*}$	$28.07 \pm 1.75^{2)*}$	$9.22 \pm 0.46^{1)*, 3)*}$

[1)]* Significant ($p < 0.05$) decrease in time finding the hidden platform relative to the first day of testing.
[2)]* Significant ($p < 0.05$) differences in parameters relative to the control group.
[3)]* Significant ($p < 0.05$) differences in parameters relative to the second group of rats with AHH.

Table 2. Effects of acute hypoxic hypoxia (AHH) and peptide geroprotectors on cognitive functions in old rats in Morris water labyrinth (c, $M \pm m$)

Group	Sum of four attempts		
	first day of testing	second day of testing	third day of testing
First, control ($n = 32$)	34.78 ± 1.12	$5.34 \pm 0.22^{1)*}$	$9.16 \pm 0.42^{1)*}$
Second, AHH ($n = 32$)	$43.03 \pm 2.05^{2)*}$	$15.03 \pm 0.67^{1)*, 2)*}$	$16.67 \pm 0.74^{1)*, 2)*}$
Third, cortexin ($n = 32$)	$33.11 \pm 0.97^{3)*}$	$21.44 \pm 0.94^{1)*, 2)*, 3)*}$	$16.01 \pm 0.76^{1)*, 2)*}$
Fourth, cortexin + AHH ($n = 32$)	$53.33 \pm 2.14^{2)*, 3)*}$	$14.42 \pm 0.64^{1)*, 2)*}$	$10.58 \pm 0.47^{1)*, 3)*}$
Fifth, pinealon ($n = 32$)	$47.42 \pm 2.32^{2)*}$	$23.91 \pm 1.10^{1)*, 2)*, 3)*}$	$9.51 \pm 0.38^{1)*, 3)*}$
Pinealon + AHH ($n = 32$)	$56.78 \pm 2.41^{2)*, 3)*}$	$30.89 \pm 1.48^{1)*, 2)*, 3)*}$	$7.33 \pm 0.32^{1)*, 3)*}$

Footnotes are as in Table 1.

sure chamber at a pressure of 66.41 kPa (3500 m above sea level) for three hours.

The activity of caspase-3 in the brain was evaluated by the fluorometric method [10, 14], calculated by the difference between the rates of the accumulation of free 7-amino-4-methylcumarine in the samples (Sigma, United States), and expressed in pmol/min per 1 mg of the protein. The caspase-3 content was evaluated by ELISA using the Biosource test system (Belgium).

Statistical treatment of the results was performed using the Statistica for Windows 6.5 package program.

RESULTS AND DISCUSSION

While studying the influence of AHH on the latent training of young rats, it was observed that the time needed to find the hidden platform decreased by the third day of testing. Over the entire testing period, the search time for the hidden platform was higher for animals with AHH as compared with the control group (Table 1).

The administration of cortexin and pinealon (groups 3 and 5) facilitated a decrease in the time spent finding the hidden platform by the third day of the experiment, with pinealon showing a higher efficiency than cortexin. On the third day of testing in rats treated with pinealon, an indicator of learning in the Morris labyrinth significantly differed for the control group, especially for rats of the third group treated with cortexin ($p < 0.05$).

In the AHH model, the administration of the peptide geroprotectors reduced the influence of stress on the animals' learning, which was especially pronounced when administrating pinealon. On the first and the third days of the experiment, rats of group 6 treated with pinealon before AHH modelling spent the same amount of time finding the hidden platform as rats of the control group (group 1).

The search time for the hidden platform in old rats with AHH was significantly higher than in the control group of animals (Table 2). Preliminary administration of cortexin before AHH reduced the search time in rats on the third day of testing by 36.5% ($p < 0.05$) as compared with rats of group 2 with AHH. The administration of pinealon in old rats with AHH led to a decrease in the search time on the third day of the

experiment by 56% ($p < 0.05$) as compared with animals of group 2 with AHH.

The search time for old rats from the control group was increased by 22% ($p < 0.05$) on the first day of the experiment and decreased by 54% on the second day ($p < 0.05$) as compared with young rats from group 1. By the third day of the experiment, this parameter for control rats of different ages did not differ.

At the same time, the effect of the peptide geroprotectors on the latent learning of old animals of groups 3 and 5 was similar to that for young rats after the administration of cortexin and pinealon. It should be noted that the administration of tripeptide pinealon to old rats led to an increase in the time finding the hidden platform on the first day of the experiment relative to the control group of the old animals, and this parameter did not differ on the third day. The search time for old animals treated with cortexin increased on the second and third day of the experiment by 301% ($p < 0.001$) and 75% ($p < 0.05$), respectively, relative to the control group.

Thus, we have found age-related differences in the effects of cortexin and pinealon on the learning rats in the Morris labyrinth. Presumably, this is due to the different level of metabolic processes in the brain of young and old rats. It is known that the production of free radicals normally decreases with aging against the background of the low activity of the antioxidant defense system, while in pathological states, the prooxidant status increases, and the antioxidant defense system is exhausted [19]. Under the normal physiological state, peptide preparations provide an increase in the intensification of free radical processes because of their preadaptation effect [5]. On the contrary, the administration of the peptides against a background of stress leads to a decrease in the enhancement of free radical processes because of the increase in the antioxidant status in a body [3]. The effects of a number of peptides on replication of the genome [9] and proteolytic activity in the brain [21] were also found. These facts suggest that peptides influence a change in the activity of replication processes and the level of proteolytic processes in order to change intra- and intercellular signaling processes under stress toward the most favorable outcome for the entire cell population. The change in the activity of proteolytic enzymes (in particular, caspase-3 and calpains) and in the neuronal cytoskeleton under the action of peptides in the norm and pathology [6] can be a consequence of the adaptive rearrangements in networks of neurons responsible for the adaptation to the new conditions.

Many cytoskeletal proteins (laminin, G-actin, fodrin, presenilin), DNA repair enzymes, cell cycle regulators (PARP, pRb), and protein kinases (MEKK 1, FAK, PAK 2) are known to be substrates for caspase-3 (so-called "death substrates") [25]. When administrating cortexin and pinealon to young rats (groups 3 and 5), we observed an increase in the activity of caspase-3 in the stem structures, and the content of the active caspase-3 in the brain structures increased only under the influence of tripeptide pinealon (Table 3). The administration of cortexin led to a decrease in the content of caspase-3 in the brainstem structures as compared to the control group of young rats. This allows one to assume that the effect of cortexin leads to a decrease in the synthesis of caspase-3 in the stem structures of young animals against a background of favorable conditions for the enhanced activity of the corresponding enzyme [8]. Cortexin probably influences the RGD sequence (arginine–glycine–aspartate) and, thereby, changes the conformation of the proenzyme molecule, which cannot provide the protease activity [16]. Cortexin can also ensure a certain range of the pH values, where caspase-3 has the highest activity. The maximum activity of caspase-3 is at close to neutral pH values [12]; at the same time, this protease shows a certain activity at acidic pH [22, 27].

Table 3. Activity and content of active caspase-3 in brain of young rats in the model of acute hypoxic hypoxia (AHH), $M \pm m$

Group	Cerebral cortex	Stem structures
Activity of caspase-3		
First, control	3.95 ± 0.31	2.85 ± 0.16
Second, AHH	4.31 ± 0.29*	4.52 ± 0.24*
Third, cortexin	4.76 ± 0.24	4.59 ± 0.21*
Fourth, cortexin + AHH	4.02 ± 0.15	3.28 ± 0.13
Fifth, pinealon	3.79 ± 0.13	3.68 ± 0.14*
Pinealon + AHH	3.22 ± 0.16	2.58 ± 0.11
Content of caspase-3		
First, control	10.10 ± 0.42	12.03 ± 0.46
Second, AHH	11.75 ± 0.51	12.04 ± 0.56
Third, cortexin	12.28 ± 0.57	8.47 ± 0.32*
Fourth, cortexin + AHH	11.75 ± 0.42	12.11 ± 0.59
Fifth, pinealon	13.45 ± 0.61*	16.73 ± 0.72*
Pinealon + AHH	12.04 ± 0.54	15.51 ± 0.69

* Significant differences in parameters relative to control group at $p < 0.05$.

The activity of caspase-3 significantly increases after AHH in the stem structures of young rats, while the content of this protease in the brain does not change in comparison with the control group. This can be due to the ability of caspases for rapid activation in a large amount in the response to inducing signals [17], which is probably observed in AHH. The activity of caspase-3 increases in ischemia/hypoxia conditions as a result of autoregulatory processes in geometric progression, which leads to cell death [15, 24]. This can probably explain why destructive changes in the brain structures of young and old rats after AHH lead to a decrease in their learning ability in the Morris lab-

Table 4. Activity and content of caspase-3 (ng/g of tissue) in brain structures of old rats under acute hypoxic hypoxy (AHH), $M \pm m$

Group	Cerebral cortex	Stem structures
Activity of caspase-3		
First, control	0.82 ± 0.04	1.03 ± 0.14
Second, AHH	1.89 ± 0.06*	1.87 ± 0.31*
Third, cortexin	0.63 ± 0.08*	1.16 ± 0.21
Fourth, cortexin + AHH	0.92 ± 0.07	1.04 ± 0.16
Fifth, pinealon	1.18 ± 0.12*	1.31 ± 0.13*
Pinealon + AHH	0.99 ± 0.11	1.42 ± 0.38*
Content of caspase-3		
First, control	9.79 ± 0.39	11.86 ± 0.42
Second, AHH	11.61 ± 0.46	11.47 ± 0.40
Third, cortexin	13.54 ± 0.54*	15.42 ± 0.61*
Fourth, cortexin + AHH	11.78 ± 0.42	13.21 ± 0.55
Fifth, pinealon	12.63 ± 0.61	13.64 ± 0.63
Pinealon + AHH	12.37 ± 0.58	13.22 ± 0.50

* Significant differences in parameters relative to control group at $p < 0.05$.

yrinth. At the same time, the activity and content of active caspase-3 in young rats did not differ from the control level after the administration of cortexin and pinealon before modelling AHH.

In old rats with AHH, the activity of caspase-3 in the cerebral cortex increased by 131% ($p < 0.001$) and in the stem structures, by 82% ($p < 0.01$), while the content of the active caspase-3 did not change relative to the control group (Table 4).

The administration of cortexin to old animals resulted in a pronounced decrease in the activity of caspase-3 in the cerebral cortex and a simultaneous increase in the content of this enzyme in the brain structures. The administration of pinealon led to the activation of this protease in the cerebral cortex and brainstem structures of old animals by 34% ($p < 0.05$) and 27% ($p < 0.05$), respectively, while the content of the active caspase-3 in the brain was the same as in the control group. These features of the effects of cortexin and pinealon on the caspase-3 system may underlie their action on latent learning.

When cortexin and pinealon were administrated before AHH, the activity of caspase-3 in the cerebral cortex of old rats corresponded to that in the control group. For example, the activity of caspase-3 in the stem structures of rats treated with pinealon before AHH increased by 38% ($p < 0.05$) relative to the first group of old rats.

CONCLUSIONS

Thus, the identified age-related effects of cortexin and pinealon on the latent learning of young and old rats can be explained by the change in the protease activity in the brain structures of these animals. Presumably, cortexin affects the caspase-3 system only in the stem structures of young rats (blocks its synthesis or the formation of active caspase-3 from procaspase-3 and increases the activity of the enzyme), while pinealon significantly increases the content of active caspase-3 in the brain against a background of the less pronounced activation of the protease in the stem structures as compared to cortexin. At the same time, cortexin leads to the accumulation of active caspase-3 and pinealon leads to an increase in its activity in the brain structures of old animals. In both cases, the enhancement of the animal learning ability comes amid changes in the functional state of caspase-3 in the cerebral cortex. Premedication with peptide geroprotectors in conditions of active hypoxic hypoxia reduces the effect of the stress factors on the proteolytic activity and the content of active caspase-3 and thereby improves the latent learning of old and young animals.

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