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Age- and Sex-Related Differences of Hypothalamic Neuroendocrine Center Response to α-Tocopherol Acetate and Thymalin Preparation

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Abstract—The effect of thymalin preparation, α-tocopherol acetate, and stress on hypothalamic centers related to the regulation of the gonadotropic function and age-specific features of such influence have been investigated in experiment. Experimental results indicate the presence of the reaction of neurons of the rostral preoptic area and arcuate nucleus for the experimental effects. The age-specific feature of the response of neurons of the rostral preoptic area and arcuate nucleus of the hypothalamus of white rats to α-tocopherol acetate injection is a reduction in the degree of response. Thymalin reduces the threshold of sensitivity of arcuate center neurons to the action of stress.

Keywords: age, preoptic area, arcuate nucleus, α-tocopherol acetate, thymalin

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INTRODUCTION

Reproductive senescence occurs in the course of aging. Experimental works on rodents show that this is not connected with gonad depletion or hypophysis sensitivity loss to gonadotropin-releasing hormone (GnRH). With advancing age, there is a change in the sensitivity of the feedback mechanism between sex hormones and neurons producing GnRH changes [22]. The neurons in rats are in the rostral preoptic area (RPA) of the hypothalamus [20]. The majority of investigations on rats have not revealed the difference in the number of GnRH cells in young and old animals [23]; however, age-related changes occur in the neuron ultrastructure and in protein biosynthesis [24].

The GnRH of neurons binds both the receptors of the sex steroids and other membrane receptors by means of neurotransmitters; some of them are produced by the neurons of the arcuate center (arcuate nucleus, AN) of the hypothalamus.

Axon terminals of GnRH neurons border on portal capillaries of the third ventricle area. With advancing age, the number of immune reactive GnRH axons in the median eminence is reduced [25]. The regulation of the GnRH neuron terminals by estrogens can occur at the level of the tanycytes of the third ventricle [27].

As the investigation have shown [13], additional injection of α-tocopherol acetate (TP) into rats alters the morphometric parameters of the hypothalamic centers affecting the functioning of the reproductive system; however, TP participation in the functioning of neurosecretory cells has not been finally ascertained. It is supposed that the corresponding receptors of these cells can be sensitive not only to steroids but to TP as well. The hypothalamic center of AN regulates a lot of an organism's functions including glucocorticoid homeostasis; therefore, it is necessary to study the cell function of this nucleus with the neurons of RPA.

Experimental studies have revealed the ability of preparations of the pineal body and the thymus to increase the lifetime of mice and rats [9]. The mechanism of geroprotective action of epiphysis peptide preparations is attributed not only to the decrease in intensity of peroxidation in tissues through the influence on melatonin production, but also to the retrieval of the hypothalamic sensitivity threshold to steroid hormones, deceleration of the age-related decrease in immune reactions, and the increase in hormone production by the thymus [2].

Preparations of the pineal body can alter the functional activity of the hypothalamic centers connected with reproductive function [1]. Similar alterations for preparations of the thymus have not yet been described.

This investigation studied age-related special features of the morphofunctional condition of neurons of RPA, the arcuate center of the hypothalamus of white rats of different age and gender in a normal state in the case of injection of thymalin preparation and TP, and under action of acute stress.

MATERIALS AND METHODS

The experiments were performed in winter using young and old sexually mature white rats of the Wistar line; young 4-month-old animals had an average body weight of males and females of 250 and 220 g, respec-

Table 1. Effect of thymalin, α-tocopherol acetate and stress on the change in area of nuclei of RPA neurons of the hypothalamus of young and old rats

Group	Gender	Young rats		Old rats	
		nuclei S, μm^2	changes, %	nuclei S, μm^2	changes, %
Control	Females	94.3 ± 3.60	No	96.2 ± 2.70	No
	Males	102.8 ± 3.80	No	100.4 ± 3.62	No
TP	Females	64.4 ± 1.84*3)	32	81.6 ± 2.44*2)	15
	Males	64.7 ± 2.39*3)	37	82.3 ± 2.86*2)	18
Stress	Females	89.1 ± 2.98	No	95.4 ± 3.48	No
	Males	108.7 ± 4.42	No	98.6 ± 3.24	No
TP + stress	Females	65.2 ± 2.53*3)	31	80.6 ± 2.62*2)	16
	Males	65.51 ± 1.96*3)	36	83.2 ± 2.74*2)	17
TM	Females	110.6 ± 3.32*2)	+17	98.3 ± 2.64	No
	Males	118.2 ± 3.78*2)	+15	101.9 ± 3.46	No
TM + stress	Females	111.3 ± 2.66*2)	+18	97.2 ± 2.56	No
	Males	114.1 ± 3.28*1)	+11	102.2 ± 3.12	No

Here and in Table 2: confidence of difference with the control group is *1) $p \le 0.05$; *2) $p \le 0.01$; *3) $p \le 0.001$.

tively, and old 20-month-old animals had an average body weight of males and females of 350 and 280 g, respectively.

Males and females were kept separately in standard conditions in a vivarium at 22°C. In the course of the experiment and just before its end, vaginal smears were studied to determine the estrous cycle phase. The investigations of females were performed on the phases of diestrus and metestrus. The experimental animals were divided into 12 groups, 6 variants separately for males and females (10 animals per group).

The first group was the control without any treatment. Animals in the second group (TP) were administered per os 10% solution of α-tocopherol acetate in a dose of 1 mg/100 g of animal mass for 14 days (before decapitation) once daily in the morning. The rats of the third group underwent immobilization stress (stress); the last 5 days before the end of the experiments the animals were placed into a plastic box in order of body size restricting free movements but not preventing air intake daily at 11 a.m. for 1 h. The fourth group underwent 5-day-long stress in combination the with 2-week TP administration (TP + stress). Animals in the fifth group were injected by thymalin intraperitoneally in a dose of 1.7 mg/100 g of animal mass for 5 days. The injections were stopped 2 days before the end of the experiments. The dose was calculated on the basis of experimental works on mice [10]. The animals in the sixth group underwent 5-day-long stress in combination with thymalin injection (as did the animals of the fifth group)—TP + stress.

The animals were decapitated in accordance with the guidelines for the use of laboratory animals under intraperitoneal chloral hydrate narcosis (2.5% solution, 1 ml/100 g of the animal's body weight).

The brain was fixed using the Karnua method; celloidin–paraffin sections were colored by 0.1% water solution of cresyl violet [4]. Morphometry of nuclei and nucleoli of the hypothalamic centers was performed using a MOV-1 15× eyepiece micrometer with a 40× lens. The nucleus square (S) in μm^2 was calculated from the formula $S = \pi/4d_1d_2$, where d_1 and d_2 were the mutually perpendicular diameters of the nucleus. The diameter of nuclei was measured in the plane of the optical section passing through the nucleolus. The number of analyzed cells in the control and in each experiment was from 40 to 50.

A 10% oil solution of α-tocopherol acetate (produced by the Pokrov biopharmaceutical factory) and thymalin preparation, which is a complex of polypeptide fractions obtained from cattle thymus (produced by Samson-Med OOO, St. Petersburg, Russia) were used in the experimental work. α-Tocopherol acetate was injected at a dose of 1 mg/100 g of animal mass. The dose was selected according to the data on optimal action of the vitamin as an antioxidant in a once daily injection and did not exceed 5 mg per animal with the average mass at 200 g [26].

The experimental data were analyzed using the alternative analysis and Student t-test. The statistical analysis was performed using licensed standard software.

RESULTS AND DISCUSSION

Morphometric analysis of hypothalamic RPA nuclei of young animals (Table 1) revealed the absence of sex-related differences in that feature ($p > 0.05$). It was ascertained that the area of nuclei in rat males

Table 2. Effect of thymalin, α-tocopherol acetate and stress on the change in area of nuclei of AN neurons of the hypothalamus of young and old rats

Variants	Gender	Young rats		Old rats	
		nuclei S, μm^2	changes, %	nuclei S, μm^2	changes, %
Control group	Females	50.7 ± 0.76	No	51.4 ± 1.35	No
	Males	52.6 ± 1.51	No	50.8 ± 1.42	No
TP	Females	37.9 ± 1.51*3)	25	49.3 ± 1.25	No
	Males	44.6 ± 1.76*2)	15	45.7 ± 1.64*1)	10
Stress	Females	40.9 ± 1.82*3)	19	43.2 ± 1.71*3)	16
	Males	45.3 ± 1.80*2)	14	42.7 ± 1.81*3)	16
TP + stress	Females	38.2 ± 1.31*3)	25	43.7 ± 1.38*2)	15
	Males	46.2 ± 1.84*2)	12	43.5 ± 1.45*2)	14
TM	Females	58.3 ± 1.68*2)	+15	56.5 ± 1.68*1)	+10
	Males	61.0 ± 1.24*2)	+16	56.9 ± 1.76*2)	+12
TM + stress	Females	41.5 ± 1.60*3)	18	41.1 ± 1.44*3)	20
	Males	43.1 ± 1.46*2)	16	40.9 ± 1.7*3)	19

and females that were administered TP significantly decreased, from 37 and 32%, respectively ($p < 0.001$).

Under the action of experimental stress, the morphometric parameters studied did not change significantly ($p > 0.05$).

The TP injection, combined with stress, caused a decrease in neuron nuclei area in females for 31% and in males for 36% ($p < 0.001$). The portion of the revealed changes is close to the isolated action of TP.

Under the action of thymalin, the area of RPA neuron nuclei increased 15% in young females and 16% in males ($p < 0.01$). In case of combined action of thymalin and stress, the increase in nuclei size was close to the action of thymalin alone ($p < 0.01$ for females and $p < 0.05$ for males).

The area of neuron nuclei in the control group of old animals was close to the value of the young ones ($p > 0.05$); no sex-related differences were found.

The TP injection resulted in a decrease in the RPA neuron nuclei area by 15% in old females and 18% in old males ($p < 0.01$)

Stress did not significantly affect the morphometric parameters of the neuron nuclei of old animals ($p > 0.05$).

In case of TP injection combined with stress, the decrease in the RPA nuclei area in old animals was connected only with the effect of TP.

Reliable alteration of the nuclei size of RPA cells in old animals was not found either in the case of isolated thymalin injection or of TM + stress.

AN of the hypothalamus. Morphometric analysis of the hypothalamic AN nuclei areas (Table 2) in young animals showed the absence of reliable sex-related differences of the studied parameter ($p > 0.05$).

The TP injection caused a significant decrease of the neuron nuclei area of 15% ($p < 0.01$) in males and 25% ($p < 0.001$) in females.

Under the action of stress, the size of the neuron nuclei decreased 19% in females and 14% in males ($p < 0.001$). The TP injection combined with stress also promoted a decrease in the area of neuron nuclei (25% in females and 12% in males, $p < 0.001$).

After a TP injection into young animals, an increase of the nuclei area of AN neurons was noted ($p < 0.01$). However, in combined action of stress and thymalin, the size of nuclei decreased in both females ($p < 0.001$) and in males ($p < 0.01$).

The area of nuclei of AN neurons in old males is 3.4% less than in young animals. However, the parameter for old females was a little higher than for young ones (1.2%); moreover, the statistically significant difference of this parameter was not revealed for either males or females ($p > 0.05$). In case of old animals, the area of neuronal nuclei decreases for 10% only in males ($p < 0.05$).

Stress caused a significant decrease in the nuclei area in males regardless of age ($p < 0.001$ in both cases), but this reaction in old females was considerably less than in young ones. In the case of combination of stress with TP injection, the nuclei square also decreased ($p < 0.01$).

Under the action of thymalin, the size of AN neurons nuclei in old animals increased less in comparison with young ones ($p < 0.05$ for females and $p < 0.001$ for males). The combined action of stress and thymalin caused a decrease in the size of AN nuclei, but in this case thymalin lowered the threshold of neuronal sensitivity to stress in old animals.

Analysis of the experimental results indicates the presence of the response of RPA and AN neurons to experimental influences. The increase in the size of nuclei of RPA neurons was found in response to thymalin action but only in young animals. TP injection

caused a decrease in nuclei of RPA cells. At the same time, immobilization stress did not affect RPA neurons. Integration of activating and inhibitory external and internal hypothalamic signals occurs in the arcuate center. AN has a receptive field for steroids and other hormones and participates in regulating energetic homeostasis [21].

The decrease in nuclei of AN neurons was noted in our experiment after stress and TP injection to young animals but without gender differences. The response of AN neuronal nuclei of old animals changed after the TP injection—a moderate decrease in nuclei size occurred only in females. The decrease in nuclei size of the AN in response to stress remained remarkable; moreover, the response of old animals was significantly lower than the response of young rats.

As the results of the study showed, a special feature of the response to the TP injection to the control animals was the significant inhibition of the functional activity of cells of neuroendocrine centers. The observed TP effect did not depend on animal age and was more pronounced in males. The reaction obtained was connected with the formation of prooxidant situations in neuroendocrine areas of the hypothalamus initially possessing the physiological optimum of pro- and antioxidant balance [3]. Depression of the antioxidant system occurs with advancing age; morphological changes caused by TP injection are less pronounced in hypothalamic neurons of old animals.

A remarkable difference in the reaction of neuroendocrine cells of RPA and AN under stress found in the experiments is probably related to significant differences in antioxidant reserves of neurons of these hypothalamic regions. The absence of the TP stress protective effect in both young and old animals is further evidence of the regulatory properties of TP, which is capable of exhibiting both antioxidant and prooxidant properties depending on the state of the oxidant–antioxidant system at the cellular level [5, 8].

As we know, the change in the size of neuroendocrine cells is interrelated to protein synthesis [12]. Neurotransmitters modulate the electrical activity of neurons of the hypothalamic nuclei and change their neurosecretory activity. The study of the TP properties at the cellular level revealed its influence on nucleus functioning and gene expression [18, 19]. However the neurophysiological effects of vitamin E need to be studied further; it particularly caused difficulties in analyzing its effect on the neuroendocrine system observed in our experiments.

The experiments also showed that thymalin injection caused an increase in nuclei size of AN neurons and marked enhancement of the response of these cells to stress in old animals. Thymalin injection probably promotes alteration of the sensitivity of the receptors presented in the AN to glucocorticoids [17, 27]. The age-specific feature of the response of RPA and AN neurons of hypothalamus of white rats to TP injection is a decrease in response.

Differences in reactions of AN and RPA neurosecretory cells to thymalin depending on the age of animals observed in the experiments are of crucial importance: enhancement in the functional activity of RPA neurosecretory cells in young animals and the absence of any differences in old animals were found using morphometric methods. As is known [16], the sensitivity of target cells to regulatory peptides changes with increasing age. In our experiments, the exogenic influence of thymalin did not restore the depressed function of RPA neurosecretory cells, which is probably defined (in contrast to AN neurosecretory cells) by the increase in the sensitivity threshold of these cells to neuropeptides and inhibition of the protein synthesis in neurosecretory cells, which is characteristic of involution. As the experimental results showed, thymalin increased the functional activity of AN neurosecretory cells independent of age and gender of animals. The thymalin effects in old rats are probably defined by thymalin's ability to maintain the protein synthesis of neurosecretory cells at a level that is characteristic of young animals. According to the data [11, 14, 15], peptide bioregulators possess the property to control gene expression and protein biosynthesis in cells; it is possible that the change of sensitivity of hypothalamic neurosecretory cells to glucocorticoids occurs under their action [6, 7].

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