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ACTIVITY OF HYPOTHALAMUS-PITUITARY-THYROID AXIS IN RATS OF DIFFERENT AGES AFTER LONG-TERM COLD EXPOSURE

Key words: *thyroid-stimulating hormone, thyroid hormones, cold adaptation, age, rats.*

In order to enhance the cold tolerance in ho-meothermic organism, both short- and long-term cold exposures of varying intensities are used [7]. Cer-tain cooling regimens are applied in medicine to accelerate adaptation, however some of them can cause cold injuries and even lead to death of an organism.

The hypothalamus-pituitary-thyroid (HPT) axis, which controls the thyroid function, maintains thyroid status and the body's metabolic balance by regulating positive and negative feedback loops that depend on the thyrotropin-releasing hormone (TRH), thyroid-stimulating hormone (TSH), and thyroid hormones (TH) levels. Thyroid function is associated with the body's adaptation to low temperatures, during which the thyroxine (T4) is deiodinated and triiodothyronine (T3) concentration in the blood increases. At physiological concentrations, TH stimulate anabolism, but when their levels rise, catabolic processes predominate, heart rate and blood circulation, especially in skin to dissipate heat, are accelerated, and respiratory depth increases [1, 3, 8].

This study aimed to explore the effects of long-term cold exposure on the activity of central and peripheral components of the hypothalamus-pituitary-thyroid axis (as measured by serum thyroid-stimulating hormone and thyroid hormone levels, respectively) in rats of different ages.

The experiments were approved by the Bioethics Committee of the Institute for Problems of Cryobiology and Cryomedicine of the National Academy of Sciences of Ukraine (Protocol N 2 of March 11, 2020) and were conducted in accordance with the Law of Ukraine "On the Protection of Animals Against Cruelty" (No. 3447-IV of February 21, 2006) and the provisions of the "European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes" (Strasbourg, 1986).

The study was performed in 6-, 12-, and 24-month-old male white outbred rats, which were housed in animal facility on a standard diet prior to experiment.

Long-term cold exposure (LTCE) was simulated by keeping animals in a cold room at 5 °C for one month under natural light conditions. Rats were housed individually in the cages lined with regularly replaced sawdust bedding, and provided with *ad libitum* access to food and water.

Rats in each age group were divided into a control group (intact animals) and a post-LTCE group (n = 5 in each group). Animals were sacrificed by decapitation.

Blood samples were centrifuged at 5000 g at 22 °C for 15 min in an MPW–311 centrifuge (Me-

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chanika Precyzyjna, Poland); the serum was then separated and stored at –18 °C until analysis.

The serum concentrations of TSH, T4, T3, and their free forms (FT4 and FT3) were measured by enzyme-linked immunosorbent assay (ELISA) using standard kits: TSH-ELISA, T4-ELISA, T3-ELISA, Free T4-ELISA, and Free T3-ELISA (XEMA, Ukraine) in accordance with the manu-facturer's instructions. Optical density was assessed using an enzyme-linked immunosorbent photo-meter-analyzer "Humanreader" (Human, Ger-many). We also calculated the integrated thyroid index (ITI) ((FT3 + FT4)/TSH), the FT3/FT4 ratio, and the systemic coefficients T3/TSH and T4/TSH [4, 5].

Results were statistically processed using the ANOVA method with the Social Science Statistics software package (https://www.socscistatistics.com/). The data are presented as $M \pm SE$.

The age-related characteristics of the HPT axis activity, which we described earlier [5], are consistent with the findings of R.P. Peeters [9], *i. e.* the TSH and FT3 serum concentrations decrease with age, while FT4 concentrations remain unchan-ged. Functional rearrangements in the HPT axis occur as a natural adaptation to age-related changes [2].

Following LTCE, which is the most natural cold stress, the TH concentration remained unchanged in 6-month-old rats; in 12-month-old animals, the T3, T4, and FT4 concentrations reduced, and in 24-month-old ones, a decrease in T3 (trend) and T4 was observed. The TSH concentration decreased in 6- and 12-month-old rats; in 24-month-old ones, conversely, it increased (Table). It is known that high concentrations of FT4 and FT3 inhibit, while low concentrations stimulate TSH secretion via negative and positive feedback mechanisms. It is

Indices of functional activity of the hypothalamus-pituitary-thyroid axis components in rats after long-term cold exposure

Age, months					
6		12		24	
Control	LTCE	Control	LTCE	Control	LTCE
T3, nmol/L					
1.3 ± 0.1	1.4 ± 0.04	7.9 ± 3.5 **	1.5 ± 0.04 *	2.5 ± 1.5	1.5 ± 0.03
FT3, pmol/L					
6.8 ± 0.4	5.8 ± 0.2	5.9 ± 0.1	5.5 ± 0.2	6.0 ± 0.2	6.7 ± 0.3
T4, nmol/L					
25.3 ± 7.1	22.9 ± 3.1	27.2 ± 4.8	18.9 ± 4.5 *	17.0 ± 1.7	11.2 ± 2.7 * **
FT4, pmol/L					
12.2 ± 1.8	13.7 ± 1.3	11.2 ± 0.7	4.3 ± 2.1 * **	9.8 ± 0.8	10.5 ± 0.7
TSH, mIU/L					
5.3 ± 0.6	1.1 ± 0.1 *	7.9 ± 3.5	1.5 ± 0.04 *	2.5 ± 1.5	4.6 ± 1.03 **
FT3/FT4 ratio					
0.6 ± 0.04	0.4 ± 0.04	0.5 ± 0.03	0.4 ± 0.03	0.6 ± 0.05	0.6 ± 0.03
Integral thyroid index (FT3 + FT4)/TSH					
3.8 ± 0.8	18.6 ± 0.9 *	6.9 ± 4.8	12.9 ± 4.6 * **	12.5 ± 4.6 **	3.1 ± 0.4 *
T3/TSH systemic coefficient					
0.3 ± 0.05	1.1 ± 0.4 *	0.6 ± 0.4	0.8 ± 0.3 **	1.2 ± 0.5 **	0.5 ± 0.2 *
T4/TSH systemic coefficient					
5.1 ± 1.7	20.4 ± 1.1 *	6.6 ± 3.9	15.4 ± 7.0	9.6 ± 3.2 **	2.8 ± 0.5 * **

Notes: * – differences are significant compared to the control group of rats of corresponding age, $p < 0.05$; ** – differences are statistically significant compared to 6-month-old rats, $p < 0.05$.

interesting to compare these data with the effect of an extremely low temperature of –120 °C in a cryochamber for 90 seconds [6], when in 6-month-old rats the blood TSH concentration decreases, while in 18-month-old ones it remains unchanged, which occurs against a background of unaltered TH concentration in young rats and increased T4 and FT4 levels in aged ones.

The FT3/FT4 ratio, which indicates the conversion rate of T4 into T3 and reflects the activity deiodinase [4], *i. e.* the deiodination index, did not change significantly either with age or following LTCE (Table).

The ratio of free TH to their pituitary regulator TSH (ITI) in 6- and 12-month-old rats increased by 4.9- and 1.8 times, respectively, while in 24-month-old ones it decreased by 3.1 times, thereby indicating either activation or suppression of thyroid function, respectively (Table).

The dynamics of T3/TSH and T4/TSH systemic coefficients, *i. e.* the amount of TH per unit of TSH, showed that the T3/TSH coefficient increased in 6-month-old rats, decreased in 24-month-old animals, and remained unchanged in 12-month-old ones. The T4/TSH coefficient increased sharply (almost fourfold) in 6-month-old rats, showed a similar trend in 12-month-old rats, and decreased almost threefold in 24-month-old ones (Table).

As we have shown previously, after short-term cold exposures (STCE) at temperatures of 10 and –12 °C, which are used to accelerate cold adaptation, the TSH and T4 concentrations change in a similar way as after LTCE [4]; the FT3/FT4 ratio remains unchanged in 6-month-old animals (as after LTCE), increases in 12-month-old (after STCE at –12 °C) and 24-month-old rats (after both STCE regimens), the ITI increases in 6-month-old animals by 2.2 times at 10 °C and by 2.6 times at –12 °C (as after LTCE). The T4/TSH coefficient also augments after STCE in 24-month-old animals, unlike after LTCE.

Thus, the response of the HPT axis to long-term cold exposure depends on animal age. The activity of HPT axis central component (as measured by serum TSH concentration) decreased in 6- and 12-month-old rats after LTCE, while it remained unchanged in 24-month-old ones.

The activity of the HPT axis peripheral component, as measured by the serum concentrations of TH and their free forms, did not change in 6-month-old rats, reduced in 12-month-old rats (due to a decrease in T3, T4, and FT4), and in 24-month-old ones (owing to T4 lowering).

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АКТИВНІСТЬ ЛАНОК ГІПОТАЛАМО-ГІПОФІЗАРНО-ТИРЕОЇДНОЇ СИСТЕМИ ЩУРІВ РІЗНОГО ВІКУ ПІСЛЯ ТРИВАЛОГО ХОЛОДОВОГО НАВАНТАЖЕННЯ

Ключові слова: тиреотропний гормон, тиреоїдні гормони, холодова адаптація, вік, щури.