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CHANGES IN THE CONTENT OF INTERLEUKINS IN THE SERUM Of MALE AND FEMALE RATS WITH DIFFERENT RESISTANCE TO ACUTE HYPOXIC HYPOXIA UNDER IMMOBILIZATION STRESS

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Abstract

Attention of scientists of the world are study the features of increased resistance to hypoxia and stress, different development inflammation in them.

The aim of the study was to trace the features of interleukins level in high- and low-resistance to hypoxic hypoxia rats (HR, LR) in different models of immobilization stress.

Material and methods of investigation. The experiments were performed on 144 outbred HR and LR aged 5.5-6 months, dividing into 3 groups – control and 2 with different model of immobilizing stress. Determine concentration of interleukins 1beta, 2, 4, 6, 10 (IL-1beta, IL-2, IL-4, IL-6, IL-10), tumor necrosis factor alpha (TNF-alpha) in the blood serum.

Results. High congenital resistance to hypoxia is associated with an increased content of anti-inflammatory cytokines (in males - IL-4 on 24.73% ($p<0.001$), in females - IL-10 on 37.36%; $p<0.001$). Intact males, compared with females, have a higher production of pro-inflammatory cytokines (IL-6, TNF-alpha). Intact males, compared with females, are dominated by the content of pro-inflammatory cytokines in the blood. In HR males, compared with females, a higher content of IL-6 (49.93%; $p<0.01$), TNF-alpha (10.85%; $p<0.01$), IL-4 (33.92%; $p<0.001$), lower - IL-1beta (37.91%; $p<0.02$) and IL-10 (93.06%; $p<0.001$). In LR

males, compared with females, higher blood levels of IL-1beta (42.98%; p<0.001), IL-6 (54.32%; p<0.001), TNF-alpha (16,40%; p<0,002), the highest level of pro-inflammatory cytokines under stress with a frequency of every 24 hours. In HR males under these conditions, the level of IL-1beta increases (in 2.35 times; p<0.001), TNF-alpha (in 8.20 times; p<0.001). In exposed to stress every 72 hours in HR males, the level of TNF-alpha increases in 2.88 times (p<0.002), and in LR males in 5.18 times (p<0.001). At the same time, in HR females its level increases in 3.25 times (p<0.01), in LR females - in 5.73 times (p<0.01).

Conclusion. The experiments revealed the changes of pro- and anti-inflammatory interleukins, which depends of sex, resistance to hypoxy and kinds of stress.

Key wards: interleukins; immobilizing stress; resistence to hypoxia; sex; rats

Introduction. Interleukins play a significant role in stress. They cause chronic low-grade inflammation [1]. There are data in the literature on the growth of interleukin 6 in chronic stress [2], the increase of which is associated with increased secretion of norepinephrine [3]. Interleukin (IL) -10 is a potent activator of the hypothalamic – pituitary – adrenal axis and immobilization stress may induce an increase in rat cytokine IL-10 [4].

Recently, more and more attention of scientists of the world are study the features of increased resistance to hypoxic hypoxia [5, 6, 7, 8]. Hypoxia is lying in the bases of all diseases. It is known that females are more resistant to hypoxia [9, 10, 11]. Also, both healthy individuals and patients have long been in a state of limited physical activity. Under conditions of stress, the inflammation can be developted. In the experiment, it is advisable to study the mechanisms of increased resistance of persons of different sexes under different models of immobilization stress.

The aim of the study was to trace the features of interleukins level in high- and low-resistance to hypoxic hypoxia rats (HR, LR) in different models of immobilization stress.

Material and methods of investigation. The experiments were performed on 144 outbred high- and low-resistance to hypoxia rats (HR, LR) aged 5.5-6 months. Animals were divided into three groups – control and two experimental (who underwent different modes of immobilization stress). Each group had 12 males and 12 females. Isolation from the general cohort of animals with different resistance to hypoxia was performed according to the method of Berezovsky V.Ya. (1978) [12]. Stress was simulated 4 times by one-hour immobilization of rats on the back down with an interval of 24 hours between immobilization episodes (stress 1) and 72 hours (stress 2) [13]. All animals kept in one room on a standard diet and vivarium regime.

All experiments were performed in the morning in a specially designated room at a temperature of 18-22 ºC, a relative humidity of 40-60% and an illumination of 250 lux. The experiments were performed in compliance with the norms of the Council of Europe Convention on the Protection of Vertebrate Animals Used for Research and Other Scientific Purposes (Strasbourg, 18.03.1986), the resolution of the First National Congress on Bioethics (Kyiv, 2001) and the Ministry of Health of Ukraine № 690 of 23.09 .2009 p.

Euthanasia of rats was performed by total bloodletting from the heart after previous thiopental-sodium anesthesia (60 mg/kg of body weight intraperitoneally). For further experimental study in the blood serum were determined the concentration of interleukins 1beta, 2, 4, 6, 10 (IL-1beta, IL-2, IL-4, IL-6, IL-10), tumor necrosis factor alpha (TNF-alpha) [14]. Determination of interleukin levels was performed in blood serum using reagent of the "VECTOR-BEST Ukraine" company on the analyzer STAT FAX 303 plus.·

Statistical processing of digital data was performed using the program "STATISTICA" 6.0 ("Statsoft", USA).

Results and discussion. In control HR rats-males, compared with LR, the lower level of the concentration of IL-1beta in 2.06 times ($p<0.001$) and higher level of IL-4 on 24.73% ($p<0.001$) were determined (Tables 1, 2).

At stress 1, compared with the control, in HR male-rats noted significant increase of IL-1beta in 2.35 times ($p<0.001$), TNF-alpha in 8.20 times ($p<0.001$), IL-10 on 40.19% ($p<0.01$), IL-4 on 22.68% ($p<0.001$). In LR rats-males it was considered significant decrease of IL-1beta on 54.74% ($p<0.001$), IL-6 on 51.33% ($p<0.001$), IL-4 on 20.40% ($p<0.01$), increase of TNF-alpha in 5.86 times ($p<0.001$). Compared that two groups with different resistance to hypoxia was determined in HR increase of IL-1beta on 60.38% ($p<0.001$), IL-6 on 33.97% ($p<0.001$), IL-4 on 22.49% ($p<0.01$).

Proinflammatory cytokine IL-1 beta responds to any stressor in the body, it is an activator of T cells, NK cells, NKT cells, stimulates the formation of cytokines by T cells, that indicating tissue damage.

High levels of serum TNF-alpha are a marker of the risk of heart damage. It stimulates the activity of leukocytes, the production of cells IL-1 beta, IL-6 and has a destructive effect on tissues. The increase of TNF-alpha and IL-1 beta was obtained under stress 1 in HR male-rats.

At stress 2, compared with the control, in male HR there was a significant decrease in IL-1 beta on 22.74% ($p<0.02$) and IL-4 – on 31.98% ($p<0.001$).

Table 1 - Changes in proinflammatory interleukins in the serum of high- and low-resistance to hypoxia rats of different sexes caused by stress, M ± m (n=12)

Group	Index		
	IL-1beta, pg/ml	IJI-2, $x10^{-3}$, pg/ml	IJI-6, pg/ml
High-resistance to hypoxia male-rats			
Control	40.45 ± 3.83	6.92 ± 1.11	3.33 ± 0.57
Stress 1	95.11 ± 2.77*	6.00 ± 0.61	2.47 ± 0.08
Stress 2	31.25 ± 0.62*	7.75 ± 0.82	3.73 ± 0.46
Low-resistance to hypoxia male-rats			
Control	83.26 ± 5.23**	8.25 ± 1.42	3.35 ± 0.39
Stress 1	37.69 ± 5.38*,**	5.00 ± 0.88	1.63 ± 0.11*,**
Stress 2	50.34 ± 3.71*,**	6.67 ± 0.80	1.86 ± 0.10*,**
High-resistance to hypoxia female-rats			
Control	55.79 ± 5.04#	8.08 ± 0.84	1.67 ± 0.24#
Stress 1	39.07 ± 3.10*,#	7.17 ± 0.50	0.67 ± 0.05*,#
Stress 2	70.25 ± 3.91*,#	5.00 ± 0.68*,#	0.62 ± 0.07*,#
Low-resistance to hypoxia female-rats			
Control	47.48 ± 2.28#	9.50 ± 0.73	1.53 ± 0.11#
Stress 1	42.38 ± 4.74	11.08 ± 0.72**,#	1.23 ± 0.19**
Stress 2	60.24 ± 3.85*	9.83 ± 1.20**,#	1.19 ± 0.15**,#
1. * – indexes are reliable, compared to control; 2. ** – indexes are reliable, compared to HR rats; 3. # – indexes are reliable, compared to male-rats.			

Table 2 - Changes in the concentration of interleukins caused by stress in the serum of high- and low-resistant to hypoxia animals of different sexes, M ± m (n=12)

Group	Index		
	TNF-alpha, pg/ml	IL-4, pg/ml	IL-10, pg/ml
High-resistance to hypoxia male-rats			
Control	0.063 ± 0.002	1.74 ± 0.08	7.79 ± 0.67
Stress 1	0.517 ± 0.088*	1.35 ± 0.03*	10.92 ± 0.85*
Stress 2	0.181 ± 0.037*	1.18 ± 0.01*	9.79 ± 0.49*
Low-resistance to hypoxia male-rats			
Control	0.068 ± 0.002	1.31 ± 0.03**	9.17 ± 0.61
Stress 1	0.396 ± 0.056*	1.04 ± 0.100*,**	9.82 ± 0.62
Stress 2	0.350 ± 0.085*	0.97 ± 0.03*,**	13.47 ± 0.43*,**
High-resistance to hypoxia female-rats			
Control	0.056 ± 0.001#	1.15 ± 0.07#	15.04 ± 1.00#
Stress 1	0.189 ± 0.048*,#	1.14 ± 0.06#	15.61 ± 1.35#
Stress 2	0.183 ± 0.047*	1.13 ± 0.03	16.66 ± 1.45#
Low-resistance to hypoxia female-rats			
Control	0.056 ± 0.003#	1.21 ± 0.06	9.42 ± 0.86**
Stress 1	0.411 ± 0.093*,**	1.07 ± 0.06	12.97 ± 0.50*,#
Stress 2	0.324 ± 0.084*	1.34 ± 0.03*,**,#	10.31 ± 0.48**,#
1. * – indexes are reliable, compared to control; 2. ** – indexes are reliable, compared to HR rats; 3. # – indexes are reliable, compared to male-rats..			

In this kind of stress in HR there was an increase of TNF-alpha in 2.88 times ($p<0.002$), IL-10 – on 25.60% ($p<0.02$). In LR males-rats there was a significant decrease of IL-1 beta on 39.54% ($p<0.001$), IL-6 – on 44.54% ($p<0.001$), IL-4 – on 25.93% ($p<0.001$), increase of TNF-alpha in 5.18 times ($p<0.001$), IL-10 – on 46.90% ($p<0.001$). In HR males-rats, compared with LR, were lower concentrations of IL-1beta in 1.61 times ($p<0.001$), IL-10 - on 37.61% ($p<0.001$), higher level of IL-6 on 50.16% ($p<0.001$), IL-4 – on 18.03% ($p<0.001$).

In control HR females, compared with LR, found a higher on 37.36% ($p<0,001$) concentration of IL-10. At stress 1, compared with the control, in HR rats there was an increase in TNF-alpha in 3.37 times ($p<0,01$), a decrease in IL-1beta - on 29.96% ($p<0,001$), IL-6 - on 59.93% ($p<0.01$). In LR rats there was a significant increase of IL-10 on 37.67% ($p<0.001$), TNF-alpha in 7.28 times ($p<0.001$). Moreover, in HR females, compared with LR females, were lower IL-2 on 54.65% ($p<0.001$), IL-6 - on 83.55% ($p<0.001$), TNF-alpha – in 2.17 times ($p<0.05$).

Under influences of stress 2, compared with the control, in HR female there was an increase of IL-1beta on 25.92% ($p<0.05$), TNF-alpha – in 3.25 times ($p<0.01$), a decrease of IL-2 on 38.14% ($p<0.01$), IL-6 - on 62.74% ($p<0.001$). In LR there was a significant increase in the concentration of IL-1 beta on 26.89% ($p<0.05$), IL-4 - on 10.84% ($p<0.001$), TNF-alpha – in 5.73 times ($p<0,01$). In HR female, compared with LR, were lower content of IL-2 on 96.67% ($p<0.001$), IL-6 – on 91.84% ($p<0.001$), IL-4 - on 18.50% ($p<0.001$), higher level of IL-10 on 38.13% ($p<0.001$).

In control HR males, compared with HR females, were lower content of IL-1beta on 37.91% ($p<0.02$) and IL-10 on 93.06% ($p<0.001$), higher level of IL-4 on 33.92% ($p<0.001$), IL-6 on 49.93% ($p<0.01$), TNF-alpha on 10.85% ($p<0.01$). In control LR males, compared with LR females, IL-1beta was higher on 42.98% ($p<0.001$), IL-6 - on 54.32% ($p<0.001$), TNF-alpha – on 16.40% ($p<0.002$).

At stress 1 in HR males, compared with HR females, were lower contents of IL-10 on 42.91% ($p<0.02$), higher level of IL-1beta on 58.92% ($p<0.001$), IL-4 - on 15.31% ($p<0.02$), IL-6 - on 72.94% ($p<0.001$), TNF-alpha - on 63.41% ($p<0.002$). At stress 1 in LR males, compared with LR females, were lower contends of IL-10 on 32.08% ($p<0.001$), IL-2 – in 2.22 times ($p<0.001$).

At stress 2 in HR male, compared with HR female, were lower contents of IL-1beta in 2.25 times ($p<0.001$) and IL-10 - on 70.27% ($p<0.001$), higher IL-2 on 35.48% ($p<0.01$), IL-6 - on 83.34% ($p<0.001$). At stress 2 in LR males, compared with LR females, were higher

level of IL-10 on 23.44% (p<0,001), IL-6 - on 35.87% (p<0,001), lower - IL-2 on 47.50% (p<0.05), IL-4 - on 38.07% (p<0.001).

In comparing the level of IL-1beta in two models of immobilization stress, different changes were found in animals, which depended on the period of time between the immobilizations, sex and resistance of animals to hypoxia. In HR male, the contents increased significantly in stress 1, decreased - in stress 2. Moreover, under stress 1, the indicator was in 3.04 times higher (p<0.001) compared to stress 2. In LR males, the indicator was significantly reduced for both models of stress, but no statistically significant difference in values was found.

In females, the content of IL-1beta decreased significantly compared with the control under immobilization stress 1 only in HR, and increased under stress 2 in both HR and LR. At stress 1, it was significantly lower than in stress 2, in HR on 44.38% (p<0.001), in LR - on 29.65% (p<0.02). The development of inflammation can be said only in HR males at stress 1 and in HR and LR females under at stress 2.

When comparing the values of IL-2 in the two models of immobilization stress, a significant difference between the indexes was not found in males and LR females. In HR female were lower on 43.33% (p<0.05) rates of stress 2.

When comparing the values of IL-4 in the two models of immobilization stress, a significant difference between the indexe in LR males and HR females was not detected. In HR males were lower level on 13.67% (p<0.001) the value at stress 2, and in LR females were lower on 19.97% (p<0.001) the value in stress 1.

When comparing the values of IL-6 in the two models of immobilization stress, no significant difference between the indexes was found in LR males and all females. In HR males were lower on 33.77% (p<0.02) the value of the indexes at stress 1, compared to stress 2. There was a significant decrease of IL-6 in LR males and HR females, regardless of stress.

When comparing the indexes of TNF-alpha in two models of immobilization stress, a significant difference in the LR males and all females between the indexes was not detected. In HR male, the values were in 2.85 times smaller (p<0.002) ate stress 2.

When comparing the values of IL-10 in two models of immobilization stress, changes were found only in LR rats. It was found that in males at stress 2 the indexe was higher than at stress 1, on 27.07% (p<0.001). In females at stress 1, the rate was higher than at stress 2, on 25.82% (p<0.002). It is special that in HR female the concentration of IL-10 in the control was the highest and remained consistently high in all studied stress. At stress 1 were high in LR females (no dependence on resistance to hypoxia). At stress 2, the values of IL-10 also

increased in LR males, they did not differ significantly from the values of LR females and HR females.

The difference in interleukin content when comparing values at different models of immobilization stress was insignificant. A greater increase in the content of IL-1beta was in HR males atr stress 1, and females at stress 2. IL-6 was greater only at stress 2 in HR males. TNF-alpha in HR male increased more at stress 1, compared with stress 2. IL-10 values were significantly higher at stress 2 in LR males, and at stress 1 in LR females, in HR females it were always at a high level. The obtained data indicate the dependence of the development of the injury from sex, resistance to hypoxy and the model of immobilization stress.

Thus, intact male rats have a higher pro-inflammatory potential compared to females, and it is greater in LR animals compared to HR, regardless of sex. Most anti-inflammatory interleukins were observed in female HR, which persisted under stress 1. The maximum activity of pro-inflammatory cytokines in the control was observed in LR males. Activation of proinflammatory cytokines under stress 1 occurred in HR male. At stress 2 in males the content of anti-inflammatory cytokines increased and pro-inflammatory – decreased. In females, an increase in proinflammatory interleukins was observed with stable anti-inflammatory parameters.

Conclusion. High congenital resistance to hypoxia is associated with an increased content of anti-inflammatory cytokines (in males – IL-4, in females – IL-10). Control males, compared with females, have a higher production of pro-inflammatory cytokines (IL-6, TNF-alpha). The experiments revealed the changes of pro- and anti-inflammatory interleukins, which depends of sex, resistance to hypoxy and kinds of stress.

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