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DEVELOPMENT OF OXIDATIVE STRESS IN RATS OF DIFFERENT SEXES SUBJECTED TO IMMOBILIZATION STRESS OF DIFFERENT DURATIONS

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Abstract

Introduction. The problem of stress is currently one of the main problems not only in Ukraine, but also in the whole world. True, stress does not lead to damage in everyone, some adapt to stress. Studying the impact of different durations of stress, in particular, clarifying the mechanisms of their impact on the body, is relevant.

The purpose of the study is to evaluate the processes of lipid peroxidation in the blood of rats of different sexes that have experienced stress of different durations.

Materials and methods. Experiments were performed on 56 white male Wistar rats aged 3.5-4 months. The animals were divided into 4 groups: 1 – control, 2 – stress during 1 hour (Stress 1), 3 – stress during 2 hours (Stress 2), 4 – stress during 3 hours (Stress 3). Stress was induced by gently fixing the animal's on a plate with its back down. Superoxide dismutase and catalase activity (SOD, CAT), the concentration of diene and triene conjugates (DC, TC), Schiff's bases (SB), TBA-active products (TBA-ap) were determined in blood serum.

Research results and their discussion. In male rats with Stress 1, compared to the control, there was an increase of DC by 11.1%, TC – by 14.9%, SB – by 26.3%, TBA-ap – by 27.5%, SOD – by 12.5%, CAT – by 31.3%. In female rats with Stress 1, compared to the control, there was an increase in DC by 4.2%, TC – by 8.9%, SB – by 45.2%, TBA-ap – by 14.1%, SOD – by 15.5%, and CAT – by 7.3%. Such results indicated greater activation of lipid peroxidation processes in males and greater activation of antioxidants in females.

In males with Stress 2, compared to the control, the content of DC increased by 83.1%, TC – by 97.4%, SB – by 65.1%, TBA-ap – by 2.6 times, SOD decreased by 9.5%, CAT increased by 44.5%. In females with Stress 2 the content of DC increased by 30.3%, TC – by 28.2%, SB – by 35.5%, TBA-ap – by 85.6%. SOD was at the same level as in the control, CAT increased by 23.1%.

Such results indicated a greater activation of lipid peroxidation processes in males and a decrease in their antioxidant activity.

In males with Stress 3, compared to the control, the content of DC increased by 2.6 times, TC – by 2.8 times, SB – by 75.8%, TBA-ap – by 3.2 times. SOD decreased sharply by 41.9%, and CAT increased by 66.6%. In females with Stress 3 the content of DC increased by 2.5 times, TC – by 2.5 times, SB – by 45.2%, TBC-ap – by 2.9 times. SOD sharply decreased by 19.6%, and CAT increased by 35.4%. Such results indicated a significant activation of lipid peroxidation processes when antioxidant activity was depleted in males.

It was also noted the presence of erosions on the mucous membrane precisely during stress lasting 3 hours, when spot hemorrhages appeared after 2 hours, and hyperemia of the gastric mucosa - after 1 hour.

Conclusion. Animals adapt to stress lasting 1 hour. With stress that lasts for 2 hours, disadaptation of animals occurs. With stress that lasts for 3 hours, the animal's body becomes exhausted. In male rats, lipid peroxidation processes occur at a higher level, compared to females.

KEY WORDS: lipid peroxidation, antioxidant activity, stress, blood, rats.

Introduction. The problem of stress is one of the main problems today not only in Ukraine, but also in the whole world [1]. True, stress does not lead to damage in all people, some of us adapt to stress [2]. It depends not only on the individual perception of a stressful situation, genetic factors [3], but also on the duration of stress, the strength of the stressor, and the frequency of repetition of the stressor. Depending on this, damage occurs in various organs and tissues. Studying the state of the body, in particular cell membranes, is important because it can cause the development of cardiovascular pathology, impaired immunological reactivity, cause endocrine dysregulation, disorders of the functioning of the gastrointestinal tract. Studying the impact of stress models of

different durations, in particular, clarifying the mechanisms of their impact on the body, is relevant. The gender aspect in modern conditions is gaining more and more relevance [4].

The purpose of the study is to evaluate the processes of lipid peroxidation in the blood of rats of different sexes that have experienced stress of different durations.

Materials and methods. Experiments were performed on 56 white Wistar rats aged 3.5-4 months. The animals were divided into 4 groups: 1 – control, 2 – stress during 1 hour (Stress 1), 3 – stress during 2 hours (Stress 2), 4 – stress during 3 hours (Stress 3). Stress was induced by gently fixing the animal on a board with its back down [5]. Superoxide dismutase and catalase activity [6], concentration of diene and triene conjugates (DC, T), Schiff's bases (SB), TBA-active products [6, 7] were determined in blood serum.

Euthanasia of rats was performed by total bleeding from the heart after previous thiopental-sodium anesthesia (60 mg·kg⁻¹ of the animal's body weight intraperitoneally).

All experiments were carried out in the first half of the day at a temperature of 18-22 °C, relative humidity of 40-60% and 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, March 18, 1986), the resolution of the First National Congress on Bioethics (Kyiv, 2001) and the order of the Ministry of Health of Ukraine No. 690 dated September 23 .2009.

The significance of the obtained differences between the results (minimum level of significance $p < 0.05$) was assessed using the Kruskal–Wallis and Newman–Keuls tests (BioStat program, AnalystSoft Inc.).

Research results and their discussion. In male rats with Stress 1, compared to controls, an increase in DC by 11.1% ($p < 0.01$) and TC by 14.9% ($p < 0.001$) was noted (table 1). In males with Stress 2, compared to the control,

the content of DC increased by 83.1% ($p < 0.001$), and was higher than the animals of group 2 by 64.7% ($p < 0.001$). TC in this group of animals was higher compared to control rats by 97.4% ($p < 0.001$) and was larger compared to group 2 rats by 71.7% ($p < 0.001$). In males with Stress 3, compared to the control, the content of DC increased by 2.6 times ($p < 0.001$), and was higher, compared to the 2 group of rats, by 2.3 times ($p < 0.001$), and higher than animals 3 group by 40.5% ($p < 0.001$). TC in this group of animals was 2.8 times higher ($p < 0.001$) compared to control rats, and was 2.4 times higher ($p < 0.001$) compared to group 2 rats, and higher than animals 3 group by 42.1% ($p < 0.001$).

Table 1 – Changes in indicators of lipid peroxidation in blood serum of rats ($M \pm \sigma$, $n=7$)

A group of animals	Indicator	
	ДК, $\mu\text{M.OD./ML}$	ТК, $\mu\text{M.OD./ML}$
	Males	
1 Control	1.023 ± 0.014	1.032 ± 0.015
2 Stress 1	$1.137 \pm 0.026^*$	$1.186 \pm 0.024^*$
3 Stress 2	$1.873 \pm 0.035^{*,**}$	$2.037 \pm 0.036^{*,**}$
4 Stress 3	$2,631 \pm 0,043^{*,**,***}$	$2,895 \pm 0,048^{*,**,***}$
	Females	
1 Control	0.981 ± 0.011	1.018 ± 0.016
2 Stress 1	$1.022 \pm 0.023^{*,\#}$	$1.109 \pm 0.026^{*,\#}$
3 Stress 2	$1.278 \pm 0,032^{*,**,\#}$	$1.305 \pm 0,032^{*,**,\#}$
4 Stress 3	$2.434 \pm 0.043^{*,**,***,\#}$	$2.598 \pm 0.041^{*,**,***,\#}$

Notes: 1. * – probable differences with the control; 2. ** – probable differences with animals of series 2; 3. *** – probable differences with animals of series 3; 4. # – probable differences with males.

In female rats with Stress 1, compared to controls, an increase in DC by 4.2% ($p < 0.05$) and TC by 8.9% ($p < 0.001$) was noted (table 1). In females with Stress 2, compared to the control, the content of DC increased by 30.3% ($p < 0.001$), and was higher than the animals of group 2 by 25.0% ($p < 0.001$). TC in this group of animals was higher compared to control rats by 28.2% ($p < 0.001$) and was larger compared to group 2 rats by 17.7% ($p < 0.001$). In females with Stress 3, compared to the control, the content of DC increased by 2.5 times ($p < 0.001$), and was higher, compared to the 2 group of rats, by 2.4 times ($p < 0.001$), and higher than animals 3 group by 90.4% ($p < 0.001$). TCs in this group of animals were 2.5 times higher compared to control rats ($p < 0.001$), and were 2.3 times higher compared to group 2 rats ($p < 0.001$), and higher than animals 3 group by 99.1% ($p < 0.001$).

It should be noted that males have higher rates compared to females. In controls, DC were 4.1% higher in males ($p < 0.05$). In rats with Stress 1, the content of DC was higher by 11.2% ($p < 0.01$), TC – by 6.9% ($p < 0.05$). In rats with Stress 2, the content of DC was higher by 10.1% ($p < 0.05$), TC – by 6.5% ($p < 0.05$). In males with Stress 3, the content of DC was higher by 7.5% ($p < 0.05$), TC – by 10.3% ($p < 0.05$).

In male rats with Stress 1, compared to controls, there was an increase in SB by 26.3% ($p < 0.001$), and TBA-active products – by 27.5% ($p < 0.001$) (table 2). In males with Stress 2, compared to controls, the content of SB increased by 65.1% ($p < 0.001$), and was higher than animals of group 2 by 28.0% ($p < 0.001$). TBA-active products in this group of animals were higher, compared to control rats by 2.6 times ($p < 0.001$) and were greater, compared to group 2 rats, by 2.0 times ($p < 0.001$). In males with Stress 3, compared to the control, the content of SB increased by 75.8% ($p < 0.001$), and was higher, compared to the 2 group of rats, by 39.2% ($p < 0.001$), and higher than animals 3 group by 6.5% ($p < 0.05$). TBA-active products in this group of animals were higher, compared to control

rats, by 3.2 times ($p < 0.001$), and were higher, compared to group 2 rats, by 2.5 times ($p < 0.001$), and greater from animals of 3 groups by 24.7% ($p < 0.001$).

Table 2 – Changes in secondary indicators of lipid peroxidation in blood serum of rats ($M \pm \sigma$, $n=7$)

A group of animals	Indicator	
	ОШ, ум.од./мл	ТБК-ап, мкмоль/л
	Males	
1 Control	1.131 ± 0.017	1.034 ± 0.012
2 Stress 1	$1.428 \pm 0.022^*$	$1.318 \pm 0.024^*$
3 Stress 2	$1.867 \pm 0.034^{*,**}$	$2.682 \pm 0.036^{*,**}$
4 Stress 3	$1.988 \pm 0.031^{*,**,***}$	$3.345 \pm 0.034^{*,**,***}$
	Females	
1 Control	1.153 ± 0.014	$0.984 \pm 0.013^{\#}$
2 Stress 1	$1.437 \pm 0.024^*$	$1.123 \pm 0.022^{*,\#}$
3 Stress 2	$1.562 \pm 0.037^{*,**,\#}$	$1.826 \pm 0.031^{*,**,\#}$
4 Stress 3	$1.674 \pm 0.026^{*,**,***,\#}$	$2.875 \pm 0.032^{*,**,***,\#}$
Notes: 1. * – probable differences with the control; 2. ** – probable differences with animals of series 2; 3. *** – probable differences with animals of series 3; 4. # – probable differences with males.		

In female rats with Stress 1, compared to controls, there was an increase in SB by 45.2% ($p < 0.001$), and TBA-active products – by 14.1% ($p < 0.001$) (table 2). In females with Stress 2, compared to the control, the content of SB increased by 35.5% ($p < 0.001$), and was higher than the animals of group 2 by 8.7% ($p < 0.05$). TBA-active products in this group of animals were higher compared to control rats by 85.6% ($p < 0.001$) and were larger compared to group 2 rats by 62.6% ($p < 0.001$). In females with Stress 3, compared to the control, the content of SB increased by 45.2% ($p < 0.001$), and was higher, compared to the 2

group of rats, by 16.5% ($p<0.001$), and higher than animals 3 group by 7.1% ($p<0.05$). TBA-active products in this group of animals were higher, compared to control rats, by 2.9 times ($p<0.001$), and were higher, compared to group 2 rats, by 2.6 times ($p<0.001$), and greater from animals of 3 groups by 57.4% ($p<0.001$).

It should be noted that males have higher rates compared to females. In the control, TBA-active products were higher in males by 4.8% ($p<0.05$). In rats with Stress 1, the content of TBA-active products was higher by 14.8% ($p<0.01$). In males with Stress 2, the content of SB was higher by 16.3% ($p<0.001$), TBA-active products – by 31.9% ($p<0.001$). In males with Stress 3, the content of SB was higher by 15.8% ($p<0.001$), TBA-active products – by 14.0% ($p<0.01$).

In males with Stress 1, compared to controls, an increase in superoxide dismutase activity by 12.5% ($p<0.01$) and catalase activity by 31.3% ($p<0.001$) was noted (table 3). In animals with Stress 2, compared to control, superoxide dismutase activity decreased by 9.5% ($p<0.05$), and was lower, compared to animals of group 2, by 19.6% ($p<0.001$). Catalase activity, compared to the control, increased by 44.5% ($p<0.001$), and was greater, compared to animals of group 2, by 10.0% ($p<0.05$). In animals with Stress 3, compared to the control, superoxide dismutase activity sharply decreased by 41.9% ($p<0.001$), and was lower, compared to the Stress 1 group, by 48.4% ($p<0.001$), and Stress 2, – by 35.8% ($p<0.001$). Catalase activity, compared to the control, increased by 66.6% ($p<0.001$), and was greater, compared to animals of group 2, by 26.8% ($p<0.001$), and with animals of group 3 – by 15.3% ($p<0.01$).

Table 3 – Changes in antioxidant activity in blood serum of rats ($M \pm \sigma$, n=7)

A group of animals	Indicator	
	Супероксиддисмутазна активність, ПИТ.ОД./мл	Каталазна активність, мкат/л
	Males	
1 Control	1.126 ± 0.016	1.021 ± 0.022
2 Stress 1	1.267 ± 0.024*	1.341 ± 0.026*
3 Stress 2	1.019 ± 0.037*,**	1.475 ± 0.038*,**
4 Stress 3	0.654 ± 0.034*,**,***	1.701 ± 0.051*,**,***
	Females	
1 Control	1.234 ± 0.021 [#]	1.038 ± 0.012
2 Stress 1	1.425 ± 0.035*, [#]	1.114 ± 0.028*, [#]
3 Stress 2	1.210 ± 0.037**, [#]	1.278 ± 0.034*,**, [#]
4 Stress 3	0.992 ± 0.028*,**,***, [#]	1.406 ± 0.048*,**,***, [#]
Notes: 1. * – probable differences with the control; 2. ** – probable differences with animals of series 2; 3. *** – probable differences with animals of series 3; 4. # – probable differences with males.		

In females with Stress 1, compared to controls, an increase in superoxide dismutase activity by 15.5% ($p < 0.01$) and catalase activity by 7.3% ($p < 0.05$) was noted (table 3). In animals with Stress 2, compared to the control, superoxide dismutase activity was at the same level, and was lower, compared to animals of group 2, by 15.1% ($p < 0.001$). Catalase activity, compared to the control, increased by 23.1% ($p < 0.001$), and was greater, compared to the animals of group 2, by 14.7% ($p < 0.01$). In animals with Stress 3, compared to the control, superoxide dismutase activity sharply decreased by 19.6% ($p < 0.001$), and was lower, compared to the Stress 1 group, by 30.4% ($p < 0.001$), and Stress 2, – by 18.0% ($p < 0.001$). Catalase activity, compared to the control, increased by 35.4% ($p < 0.001$), and was greater, compared to animals of group 2, by 26.2% ($p < 0.001$), and with animals of group 3 – by 10.0% ($p < 0.05$).

It should be noted the higher indicators of antioxidant superoxide dismutase activity in females compared to males in the control: the activity was higher by 9.6% ($p < 0.05$). In Stress 1 rats, female superoxide dismutase activity was 12.5% higher ($p < 0.01$), and catalase activity was 16.9% lower ($p < 0.001$). In Stress 2 rats, superoxide dismutase activity in females was 18.7% higher ($p < 0.001$), and catalase activity was 13.4% lower ($p < 0.01$). In males with Stress 3, superoxide dismutase activity in females was 51.7% higher ($p < 0.001$), and catalase activity was lower by 17.3% ($p < 0.001$).

So, stress causes the activation of lipid peroxidation processes. Stress 1, in addition to an increase in lipid peroxidation products, causes an increase in antioxidant activity in the blood. At the same time, the end products of lipid peroxidation increase, which can be estimated as the rapid neutralization of primary and secondary products of lipid peroxidation by antioxidants. Stress 2, in addition to an increase in lipid peroxidation products, causes a greater increase in the end products of lipid peroxidation, which can be evaluated as a rapid neutralization of primary and secondary products of lipid peroxidation. Superoxide dismutase activity remains at the control level, and catalase activity increases. It is possible that the products of lipid peroxidation are neutralized by other antioxidants, or this indicates a significant accumulation of the end products of lipid peroxidation as a result of their less neutralization, which can lead to damage to various organs and tissues of the body. Stress 3, in addition to the greatest increase in the products of lipid peroxidation, causes a significant increase in the end products of lipid peroxidation, a decrease in superoxide dismutase activity, and an increase in catalase activity, which can be evaluated as less neutralization of products of lipid peroxidation at intermediate stages. Such results indicate the greatest damage to the body of rats. It was also noted the presence of erosions on the mucous membrane precisely during stress lasting 3 hours, when spot hemorrhages appeared after 2 hours, and hyperemia of the gastric mucosa - after 1 hour. The results can be evaluated as follows. Animals

adapt to stress lasting 1 hour. With stress that lasts for 2 hours, disadaptation of animals occurs. With stress that lasts for 3 hours, the animal's body becomes exhausted.

Conclusion. Animals adapt to stress lasting 1 hour. With stress that lasts for 2 hours, disadaptation of animals occurs. With stress that lasts for 3 hours, the animal's body becomes exhausted. In male rats, lipid peroxidation processes occur at a higher level, compared to females.

Perspectives of further research consist in studying further the lipid peroxidation in different organs under different duration of stress.

Supplementary Materials

Table S1 – Changes in indicators of lipid peroxidation in blood serum of rats ($M \pm \sigma$, $n=7$)

Table S2 – Changes in secondary indicators of lipid peroxidation in blood serum of rats ($M \pm \sigma$, $n=7$)

Table S3 – Changes in antioxidant activity in blood serum of rats ($M \pm \sigma$, $n=7$)

Author Contributions

Conceptualization, O.V. Denefil.; writing—original draft preparation A.O. Pokryshko; writing—review and editing - O.V. Denefil; project administration O.V. Denefil i A.O. Pokryshko.

All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement

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, March 18, 1986), the resolution of the First National Congress on Bioethics (Kyiv, 2001) and the order of the Ministry of Health of Ukraine No. 690 dated September 23, 2009.

Informed Consent Statement

Not applicable.

Data Availability Statement

Publicly available datasets were analyzed in this study. This data can be found here: That work did in University Laboratory of I. Ya. Horbachevsky Ternopil National Medical University

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Conflicts of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

References

1. Kramer A.C., Neubauer A.B., Stoffel M., Voss A., Ditzen B. Tomorrow's gonna suck: Today's stress anticipation predicts tomorrow's post-awakening cortisol increase // *Psychoneuroendocrinology*. – 2019. – Vol. 106. – P. 38-46. doi: 10.1016/j.psyneuen.2019.03.024
2. Kristensen T.N., Ketola T., Kronholm I. Adaptation to environmental stress at different timescales // *Ann N Y Acad Sci*. – 2020. – Vol. 1476(1). – P. 5-22. doi: 10.1111/nyas.13974
3. Szyf M. The epigenetics of perinatal stress // *Dialogues Clin Neurosci*. – 2019. – Vol. 21(4). – P. 369-378. doi: 10.31887/DCNS.2019.21.4/mszyf.
4. Children's stress regulation mediates the association between prenatal maternal mood and child executive functions for boys, but not girls / R. Neuenschwander, K. Hookenson, U. Brain [et al.] // *Dev. Psychopathol*. – 2018. – No. 30 (3). – P. 953–969.
5. Denefil, O. V., Ordynskyi, Iu. M., Nebesna, Z. M. (2019). Vplyv riznych modelej immobilizatsijnoho srtesu na morpholohichni zminy u sertsii vysoko- I nuzkostijkych do hostroi hipoksychnoi hipoksii shchuriv riznoi stati –

The influence of different models of immobilizational stress on morphological changes of high and low-resistance to acute hypoxic hypoxia rats of different sex. *Zdobutky Klinichnoi s experimentalnoi Medycyny – Achievements of Clinical and Experimental Medicine*. 2019. – 2. – P. 112-118. [in Ukrainian].

6. Vlizlo, V. V. (2012). *Laboratory methods of research in biology, animal husbandry and veterinary medicine: reference book*. / edited by V. V. Vlizlo. – 2012. Lviv: SPOLOM. – 764 p. [in Ukrainian].

7. Stefanova, O. V. (Ed.). (2001). *Preclinical studies of medicinal products: methodical recommendations*. 2001. K.: Avicenna. – 527 p. [in Ukrainian].