DYNAMICS OF FREE RADICAL OXIDATION INDICES AND ANTIOXIDANT PROTECTION IN MALE RATS’ HEART UNDERGONE VARIOUS TYPES OF STRESS

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SUMMARY. Any change of external or internal environment causes body’s responses that facilitate its adaptation. Pathogenesis of stress reaction study is always topical as it is aimed at the investigation of optimal adaptation mechanisms to impaired agents.

The aim – to study the dynamics of free-radical oxidation indices and antioxidant protection in male rats’ heart undergone chronic prenatal, postnatal stress and their combination.

Material and Methods. The study was performed on 44 white non-pedigreed pubertal three-month-rats. Prenatal stress was induced by keeping pregnant female rats in narrow pencil case for an hour. Postnatal stress was induced in 1.5 to three-month- rats by keeping them in cages with living space restriction twice as much. Combined stress comprised using two methods. Contents of peroxide lipids oxidation products and antioxidant protection were determined in the heart.

Results. Psycho-emotional stress in three-month-rats causes activation processes of peroxide lipids oxidation and antioxidant protection regardless of stressor’s period action. On the basis of correlations study it was proved that development mechanisms of chronic stress depend on life-period when stress was sustained.

Conclusions. Processes of peroxide lipids oxidation in all modelling types of stress are intensified in young male-rats’ heart during the effect of chronic psycho-emotional stress. Activity of enzyme part of antioxidant protection increases simultaneously with the increase of LPO products in the heart. It is more manifested in prenatal and postnatal stress. Intensification of glutathione system action and disturbances of its activity, especially in animals that undergone prenatal stress were also marked.

KEY WORDS: prenatal and postnatal stress; rats; antioxidant system; lipid peroxide oxidation.

Introduction. The question of acute and chronic stress, functional disorders of physiological systems of the body that are associated with it are under consideration of many practitioners and theoretical experts. It should be noted that stress and its consequences are the most frequent causes of morbidity and mortality in the developed countries [1; 2].

Cardiovascular, endocrine, immune systems are mostly affected in chronic stress and higher nervous activity is disturbed [3; 4; 5].

Activation of sympatho-adrenal and hypothalamo-pituitary-adrenal axis systems is the main trigger action of body’s response reaction on stress. Prolonged glucocorticoids release is observed in chronic stress. Excessive and constant high concentration of glucocorticoids in blood causes immunosuppression [6], development of thymus atrophy, gastrointestinal ulcers [7; 8; 9], cardiovascular pathology, depression [10; 11].

Stress effect on pregnant and their descendants, children, adolescents is harmful. Chronic diseases of various organs and systems occur due to hypothalamo-pituitary-adrenal axis regulation in prenatal and postnatal stress as well. These diseases may develop in any age and even have stressor remote effect [12; 13].

The increase of diseases caused by stress as a general pathogenetic agent determines permanent significance and topicality of this study. The investigation of general pathogenetic agents of stress-induced diseases is aimed at the development of general measures of their correction.

The aim – to study complex action of chronic psycho-emotional stress on young male rats’ bodies experienced in prenatal and postnatal periods of their development. Estimation of principal pathogenetic factors of chronic psycho-emotional stress impact in trials permits to extend existing conceptions about its development mechanism on these conditions and proves possible ways of disorders correction.

Material and Methods. The research was carried out on the base of Central Scientific Laboratory of I. Horbachevsky Ternopil National Medical University (attestation certificate No. 053/13 of 03.04.2013).

All trials were carried out before noon in specialized premises at 18–22 C, relative humidity 40–60 % and brightness 250 lux. Experiments were carried out according to European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes (Strasbourg, 03.18.1986), decision of the First National Bioethics Congress (Kyiv, 2001) and the Decree of Public Health Care Ministry of Ukraine No 690 of 09.23.2009. Bioethics Commission of I. Horbachevsky Ternopil National Medical University (Minutes No. 35 of 05.05.2016) did
Research was carried out on 44 white non-pedigreed pubertal three-month-male rats, weight from 85 to 155 g, keeping in the same premises on basic diet and vivarium regime [14].

Prenatal stress was induced in pregnant female-rats by standard methods [15]. Rats in the third trimester of pregnancy – from 15 to 21 days were kept in narrow pencil cases for an hour in order to reproduce the experiment. The number of newborns was 7–8, and 8–10 newborns were in the female group with non-induced stress. Death of some infant rats was marked among newborns at the age of 1–3 days in the female group with induced stress during pregnancy. Mortality was 20 %. Infant-rats were kept in standard conditions, on standard diet of vivarium till month age with female-rats. Then rats were isolated from female-rats and seated apart in separate cages for three months.

Postnatal stress was induced in 1.5–3 month-rats. Rats were kept in cages with living space restriction twice as much (the requirement for adult-rats is 8–10 cm² per rat). They were fixed backs downwards for an hour in the day of experiment whereupon investigation was carried on [16].

Combined stress included the usage of two methods: prenatal and postnatal stress. Randomized selected rats of prenatal induced stress group, 1.5 months of age were in the conditions of living space restriction for three month period. They were fixed backs downwards for an hour in the day of experiment whereupon investigation was carried out.

Infant rats born from non-induced stress female rats that were in standard vivarium conditions on ordinary diet till three months of age were selected for control group.

Euthanasia of rats in all experiments was performed by total heart blood letting after previous sodium thiopental anaesthesia (60 mg/kg of animal body weight intraperitoneally).

Indices evaluation of LPO in animals’ heart was performed at DC and TC concentration levels and evaluated by the method [17]. SOD activity in homogenate of the heart was determined by the method [20]. Catalase activity in heart homogenate was determined by the method [21]. GSH concentration by the method [22]. GP activity and GR activity was determined by the method described in the study [23].

Statistical processing of digital data was done by means of Excel ("Microsoft", USA) and STATISTICA 6.0. ("Statsoft", USA) using parametric and nonparametric methods of received data evaluation in System Statistical Research Division of I. Horbachevsky Ternopil National Medical University.

Arithmetic mean value of sampling (M), its dispersion and error of the mean (m) were calculated for all indices. Accuracy of value differences between independent quantity was determined at normal distribution by Student criterion, otherwise – by U criterion of Mann-Whitney test, correlations – by Pearson criterion (r) [24].

Results and Discussion. Activation of LPO is non-specific response to any kind of stress effect. Thereby we assessed changes of LPO indices in the heart of young male-rats during chronic psycho-emotional stress sustained in prenatal, postnatal periods of their development and in the combined stress periods.

Significant activity of LPO processes was marked in male-rats hearts during all methods of chronic stress investigation (Figure 1). So, DC index was increased in male-rats that sustained prenatal stress comparing with control group up to 53.1 % (p<0.001), postnatal – up to 44.4 % (p<0.001), combined – up to 56.1 % (p<0.001).

Concentration of TC was respectively increased to DC: in male-rats sustained prenatal stress comparing with control group up to 56.6 % (p<0.001), postnatal – up to 45.3 % (p<0.001), combined – up to 57.1 % (p<0.001).

TBA-active products concentration was also increased during all types of stress (Figure 1). The content of TBA-active products in male-rats that sustained prenatal stress was increased comparing with control up to 124.1 % (p<0.001), postnatal stress-up to 85.7 % (p<0.001), combined stress – by 136.3 % (p<0.001).

Indices of antioxidant system were changed together with LPO activation. The activity of enzyme part of antioxidant system in rats’ heart was considerably increased (Figure 2). SOD activity in male-rats sustained prenatal stress comparing with control rose in 2.6 times (p<0.001), postnatal stress – in 2.9 (p<0.001), combined – in 2.2 (p<0.001). Minimal (the lowest) activity incremen of catalase was marked in animals with combined stress (comparing with prenatal – by 16.7 % (p<0.01), postnatal – by 30.1 % (p<0.001).

Catalase activity in all experimental groups was also increased comparing with control. Enzyme activity in male-rats after sustained prenatal stress rose in 2.6 times (p<0.001), postnatal stress – in 2.9 (p<0.001), combined – in 2.2 (p<0.001). Minimal (the lowest) activity incremen of catalase was marked in animals with combined stress (comparing with prenatal – by 16.7 % (p<0.01), postnatal – by 30.1 % (p<0.001).

In estimation of glutathione system indices in rats’ heart (Figure 3) it was determined that in male-rats with prenatal stress comparing with control the increase of GSH concentration is marked by 47.9 % (p<0.001). GP activity in male-rats was also reliably increased by 69.9 % (p<0.001).
Figure 1. LPO indices changes in the heart of male-rats sustained various types of chronic stress.

Note: * – indices are significant comparing with control.

Figure 2. Indices changes of enzyme part of antioxidant system in rats’ heart sustained various types of chronic stress.

Notes: 1. * – indices are significant comparing with control; 2. ** – indices are significant comparing with prenatal stress; 3. ## – indices are significant comparing with postnatal stress.

Figure 3. The changes of glutathione system indices induced by chronic stress in animals’ heart.

Notes: 1. * – indices are significant comparing with control; 2. ** – indices are significant comparing with prenatal stress.
GSH was increased by 19.19% (p<0.05) in postnatal-stress rats comparing with control. Values were less (smaller) in this group of male-rats than in prenatal stress animals by 24.1% (p<0.02), and comparing also with female-rats sustained postnatal stress by 16.2% (p<0.01). GP activity was significantly increased comparing with control in male-rats (by 72.2 %, p<0.001). GR activity was increased by 57.8 % (p<0.01).

Table 1. Correlations between lipid peroxide oxidation products and antioxidant system in intact male-rats

<table>
<thead>
<tr>
<th>Index</th>
<th>Correlation coefficient</th>
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<tbody>
<tr>
<td></td>
<td>DC</td>
<td>TC</td>
<td>TBA-active products</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>SOD</td>
<td>-0.14</td>
<td>p&gt;0.05</td>
<td>0.01</td>
<td>p&gt;0.05</td>
<td>-0.32</td>
<td>p&gt;0.05</td>
</tr>
<tr>
<td>Catalase</td>
<td>-0.10</td>
<td>p&gt;0.05</td>
<td>-0.12</td>
<td>p&gt;0.05</td>
<td>-0.38</td>
<td>p&gt;0.05</td>
</tr>
<tr>
<td>GSH</td>
<td>0.58</td>
<td>p&gt;0.05</td>
<td>0.41</td>
<td>p&gt;0.05</td>
<td>-0.48</td>
<td>p&gt;0.05</td>
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<tr>
<td>GP</td>
<td>-0.23</td>
<td>p&gt;0.05</td>
<td>-0.30</td>
<td>p&gt;0.05</td>
<td>-0.21</td>
<td>p&gt;0.05</td>
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</table>

Strong negative correlations between LPO and SOD experimental products were revealed in male-rats during prenatal stress. Thus, between DC and SOD – r=-0.76 (p<0.05), between TC and SOD – r=0.73 (p<0.05), between TBA-active products and SOD – r=-0.81 (p<0.05). Strong negative correlations between TBA-active products concentration and GP were also established – r=0.75 (p<0.05) (Table 2).

Table 2. Correlations between lipids peroxide oxidation products and antioxidant system in prenatal stress conditions

<table>
<thead>
<tr>
<th>Index</th>
<th>Correlation coefficient</th>
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<tr>
<td></td>
<td>DC</td>
<td>TC</td>
<td>TBA-active products</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SOD</td>
<td>-0.76</td>
<td>p&lt;0.05</td>
<td>-0.73</td>
<td>p&lt;0.05</td>
<td>-0.81</td>
<td>p&lt;0.05</td>
</tr>
<tr>
<td>Catalase</td>
<td>-0.47</td>
<td>p&gt;0.05</td>
<td>-0.37</td>
<td>p&gt;0.05</td>
<td>-0.30</td>
<td>p&gt;0.05</td>
</tr>
<tr>
<td>GSH</td>
<td>-0.26</td>
<td>p&gt;0.05</td>
<td>-0.21</td>
<td>p&gt;0.05</td>
<td>-0.22</td>
<td>p&gt;0.05</td>
</tr>
<tr>
<td>GP</td>
<td>-0.50</td>
<td>p&gt;0.05</td>
<td>-0.55</td>
<td>p&gt;0.05</td>
<td>-0.75</td>
<td>p&lt;0.05</td>
</tr>
</tbody>
</table>

Strong positive correlations between LPO products and catalase were revealed in male-rats during postnatal stress (Table 3). Thus, between DC and catalase – r=0.83 (p<0.05), between TC and catalase– r=0.83 (p<0.05), between TBA-active products and catalase– r=0.91 (p<0.05).

Table 3. Correlations between lipids peroxide oxidation products and antioxidant system caused by postnatal stress conditions in experimental animals

<table>
<thead>
<tr>
<th>Index</th>
<th>Correlation coefficient</th>
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<tbody>
<tr>
<td></td>
<td>DC</td>
<td>TC</td>
<td>TBA-active products</td>
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<tr>
<td>SOD</td>
<td>-0.03</td>
<td>p&gt;0.05</td>
<td>-0.02</td>
<td>p&gt;0.05</td>
<td>0.27</td>
<td>p&gt;0.05</td>
</tr>
<tr>
<td>Catalase</td>
<td>0.83</td>
<td>p&lt;0.05</td>
<td>0.83</td>
<td>p&lt;0.05</td>
<td>0.91</td>
<td>p&lt;0.05</td>
</tr>
<tr>
<td>GSH</td>
<td>-0.16</td>
<td>p&gt;0.05</td>
<td>-0.17</td>
<td>p&gt;0.05</td>
<td>-0.23</td>
<td>p&gt;0.05</td>
</tr>
<tr>
<td>GP</td>
<td>0.44</td>
<td>p&gt;0.05</td>
<td>0.44</td>
<td>p&gt;0.05</td>
<td>0.32</td>
<td>p&gt;0.05</td>
</tr>
</tbody>
</table>

Strong negative correlations between all experimental products of LPO and catalase were revealed in male-rats sustained combined stress namely between DC and catalase – r=0.94 (p<0.05), between TC and catalase – r=0.94 (p<0.05), between TBA-active product and catalase – r=0.99 (p<0.05) (Table 4).

Thus, despite of indices increase of antioxidant system activity in chronic stress it was marked their involvement in antiradical, antioxidant and antioxidation protection.

**Conclusion.** Processes of lipids peroxide oxidation in all modelling types of stress are intensified in young male-rats’ heart during the effect of chronic
psycho-emotional stress. Activity of enzyme part of antioxidant protection increases simultaneously with the increase of LPO products in the heart. It is more manifested in prenatal and postnatal stress (superoxide dismutase and catalase activity is increased). Enzyme part of blood antioxidant system is also activated (ceruloplasmin activity increases, peroxidase blood activity), that is more manifested during postnatal stress. Intensification of glutathione system action and disturbances of its activity, especially in animals that undergone prenatal stress were also marked.

LITERATURE

REFERENCES


Огляд літератури, оригінальні дослідження, погляд на проблему, випадок з практики, короткі повідомлення


ДИНАМІКА ПОКАЗНИКІВ АнТІОКСИДАНТНОГО ЗАХИСТУ Та ВІЛЬНОРАДИКАЛЬНОГО ОКИСНЕННЯ В Серці ЩУРІВ-САМЦІВ, які ЗАЗНАЛИ ВПЛИВУ РІЗНИХ ВИДІВ СТРЕСУ

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РЕЗЮМЕ. На будь-яку зміну зовнішнього чи внутрішнього середовища організм відповідає реакцями, які сприяють пристосуванню до неї. Проблема вивчення патогенезу стресової реакції є актуальною і на сьогодні у зв'язку з необхідністю дослідження оптимальних механізмів адаптації до різних пошкоджуючих агентів.

Мета – вивчити динаміку показників антиоксидантного захисту та вільнорадикального окиснення в серці щурів-самців, які зазнали хронічного пренатального, постнатального стресу та їх поєднання.

Матеріал і методи. Дослідження виконано на 44 білих безпородних статевозрілих щурах-самцях віком 3 місяців. Хронічний пренатальний стрес викликали шляхом утримування вагітних самиць протягом 1 години в тісних пеналах. Після народження однорічних тварин відлучали від самиць і відсаджували в окремі клітки. Хронічний постнатальний стрес у щурів викликали з 1,5 до 3-місячного віку шляхом утримування у клітках з обмеження життєвого простору вдвічі, у день досліду їх фіксували протягом 1 години спинкою донизу, після чого проводили дослідження. Поєднаний стрес включав застосування на одних щурях двох методик: пренатального і постнатального стресу.

Результати. Хронічний пренатальний стрес викликає активізацію процесів перекисного окиснення ліпідів, антиоксидантної захисту незалежно від періоду дії стресора. На підставі вивчення кореляційних зв'язків доведено, що механізми розвитку хронічного стресу залежать від періоду життя, коли був перенесений стрес.

Висновки. У серці молодих щурів-самців при дії хронічного психоемоційного стресу інтенсифікуються процеси перекисного окиснення ліпідів, а також активність ферментної ланки антиоксидантного захисту.

КЛЮЧОВІ СЛОВА: пренатальний і постнатальний стрес; щури; антиоксидантна система; перекисне окиснення ліпідів.
Огляди літератури, орігінальні дослідження, погляд на проблему, випадок з практики, короткі повідомлення
налах в течінне 1 часа. Попосле рождения одномесячных животных отлучали от самок и помещали в отдельные клетки. Хронический постнатальный стресс у крыс вызывали с 1,5 до 3-месячного возраста путем содержания в клетках с вдвое ограниченным жизненным пространством, в день опыта их фиксировали в течение 1 часа спинной вниз, после чего проводили исследования. Комбинированный стресс включал применение на одних крысах пренатального и постнатального стресса. В сердце определяли содержание продуктов перекисного окисления липидов, антиоксидантной системы.

Результаты. Хронический психоэмоциональный стресс вызывает активацию процессов перекисного окисления липидов и антиоксидантной защиты независимо от периода действия стрессора. На основании изучения корреляционных связей доказано, что механизмы развития хронического стресса зависят от периода жизни, когда был перенесен стресс.

Выводы. В сердце молодых крыс-самцов при воздействии хронического психоэмоционального стресса интенсифицируются процессы перекисного окисления липидов. Одновременно с увеличением в сердце продуктов ПОЛ возрастает активность ферментного звена антиоксидантной защиты, больше при пренатальном и постнатальном стрессе, и активируется ферментное звено антиоксидантной системы крови, наиболее выраженное при постнатальном стрессе. Также отмечена интенсификация работы системы глутатиона, нарушение ее деятельности, особенно у животных, подвергшихся пренатальному стрессу.

КЛЮЧЕВЫЕ СЛОВА: пренатальный и постнатальный стресс; крысы; антиоксидантная система; перекисное окисление липидов.

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