

Sverediuk Yu. A. Effects of dexamethasone with sodium chloride load on the nitrite anion content in the rat's blood serum, heart atria and ventricles. *Journal of Education, Health and Sport*. 2020;10(1):208-217. eISSN 2391-8306. DOI <http://dx.doi.org/10.12775/JEHS.2020.10.01.023> <https://apcz.umk.pl/czasopisma/index.php/JEHS/article/view/JEHS.2020.10.01.023> <http://dx.doi.org/10.5281/zenodo.3742215>

The journal has had 5 points in Ministry of Science and Higher Education parametric evaluation. § 8. 2) and § 12. 1. 2) 22.02.2019.
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The authors declare that there is no conflict of interests regarding the publication of this paper.

Received: 20.12.2019. Revised: 25.12.2019. Accepted: 31.01.2020.

Effects of dexamethasone with sodium chloride load on the nitrite anion content in the rat's blood serum, heart atria and ventricles

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Abstract

The consequence of prolonged treatment with corticosteroids is development of hypertension and metabolic disorders. On the other hand, high levels of sodium chloride in the diet is able to amplify their risks. In a result of that disorders may be development metabolic cardiomyopathy. One of mechanisms that is capable to preventing hypertension and metabolic disorders is nitric oxide system. The research of that system, account short lifetime of the nitric oxide and features its metabolism, can be performed by analysis of nitrite anion (NO_2^-). It is mainly inactive metabolites in a result of the molecules NO. L-carnitine, as an antioxidant and having the ability to influence to energetic metabolism, apoptosis and transcription of DNA may be a promising means for correction disorders in the nitric oxide system. The purpose of our study was to investigate the NO_2^- levels in the animals serum and heart tissue of both sex for long-term action of dexamethasone in conditions of high concentration of 4 % solution sodium chloride in water and use L-carnitine in order to

correction data. The experiment was performed with the use of 96 mature white nonlinear rats. It was established that long-term use and high salt content and especially their combination conducted reduce of the NO_2^- level in the serum and ventricular myocardium. The high changes dominated in the males. L-carnitine demonstrates the ability to recover the metabolite level. The characterised changes occur between both sex in animals with intake of usual quantity sodium chloride in water. The usage water with high content of sodium chloride caused more intensive negative changes but the difference between the sex was not as pronounced. The difference of content NO_2^- in the atria was found only between males and females. The dexamethasone or sodium chloride didn't exert influence of the NO_2^- metabolism in that tissue.

Key words: NO_2^- ; dexamethasone; sodium chloride load; L-carnitine.

Introduction

Cardiomyopathy is a disorder of structure and function of heart muscle. It includes various disorders in the myocardium, are manifested by various structural and functional changes. They are often genetic, but may also be caused by various diseases owing to hypertension (AH), coronary heart disease or valvular insufficiency or metabolic diseases [7, 16]. There are pathologies that are associated with hormonal status or result from use of certain drugs. For example, glucocorticoids can cause metabolic damage in organs or hypertension, which is often used in various inflammatory diseases [12]. At the same time excessive sodium chloride intake can cause increasing the blood pressure and also contribute to cardiomyopathy development [10, 13]. For example, in hypertension left ventricular mass and wall thickness are increased by interstitial infiltration or intracellular accumulation of metabolic substrates. Metabolic cardiomyopathy develops in the wide range of pathological conditions and mainly is associated with systemic metabolic defects that were acquired in adulthood. The cardiomyopathy is characterized by structural (decreased capillary density and increase the distance between them, the increased diameter of hypertrophic cardiomyocytes) and functional changes (hypoxia), interstitial fibrosis for reducing energy supply without coronary artery disease or hypertension [10, 15]. Glucocorticosteroids are the most effective drugs in anti-inflammatory therapy of some diseases as rheumatoid arthritis, systemic lupus erythematosus, asthma. However, with prolonged use, possible negative effects as Cushing's syndrome, adrenal suppression, hyperglycemia, dyslipidemia, cardiovascular diseases, osteoporosis, mental disorders and immunosuppression [8, 9, 12].

In the cardiovascular system, one of mechanisms that can cause both general and local vasodilation and consequently prevent hypertension and metabolic disorders that arise owing to hypoxia is a system of nitric oxide [2, 3]. In addition to the regulation of vascular tone NO performs an important role in the ability to reduce, vascular reconstruction and baroreceptors function. This indicates that resistive type vessel under influence of modulating NO vasodilatory action [14]. Nitric oxide, considering its short lifetime and metabolic characteristics, is usually determined by the level of nitrite anion (NO_2^-), so far as it is mainly inactive metabolites in the disintegration of NO molecules [1, 4, 5, 6].

L-carnitine is a very important amino acid that has important functions in the cell. For example, in the mitochondria is involved in generating energy in the oxidation of fatty acids, also known antioxidant effects of L-carnitine, neuromodulatory properties, the ability to influence the process of apoptosis in myopathy and transcription of DNA. These effects can influence to metabolism of nitric oxide.

Purpose of the research:

The studying of nitric oxide metabolism in the both sex animals under influence of dexamethasone and high concentration of sodium chloride in drinking water and the correction of the disorders by L-carnitine.

Materials and methods

The experimental studies were performed with the use of 96 mature white nonlinear rats aged 5-6 months, weighing 0.17-0.23 kg. The glucocorticoids dexamethasone was introduced in a dose 350 mg / kg for 15 days. Half of the animals taken 4 % sodium chloride solution in drinking water. The correction of the pathological changes was performed by using L-carnitine ("Ahvantar" Mr. oral 20 % Ersel Pharma) in a dose 200 mg / kg for 19 days. The significance of differences between the results (minimum significance of $p < 0,05$) was assessed using criteria by Newman-Keuls (software BioStat, AnalystSoft Inc., version 6). The content of nitrite anion was determined in serum, atria and ventricles homogenates by method Gris.

Results and discussion

Significant differences of the nitrite anion content were found in the males and the females serum of intact group animals. Introduction of L-carnitine to the females as opposed to males conduce to a significant increase of nitrite anion in the serum (by 10.7 %). The difference between the males and the females under the action of L-carnitine formed 6.9 %. Admission of the dexamethasone to the animals for 14 days conduced significant change of nitrogen monoxide metabolite – nitrite anion level. Thus, the nitrite anion content in the

female's blood serum of intact animals was decreased by 34 % and the males – by 50.3 %. At the same time difference nitrite anion levels between the sex was formed by 26.8 % (in the males level was lower than in the females). The use of L-carnitine on purpose to correction of that disturbances caused with dexamethasone in the females led to the increase of index (by 64.5 %), which was higher (by 8, 7 %) than the average rate in the control animals (as the animals that taken only L-carnitine). The correction of negative effects of the dexamethasone caused increase of the nitrite anion content in the males (by 2 times) and removal of differences as compared to intact animals. In general, in comparing animals of different sex for the correction of L-carnitine was found that the investigated metabolite were lower (by 11.6 %) in the males as compared to the females.

Table 1. Serum nitrite anion value in the experimental animals

Rats	Groups of animals:							
	Control		L-carnitine		Dexamethasone		Dexamethasone + L-carnitine	
Sex:	female	male	female	male	female	male	female	male
The form of experiment	NO ₂ ⁻ level in blood serum, 10 ⁻⁵ mol/L, M ± σ							
Intake of usual quantity NaCl	1,448 ± 0,082	1,410 ± 0,071§	1,602 ± 0,084*	1,492 ± 0,083*§	0,956 ± 0,080*	0,700 ± 0,080*§	1,573 ± 0,086*	1,391 ± 0,076§
Intake of 4 % solution NaCl	1,008 ± 0,075#	0,897 ± 0,069#§	1,402 ± 0,087*#	1,387 ± 0,081*§	0,499 ± 0,077*#	0,445 ± 0,067#	1,445 ± 0,078*#	1,152 ± 0,075*#§

Note: * p<0,05– significance of differences in relation to intact animals;
 # p<0,05 – significance of differences in relation to animals with normal level of sodium chloride;
 § p<0,05 – significance of differences between the male and the female rats.

The high content of sodium chloride in drinking water caused changes of nitrite anion content in the animal's serum of both sex. In particular, content of the nitrite anion in the control animals was decreased in the females (by 30.4 %), the males (by 36.4 %) as compared to the intact animals, but it was lower (by 11.1 %) in the males than in the females. The L-carnitine use in the female rats increased the nitrite anion content (by 39.1 %), but it was still lower (by 12.5 %) than in the animals that did't take sodium chloride. At the same time, the L-carnitine usage increased the nitrite anion content in the male's serum (by 54.7 %) and as a result its did't different of the animals without sodium chloride intake. Admission of the dexamethasone for 14 days on the background of the sodium chloride high content in the water caused significant changes of the nitrite anion content in the rats. The content of

investigated metabolite in the animals both sex was significantly decreased (by 50.5 %) but it was lower (by 47.8 %) in the females and the males (by 36.5 %) than in the animals that did't take sodium chloride. The difference between both sex in this case was leveled. The use of L-carnitine for therapeutic purposes in the animals on the background of the sodium chloride high content and dexamethasone was effective in the both sex. So, nitrite anion content in the female's serum was increased (by 2.9 times) and was higher (by 43.4 %) than level in the control animals, and below (by 8.2 %) of the animals level with L-carnitine and dexamethasone, but did't treat with sodium chloride, and also insignificantly different from the intact animals.

In general, changes in the males was observed similar, but less pronounced reaction on the correction with L-carnitine of dexamethasone effects on the background of sodium chloride high concentration in water. The L-carnitine led to the increase of the nitrite anion (by 2.6 times), what was (by 28.4 %) higher as compared with the control animals with a high content of sodium chloride in water, but lower (by 17.2 %) than in the treated males with L-carnitine and dexamethasone with usual intake sodium chloride and by 18.4 % in relating of the intact animals. In general, the content of nitrogen monoxide metabolite in the males who had elevated sodium chloride content in water and treated with L-carnitine and dexamethasone, was lower than in the females (by 20.3 %), but lower (by 17.2 %) than in the treated males with L-carnitine and dexamethasone with usual intake sodium chloride and (by 18.4 %) on the intact animals. In general, the content of nitrogen monoxide in the investigated males who had elevated sodium chloride content in water and treated with L-carnitine and dexamethasone, was lower than in the females (by 20.3 %). but lower (17.2 %) than in the treated males with L-carnitine and dexamethasone with usual intake sodium chloride and as compared to the intact animals (by 18.4 %). The content of the nitrogen monoxide metabolite in the investigated males who intaken elevated sodium chloride content in water and was treated with L-carnitine and dexamethasone, was lower than in the females (by 20.3 %).

The content of nitrite anion in the atrial myocardium of the intact animals was significantly lower (by 12.7 %) in the males as compared to the females, and on the background of the L-carnitine intake – 13.5 %. The usage of the dexamethasone in the males caused decrease of the nitrite anion content as compared with the females (by 13.4 %), and use of L-carnitine on the background for long-term administration of the dexamethasone the difference was 13.9 %.

Table 2. Nitrite anion content⁻ in the atrial heart tissue for the experiment

Rats	Groups of animals:							
	Control		L-carnitine		Dexamethasone		Dexamethasone + L-carnitine	
Sex:	female	male	female	male	female	male	female	male
The form of experiment	NO ₂ ⁻ level in the atrium heart tissue, 10 ⁻⁵ mol/kg, M ± σ							
Intake of usual quantity NaCl	1,899 ± 0,083	1,657 ± 0,092§	1,934 ± 0,075	1,672 ± 0,070§	1,847 ± 0,088	1,600 ± 0,094§	1,923 ± 0,075	1,655 ± 0,091§
Intake of 4 % solution NaCl	1,896 ± 0,093	1,638 ± 0,078§	1,941 ± 0,076	1,667 ± 0,070§	1,820 ± 0,075	1,550 ± 0,079§	1,939 ± 0,072	1,679 ± 0,072§

Note: § p < 0,05 – significance of differences between the male and the female rats.

Significant difference of investigated metabolite content in the animals of different sex was found in conditions of intake higher concentrations of the sodium chloride in water. That index in the control males was lower (by 13.6 %) as compared to the females, in the treated animals with L-carnitine difference was 14.1 %, on the dexamethasone – 14.9 %, the combination of L-carnitine and dexamethasone – 13.4 %. L-carnitine, dexamethasone and increased content of sodium chloride in the water didn't cause significant changes on the nitrite anion content in the atrial myocardium.

Table 3. Nitrite anion content⁻ in the ventricular heart tissue for the experiment

Rats	Groups of animals:							
	Control		L-carnitine		Dexamethasone		Dexamethasone + L-carnitine	
Sex:	female	male	female	male	female	male	female	male
The form of experiment	NO ₂ ⁻ level in the ventricular heart tissue, 10 ⁻⁵ mol/kg, M ± σ							
Intake of usual quantity NaCl	1,677 ± 0,075	1,515 ± 0,084§	1,790 ± 0,117*	1,604 ± 0,075§	1,249 ± 0,077*	1,194 ± 0,095*	1,765 ± 0,077	1,530 ± 0,070§
Intake of 4 % NaCl	1,590 ± 0,077	1,460 ± 0,087§	1,821 ± 0,078*	1,581 ± 0,081*§	1,210 ± 0,074*#	1,105 ± 0,070*#§	1,698 ± 0,064*#	1,491 ± 0,074*#§

Note: * p < 0,05 – significant differences in relation to the control group;
 # p < 0,05 – significant differences in relation to the group with normal level of sodium chloride;
 § p < 0,05 – significant differences between the male and the female rats;

The determination of the nitrite anion in the ventricular myocardium showed that index in the intact males was lower (by 9.7 %) as compared to the females. Daily intake of the

L-carnitine contributed to the insignificant increase of the studied metabolite (by 6.7 %), in the males insignificant changes were found (the difference between different animals sex was by 10.4 % (lower level in the males)). Long-term use of the dexamethasone caused reduction of the nitrite anion content in ventricular myocardium in the animals of both sex – the females 25.5 %, the males – 21.2 %.

The use of the L-carnitine on the background of long-term use of the dexamethasone was resulted in increased level of the nitrite anion (by 41.3 %) in the females and the males (by 28.2 %) and accordingly restore its contents to relative the intact animals in respect of sex. The difference in the levels of the studied parameters in this case between sex – in the males that index was lower (by 13.3 %) than in the females.

The content of the nitrite anion unchanged as compared to the intact animal that intake water with increased sodium chloride content. The content of this metabolite in the males under these conditions was lower (by 8.2 %) than in the females. The L-carnitine on the background of the high content of sodium chloride in the water contributed to a significant increase this parameter in the females (by 14.5 %), the males (by 8.2 %) (in the females it was higher (by 13.2 %) as compared to the males.

These groups also did't differ from the animals group that intaken usual quantity sodium chloride and the L-carnitine. The dexamethasone on the background of the high sodium chloride content in the water as compared to the control group resulted to the decrease of nitrite anion content (by 23.9 %) in the females and males (by 24.4 %) that in the females corresponded to the level of the animals that did't intake increased amounts of sodium chloride, and in the male was still lower (by 7.5 %) than the corresponding group with usual intake sodium chloride. The anion nitrite level in the male in this situation was significantly lower (by 8.7 %) than in the females. The use of L-carnitine resulted to significant increase of the studied metabolite level in rat's ventricular myocardium in the females – 40.3 %. This level was higher (by 6.8 %) as compared with control animals that intaken water with high content of sodium chloride, but did't differ significantly from level of the animals with L-carnitine correction on the background prolonged use of the dexamethasone, did't take high concentration of sodium chloride and the intact group. Males that intaken sodium chloride and dexamethasone with the L-carnitine led to increased level of nitrite anion in ventricular myocardium (by 35 %), which in turn graded as negative effects of sodium chloride and dexamethasone as well as on this metabolite, The nitrite anion content in the females as compared to the males significant was higher (12.2 %).

Conclusions

1. Long-term use of dexamethasone as the high content of sodium chloride in the water, lead to a sharp reduction of nitrite anion content in the rat's blood serum. The combination of these two factors potentiate action of one another in regard to content of the studied metabolite, the changes are more expressed in the males. L-carnitine conducted to increase the content of nitrite anion in the of animal's blood serum of both sex. The nitrite anion content in blood serum unnormalized only in the males group with high level of sodium chloride in water and prolonged exposure to dexamethasone. The difference of the nitrite anion content between animals of different sex display in the conditions of use the L-carnitine, or a combination of dexamethasone with usual content of sodium chloride in water.

2. The level of nitrite anion in atrial myocardium lower in the males as compared to the females, but its independent of the action of L-carnitine, dexamethasone or high content of sodium chloride in the water.

3. As long-term use of dexamethasone and high content of sodium chloride in the water conduce to reduce of nitrite anion level in the ventricular myocardium. The use of L-carnitine as a correction of that disorders proved effective. The difference in the levels of the studied metabolites between animals of different sex absent only in chronic administration of dexamethasone, in other cases it is always significantly lower in the males as compared to the females.

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