G E O R G I A N
MEDICAL
NEWS

ISSN 1512-0112

No 12 (237) Декабрь 2014

ТБИЛИСИ - NEW YORK

ЕЖЕМЕСЯЧНЫЙ НАУЧНЫЙ ЖУРНАЛ

Медицинские новости Грузии

GEORGIAN MEDICAL NEWS

No 12 (237) 2014

Published in cooperation with and under the patronage
of the Tbilisi State Medical University

Издается в сотрудничестве и под патронажем
Тбилисского государственного медицинского университета

ЕЖЕМЕСЯЧНЫЙ НАУЧНЫЙ ЖУРНАЛ
ТБИЛИСИ - НЬЮ-ЙОРК

ENERGY METABOLISM DISORDERS IN RAT SALIVARY GLANDS TISSUES IN CONNECTION WITH CHRONIC SODIUM NITRATE INTOXICATION

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The research has been carried out within the research scientific work, entitled "Inborn and Acquired Morphofimctional Disorders of Dentition, Organs, Head and Neck Tissues, Their Diagnostics, Surgical and Conservative Treatment", State Registration No. 0111U00630 II.

Salivary glands are highly sensitive organs, which respond to the major lesions in human organism both in physiologi-cal and pathological conditions [4,10]. Nitric oxide(NO) is known as a strong bioregulator. In excessive entering,nitric oxide disturbs functional activity of iron- and copper-bearing proteins-enzymes and generates vigorous prooxidant, i.e., peroxinitrite, while reacting with active forms of oxygen [3,9]. Aggregated nitric oxide has an impact on activation of peroxidation, inhibits energy metabolism, and causes the development of hemic hypoxia. Currently, lesions in salivary glands in excessive production of nitric oxide associated with chronic sodium nitrate intoxication have little been studied. Uninvestigated are mechanisms of salivary glands damage associated with excessive production of nitric oxide "depot", i.e., dinitrosyl iron complexes, in excessive entering of nitrates with drinking water. In case of inflammation, the additional source of nitric oxide production is formed due to increased activity of inducible NO- synthase [6]. Cumulative effect of nitric oxide, produced by exogenous precursors and de novo in various morbid conditions of the organism has little been studied, too. Expansion of nitrates in the environment and their overall impact on the human organism is one of the problems for the nation with developed agricultural sector [2]. The least studied is the response of salivary glands with nitrate reductase activity in intensified production of nitric oxide from ectogenous and endogenous sources. On the ground of pathobiochemical characteristics of excessive production of nitric oxide it is possible to study the effect of oxygenic and copper-bearing couplings, modifying the activity of oxygenic couplings of nitrogen and NO effect [5,11]. Nitric oxide is a strong bioregulator; however, generation of great amount of this compound may cause negative changes in the human organism, related to free - radical characteristics of its molecule, providing unpaired electron, thereafter disturbing the activity of iron- and copper-bearing bio molecules of aconitase, cytochromes [1], resulted in reaction with active forms of oxygen and vigorous prooxidant, i.e., peroxinitrite [1,7]. The effect of nitrates in low doses leads to tissue hypoxia-related chronic intoxication [8].

The purpose of the research was to study the mechanisms of toxic effect of excessive sodium nitrate on the energy metabolism of salivary glands.

Material and methods. The study has been carried out on 100 Wistar rats, weighing 160-250 g, which were divided

into following groups: the 1st group consisted of intact rodents (control); the 2nd group consisted of experimental rodents, where chronic nitrate intoxication has been reproduced during 14, 30, 60 and 90 days. Animal housing and experiments on them have been carried out in compliance with the requirements of international principles of the "European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes" (Strasbourg, 1985) and "General Ethic Rules for Conducting Experiments on Animals", approved by the First National Congress on Bioethics (Kiev, 2001).

Rodents were administered with sodium nitrate in a doze of 200 mg/Kg of body weight daily through special tube intragastrally in a form of water solution.

Tissue sample (salivary glands) were minced, squeezed through chilled press, washed in quintupled outflow medium twice, and then homogenized by Teflon pestle during 30-90 sec. in 50 ml of the outflow medium. Homogenate of salivary glands was centrifuged in angular rotor of TSLR-1 cold centrifuge during 10 min. in 600 g for sedimentation of cells, which were not disintegrated during the process of homogenization. Supernatant was centrifuged during 20 min. in 8000 rev/min. At the same time, suspension protein concentration constituted 40-60mg/ml. All operations, related to homogenate production and mitochondria outflow, have been carried out at 0...+2°C. Respiratory and phosphorylic activity of mitochondria has been measured according to Chance B. and Williams G.R. [12] by means of platinum Clark oxygen electrode on the LP -7E polarograph. The respiration rate has been measured at +24°C in 1 ml of incubation medium, containing 120 M of saccharose, 60 mM KC1, 5 mM KH_2P0_4, 8 mM of succinate, 30 mM of tris-HCl, 5 mM $MgS0_4$, 0,25 mM EDTA, pH 4,7. The essence of the method is in the rate of oxygen uptake by mitochondria, with ADP (250 mcM) present. The phosphorylating respiration rate of the V_3 control (V_3 in the control state 3, according to Chance, with oxygen substrate and ADP present) and V_4 metabolic (V_4 in the metabolic state 4, according to Chance, after phosphorylation of total amount of add ADP) respiration has been calculated on the oxygen uptake curves. Additionally, values, specifying the degree of oxidation and phosphorylation have been calculated:

- respiratory control (RC) has been measured according to Chance B. and Williams G.R. [13], i.e., respiration rate with ADP present to respiration rate after total amount of add ADP had been phosphorylated in ATP ratio: RC = V3/V4;

- phosphorylation efficiency ratio (ADP/O) is the amount of add ADP (in np) to the amount of oxygen uptake during the period of phosphorylation of add ADP [12].

Results and their discussion. The results, shown in Table 1, are proved by the findings, obtained during the analysis of tissue respiration and oxidative phosphorylation, made by the polarographic method. Thus, the increase of phosphorylating respiration (V_3) by 27,6% has been admitted while administering sodium nitrate to rats during 14 days. After more prolonged poisoning a significant lowering has been observed in comparison with intact animals: V_3 after 3O-, 60-, 90-day poisoning by 21,1%, 43,9%, 53,3%, respectively. As for the V_4 values, specifying the tissue respiration with oxygen and substrate present, but without ADP, a significant lowering by 16,0% has been admitted after 90-day intoxication. At the same time, after 60- and 90-day of poisoning the respiratory control lowered by 36,6%, 44,4%, respectively, in comparison with intact animals; after 14-, 30-, 60-, 90-day ofpoisoning the ADP/O lowered by 15,2%, 25,0%, 31,1% and 37,8%, respectively. After 30-, 60- and 90-day of poisoning the activity of cytochromeoxidase (CCHO) lowered by 11,9%, 17,9% and 22,4%, respectively. Inflammation significantly lowers the following values: rate of phosphorylating respiration (V_3) by 62,3%, V_4 by 18,0%, respiratory control by 54,0%, CCHO by 26,4%, ADP/O by 41,5%.

Inhibition of V_3 indicates about the lowering of phosphorylating respiration rate. It inhibits pathways for oxidation substrate transport to mitochondria inner membrane. Substrate transport disorder and oxidation in mitochondria respiratory chain, stimulated by inhibition of Krebs cycle enzymes, causes the development of hypoergasia dissimilation. It has been established that chronic nitrate intoxication leads to the development of hemic hypoxia, resulted in the development of tissue hypoxia as chain reaction in salivary glands tissues, leading to energy metabolism disorder and inhibition of oxidation process in them. Biotransformation of nitrates into nitric oxide inhibits glyceraldehydes-3- phosphatdehydrogenase in connection with nitrosolation of enzyme [9], which limits the ability to compensate bioenergy insufficiency in tissues [1,9]. Lowering of phosphorylating respiration rate (in metabolic state 3 according to Chance) is the major chain in ATP synthesis disorder [12], leading to lowering of ATP- synthase activity, resulting in oxidation and phosphorylation process disorder. During the lowering of respiratory control activity, the mitochondrial respiration control is disturbed as well as interaction between the level of reproduced equivalents and the state of phosphorylation [5,7]. Mitochondrial respiration and phosphorylation disorder in salivary glands tissues in chronic nitrate intoxication is associated with the effect of nitrites to nitric oxide biotransformation product. These processes lead to macroergic couplings biosynthesis decay, consequently inhibiting the processes of anabolism, resulted in disorders of salivary glands functioning.

Table. Changes in rates of respiration and oxidative phosphorylation of mitochondria of rat salivary glands in chronic nitrate intoxication

Study rates	Statistical measurements	Intact animals	14 days	30 days	60 days	90 days
V„ natom *Ol* min. x mg	M ±m	22,*4*± 1,30 .	27,6± 2,96 P <0,01	17,67±1,04 P<0,01	12,56±0,72 P <0,001	10,47±0,60 P <0,001
V_4, natom 0/ min. x mg	M ±m	18,06±1,07	18,56±1,08	17,16±1,02 i	15,90±0,89	15,17±0,86 P <0,05
Respiratory control	M ±m	1,24±0,13	1,54±0,16	1,03±0,11 1	0,79±0,08 P <0,01	0,69±0,07 P <0,001
ADP/O, ADP nmole/natom 0	M ±m	1,64±0,07	1,39±0,06 p<0,01 ;	1,23±0,05 p <0,001 1	1,13±0,05 P <0,001 í	1,02±0,04 P <0,001
/ CCHO u.,act.	M ±m	16,64±0,65	15,35±0,60	14,66±0,58 P <0,05	13,66±0,53 P<0,01	12,91±0,50 P <0,001

note: P - data are assumed only for reliable data in comparison with intact animals

Thus, it has been established that prolonged intake of nitrates in low doses leads to their accumulation in salivary glands tissues that causes tissue respiration disorder at the cellular level.

Perspectives. It is planning further investigation of nitrites effect on salivary glands functioning in rats during prolonged entering to the organism in low doses in the form of water solution.

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SUMMARY

ENERGY METABOLISM DISORDERS IN RAT SALIVARY GLANDS TISSUES IN CONNECTION WITH CHRONIC SODIUM NITRATE INTOXICATION

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The purpose of the research was the study of nitrite impact on energy metabolism in salivary gland tissues in connection with chronic nitrate intoxication. The study has been

carried out on 100 Wistar rats, weighing 160-250 g, which were divided into following groups: the 1st group consisted of intact rodents (control); the 2nd group consisted of experimental rodents, where chronic nitrate intoxication has been reproduced during 14,30,60 and 90 days. Intoxication leads to hypoxia, which complications cause tissue hypoxia. Has been established that prolonged intake of nitrates in low doses leads to their accumulation in salivary glands tissues all these result in derangement of metabolism at the intermembrane cellular level. At the same time energy metabolism in salivary glands is inhibited, resulting in their dysfunction at the excretory and endocrine levels.

Keywords: nitrites, mitochondrial respiration, salivary glands, energy metabolism, phosphorylation.

РЕЗЮМЕ

НАРУШЕНИЕ ЭНЕРГЕТИЧЕСКОГО МЕТАБОЛИЗМА В ТКАНЯХ СЛЮННЫХ ЖЕЛЕЗ КРЫС В УСЛОВИЯХ ХРОНИЧЕСКОЙ ИНТОКСИКАЦИИ НИТРАТОМ НАТРИЯ

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Целью исследования явилось изучение влияния нитритов на энергетический метаболизм в тканях слюнных желез на фоне хронической нитратной интоксикации. Исследования проведены на 100 крысах линии Вистар, весом 160-250 гр, которые были разделены на группы: 1 группа -интактные животные (контроль), II группа - опытная, в которой моделировали хроническую нитратную интоксикацию в течение 14, 30, 60, 90 суток.

В результате проведенных исследований установлено, что на фоне интоксикации развивается гемическая гипоксия, осложнения которой ведут к тканевой гипоксии. При длительном поступлении нитратов в малых количествах происходит их накопление в тканях слюнных желез, что приводит к нарушению обменных процессов на межмембранном уровне клеток, угнетается энергетический метаболизм в слюнных железах, нарушаются экскреторная и эндокринная функции.