9. Sonu Y, Avinash SS, Sreekantha, Arun KK, Malathi M, Shivashankara AR. Effect of estrogen on altering the serum and urinary levels of calcium, phosphate and magnesium in hysterectomised women compared to natural menopausal south indian women: a case control study. Indian journal clinical biochemistry. 2016;31(3):326–331. doi: 10.1007/s12291-015-0532-y.
10. Shugurov OO. The evoked potentials of spinal cord under influence of mechanical stimuli. Regulatory Mechanisms in Biosystems. 2011; 2(1): 125–129. doi: 10.15421/021120.
11. Zhang Y, Xiao X, Zhang XM, Zhao ZO, Zhang YO. Estrogen facilitates spinal cord synaptic transmission via membrane-bound estrogen receptors. Journal of Biological Chemistry. 2012;287(40):33268–33281. doi:10.1074/jbc.M112.368142
12. Zhong Y.Q. K.C. Li, X. Zhang. Potentiation of excitatory transmission in substantia gelatinosa neurons of rat spinal cord by inhibition of estrogen receptor alpha. Molecular Pain. 2010;6.92. doi: 10.1186/1744-8069-6-92

Стаття надійшла 11.10.2020 р.

DOI 10.26724/2079-8334-2021-4-78-246-250
UDC 591.1./5:616-07:616.316-06:[616-056.52+616.45-001.1/3]-092.9

Y.O. Chyzhanska, N.M. Slobodinyk, A.Ye. Omelchenko, K.S. Neporada
Poltava State Medical University, Poltava

INFLUENCE OF BEHAVIORAL PHENOTYPING ON THE DEVELOPMENT OF PATHOLOGICAL CHANGES IN THE SALIVAL GLANDS OF RATS AGAINST THE BACKGROUND OF OBESITY AND STRESS

e-mail: neporadaks@gmail.com

Pathological changes in rat salivary glands depending on behavioral phenotyping were analyzed in a model of glutamate-induced obesity and the combined effects of obesity and stress. The study found that under conditions of modeling immobilization stress and the combined effects of obesity and stress, probable changes in the concentrations of thiobarbituric acid reactants, oxidative modified proteins and catalase in the salivary glands of stress-resistant and non-stress-resistant rats compared to controls. A probable difference between the studied parameters in the salivary glands is observed between rats with different stress resistance only in the content of thiobarbituric acid reactants. Thus, under conditions of glutamate-induced obesity and chronic stress in the salivary glands of rats with different behavioral phenotyping, a significant increase in the number of thiobarbituric acid reactants, an increase in oxidative modified proteins and a decrease in catalase activity, indicating a decrease in antioxidant system activity.
Key words: stress resistance, oxidative stress, sodium glutamate.

Ю.О. Чижанська, Н.М. Слободяник, О.Є. Омельченко, К.С. Непорада

ВПЛИВ ПОВЕДІНКОВОГО ФЕНОТИПУВАННЯ НА РОЗВИТОК ПАТОЛОГІЧНИХ ЗМІН У СЛИННИХ ЗАЛОЗАХ ЩУРІВ НА ТЛІ ОЖИРІННЯ ТА СТРЕСУ

На моделі глутамат-індукованого ожиріння та поєднаної дії ожиріння і стресу проаналізовані патологічні зміни у слинних залозах щурів в залежності від поведінкового фенотипування. Дослідження виявило, що за умов моделювання іммобілізаційного стресу та сполученої дії ожиріння і стресу були виявлені вірогідні зміни концентрацій реактантів тіобарбітурової кислоти, окисно-модифікованих білків та каталази у слинних залозах щурів стресостійкого та стресонестійкого типу порівняно з контролем. Вірогідна різниця між досліджуваними показниками у слинних залозах спостерігається між різними за стресостійкістю щурами лише за вмістом реактантів тіобарбітурової кислоти. Таким чином, за умов глутамат-індукованого ожиріння та хронічного стресу у слинних залозах щурів з різним поведінковим фенотипуванням визначено достовірне зростання кількості реактантів тіобарбітурової кислоти, підвищення вмісту окисно-модифікованих білків і зниження активності каталази, що свідчить про зниження активності антиоксидантної системи та розвиток оксидативного стресу.
Ключові слова: стресостійкість, оксидативний стрес, глутамат натрію.

The study is a fragment of the research project "Features of pathological changes development in the digestive system under different conditions and the development of methods for their correction", state registration No. 0120U100502.

The World Health Organization and other international organizations consider obesity a pandemic of this century. Obesity plays a crucial pathophysiological role in the development of insulin resistance, dyslipidemia and hypertension, which leads to type 2 diabetes and increased risk of cardiovascular disease, obstructive sleep apnea, osteoarthropathy and others [7, 8, 11].

New accents of the classical concept of stress, which were proposed at the end of the last century by Sudakov K.V. argue that the basis of stress syndrome in addition to non-specific mechanisms of its implementation are specific systemic hormonal and tissue mechanisms. In the works of most researchers analyzing the various manifestations of stress syndrome are used, as a rule, averaged physiological and biochemical parameters without taking into account the individual typological characteristics of the body.

However, back in 1992 Simonov P.V. proved that stress is an indicator of individual-typological differences. Fedorenko Y.V., Grzegotski M.R. [6] note that the formation of adaptive reactions of the body

has an individual specificity that correlates with characteristics of the nervous system. Individual properties of the CNS determine the variety of forms of sensitivity and stress resistance of the body to stressors and factors, behavior, the nature of the functional activity of body systems, the state of internal organs, immunobiological reactions etc.

We have previously shown that under conditions of acute and chronic stress, the intensity, direction and change over time of biochemical parameters of the oral cavity tissues and pancreas depends on individual typological characteristics of animal behavior [4,5].

One of the main organs and tissues affected by obesity and its consequences are the salivary glands, which are responsible for the secretion of enzymes, growth factors necessary for oral cavity homeostasis, as well as for its protection. To date, the results of studies indicate the possible impact of obesity on the morphology and function of the salivary glands, although it has been reported in relation to oral diseases such as periodontitis, dental caries and others closely related to saliva and salivary gland function [10].

The pathogenesis of salivary gland damage in obesity is not fully understood, although the effects of oxidative stress are emphasized. According to the authors [1, 9], it was proved that obesity in adults is associated with disorders of antioxidant systems and oxidative modification of proteins in saliva. Also, at present, the question of the effect of the combined action of obesity and stress on the salivary glands remains open.

The purpose of this study was to study the effect of the combined action of obesity and stress on the salivary glands of rats depending on behavioral phenotyping.

Materials and methods. Experimental studies were performed on 51 white nonlinear rats of both genders, which were simulated glutamate-induced obesity and chronic stress. Obesity simulations were replicated by postnatal subcutaneous administration of sodium glutamate to newborn rats [3] at the dose of 4 mg/g on the 2nd, 4th, 6th, 8th and 10th days after birth. Rats of the control group were administered subcutaneous saline at the dose of 8 μl/g. For 4 months, the animals were kept on the usual diet of the vivarium. Weight, BMI, and Lee index were monitored throughout the experiment. Chronic stress was modeled by G. Selye by immobilizing animals on their backs for 5 hours during the last week before slaughter. The neuroethological Open Field test and the factor-analytical method were used to study the behavioral phenotyping of rats. The neuroethological test was performed in a month-old rats at a well-defined time (10 a.m.) for 6 minutes, analyzing Open Field variables: latency of the first movement, number of squares crossed, number and time in the center of the field, rearing, grooming and number of boluses. Stress-resistant rats had a high rate of adaptation, low rates of motor research behavior and autonomic balance, and stress-unbalanced rats included animals with low adaptation rates, high motor research behavior and autonomic balance indices [2].

Slaughter of 4-month-old animals was performed 2 hours after immobilization stress under thiopental anesthesia by bloodletting, removal was performed of salivary glands, stomach, thymus, adrenal glands and visceral fat.

The concentration of TBA-active products in the salivary gland homogenate was determined by the method of I.D. Stalnaya and T.G. Garishvili (1977), catalase activity – by the method of M.A. Korolyuk et al. (1988) and the content of oxidatively modified proteins (OMB) –by the method of O.Yu. Dubinina.

The obtained results were subjected to statistical processing using Microsoft Office Excel and extending it to Real Statistics 2019. To determine the statistically significant difference between the groups the Mann-Whitney test was used.

Results of the study and their discussion. Oxidative stress, defined as an imbalance between the generation of reactive oxygen species and antioxidant protection in the human body, is a risk factor that plays a significant pathogenetic role for noncommunicable diseases. Integral indices that reflect its development include the content of TBA-reactants as secondary products of lipid peroxidation and the content of oxidatively modified proteins, which reflects the degree of carbonyl stress.

We found that under the conditions of modeling of immobilization stress and the combined effect of obesity and stress, probable changes in the concentrations of TBA-active products in the salivary glands of rats were detected compared to the control (table 1).

Under conditions of chronic stress, the number of TBA reactants in the salivary glands of stress-resistant rats increased by 26 % compared to the control group of animals of the respective type of response. The content of TBA reactants in the salivary glands of stress-resistant rats under stress against the background of obesity was significantly higher by 1.2 times compared to the group of stress-resistant rats under the same conditions. Thus, the activation of lipid peroxidation in the salivary glands of animals under the combined effects of glutamate-induced obesity and immobilization stress depends on the individual typological characteristics of the body.

Table 1

The content of TBA-reactants in the salivary glands of rats, dependingon stress resistance of the body, μmol/g (M±m)

Groups of animals	Stress resistant	Stress-unbalanced
1. Control	16.07±1.30 (n=5)	15.48±1.80 (n=5)
2. Obesity	16.96±2.32 # (n=8)	22.13±2.01 (n=8)
3. Stress	20.75±2.01 (n=5)	24.79±2.34*^ (n=5)
4. Obesity+ stress	21.66±3.10 (n=8)	26.77±2.67*^ (n=8)

Note: in this and the following tables: * -compared to the stress-resistant type; ^ - compared to the control of the respective type of response; # - compared to a group of rats with glutamate-induced obesity and stress.

According to our data in table 1, the amount of TBA reactants in the salivary glands in the group of stress-resistant rats under obesity and stress was by 20 % higher than in the group of stress-resistant rats, indicating increased oxidative stress depending on behavioral phenotyping.

Studies by Zalewska A. et al. [14] showed that increased production of free radicals exceeds the antioxidant system's abilities of adolescents with obesity and overweight both at the central level and in the salivary glands, which was observed in increased concentrations of oxidative modification products in plasma and unstimulated and stimulated saliva. They note that enhanced oxidative modifications show a tendency in obese adolescents compared to their overweight peers and argue that oxidative damage occurred in the salivary glands of overweight adolescents and was more pronounced in obese individuals. According to the authors, this is a disturbing phenomenon that may indicate the beginning of subclinical ineffectiveness of antioxidant mechanisms, which will lead to increased oxidative damage to the salivary glands of obese adolescents.

It is known that the earliest sign of the oxidative stress development are the products of oxidative lipid modification, because the cell membrane and its lipids are the first to be exposed to free radicals. As the concentration of ROS further increases, the concentration of lipid peroxidation products increases, as well as proteins that undergo oxidative modification.

According to the data shown in table 2, the content of oxidatively modified proteins in the salivary glands of rats with glutamate-induced obesity and chronic stress is significantly higher compared to the group of the control rats. No differences in the content of oxidatively modified proteins in the salivary glands between the groups of stress-resistant and stress-unbalanced rats were found.

Table 2

The content of oxidatively modified proteins in the salivary glands of rats depending on behavioral phenotyping, RU (M±m)

Groups of animals	Stress resistant	Stress-unbalanced
1. Control	0.129± 0.003 (n=5)	0.155±0.011* (n=5)
2.Obesity	0.206±0.031^ (n=8)	0.221±0.028^ (n=8)
3.Stress	0.173± 0.007^ (n=5)	0.222±0.037^ (n=5)
4. Obesity + stress	0.221± 0.020^ (n=8)	0.234±0.030^ (n=8)

Analyzing the baseline level of oxidatively modified proteins in the salivary glands of the control animals, we found probable changes in this index between stress-resistant and stress-unbalanced rats, which indicates individual typological features of redox balance.

Assessing the antiradical protection of salivary glands by catalase activity, we found that in the group of stress-resistant rats under glutamate-induced obesity and stress, catalase activity decreased by 38 % compared to the control group of rats of this type (fig. 1).

Under the comorbidity of obesity and stress factors, catalase activity in the salivary glands of stress-resistant rats decreased by 37 % compared to the control group of rats of the respective type, indicating a decrease in antioxidant protection. Thus, the activity of antiradical protection in the tissues of the salivary glands under the combined action of obesity and stress is not affected by individual-typological features of the rats' behavior.

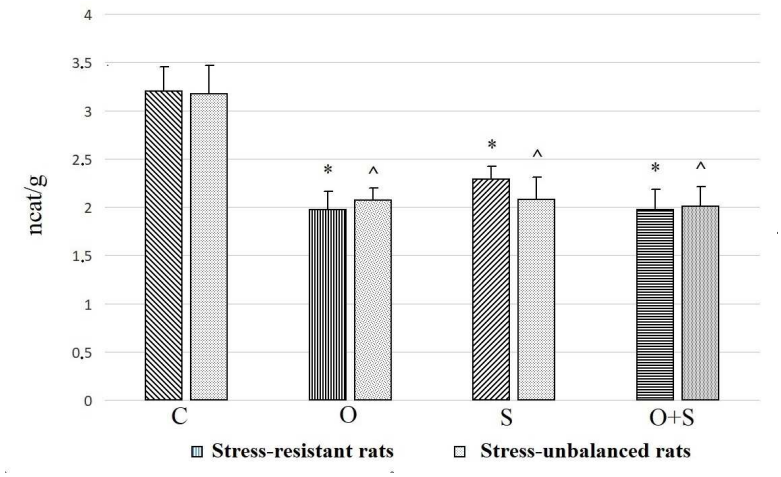

Fig. 1. Catalase activity (M±m) (n=8)

C – control group of rats; O – group of rats with glutamate-induced obesity; S – group of rats exposed to stress; O+S is a group of glutamate-induced obese stress-unbalanced rats. Note: * compared to the control of stress-resistant rats; ^ - compared to the control of stress-unbalanced rats.

Increased reactive oxygen species in the salivary glands and their cytopathological consequences are associated with the onset and development of periodontal tissue, precancerous conditions of the oral cavity, as well as diabetes, insulin resistance and obesity [9].

A high-calorie, high-fat diet causes mitochondrial dysfunction in the salivary glands of animals, increasing ROS production and reducing antiradical protection [15].

Interestingly, in our modern society, the obesity pandemic coincides with an increase in factors that enhance cortisol production, such as chronic stress, consumption of foods with a high glycemic index and decreased sleep. This indicates a vicious circle where the strengthening of glucocorticoids, obesity and stress interact and enhance each other [12]. This hypothesis is supported by recent studies showing a significant correlation between obesity and long-term cortisol levels measured in hair in both adults and children [13].

Conclusion

Thus, under conditions of glutamate-induced obesity and chronic stress in the salivary glands of rats with different behavioral phenotyping, a significant increase in the number of thiobarbituric acid reactants, increased content of oxidatively modified proteins and decreased catalase activity were revealed, indicating reduced antioxidant activity. The probable difference between the studied parameters in the animals' salivary glands under stress against the background of obesity is observed between rats with different stress resistance only in the content of TBA reactants, i.e., intensification of lipid peroxidation more depends on behavioral phenotyping than on carbonyl stress and antioxidant defence.

References

1. Hordiyenko LP, Berehova TV, Neporada KS, Falalyeyeva TM. Rozvytok oksydatyvnoho stresu v tkanynakh slynnykh zaloz shchuriv za umov hlutamatindukovanoho ozhyrinnya. Fiziolohichnyy zhurnal. 2014; 60 (4): 105–7. [in Ukrainian] DOI: https://doi.org/10.15407/fz60.04.105

2. Mayorov OYU. Neyrodinamicheskaya struktura sistemnykh mekhanizmov ustoychivosti k emotsional'nomu stressu [dissertatsiya]. Moskva; 1988. 45 s. [in Russian]

3. Rudyk MP, Pozur VV, Opeyda YEV, Voyeykova DO, Khranovska NM, Fedorchuk OH. ta in. Modulyatorni efekty hlutamatu natriyu na funktsiyi tsyrkulyuyuchykh fahotsytarnykh klityn shchuriv in vivo ta in vitro. Dopov. Nats. akad. nauk Ukr. 2017; 5: 89–95. [in Ukrainian]

4. Slobodyanyk NM. Patohenetychne obgruntuvannya korektsiyi patolohichnykh zmin u pidshlunkoviy zalozi v zalezhnosti vid stresostiykosti tvaryn [dysertatsiya]. Kharkiv: Kharkivskyy natsionalnyy medychnyy universytet; 2018. 22 s. [in Ukrainian]

5. Tarasenko LM, Bilets MV. Mekhanizmy stresornykh ushkodzhen tkanyn porozhnyny rota (korotkyy ohlyad literatury i vlasnykh doslidzhen). Ukrayinskyy stomatolohichnyy almanakh. 2016; 1(2): 23–5. [in Ukrainian]

6. Fedorenko YUV, Hzhehotskyy MR. Vplyv poyednanoyi diyi immobilizatsiynoho stresu i kadmiyu na dynamiku biokhimichnykh pokaznykiv u shchuriv z aktyvnym i pasyvnym typom povedinky. Fiziolohichnyy zhurnal. 2020; 66(1): 44–51. [in Ukrainian] DOI: https://doi.org/10.15407/fz66.01.044

7.Cuevas AG, Chen R, Thurber KA, Slopen N, Williams DR. Psychosocial Stress and Overweight and Obesity: Findings From the Chicago Community Adult Health Study. Ann Behav Med. 2019;53(11):NP. doi:10.1093/abm/kaz008

8. Hernández Bautista RJ, Mahmoud AM, Königsberg M, López Díaz Guerrero NE. Obesity: Pathophysiology, monosodium glutamate-induced model and anti-obesity medicinal plants. Biomed Pharmacother. 2019 Mar;111:503–516. doi: 10.1016/j.biopha.2018.12.108.

9. Kołodziej U, Maciejczyk M, Miąsko A, Matczuk J, Knaś M, Żukowski P, et al. Oxidative Modification in the Salivary Glands of High Fat-Diet Induced Insulin Resistant Rats. Front Physiol. 2017 Jan 26;8:20. doi: 10.3389/fphys.2017.00020.

10. Roa I, Del Sol M. Obesity, salivary glands and oral pathology. Colomb Med (Cali). 2018 Dec 30;49(4):280–287. doi: 10.25100/cm.v49i3.3919.

11. Rangel-Huerta OD, Pastor-Villaescusa B, Gil A. Are we close to defining a metabolomic signature of human obesity? A systematic review of metabolomics studies. Metabolomics. 2019;15(6):93. Published 2019 Jun 13. doi:10.1007/s11306-019-1553-y

12. van der Valk ES, Savas M, van Rossum EFC. Stress and Obesity: Are There More Susceptible Individuals? Curr Obes Rep. 2018 Jun;7(2):193–203. doi: 10.1007/s13679-018-0306-y.

13.van Rossum EF Obesity and cortisol: New perspectives on an old theme. Obesity (Silver Spring). 2017 Mar; 25(3):500–501.

14. Zalewska A, Kossakowska A, Taranta-Janusz K, Zięba S, Fejfer K, Salamonowicz M, et al. Dysfunction of Salivary Glands, Disturbances in Salivary Antioxidants and Increased Oxidative Damage in Saliva of Overweight and Obese Adolescents. J Clin Med. 2020 Feb 17;9(2):548. doi: 10.3390/jcm9020548
15. Zalewska A, Ziembicka D, Żendzian-Piotrowska M, Maciejczyk M. The Impact of High-Fat Diet on Mitochondrial Function, Free Radical Production, and Nitrosative Stress in the Salivary Glands of Wistar Rats. Oxid Med Cell Longev. 2019;2019:2606120. Published 2019 Jul 4. doi:10.1155/2019/2606120

Стаття надійшла: 14.12.2020 р.

DOI 10.26724/2079-8334-2021-4-78-250-254
УДК 572.786-546.175

O.Yu. Chumachenko, O.G. Redka[1], V.S. Cherno[1]
Separate structural subdivision of the of Higher Education Institution "Open International University of Human Development "Ukraine", Mykolayiv Institute of Human Development, Mykolayiv; [1]Petro Mohyla Black Sea National University, Mykolayiv

ULTRASTRUCTURAL CHANGES OF THE ADENOGIPOPHYSIS INTERMEDIATE PART UNDER LONG-TERM ACTION OF NITRATES AND MEANS OF CORRECTION BY METHYLENE BLUE

e-mail: youngmykolayiv@gmail.com

The perpose of the work was to study the ultrastructural changes of melanotropic cells in the intermediate part of the adenohypophysis in 14-, 45- and 90-day-old rats under the action of nitrates and correction with methylene blue. The results of the study showed that the simultaneous action of nitrates and methylene blue had a positive effect on the structure and function of melanotropic cells of the intermediate lobe of the adenohypophysis in all periods of the study. Methylene blue mitigated their toxic effect on melanotropics of the adenohypophysis intermediate and was a kind of protector of nitrates, reducing the manifestation of the depletion stage of the general adaptation syndrome and helping to restore hormonal balance in the intermediate adenohypophysis. The obtained results open the prospect of further study of structural and functional changes patterns in the endocrine glands under nitrate intoxication and provide a basis for developing effective and safe preventive measures to correct endocrine disorders in humans as a result of professional activities.

Key words: nitrate intoxication, simultaneous exposure, melanotropic cells, ultrastructural changes, protective effect.

О.Ю. Чумаченко, О. Г. Редька, В.С. Черно

УЛЬТРАСТРУКТУРНІ ЗМІНИ ПРОМІЖНОЇ ЧАСТКИ АДЕНОГІПОФІЗА ЗА УМОВ ТРИВАЛОЇ ДІЇ НІТРАТІВ ТА ЗАСОБІВ КОРЕКЦІЇ МЕТИЛЕНОВИМ СИНІМ

Метою дослідження було вивчення ультраструктурних змін меланотропних клітин проміжної частки аденогіпофіза у щурів 14-, 45- і 90- добового віку під дією нітратів та корекції метиленовим синім. Результати дослідження показали, що одночасна дія нітратів і метиленового синього позитивно впливала на структуру і функцію меланотропних клітин проміжної частки аденогіпофіза в усі періоди дослідження. Метиленовий синій на фоні надходження в організм нітратів зм'якшував їх токсичний ефект на меланотропи проміжної частки аденогіпофіза та був своєрідним протектором дії нітратів, знижуючи прояв стадії виснаження загального адаптаційного синдрому і сприяючи відновленню гормональної рівноваги в проміжній частці аденогіпофіза. Отримані результати відкривають перспективу подальшого вивчення закономірностей структурно-функціональних змін залоз внутрішньої секреції в умовах дії нітратної інтоксикації та представляють підґрунтя для розробки ефективних та безпечних профілактичних заходів корекції уражень ендокринної системи, що виникають у людей у результаті професійної діяльності.

Ключові слова: нітратна інтоксикація, одночасний вплив, меланотропні клітини, ультраструктурні зміни, протекторна дія.

The study is a fragment of a research project "Histophysiological state of the endocrine system under the influence of adverse environmental factors", state registration No. 0120U002026.

Currently, in various regions of Ukraine there is significant contamination of soils, groundwater, which in combination with inflated levels of pesticides, radionuclides and other substances poses a real threat to all parts of the ecosystem [4, 8]. However, unlike other xenobiotics, the danger of nitrates on the homeostasis of humans and animals is still not fully understood by society today.

The effects of nitrates are complex and multifaceted. They are manifested in acute poisoning, malignant neoplasms, congenital malformations, infant mortality. This diverse action of nitrates causes significant structural and functional changes in various organs and systems of the body and especially in the endocrine glands, which are known to provide a complex set of compensatory-adaptive processes in the body under any extreme effects [11, 15].

It is known that the pathogenetic basis of eco-dependent diseases are violations in the process of the organism adaptation [14]. The main mechanism of damaging action of nitrates is free radical oxidation