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## THE GINGIVAL TISSUES REACTION ON HYPOBARIC HYPOXIA ACTION ACCORDING TO VARYING DURATION PHOTOPERIOD IN IMMATURE FEMALE RATS

**Key words:** *tissue proteolysis, photoperiod, hypobaric hypoxia, gingival tissues, immature female rats.*

**Abstract.** *This study showed peculiarities of the reaction of proteolytic processes indices on hypobaric hypoxia, influenced by constant light, in the gingival tissues in immature female rats. It has been established that modeling of the hypobaric intermittent hypoxia equal the altitude 4000 meters (2 hours per day for 14 days) decreases proteolysis intensity in the gingival tissues in immature female rats. The pineal gland hypofunction by means of a constant illumination significantly influences upon the character of changes of the proteolytic processes in the gingival tissues, caused by systemic hypobaric hypoxia at a combined use of the indicated influences.*

### Introduction

In recent years it has become apparent in dental research that in periodontal health and disease environmental factors play an important role in physiological and pathological situations [16]. Hypoxia, as an environmental factor, is one of the condition of usual mode of life and, at the same time, growth factor levels of reactive oxygen species (ROS) [15]. The presence of polyorganic, morpho-functional disturbances, formed at participation of the neuro-endocrine system [10] and the manifestations of which depend on the age, sex and the presence of influence of other environmental factors is a peculiar feature for systemic adaptation to hypoxia. Hypoxia results in decrease of energetic potential, macroergic phosphate and pyruvate dehydrogenase content [6]. The intensity of the response of organs and tissues of the body depends on the hypoxia level and sensitivity of the tissue to hypoxia [13, 17]. Possibility of the development of cross-adaptation according to hypoxia enables to use moderate hypoxic effects for the purpose hypoxic preconditioning of pathological changes caused by other factors [3, 11]. Hypobaric hypoxia influence upon expression of gene of sensitivity to hypoxia that may create protective effect under various pathologies, including periodontal [4, 9]. The nature of proteolytic activity in peripheral tissues changes in the process of reaction to a variety of environmental factors, that may be both a manifestation of regenerative processes, and their involvement in the mechanisms of apoptosis [7].

Hormones have potent effects on the major determinants of the developments and functions of oral cavity organs and tissues including gingiva. They can influence the periodontium at different life time. Peri-

dontal glucocorticoid system and its possible role in the periodontum diseases are described, testosterone receptors are found in periodontal tissues and results showed that testosterone may have anti-inflammatory effects on the periodontum [1]. Sex hormones play an important role in periodontal health and disease. It is clear that endogenous sex steroid hormones play significant roles in modulating the periodontal tissue responses [14].

Melatonin is the main hormone of the pineal gland of the brain, which not only synchronizes the rhythms of peripheral tissues but also has anti-stress and antioxidant defenses and provides the body adapt to the dangerous effects of the environment, including hypoxia [5], and it has a significant effect on the reproductive system [8]. Among the endocrine glands, which are studied in connection with periodontal health and disease epiphysis does not deserve enough attention of researchers.

Specific characteristics of the reaction proteolytic activity in the gingival tissues in immature female rats on hypoxic hypoxia under conditions of the altered regime of illumination remain not studied.

### The aim of the research

To study of specific characteristics of fibrinolysis in the gingival tissues in immature female rats under ordinary conditions of holding and hypoxic conditioning by systemic intermittent hypobaric hypoxia of changes, caused by permanent lighting.

### Material and methods

Experiments were carried out on 40 female immature white laboratory rats aged 1 month with an average body weight of 0,052 kg. The changes of

photoperiod duration were modeled by permanent day-and-night lighting (500 lux light intensity) a model of decreasing of melatonin-producing function of the pineal gland (group 3, and 4). Hypobaric hypoxia was created in the flowing pressure chamber by means rarefaction of air till the value corresponding to a height of 4000 m above sea level with "lifting" velocity 0.4 km / min [12]. Under hypoxic conditions the animals were held for 2 hours daily during 14 days (group 2 and 4). Animals of group 4 were held at simultaneous action of hypobaric hypoxia and constant lighting. Intact rats (group 1) holding under conditions of natural light and normal atmospheric pressure, were used as control ones. The following day after the completion of experimental influence all animals were decapitated under slight diethyl ether narcosis. The gingival tissues was taken out on cold immediately after the decapitation and the weighted samples were homogenized in 2.0 ml of cooled borate buffer (pH 9.0). The homogenate was used in biochemical analysis. The proteolytic activity was determined according azoalbumin, azokazein and

azokol lysis [2].

Statistical analysis of the results was performed by the method of variational statistics using Student's test.

Experiments have been carried out in compliance with the European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes (Strasbourg, 1986).

### Discussion of the Results

Modeling of chronic hypobaric hypoxia under conditions of natural light in immature female rats resulted in a significant restructuring of the nature of the processes of proteolysis in the gingival tissues (Table 1). In particular, lysis of low molecular weight proteins (LMWP) in the gingival tissues decreased by 15,8 % in comparison with the control, lysis of high molecular weight proteins (HMWP) - by 42,8 %. Lysis of collagen mostly decreased the - by 59,2 % compared with the group that was under conditions of normoxia.

Table 1

Reaction of proteolytic process in the gingival tissues of immature female rats under conditions of hypobaric hypoxia and modified duration of photoperiod (M±m).

№ of group	Conditions of research		Lysis of LMWP, мкг азоalbumin/1g of tissue per 1 hour	Lysis of HMWP, мкг азоказеїн/1g of tissue per 1hour	Lysis of collagen, мкг азокол/1g of tissue per 1hour
1	Natural lighting, normoxia, n=10	M±m	43,71±3,09	37,38±2,90	8,53±0,847
2	Natural lighting, hypoxia, n=10	M±m	36,82±6,52	21,36±1,80	3,48±0,331
		p	0,187	0,000	0,000
3	Permanent lighting, normoxia, n=10	M±m	70,08±5,37	46,03±3,78	15,33±1,17
		p	0,000	0,049	0,000
4	Permanent lighting, hypoxia n=10	M±m	47,90±4,12	25,10±1,97	4,970±0,406
		p	0,222	0,001	0,001
		p <sub>1</sub>	0,002	0,000	0,000
		p <sub>2</sub>	0,051	0,037	0,000

Note in table 1: p – criterion of probability of difference in comparison with group 1; p<sub>1</sub> - criterion of probability of difference in comparison with group 3; p<sub>2</sub> - criterion of probability of difference in comparison with group 2

Processes of proteolysis in the gingival tissues of females were undergone of opposite changes by application of constant light. Thus, LMWP lysis increased by 1,6 times, lysis of azokazein - by 23,1 %, and lysis of collagen - increased by 1,8 times in comparison with the control one. Compared with indices of animals held in hypoxia only, all indicators have increased even more: respectively by 1,9 times for LMWP lysis, 2,2 times for HMWP lysis and maximally by 4,4 times for collagen lysis. Thus, constant lighting is more stressful and damaging

environmental factor then hypoxia level used. Simultaneous action of hypobaric hypoxia and constant lighting led to decrease of proteolysis indicators concerning all kinds of protein molecules, comparing to group of animals held in constant lighting and normoxia, and indicators were practically equal to group of animals held in hypoxia only, excluding LMWP lysis, which intensity was higher, then in only hypoxic condition. Thus, it can be argued that hypoxia does a compensatory effect on the lysis of high molecular weight proteins and

collagen, increased in melatonin deficiency caused by constant illumination. But, low molecular weight proteins permanently are damaged by effect of the constant lighting, in hypoxia as well as in normoxia conditions.

Draws attention that intensity of tissue proteolysis significantly increases under conditions of constant illumination both with hypoxia, that may be an evidence of intensification of elimination of oxidation-modified protein molecules, formed by reduction of antioxidant potential of tissue at melatonin deficiency [5].

Detected peculiarities of the reaction of the tissues proteolysis indices in the gingival tissues in immature female rats to the applied factors and their combinations, indicate different intensity of the process of adaptation under various conditions. Consequences of hypobaric hypoxia influence in case of hypoxic conditioning of photoperiodic changes are leveled by hypofunction of the pineal gland, that result in "normalization" of indices, that is their approaching to the control ones. However, this may be also indicate of disturbance of adaptation process, that subsequently may lead to the development of pathology.

### Conclusions

1. Modeling of chronic hypobaric hypoxia under conditions of natural light in immature female rats resulted in a decreases of intensity of the lysis of LMWP in the gingival tissues by 15,8 %, lysis of HMWP - by 42,8 %, and lysis of collagen by 59,2 % in comparison with the control.

2. Modeling of the decreased melatonin-producing function of the pineal gland by application of constant lighting resulted in significant increase of the activity of proteolytic processes in the gingival tissues in immature female rats, that may testify to intensification of elimination of oxidation-modified protein molecules, formed by reducing of tissues antioxidant capacity according to melatonin deficiency.

3. Simultaneous action of hypobaric hypoxia and constant lighting led to decrease of proteolysis indicators concerning all kinds of protein molecules, comparing to group of animals held in constant lighting and normoxia, and indicators were practically equal to group of animals held in hypoxia only, excluding LMWP lysis, which intensity was higher, then in only hypoxic condition, that probably indicates of the hypoxic compensatory effect on the lysis of high molecular weight proteins and collagen in female rats gingival tissues, increased in melatonin deficiency caused by systemic effect of the constant illumination.

### Perspectives of the research

Mechanisms of participation of proteolysis processes in their interaction with the processes of peroxidation of lipids and proteins and their role in ensuring gingival tissues reaction under conditions of hypoxic preconditioning of the damaging effects of the modified duration of photoperiod by moderate hypobaric hypoxia require further investigation and comprehensive analysis.

**Literature.** 1. Білоклицька Г.Ф. Структура захворювань тканин пародонта у жінок з фізіологічною та хірургічною менопаузою, ускладненою посткастраційним синдромом / Г.Ф. Білоклицька, Г.В. Погребняк // Галицький лікарський вісник.-2004, Т.11, №1.-С.133-136. 2. Веремеєнко К.Н. Белковые ингибиторы плазмы крови - регуляторы активности протеолитических ферментов / К.Н. Веремеєнко // Системная энзимотерапия. Теоретические основы, опыт клинического применения. -К.: МОРИОН, 2000. - С. 21-53. 3. Горбань Е.Н. Влияние хронической гипоксической тренировки на уроне стабильных метаболитов в крови, тканях сердца и аорты взрослых и старых крыс в отдалённые сроки после облучения / Е.Н. Горбань, Е.В. Подъяченко, Н.В. Топольникова // Пробл. старения и долголетия.- 2013.-Т.22, прилож. - С.21-22. 4. Горбань Е.Н. Уровни экспрессии мРНК HIF-1 $\alpha$  и HIF-3 $\alpha$  в ткани сердца взрослых и старых крыс после однократного облучения и предшествующей интервальной гипоксической тренировки / Е.Н. Горбань, Т.И. Древицкая, Н.В. Топольникова // Современные аспекты геронтологии и гериатрии: от теории к практике: научн.-практ. конф. и школа, посвящ. 90-летию со дня рождения акад. В.В. Фролькиса: Мат. конф. (Киев, 26-27 мая 2014 года). - К., 2014. - С.45-46. 5. Заморский И.И. Участие мелатонинергической системы организма в механизмах немедленной адаптации к острой гипоксии/ И.И. Заморский // Кліні. та експерим. патол.-2012.-Т.ХІ, №3 (41), Ч.1.-С.74-76. 6. Карпов Л.М., Возможность коррекции витаминными группы В та їх похідними енергетичного обміну у мишей в умовах гіпобаричної гіпоксії / Л.М. Карпов, Л.Г. Савлущинська, Н.В. Полтавцева, Л.А. Преснова, В.В. Пеню // Вісник Харківського національного університету ім.В.Н. Каразіна. Серія: біологія. Вип. 18, № 1079, 2013.- С. 24-28. 7. Мойбенко А.А., Досенко В.Е., Нагибин В.С. Ферментативные механизмы апоптоза // Патолог. физиология и експерим. терапия.-2005.-№3.-С.17-26. 8. Пішак В.П. Фотоперіодизм і функціонування репродуктивної системи у людини / В.П. Пішак // Міжнародн. ендокрин. журн.-2013.-№2(50).-С.77-80. 9. Подгаєцька О.С. Пошкодження тканин пародонта при експериментальному стресі та його корекція за допомогою інтервальних гіпоксичних тренувань / О.С. Подгаєцька, І.М. Маньковська, К.В. Розова [та ін.] //Фізіол. журнал.-2006.- Т.52, №2.- С.152. 10. Сазонтова Т.Г., Архипенко Ю.В. Роль свободнорадикальних процесів и редокс-сигналізації в адаптації організму к изменению уровня кислорода // Росс. физиол. журнал им. И.М. Сеченова.-2005.-Т.91, №6.-С.636-656. 11. Топольникова Н.В. Влияние интервальной гипоксической тренировки на показатели углеводного и липидного обменов у взрослых и старых крыс после однократного облучения / Н.В. Топольникова, Е.Н. Горбань, Е.В. Подъяченко // Современные аспекты геронтологии и гериатрии: от теории к практике: научн.-практ. конф. и школа, посвящ. 90-летию со дня рождения акад. В.В. Фролькиса: Мат. конф. (Киев, 26-27 мая 2014 года). - К., 2014. - С.85-86. 12. Ясінська О.В. Особливості фотоперіодичних змін прооксидантних процесів, антиоксидантної системи та надниркових залоз за умов екзогенної гіпоксії: автореф. дис. на здобуття наук. ступеня канд. мед. наук: спец.14.03.03 "Нормальна фізіологія" / О.В. Ясінська.-Вінниця, 2007.-24 с. 13. Groenendaal F. Selection of babies for intervention after birth asphyxia / F. Groenendaal, L.S. de Vries // Semin. Neonatale.-2000.-Vol.5, N1.- P.17-32. 14. Гьнсь G.N. Effects of endogenous sex hormones on the periodontium - Review of literature / G.N. Гьнсь, T.F. Тизъм, F. 3 aglayan // Australian Dental Journal.-2005; 50: (3):138-145. 15. Mark W. Dewhirst Cycling hypoxia and free radicals regulate angiogenesis and radiotherapy response / Mark

W. Dewhirst, Yiting Cao, Benjamin Moeller // Nature Reviews Cancer.-2008.-P.425-437. 16.Periodontitis and systemic diseases - proceedings of a workshop jointly held by the European federation of periodontology and American academy of periodontology // Journal of periodontology Online.-2013, Vol.84, №4-s. - S.164-S.209. 17.Zayour Dany Endocrine changes in a rat model of chronic hypoxia mimicking cyanotic heart disease / Zayour Dany, Azar Sami T., Bitar Fadi F. // Endocr.Res.-2003.- 29, № 2. - С.191-200.

**РЕАКЦИЯ ТКАНЕЙ ДЕСЕН НЕПОЛОВОЗРЕЛЫХ САМОК КРЫС НА ДЕЙСТВИЕ ГИПОБАРИЧЕСКОЙ ГИПОКСИИ ПОД ВЛИЯНИЕМ ИЗМЕНЕННОЙ ДЛИТЕЛЬНОСТИ ФОТОПЕРИОДА**

*Р.Р. Дмитренко, Г.И. Ходоровский, Е.В. Ясинская*

**Резюме.** Изучены особенности реагирования показателей протеолиза в тканях десен неполовозрелых самок крыс на действие гипобарической гипоксии в условиях самостоятельного её применения и одновременно с постоянным освещением. Установлено, что моделирование гипобарической прерывистой гипоксии, эквивалентной высоте 4000 м над уровнем моря (по 2 часа ежедневно в течение 14 суток) снижает интенсивность процессов протеолиза в тканях десен неполовозрелых самок крыс. Гипофункция эпифиза, обусловленная постоянным освещением, значительно влияет на характер изменений процесса протеолиза в тканях десен неполовозрелых самок крыс, вызванных системной гипобарической гипоксией при условии одновременного применения указанных влияний.

**Ключевые слова:** тканевой протеолиз, фотопериод, гипобарическая гипоксия, ткани десен, неполовозрелые самки

крыс.

**РЕАКЦІЯ ТКАНИН ЯСЕН СТАТЕВОНЕЗРІЛИХ САМИЦЬ ЩУРІВ НА ГІПОБАРИЧНУ ГІПОКСІЮ ПІД ВПЛИВОМ ЗМІНЕНОЇ ТРИВАЛОСТІ ФОТОПЕРІОДУ**

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**Резюме.** В даному дослідженні вивчені особливості реагування показників протеолізу в тканинах ясен статевонезрілих самиць щурів на дію гіпобаричної гіпоксії за умов самостійного її застосування та поєднано із постійним освітленням. Встановлено, що моделювання гіпобаричної переривчастої гіпоксії, еквівалентної висоті 4000 м над рівнем моря (по 2 години щоденно протягом 14 діб) знижує інтенсивність процесів протеолізу в тканинах ясен статевонезрілих самиць щурів. Гіпофункції епіфіза, зумовлена постійним освітленням, впливає на характер змін процесу протеолізу в яснах щурів за системної гіпоксії при поєднанні застосованих впливів.

**Ключові слова:** тканинний протеоліз, фотоперіод, гіпобарична гіпоксія, тканини ясен, статевонезрілі самиці щурів.

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