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PHOTOPERIOD-INDUCED ALTERATIONS OF CARBONYL DERIVATIVES OF OXIDATIVELY MODIFIED PROTEINS IN THE HEPATIC TISSUE OF MALE RATS OF DIFFERENT AGES AND PHYSIOLOGICAL REACTIVITY

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There are individuals in animal populations and humans that significantly differ in the sensitivity to oxygen deficiency. The reasons for the differences in the resistance of organisms to oxygen deficiency have been comprehensively analyzed by some authors (Lukyanova and Kirova, 2015; Krzywinska and Stockmann, 2018) but are still not quite clear. It has been shown that resistance to hypoxia depends on the intensity of oxygen consumption, metabolism peculiarities, behavioral reactions, and many other individual differences. In a multidisciplinary study of adaptative reactions in animal and human organisms to environmental factors, M.A. Veloso (1981) concluded that such reactions in populations from different regions are not associated with genetic features. In contrast to these data, other authors concluded that genetic individuality predetermines the features of organism reactivity in acute hypoxia (Serebrovskaya and Xi, 2012). The genetic determination of resistance to hypoxia is confirmed by findings on numerous polymorphisms of the HIF1A gene (Kobayashi et al., 2013; Strauss et al., 2015).

Findings reported by various authors have revealed daily rhythmic sensitivity of animals to oxygen deficiency: in the evening and night hours, the lifetime in hypobaric hypoxia is lower than in the daytime (Masukawa and Tochino, 1993). There are seasonal fluctuations in hypoxia resistance, i.e. the minimum number of low-resistant individuals is registered in the autumn-winter period, and the maximum number – in summer (Chernobayeva and Lukyanova, 1989). It was shown that glucocorticoids increase the expression of genes responsible for the development of adaptation mechanisms in response to hypoxia (Kodama et al., 2003).

Thus, the aim of our study was the evaluation of carbonyl derivatives of protein oxidation in the liver of male rats of different ages with high (HR) and low resistance (LR) to hypoxia in four photoperiods (winter, spring, summer, autumn). In the current study, we observed that rats with low and high resistance to hypoxia had different age-related levels of oxidatively modified proteins, depending on the photoperiods.

Protein carbonyl content is the most widely used marker of oxidative modification of proteins. It helps to demonstrate that oxidative damage to proteins correlates well with aging and the severity of some diseases. A commonly measured modification is carbonyl formation, which can occur in lysine, arginine, serine, threonine, and proline residues following metal-catalyzed oxidation or hypochlorous acid attack. Carbonyl groups react with DNPH (2,4-dinitrophenylhydrazine) and other aldehyde reaction probes, such as N'-amino-oxymethylcarbonylhydrazino-D-biotin, offering the potential for colorimetric detection or selective enrichment approaches (Verrastro et al., 2015).

The study was carried out on 96 white nonlinear male rats divided into 16 groups by resistance to hypoxia (LR, low resistance, HR, high resistance) and age, i.e. 6 and 21 months old. Rats were randomly assigned to sixteen groups. There were six animals in each group. The studies were conducted at four seasonal points: winter (January), spring (March), summer (July), autumn (October). The ratio of day/night in different seasons was: winter – 8:16, spring – 12:12, summer – 16:8, autumn – 10:14.

Previously, the animals were divided into 2 groups: LR and HR. The resistance of rats to hypoxia was evaluated as survival time (min) in the altitude chamber 11,000 m above sea level. Survival time was measured after achieving the altitude. Cessation of breathing served as the criterion for resistance to hypoxia (Kurhaluk et al., 2018; Dzhalilova et al., 2018; Lukyanova et al., 2018). Animals with a maximum survival time after the second agonistic breath were classified as high-resistance animals, and those with a minimal survival time as low-resistance animals. After the survival assessment, animals were housed for at least 3 weeks in vivarium conditions to adapt.

The male rats were housed at a constant temperature of 20 ± 2 °C. The animals had free access to feed and water throughout the experiments. During the study, animals were kept on a standard diet and temperature conditions under natural lighting. The influence of artificial light sources was prevented. Blood sampling was chosen for the peak secretion of melatonin it is from 2.00 to 4.00 AM.

Tissues were removed from rats after decapitation. One rat was used for each homogenate sample. The liver was excised, weighed, and washed in an ice-cold buffer. The Bradford method (1976) with bovine serum albumin as a standard was used for the quantification of proteins. Absorbance was recorded at 595 nm.

The rate of aldehydic (AD) and ketonic derivatives (KD) of protein oxidative destruction was estimated from the reaction of the resultant carbonyl derivatives of amino acid reaction with 2,4-dinitrophenylhydrazine (DNFH) as described by Levine et al. (1990) and modified by Dubinina et al. (1995). DNFH was used for determining carbonyl content in soluble and insoluble proteins. Carbonyl groups were determined spectrophotometrically from the difference in absorbance at 370 nm (aldehydic derivatives, OMP_{370}) and 430 nm (ketonic derivatives, OMP_{430}).

The results were expressed as mean ± S.D. Before analysis, all variables were tested for normal distribution using the Kolmogorov-Smirnov test ($p>0.05$), and homogeneity of variance was assessed using Levene's test. The significance of differences in the level of amino acid carbonyl derivatives value between all examined groups was determined using one-way analysis of variance (ANOVA) according to Zar (1999). Differences were considered significant at $p<0.05$. All statistical calculation was performed on separate data from each individual with Statistica 13.3 software (StatSoft Inc., Poland).

In the groups of adult rats with LR, the minimum level of aldehydic derivatives (AD) of oxidatively modified proteins (OMP) was noted in autumn, while their maximum content was recorded in winter, respectively. A similar trend of these markers was observed in the groups of the adult animals with HR: the minimum level of aldehydic derivatives was observed in autumn, and the maximum value was obtained in winter. Aging in the rats with LR was accompanied by increased protein oxidation and formation of aldehydic derivatives, compared to both the adult animals and the groups of individuals with HR. The minimum level of OMP in the hepatic tissue was observed in this group in spring, and the maximum amount was noted in winter and autumn. A lower level of protein oxidation biomarkers was observed in the old animals with HR, i.e. the lowest level of aldehydic derivatives was shown in summer, and the highest content was recorded in autumn (table).

Table – Aldehydic and ketonic derivatives of protein oxidation (nmol·mg-1 protein) in the hepatic tissue of male rats of different ages (Adult – 3 months old, Old – 21 months old) and physiological reactivity (LR – low resistance to hypoxia and HR – high resistance to hypoxia) in different photoperiods (winter, spring, summer, autumn)

Photoperiods/Parameters	Adult rats		Old rats	
	LR	HR	LR	HR
	Aldehydic derivatives, nmol·mg^{-1} protein			
Winter	5.51±0.23	5.52±0.48	10.17±0.99$^{\#}$	7.49±2.76
Spring	2.97±0.45$^{a'}$	2.60±0.88	3.65±0.97	5.00±0.68$^{*\ \#}$
Summer	5.32±2.03$^{d'}$	3.99±1.51	5.09±0.58	3.64±0.57
Autumn	2.28±0.51$^{f'}$	1.92±0.48	10.20±0.67$^{\#}$	10.60±0.51$^{\#}$
	Ketonic derivatives, nmol·mg^{-1} protein			
Winter	8.70±0.19	8.78±0.37	17.30±2.82$^{\#}$	14.31±1.39$^{\#*}$
Spring	4.01±0.98$^{a'}$	2.92±0.89$^{*\ a'}$	6.56±1.27$^{\#\ a''}$	4.64±1.41$^{*\#\ a''}$
Summer	6.42±0.62$^{b'd'}$	3.99±1.51$^{*\ b'}$	5.98±1.71$^{b''d''}$	4.08±0.61$^{b''}$
Autumn	2.72±0.83$^{c'e'f'}$	1.84±0.53$^{c'e'f'}$	14.33±1.07$^{\#e''f''}$	13.57±2.41$^{\#e''f''}$

The results are expressed as mean ± S.D. The differences between the experimental groups ($n = 6$) were analyzed using one-way ANOVA and Bonferroni post-hoc test. The differences were considered statistically significant at $p < 0.05$.

Significant differences between groups are designated as follows: *– Low resistant group vs. High resistant group in one photoperiod; # – Adult group vs. Old group in one photoperiod. In the Adult groups: a'– Winter group vs. Spring group; b' –

Winter group vs. Summer group; c' – Winter group vs. Autumn group; d' – Spring group vs. Summer group; e' – Spring group vs. Autumn group; f' – Summer group vs. Autumn group. Into the Old groups: a'' – Winter group *vs.* Spring group; b'' – Winter group *vs.* Summer group; c'' – Winter group *vs.* Autumn group; d'' – Spring group *vs.* Summer group; e'' – Spring group *vs.* Autumn group; f'' – Summer group *vs.* Autumn group.

Adult animals with LR had a minimum level of ketonic derivatives in autumn, while a maximum level was recorded in winter. It should be noted that the difference in the ketonic derivatives of OMP in the groups of the adult animals with HR was higher in winter, and minimum levels were observed in the autumn, which may indicate effective mechanisms of prevention of oxidative stress and systems of the proteolytic breakdown of oxidatively modified proteins. The age-related changes in the rats with LR were accompanied by statistically significant intensification of protein oxidation with maximum and minimum levels in winter and summer, respectively. The old rats with HR were characterized by a minimum level of ketonic derivatives of OMP in summer and a maximum value in winter.

Our current study focused on clarification of the mechanisms of photoperiodicity reflected in the rhythmic functions of the organism influenced by the age and individual physiological reactivity estimated by resistance to hypobaric hypoxia on protein oxidation (aldehydic and ketonic derivatives of oxidatively modified proteins) in the hepatic tissue of rats. Our studies have shown that photoperiods are related to the redox characteristics of pro-oxidative processes and antioxidant defenses against oxidative modification of proteins in old rats with a low resistance to hypoxia, compared to a group of highly resistant adults. Carbonyl derivatives of oxidatively modified proteins depend on metabolic processes in hepatic tissue and exhibit photoperiodical variability in adult and old rats.

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STRESS-INDUCED MITOCHONDRIAL FUNCTIONING IN RAT CARDIAC TISSUE: ROLE OF K_{ATP} CHANNEL MODULATORS AND INTERMITTENT HYPOXIC TRAINING

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Mitochondrial dysfunction and oxidative stress are largely involved in metabolic disorders, aging, cancer, age-related neurodegenerative syndrome, etc. (Bhatti et al., 2017). ATP-sensitive potassium channels (K_{ATP} channels) are a major contributor to