BIOGERONTOLOGY

Effect of Delta Sleep-Inducing Peptide on Oxidative Modification of Proteins in Rat Tissues and Blood during Physiological Aging

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Accumulation of oxidized proteins (evaluated by the levels of carbonyl and SH groups) in tissues of 2-24-month-old rats (spleen>myocardium>testicles>liver>skeletal muscles) has been demonstrated. Exogenous delta sleep-inducing peptide injected subcutaneously to rats of different age in a dose of 100 μg/kg by monthly 5-day courses protected proteins of the studied tissues from oxidation; its effect was tissue-specific. Delta sleep-inducing peptide exhibited a hypoglycemic effect: it prevented nonenzymatic glycosylation of hemoglobin and reduced the level of defective protein molecules during aging.

Key Words: *delta sleep-inducing peptide; aging; oxidative modification of proteins*

Prolongation of human life span in countries with well-developed economy and the desire to improve its quality led to high demand of new effective drugs preventing early aging and age-associated diseases. Recent studies demonstrated good prospects of peptide bioregulators as geroprotectors [11]. One of interesting agents is synthetic analog of delta sleep-inducing peptide (DSIP) with the following amino acid composition: WAGGDASGE. A wide spectrum of its biological effects has been demonstrated [5], including the geroprotective effect [14]. The molecular mechanisms of these effects in aging are unknown and deserve detailed studies.

Aging is an age-associated fractal increase in the number of deviations from homeostasis at the molecular, subcellular, cell and tissue, and systemic levels. Homeostasis disorders promote the development of age-associated diseases (oncogenesis, autoimmune and neurodegenerative diseases, *etc.*) [6]. Aging and age-associated diseases are often caused by accumulation with aging of oxidative lesions of DNA, proteins, lipids, and other macromolecules essential for life [1]. Oxidative damage to DNA and lipids, caused by active oxygen and nitrogen species, are sufficiently well studied. Free-radical modification of proteins is seemingly not as important for cell survival as lipid oxidation (which can cause cell lysis) or DNA oxidation (which can cause mutations). However, free-radical modification can lead to loss of protein function; moreover, living organisms can use protein modification of this kind for signaling, cell-cell communication, and for getting information from the environment [4]. Evaluation of oxidized protein content is widely used for evaluating the intensity of age-associated oxidative stress *in vivo*. Geroprotective functions of the host should be improved at a total systems level for effective control

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of aging. A characteristic feature of unfolding aging process is different onset of the process in different tissues, organs, systems and its heterokinetic nature (development of age-associated changes with different velocity: in some tissues these changes emerge rather early and progress slowly and more or less smoothly, while in others they start later, but progress more rapidly) [9].

We studied the effects of DSIP on oxidative modification of proteins (OMP) in rat tissues and blood during physiological aging.

MATERIALS AND METHODS

Experiments were carried out on 2-24-month-old outbred male albino rats.

Animals matched for age were monthly injected with DSIP from the age of 2 months. The drug was injected subcutaneously in a dose of 100 μg/kg by 5-day courses. Two-month-old animals served as controls for intact 2-24-mointh-old animals. Intact animals of the corresponding age served as controls for animals treated with DSIP. The animals were kept under standard vivarium conditions on standard diets with free access to water and food. All rats were weighed monthly. The mean body weights and errors of the means were calculated for each group. Animals with tumor growth or inflammatory processes were excluded from the experiment. The time course of age-associated biochemical values was traced for each animal. The tasks of the study were as follows: isolation and purification of total proteins of different tissues (liver, skeletal muscles, myocardium, spleen, testicles) of control and experimental animals [8]; measurements of carbonyl groups in specimens of total proteins of rat tissues by the spectrophotometric method with 2,4-dinitrophenylhydrasine [3], of protein SH groups by reaction with Ellman's reagent [15], and of nonenzymatic glycosylation of hemoglobin in erythrocyte hemolysate by reaction with TBA using GHB-100 kit (PLIVA-Lachema Diagnostica). Plasma glucose was measured by the glucose oxidase method (Glucose-FKD kit), protein was measured by modified Lowry's method [16].

The significance of differences between experimental and control groups was evaluated by Student's *t* test.

RESULTS

The data on the levels of carbonyl and SH groups in proteins from various tissues of rats of different age are summed up in Figs. 1 and 2. Accumulation of oxidized proteins is tissue-specific. By the content of these proteins, the studied tissues can be presented as

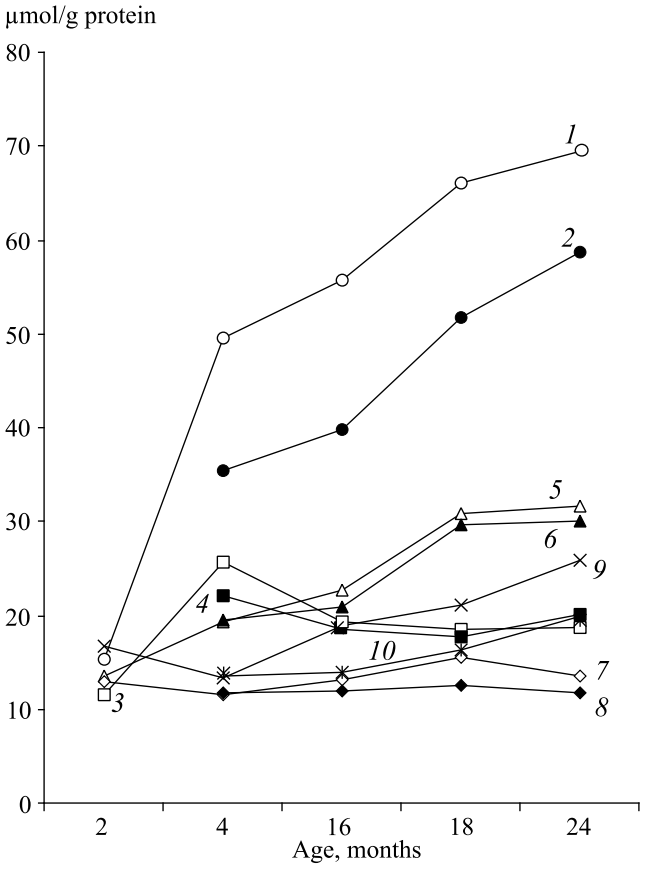

Fig. 1. Effects of DSIP on content of carbonyl groups in total tissue proteins of rats of different age. Here and in Fig. 2: *1*) spleen; *2*) spleen+DSIP; *3*) testicles; *4*) testicles+DSIP; *5*) myocardium; *6*) myocardium+DSIP; *7*) skeletal muscle; *8*) skeletal muscle+DSIP; *9*) liver; *10*) liver+DSIP.

follows: spleen>myocardium>testicles>liver>skeletal muscles. Oxidation of amino acid residues with subsequent formation of carbonyl groups deserves special attention. These groups are formed in oxidation of lysine, arginine, histidine, proline, threonine, glutamic and asparaginic acids. The content of carbonyl groups in splenic proteins of 4-month-old rats increased by 223%, at 16 months by 264%, at 18 months by 331%, and at 24 months by 354% in comparison with their level in 2-month-old animals. Significant changes in the myocardium were found in animals aged 18 and 24 months (by 127 and 132% in comparison with the control). In the testicular proteins the content of carbonyl groups was elevated significantly (by 123%) at the age of 4 months, after which it reduced and surpassed the control levels by 68, 62, and 64% in animals aged 16, 18, and 24 months, respectively. The increment of carbonyl groups in liver proteins of 16-month-old animals was 13%, in 18-month animals 26%, and at 24 months 54% *vs.* control. For skeletal muscles the increase of 19% was found in only 18-month-old animals. Lipid peroxides and products of lipid metabolism (for ex-

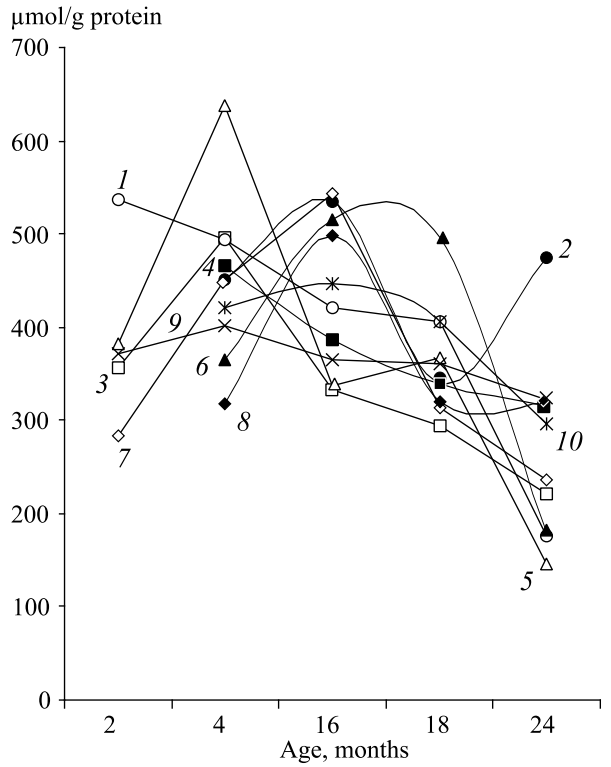

Fig. 2. Effects of DSIP on the content of SH groups in total proteins from tissues of rats of different rats.

ample, MDA) can be responsible for the appearance of additional carbonyl groups in proteins. Reduction of the activities of proteases (particularly cleaving oxidized proteins) can lead to a similar result. Oxidative modification of proteins can be also induced by an increase of free iron concentration and reduction of antioxidant defense [7]. Injection of DSIP to animals of different age leads to reduction of carbonyl group level in splenic proteins by 28% in 4-montn animals, by 29% in animals aged 16 months, by 22% at the age of 18 months, and by 20% at 24 months. For liver proteins the reduction was 27% at 16 months, 23% at 18 and 24 months. In skeletal muscle proteins the parameter decreased by 9% at 16 months, by 18% at 18 months, and by 13% at 24 months in comparison with animals of the same age without DSIP infection. The drug caused virtually no changes in the levels of carbonyl groups in myocardial and testicular proteins.

Cysteine residues in the proteins are particularly sensitive to oxidation of active oxygen, nitrogen, and chlorine. On the other hand, oxidized cysteine forms can be reduced. These processes are supported by special enzymatic systems. Cyclic oxidation/reduction constitues the base for their functioning as regulators of cell processes or as antioxidants. The content of SH groups in splenic proteins reduces with aging: by 22% in animals aged 16 months, by 24% in those aged 18 months, and by 67% in animals aged 24 months (Table 1). The levels of SH groups in the myocardial, testicular, and skeletal muscle proteins of 4-month-old rats increase by 67, 39, and 59%, respectively, while later the parameter reduces: by 62% in myocardial proteins, by 17% in the skeletal muscle, and by 13% in the liver of 24-month-old rats; the levels of SH groups in testicular proteins reduce by 18 and 38% in rats aged 18 and 24 months, respectively, in comparison with the parameter at 2 months. Life-time DSIP treatment leads to increase of SH group levels in comparison with their levels in animals of the same age without DSIP treatment. The levels of SH groups in splenic proteins increase by 27% at 16 months, by 170% at 24 months. In the myocardial proteins the parameter increased by 54% at 16 months, by 35% at 18 months, and by 24% at 24 months. In the testicular proteins SH group levels increase by 16% at 16 months, by 15% at 18 months, and by 44% at 24 months. In the skeletal muscle proteins the parameter increases by 35% in animals aged 24 months. Hence, our results indicate a lesser oxidation of proteins in tissues of rats of different age treated by DSIP. Presumably, this effect is a result of antioxidant activity of DSIP [2].

Glycation is now suggested to be referred to OMP. Along with lipid peroxides, glucose is an active modifier, reacting with proteins, amino acids, and nucleic acids. Reduction of glucose concentration leads to reduction of the levels of glycosylated proteins and LPO. Nonenzymatic glycosylation of biologically significant molecules is now becoming a more and more important trend in studies of diabetes and normal aging [12]. The best studied glycosylated protein is glycosylated hemoglobin, widely used as integral indicator of glycemia [10]. Our findings on age-associated time course of the concentrations of glucose and glycosylated hemoglobin are presented in Table 1.

Plasma glucose levels in animals aged 6, 8, 12, 16, 18, 20, 22, and 24 months increased by 15, 19, 25, 17, 26, 31, and 43%, respectively, in comparison with animals aged 2 months. Blood levels of glycosylated hemoglobin also increased with aging. We found an elevation of this parameter by 16% in animals aged 8 months, by 23% at 12 months, by 50% at 16 months, by 57% at 18 months, by 51% at 22 months, and by 79% at 24 months in comparison with 2-month-old rats.

No appreciable changes in glycosylated hemoglobin levels were found in animals aged 4 and 6 months in comparison with 2-month-old rats. Treatment of animals of different age by DSIP promoted a reduction of plasma glucose concentrations by 21% at 12 months, by 17% at 16 months, by 25% at 18 months, by 19% at 20 months, by 27% at 22 months, and by 23% at 24 months in comparison with intact animals of the same age groups. The levels of glyco-

TABLE 1. Plasma Glucose and Glycosylated Hemoglobin in Erythrocytes in Rats of Different Age Injected and Not with DSIP (*M*±*m*, *n*=4-10)

Group, age	Glucose, mmol/liter	Glycosylated hemoglobin, μmol fructose/g Hb
2 months	4.73±0.08	4.15±0.14
4 months	4.58±0.16	4.09±0.19
4 months+DSIP	4.95±0.17	4.55±0.19
6 months	5.42±0.14*+	4.53±0.25
6 months+DSIP	5.14±0.15	4.32±0.10
8 months	5.61±0.17*	4.82±0.22*
8 months+DSIP	5.30±0.13	4.38±0.11
12 months	5.93±0.14*	5.09±0.27*
12 months+DSIP	4.70±0.22x	4.78±0.31
16 months	5.53±0.18*	6.22±0.16*+
16 months+DSIP	4.62±0.16x	4.33±0.33x
18 months	5.98±0.18*	6.50±0.38*
18 months+DSIP	4.50±0.15x	5.17±0.37x
20 months	6.18±0.14*	6.30±0.26*
20 months+DSIP	4.98±0.14x	4.94±0.25x
22 months	6.88±0.27*+	6.27±0.50*
22 months+DSIP	5.05±0.17x	5.10±0.27x
24 months	6.74±0.20*	7.43±0.20*+
24 months+DSIP	5.21±0.10x	5.15±0.22x

Note. $p<0.05$-0.001 in comparison with: *2-month-old animals, +previous age group, xanimals without DSIP treatment.

sylated hemoglobin reduced under the effect of DSIP: by 30% at 16 months, by 21% at 18 months, by 22% at 20 months, by 19% at 22 months, and by 31% at 24 months in comparison with intact animals of the same age. Treatment by DSIP caused no appreciable changes in these parameters in animals aged 4, 6, and 8 months. Presumably, DSIP is involved in regulation of pancreatic hormones (insulin and glucagon) secretion and release. Intravenous ^{3}H-DSIP accumulates in peripheral tissues, the highest radioactivity found in the pancreas [13]. Elevation of blood insulin concentration under conditions of DSIP treatment has been described. In addition, DSIP effects on the plasmatic membrane presumably stimulate the activities of glucose transmitter proteins, this stimulating cell sensitivity to insulin, which causes redistribution of transporter proteins from the cytoplasm to the membrane.

Hence, our results indicate that DSIP protects tissue and blood proteins from oxidative modification during physiological aging, and its effect is tissue-specific.

REFERENCES

1. V. N. Anisimov, *Molecular and Physiological Mechanisms of Aging* [in Russian], St. Petersburg (2008).
2. T. I. Bondarenko, N. A. Zhigalova, E. A. Maiboroda, and I. I. Mikhaleva, *Vopr. Biol. Med. Farm. Khim.*, No. 1, 38-43 (2010).
3. E. E. Dubinina, S. O. Burmistrov, D. A. Khodov, and G. E. Borotov, *Vopr. Med. Khim.*, **41**, No. 1, 24-26 (1995).
4. V. I. Lushchak, *Biokhimiya*, **72**, No. 8, 995-1017 (2007).
5. I. I. Mikhaleva and B. O. Voitenkov, *Nov. Lek. Prep.*, No. 3, 6-20 (2007).
6. A. A. Moskalev, *Uspekhi Gerontol.*, **23**, No. 1, 9-20 (2010).
7. L. E. Muravleva, V. B. Molotov-Luganskii, and D. A. Klyuev, *Fundamental'n. Issled.*, No. 1, 74-77 (2010).
8. N. V. Pushkina and A. I. Lukash, *Izv. SKNTsVSh., Natural Sciences*, No. 2, 95-97 (1976).
9. G. A. Ryzhak and S. S. Konovalov, *Geroprotectors and Prevention of Age-Associated Diseases* [in Russian], St. Petersburg (2004).
10. E. V. Tereshina, *Manual of Gerontology* [in Russian], Moscow (2005).
11. V. Kh. Khavinson and V. N. Anisimov, *Uspekhi Gerontol.*, **22**, No. 1, 11-23 (2009).
12. F. S. Facchini, N. W. Hua, C. M. Reaven, and R. A. Stoohs, *Free Radic. Biol. Med.*, **29**, No. 12, 1302-1306 (2000).
13. M. V. Graf, H. P. Lorez, D. Gillessen, *et al.*, *Experientia*, **37**, No. 6, 625-627 (1981).
14. I. G. Popovich, B. O. Voitenkov, and V. N. Anisimov, *Mech. Ageing Dev.*, **124**, No. 6, 721-731 (2003).
15. P. W. Riddles, R. L. Plakeley, and B. Zermer, *Anal. Biochem.*, **94**, No. 2, 75-81 (1979).
16. G. R. Schacterle and R. L. Pollack, *Anal. Biochem.*, **51**, No. 2, 654-655 (1973).